PREVENTION AND TREATMENT OF AIDS

Organizer: Dani P. Bolognesi

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Virus/Host Cell Interactions

Q 001 INFECTION OF CD4-NEGATIVE CELLS BY HIV-2, Robin A. Weiss and Paul R. Clapham, Chester Beatty Laboratories, Institute of Cancer Research, London, England.

A subset of CD4-negative human cell lines is sensitive to infection and cell-fusion by HIV-2, but not by SIV or HIV-1. Most, but not all, HIV-2 strains require activation by soluble CD4 (sCD4) in order to effect fusion, indicating that conformational changes in the envelope are required before CD4-independent infection can occur. Soluble CD4-IgG chimeric molecules, and sCD4 complexed with monoclonal antibodies to the D3 domain block HIV-2 infection, even by the strain (LAV-2/B) which is not dependent on sCD4 activation. The CD4-IgG molecules do not prevent HIV-2 binding to CD4 cells but block fusion after virion attachment. Certain $CD4^+$ T-cell lines have previously been found to have enhanced sensitivity to HIV-2 infection following sCD4 treatment. The infection of these cells by LAV-2/B is not blocked by anti-CD4 antibodies or by sCD4, indicating that they express a distinct receptor for HIV-2 entry. The identity of the alternative HIV-2 receptor on CD4-negative and CD4-positive cells is under investigation.

Workshop

Q 002 A BIOLOGICAL ASSAY FOR DETECTION OF CELL FUSION INDUCED BY CD4-HIVenv INTERACTION. APPLICATION TO MURINE-HUMAN CELL HYBRIDS . Tanya Dragic[1], Pierre Charneau[2], Marc Alizon[1]. [1]ICGM, [2] Institut Pasteur, Paris, France.

Recognition of CD4 by the envelope glycoprotein of HIV (gp120) is sufficient to initiate viral entry in human cells, but not in murine or any other animal cell lines (and also notably in two cell lines of human origin, U87 and SCL1). In all these cases, the block to HIV entry resides at the level of fusion of viral envelope and plasma membrane. Several mechanisms can be proposed to explain this difference of behavior --- permissivity vs. resistance to HIV entry of human vs. animal cells. One usually imagines the need for additional factors only expressed in human cells, and acting in collaboration with CD4, or involved in downstream events. Less likely is the expression in "resistant" cells of specific inhibitors, but no clear evidence for one or the other possibility has yet been provided.

We have attempted the genetic complementation of murine 3T3-CD4 cells by transfection of human genomic DNA, followed by infection with HIV-derived retroviral vectors able to transduce a drug resistance gene. Clones of murine cells carrying an HIVneo vector were selected in several experiments, but none could be reinfected by HIV, which could be due either to unspecific entry of the HIVneo vector, or also to loss or inactivation of the human DNA transferred. To rule out possible elimination of human DNA during the course of selection, we are currently repeating these experiments using human cDNA libraries cloned in expression vectors instead of genomic DNA. Also human U87-CD4 are used as well as 3T3-CD4 as recipient cell lines. But this type of experiment cannot be conclusive unless a DNA fragment or cDNA able to specifically confer HIV permissivity is isolated. A negative result can always be due to technical limitations rather than to an intrinsic unability to complement .

We have therefore taken another angle of investigation by studying heterocaryons made between murine and human cells. Most of these hybrids, obtained by PEG fusion, are growth-arrested, which prevents retroviral integration and forbids to use assays for viral entry based on HIV expression. To circumvent this problem, we have devised a simple assay based on the transactivation of a lacZ reporter gene placed under control of the HIV LTR by the *tat* protein. The source of *tat* is a human lymphoblastic cell line, 8E5, harbouring a HIV provirus defective for reverse transcriptase, but expressing all other viral proteins, including *tat* and *env*. Therefore if 8E5 cells engage fusion with a LTR lacZ containing cell or heterocaryon, transactivation turns it blue in an Xgal assay, and the event can be easily scored. When 3T3-CD4 and HeLa LTR-lacZ cells are fused with PEG, blue heterocaryons can be observed after overnight incubation with 8E5. In contrast, the X-gal assay is negative if 3T3, or other CD4-negative cells are used. In a similar test U87-CD4 / HeLa LTR-lacZ hybrids can fuse to 8E5. These experiments indicate that murine cells, or human U87, do not contain dominant negative inhibitors of HIV entry. To further investigate the genetic basis of complementation, we have constructed 3T3-CD4-LTRlacZ cells, to be fused with somatic hybrids containing various amounts of the human genome. It should be possible to define which human chromosome(s) is (are) necessary to render murine cells permissive to HIV entry.

Q 003 RECEPTOR-MEDIATED ACTIVATION EVENTS IN HIV/SIV VIRAL FUSION, Jonathan S. Allan[1], Evelyn Whitehead[1], Mary Short[1], Timothy Hart[2], Peter Bugelski[2], Raymond Dunham[1], and Patrick Kanda[1], [1]Southwest Foundation for Biomedical Research, San Antonio, TX 78228, [2]SmithKline Beecham Pharmaceuticals, King of Prussia, PA.

The process of viral entry among immunodeficiency viruses begins with receptor-binding and culminates in viral fusion. Our studies have focused on an HIV-related virus from African green monkeys (SIVagm). In contrast to efficient neutralization of HIV-1 infection by soluble forms of its receptor (sCD4), we observed receptor-induced enhancement SIVagm infection (1). This enhancement is a direct consequence of sCD4 binding and the induction of conformational changes on the viral envelope which we have termed "receptor-mediated activation" (2). Incubation of sCD4 with SIVagm led to a 100 fold increase in infectivity and an acceleration in the kinetics of giant cell formation. Study of CD4 mutations has allowed us to determine the regions within the CD4 protein that are required for this enhancement. In general, well-defined HIV-1 gp120 binding regions within the D1 loop of CD4 are also necessary for SIVagm gp120 binding and viral enhancement.

The mechanism of sCD4 neutralization of HIV-1 involves both receptor-induced shedding of gp120 and direct competitive inhibition with cellular CD4 for binding. We theorized that the differential effect of sCD4 on SIVagm infection was related to differences in the intrinsic stability of the viral envelope (2). A more stable heterodimeric association of gp120 with its transmembrane protein for SIVagm might therefore allow for the induction of a stable transitional state that would act in facilitating viral entry whereas a less stable association for HIV-1 results in a loss of important conformations necessary for viral fusion. Our studies thus far have shown that the heterodimers of SIVagm are remarkably stable and resist dissociation in the presence of detergent concentrations which solubilize most protein-protein associations. Furthermore, electron microscopic analysis and biochemical studies revealed that no shedding of gp120 occurred upon sCD4 binding suggesting that the opposing effects of sCD4 on viral entry are at least in part related to differences in the integrity of the viral envelope.

Although neutralizing antibodies have been demonstrated in HIV-1 infected individuals and hyperimmunized animals, there is an apparent lack of available neutralizing epitopes on the envelope of SIVagm. Surprisingly, we have found that sCD4 induces the exposure of cryptic neutralizing domains which are associated with viral fusion. Naturally infected monkeys have neutralizing titers as high as 1:10,000, these antibodies neutralizing sCD4-enhancement (1). Hyperimmune sera generated in rabbits immunized with SIVagm has allowed us to further define these neutralizing responses. Furthermore, this neutralizing effect was demonstrated with Fab fragments from IgG fractions indicating that specific binding to these cryptic sites are required for neutralization. We have mapped the neutralizing epitope(s) to gp120 suggesting that further changes are required in the viral envelope before insertion of the fusogenic domain of gp41. Our recent studies have focused on defining these neutralizing domains with both monoclonal and polyclonal antibodies and to understand the role that these epitopes play in subsequent events in viral fusion. In summary, we hope to exploit these novel neutralizing domains in both vaccine and therapeutic strategies for preventing infection and disease associated with HIV.

1. Allan, J.S., Strauss, J., and Buck, D. (1990) Enhancement of SIV infection with soluble receptor molecules. Science 247:1084-1088.
2. Allan, J.S. (1991) Receptor-mediated activation of immunodeficiency viruses in viral fusion. Science 252: 1322.

Q 004 STRUCTURAL COMPONENTS OF THE HIV ENVELOPE THAT INFLUENCE VIRUS-INDUCED MEMBRANE FUSION AND VIRUS ENTRY, John Dubay, David Einfeld, Patrick Johnston, Susan Roberts, Karl Salzwedel and Eric Hunter. Dept. of Microbiology and UAB Center for AIDS Research, University of Alabama at Birmingham, Birmingham, AL 35294

The biosynthesis and processing of a retroviral glycoprotein involves a series of interactions between the virally-encoded protein and a complex vesicular pathway to the plasma membrane. Immediately following synthesis the core-glycosylated protein must form an oligomeric structure in order to be transported out of the endoplasmic reticulum. By constructing a truncated *env* gene in which the SU coding domain has been deleted, we have determined that the TM protein alone can oligomerize and be transported to the plasma membrane of the cell. This localizes the domain responsible for oligomerization to the TM protein. A potential site for such intermolecular interactions between TM proteins is a recently described leucine zipper-like motif in the external domain of gp41. We have constructed a series of mutations that change the middle *ile* of the proposed leucine zipper to either *leu, val, ala, glu, asp, gly* or *ser*. Pulse-chase experiments indicate that the mutations have no effect on the synthesis, oligomerization, transport or processing of the mutant glycoproteins. The leucine and valine mutants have a wild-type phenotype when assayed in a HeLa T4 fusion assay or a T-cell infection assay. In contrast, all of the other mutations except the change to alanine completely inhibit the ability of the glycoprotein to fuse CD4 expressing cells and mutant virus to infect T cells. The alanine change resulted in an intermediate phenotype in which both cell fusion and infectivity were significantly reduced. Since gp120/CD4 binding is unchanged in the mutants, these results point to an important post-binding role for this region in HIV-1 infection.

Point mutations have been inserted into the 3'-end (C-terminal domain) of the HIV-1 env gene sequence which introduce premature stop codons into the env open reading frame. They result in the synthesis of progressively truncated proteins lacking from 6 to 191 amino acids. None of the mutations prevented normal levels of env gene product biosynthesis, or the assembly and release of virus at wild-type levels, even though proteins truncated by 172 and 191 amino acids were secreted from the cell. When the mutant env genes were expressed in a pol-deficient HIV-1 genome in HeLa-T4 cells, we showed that all of the glycoproteins which retained the membrane spanning domain were able to induce large syncytia. Thus gp41 molecules truncated by 6 to 138 amino acids can still be inserted in a biologically active form in the membrane. Nevertheless, all of the truncations in the C-terminal domain of gp41 abrogated infectivity of the mutant viruses in susceptible T cell lines when culture supernatants were followed for RT activity. In contrast, replication competent forms of both SIV and HIV-2 have been isolated in which the cytoplasmic domain is truncated more than 135 amino acids. We have examined whether the remaining cytoplasmically located amino acids play a role in glycoprotein function by inserting termination codons that eliminate portions or all of this region. Surprisingly, glycoprotein molecules in which the entire predicted cytoplasmic domain has been deleted retain the ability both to induce cell-cell fusion and confer infectivity upon incorporation into virions. Moreover, such mutants retain the ability of those SIV isolates with truncated envelope genes to grow efficiently on human T-cell lines.

In an effort to further clarify the role of the membrane spanning region of the HIV-1 gp41 we have engineered a chimeric *env* gene in which this region is replaced by a phospholipid anchor. The lipid linked protein is efficiently exported to the Golgi complex, proteolytically cleaved and then transported to the plasma membrane. Treatment of cells expressing the chimeric protein with a phospholipase results in efficient removal of the molecules from the surface. Nevertheless, the lipid linked molecules are unable to induce fusion of HeLa-T4 cells, indicating a crucial role for the native membrane anchor in the process of membrane fusion.

Q 005 CD4-INDUCED CONFORMATIONAL CHANGES IN THE HIV ENVELOPE, John Moore[1], Linda Burkly[2], Richard Fisher[2], Sarah Turner[2], Thomas Schulz[1], Jane McKeating[1], Brad Jameson[3], David Ho[4], Robin Weiss[1] and Quentin Sattentau[5], [1]Chester Beatty Labs, London, UK, [2]Biogen, Cambridge, UK, [3]Temple University, Philadelphia, [4]Aaron Diamond, AIDS Research Center, New York, and [5]Centre d'Immunologie de Marseille-Luminy, France.

CD4 serves not only as the principal receptor for HIV, but also as the activator of the virus-cell fusion reaction. Thus a consequence of HIV binding to CD4 is the induction of conformational changes in the viral envelope that presumably prime the fusogenic potential of gp41. Such changes can be observed after sCD4 binding to HIV-1 virions and to the surface of HIV-1-infected cells. They include modulation of V3 loop exposure on gp120, assessed by accessibility to a proteinase and by antibody binding; increased exposure of gp41 epitopes; and in some instances complete dissociation of gp120 from gp41. All of these changes can be induced by sCD4 and also by a cyclised, non-derivitised peptide corresponding to residues 83-101 of CD4 (the CDR-3 loop), which implicates this region of CD4 in post-binding events. Antibodies mapping within CD4 D2 and D3 do not block sCD4 binding to HIV but inhibit several of these conformational changes and prevent HIV infectivity and syncytium formation. Different HIV-1 isolates change their conformations in different ways post-CD4 binding, exemplified by altered susceptibility to inhibition by CD4 mabs mapping outside D1, and by alterations in the rates and temperature dependences of gp120 dissociation from virions after sCD4 binding. In particular, virions of 5 primary HIV-1 isolates relatively resistant to sCD4 neutralisation bind sCD4 with reduced affinity but retain gp120 more strongly post-binding. Weak retention of gp120 on the virion correlates with the adaptation of 2 other primary HIV-1 isolates to growth in transformed T cells. Sequence analysis of these 2 primary viruses and their passaged counterparts indicates that a limited number of amino acid changes may be necessary to confer the gp120 shedding phenotype. We are investigating whether these changes may affect a 'knob and socket' gp120-gp41 linkage structure that we suggest is conserved in all retroviruses and may serve to transduce conformational signals from the SU glycoproteins to activate the fusion potential of the TM glycoproteins.

Q 006 HIV ENVELOPE INTERACTIONS WITH THE HUMAN CD4 RECEPTOR, Martin Rosenberg and Ray Sweet, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

The interaction of the HIV envelope complex with CD4 is a major determinant of viral tropism and viral pathogenicity. Over the past few years, we have examined the nature of this interaction with a goal of developing antagonists of HIV infection. The demise of soluble CD4 (sCD4) and other CD4 analogues in clinical trials is attributed in part to the in vitro resistance of several fresh isolates to inhibition by these agents. However, in collaboration with Irv Chen's laboratory, we found that this resistance contrasted with the high affinity of their soluble gp120 proteins for sCD4. An emerging theme to account for this dicotomy is that the affinity between soluble gp120 and CD4 proteins does not necessarily reflect the affinity of the envelope complex for cell surface CD4. To potentially overcome this and other barriers confronting protein-based strategies, we have focused on low molecular weight antagonists of the interaction of CD4 with gp120. The design of such antagonists is predicated on a molecular definition of this binding site. Our previous studies, and those of others, (1) localized the primary determinants of binding to residues 40-60 within the first domain (D1) of CD4 and (2) revealed that gp120 proteins of divergent isolates of HIV-1, HIV-2 and SIV display a wide range of affinities for CD4. We have now utillized two complementary strategies (detailed in companion poster abstracts) to refine the determinants on CD4 for recognition of gp120. For the 3B/BH10 isolate, in conflict with other recent mutational studies, our complementary strategies identified only 2 probable contact residues which define a highly localized binding site. Extending this analysis to other isolates of HIV-1 and HIV-2, the differential affinity of their soluble gp120 proteins for CD4 is reflected in apparent subtle differences in their recognition of a common binding site. These results suggest a strategy for the design of low molecular weight antagonists.

Molecular Biology

Q 007 REGULATION OF HIV GENE EXPRESSION BY TAT AND TAR.

Michael B. Mathews, Shobha Gunnery, Mark Kessler and Michael Laspia. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

The Tat protein of HIV-1 is a powerful activator of transcription from the viral promoter. Its target is an RNA stem-loop structure encoded by the TAR element located immediately downstream from the transcription start site. A large body of data suggests that TAR RNA serves to draw the Tat protein to the vicinity of the transcriptional initiation site, thereby increasing both the frequency of transcriptional initiation and the efficiency with which transcription complexes successfully elongate through the transcription unit. We have proposed that Tat affects the composition and properties of the transcription complex that assembles at the HIV promoter site in such a way as to enhance both initiation and elongation. The extent to which it modulates these two processes is influenced by the basal transcription rate, such that the initiation effect is small when the basal transcription rate is high (as in the presence of other transcriptional activators or a suitably positioned SV40 DNA replication origin). To explore the mechanism of transcriptional activation by Tat we have used a cell-free transcription system from uninfected HeLa cells. In this system, Tat increases elongation, but (so far) not initiation, in a promoter- and TAR-dependent fashion. Experiments with the detergent sarkosyl suggest that Tat relieves transcriptional repression and counteracts the tendency of transcribing complexes to pause during elongation. In run-on transcription assays, pausing can occur at a large number of sites and appears to give rise to short RNA molecules which accumulate in the cytoplasm after processing. These truncated transcripts have the ability to stimulate protein synthesis *in vitro* by blocking the activation of the protein kinase DAI. DAI, the double-stranded activated inhibitor of translation, is induced by interferon. Thus, the short transcripts may serve to counteract an aspect of the interferon-induced host anti-viral defenses.

Workshop

Q 008 GENETICALLY-DIVERSE SIMIAN-RELATED HUMAN IMMUNODEFICIENCY TYPE-2 VIRUSES IN WEST AFRICA, Feng Gao[1], Ling Yue[1], Albert T. White[1], Peter G. Pappas[1], Joseph Barchue[2], Aloysius P. Hanson[2], Bruce M. Greene[1], Paul M. Sharp[3], George M. Shaw,[1], Beatrice H. Hahn[1], [1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, [2]Liberian Institute for Biomedical Research, Robertsfield, Liberia, [3]Department of Genetics, Trinity College, Dublin, Ireland.

Current understanding of the natural history and phylogeny of human immunodeficiency virus type 2 (HIV-2) is almost exclusively derived from studies of culture-amplified viruses from urban populations experiencing epidemic spread of infection and disease. As an alternative to HIV culture as the only means to obtain sufficient amounts of virus for genetic and biological studies, we have developed a highly sensitive PCR technique which allows us to amplify genetically divergent HIV viral sequences directly from uncultured peripheral blood mononuclear cells. Using this approach, we identified and molecularly characterized HIV-2 viruses in two healthy rural Liberian agricultural workers (F0784, 2238) from whom virus cultivation was repeatedly unsuccessful, and in a symptomatic, culture-positive urban dweller from Cote d' Ivoire (7312A). Sequence comparison of envelope, polymerase, and LTR fragments revealed an unexpectedly high degree of genotypic variation, with up to 23% sequence differences in regions in which most HIV-2 viruses differ by less than 10%. Subsequent phylogenetic analysis identified one virus (HIV-2_{F0784}) to cluster with simian immunodeficiency viruses infecting sooty mangabeys and rhesus macaques (SIV_{SM}/SIV_{MAC}) rather than with other HIV-2 viruses, a second virus (HIV-2_{2238}) to branch off the tree before all previously reported HIV-2 isolates, and a third virus (HIV-2_{7312A}) to represent a likely recombinant between phylogenetically divergent strains. In addition, one subject (F0784) was found to harbor multiply-defective viral genotypes that resulted from G to A hypermutation. These results demonstrate that West African human populations are infected with highly divergent HIV-2 strains, some of which are more closely related to SIV_{SM}/SIV_{MAC} viruses than to any other known virus of human derivation. Such extensive genetic heterogeneity may contribute to observed differences in HIV-2 natural history and indicates that HIV-2 has either been present in humans for a very long time, like SIV_{AGM} in African green monkeys, or has resulted from repeated transmission of genetically divergent SIVs from nonhuman primates to man. The results of the present study would thus suggest that viruses from feral monkey populations and humans living in rural and remote jungle areas of Africa should be targeted in a search for the origins of these human and simian immunodeficiency viruses and the events leading to their recent epidemic spread.

Q 009 HIV STRUCTURAL PROTEIN GENES AND THEIR REGULATION, David Rekosh, Marie-Louise Hammarskjöld, Alan Smith, Nancy Lewis, Xiaobin Lu, Hongbo Li, N. Srinivasakumar, Helga Carlsdottir, Susan Prasad and R. Meis, Departments of Biochemistry and Microbiology, State University of New York at Buffalo, Buffalo, NY 14214

Expression of the HIV structural genes *gag, pol,* and *env* has been achieved using SV40 based vectors. These constructs were used to study HIV particle formation and regulation of *env* gene expression by rev.

For *gag* and *gag-pol*, three constructs were created to produce both the gag and gag-pol precursors together or individually. In all three cases, the encoding DNA was cloned downstream of the SV40 late promoter and upstream of the HIV rev responsive element (RRE). Expression required co-expression with rev. Simultaneous expression of both gag and gag-pol produced particles which had condensed cores characteristic of HIV-1, while expression of gag alone produced immature particles. In contrast, expression of gag-pol by itself failed to produce particles, although intracellular processing of the precursor occured. Particle formation required a functional myristic acid addition signal on gag . The presence of this signal on only gag-pol in cotransfections did not allow particles to form. However, a gag precursor containing a functional myristic acid addition signal could "rescue" a myristic acid negative gag-pol into particles, demonstrating that gag-pol was targeted into particles through an interaction with gag rather than solely through its myristic acid moiety. To further examine the interaction of gag-pol with gag we produced a series of deletion mutations within the p24 region of gag-pol. Interestingly, all of these deletion mutants still processed gag-pol in the cytoplasm when transfected alone. The ability of these molecules to enter into virus particles was also assayed in co-transfection experiments with the vector that produced gag. This has allowed us to determine regions of p24 which are necessary for targeting of gag-pol into particles.

Expression of HIV envelope proteins from the SV40 vector is also regulated by rev. This requires the presence of two *cis* acting elements in the RNA, the rev responsive element (RRE) and the upstream *tat/rev* 5' splice site. Using U1 suppressor mutants we have shown that the *env* mRNA forms a complex with U1 snRNA (PNAS 87,7598, 1990). These results suggested that rev might interact directly with factors involved in splicing to facilitate transport of intron containing RNAs. We have now performed experiments with constructs in which a complete excisable intron was placed upstream of the HIV env gene. These plasmids showed rev independent env expression. However, rev dependence was restored by insertion of a small oligonucleotide containing an additional 5' splice site into one of these constructs. This shows that a splice site can act as a CRS element to retain RNA in the nucleus. We have also been able to specifically immunoprecipitate a RRE-RNA/rev complex from transfected cells, demonstrating that rev binds to RRE-RNA *in vivo*. A significant amount of U1 snRNA was present in the immunoprecipitate. These results support the hypothesis that rev promotes dissociation of splicing complexes enabling efficient nuclear export of incompletely spliced RNA.

Viral Pathogenesis

Q 010 IMMUNOPATHOGENIC MECHANISMS OF HIV INFECTION, Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

Infection with HIV results in an invariable decline in the number of circulating CD4+ T cells. While HIV has been shown to infect both CD4+ T cells and cells of the monocyte/macrophage lineage, we have shown that the principal reservoir for HIV in the peripheral blood is the CD4+ T cell. Using polymerase chain reaction (PCR) techniques, we have found that approximately 1 in 100 circulating CD4+ T cells in AIDS patients are infected with HIV. In asymptomatic HIV-infected individuals, the viral burden is one to two logs lower. By following HIV-infected individuals over time, we have demonstrated that as disease progresses, the viral burden in the peripheral blood CD4+ T cells increases in contrast to those individuals with stable disease. Through the use of a modified polymerase chain reaction technique to detect HIV-specific mRNAs, we have found that active HIV expression can be detected in the vast majority of HIV-infected individuals at all stages of infection. The presence of gag-specific mRNA was closely associated with more advanced immunosuppression. Although progression from an asymptomatic stage to AIDS is associated with an increase in viral burden in the peripheral blood, the relatively low viral burden found early during the course of HIV infection does not explain the immune dysfunction seen early in infection, the progressive diminution of CD4+ T cells, or the lack of regeneration of CD4+ T cells. To address this problem, we have recently examined whether HIV-infected CD4+ T cells in the peripheral blood are an accurate reflection of the total pool of HIV-infected CD4+ T cells in the body. We used quantitative PCR to analyze simultaneously the HIV burden in both peripheral blood and lymphoid tissues from HIV-infected patients with early stage disease. We have found that the peripheral lymphoid tissues maintain a much greater viral burden (from 1/2 to 1 log higher) per given number of CD4+ T cells than does the peripheral blood. Furthermore, lymphoid tissue from HIV-infected individuals contained high levels of HIV-specific RNA as detected by in situ hybridization. Thus, it is very likely that the peripheral lymphoid tissues are important reservoirs of HIV and are significant sites of HIV replication, propagation and cytopathicity. One important question that has yet to be answered is how HIV causes the death of CD4+ T cells. We are presently addressing this question by extending our work into an examination of deletions of V_β subsets of T cells. These studies may explain the progressive depletion of CD4+ T cells and immune dysfunction in individuals with relatively low viral burden in the peripheral blood.

Q 011 PATHOGENESIS OF AIDS-ASSOCIATED KAPOSI'S SARCOMA (KS): ROLE OF IMMUNE STIMULATION RATHER THAN IMMUNODEFICIENCY, Robert C. Gallo, Giovanni Barillari, Luigi Buonaguro, Yanto Lunardi-Iskandar and Barbara Ensoli, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

KS is a tumor of vascular origin particularly frequent in HIV-1 infected homosexual men. Primary spindle cell cultures of vascular origin established from Kaposi's sarcoma (KS) lesions of AIDS patients produce inflammatory and angiogenic cytokines and induce KS-like lesions in nude mice. These data may explain how KS lesions form but did not clarify the role of HIV-1 infection in KS pathogenesis. The first experimental link with HIV-1 infection came from data indicating that the HIV-1 tat gene product (Tat) is released from infected T cells and promotes the growth of the KS cells, and that tat-transgenic mice develop lesions resembling KS. However, Tat had little or no effect on the growth of normal vascular cells, potential cell progenitors of the KS cells. Further, these data did not explain the frequency of KS in HIV infected gay men. Clinical-epidemiological observations suggested that immunoactivation rather than immunodeficiency is frequent in this group, and we found inflammatory cytokines, released by activated immune cells and known to be increased in sera from HIV-1 infected individuals (particularly, IL-1, IL-6, and TNF), induce several effects relevant to AIDS-KS pathogenesis. For example, we found that these cytokines induce normal vascular cells (endothelial and smooth muscle cells) to: 1) become responsive to the mitogenic effect of Tat, 2) acquire spindle cell morphology (typical feature of KS cells in vitro and in vivo), 3) promote growth of KS cells and normal vascular cells, and 4) activate HIV-1 gene expression in systems of true viral latency (rescue of Tat-defective proviruses). These effects of Tat and cytokines are synergistic and obtained at low concentrations as produced in vitro and possibly present in vivo. Further, Tat itself activates the expression of TNF in infected T cells. Thus, Tat may act directly as a vascular growth factor for KS cells and indirectly by inducing production of TNF. These results support our hypothesis that immunostimulation rather than immunodeficiency cooperates with HIV-1 infection in the induction and progression of KS in infected susceptible individuals. Finally, recent results with a new immortalized cell line combined with earlier results of many cell strains from KS specimens leads us to propose that early KS may be a hyperplasia while later stages may progress to frank malignancy.

Q 012 AMPLIFICATION FACTORS OF AIDS PATHOGENESIS, Luc Montagnier, Institut Pasteur, Unité d'Oncologie Virale, Paris.

The contrast between highly visible damages of the immune system and the low virus load during the silent phase of HIV infection suggests some indirect mechanisms amplifying the effects of the virus. We have observed an important general loss of viability of T lymphocytes from HIV infected patients, even at the asymptomatic stage, when the lymphocytes are put in short term cultures. Cell death was shown to be due to apoptosis and we have developed quantitative test to explore the phenomenon. Stimulation with some mitogens and bacterial superantigens increase the apoptotic death *in vitro* of patients lymphocytes, while incubation with a mixture of several cytokines prevent it. Abnormal signalling coming from the interaction of HIV glycoprotein with the CD4 receptor may prime the cells to die by apoptosis when their T receptor meets the appropriate antigens or superantigens, leading to clonal T4 deletion or to deletion of cells harboring particular Vβ chains in the T receptor. Among the candidate superantigens are viral proteins, bacterial proteins, including mycoplasma membrane proteins. These phenomenons are not observed with lymphocytes from chimpanzees inoculated for 7 years with HIV-1 and remaining healthy, indicating some correlation between the *in vitro* observations and AIDS pathogenesis. Similarly antibodies against nucleosomal histones are observed in a large proportion of HIV-infected individuals, in SIV-infected macaques, but not in HIV-1 infected chimpanzees nor in naturally SIV-infected mangabeys. In addition, a particular subset of CD8+ lymphocytes expressing also the CD38 surface antigen, is increased according to AIDS progression, and can be propagated in vitro as cell lines and clones in the presence of two cytokines TNF-α and IL2. The increase of circulating TNF-α in AIDS patients may therefore explain the expansion of this particular subset. Such cells often harbor a γδ phenotype and seem to have cytotoxic activity against T4 cells, even not HIV-infected. TNF-α can be itself induced in monocytes by bacterial infections, including mycoplasmas. Therefore a vicious circle created by HIV, bacterial or viral co-factors and cytokines may sterilize the bone marrow renewal of T cells, impair their differentiation in thymus and finally destroy them by apoptosis.

Workshop

Q 013 STRUCTURE/FUNCTION RELATIONSHIPS IN HIV-1 INFECTION, Cecilia Cheng-Mayer[1], Tatsuo Shioda[2], Muriel Le Guern[3], and Jay A. Levy[1], [1]Cancer Research Institute, UCSF, San Francisco, CA 94143-0128,[2]Institute of Medical Science, University of Tokyo, Tokyo, Japan.

HIV-1 isolates display a high degree of *in vitro* biologic heterogeneity which may be linked to different clinical manifestations of AIDS. The isolates differ in their replication rate and yield, host range tropism, and the ability to induce syncytium formation in the infected cells. Structure/function studies of recombinant viruses generated between HIV-1 have shown that only two amino acid (aa) changes in the *tat* gene are associated with replication rate in some cell types and small changes (10-12aa) in the region of the envelope gp120 that encompasses the V3 domain can determine T-cell line and macrophage tropism, and cytopathicity of the virus. Site-directed studies indicate that a single aa change in the *tat* gene affects its transactivation ability and, hence, the replicative rate of the virus. Single aa change in the V3 hypervariable domain of a virus isolate can affect its cytopathicity and the infection of some T-cell lines. However, modifications in the overall conformation of the V3 loop as a result of aa changes appear to be the major factor involved in determining host range tropism of HIV-1.

Q 014 PREVENTION OF HIV-1 INFECTION IN hu-PBL-SCID MICE, Donald E. Mosier[1], Richard J. Gulizia[1], Paul MacIsaac[1], Bruce E. Torbett[1], Lawrence Corey[2], and Phillip Greenberg[2]; [1]Medical Biology Institute, 11077 North Torrey Pines Road, La Jolla, CA 92037; [2]University of Washington, Seattle, WA 98195

Immunodeficient SCID mice accept xenografts of human peripheral blood leukocytes to yield hu-PBL-SCID mice. Human T cells persist in these mice for several months, where they comprise the majority of cells recovered from the peritoneal cavity (the site of injection), and 1-5% of cell in the spleen, lymph nodes, and peripheral blood. The majority of $CD4^+$ T cells also express CD45RO, indicating that they belong to the activated/memory subset. hu-PBL-SCID mice are susceptible to infection by all tested laboratory and clinical isolates of HIV-1 tested to date (16), and the minimal infectious dose of HIV-1_{IIIB} is 10 $TCID_{50}$. HIV-1 infection of hu-PBL-SCID mice results in increased human immunoglobulin levels and a strain-dependent decrease in CD4 T cells. These features make it possible to evaluate the ability of vaccines and antivirals to reduce viral burden and to reverse the decline in CD4 T cells. We have evaluated protection from virus infection in hu-PBL-SCID mice reconstituted with PBL from vaccinated donors. Four normal donors vaccinated originally with gp160-vaccinia and subsequently boosted repeatedly with recombinant gp160 have been evaluated at 1-2, 10-11, and >15 mo. intervals after boosting in the hu-PBL-SCID model. PBL were injected into 3-14 SCID mice/donor, and 2-4 weeks later, the hu-PBL-SCID animals were challenged with 10^{3-4} $TCID_{50}$ of homologous HIV-1_{IIIB}. Infection with HIV-1 was assessed by virus isolation using co-culture and amplification of HIV-1 proviral *gag* sequences using PCR. For donor 1, 60% (6/10) resisted infection at 1-2 mo. post-boost, 50% at 10 mo. post-boost, and all mice became infected at >15 mo. post-boost. For donor 2, 42% (5/12) of mice resisted infection at 1-2 mo. post-boost, and all animals were infected at 11 and 18 mo. post-boost. Both donor 1 and 2 had strong T cell proliferation responses, but only donor 2 had neutralizing antibody. Donors 3 and 4 had weak T cell responses and no neutralizing antibody, and mice derived from these donors showed minimal (non-significant) protection only at the 1 month post-boost interval. These results suggest that adoptive transfer of protective immunity from vaccinees to hu-PBL-SCID mice is possible, but that cells capable of such transfer persist for <1 year after boosting. Transfer of protective immune responses to hu-PBL-SCID mice correlates with *in vitro* assays of T cell immunity but not with neutralizing antibody levels in the donor vaccinee.

Immune Responses to HIV/SIV

Q 015 EVOLUTION OF ANTIGENIC AND VIRULENCE V3 DETERMINANTS DURING THE CURRENT AIDS EPIDEMIC, Jaap Goudsmit[1], Tom Wolfs[1], Gabriël Zwart[1], Jean-Jacques de Jong[1], Tony de Ronde[2], Thijs Tersmette[3], and Carla Kuiken[1], [1]Human Retrovirus Laboratory, AMC, Amsterdam, [2]State University of Utrecht, Utrecht and [3]Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.

The third variable domain (V3) of the HIV external envelope protein gp120 contains determinants for type specificity of neutralization as well as for distinction in biological phenotype.

To identify amino acids defining antigenic variants, genomic RNA was isolated from serum of HIV infected individuals. Around seroconversion, genomic RNA levels reached a peak and the population of sequences was highly homogenous. In the course of infection, the number of amino acid substitutions accumulated. Fixation of a substitution at amino acid position 308 ($IH^{308}I$) was frequently observed. With the use of 16-meric synthetic peptides, differing only at the 308 position, antibody binding specificity was found to be dependent on this difference. Only in 6% (3 of 47 seroconversions) we observed a switch in antibody specificity at later stages of infection. After the decline of V3-specific antibodies, the simultaneous increase in genomic RNA levels and progression to AIDS, new V3 variants with additional changes occurred, again forming a homogenous population of sequences. These changes coincided with a shift in the virus phenotype from non-syncytium forming to syncytium forming.

Subsequently, chimeric molecular clones, differing only in the V3 domain, were constructed. The V3 regions were derived from two HIV-1 isolates with a non-syncytium inducing (non-SI) non-T-cell tropic and two HIV-1 isolates with a syncytium inducing (SI) T-cell tropic phenotype. When assayed in SupT1 and A301 cells, chimeric viruses with a V3 region derived from the non-SI isolates did not induce syncytia and showed a low level of replication. The chimeric viruses with a V3 region derived from SI isolates did induce syncytia and replicated efficiently. *In vitro* mutagenesis showed that an increase in the capability to induce syncytia and replication rate depended upon changes in the V3 domain at both sites of the tip of the loop.

Data will be presented on the spread of antigenic and virulence V3 determinants in transmitter and recipient pairs and in the population as a whole.

Q 016 IMPORTANT B CELL ANTIGENIC SITES IN HIV AND SIV, Erling Norrby[1], Ewa Björling[1], Astrid Samuelsson[1], Francesca Chiodi[1], Per Putkonen[2], and Gunnel Biberfeld[2], [1]Department of Virology, Karolinska Institute, School of Medicine, Stockholm and Department of Immunology, National Bacteriological Laboratory, Stockholm, Sweden.

The development of means for serology reflecting defined cross-reactivity and for immune interventions in reference to primate lentiviruses demands precise knowledge of the immunochemistry of critical virus components. Linear antigenic sites can be probed with synthetic peptides whereas characterization of conformational antigenic sites requires more complicated analysis. Several dominating antigenic sites have been identified in the glycoproteins as well as in other structural components of HIV types 1 and 2 and SIV. Synthetic peptides have been identified, which can be used as antigens in site-specific serology to distinguish strain, type and type cross-reactive antibody responses. For both HIV-1 and HIV-2/SIV types, the glycoproteins represent components playing a dominant role in immune protection. Both the large glycoprotein of HIV-1, gp 120, and the corresponding component of HIV-2, gp 125, contains variable as well as conserved regions. One of the variable regions, V3, of HIV-1 includes the principal neutralizing domain (PND) which induces the dominating early strain-specific neutralizing antibody response. The corresponding region of HIV-2/SIV is less variable. Still, there are immunodominant linear sites in the V3-homologous region of these viruses as well, although they may have a more carboxy terminal position. This was evidenced by immunization of guinea pigs with KLH-coupled peptides representing selected parts of the large glycoproteins of HIV-2 strain SBL6669, as well as SIV. The HIV-2 immunodominant site reacting with antibodies giving neutralization as well as antibody dependent cell lysis was located at amino acids 311-337. Guinea pig antibodies against peptides representing this site could neutralize not only the homologous virus strain, but also other HIV-2 strains. Two overlapping peptides representing this HIV-2 site or the corresponding SIV site were used alone or together with other peptides with a capacity to induce neutralizing antibodies for primary immunization of cynomolgus macaque monkeys. The two HIV-2 peptides were also used for boostering of animals previously immunized with a vaccinia HIV-2 SBL6669 env construct or with non-replicating whole virus. So far protection against replication was demonstrated under the latter condition of immunization in three out of four monkeys. In order to further characterize the important neutralization site in HIV-2, antipeptide murine monoclonal antibodies were produced. Antibodies with neutralizing ability are mapped for the role of individual amino acids in the epitope with which they react. The final goal of these studies is to develop a synthetic vaccine for immunization against HIV-2 infection/disease. The observation that there is a dominating neutralizing domain in what may be a more constant region in the large glycoprotein of this virus type can be beneficial to this endeavor.

Q 017 THE ROLE OF HIV-SPECIFIC T CELLS IN THE PATHOGENESIS AND PREVENTION OF AIDS, Robert F. Siliciano, Kevin Callahan, Alice Y. Liu, Scott Hammond, and Patricia E. Stanhope, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

The normal human T cell response to the HIV-1 envelope proteins gp160 and gp120 has been analyzed in seronegative vaccine recipients using T cell cloning methods. The T cell response to candidate AIDS vaccines consisting of recombinant forms of gp160 and gp120 is comprised exclusively of $CD4^+$ T cells. However, considerable functional heterogeneity exists among vaccine-induced, *env*-specific $CD4^+$ T cell clones. This heterogeneity is revealed by differences in the ability of various clones to produce the cytokines IL2, IL4, IFN-γ, and TNF-α following antigen stimulation. In addition, heterogeneity is evident from an analysis of antigen-specific cytolytic activity. The vaccine-induced *env*-specific response includes a significant fraction of $CD4^+$ T cells with high levels of cytolytic activity. These $CD4^+$ cytolytic T lymphocytes (CTL) lyse target cells in a rapid and highly specific fashion through a mechanism that depends upon the expression of the cytolytic protein perforin. Because the HIV-1 envelope protein synthesized in infected cells can be processed for association with class II MHC gene products, these vaccine-induced $CD4^+$ CTL readily lyse autologous HIV-1-infected $CD4^+$ T lymphoblasts. The mechanism of processing of endogenously synthesized HIV-1 envelope protein has been analyzed by expression of forms of the protein with altered intracellular trafficking properties. For example, retention of the envelope protein in the endoplasmic reticulum (ER) by coexpression with a mutant form of soluble CD4 containing a C-terminal KDEL sequence results in a dramatic inhibition of presentation of the envelope protein to $CD4^+$ T cells. These results suggest that the processing occurs in a post-ER compartment.

All $CD4^+$ *env*-specific T cell clones with cytolytic activity also secrete TNF-α upon stimulation. TNF-α is produced very rapidly (1 hr) after antigen stimulation and is detectable as a 17 kd secreted form as well as a 26 kd transmembrane form. The production of TNF-α is not involved in the mechanism of cytolysis by $CD4^+$ CTL. Cyclosporin treatment of T cell clones causes the complete inhibition of the expression of membrane as well as secreted TNF-α but does not affect the cytolytic activity of the clones. However, the amounts of TNF-α produced by these cells are sufficient to induce upregulation of HIV-1 gene expression in chronically infected T cells, a result with important implications for vaccine design.

Finally, the cloning of $CD8^+$ CTL specific for gp160 from volunteers immunized with a recombinant vaccinia virus carrying the HIV-1 *env* gene will be described. These clones have been shown to lyse autologous HIV-1-infected $CD4^+$ lymphoblasts and to produce TNF-α. Some vaccine induced $CD8^+$ CTL recognize conserved epitopes in the envelope protein and cross-react on divergent HIV-1 strains. Previous studies showed that $CD4^+$ CTL specific for gp120 can lyse autologous $CD4^+$ lymphoblasts that have taken up free gp120 via CD4. Unlike gp120-specific $CD4^+$ CTL, $CD8^+$ CTL specific for gp120 do not mediate the potentially deleterious gp120-dependent lysis of non-infected "innocent bystander" $CD4^+$ T cells.

Q 018 TYPE-SPECIFIC AND GROUP-SPECIFIC ANTIBODIES TO HIV: THE DECLINE AND FALL OF AN OVERSIMPLIFIED CONCEPT, Susan Zolla-Pazner[1,2], Sylwia Karwowska[2], Jian-Yin Xu[2], Aby Buchbinder[1], and Miroslaw K. Gorny[2], [1]New York Veterans Affairs Medical Center, New York, NY 10010, [2] New York University Medical School, New York.

The majority of monoclonal antibodies (mAbs) described to date which neutralize HIV-1 are directed to the viral envelope and have been categorized as group-specific or type-specific. The former have generally included Abs to the CD4 binding domain (CD4bd) while the latter are most often identified as Abs specific for the V3 loop of gp120. Studies indicate that, generally, anti-CD4bd Abs are more broad in their reactivity than anti-V3 Abs. Abs to the V3 loop, on the other hand, have been considered to be type-specific for strains to which they are homologous or for closely related strains. To study group- and type-specificity more carefully, a panel of human mAbs to gp120 have been examined for their immunochemical and biological characteristics against various HIV-1 strains. Of a group of 13 human mAbs to the V3 loop of MN, 10 have been shown to react with epitopes spanning a 10 amino acid sequence near the tip of the loop. Immunochemical studies of these mAbs reveal that, while two are reactive in ELISA only with V3 peptides of MN, the rest are cross-reactive with V3 peptides of 2 or more HIV-1 strains. Monoclonal Ab 447-52-D is the most broadly reactive. With a specificity directed toward GPGR, it reacts with V3 peptides derived from all of eight tested American and European isolates including such divergent strains as MN, RF and IIIB. This mAb does not react with the V3 peptide of ELI, an African strain, which bears GLGQ in place of GPGR. Moreover, this mAb is capable of neutralizing several strains of HIV which contain the GPGR motif. These results suggest that Abs to the V3 loop are not necessarily type-specific; indeed, the majority of anti-V3 mAbs are cross-reactive with more than one strain and many are broadly cross-reactive. Studies by this and other groups of the second category of mAbs, those to the CD4bd, have revealed that many of these mAbs do not compete with one another, suggesting that the CD4bd is large, discontinuous and composed of several epitopes. Given the complexity of the CD4bd, it should not be surprising that any given anti- CD4bd mAb does not neutralize all HIV-1 isolates. Thus, the concept of the type- and group- specificity of anti-V3 and anti-CD4bd Abs must be modified and, given our understanding of HIV variability and the molecular basis for immunologic cross-reactivity, these concepts may, indeed, need to be discarded.

Workshop

Q 019 PROMISCUITY FOR MHC AND INDUCTION OF BROAD CROSSREACTIVITY FOR HIV-1 ISOLATES OF A DOMINANT CTL DETERMINANT OF THE HIV-1 ENVELOPE. J. A. Berzofsky[1], M. Shirai[1], Y. Nakagawa[2], C. D. Pendleton[1], R. A. Houghten[3], K. Yokomuro[2], R. N. Germain[4], and H. Takahashi[2]. [1]NCI and [4]NIAID, NIH, Bethesda, MD 20892 USA, [2]Nippon Medical School, Tokyo 113, Japan, and [3]Torrey Pines Institute for Molecular Studies, San Diego, CA 92121 USA.

We have examined the range of class I Major Histocompatibility Complex (MHC) molecules that can present a major determinant of the HIV-1 envelope protein, gp160, recognized by $CD8^+$ cytotoxic T lymphocytes (CTL) of both mice and humans, and have studied the CTL crossreactivity among diverse isolates of HIV-1. We have also developed an immunization sequence that induces broadly crossreactive CTL in mice. Previous studies showed that residues 315-329 (peptide 18 or P18) of HIV-1 gp160 IIIB (RIQRGPGRAFVTIGK) and MN (RIHIGPGRAFYTTKN) constituted an immunodominant determinant recognized by $CD8^+$ CTL from mice immunized with recombinant vaccinia virus expressing gp160, in association with the murine H-$2D^d$ class I MHC molecule, as well as by $CD8^+$ CTL from HIV-1 infected or gp160-recombinant vaccinia vaccinated humans in association with the human class I molecules HLA-A2, A3, and possibly A1 or B8. To determine whether this peptide would be recognized with multiple murine class I molecules, we immunized 10 B10 congenic strains of mice representing 10 MHC types, and found that $CD8^+$ CTL from 5 strains killed syngeneic targets in the presence of P18 IIIB. Presentation of P18 in two strains was by D^d, and in H-2^q mice was by D^q (or L^q), but in H-2^p and H-2^u mice could not be mapped to a specific MHC molecule. Nevertheless, all 4 murine MHC class I molecules that could present this peptide presented the same core region of the peptide, not distinct adjacent or slightly overlapping determinants. Thus, this peptide is promiscuously presented by both murine and human class I MHC molecules.

Murine D^d-restricted CTL specific for P18 of the IIIB and RF isolates recognized only these isolates, whereas those against P18 MN were somewhat more crossreactive. Based on data that CTL crossreactivity was strongly influenced by residue 325, we examined the effect of many substitutions at position 325 in the P18 MN peptide. IIIB-specific CTL killed targets pulsed with P18 MN variants carrying an aliphatic amino acid (V, L, I) substituted for 325Y, whereas MN-specific CTL killed targets pulsed with P18 MN variants with any aromatic (Y, F, H, W) or cyclic (P) amino acid at position 325. These results correlate with the natural HIV-1 variants crossreactively recognized by these CTL. Using this information, we devised an immunization procedure, involving priming with recombinant vaccinia and restimulation with a chimeric P18 peptide variant whose sequence did not correspond to any single reported HIV-1 isolate, to elicit broadly crossreactive CTL. Such CTL recognized P18 MN variants with hydrophilic or even charged amino acids, as well as aliphatic and aromatic ones, at position 325, and recognized natural HIV-1 sequences from isolates not recognized by P18 IIIB or P18 MN-specific CTL. Although this immunization study had to be carried out in animals, this approach may be applicable to human vaccine development aimed at induction of broadly crossreactive CTL with activity against a wide range of HIV-1 isolates.

Q 020 THE PRINCIPAL NEUTRALIZATION DETERMINANT (PND) OF SIV DEPENDS ON THE NATIVE CONFORMATION OF THE ENVELOPE PROTEIN, Kashi Javaherian*, Alphonse J. Langlois**, Karen A. Kent***, Dani P. Bolognesi**, Gregory J. LaRosa*, *Repligen Corporation, Cambridge, MA 02139, **Duke University Medical School, Durham, NC 27710, ***National Institute for Biological Standards and Control, England

In the case of HIV-1, studies have shown that the neutralizing Abs that bind to the third variable region of the external envelope glycoprotein, gp120, confer protective immunity after challenge of either actively or passively immunized chimpanzees. PND peptides elicit neutralization titers that are equivalent to those found in sera of HIV-infected individuals and this indicates that the PND of HIV-1 does not depend on the native conformation of gp120. Our efforts over the past two years have been to determine the PND of SIV envelope. Our results show that in contrast to HIV-1, the PND of SIV is dependent on the native conformation of the envelope protein. Furthermore it appears that V3 is a part of this conformation dependent PND. Utilizing a number of neutralizing Mabs, we have been able to obtain information regarding the neutralizing domains of the SIV envelope protein.

Q 021 A PREDOMINANT USAGE OF A TCR Vβ SEGMENT IN SIVmac GAG-SPECIFIC CYTOTOXIC T LYMPHOCYTES, N.L. Letvin, Z.W. Chen, and H. Yamamoto, Harvard Medical School, New England Regional Primate Research Center, Southborough Massachusetts.

To determine the extent of the diversity of the T cell receptor (TCR) repertoire of SIVmac-specific cytotoxic T lymphocytes (CTL), the anchored polymerase chain reaction (PCR) was used to isolate TCR α and β chain cDNA from SIVmac gag-specific CTL clones. Previous studies have shown that all SIVmac-infected rhesus monkeys expressing the major histocompatibility complex (MHC) class I gene Mamu-A1 will develop a gag-specific CTL response restricted only by Mamu-A1 and with specificity for a single SIVmac gag peptide (peptide 11: amino acid residues 181-191 of SIVmac gag). 6 independently generated CTL clones from a SIVmac-infected Mamu-A1+ monkey were shown to recognize peptide 11 in association with Mamu-A1. TCR-α chain sequences from these 6 CTL clones were heterogenous; they expressed 4 different Vα and 5 different Jα genes. In contrast, 5 of the 6 CTL clones utilized genes of the same Vβ family (a human Vβ23 homologue). An investigation of genomic TCR β DNA sequences by PCR amplification of rearranged VDJ genes confirmed this Vβ23 bias in the CTL clones. Furthermore, only one Jβ gene family was employed by 4 of the 6 CTL clones, indicating a significantly limited diversity of the β junctional region. These results indicate that the SIVmac gag-specific CTL response in Mamu-A1+ rhesus monkeys is mediated by CTL utilizing TCRs with limited β chain variation. This observation raises the possibility that selective manipulation of a restricted population of lymphocytes may alter the cellular immune response to the AIDS virus.

Progress in Vaccine Research

Q 022 PHASE I TRIALS OF SUBUNIT Gp160 VACCINES: A CURRENT SUMMARY. Lawrence Corey, Representing the NIAID AIDS Vaccine Evaluation Units, Johns Hopkins University, Baltimore, MD; St. Louis University School of Medicine, St. Louis, MI; University of Rochester Medical Center, Rochester, NY; and University of Washington, Seattle, WA

Several Phase I clinical trials of recombinant subunit gp160 vaccines (LAV-IIIB strain) have been conducted. The tested products have included a baculovirus derived protein in an alum adjuvant (Bac gp160) a vaccinia derived protein in an oil adjuvant (Vac gp160), a vaccinia gp160 recombinant (HIVAC-1e) and a trial using HIVAC-1e followed by boosting with the Bac gp160. All these vaccines have had excellent safety profiles. However, their immunogenicity profiles differ significantly. While antibody responses by both Western blot and whole virus EIA assays are elicited in high titers by Bac gp160, no recipients get neutralizing antibodies to LAV. The Vac gp160 appears more potent in that after two doses of 50 ug of vaccine, all recipients develop gp160 antibodies. However, neutralizing antibodies have yet to be elicited.

HIVAC-1e has been tested in both vaccinia naive and vaccinia primed individuals. Among vaccinia naive individuals, >90% achieve Western blot antibodies and 1/2 develop neutralizing antibodies to LAV. The slope of the antibody rise is slower and more sustained than with any of the recombinant subunit vaccines. While vaccinia primed individuals develop T cell priming as measured by lymphoproliferation to gp160, this is transient and antibodies to HIV envelope and neutralizing activity are not detected (0/16).

The combination of HIVAC-1e followed by subunit boosting appears to be the most immunogenic approach. Over 50% of vaccinia naive individuals who subsequently received subunit boosts have developed antibodies to the V3 loop, fusion inhibition antibodies and neutralizing antibodies to LAV. In addition, neutralizing antibodies to heterologous strains (Mn) have also been achieved. In vaccinia primed persons, about 1/3 develop neutralizing antibodies, albeit titers are lower than in vaccinia naive individuals. Adoptive transfer experiments in SCID Hu PBLs indicate partial protection with the first boost. Subsequent boosts with Bac gp160 have revealed increasing Western blot titers but decreasing neutralizing antibody titers as well as decreased protection in the SCID Hu PBL model. These data suggest that vaccinia priming is the key feature in developing subsequent neutralizing antibodies with Bac gp160. Conceptually, these early phase I trials suggest live virus vector priming followed by subunit boosting may provide the best, long lived and functional immune response to the HIV-1 envelope.

Q 023 EPITOPE-SPECIFIC ANTIBODY-MEDIATED PROTECTION AGAINST THE ESTABLISHMENT OF HIV-1 INFECTION, Emilio A. Emini[1], W.A. Schleif[1], A.J. Conley[1], J.A. Nunberg[1], Y. Eda[2,5], S. Tokiyoshi[2], S.D. Putney[3], S. Matsuchita[4], K.E. Cobb[5], C.M. Jett[5,2], J.W. Eichberg[5], and K.K. Murthy[5], [1]Merck, Sharp and Dohme Research Laboratories, West Point, PA, [2]The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan, [3] Repligen Corporation, Cambridge, MA, [4]Kumamoto University, Kumamoto, Japan and [5]Southwest Foundation for Biomedical Research, San Antonio, TX

Efforts to develop and clinically assess HIV-1 vaccines are hampered by an incomplete understanding of the immunological basis of possible protection against HIV-1 persistent infection. The absence of <u>in vitro</u> correlates of immunity does not allow for the appropriate evaluation of the protective potential of trial HIV-1 vaccines. We are using the chimpanzee model of HIV-1 infection and antibodies of defined specificity and activity to define those components of the antiviral humoral immune response that may contribute to such protection. The first of these studies investigated the protective efficacy of antibody directed to the V3 loop domain of the viral gp120 envelope glycoprotein. The results of several chimpanzee immunization studies had suggested that this antibody can prevent HIV-1 persistent infection. *Cβ1 is a monoclonal, virus-neutralizing* mouse-human IgG1 chimeric antibody directed against the V3 domain of the HIV-1 IIIb variant. In an initial study, a purified preparation of the antibody was passively administered to a chimpanzee. Twenty-four hours later, the animal was challenged intravenously with 75 chimpanzee infectious doses of the virus. The animal has remained free of apparent virus infection for over one year, post-challenge. In contrast, a simultaneously challenged untreated control chimpanzee readily developed persistent infection. A subsequent study was performed in which an identical quantity of the antibody was passively administered to a chimpanzee immediately following an equivalent virus challenge. The antibody infusion was started 10 minutes after virus inoculation and lasted for 30 minutes. This animal has also remained free of apparent virus infection. The results of these studies suggest the usefulness of anti-V3 domain antibody for pre- or post-exposure protection *against the establishment of HIV-1 infection. The results* also define virus-neutralizing, anti-V3 domain antibody as an <u>in vitro</u> correlate of a potential vaccine-elicited anti-HIV 1 protective antibody response.

Q 024 **MOLECULAR AND BIOLOGIC TRANSITIONS FROM ACUTE TO CHRONIC HIV-1 INFECTION**. George M. Shaw[1], George Pan[1], Stephen J. Clark[1], Beatrice H. Hahn[1], Michael S. Saag[1]. [1]University of Alabama at Birmingham, Birmingham, AL

Acute, symptomatic HIV-1 infection (CDC stage I) represents a dynamic period during which high level viral replication and widespread viral dissemination occur. These virologic events frequently occur in association with severe clinical symptoms and signs of immune activation and are followed by prompt resolution of plasma viremia, antigenemia, and clinical symptoms. We studied changes in plasma viremia, PBMC-associated viremia, antigenemia, *serologic response, genetic evolution of the viral quasispecies, and* changes in viral biology in three individuals with acute HIV-1 infection and the sexual contact responsible for transmission to one of them. Peripheral blood lymphocytes (uncultured) were obtained sequentially throughout the period of acute infection and subjected to nested PCR amplification and viral DNA sequence analysis in order to characterize the nature of genotypic variation occurring during this important transition period between acute and chronic infection. The results demonstrate transmission and high level replication of a relatively homogeneous viral population followed by the development of greater genotypic diversity commonly associated with established HIV-1 infection. Changes within and outside the envelope V3 loop sequence are described. In one subject who experienced a rapid *decline in circulating* CD4$^+$ *lymphocytes over a one year period* (CD4$^+$ cells falling from >500 to 50/mm^3), a highly cytopathic virus strain was isolated at the time of acute infection and eight weeks later which replicated well in both PBMCs and T-cell lines. The results of these studies will be discussed in the context of viral pathogenesis and possible interventional strategies.

Workshop

Q 025 HIV-2 VACCINES, Gunnel Biberfeld*, Per Putkonen*, Rigmor Thorstensson*, Jan Albert**, Erling Norrby***, *Department of Immunology, and **Department of Virology, National Bacteriological Laboratory, Stockholm, Sweden, ***Department of Virology, Karolinska Institute, Stockholm, Sweden.

We have established experimental HIV-2 infection in cynomolgus monkeys and successfully used this monkey model for vaccine experiments. Monkeys inoculated with HIV-2(SBL-6669) passaged once in vivo in monkeys became persistently infected but did not develop immunodeficiency or clinical symptoms. However, in vivo passage of HIV-2(SBL-K135) seemed to increase the pathogenicity of this virus. Monkeys infected with the second in vivo passage of this virus developed an early and persistent decrease of CD4 lymphocytes. Vaccine-induced protection against HIV-2 infection was first demonstrated in 2 of 2 monkeys immunized with a Triton-treated preparation of HIV-2(SBL-6669) in incomplete Freund's adjuvant. Subsequently we have shown protection against homologous virus challenge in 2 of 4 monkeys immunized 5 or 6 times with 300 μg of formalin inactivated whole HIV-2(SBL-6669) vaccine in RIBI adjuvant and in 3 of 4 monkeys, immunized 5 times with detergent-treated HIV-2 whole vaccine in iscoms and then boosted twice with synthetic peptides representing a dominating neutralizing region of HIV-2 gp125 attached to iscoms. In contrast 4 monkeys immunized 4 times with 300 μg of formalin-killed whole HIV-2(SBL-6669) vaccine in alum adjuvant were not resistant to challenge with live homologous virus. Three monkeys preinfected with a live poorly replicating HIV-2 strain developed cross-protection against SIV induced immunodeficiency and disease, although they yielded virus for a short time after SIVsm inoculation. These monkeys are still clinically healthy after 28 months whereas 4 SIVsm infected control monkeys died of immunodeficiency disease after 2-26 months. Passive immunization prevented infection with homologous live virus challenge in 2 of 3 monkeys which had received a high dose (9 ml/kg) of anti-HIV-2 serum from a HIV-2 immunized and protected monkey and in 3 of 4 monkeys which had received a high dose of anti-SIV serum from a clinically healthy SIVsm infected monkey. The demonstration of vaccine-induced protection against HIV-2 in monkeys raises hope for effective immunization against HIV infections in humans as well.

Q 026 IMMUNIZATION OF CHIMPANZEES AGAINST HIV-1 PROVIDE PROTECTION AGAINST CELL-ASSOCIATED VIRUS CHALLENGE,

Marc Girard[1], Peter Nara[2], Françoise Barré-Sinoussi[1], Abraham Pinter[3], Elizabeth Muchmore[4], Marie-Paule Kiény[5], and Patricia Fultz[6], [1]Institut Pasteur, Paris, France, [2]National Cancer Institute, Frederick, [3]Public Health Research Institute, New York, [4]Laboratory for Experimental Medicine and Surgery in Primates, Tuxedo, [5]Transgène, Strasbourg, France, [6]University of Alabama, Birmingham.

Two types of HIV-1 challenge were studied in vaccinated chimpanzees : challenge with cell-free virus and challenge with virus infected cells. Chimpanzee C-339 was immunized with inactivated whole HIV and recombinant gp160 in the presence of SAF-1 adjuvant. Chimpanzee C-499 was immunized with recombinant gp160 and p18*gag*, also in the presence of SAF-1. Both animals were boosted with synthetic peptides corresponding to the principal neutralization determinant (PND) of HIV-1 either as a KLH conjugate (C-339), or as a mixture of free PND peptides (C-499). The two chimpanzees were challenged by I.V. injection of 40 chimpanzee infectious doses of the HIV-1 IIIB strain. Both animals remained virus-free during the subsequent one-year follow-up period.

To determine whether the animals could also be protected against experimental infection with cell-associated virus, the two chimpanzees were then rechallenged with a suspension of PMBC from an HIV-1_{IIIB}-infected chimpanzee. At 4 and 8 weeks prior to challenge, C-339 was boosted with a mixture of inactived whole HIV, recombinant gp160, and PND-KLH conjugate. C-499, however, received no booster injection. A third chimpanzee, C-447, was added to the study. That animal had been immunized with recombinant vaccinia viruses and recombinant HIV antigens, then with PND peptide, but had not previously been challenged. A naive chimpanzee, C-435, served as a control. All four animals were challenged by I.V. inoculation of 5.8×10^5 PBMC from a chimpanzee that had been infected for 3 months with HIV-1_{IIIB}. This corresponded to approximately 12-25 infectious viable cells, as determined by cocultivation with normal human or chimpanzee PBMC. HIV infection was detected in the control animal at 4 weeks after challenge. In contrast, chimpanzees C-339 and C-447 remained free of any evidence of virus infection for the subsequent one year follow-up period. C-499 also remained virus-free but prematurely died 8 months after challenge from congestive heart failure. Attempts at recovering virus from its organs and tissues by cocultivation with human PMBC have remained negative. These results indicate that HIV-1 immunization can provide protection against cell-associated as well as cell-free virus challenge. Neutralizing antibody titers were the only marker that correlated with protection. Cell mediated immune response markers were variable and displayed no correlation.

Q 027 HIV SYNTHETIC PEPTIDES CONTAINING T AND B CELL EPITOPES AS VACCINE CANDIDATES, Barton F. Haynes, Mary Kate Hart[1], Richard M. Scearce[1], Dawn M. Jones[1], Thomas J. Matthews[1], Kent Weinhold[1], Alphonse J. Langlois[1], M. Anthony Moody[1], Murray B. Gardner[2], Jose V. Torres[2], Larry O. Arthur[3], Dani P. Bolognesi[1], and Thomas J. Palker[1], [1]Duke University Medical Center, Durham, NC 27710, [2]University of California Davis, Davis, CA, 95616, and [3]NCI-Frederick Cancer Research Facility, PRI/DynCorp, Frederick, MD 21702.

Immune responses that potentially will need to be elicited by HIV immunogens to protect subjects from HIV infection are anti-HIV T helper cell responses, anti-HIV neutralizing antibody (NA) responses, and anti-HIV cytotoxic T cell (CTL) responses. We have designed a series of synthetic peptides comprised of regions of HIV envelope proteins and have studied their ability to induce anti-HIV T and B cell responses in animals. The HIV env regions included in peptides are the T1 T cell epitope (aa428-443 of HIV gp120); the SP10 HIV env epitope that comprises aa 303-321 of the gp120 V3 loop; the A region, a 5 to 7 amino acid extension of the right-hand side of the V3 loop that completes an MHC Class I restricted CTL epitope; and the fusion (F) domain of env gp41 (aa519-530) that mediates fusion of HIV infected cells. In goats and rhesus monkeys, immunization with T1-SP10 peptides were capable of priming memory T cells that recognize native HIV env proteins. Studies in $H2^d$ Balb/c mice have demonstrated that both T1-SP10(A) and F-T1-SP10(A) peptides were capable of priming type-specific, MHC Class I restricted CTL <u>in vivo</u> that killed targets expressing HIV env proteins. In mice and goats, both T1-SP10(A) and F-T1-SP10(A) peptides were capable of inducing high titers of specific NA. In primates (rhesus monkeys and chimpanzees) T1-SP10(A) peptides were better immunogens compared to F-T1-SP10(A) peptides with higher titers and earlier antibody rises seen with T1-SP10(A) peptides. Thus, HIV env synthetic peptides containing T and B cell epitopes prime T and B cells <u>in vivo</u> to react against native HIV proteins with anti-HIV NA, T helper and CD8+ CTL responses.

Q 028 **VACCINATION AGAINST SIV INFECTION OF MACAQUES**

Pete A Kitchin[1], Kingston H G Mills[1], Mark Page[1], W Ling Chan[1], Frank Taffs[1], Martin Cranage[2], Arthur Baskerville[2], Peter Greenaway[2] and Jim Stott[1]. [1]National Institute for Biological Standards and Control, Potters Bar, UK. [2]Centre for Applied Microbiology and Research, Porton Down, UK.

Protection against intravenous challenge with 10 MID_{50} of SIVmac 32H was observed after immunisation with formalin-fixed SIVmac 32H or ß-propiolactone inactivated molecular cloned SIVmac BK28, formulated with either Freunds adjuvant, syntex or Ribi adjuvant systems. Formalin-inactivated SIVmac 32H in SAF-1 also protected against heterologous SIV/delta, but not HIV-2 challenge. Furthermore animals were protected against infection with homologous virus following a 3 dose immunisation schedule (0,4,8 weeks), without the need for a rest period and final boost. Formalin-fixed virus vaccines also protect against challenge by the intrarectal route; but not against an homologous intravenous challenge with cell associated virus. Three different recombinant SIV envelope protein preparations failed to protect against challenge, despite inducing high levels of neutralizing and anti env antibody.

Attempts to correlate protection with titres of neutralizing antibody or anti-SIV env antibodies were unsuccessful. Titres of antibody to cellular components in the inactivated vaccines were significantly higher in protected macaques and these may be responsible for the protection observed.

Q 029 A SIMIAN MUCOSAL MODEL OF INDUCTION OF A DUAL PERIPHERAL MUCOSAL AND CENTRAL SYSTEMIC IMMUNITY TO SIV ANTIGENS, Thomas Lehner, Lesley Bergmeier, Christina Panagiotides, Roger Brookes and Sally Adams[1]. Dept. of Immunology, United Medical and Dental Schools of Guy's and St. Thomas' Hospitals, London, England and [1]British Biotechnology Ltd, Oxford, England.

Vaginal infection with the simian immunodeficiency virus (SIV) can cause simian AIDS in macaques. The objectives of this project were to elicit a mucosal immune response by an augmented oro-vaginal route of immunization in 17 macaques. The vaccine used was SIV gag p27 protein hybridized to the yeast retrotransposon virus-like particle (Ty-VLP). In order to elicit local cervico-vaginal and oral immune responses the hybrid vaccine was covalently linked to cholera toxin B subunit (CTB). Sequential, 2 monthly immunizations of 500µg of p27 Ty-VLP linked to CTB and delivered in gelatin capsules by gastric intubation, were followed by 3 vaginal immunizations of 200µg of the vaccine applied in solution to the mucosa. The sequence was reversed in another group of macaques, with 2 vaginal followed by 3 oral immunizations. Control animals were immunized with the Ty-VLP linked to CTB, p27 Ty-VLP without CTB, p27 linked to CTB or p27 alone. IgA and IgG antibodies were assayed in serum, saliva and vaginal washings by ELISA. Vaginal IgA and IgG antibody titres increased from 0 to 1:2-1:16 in all 4 macaques immunized by the vagino-oral route with p27 Ty-VLP linked to CTB, compared with titres of 0 to1:2-1:4 immunized by the oro-vaginal route. Rectal antibodies were detected only in 2/7 macaques but an increase in salivary IgA anti-p27 antibody titre (1:8-1:64) was found in all macaques. Significant increases in serum anti-p27 antibodies of 1:200-1:1600 were found in all macaques and the titres of IgA and IgG antibodies were similar, unlike the higher IgG than IgA antibodies found in systemic immunization. A significant increase in T cell proliferative response was also found in all macaques, when stimulated in vitro with p27 but not with Ty-VLP. These are circulating $CD4^+$ cells which can induce in vitro specific IgA and IgG anti-p27 antibody synthesis, when reconstituted with B cells and macrophages and stimulated with p27 antigen. The results suggest that vagino-oral immunization with the hybrid p27 Ty-VLP linked to CTB elicits vaginal, salivary and serum IgA and IgG anti-p27 antibodies and circulating CD4 cells sensitized to p27. The combined vaginal and oral mucosal routes of immunization elicit a first line of defence against the HIV transmission at the cervico-vaginal mucosa, consisting of secretory IgA and IgG antibodies. If the HIV were to breach the mucosal defence barrier, the second or central immune response may prevent HIV infection, through the functional activities of serum IgG and IgA antibodies and sensitized T cells. The vagino-oral route of immunization in macaques can now be used to investigate prevention of SIV infection by the vaginal route.

Q 030 USE OF LENTIVIRUS-LIKE PARTICLES ALONE AND IN COMBINATION WITH LIVE VACCINIA-VIRUS BASED VACCINES, Dennis L. Panicali[1], G. Mazzara[1], Steve DiSciullo[1], J.L Sullivan[2], R. Hesselton[2], L. Shen[3], N. Letvin[3], M. Daniel[3] and R. Desrosiers[3], [1]Therion Biologics Corporation, 76 Rogers Street, Cambridge, MA 02142, [2]UMASS Medical School, Worcester, [3]New England Regional Primate Research Center, Worcester.

The immunogenicity of HIV and SIV-like particles produced by recombinant poxviruses was examined. Particles were tested in rabbits and macaques either alone or in combination with recombinant vaccinia viruses expressing multiple HIV or SIV proteins. Recombinant poxviruses expressing the gag/pol and env genes serve as efficient expression systems for producing lentivirus-like particles. These particles contain all of the appropriately modified and processed proteins encoded by the gag/pol and env genes. We have utilized both vaccinia viruses and fowlpox viruses to produce particles in cultured mammalian cells. HIV-like particles are capable of eliciting neutralizing antibodies to HIV in rabbits. Particles also efficiently boost antibody responses in rabbits following a primary vaccination with recombinant vaccinia virus. SIV-like particles have been used to immunize rhesus macaques. We have previously shown that primary vaccination with recombinant vaccinia viruses expressing the SIV gag/pol and env genes are capable of stimulating both humoral and cellular immune responses including SIV neutralizing antibodies and CD8+MHC class I restricted cytotoxic T lymphocyte (CTL) responses. Using SIV-like particles to boost animals primed with vaccinia recombinants we have demonstrated a dramatic increase in antibody responses and a significant re-stimulation of the gag specific CTL response. These studies demonstrate the poxvirus produced lentivirus like particles can serve as an effective boosting immunogen in vaccine protocols employing live recombinant viruses. In addition, these virus-like particles can also serve as a safe alternative to whole-inactivated viruses for either prophylactic or therapeutic vaccines.

New Strategies for AIDS Treatment

Q 031 ANTIRETROVIRAL THERAPY: PAST, PRESENT AND FUTURE, Samuel Broder, National Cancer Institute, Bethesda, Maryland

Therapeutic advances in AIDS relate directly to the translation of basic virologic and immunologic discoveries into clinical application. HIV, the causative agent, is a genetically complex retrovirus with at least 9 genes, including a gene encoding reverse transcriptase (RT). The panel of HIV regulatory genes modulates host cell gene expression, T cell antigenic responses, and the production of immune-active cytokines that in turn enhance HIV replication.

Knowledge of the multi-stage HIV life cycle opens many therapeutic avenues. The dideoxynucleosides, of which AZT is the prototype, act through RT inhibition. New drugs of this class (ddI, ddC, 3-TC, d4T) afford the creation of multi-drug combinations that ameliorate toxicities and prevent emergence of drug resistance. The ability to block host transcriptional factors (such as NF-kB) may offer another strategy, especially when combined with RT inhibitors. The HIV protease has a pivotal role in multiple HIV life cycle stages. The three-dimensional structure of this enzyme has been used to develop inhibitors for clinical testing. The Tat protein, through autocrine stimulation of HIV replication, may have a central role in disease progression. The specific HIV RNA sequence to which the Tat protein binds (TAR) can be attacked through inhibitors of TAR-Tat binding, i.e. TAR decoy molecules or antisense DNA constructs directed against the tat genomic RNA or mRNA. The binding of HIV envelope glycoproteins to CD4 can theoretically be abrogated by decoy molecules or immunoconjugates. The development of an experimental vaccine that stimulates humoral and cellular response against gp 160 in humans is a major advance for anti-HIV therapy and prevention. Future innovations include discovery of unique natural products, use of sophisticated biophysical tools to define the structures of key viral components and design targeted drugs, rational use of immunomodulatory cytokines or hormones, and genetic engineering to confer resistance to HIV infection or cellular destruction or to augment immune-based HIV eradication.

The momentum of progress on the molecular level is unfortunately matched by the provocative challenge of increasing numbers of aggressive HIV-associated malignancies, ironically due to lengthening survival in the deeply immuno- compromised state. The emergence of non-Hodgkin's lymphomas (NHL) as a major sequela of HIV infection bears a striking relationship to depletion of CD4 lymphocytes, particularly below 50/mm^3. The ability to interfere early in the course of active HIV infection with additional strategies that may promulgate transformed cell hyperproliferation and clonal expansion--growth factors, HIV itself or other viruses, aberrant oncogene or tumor suppressor gene expression, factors that induce genetic instability or DNA damage or alter host or viral genome repair -- might decrease the occurrence or prolong the time to development of AIDS-related malignancies. The development of antiretroviral strategies that confer long-term suppression of HIV activity and relative preservation of immune function are essential to the ultimate prevention of malignancies that arise as a consequence of HIV-induced immunosuppression.

Q 032 STRUCTURE-BASED DESIGN OF INHIBITORS FOR HIV-1 PROTEASE, John Erickson, PRI/DynCorp, NCI-FCRDC, P.O. Box B, Frederick, MD 21702

HIV-1, the causative agent of AIDS, contains a protease that processes the viral polyproteins into the structural proteins and replicative enzymes found in mature virions. Protease activity has been shown to be essential for the proper assembly and maturation of fully infectious HIV-1. Thus, the HIV protease has become an important target for the design of antiviral agents for AIDS. Based upon an analysis of the three-dimensional structures of HIV and related aspartic proteinases, two classes of C2 symmetric inhibitors have been designed, synthesized, and found to exhibit potent antiviral activity. The crystal structures of several HIV protease/symmetric inhibitor complexes have been solved. Analysis of these structures has led to the identification of a class of novel, non-peptide inhibitors.

Q 033 ENVELOPE BASED HIV VACCINE THERAPY: CONCEPTS AND CURRENT STATUS, Robert R. Redfield and Deborah L. Birx, Department of Retroviral Research, Walter Reed Army Institute of Research

The immunoregulatory mechanisms responsible for effective the in vivo post-infection control of HIV are unknown. In the setting of natural HIV infection, host directed immune responses directed against gp120 are minimal. This includes poor gp120 epitope specific antibody responses to C1, C2, and C3; lack of T cell recognition and proliferation to gp120; and minimal envelope cytotoxic T cell responses. The historical important of anti-envelope responses in the control of other viral pathogens, coupled with the paucity of anti-envelope responses elicited as a consequence of HIV infection, formed the basis for our rationale to pursued a research program which focused on post infection vaccination utilizing HIV enveloped derived products. Recently we have demonstrated the scientific feasibility of post infection vaccination with an HIV envelope based vaccine to broaden host directed HIV specific immune responses to include seroconversion to gp120, C1, C2 and C3; the induction of T cell recognition to gp120 and 160; and cytotoxic T cell responses to envelope peptides. The current status of ongoing therapeutic vaccine trials will be reviewed involving two products, a recombinant gp160 baculovirus expressed protein vaccine (MicroGeneSys) and a recombinant gp120 CHO cell produced protein vaccine (Genentech). The emphasis of the presentation will be the in vivo safety and immunogenicity of these products in adult patients with early HIV infection. Two to three year follow up data will be presented from the phase 1 rgp160 trial to examine the potential role of HIV specific vaccine therapy to alter the natural history of HIV infection. Finally, data will be presented exploring the value of post infection immunization in defining specific immune responses and their in vivo HIV immunoregulatory relevance.

Q 034 HIV VACCINATION AND AZT TREATMENT OF ASYMPTOMATIC INFECTED PERSONS, Britta Wahren, Department of Virology, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden; E. Sandström; G. Bratt; D. Birx; R. Redfield; and H. Wigzell.

40 asymptomatic HIV-infected persons were immunized with gp160 of HIV-1 BRU strain. Half of the patients received short term AZT in a blinded fashion. Inclusion criteria were HIV seropositivity and over 400 x 10^6 CD4 cells/l. Cytotoxic T-cell reactivity was measured by ^{51}Cr-release from HIV-infected Jurkat-tat$_I$ T-cells. T-cell proliferation was measured by ^{3}H-dt incorporation in DNA. Nineteen of the 40 patients had the HLA Class I A-2 phenotype and were studied for HIV-specific CTL. Nine out of nineteen persons initially demonstrated a clear Class I-associated CTL activity, while ten lacked measurable CTL. Proliferative T-cell responses appeared after the second or third injection and were strong against gp160. Affinity maturation of HIV-specific IgG was seen during immunization to MN-like V3 sequences, but not to BRU. Affinity of antibodies increased considerably, indicating more potent cross-reactive neutralization.

Workshop

Q 035 BIOLOGICAL EFFECTS OF HIV-1 TAT PROTEIN: AN OPTIMAL TARGET FOR GENE THERAPY, Barbara Ensoli, Luigi Buonaguro, Giovanni Barillari, Hsiao K. Chang, Valeria Fiorelli and Robert C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The *tat* gene product (Tat) of HIV-1 is essential for HIV-1 gene expression and virus replication. Tat is released from infected cells in a biologically active form which, acting in a paracrine manner, is able to activate HIV-1 LTR-directed gene expression, to rescue Tat-defective proviruses and to induce proliferation of cells derived from KS lesions of HIV-1 infected individuals or their cell progenitors. The growth promoting effect of extracellular Tat requires Tat domains and follows cellular pathways different from the viral effects. Tat is also capable of activating cellular gene expression in HIV-1 infected cells, namely, the TNFß. This inflammatory cytokine is increased in the sera of HIV-1 infected individuals and has activities on the immune and vascular systems, and on HIV-1 gene expression and replication. These results suggest new ways of how Tat may function in mediating virus spread and survival and of how HIV may produce human diseases. Tat may employ these biological activities to participate in the pathogenesis of the immunodeficiency and in the development of KS in HIV-1 infected individuals. Thus, Tat represents an optimal target for gene therapy against HIV-1 infection and AIDS-KS. Antisense *tat*-RNA expressing vectors have been made and used alone or in association with multiple-Tar constructs to block Tat expression and activity in *tat*-transfected or HIV-1 infected T cells. The association of "protective" genes acting by different mechanisms on the same target is effective in inhibiting more than 90% of Tat activity and in blocking HIV infection of T cells.

Q 036 INHIBITION OF HIV REPLICATION IN ACUTE AND CHRONIC INFECTION IN VITRO BY A TAT ANTAGONIST, Ming-Chu Hsu[1], Andrew D. Schutt[1], Maureen Holly[1], Lee W. Slice[1], Michael I. Sherman[1], Douglas D. Richman[2], Mary Jane Potash[3], and David J. Volsky[3], [1]Hoffmann-La Roche Inc., Nutley, NJ 07110, [2]VA Medical Center, University of California, San Diego, CA 92161, [3]St. Luke's/Roosevelt Hospital Center, Columbia University, New York, NY 10019

Ro 5-3335, 7-chloro-5-(2-pyrryl)-3H-1,4-benzodiazepin-2(H)-one, has been shown to inhibit gene expression controlled by the human immunodeficiency virus-1 (HIV-1) LTR promoter. The inhibition was specific for the viral transcriptional transactivator Tat. The compound did not inhibit the basal activity of the HIV-1 LTR or the activity of promoters not responsive to Tat. Consistent with its mode of action, Ro 5-3335 inhibited HIV-1 replication (IC_{50} = 0.1-1µM) by reducing viral RNA synthesis in acutely as well as chronically infected cells in vitro. The compound was active against both HIV-1 and HIV-2, and AZT-resistant clinical isolates. The mechanism of action of the compound will be discussed.

Q 037 NEVIRAPINE, A NON NUCLEOSIDE INHIBITOR OF HIV-1 REVERSE TRANSCRIPTASE, Vincent J. Merluzzi[1], Peter M. Grob[1], Joe C. Wu[1], Kenneth A. Cohen[1], Richard Ingraham[1], Cheng-Kon Shih[1], Karl D. Hargrave[1], Julian Adams[1], Peter Farina[1], Johanna Griffin[1], John L. Sullivan[2], Douglas Richman[3], and Alan S. Rosenthal[1], [1]Boehringer Ingelheim Pharmaceuticals, Inc., 90 East Ridge Road, Box 368, Ridgefield, CT 06877, [2] University of Massachusetts Medical School, 373 Plantation Street, Suite #318, Worcester, MA 01605 and [3]University of California San Diego & Veterans Administration Medical Center, 3350 La Jolla Village Drive, San Diego, CA 92161.

Nevirapine (BI-RG-587) a non nucleoside inhibitor of HIV-1 RT, exhibits an IC_{50} of 84nM against RT polymerase activity in an enzyme assay and 40nM against virus replication in culture. This inhibitory activity is highly specific since RT from HIV-2, SIV and FeLV are unaffected. Eukaryotic DNA polymerases alpha, beta, delta and gamma are also not inhibited by nevirapine. The inhibitor activity of nevirapine is not competitive with respect to template-primer nor nucleoside triphosphates, indicating that this inhibitor does not act directly at the catalytic site of RT. One consequence of this is that HIV-1 mutants which are resistant to AZT remain fully sensitive to nevirapine.

The binding site for nevirapine was investigated by employing a tritiated azido analog, [^{3}H]-BI-RJ-70, as a photoaffinity probe to covalently label the enzyme. These studies demonstrated that one molecule of inhibitor is sufficient to inactivate one molecule of enzyme and demonstrated that only the p66 subunit of p66/p51 heterodimeric RT is labeled. Interestingly, a TIBO derivative which inhibits HIV-1 RT also binds to this non catalytic modulatory site (RT;MS) on HIV-1 RT.

The interaction of nevirapine with RT was characterized further by utilizing the [^{3}H]-BI-RJ-70-RT photoadduct to map the inhibitor binding site. Upon tryptic digestion, the photoadduct yielded a labeled peptide which contains residues 174-199. Amino-terminus sequencing of this peptide determined that Tyr_{181} and Tyr_{188} were labeled with the probe.

The influential roles that Tyr_{181} and Tyr_{188} played in the interaction between HIV-1 RT and nevirapine were corroborated when amino acid substitutions using corresponding residues from HIV-2 RT were made at these two positions. A change at either position resulted in full HIV-1 RT polymerase activity but dramatic loss of sensitivity to nevirapine as well as to a TIBO derivative. Furthermore, the importance of the amino acids contiguous to Try_{181} and Tyr_{188} was delineated when residues 176-190 from HIV-1 RT were substituted into HIV-2 RT. The chimeric HIV-2 RT exhibited dramatic restoration of sensitivity to nevirapine.

We conclude that nevirapine (and TIBO compounds) interact with HIV-1 RT in a region containing Tyr_{181} and Tyr_{188} and that the tyrosines are crucial components of this nevirapine binding site.

Q 038 APPLICATION OF HIV PROTEASE INHIBITORS AND TNF SYNTHESIS INHIBITORS TO THE CONTROL OF HIV INFECTION AND AIDS, S. R. Petteway, Jr.[1], D. M. Lambert[1], J. Leary[1], K. Esser[1], G. B. Dreyer[1], T. Meek[1], T. J. Matthews[2], M. A. Ussery[3], P. L. Black[3,4], M. G. Lewis[4] and B. Metcalf[1], [1]Departments of Antiinfectives, Cell Sciences and Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406, [2]Department of Surgery, Duke University Medical Center, Durham, NC 27710, [3]Food and Drug Administration, Rockville, MD 20857, [4]Southern Research Institute-Frederick Research Center, Frederick, MD 21701

HIV infection can be thought of as occurring in at least two stages: an early stage in which viral cDNA is transcribed and inserted into the host genome, and a late stage in which viral nucleic acid and proteins are synthesized and assembled into progeny virions. Current therapy consists of the application of nucleoside analogs as inhibitors of the early stage event of reverse transcription. HIV protease inhibitors act at the late stage event of viral protein maturation by inhibiting viral polyprotein processing. We have investigated the effects of co-treatment of HIV infected T cells with nucleoside analogs such as AZT and HIV protease inhibitors. Depending on the cell line incorporated protease inhibitors act additively or synergistically to prevent virus spread in culture. Selected protease inhibitors were found to be active against Rmulv and SIV infection. A separate strategy for the control of HIV infection was first proposed by Anthony Fauci and colleagues (Science 239:617, 1988) and involves inhibition of TNF stimulated viral replication. We have investigated the ability of selected synthetic inhibitors of macrophage TNF synthesis to inhibit the HIV replication enhancing activity of TNF contained in the supernatants of LPS stimulated macrophages. Treatment of LPS stimulated macrophages with selected inhibitors of TNF synthesis results in a selective reduction of TNF and a loss of the HIV replication enhancement activity. The availability of selective inhibitors of cytokine synthesis makes the clinical application of this strategy feasible.

Q 039 DISCOVERY, STRUCTURE-ACTIVITY RELATIONSHIPS, AND ANTIVIRAL ACTIVITIES OF NOVEL BHAP HIV-1 RT INHIBITORS, W. Gary Tarpley, Upjohn Laboratories, Kalamazoo, MI.

Collaborative work by scientists from the Upjohn Laboratories HIV Reverse Transcriptase (RT) Team, the University of Miami, and the Mt. Sinai Medical Center of Greater Miami, resulted in the discovery of potent and selective nonnucleoside inhibitors of the HIV-1 RT. Select bisheteroarylpiperazine (BHAP) inhibitors exhibit anti-HIV activities in multiple test systems identical to those of the nucleoside analog inhibitors. Studies which led to the identification of U-87201E, a BHAP currently undergoing clinical evaluation, will be presented. (Research supported in part by Grant UOI AI25696 from the NIAID.)

Late Abstracts

NEUTRALIZATION OF LABORATORY AND CLINICAL ISOLATES OF HIV-1 BY ANTISERA RAISED AGAINST A MONOVALENT SUBUNIT VACCINE, P.W. Berman*, T. J. Matthews#, L. Riddle*, F. M. Wurm*, M. Champe*, M. R. Hobbs*, G. R. Nakamura*, J. Mercer*, D. J. Eastman*, C. Lucas*, A. Langlois#, and Tim Gregory*, *Departments of Immunobiology, Process Sciences, Cell Culture Recovery, and Assay Development, Genentech, Inc. South San Francisco, CA. 94080 and the #Department of Surgical Virology, Duke University Medical School, Durham, North Carolina.

Recombinant gp120 (rgp120) derived from the MN and IIIB isolates of HIV-1 were compared for the ability to elicit antibodies able to inhibit the binding of gp120 to CD4, react with the principle neutralizing determinant (PND) of gp120, and neutralize a diverse panel of laboratory and clinical isolates of HIV-1. While both immunogens elicited similar levels of antibodies to gp120, sera against IIIB-rgp120 reacted only with the PND of the homologous strain whereas antibodies from animals immunized with MN-rgp 120 reacted with the PND the homologous strain and those of five diverse isolates. Antibodies that blocked the binding of gp120 to CD4 were elicited by both immunogens, however both type common and type specific blocking antibodies were detected. The cross reactivity with respect to PND binding paralleled the neutralizing activity. Sera from animals immunized with IIIB-rgp 120 were effective only against the homologous virus, whereas sera to MN-rgp 120 was able to neutralize six of the nine strains tested. Sequence analysis of the clinical isolates used in the neutralization studies revealed that cross neutralization correlated with the PND sequence. These studies demonstrate that rgp 120 prepared from a strain (MN) with a PND representative of a large fraction of viruses in the U.S.A. will neutralize a large fraction randomly chosen laboratory and clinical isolates of HIV-1. While antibodies to the PND appear to be an important factor in determining whether a given antiserum will neutralize a particular virus, other activities (eg. CD4 blocking antibodies) may also be important for neutralization. These results support speculation that earlier vaccines prepared against the envelope glycoproteins of the IIIB isolate of HIV-1 were ineffective in neutralizing heterologous viruses because the IIIB/LAV-1 isolate of HIV-1 is not representative of most laboratory or clinical isolates.

IN VITRO ANALYSIS OF THE HIV-1 INTEGRATION AND CIRCULARIZATION REACTIONS

Chris M. Farnet, Cha-Gyun Shin and William A. Haseltine, Division of Human Retrovirology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115

A variety of DNA molecules derived from the viral RNA genome are synthesized in cells during a productive retroviral infection. One viral DNA form, the integrated provirus, is essential for the production of progeny virions and for viral replication. Circular unintegrated viral DNA molecules, a ubiquitous feature of retroviral replication, represent non-productive alternatives to the integrated provirus. Among the circular viral DNA molecules found in an infected cell are circles containing 1-LTR, simple 2-LTR circles formed by joining of the ends of linear viral DNA, and circular molecules that result from intramolecular integration (autointegration), in which the ends of the linear viral DNA integrate into viral DNA sequences on the same molecule. The development of a cell-free system that supports the formation of the various forms of viral DNA in vitro has allowed us to examine the factors involved in the formation of the various forms of viral DNA.

Studies using this system have demonstrated that the two most abundant forms of circular viral DNA found in vivo, 1-LTR circles and simple 2-LTR circles, result from the action of cellular recombinases on the linear viral DNA. In addition to mediating the integration of viral DNA into target DNA, the viral integrase is also responsible for the autointegration of viral DNA. The autointegration reaction, which normally occurs at a very low frequency in vivo and is lethal to the virus, can be induced at a high frequency in cell extracts.

The formation of all types of circular viral DNA in cytoplasmic extracts of infected cells requires the addition of millimolar concentrations of nucleoside triphosphates (NTPs). Formation of 1-LTR circles occurs by cell-mediated recombination between the LTRs of linear viral DNA, and specifically requires the addition of ATP. A possible role for ATP in the recombination reaction will be discussed. A variety of NTPs, in addition to ATP, are able to stimulate the autointegration reaction. In vitro experiments highlighting a possible role for NTPs in the autointegration reaction will be discussed.

HIV-1 REPLICATION IN HUMAN BLOOD DENDRITIC CELLS

Erik Langhoff, Karl H. Kalland and William A. Haseltine, Division of Human Retrovirology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115

Blood derived T helper dendritic cells are very sensitive to infection by the human immunodeficiency virus type 1 and readily support abundant virus production. The efficiency of key steps in the molecular virus life cycle was examined in T helper dendritic cells. The results show that viral reverse transcription proceeds much more rapidly and efficiently in primary T helper dendritic cell populations than in primary T cells or monocytes. The subsequent integration of viral DNA into the host, and appearance of spliced viral mRNA products also occur more rapidly in primary blood derived T helper dendritic cells than is the case in primary T cells and monocytes. The increased efficiency of the early steps of HIV-1 replication in primary blood derived T helper dendritic cells than in other blood derived mononuclear cells suggests that dendritic cells play a central role in HIV-1 infection and pathogenesis.

TRYING TO KEEP UP WITH HIV VARIATION, Simon WAIN-HOBSON, Institut Pasteur, Paris, France.

HIV sequence data is increasing at a phenomal rate. Most of the studies, with the exception of in vivo/ex vivo comparisons, all describe original phenomena. The populations of genomes (quasispecies) fluctuate in as little as 3 months and most likely less. Given the enormous proviral copy number within any infected individual (easily $>10^8$) and the fact that virtually all of these genomes are genetically distinct one wonders sometimes whether HIV sequence variation is at all important in terms of AIDS pathogenesis. Certainly neutralizing antibody can passively block experimental viral infection but what is its role in the natural setting ? Is it always on the run ? The existing data would suggest that the intense cytotoxic T cell response does not constitute an important selection pressure in vivo. The quasispecies description holds that there is a threshold beyond which there is a catastrophic breakdown in the fidelity of replication. G->A hypermutation of the HIV genome would seem to respond to such a definition. It occurs during reverse transcription and predominantly during DNA(-) strand synthesis via a dislocation of the nascent strand with respect to the template. This could be shown by isolating the dislocated intermediate in the nascent strand. G->A hypermutation is most likely not due to a mutant polymerase but the interaction of the virus with the cell. Extensive analyses of HIV-1 env quasispecies in vivo show that even within the hypervariable regions there are preferred sites of base substitution. Using the exquisite sequence and length polymorphisms inherent to the V1 and V2 regions we sought double infections to no avail. The work highlighted the fickleness of extrapolating from V3 derived sequence to the rest of the genome.

Virus/Host Cell Interactions

Q 100 CHARACTERIZATION OF AN *IN VIVO* SELECTED BROADLY NEUTRALIZATION RESISTANT HIV-1 STRAIN. Nicole K.T. Back[1], Lia Smit[1], Chitra Ramautarsing[1], Pete L. Nara[2], Matthijs Tersmette[1], and Jaap Goudsmit[1]. [1]Human Retrovirus Laboratory, Academic Medical Centre, Amsterdam, The Netherlands.[2] Laboratory of Tumor Cell Biology, National Cancer Institute, FCRF, Frederick, MD

Aim: To analyze the mechanism of neutralization resistance of the V32 HIV-1 strain, recovered from an HTLV IIIB immunized chimpanzee challenged with homologous virus.

Methods: Neutralization of the resistant virus (V32) by a panel of V3 antibodies and human sera was investigated. Effects of virus stock preparation on neutralization was investigated with a panel of plaque-purified clones. The V3 sequences of these clones were analyzed and binding of V3 monoclonal antibodies to the neutralization sensitive HX10 clone and to the resistant clones were compared in a gp120 capture EIA.

Results: V32 was resistant to neutralization by a panel of V3 antibodies and broadly neutralizing human sera. Clones from V32 with a similar neutralization profile were obtained. Resistance to neutralization was still observed when stocks of these clones were used with varying ratios of virion-bound/ soluble gp120, prepared in various cell lines. The V3 sequences of these clones and λBH10 were identical. V3 monoclonal antibodies bound to gp120 of the V32 clones and HX10 with comparable affinity.

Conclusion: The resistance to V3-mediated neutralization of V32 apparently results from conformational changes of gp120 which might e.g. affect the availability of the V3 domain. Differential gp120 shedding did not appear to be involved. The escape of V32 from broadly neutralizing human sera suggests that these conformational changes also affect group-specific neutralization epitopes.

Q 101 CARBOHYDRATE PROCESSING OF THE HIV-1 ENVELOPE GLYCOPROTEIN Helene B. Bernstein, Simon P. Tucker and Richard W. Compans, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

We have observed a time dependent decrease in the apparent molecular weight of HIV-1 gp160 expressed via a vaccinia virus recombinant in BHK-21 cells. To investigate whether this decrease in molecular weight was due to carbohydrate processing we treated the glycoprotein with several enzymes which hydrolyze N-linked glycans. N-Glycosidase F (PNGase F) which hydrolyzes the aspartylglucosamine linkage of N-linked glycans was found to cause a decrease in the apparent molecular weight of gp160, but the time dependent difference in apparent molecular weight was still observed. The time dependent size difference also remained after treatment with endoglycosidase F (Endo F) which hydrolyses the diacetylchitobiose linkage of N-linked carbohydrates. Sequential digests of gp160 with Endo F followed by PNGase F produced deglycosylated proteins of a slightly lower molecular weight than were observed after independent digestion with each enzyme. However, the time dependent size decrease in the molecular weight of gp160 remained evident following these treatments. Digestion with Endoglycosidase H (Endo H), an enzyme which also hydrolyzes the diacetylchitobiose linkage of N-linked carbohydrates but is restricted to high-mannose type carbohydrates, yielded a protein of lower apparent molecular weight than was observed with any of the treatments described above. This indicates that some sugar residues which are sensitive to Endo H are not cleaved by Endo F or PNGase; perhaps as a consequence of steric hindrance. Additionally, preliminary results indicate that further digestion with neuraminidase and O-glycanase results in an additional decrease in the molecular weight of gp 160 and eliminates the time dependent decrease in it's apparent molecular weight. This result suggests that gp160 may be at least transiently O-glycosylated.

Q 102 LOCALIZATION OF DETERMINANTS THAT DETERMINE HIV-1 CYTOPATHICITY ON THE VIRAL ENVELOPE GLYCOPROTEINS

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Lab. of Immunobiology, Natl. Institute for Public Health and Env. Protection, Bilthoven and * Lab. of Viro- Immunology, Central Lab. of the Netherlands Blood Transfusion Service, Amsterdam, the Netherlands

HIV-1 variants differ in their replication rates *in vitro*, in their capacity to grow in continuous cell lines and in their capacity to induce syncytia. We have concentrated on the envelope genes of HIV-1 variants with differing syncytium inducing capacities to study the genetic basis underlying the observed biological differences. The envelope genes of eight biological clones of HIV-1 were cloned and expressed using recombinant vaccinia viruses (rVV) and we have demonstrated that the syncytium inducing capacities of the individual env egene products correlated fully with that of the parental clones. Making use of a different cloning/expression system we have now generated over 25 intragenic recombinants between syncytium-inducing and non syncytium-inducing envelope genes to determine the localization of determinants that control HIV-1 envelope cytopathicity. The results of these exchange experiments have allowed us to conclude that:
1) at least two different regions of the envelope glycoproteins independently control HIV-1 envelope cytopathicity
2) the V3 loop is not a major determinant in determining syncytium-forming capacity

Q 103 CD4 TRANSLATION IS INHIBITED WHEN COTRANSLATED IN THE PRESENCE OF HIV-1 ENV mRNA, Stephane Bour, Romas Geleziunas, and Mark A. Wainberg, Lady Davis institute-Jewish General Hospital and Mc Gill AIDS Centre, Mc Gill University, 3755 chemin Côte Ste- Catherine, Montreal, Quebec, Canada, H3T 1E2

Infection of CD4+ cells generally leads to cell surface depletion of CD4 glycoprotein. The three levels of CD4 down-regulation, transcriptional, translational, and post-translational were studied using the promonocytic cell line U937 infected with the IIIB strain of HIV-1. This work led us to the conclusion that a translational down regulation of CD4 occured in this model. In order to better define the mechanisms leading to such a down regulation, in vitro cotranslation of CD4 and gp160 mRNA was performed. When translated alone, each mRNA gave rise to proteins recognized by monoclonal antibodies directed against both CD4 and gp160. The use of pancreatic membranes in this system allowed for the production of the glycosylated versions of each protein and for the cleavage of gp160 to gp120 and gp41 was also occuring in this system. When a 1:1 ratio of CD4 and gp160 mRNA are cotranslated in the presence of membranes, a complete inhibition of CD4 production is observed. This inhibition could only be abrogated at a CD4:gp160 ratio of 4:1 which is, at least, 20 times higher than the ratio observed in vivo. The cotranslational inhibition of CD4 is specific, yet has no effect on CD8 translation. Moreover, we show that translation of a CD4 protein mutated in the gp160 binding site (CD4-M1) is 4 times less sensitive to cotranslational inhibition than the wild-type CD4. In order to address whether presynthesized gp160 glycoprotein may affect CD4 translation, pancreatic membranes were first loaded with gp160, rinsed, and used for CD4, CD4-M1, and CD8 translation. Such sequential translations did not impair either CD4 or CD8 synthesis attesting that inhibition of CD4 production by gp160 was cotranslational rather than post-translational. In an effort to identify the molecular mechanisms leading to the inhibition of CD4 translation, we are presently investigating the influence of the regions 5' to the AUG of each mRNA.

Q 104 CD4 MOLECULES WITH A DIVERSITY OF MUTATIONS IN THE CDR3 REGION EFFICIENTLY MEDIATE CELL-CELL FUSION,
Christopher C. Broder and Edward A. Berger, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892
The CDR3 region within the first domain of CD4 has been suggested to play a critical role in membrane fusion mediated by the CD4/HIV-1 envelope glycoprotein interaction. To analyze in detail the role of the CDR3 region in fusion events, we constructed a panel of 30 site-directed mutations between residues 79-92 of the full-length CD4 molecule, using recombinant vaccinia virus expression vectors. Cells expressing the mutant molecules were assayed for their ability to form syncytia when mixed with cells expressing the HIV-1 envelope glycoprotein; they were also analyzed for cell surface CD4 expression, gp120 binding activity, and overall structural integrity as assessed by reactivity with a battery of anti-CD4 monoclonal antibodies. Surprisingly, most of the mutations had little effect on syncytia-forming activity. No fusion impairment was detected in 21 mutants including: substitution of human residues 81-92 with the corresponding sequences from either chimpanzee, rhesus, or mouse CD4; a panel of ser-arg double insertions after each residue from 86-91; a number of other charge, hydrophobic, and proline substitutions and insertions within this region. Three mutations causing some impairment of gp120 binding activity and overall structural integrity still showed fusion activity, albeit with a slightly lower kinetic efficiency. Only 6 mutants were completely fusion negative, but these also displayed a loss of surface expression, gp120 binding activity, and overall structural integrity. In further contrast to previous reports of others, we observed that Hela transfectant cell lines expressing native chimpanzee or rhesus CD4 molecules efficiently mediated syncytia formation with cells expressing vaccinia-encoded envelope glycoprotein. These data suggest that if the CDR3 region participates in membrane fusion activity of CD4, a wide variety of sequence variation can be tolerated.

Q 105 EFFECTS OF DELETIONS IN THE V3 LOOP ON HIV-1 ENVELOPE GLYCOPROTEIN FUNCTION
Shiow-Her Chiou, Eric O. Freed*, Antonito Panganiban* and William R. Kenealy, Department of Biochemistry; and *Department of Oncology, University of Wisconsin-Madison, Madison, WI 53706
The V3 loop of the HIV-1 surface envelope glycoprotein gp120 is a principal neutralizing domain of HIV-1. Neutralizing antibodies against this region generally neutralize viral infectivity and block cell fusion in a type-specific manner, while not preventing virus binding to the cellular receptor CD4. Mutations within the V3 loop block or greatly reduce the ability of the HIV-1 envelope glycoprotein to induce cell fusion in CD4+ HeLa cells without influencing its other functions. However, when either one cysteine or both cysteines forming the V3 disulfide bridge were mutated, the resultant glycoproteins could not mediate cell fusion, undergo proteolytic cleavage, or bind CD4. Elimination of the V3 disulfide linkage may greatly change envelope glycoprotein conformation such that many of its functions are impaired. To test this hypothesis, we deleted the entire V3 loop region of the *env* gene of the HIV-1 proviral clone pNL4-3. The resultant glycoprotein could not mediate cell fusion in the CD4+ HeLa T4 cell line and no proteolytic cleavage of gp160 or CD4 binding could be detected. To test if a domain in the V3 loop is involved in proper envelope glycoprotein conformation for proteolytic processing and CD4 binding, we introduced a series of partial V3 loop deletions into an HIV-1 envelope glycoprotein expression vector and analyzed the abilities of the mutant envelope glycoproteins to induce cell fusion, to undergo proteolytic processing and to bind CD4. Our results indicated that most of the residues within the V3 loop can be removed without perturbing the conformation required for gp160 processing and CD4 binding. We conclude that the cysteines in the V3 loop or the disulfide bond are important for the proper folding and processing of the protein. Because many of these mutant proteins do not contain the type-specific neutralizing determinant of HIV-1, they may be exploited as potential reagents to bind group-specific neutralizing antibodies or to elicit a group-specific neutralizing response against HIV-1.

Q 106 PCR ANALYSIS OF HIV PROVIRUS FORMATION IN PRIMARY HUMAN MACROPHAGES, Matt Collin and Siamon Gordon, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK
Macrophages (MØ) are widely distributed in the body and have the ability to support HIV replication, suggesting that they are involved in the pathogenesis of AIDS. MØ have a very low proliferative capacity, a property which has been clearly shown to restrict retroviruses in most other cell types, through failure to form a complete, integrated provirus. In this study, we have investigated the kinetics of reverse transcription in MØ, to determine what differences exist compared with the same process in T cells.
A PCR-based strategy has been developed to analyse the formation of provirus in cultured monocyte-derived MØ. It is possible to measure the copy number of several DNA intermediates in reverse transcription over the time span of a single cycle of infection. This analysis reveals that there is marked attenuation of reverse transcription in macrophages; for every copy of full length provirus that is completed, about 1000 transcripts have been initiated. Studies are in progress to determine if similar attenuation occurs in T cells; it is likely that reverse transcription is slower or less efficient in MØ, in view of the very low levels of nucleotide triphosphates that these cells contain, compared with T cells. This work will increase our understanding of the extent to which MØ are permissive to HIV, and may contribute to defining their role in the pathogenesis of AIDS.

Q 107 GENERATION AND CHARACTERIZATION OF AN INFECTIOUS MOLECULAR CLONE OF A HIGHLY MACROPHAGE-TROPIC AND CYTOPATHIC HIV-1
Ronald Collman, John Balliet, Susan A. Gregory, Harvey Friedman, Neal Nathanson and A. Srinivasan. University of Pennsylvania Medical Center and the Wistar Institute, Philadelphia, PA 19104

Work in several laboratories has identified regions of the *env* gene involved in the ability of HIV to infect macrophages (macrophage tropism). We want to identify viral elements involved in permissiveness for replication and cytopathic effect in macrophages, and to examine the role of HIV-1 regulatory genes in modulating viral replication in macrophages.

We isolated an HIV-1 strain from mixed PBMC of an individual with AIDS which is highly tropic for and cytopathic in macrophages. From this isolate three full-length clones were generated which differ in a number of restriction sites. One of these (p89.6) is an intact infectious clone and has been extensively characterized. Cloned HIV-1/89.6 replicates to high titer in macrophages and results in extensive syncytia formation without cell death. Like other macrophage-tropic isolates it also replicates in PBL but, unlike many other macrophage-tropic isolates described, is highly syncytia-inducing in lymphocytes as well.

We have constructed a panel of recombinant viruses between p89.6 and cloned non-macrophage-tropic HIV-1 isolates, introducing regions of the p89.6 genome into the non-macrophage-tropic clone as well as corresponding reciprocal recombinants. Studies are under way examining the ability of recombinant viruses to replicate in macrophages, level of permissiveness, and degree of syncytia formation. In addition, we are utilizing this infectious clone to introduce mutations into accessory genes of p89.6 in order to determine their role in regulating viral replication in macrophages.

Q 108 PARADOXICAL RELATIONSHIP BETWEEN CD4 EXPRESSION AND SUSCEPTIBILITY OF MONOCYTES TO HIV INFECTION, A Colvin, S Uren*, A Violo*, S Sonza, W Boyle* and S Crowe. Macfarlane Burnet Centre for Medical Research, Fairfield 3078, *University of Melbourne, Parkville 3052, Victoria Australia.

We have compared the level of CD4 expression on the surface of monocyte/macrophages (MØ) with susceptibility to HIV infection and have determined the kinetics of CD4 expression post infection. Monocytes were isolated from HIV seronegative donors by density gradient centrifugation and counter current elutriation. Cells were maintained in suspension (teflon), supplemented with 10% Human AB+ serum. MØ were infected with HIV-1 (DV strain) on day 0 and 7 of culture and stained for CD4 on the day of infection and day 7 post infection. HIV infection was quantitated, 14 days post infection, by p24 monoclonal antibody staining and flow cytometric analysis. Northern analysis was performed using RNA from $1x10^6$ cells and a CD4 probe, pT4B.

Although freshly isolated monocytes have higher levels of CD4 expression, they are less susceptible to HIV infection (CD4 +ve: mean 73.5%, p24 +ve: mean 4.5%) than MØ from the same donor cultured for 7 days prior to infection (CD4 +ve: mean 14.4%, p24 +ve: mean 38.0%). In MØ, CD4 expression is decreased still further by infection with HIV. A 2-4 fold decrease was found within 24 hours of infection and thereafter remained at this low level. These data were confirmed by Northern analysis.

There appears to be a paradoxical relationship between CD4 expression on MØ and susceptibility to HIV infection, suggesting that maturation may contribute to susceptibility. Down-regulation of CD4 surface expression occurs within 24 hours of infection and does not recover.

Q 109 INTERACTIONS OF GP120 WITH GALACTOSYL CERAMIDE, David G. Cook, Kenneth Ugen, David Weiner, Steven L. Spitalnik, Francisco Gonzalez-Scarano, University of Pennsylvania Medical Center, Philadelphia, PA 19104-6146

It is well established that the HIV-1 receptor is the CD4 molecule, which is bound by the surface glycoprotein gp120. However, a number of other cell types, including the neurally derived cell lines SK-N-MC and U373 which do not express CD4 on their surface, can be infected with one or more strains of HIV-1 and support low levels of viral replication. Recent work has indicated that the glycolipid, galactosyl ceramide (Gal-Cer) plays a role in facilitating infection of these CD4 negative cell lines since antibodies against Gal-Cer block infection by HIV-1 and recombinant gp120 binds Gal-Cer, but not other closely related glycolipids.

We have extended these studies by examining the interaction between Gal-Cer and gp120 in more detail. Specifically, we have asked which site(s) on gp120 are critical for binding to Gal-Cer.

To accomplish this we have assayed the binding of gp120 (derived from a drosophila expression system and kindly provided by Raymond Sweet, SmithKline Beecham) to Gal-Cer immobilized on HPTLC plates. We have found that gp120 binding to Gal-Cer is not mediated by the CD4 binding site because incubation of gp120 with soluble CD4 prior to exposure to Gal-Cer does not block gp120/Gal-Cer binding. Furthermore, soluble CD4 binds to gp120 already bound to Gal-Cer, indicating that gp120 simultaneously binds both CD4 and Gal-Cer.

We are currently testing a panel of monoclonal antibodies against gp120 to determine whether it is possible to block the binding of gp120 to Gal-Cer. Preliminary findings suggest that some but not all of these antibodies interfere with gp120 binding to Gal-Cer.

Q 110 KINETICS OF INTERACTIONS OF sCD4 AND $CD4^+$ CELLS WITH HIV-1 ENVELOPE EXPRESSING CELLS, Dimiter S. Dimitrov[1], Robert Blumenthal[1] and Hana Golding[2], [1]NCI, NIH and [2]CBER, FDA, Bethesda, MD 20892

The kinetics of binding of sCD4 to HIV-1 envelope expressing cells was studied by flow cytometry. Binding was very slow at low sCD4 concentrations (< 0.2 μg/ml). At high sCD4 concentrations (> 2 μg/ml), equilibrium was reached within 10-30 min. Dissociation of prebound sCD4 was faster than fusion of cell membranes and syncytia formation. The rate constant for association at 37°C ($1.5x10^5$ 1/Ms) was 14-fold higher than at 4°C, but the affinity of sCD4 to membrane bound gp120/gp41 was not significantly affected by temperature. The activation energy at higher temperatures (28°C-37°C) was several fold smaller than at low temperatures (4°C to 13°C). After 2-6 hrs of incubation with high sCD4 concentrations, up to 70% of the surface gp120 was shed. Inhibition of syncytia formation after preincubation with sCD4 correlated with the sCD4 binding, but not with the gp120 shedding. Covering of 22% of the surface gp120 with sCD4 resulted in 70% inhibition of syncytia formation when sCD4 remained during the syncytia formation. However, no inhibition of syncytia formation was observed when 54% of the gp120 molecules were shed by preincubation with high sCD4 concentrations and non-bound sCD4 was washed after the preincubation. Instead, an enhancement of syncytia formation occured. The lack of inhibition of syncytia formation when more than half of the gp120 molecules were released from the cell surface may indicate existence of a long-lived fusogenic state. The slow rate of sCD4 binding at low sCD4 concentrations, the fast rate of its dissociation, the existence of activation energy barriers and long-lived fusogenic states are important for the mechanisms of gp120/41-mediated membrane fusion and for the therapy of AIDS.

Q 111 STUDIES ON THE ASSEMBLY AND MULTIMERIC CD4 BINDING EXHIBITED BY HIV-1 ENV PROTEIN DIMERS, Patricia L. Earl, Robert W. Doms, and Bernard Moss, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, Md. 20892.

Previous work has shown that the amino terminal 129 amino acids of gp41 constitute the assembly domain required for HIV-1 env oligomer formation and stability. We have made point mutations in several highly conserved elements within this region. These included a leucine zipper motif, Cys residues, and potential N-linked glycosylation sites. In addition, several C-terminal truncations were made. Proteins were expressed using the vaccinia virus/T7 RNA polymerse system and were analyzed by sucrose gradient centrifugation and chemical cross-linking. Results indicated that no single point mutation had adverse effects on the oligomerization potential of the env protein. However, further C-terminal truncations of the gp41 assembly domain resulted in inefficient oligomerization.

To examine the functional consequences of env protein assembly, we determined whether env protein oligomers exhibited multimeric CD4 binding. We found that full length env protein, expressed in oligomeric form on the surface of infected cells, bound at least 2 CD4 molecules. In contrast, monomeric gp120 bound only a single CD4 molecule.

Q 112 PLASMA VIRAEMIA IN SERONEGATIVE HIV-1-INFECTED INDIVIDUALS, Fabrizio Ensoli[1], Valeria Fiorelli[2], and Fernando Aiuti*, *Department of Allergy and Clinical Immunology, University of Rome, Italy, Current Address: [1]Pediatric Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md, 20892, [2]Laboratory of Tumor Cell Biology, National Cancer Institue, National Institutes of Health, Bethesda, Md, 20892.

The period of latency between HIV-1 infection and the production of antibodies against the virus is sometimes prolonged for > 6 months. However, the data supporting this observation are still controversial, and it is not known whether these individuals are actually infectious, especially through body fluids. We have performed a prospective study of 65 high-risk HIV-1-antibody-negative individuals selected from 542 seronegative previous intravenous drug users (IVDU) and 90 seronegative partners of HIV-positive subjects, who were followed-up for a period of at least 1 year. Twelve of these individuals were shown to be carriers of HIV-1 proviral sequences by the polymerase chain reaction (PCR). The virus was isolated from lymphocytes in five out of 10 PCR-positive subjects and from cell-free plasma in two. Our data indicate that in some cases delayed seroconversions may be associated with productive infection, suggesting that mechanism(s) other than viral latency may be responsible for the absence of antibody responses to HIV-1 proteins.

Q 113 PHENOTYPE ASSOCIATED SEQUENCE VARIATION IN THE HIV-1 PND, Ron A.M. Fouchier, Martijn Groenink, Neeltje A. Kootstra, Thijs Tersmette, Han G. Huisman, Frank Miedema and Hanneke Schuitemaker, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam, The Netherlands.

The principal neutralizing determinant (PND) of HIV-1 gp120 elicits type specific neutralizing antibodies, and contains a type specific T-cell epitope. The PND is critical for gp120 function, and has been implied in determining the virus phenotype including fusion capacity and monocytotropism. Comparison of small numbers of isolates have not provided insight as how the high degree of sequence variability may give rise to large subgroups of HIV-1 strains. PND sequence analysis using a large panel of primary isolates with well defined biological phenotypes revealed that fast-replicating, syncytium-inducing (SI)/non-monocytotropic isolates contained PND sequences with a significantly higher positive charge than those of slow-replicating, non-syncytium-inducing (NSI)/monocytotropic isolates. On either side of the loop, midway the cysteine and the central GPG motif, a highly variable amino acid residue is located that was negatively charged or uncharged in NSI/monocytotropic isolates whereas in SI/non-monocytotropic isolates either one or both were positively charged. These substitutions result in changes in predicted secondary structure of the PND. Our data indicate that the PND is an important determinant for the biological phenotype of HIV-1 isolates and further suggest that an efficacious AIDS vaccine conferring protection to the relevant HIV-1 variants should include PND sequences specific for NSI/monocytotropic in addition to the now generally used SI/non-monocytotropic isolates.

Q 114 CHARACTERIZATION OF SOOTY MANGABEY MONKEY CONTINUOUS T-CELL LINES COINFECTED WITH SIVsmm AND STLV-I, Patricia N. Fultz, Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294.

T-cell lines were established from peripheral blood mononuclear cells obtained from a sooty mangabey monkey naturally infected with both STLV-I and SIVsmm. Following stimulation with PHA, superinfection with SIVsmm9 or SIVsmmPBj14, and cultivation with medium containing human recombinant IL-2, continuously proliferating cultures evolved and were monitored periodically for viability, total cell number and reverse transcriptase (RT) activity. After approximately 1 year in continuous culture, the cells were characterized for phenotypic and biologic properties both *in vitro* and by inoculation of rhesus and pig-tailed macaques. Cells in the culture originally superinfected with smmPBj14 produced high levels of cell-free RT activity, which was attributed to virus particles with morphologic characteristics of both lenti- and C-type retroviruses, as detected by electron microscopy. The cells expressed surface antigens recognized by monoclonal antibodies to CD2, CD25, and HLA-DR, but not to CD4, CD8, CD20 or CD14. In addition, radioimmunoprecipitation assays performed with SIV- and HTLV-1-specific antisera showed equivalent expression of *gag* antigens from both retrovirus subfamilies. Current studies are directed at obtaining both infected and uninfected cloned cell lines with different phenotypic properties, characterizing the viruses produced by these cell lines, and assessing infectivity of the viruses for macaques. These cell lines may prove valuable in (1) elucidation of factors that influence the inherent resistance of sooty mangabeys to SIV-induced AIDS; (2) defining modulation of SIV infection by STLV-1 (and vice versa); and (3) the generation of continuous uninfected T-cell lines of simian origin.

Q 115 GENETICALLY-DIVERSE SIMIAN-RELATED HUMAN IMMUNODEFICIENCY TYPE-2 VIRUSES IN WEST AFRICA, Feng Gao[1], Ling Yue[1], Albert T. White[1], Peter G. Pappas[1], Joseph Barchue[2], Aloysius P. Hanson[2], Bruce M. Greene[1], Paul M. Sharp[3], George M. Shaw,[1], Beatrice H. Hahn[1], [1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, [2]Liberian Institute for Biomedical Research, Robertsfield, Liberia, [3]Department of Genetics, Trinity College, Dublin, Ireland. Current understanding of the natural history and phylogeny of human immunodeficiency virus type 2 (HIV-2) is almost exclusively derived from studies of culture-amplified viruses from urban populations experiencing epidemic spread of infection and disease. As an alternative to HIV culture as the only means to obtain sufficient amounts of virus for genetic and biological studies, we have developed a highly sensitive PCR technique which allows us to amplify genetically divergent HIV viral sequences directly from uncultured peripheral blood mononuclear cells. Using this approach, we identified and molecularly characterized HIV-2 viruses in two healthy rural Liberian agricultural workers (F0784, 2238) from whom virus cultivation was repeatedly unsuccessful, and in a symptomatic, culture-positive urban dweller from Cote d' Ivoire (7312A). Sequence comparison of envelope, polymerase, and LTR fragments revealed an unexpectedly high degree of genotypic variation, with up to 23% sequence differences in regions in which most HIV-2 viruses differ by less than 10%. Subsequent phylogenetic analysis identified one virus ($HIV\text{-}2_{F0784}$) to cluster with simian immunodeficiency viruses infecting sooty mangabeys and rhesus macaques (SIV_{SM}/SIV_{MAC}) rather than with other HIV-2 viruses, a second virus ($HIV\text{-}2_{2238}$) to branch off the tree before all previously reported HIV-2 isolates, and a third virus ($HIV\text{-}2_{7312A}$) to represent a likely recombinant between phylogenetically divergent strains. In addition, one subject (F0784) was found to harbor multiply-defective viral genotypes that resulted from G to A hypermutation. These results demonstrate that West African human populations are infected with highly divergent HIV-2 strains, some of which are more closely related to SIV_{SM}/SIV_{MAC} viruses than to any other known virus of human derivation. Such extensive genetic heterogeneity may contribute to observed differences in HIV-2 natural history and indicates that HIV-2 has either been present in humans for a very long time, like SIV_{AGM} in African green monkeys, or has resulted from repeated transmission of genetically divergent SIVs from nonhuman primates to man. The results of the present study would thus suggest that viruses from feral monkey populations and humans living in rural and remote jungle areas of Africa should be targeted in a search for the origins of these human and simian immunodeficiency viruses and the events leading to their recent epidemic spread.

Q 116 *NEF* AND P56^{lck} SHARE AN OVERLAPPING REGION OF CD4 FOR THEIR FUNCTION

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Using a Moloney murine leukemia virus-based retroviral vector, the *nef* gene of HIV-1 was expressed in three lymphocytic cell lines expressing human (h) CD4. In all cases cell surface hCD4 expression was inversely related to *nef* expression. However, *nef* did not alter steady state levels of hCD4 RNA nor hCD4 protein. In cells expressing *nef*, hCD4 is found to be localized inside the cell as determined by immunofluorescence and confocal microscopy. *Nef* was also capable of downregulating a hCD4 triple mutant (ser ->ala) that is not phosphorylated nor down regulated by phorbol esters, indicating a different mechanism for *nef* action (*Nature* (1991) 350, 508).

In order to map the determinants in CD4 responsible for downregulating by *nef*, we first determined if *nef* could downregulate murine (m) CD4. *Nef* expression correlates with a decrease in the mCD4 levels of the AKR1-G1 murine T cell line while the levels of two unrelated cell surface antigens (CD3 and MLV gp70) were not affected. These results suggests that some of the conserved sequences between human and mouse CD4 are responsible for its downregulation by *nef*. The most conserved portion of the CD4 molecule is the cytoplasmic tail. The fact that a hCD4 mutant with a truncated intra ceilular domain is not downregulated by *nef* indicates that the cytoplasmic portion of CD4 is required for its downregulation. The specific amino acid sequences needed for CD4 downregulation were further mapped by testing three mCD4 mutants that extend to amino acids 397, 408 and 421 (L3T4-T1, T2 and T3 respectively). Only the L3T4-T3 mutant is downregulated by *nef* indicating that the residues between amino acids 408 and 421 are required for CD4 downregulation. Interestingly, these same amino acids have been shown to be required for p56^{lck} binding to CD4. These results suggest that both p56^{lck} and Nef might interact with the same region of CD4. p56^{lck} also binds to CD8 and we have previously shown that hCD8 is not downregulated by *nef*. However, under conditions in which hCD8-alpha is not downregulated by *nef*, mCD8-alpha is, although to a lesser extent when compared to CD4. These results indicate that differences between the cytoplasmic domains of human and mouse CD8-alpha might be responsible for this differential effect.

Our results indicate that HIV is capable of downregulating expression of its own receptor via the *nef* gene. Downregulation of cell surface CD4 by *nef* would be advantageous for the survival and spread of the virus both *in vivo* and *in vitro* and might have significant implications for pathogenesis.

Q 117 HIV-1 MIMICRY OF MAMMALIAN HORMONE PRECURSORS

John D. Glass, Medical Department, Brookhaven National Laboratory, Upton, New York, 11973. All HIV-1, HIV-2, and SIV p17 core proteins contain a seven residue sequence motif that closely mimics the amidation site of mammalian gondadoliberin (LHRH) precursor proteins. A second sequence that mimics the SP2 amidation site in proglucagon occurs in the transmembrane protein of HIV-1, but not at the corresponding sites in the HIV-2 and SIV proteins. The amidation sites include post-translational processing signals that are not present in the mature hormone structure. The mimic sequences stand out clearly using a sequence analysis method based on pathways of peptide amide hormone biosynthesis, although they are nearly indistinguishable above statistical background using conventional methods based on homology with mature hormones. Strong conservation of post-translational processing signals (especially the absolute conservation of the amidation signal in the LHRH precursor mimic sequence) suggest enzymatic processing in the viral context, perhaps opening new opportunities for pharmaceutical intervention in the replication of the AIDS virus. The hormone precursor mimic sequences and their implied metabolic products may also provide the basis for synthetic vaccines -- especially the LHRH precursor mimic sequence, which overlaps a reported neutralizing epitope for HIV. In the context of known peptide amide hormone precursor expression and metabolism in lymphocytes, the mimic sequences may provide a mechanistic explanation for recent observations that rhesus macaques immunized with uninfected lymphocytes are sometimes protected against SIV.

The sequence analysis strategy used in the present study is relatively simple and is capable of recognizing only a limited subset of hormone precursor mimic sites. The prospects for design of a more sophisticated search pattern, perhaps capable of finding additional mimic sites, will be discussed along with the potential importance of hormone mimicry for such viral mechanisms as variable infectivity of individual viral strains in specific types of host cells (tropism).

Q 118 REDUCED SUSCEPTIBILITY TO HIV-1 INFECTION OF EMS-SELECTED CEM SUBCLONES, CORRELATES WITH A REDUCED PKC-INDUCED NFkB DNA BINDING.

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The chemical mutagen ethyl-methanesulfonate (EMS) was used to treat the CD4$^+$ human T cell line CEM. Several subclones were selected, which express CD4 and bind soluble gp120, yet show reduced susceptibility to infection by multiple HIV-1 strains as judged by syncytia and RT assays. Quantitative PCR suggested that the block occurs during or shortly after viral entry. TNFa added 24 hr after infection, could only partially increase syncytia formation, suggesting that viral reactivation is also reduced in these subclones. Treatment with the phorbol ester PMA lead to induction of the IL2R and CD3 receptors in the CEM line, but not in the HIV-1 resistant subclones. Analysis of protein kinase C (PKC) activity showed similar total cellular enzymatic activity in the CEM and the subclones. Phosphrylation of membrane receptors following PMA treatment was only partially reduced. The induction of NFkB DNA binding proteins by PMA was found to be markedly reduced in these subclones as judged by several criteria: (a) HIV-1-LTR-CAT transfection and PMA treatment did not induce CAT activity (while actin-CAT plasmid was expressed). (b) Gel retardation assays using ^{32}P-NFkB probe showed much reduced NFkB binding proteins in the nuclear extracts from these subclones following induction with either PMA or TNF-alpha. Deoxycholate treatment of cytoplasmic extracts from these subclones released very low levels of NFkB binding proteins from their complexes with IkB. These subclones will allow identification of cellular genes required for HIV-1 infection.

Q 119 USE OF VIRUS PSEUDOTYPES TO STUDY THE ROLE OF VIRUS ENTRY IN MACROPHAGE INFECTION BY HIV-1

Susan A. Gregory, Ronald Collman, Neal Nathanson. University of Pennsylvania Medical Center, Philadelphia, PA 19104.

Recent evidence points to the envelope gene as one of the primary determinants of the ability of HIV-1 to infect macrophages. Our goal is the development of a biological system that will allow the study of the early events in macrophage infection by HIV-1.

We have developed a system using virus pseudotypes to isolate viral entry from other steps in the retrovirus life cycle. Pseudotypes are produced by co-infection of CEMx174 cells with HIV-1 and Cocal virus (COV), a rhabdovirus. Virions are produced which contain the genome and core proteins of COV surrounded by the HIV-1 envelope, designated COV(HIV). In subsequent infection by COV(HIV) pseudotypes, the HIV-1 envelope mediaties entry but subsequent rounds of replication are dependent on COV.

Entry mediated by the HIV-1 envelope correlates with the presence of viral plaques in a cell monolayer or in an infectious center assay. Monolayer cells such as CD4-positive and CD4-negative HeLa cells are the target cell in a viral plaque assay. This system can also be applied in an infectious center assay utilizing either suspension cells (such as CEMx174) or adherent cells which do not form a confluent monolayer (such as monocyte-derived macrophages). HIV-1 isolates of differing phenotypes can be used to generate pseudotype virus. In addition, we are utilizing chimeric HIV-1 generated by recombination between cloned isolates of HIV-1 that differ in host cell tropism.

We have developed a system using COV(HIV) pseudotypes which provides a model for the study of the early events in HIV-1 infection. This assay detects phenotypic differences in the HIV-1 envelope that may enhance or restrict viral entry into different cells, including macrophages, and will provide a tool to examine the mechanism and molecular basis of host cell tropism.

Q 120 SOLUBLE CD4 (sCD4) INDUCES AGGREGATION OF VIRIONS AND PROTEOLYTIC CLEAVAGE OF ENVELOPE gp120 IN VARIANTS OF SIV-BK28. Timothy K. Hart[1], Celia C. LaBranche[2], Peter J. Bugelski[1], Raymond W. Sweet[3], and James A. Hoxie[2], Dept. of Exp. Path.[1] and Mol. Gen.[3], SmithKline Beecham Pharm., King of Prussia, PA and Univ. of Penn.[2], Philadelphia, PA.

A soluble form of the cell surface receptor for human and simian immunodeficiency viruses, sCD4, may either block or enhance infection of T-cells by these viruses. We and others have recently shown that sCD4 interacts with HIV-1 gp120 to initiate a conformational change resulting in release of gp120 from virus, exposure of gp41, and inactivation of virus. In the present study, we evaluated the biochemical, morphological, and biological effects of sCD4 on two highly related but biologically distinct isolates of SIV-BK28. One isolate, NC-MAC infects Sup-T1 cells slowly and without cytopathic effects, while the second, CP-MAC, infects Sup-T1 cells with accelerated kinetics and cytopathicity. In contrast to HIV-1, sCD4 did not induce release of gp120 from either CP-MAC or NC-MAC. However, as observed by negative staining, electron microscopy and Western blot analysis, sCD4 induced aggregation of CP-MAC and NC-MAC virions in the presence or absence of Sup-T1 cells. Viral aggregates easily pelleted with cells at 1000 x g centrifugation. gp120 Western blots of cell and viral lysates from CP-MAC-infected Sup-T1 cells but not NC-MAC-infected cells incubated with sCD4 also revealed a dose-dependant proteolytic cleavage of viral gp120 into 90 and 35 kD fragments. Proteolytic cleavage was blocked by leupeptin but not pepstatin nor TLCK or TPCK indicating the probable involvement of a thiol protease. In infectivity assays, incubation of CP-MAC with sCD4 blocked infection of Sup-T1 cells. In contrast, sCD4 markedly enhanced the kinetics of infection of Sup-T1 cells by NC-MAC. These data add the growing body of reports to suggest that the structural events which occur subsequent to binding of sCD4 to viral gp120 are important in determining the phenotypic expression of both human and simian immunodeficiency viral isolates.

Q 121 HIV-1 V3 LOOP PEPTIDES BIND TO THE CELL SURFACE OF MOLT-4 CLONE 8 CELLS IN A POSITIVE COOPERATIVE FASHION

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A principal neutralizing determinant (PND) in V3 loop of gp120 of HIV-1 plays a critical role for infection of HIV-1 by interacting with cell surface protease-like molecules (Hattori, T., *et al. FEBS Letters* 248, 48, 1989 Murakami, T., *et al. BBA* 1079, 279, 1991). Biotinylated V3 loop peptides derived from two different HIV-1 strains, namely HTLV-IIIB (42-mer; V3IIIB) and LAV_{ELI} (43-mer; V3ELI) were made, to assess the mode of binding of V3 loop to the cell surface of Molt-4 clone 8 cells, which is a highly susceptible cell line for HIV infection. Two cysteine residues in the synthesized peptides were linked by an air oxidation to make an intramolecular disulfide bond. Then, two carboxyl groups of each loop peptide (side chain of Glu at N-terminal and C-terminal) were biotinylated. ELISA showed that Biotin-V3IIIB binds to a plate-coated murine monoclonal antibody directed against a native V3 loop of HTLV-IIIB (0.5β), than the 24 mer Biotin-PNDIIIB dose. Biotin-V3IIIB (2.5-85 μg/ml) was incubated with Molt-4 clone 8 cells ($2x10^5$) for 30 min at 4°C followed by washing. The cells were further reacted with FITC-avidin and analyzed by a flow cytometry. The significant binding of Biotin-V3IIIB to the cell surface was detected, when more than 20 μg/ml of the peptide was used. The binding of Biotin-V3ELI was also detected at the same concentration, though that of Biotin-insulin chain B was not detected. That of Biotin-V3IIIB was specifically inhibited by 0.5β, in a dose dependence manner. Interestingly, the binding of Biotin-V3IIIB increased by the simultaneous presence of non-labelled V3IIIB. In addition, the increase was still observed when the cells, which were preincubated with non-labbIled V3IIIB peptide followed by washing, were used. Taken together, these findings indicate that V3 loop binds to Molt-4 clone 8 cells in a positive cooperative fashion.

Q 122 HIV INFECTION OF PRIMARY MONOCYTES IS REGULATED BY MULTIPLE GENETIC DETERMINANTS. Timothy J. Henkel, Peter Westevelt, and Lee Ratner. Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110.

HIV-1 infection of monocytes is thought to be important in the establishment of virus latency, the pathogenesis of primary CNS disease, and in the dissemination of virus. Three phenotypes of viral replication were observed after infection of primary monocytes with virus generated by transfection of COS-7 cells with full-length proviral clones: 1) no infection, 2) silent infection, with little or no virus detectable by reverse transcriptase activity, but recovery of virus after co-cultivation with peripheral blood mononuclear cells, and 3) productive infection. A portion of the envelope glycoprotein, the V3 loop, has been previously identified as an essential determinant of monocyte tropism. Proviral clones containing the *env* determinant from a monocyte-tropic isolate and functional *vpr* and *vpu* genes generated virus capable of productive infection of monocytes. Inactivation of either *vpr* or *vpu* resulted in some attenuation of the level of replication, while inactivation of both *vpr* and *vpu* caused a change in phenotype from productive to silent infection. These findings confirm the necessity of accessory genes *vpr* and *vpu* in addition to *env* in the determination of monocyte tropism and raise the possibility of functional complementation by these genes.

Q 123 LETHAL MUTATION IN THE V3 LOOP OF HIV-1 gp120 IS OVERCOME BY REVERSION IN TISSUE CULTURE. L. Ivanoff[1], L. Gutshall[1], E. Sternberg[1], J. Morris[1], E. Hunter[2], T. J. Matthews[3] and S. R. Petteway, Jr.[1], [1]Department of Antiinfectives, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406, [2]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, [3]Department of Surgery, Duke University Medical Center, Durham, NC 27710

Single amino acid substitutions in the V3 loop region of the HIV-1 gp120 protein have been shown to alter virus infectivity and syncytium formation. In this study, we have replaced the glycine codon (GGA) at position 312, which is the first residue of the conserved GPGR tetrapeptide sequence in the V3 loop region of gp120, with a threonine codon (ACT) by site-specific mutagenesis. The processing, transport and CD4-binding of the G312T mutant envelope was qualitatively normal, but syncytium formation in the Hela-T4 system was impaired. To study virus infectivity, cos-1 cells were transfected with the mutant proviral DNA followed by cocultivation with SupT1 T-cell. In 4 separate coculture experiments, a mutant virus was detected 15-20 days after peak RT levels of the control clone were observed. The env region was amplified from the mutant cocultures by PCR and DNA sequence analysis revealed that the original G312T mutation (ACT) had reverted to GCT, which encodes an alanine residue. In end-point dilution titrations, the revertant (G312A) virus was found to be as infectious as the control virus on SupT1 cells, but exhibited a reduced ability to infect AA5 cells. These results indicate (1) that the presence of a threonine at position 312 in the V3 loop is not well-tolerated by the virus; (2) that to overcome the G312T defect (fusion?), selection involving the V3 loop occurred in tissue culture generating the revertant G312A virus; and (3) that the target cell specificity of the virus can be strongly influenced by changes in the V3 loop sequence.

Q 124 RECOVERY OF INFECTIOUS HIV FOLLOWING THE PASSAGE OF APOPTOTIC CELLULAR DEBRIS THROUGH PHAGOCYTIC MACROPHAGES. Richard S. Kornbluth and Douglas D. Richman, Depts. of Medicine and Pathology, Univ. Calif. San Diego, and the V. A. Medical Center, San Diego, CA 92161

We have previously shown that HIV can directly induce apoptosis in T cells following several rounds of re-infection and the accumulation of unintegrated viral DNA (J. Clin. Invest. 87:1710, 1991). Using ficoll-hypaque to separate the trypan blue-positive dead cells from a population of HIV-1_{BRU}-infected CEM cells, most of the viral DNA was contained in this fraction. In spite of the extensive fragmentation of host cell DNA, the viral DNA remained intact (similar to the results obtained by Weller and Temin for RAV-2, J. Virol. 39:713, 1981). Although HIV-1_{BRU} virions (clarified by centrifugation) were unable to infect monocyte-derived macrophages (MDM) and did not generate viral DNA in these cells detectable by PCR, debris from dead T cells that already contained viral DNA was taken up by MDM. Despite extensive washing, MDM exposed to this debris continued to release p24 antigen and infectious HIV. If these MDM were pretreated with AZT, they still produced HIV (although the virions are susceptible to AZT inhibition in T cells), raising the possibility that the endocytosed HIV DNA is newly transcribed in the phagocytosing macrophages. Treatment with certain cytokines can affect the efficiency of this process, suggesting that the state of the host MDM is important. To evaluate the role of viral factors, we serially passaged recent clinical isolates through PHA plus IL-2-stimulated PBMC cultures, and inoculated MDM cultures with the resulting dead cells. The progeny were then used to infect new PBMC cultures. One SI-type isolate survived three such cycles (without directly infecting MDM), whereas HIV-1_{BRU} and a NSI-type isolate did not, suggesting a role for an HIV gene(s) in this transfer. Experiments in progress will differentiate whether HIV virions are simply endocytosed by MDM and then regurgitated, or whether viral DNA is reutilized in MDM for new rounds of transcription (which would constitute a non-virion salvage pathway for the HIV genome). Regardless of the mechanism, these data indicate that HIV-infected cells killed by virally induced apoptosis (and perhaps cytotoxic lymphocyte-induced apoptosis) may still contribute to ongoing HIV infection even after the death of the initial host cell.

Q 125 CHANGES IN THE ENVELOPE GLYCOPROTEINS OF SIV ASSOCIATED WITH INCREASED INFECTIVITY AND CD4 DOWN-MODULATION, Celia C. LaBranche[1], Timothy K. Hart[2], Peter J. Bugelski[2], Patricia J. Vance[1], and James A. Hoxie[1];[1]University of Penn., Phila., PA; [2]SmithKline Beecham, King of Prussia, PA.
In contrast to its cytopathic effects on HUT-78 cells, SIV-BK28 has been shown to infect Sup-T1 cells slowly and without inducing cell killing, syncytium formation, or down-modulation of CD4. We derived a variant of this virus that infected Sup-T1 cells with rapid kinetics and induced CD4 down-modulation. Ultrastructural and biochemical comparison of the less infectious parental virus in SUP-T1 cells (NC-MAC) and the more infectious variant virus (CP-MAC) showed marked differences in their envelope glycoproteins. Analysis of envelope spikes from electron micrographs revealed that CP-MAC virions had a significantly greater number of envelope spikes (63.0 $\pm$ 17.0; mean $\pm$S.D. spikes/μm viral membrane) in comparison to NC-MAC virions (29.2 $\pm$ 16.0), suggesting an increased stability of transmembrane-gp120 (TM-SU) complexes for CP-MAC. Immunoprecipitation of envelope glycoproteins from viral lysate with an anti-TM monoclonal antibody, anti-serum to SU, or recombinant soluble CD4 (sCD4), demonstrated that TM and SU of CP-MAC (but not NC-MAC) co-precipitated, consistent with a stable association of these molecules in detergent. Moreover, an HIV-2/SIV SU-specific monoclonal antibody reactive with a potential TM contact site on gp120 precipitated SU from NC-MAC lysates, but reacted with CP-MAC SU only after the SU-TM complexes were disrupted by boiling. Western blot analysis identified a smaller TM for CP-MAC than for NC-MAC (28 kDa vs. 32 kDa). The size difference was still apparent after N-glycanase digestion, indicating that altered glycosylation could not explain this change. Nucleotide sequence analysis of a PCR-amplified fragment from the env gene showed that CP-MAC had acquired a new stop codon upstream of the premature stop codon present in the parental BK28 sequence. Additionally, quantitative analysis of fluoresceinated CD4 binding to the viral envelope expressed on the surface of infected cells demonstrated an affinity for CP-MAC SU similar to that determined for HIV-1/IIIB-infected Sup-T1 cells (10-15 nM). These findings suggest that changes in envelope glycoproteins, which increase the stability of the TM/SU association on CP-MAC, may contribute to its increased envelope spike density on virions, sCD4 binding affinity, and infectivity. Studies are in progress to identify molecular determinants for the phenotypic differences between these two viruses, including the possible role of the new TM stop codon.

Q 126 IN VIVO ROLE FOR COMPLEMENT RECEPTOR 2 (CR2) IN HIV INFECTION Alan L. Landay, Mark Scott, Thomas F. Lint, and Gregory T. Spear, Department of Immunology/Microbiology, Rush Medical Center, Chicago, IL 60612.
Our laboratory has previously performed flow cytometric studies on peripheral blood lymphocytes (PBL) from healthy volunteers and shown that $CD4^+$ and $CD8^+$ lymphocytes express low levels of CR2. Evaluation of $CD4^+$ cells in HIV^+ individuals showed that CR2 coexpression was decreased by 50-80% whereas the proportion of $CR2^+$ $CD8^+$ cells was unchanged. The current study was undertaken to evaluate CR2 expression on B cells in HIV^+ individuals. The B cell population ($CD20^+$) was evaluated using multiparameter flow cytometry for their coexpression of CD19, CD21 (CR2), CD69 (activation antigen), and HLA-DR. The expression of the CR2 receptor was significantly reduced on B cells from HIV^+ individuals (68% $CR2^+$) as compared to HIV^- controls (95% $CR2^+$) but fluorescence intensity was the same. This decrease in CR2 expression did not correlate with $CD4^+$ cell number, but appeared to correlate with advancing disease stage of the invididuals studied. In contrast, the expression of CD19 on $CD20^+$ B cells did not differ significantly between control (98% CD19 positive) and HIV^+ individuals (95% CD19 positive). The percent $HLA\text{-}DR^+$ B cells was also similar between the control and HIV^+ individuals as was the fluorescence intensity of the HLA-DR antigen. The CD69 activation antigen was expressed on B cells from 2 of 16 HIV^+ individuals and 1 of 15 HIV^- controls. CR2 has been implicated in B cell activation and its decreased expression is likely to be correlated with B cell dysfunction in HIV infection. Taken together, these data indicate a potential role for CR2 bearing cells in HIV disease pathogenesis.

Q 127 SPONTANEOUS SHEDDING OF gp120 FROM HIV-1 MODULATES INFECTIVITY, Scott P. Layne[1], Michael J. Merges[2], Hans Gelderblom[3], and Peter L. Nara[2], [1]Los Alamos National Laboratory, Los Alamos, NM 87545; [2]National Cancer Institute, Frederick, MD 21701; [3]Robert Koch-Institute, Berlin, Germany
HIV particles are highly labile at physiological conditions, and this property may vary between lab strains and field isolates. Various processes are responsible for this lability, however, their relationships to neutralization are not known. We have therefore undertaken extensive physical and biological decay analyses on HIV-1HXB3.

Quantitative syncytium-forming infectivity assays, gp120 and p24 ELISA, quantitative electron microscopy, and reverse transcriptase (RT) assays were used to collect parallel data sets from HIV-1HXB3 stocks at 37°C. Kinetic models of HIV infection were used to analyze the viral decay data.

The spontaneous shedding of gp120 from viral particles obeys first order kinetics (half life ~30 hrs). The spontaneous dissolution of p24 from viral particles and their physical breakup is slow (half life >100 hrs) compared to gp120 shedding. The decay of RT polymerase activity obeys a first order process (half life ~25 hrs). The ratio of infectious to non-infectious viral particles ranges from 10^{-3} to 10^{-7} in viral stocks containing 10^9 to 10^{10} particles per ml. For freshly harvested stocks, there are relatively few gp120 knobs per virion, which disappear at a rate corresponding to spontaneous shedding. Each HIV particle contains ~$5x10^{-17}$ g of p24, corresponding to 1250 molecules. HIV decay obeys multi-hit kinetics at 37°C. At short preincubation times, the loss of infectivity correlates with gp120 spontaneous shedding. At longer times, however, an accelerating decay rate indicates that HIV requires a minimal number of gp120 molecules for efficient infection of $CD4^+$ cells. The blocking activity of soluble CD4 and immunoglobulins depends on the physical state of viral particles. Particles with more than a minimal number of gp120 on their surface are blocked in proportion to complex formation. Blocking activity depends on preincubation time and the minimal number of gp120.

These results demonstrate that the physical state of viral particles greatly influences infectivity. The knowledge gained from our findings enhances the reliability of *in vitro* assays and improves our understanding of *in vivo* infections.

Q 128 CELL CYCLE DEPENDENCE OF CIRCULAR HIV DNA FORMATION, Peng Li, Lara Kuiper, Alice Stephenson, and Christopher Burrell, National Centre for HIV Virology Research, Institute of Medical and Veterinary Science, Frome Road, Adelaide SA 5000, Australia

Recent *in vitro* studies suggest that linear free viral DNA may be the immediate precursor to integration in the retrovirus life cycle. On the other hand, supercoiled and relaxed circular HIV DNA may constitute 80-90% of the total unintegrated viral DNA late after infection. We therefore investigated the mechanisms and rationale involved in circular HIV DNA formation using a synchronized one-step cell-to-cell infection model.

When growing H9-HTLV IIIB (H3B) cells were used as virus-donor cells and HUT78 or PHA-stimulated human peripheral blood mononuclear cells (PBMC) as virus-recipient cells, following the synthesis of linear viral DNA, circular HIV DNA appeared 8-12 hr post infection and became the majority species in the total unintegrated viral DNA population 24-48 hr post infection. When resting PBMC (G0/G1) or aphidicolin-arrested HUT78 cells (G1) were infected with G1-arrested H3B cells, linear viral DNA was produced but circular DNA formation was inhibited. When both the virus-donor cells and virus-recipient cells were arrested at S phase of the cell cycle by DNA topoisomerase II inhibitors Vm26 and Vp16, again only circular viral DNA formation was inhibited. When lower concentrations of Vm26 and Vp16 were used and cells were arrested at G2/M phases, circular viral DNA formation was only partially suppressed. Interestingly, there seems to be a correlation between circular, but not linear, viral DNA formation and progeny virus production as judged by supernatant reverse transcriptase (S/N RT) activity: Infection of G0/G1 or S phase arrested cells, where very little circular viral DNA could be detected, produced base-line S/N RT activity. Whereas the same drugs when used at concentrations that led to the arrest of cells in G2/M phases and accumulation of reduced amounts of circular viral DNA upon infection gave rise to reduced S/N RT activity.

Q 129 THE GENOTYPIC COMPLEXITY OF HIV-1 *IN VIVO*, Yuexia Li[1], Huxiong Hui[1], John C. Kappes[1], Joan A. Conway[1], Richard W. Price[2], George M. Shaw[1], and Beatrice H. Hahn[1], [1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, [2]Department of Neurology, University of Minnesota, Minneapolis, MN 55455. All presently available replication competent proviral clones of HIV-1 are derived from cell culture amplified virus. Since tissue culture is highly selective for viral strains with an *in vitro* growth advantage, such clones may not be representative of the biologically relevant virus present *in vivo*. In this study, we report the molecular cloning and genotypic characterization of ten HIV-1 genomes directly from uncultured brain tissue of a patient with AIDS dementia complex. Nucleotide sequence analysis of these clones demonstrated chromosomal integration, circle formation, genomic inversion, and LTR-mediated autointegration of HIV-1 genomes *in vivo*. Transfection of four full-length HIV-1 genomes identified one clone (YU2) to be replication competent and to exhibit growth characteristics similar to tissue culture-derived macrophage tropic strains of HIV-1. Three other clones (YU10, 21, 32), each of which alone was defective, led to a productive viral infection when co-transfected in any combination. Two full-length proviral clones (YU2, 10) were subsequently sequenced in their entirety and their genomic organizations defined. The results showed that, in contrast to YU2, YU10 contained a single base-pair deletion in the *pol* gene, rendering this provirus replication defective. These data demonstrate, for the first time, that replication competent HIV-1 genomes and complex mixtures of minimally-defective viral forms persist *in vivo*. Finally, comparative sequence analysis of PCR-derived viral sequences from brain and spleen of two independent subjects demonstrated that viral populations within the CNS exhibit significantly less genotypic complexity than in peripheral lymphoid tissues. These studies of brain-derived viral clones are expected to prove valuable for future analysis of macrophage and neurotropism as well as other viral properties which are subject to *in vitro* selection pressures.

Q 130 High Level of Surface CD4 on T Cell Transfectants Prevents Persistent HIV Infection *In Vitro*, William L. Marshall, David C. Diamond, and Robert W. Finberg, Dana-Farber Cancer Institute, Boston, MA 02115.

Transfection of the T cell line, HSB, with CD4 DNA allowed us to select a series of CD4 positive clones which varied in their susceptibility to HIV-1 infection. Surprisingly, while clones which expressed low levels of CD4 were infectible by HIV, high expresser clones were not. Cell lines with high CD4 expression displayed only a transient rise in p24 antigen following exposure to several different HIV-1 isolates:HIV-1_{MN}, HIV-1_{IIIB} and HIV-1_{NL4-3}.

Early after infection, HIV proviral DNA was detectable by PCR in equal amounts in both the high and low CD4 expressers This indicated that viral entry was not significantly blocked in the high expressers. Several days following infection, however, no proviral DNA was present in the high expressers, coincident with a loss of ability to isolate virus either by coculture with permissive cells, or by p24 antigen(Ag) detection. Upon rechallenge of high expressers with virus, the same process of transient p24 Ag and provirus production occurred. This ruled out the possibility that the observed phenomenon resulted from an infected subpopulation of cells which would have been eliminated during the first infection.

When infection was initiated by transfection of proviral DNA, high and low CD4 expressers initially produced p24 Ag at the same level. Two weeks following transfection the high expressers ceased producing viral p24 Ag. As demonstrated by coculture, high expressers produced virus only during the first few days following transfection, while low CD4 expressers transfected with HIV continued to produce virus beyond 6 weeks.

The implication of these results is that cells which express high levels of surface CD4 exert a post-entry block upon HIV infection, yet such cells are transiently capable of replicating virus.

Q 131 SYNTHETIC PEPTIDE ANALOGS OF ICAM-1 INHIBIT HIV-1 REPLICATION. D. McPhee[1], N. Pavuk[1], K. Silburn[1], D. Read[1], A. Mansell[2], A. Boyd[3], S. Kent[4], and J. Fecondo[2,3] [1]Macfarlane Burnet Centre for Medical Research, Melbourne, [2]Swinburne Institute of Technology, Melbourne, [3]Walter and Eliza Hall Institute, Melbourne, Australia and [4]California Institute of Technology, Pasadena, U.S.A.

It has been suggested that LFA-1 has a possible role in HIV-1 infection. Given that ICAM-1 is the ligand for LFA-1, we have explored the possibility that this molecule may be involved in the HIV-1 infection process. Replication was assessed in PBMCs and MT-2 cells, both susceptible to HIV infection, but differing in their ability to form syncytia. Surface expression of the ICAM-1 and LFA-1 molecules was quite different for these two cell types in that ICAM-1 was very high with MT-2 cells (80-95%) compared with PBMCs (10-50%). In contrast, LFA-1 expression was very high with PBMCs (75-95%) compared with MT-2 cells (10-20%). A peptide corresponding to a unique region of the ICAM-1 sequence had little inhibitory effect on virus replication, despite it's ability to inhibit cell-cell adhesion (Fecondo et al, 1991). However, an N-terminal peptide markedly inhibited virus replication in MT-2 cells but not in PBMCs as measured by cell free RT activity, p24 antigen production and syncytium formation. With MT-2 cell infection the inhibitory levels of peptide required (10.3uM for 50% inhibition) were at least 10 fold more effective than inhibitory CD4 peptides (Jameson et al, 1988). Minimal cytotoxic effects were observed with test and control peptides at levels tested. These results indicate that we have identified a possible role for ICAM-1 in HIV-1 infection, and that the functional region, if ICAM-1 is involved in this process, is separate to the previously identified sites of interaction with LFA-1. Additionally, the effect on virus replication was variable depending on levels of surface expression of the molecule and syncytium formation.

Fecondo, J. et al (1991) Proc. Natl Acad, Sci, USA **88** 2879-2882.
Jameson, B. et al (1988) Science **240** 1335-1339.

Q 132 MULTIPLE MECHANISMS FOR THE INTERFERON INHIBITION OF HIV REPLICATION IN MACROPHAGES. **Pascal R.A. Meylan, Guatelli J.C., Munis J.R., Kornbluth R.S., Richman D.D. University of California and VAMC, San Diego, CA 92161.**

Objective: Interferons (IFNs) display a potent antiviral effect on HIV-1 replication in macrophages (MΦ). A high multiplicity of infection (MOI) system was used to investigate which steps of the life cycle are affected by IFNs.

Methods: Monocyte-derived MΦ from seronegative donors were infected with the BaL strain. Viral replication was assessed by measuring (i) proviral DNA synthesis by PCR for an *env* sequence, (ii) HIV transcript levels by RT-PCR with primers bracketing the 5' introns of HIV-1. This technique selectively detects *tat, rev, nef* and *env* mRNAs. Both amplification systems were shown to be quantitative by titration curves. (iii) Virus production was assessed by measuring the p24 antigen (Ag).

Results: MΦ pretreated for 18 h with 1000 U/ml of IFN-α,-β or -γ were infected at a MOI=3. IFNs reduced the *env* proviral DNA signal by 2-10 fold while the spliced RNA signal was essentially abolished, and no virus was produced. To test whether IFNs can reduce HIV transcripts in "chronically" infected MΦ, MΦ were infected at a low MOI (0.01-0.1) and cultured 10 days before IFN treatment. No effect of IFNs on viral DNA and RNA was observed in 2/4 experiments, while in the 2/4 other experiments, IFNs reduced the viral DNA and RNAs, and the p24 Ag production. This was compatible with IFNs preventing MΦ reinfection. To assess whether IFNs had a direct effect on viral RNA independent from reinfection, MΦ were infected at a MOI=3, cultured for 2 days and then treated with AZT (to prevent reinfection) ± IFNs. A similar decrease of proviral DNA and spliced RNA signal was observed in AZT and AZT+IFN-treated MΦ. Both cell-associated and extracellular p24 Ag were consistently more reduced by AZT+IFN than by AZT alone.

Conclusion: IFNs prevented proviral DNA synthesis, and subsequent transcription. No separate direct effect on transcript levels was observed. While IFNs had no more effect than AZT on provirus formation or transcripts levels, they produced an additional reduction of p24 Ag, suggesting a post-transcriptional effect.

Q 133 Mechanisms of Activation of Latent HIV from Monocytes, [1]J.A. Mikovits, [2]N.C. Lohrey and [2]F.W. Ruscetti. [1]BCDP, PRI/DYNCORP, [2]LMI, BRMP, FCRDC, Frederick, MD. 21702.

Human Immunodeficiency virus-1 (HIV), a prototype lentivirus, can persist despite a strong host immune response, in part because the monocyte can harbor the viral genome in a latent state. To better understand the mechanisms involved in activation of latent HIV from monocytes, latently infected monocytoid cell lines U937 and THP-1 were used as models. U937, THP-1 and several clones of THP-1 containing latent HIV (no RNA expression as measured by PCR) could be induced to express infectious virus by coculture with Con A-activated T cells, isolated from normal, HIV- donors. Fractionation of these T cells into CD4+ and CD8+ subsets showed the CD4+ cells were responsible for the induction of expression of latent HIV. HIV expression was not induced by Con-A alone, resting T cells, Con-A-activated T cell lines (HUT-102) or T cell supernatants. However, plasma membranes isolated from Con-A-activated T cells stimulated expression from latent THP-1, suggesting that cell contact induces factor(s) in monocytes capable of overcoming latency. In addition to this immune activation, two other treatments can induce latently infected THP-1 and U937 to express infectious HIV: treatment with the hypomethylating agent, 5-azacytidine and transient expression of the viral regulatory proteins Tat and Tev in the these cell lines. Thus, monocytes harboring latent HIV are inducible in several distinct ways: immune activation, hypomethylation and HIV trans-acting factors. HIV produced by such monocytes can infect T cells and induce pathology suggesting that latently infected monocytes play a role in the pathogenesis of AIDS.

Q 134 *IN VITRO* AND *IN VIVO* SUSCEPTIBILITY OF BONE MARROW AND LYMPH NODE DERIVED CELLS FROM CHIMPANZEES TO HIV-1 INFECTION, Krishna K. Murthy, James Hastings and Scott R. Rouse, Department of Virology and Immunology, Center for AIDS Research, Southwest Foundation for Biomedical Research, San Antonio, TX 78228

Several recent studies are of the opinion that infected peripheral blood cells are not true indicators of virus load, because HIV tends to sequester in bone marrow (BM) and lymph nodes (LN). Therefore, we have evaluated the *in vitro* susceptibility of BM derived cells from seronegative chimpanzees. In addition, peripheral blood, BM and LN derived cells from HIV-1 infected chimpanzees were subjected to virus isolation and polymerase chain reaction (PCR) analysis. Cells were cultured with 0.5 - 1.0 μg/ml phytohemagglutinin for three days and then cultured in medium containing either 10% interleukin-2 (IL-2) or 10 ng/ml granulocyte-monocyte colony stimulating factor (GMCSF). BM derived cells from 6/7 normal chimpanzees were susceptible to *in vitro* infection in a descending order of magnitude with IIIB, RF, MN and SF2 isolates of HIV-1. In contrast, 1/4 BM and 3/4 LN samples from HIV-1 infected chimpanzees were positive for virus isolation. Culture conditions appear to influence the selection of particular cell types during *in vitro* culture of BM cells. In the presence of IL-2, the predominant cell type observed during a 4 to 6 wk culture period was CD3+ T cells, whereas substitution with GMCSF resulted in the selection of LeuM5+ monocyte/granulocyte cell types.

Q 135 LPS MEDIATES THE DOWNMODULATION OF MONOCYTE CD4 IN WHOLE BLOOD *EX VIVO*.

Elizabeth V. Neate*, William Boyle+ and Dale A. McPhee*.
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+University of Melbourne, Parkville, Vic., Australia.

Monocyte/macrophages are important cellular targets for HIV-1 infection. Kazazi et al have shown that freshly isolated monocytes were more susceptible to infection than cultured macrophages and that surface CD4 expression was down-modulated on overnight culture (1). We have examined the possibility that trace levels of LPS (below detection limits of LAL assays) can downmodulate monocyte surface CD4 during purification rendering cells less susceptible to HIV-1 infection. Whole blood was cultured *ex vivo* (2) with titrations of LPS and monocyte surface antigen expression assessed by dual colour flowcytometry. Culture supernates were assayed for TNFα. LPS selectively downmodulated monocyte surface CD4 expression and induced CD25 expression in a dose dependent manner (pg-ng range) with all donors tested (n=8). Primary T-cell CD4 surface expression was not downmodulated. Induction of CD25 expression and TNFα release preceded CD4 loss. Separation of PBMC over Ficoll also resulted in loss of monocyte CD4 (PBMC cultured in plasma without added LPS) and was shown not to be due to separation technique. Attempts to block CD14, the high affinity LPS receptor, with monoclonal antibody were unsuccessful. Removal of CD14 from purified monocytes using phosphatidyl specific phospholipase C (PI-PLC) did not inhibit CD4 loss indicating that the signal/s to downmodulate CD4 was delivered prior to PI-PLC treatment or that LPS receptors other than CD14 were utilized. Thus, monocyte surface CD4 can be downmodulated by LPS *ex vivo* with coincidental activation. Protection of cultured macrophages from HIV-1 infection by trace levels of LPS (3), present in media and viral inoculums, may well be explained by the loss of CD4 surface expression.

1. Kazazi F et al (1989) 70:2661-2672. 2. Decsh CE et al (1989) 8:141-146.
3. Kornbluth RS et al. (1989) J Exp Med 169:1137-1151.

Q 136 PRIMARY HUMAN BRAIN CAPILLARY ENDOTHELIAL CELLS ARE PERMISSIVE FOR HIV INFECTION, Jay A. Nelson[1], Floyd E. Bloom[2], C. David Pauza[3], and Ashlee V. Moses[1], [1]Department of Immunology, [2]Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037, and [3]Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI 53706.

Primary cultures of adult human brain capillary endothelial cells were studied for their permissiveness to infection by the LAV-1 strain of human immunodeficiency virus (HIV). Up to 50% of cells in low passage cultures expressed cytoplasmic p24 gag protein as determined by indirect immunofluorescence. Infected cells were confirmed as endothelial cells by double labeling with von Willebrand factor (Factor VIII). Infectious virus was first detectable in culture supernatants at one week post infection. Cultures remained positive after continued passage. Despite supporting a significant level of virus infection, endothelial cells displayed no cytopathic effect and the expression of Factor VIII remained unchanged. Endothelial cells were negative for surface CD4, suggesting a CD4-independent mechanism of virus entry. Given the fact that brain endothelial cells have been identified as an infected cell type in AIDS patients and are permissive for HIV infection *in vitro*, they may provide a route of entry of HIV into the CNS without physical disruption of the blood-brain barrier. Despite the absence of an overt cytopathology, infected cells may secrete toxic metabolites or display an altered permeability, thus perturbing the integrity of the blood-brain barrier. Direct infection of brain endothelial cells may thus contribute to the neuropathological consequences of AIDS.

Q 137 TWO MECHANISMS OF SOLUBLE CD4 (sCD4)-MEDIATED INHIBITION OF HIV-1 INFECTIVITY AND THEIR RELATION TO sCD4 RESISTANCE BY FRESH HIV-1 ISOLATES, Sherry L. Orloff, M. Susan Kennedy, Paul J. Maddon, and J. Steven McDougal. Immunology Branch, Division of HIV/AIDS, Centers for Disease Control, Atlanta, GA 30333 and Progenics Pharmaceuticals, Tarrytown, NY 10591.

Two assays that represent separate mechanisms by which sCD4 blocks HIV-1 infectivity are described. The assays differ procedurally, and results of experiments where sCD4/HIV-1/cell concentrations and sequence of combination, noninfectious/infectious particle ratio, and temperature were varied support the conclusion that sCD4 inhibits HIV-1 infection by two mechanisms: reversible blockage of receptor binding and irreversible inactivation of infectivity. Fresh isolates obtained from HIV-1-infected people were found to be relatively resistant to sCD4 inhibition in the neutralization assay (as has been reported) and also in the inactivation assay. Binding studies with sCD4 revealed similar affinities for sCD4 in detergent lysates of sensitive and resistant strains at both 4°C and 37°C. However, the avidity of intact virions for sCD4 was lower at 4°C than at 37°. Compared to the intrinsic or whole virion Kd, neutralization and inactivation by sCD4 occur at dose ranges of sCD4 that are one and two orders of magnitude higher, respectively. Relative resistance to sCD4 is not related to differences in intrinsic affinity of gp120 for sCD4. Rather, at saturating levels of sCD4, relative resistance represents differences in access to gp120 or differences in secondary effects of sCD4 on the virion.

Q 138 HSV-1 ACTIVATES HIV-1 PROVIRUS BY NF-κB-DEPENDENT AND -INDEPENDENT MECHANISM, Paula M. Pitha and Jaromir Vlach, Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD 21231

Herpes simples virus type 1 (HSV-1) infection induces expression of the human immunodeficiency virus type 1 (HIV-1) provirus in the chronically infected T-cell line, ACH-2. The HSV-1-mediated induction correlates with the appearance of regulatory proteins binding to two HIV-1 LTR, κB enhancer region and to the untranslated leader sequence consisting of three LBP-1 binding sites. HSV-1 infection induced presence of two NF-κB-binding proteins of m.w. 55- and 85-kilodalton (kDa) in the nucleus; these proteins presumably represent the alternatively processed form of NF-κB p50 and the c-rel protooncogene, respectively. The leader sequence is a target for binding of a novel protein, designated HLP-1 that is present exclusively in HSV-1-infected, but not in 12-0-Tetradecanoylphorbol 13-acetate- (TPA) or tumor necrosis factor- (TNF) treated ACH-2 cells. Both the NF-κB and LPB-1 target sequences, when inserted either alone or together 5' of a heterologous (TK) minimal promoter, can confer inducibility by HSV-1 infection. Thus, it appears that the HSV-1-mediated activation of HIV-1 provirus proceeds through the binding of both NF-κB and HLP-1 proteins. Purification and further characterization of the HLP-1 protein will be discussed.

Q 139 A C-TERMINAL DOMAIN OF THE HIV-1 GAG PRECURSOR PROTEIN FACILITATES ITS STABLE ASSOCIATION WITH CELLULAR MEMBRANES. Emily J. Platt and Omar K. Haffar, Virology Department, Bristol Myers-Squibb Pharmaceutical Research Institute, 3005 First Avenue, Seattle, WA. 98121.

Association of the HIV gag polyprotein precursor with cellular membranes is a crucial step in the assembly of HIV virions. We have used the rabbit reticulocyte lysate (RRL) translation system to in vitro synthesize HIV gag in order to study its association with isolated cellular membranes. RRL programmed with gag mRNA incorporated [^{35}S]-methionine into two predominant species of 55 kDa and 40 kDa. Both were radioimmunoprecipitated with HIV specific rabbit polyclonal sera and visualized by SDS-PAGE. Substituting [^{3}H]-myristate for [^{35}S]-methionine in the translation reaction revealed that both the 55 kDa and the 40 kDa gag specific proteins were myristylated in the reticulocyte lysate system. A monoclonal antibody specific for p17, the amino terminal portion of the gag precursor, recognized both the 55 and 40 kDa gag specific proteins, suggesting that the 40 kDa protein lacks C-terminal sequences and may arise by proteolytic cleavage or premature termination. The 55 kDa protein represents the HIV polyprotein precursor (Pr55gag). Incubation of in vitro translated gag proteins with membranes, followed by centrifugation at 100,00x g, resulted in a majority of Pr55gag being distributed to the pellet fraction. The membrane binding of Pr55gag could be disrupted by treatment with 500mM NaCl. In contrast, the 40 kDa protein remained in the supernatant both in the absence and presence of membranes. This suggests that a C-terminal domain in the HIV gag precursor may facilitate stable association of the HIV gag precursor with membranes. Other truncated gag proteins synthesized in vitro (RRLs were programmed with truncated gag mRNAs generated by linearizing the gag template within the gag coding sequence) indicate that this putative domain resides N-terminal to amino acid residue 436.

Q 140 HIV-1 INFECTION OF MAMMARY EPITHELIAL CELLS, Kimber L. Poffenberger, Gary Riordan, Sherwin Lee, Jay Epstein, and Indira Hewlett. Retrovirology Laboratory, Division of Transfusion Science, Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892.

The breast milk of seropositive mothers is known to harbor the HIV virus and may be a determinant in mother-infant transmission. The source of virus in breast milk has not been defined; virus could pass into milk in infected lymphocytes and macrophage cells or may be produced by infected mammary secretory or ductal cells which contact milk. We are studying cell lines isolated from both normal breast and breast carcinomas to determine whether mammary epithelial cells can be productively infected by HIV-1. We report that mammary epithelial cells can support the growth of HIV-1 (MN). Viral p24 antigen can be detected in the culture supernate of infected cells, although at levels far below those of H9 cells infected at the same multiplicity. MN DNA is detectable by PCR in mammary epithelial cells, again at decreased levels relative to that in H9 cells. The profiles of p24 production over time after infection and the relative amounts of p24 antigen produced vary among different cell lines. Cells from carcinomas appear to produce more viral antigen than cells from normal tissue. We will report our ongoing characterization of viral products made during infection and of cellular factors which may alter virus growth in different mammary cell lines.

Q 141 HTLV-I AND HTLV-II "PROBLEM SPECIMENS" BY IMMUNOBLOT AMONG INTRAVENOUS DRUG USERS IN BALTIMORE, MD. Proietti F* **, Vlahov D*, Alexander S***, Farzadegan H*, Kalfaglou C*, Cohn S*, Saah A*. *Dept of Epidemiology, Johns Hopkins School Hygiene Public Health, Baltimore, MD; ** Federal University Minas Gerais, Brazil; *** Cambridge Biotech, Rockville, MD.

OBJECTIVE: To characterize PCR confirmed HTLV-I and HTLV-II infections with equivocal immunoblot (IB) results among IVDUs in Baltimore.

METHODS: 2,801 IVDUs recruited by extensive community outreach underwent serological testing by ELISA (Cambridge BioScience); reactive samples were submitted to IB testing that incorporates a recombinant HTLV-I transmembrane envelope p21E. HTLV-I versus HTLV-II was determined by the relative intensity of p19 and p24 bands, given p21E positivity. Polymerase chain reaction (PCR) was used to confirm HTLV-I or HTLV-II infection.

RESULTS: Of 2,802 IVDUs, 201 (7.1%) were HTLV-I/II (+) by ELISA and IB confirmed. 71 "IB problems samples", requiring rereading of the IB were tested by PCR (HTLV-II = 23; HTLV-I = 8; negative = 19; indeterminants = 21, by the IB criteria).

IB	PCR			
	HTLV-I	HTLV-II	Negative	Total
HTLV-I	3 (38%)	3 (38%)	2 (24%)	8 (100%)
HTLV-II	—	19 (83%)	4 (17%)	23 (100%)
Neg.	—	2 (11%)	17 (89%)	19 (100%)
Indet.	—	6 (29%)	15 (71%)	21 (100%)

The classification of the samples as HTLV-I, HTLV-II, negative or indeterminant by the IB was independent of HIV-1 serology.

CONCLUSION: The IB showed limited ability to accurately discriminate between HTLV-I and HTLV-II infection in the "problem samples". These samples were further characterized using other recombinant and synthetic peptides.The IB results were not affected by HIV-1 serology.

Q 142 STUDIES ON HIV-1 ENTRY, H.L. Robinson, R. Fernandez-Larsson and S. Lu, Department of Pathology, University of Massachusetts Medical Center, Worcester, MA 01655

We have been using single cycle infections in T-cell lines and PBMCs to determine which steps in the HIV-1 life cycle determine differences in the permissiveness of infection. Each of the differences we have found has involved entry. 50% entry times have ranged from <20 minutes to 6 hours. Both the virus isolate and the cell type have affected entry. Viruses exhibiting the most rapid entry have been rapid/high patient isolates and the one exhibiting the slowest entry, a slow/low patient isolate. A lab strain, NL4-3, has intermediate entry characteristics. C8166 cells have been the most permissive for entry; PBMCs, intermediate in permissiveness and H9, A3.01 and Jurkat cells, the least permissive.

To learn which steps in entry are determining differences we have begun to screen various agents for the time course with which they block entry. The first of these studies has compared the time course with which NL4-3 escapes the ability to escape a "wash" block, block by a neutralizing antibody to the V3 loop (0.5ß), and block by an antibody to the gp120 binding domain on CD4 (leu3a). Unexpectedly, the escape from the 0.5ß block was more rapid than escape from the leu3a block. In C8166 infections 50% escape from the 0.5ß block occurred simultaneously with escape from the wash block (requiring 20 minutes) while escape from the leu3a block required 40 minutes. In H9 cells, escape from the wash block required about one hour, escape from 0.5ß, about 2 hours, and escape from leu3a about 3 hours. These studies demonstrate that anti-CD4 antibodies (such as leu3a) have a longer window of time in which they can block entry than anti-V3 loop antibodies.

Q 143 CD4 INDUCED CONFORMATIONAL CHANGES IN THE HIV ENVELOPE GLYCOPROTEINS

Quentin J. Sattentau*, Jane A. McKeating**, Brad Jameson*** Robin A. Weiss** and John P.Moore**. *Centre d'Immunology de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France, **Chester Beatty Laboratories, 237 Fulham Road, London SW3, England. ***Jefferson Cancer Institute, 233 South Tens Street, Philadelphia, PA 19107.

Infection of CD4+ cells by HIV is initiated by the high affinity binding of the outer envelope glycoprotein, gp120, to CD4. Although the regions of CD4 involved in gp120 binding have been precisely defined, the role of CD4 in virus entry is little understood. We have used recombinant, soluble CD4 (sCD4) to model the interaction between CD4 and gp120 at the surface of virions and HIV-infected cells. sCD4 binding induces modulation of gp120/V3 loop exposure, increased exposure of cryptic epitopes on gp41, and the dissociation of gp120 from gp41. Cyclised peptides synthesised from the sequence of the CDR-3-like loop of CD4 domain 1 mimic sCD4 in this activity, and block HIV infection and syncytium formation. Certain monoclonal antibodies binding outside the first domain of CD4 inhibit HIV infection and syncytium formation but not virus binding, and prevent sCD4 induced conformational changes in the HIV envelope glycoproteins. Thus regions of CD4 outside domain 1 may participate in HIV entry. We conclude that CD4 is involved not only in virus binding, but is important for subsequent events leading to HIV induced membrane fusion.

Q 144 A SIMPLE AND SENSITIVE FLOW CYTOMETRIC METHOD TO MONITOR THE BINDING OF HIV VIRIONS TO $CD4^+$ CELLS AND THE INHIBITORY EFFECTS OF ANTIVIRAL COMPOUNDS ON VIRUS-CELL BINDING, Dominique Schols and Erik De Clercq, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

PKH-2 is a stable and reproducible cell labelling dye developed by Zynaxis Cell Science. It is incorporated through a highly aliphatic reporter molecule into the lipid bilayer of cytoplasmic membranes containing a fluorochrome head group. The probe is trapped once incorporated into the membrane, because of its inherent insolubility in aqueous environment. This dye is not only suitable for cell tracking (P. Horan *et al.*, Methods Cell Biol. 33: 469-490, 1990) but can also be used for viral tracking. MT-4 cells were infected with human immunodeficiency virus (HIV) at 100 $CCID_{50}$ and stained two days later with PKH-2 at 2 x 10^{-6} M for 10 min at room temperature. After two washing steps, the cells were further cultured for three days, upon which the supernatant was collected, and the virus particles pelleted. As a control, uninfected cells were treated by the same procedure. Fluorescent-labeled viral particles produced from the HIV-infected cells could be detected by FACS analysis following exposure of these particles for 30 min at room temperature to $CD4^+$ cells. This technique is very simple, reproducible and avoids the use of either radiolabeled virus particles or specific antibodies for measuring virus-cell binding. The present method permits a direct quantitative determination of the inhibitory effects of anti-HIV agents on the virion-cell binding process.

Q 145 MODULATION OF CELL SURFACE MOLECULES DURING HIV-1 INFECTION OF H9 CELLS, Henk-Jan Schuurman, Timo Meerloo, Piet Joling, Albert DME Osterhaus, and Jaap Goudsmit, Department of Pathology, University Hospital, 3508 GA Utrecht, Natl Inst Public Health and Environm Hygiene, Bilthoven; Academic Medical Center, Amsterdam, The Netherlands

We performed immunogold electron microscopy of H9 T-cells in a chronic state of infection and cells 2 days after infection with HIV-1 IIIB, both for cell surface molecules and HIV-1 encoded antigens. Infected cells manifested HIV-1 proteins *gag* p24, *pol*, *env* gp41 and gp120 in clusters in the extracellular area and in budding particles on the cell surface. Cell surface molecules including CD3, CD4, CD5, CD25, CD30 antigens and HLA-DR are present in a random distribution along the cell surface. The CD63 antigen, a lysosomal membrane glycoprotein, was mainly located in the cytoplasm of uninfected cells. Cells 2 days after infection showed CD4 labeling on sites were virions were budding from or attached to the cell surface and on free virions: this was confirmed in double labeling for HIV-1 *gag* p24 and CD4. Virions showed also labeling by CD3, CD5, CD25, CD30 and CD63 antibodies and anti-HLA-DR. In quantitative assessment, the cell membrane of infected H9 cells showed a significantly lower density of CD4, CD5 and anti-HLA-DR than the membrane of uninfected cells. The labeling density by CD63 was significantly higher. We conclude that during the first phase of infection host cell molecules concentrate on budding structures and newly generated HIV-1 virions. This phenomenon contributes to the disappearance of these molecules (like the CD4 molecule) from the cell membrane after infection.

Q 146 A Novel, Sensitive Radioimmunoprecipitation Assay For The Detection of Human Immunodeficiency Virus-Type 2 (HIV-2)

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Laboratory of Retrovirology, Division of Transfusion Science,Center for Biologics and Evaluation Research, FDA, Bethesda, MD-20892.

We have developed a novel, sensitive, and efficient radioimmunoprecipitation assay (RIPA) which provides confirmation for ELISA assays and is an alternative to Western blot assays for characterizing antibodies against human immunodeficiency virus type-2 (HIV-2). For labelling, we used a solubilized 1000X preparation of the purified NIH-Z (HIV-2) virus strain spiked with purified recombinant HIV-2 gp105, produced in the baculovirus expression system. Reaction of the spiked lysate with ^{125}I-labelled Bolton-Hunter reagent efficiently labels (around 80%) all the major virus-specific proteins including gp140K, gp110K, gp82 (dimer of gp41), p66, p34, p31, and p24. These labelled proteins are effectively immunoprecipitated by hyperimmune human sera, even at the early stages of seroconversion. The proteins can also be precipitated by monospecific sera derived from hyperimmunized animals. A complete HIV-2 serum panel was analyzed using an existing ELISA system. The ELISA findings were confirmed using our newly developed RIPA and Western blot. This new assay system proved to be simpler and more sensitive than Western blotting for characterizing and titering antibodies against HIV-2 and is equivalent to the Western blot in specificity and efficiency. Additionally, viral proteins labelled with Bolton-Hunter reagent are well suited for biochemical studies. The number of complex oligosaccharide side chains of gp110K was determined by examining the sensitivity of these viral proteins, labelled by the Bolton-Hunter procedure, to N-Glycanase and Endo-H-glycosidase enzymes.The envelope bands of both RIPA and Western blot assays of the HIV-2 serum panel were scanned by densitometer and quantitated in terms of pixel units.

Q 147 ANALYSIS OF NON-CD4 MEDIATED HIV-1 INFECTION USING SELECTIVE INHIBITORS, Mandal. K. Singh, Yaoxing Huang, and David D. Ho, Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY 10016.

Many human cell lines of neural, hepatic and epidermal origin do not express the major HIV-1 receptor CD4 but are nevertheless infectable in vitro suggesting an alternate receptor. We previously reported productive HIV-1 infection of a CD4-negative neuronal cell line, SK-N-MC (J. Virology 64, 1383-7, 1990). We now present a detailed analysis of the infection process using anti-gp120 antibodies and other specific reagents. Monoclonal antibodies directed against the V3 loop or the CD4-binding domain have no effect on the infection of SK-N-MC cells, despite showing good neutralizing activity in T-cell infection. Similarly, soluble CD4 also failed to block SK-N-MC infection. Several sugars, including D-galactose, D-mannose, N-acetylgalactosamine, N-acetylglucosamine and mannan, also have no effect on HIV-1 infection of SK-N-MC cells. Interestingly, nucleoside analogs AZT and ddI have only a small inhibitory effect, whereas a protease inhibitor and dextran sulfate effectively blocked the infection. Our findings therefore indicate that for SK-N-MC infection by HIV-1, both CD4 and the known functional domains of gp120 (V3 loop and the CD4-binding site) are non-essential.

Q 148 CONSTRUCTION OF A BINDING SITE FOR HIV-1 GP120 IN RAT CD4, Chamorro Somoza, Gérard A. Schockmel, Simon J. Davis, Alan F. Williams and Don Healey*. MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE. *Department of Pathology, Cambridge University, Cambridge, CB2 1QP.

Previous studies of the gp120 binding site of CD4 have demonstrated that mutations in various regions of domain 1 lead to the loss of gp120 binding. In the present study an approach was taken in which a gp120 binding site was constructed in rat CD4 by replacing rat with human CD4 sequence. Initial experiments showed that human residues 36-62 were sufficient to generate a gp120 binding site in rat CD4. However the relative affinity of this mutant for gp120 was determined to be 60-70 fold less than that of human CD4, which indicated that the binding site for gp120 was not fully constituted. Based on the inspection of the X-ray crystallographic structure of human CD4 additional mutants were constructed. In two mutants rat CD4 residues were restored at positions 60-61 and 59-61, and in both the gp120 binding affinity was slightly improved, which argues against residues 59-61 being centrally involved in the gp120 binding site. In a third mutant, the rat residue Gln-80 was substituted for the Asp present in human, but this had only a small beneficial effect on the binding of gp120. In a final mutant the humanized region was extended to the conserved Gln at position 33 and this mutant had an affinity for gp120 only two-fold less than that of human CD4. In conclusion these experiments strongly indicate that the gp120 binding site of human CD4 is constituted by residues 33-58 and highlights the importance of the C, C' turn in forming the binding site. Studies to test the ability of the mutants to mediate HIV-1 infection are in progress.

Q 149 ***USE OF A HYBRID CELL LINE TO ANALYZE THE TROPISM OF HIV-1 STRAIN 89.6,*** Kelly A. Stefano, Ronald Collman, James A. Hoxie, Neal Nathanson, F. Gonzalez-Scarano, University of Pennsylvania Medical Center, Philadelphia, PA. 19104.

Strains of HIV-1 differ in their ability to infect and replicate in CD4-positive primary and transformed cell types. This suggests that other cellular factors, in addition to CD4, are involved in permissiveness for HIV-1 infection and replication.

HIV-1 strain 89.6 productively infects both peripheral blood lymphocytes (PBL) and primary monocyte-derived macrophages (MDM), but is restricted in most transformed cell lines. In contrast, the prototype strain 3B productively infects PBL and most CD4-positive transformed cell lines but not MDM. While 89.6 is restricted in most transformed cell lines, we identified a hybrid cell line, CEMx174, which is permissive for productive infection by both 89.6 and 3B. This CD4 positive hybrid cell line is derived from the fusion of the CD4 negative B cell line 721.174 and the CD4 positive T cell line CEM. While 89.6 can productively infect the CD4+ hybrid cell, it cannot productively infect either of the two parental cell lines, even though CEM cells are CD4 positive. This is in contrast to 3B, which productively infects both CD4 positive cell lines, but not the CD4 negative 721.174 cell line. These cell lines provide a model in which to examine cellular factors governing permissiveness and restriction of viral infection.

To determine the role of early versus late events in viral restriction, we transfected the infectious proviral DNA clone of 89.6 into 721.174, CEM and CEMx174 cells. Transfection of all three lines resulted in viral p24 antigen production, suggesting that restriction of 89.6 in CEM cells does not lie at the level of viral replication, but in earlier events. To determine if the cell-specific restriction reflects differences in viral entry, we utilized a sensitive assay which measures virus internalization. 89.6 was efficiently internalized by CEMx174, but not by CEM cells. In contrast, 3B was efficiently internalized by both CD4 positive cell lines. Neither virus was internalized by the CD4 negative 721.174 cell line.

Thus, the closely-related CEM and CEMx174 cell lines differ in ability to support productive infection by HIV-1 isolates 3B and 89.6, and this difference is determined, at least in part, at the level of viral entry. This suggests that HIV-1 strains differ in the cellular elements, in addition to CD4, necessary for efficient viral entry. In addition, the data suggest that CEMx174 cells posess some cellular factor(s), derived from the B cell parent, which is (are) essential for entry of some but not all isolates.

Q 150 Molecular Detailing of the gp120 Binding site on CD4 through Site-Directed Mutagenesis. R. Sweet, K. Deen, M. Chaikin, P. Ryan, D. Krawczyk, A. Truneh and M. Rosenberg. SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19004.

The design of small molecule antagonists of the interaction of gp120 with CD4 is predicated on the molecular definition of this binding site. In previous studies, we and others localized the primary determinants of the gp120 binding site on CD4 to residues 40-60 within the first extracellular domain (D1). However, subsequent reports have suggested a more extensive surface of interaction. Recently in collaboration with the laboratory of Wayne Hendrickson, the structure of the 2 N-terminal domains of CD4 was solved. Overlay of the recent mutational data onto this structure presented a topological puzzle in which putative components of the gp120 binding site were widely separated in space. Against this background, we systematically introduced more than 60 substitution mutations into the D1 domain of soluble CD4 and quantitatively assessed their effect on recognition of gp120 from the 3B/BH10 isolate and of several α-CD4 antibodies. Locally disruptive mutants with decreased, increased, and unaltered binding were observed which defined the closely apposed side chains of phe43 and arg59 as probable contact sites. This analysis was extended to the gp120 proteins of other HIV-1 and HIV-2 isolates and revealed limited differences in molecular recognition in the context of a common binding site.

Q 151 ROLE OF CD4 MOLECULE IN HIV LATENCY, [1]Michel Tremblay, [2]Sylvain Meloche, [3]Mark A. Wainberg, and [4]Rafick-P. Sékaly, [1]Laboratory of Infectious Diseases, Centre Hospitalier de l'Université Laval, [2]Hôtel Dieu de Montréal, Montréal, [3]Lady Davis Institute, Montréal, [4]Laboratory of Molecular Immunology, Institut de Recherches Cliniques de Montréal, Montréal, Canada.

A biologic feature of HIV is its ability to remain latent once integrated into host cell genome. Mechanisms responsible for this latent state are still unknown. Several studies have suggested that interactions of the virus with both host cell factors and specific viral regulatory mechanisms may induce such a state of quiescence. We will present data which indicate that the CD4 glycoprotein could participate in the latency period of HIV. The full-length CD4 molecule (wt-CD4) and a truncated form of CD4 (t-CD4), lacking most of the cytoplasmic domain, were introduced by a retroviral vector into a CD4-negative T cell line (A2.01). Thereafter, such cells were infected with different strains of HIV-1. Viral protein expression and production of infectious particles were monitored by immunofluorescence and coculture experiments. Higher levels of viral replication were detected in t-CD4 cells. In addition, Western and northern blot studies have demonstrated exclusively quantitative changes of protein synthesis and of mRNA species following HIV-1 infection of cells expressing wt-CD4 or t-CD4. We next evaluated the extent of viral entry into such cells. The level of total virus load, present in cells early after infection, was monitored by an enzymatic assay and a quantitative PCR to detect p24 antigen and viral DNA, respectively. Results from both studies have indicated that similar levels of HIV-1 were entering such cells. Experiments were then initiated with antiviral agents such as azidothymidine and soluble CD4. Data from studies using these inhibitory agents have demonstrated that the CD4 molecule could affect negatively expression of integrated viral DNA.

Q 152 High Efficiency Phage Plaque Screen for Genetic Dissection of Protein/Ligand Interactions.

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SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

A λ phage expression methodology was adapted to efficiently dissect protein/ligand interactions through the creation and rapid screening of large numbers of mutants. The method was applied to the fine mapping of the residues of CD4 involved in the high affinity interaction with the external envelope glycoprotein of the human immunodeficiency virus (HIV-1), gp120. Random substitutions were introduced throughout the gp120 binding region (amino acids 38-62) in the amino terminal domain of CD4 by oligonucleotide mutagenesis. These mutations were expressed within phage plaques and directly screened for their effect on binding of gp120 using a modified phage plaque lift procedure. Plaques showing increased, decreased, and no effect on binding were identified and mutations were verified by sequence analysis. A new site was identified at which mutations reduced binding to gp120 and several novel amino acid substitutions were defined at sites previously implicated in binding. Of particular interest, this in vitro genetic approach identified a mutation which increased binding to gp120. The phenotypes of several of these mutants were further characterized by quantitative measurement of their binding affinity. The results confirmed the accuracy of the phenotypic selection and demonstrated that the sensitivity of the system allowed detection of a 3-4 fold increase or decrease in affinity. In the context of the recently determined atomic structure of CD4, these results further implicate residues in the CDR2-like region and in an adjacent loop in recognition of gp120. This methodology should be generally applicable to other high-affinity protein/ligand interactions, such as antibody/antigen recognition, that are compatible with expression in E. coli.

Q 153 GP120 CONFORMATIONAL CHANGES INDUCED BY SOLUBLE AND CELLULAR CD4 BINDING AS STUDIED BY BIOSPECIFIC INTERACTION ANALYSIS AND FLOW CYTOMETRY

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Multiple sequential antibody and rCD4 binding interactions with conformationally intact gp120 have been studied using Biospecific Interaction Analysis (BIAcore) and flow cytometry in hopes of gaining better insight into the interaction of polyclonal serum with gp120. Initially monospecific antibodies were studied. The conformation of gp120 immobilized within the BIAcore biosensor surface retains high binding affinity to rCD4 (~8 nM) as determined by a kinetic analysis measuring the association (k1) and dissociation (k-1) rate constants. This conformationally intact, yet restrained, gp120 is able to simultaneously bind multiple monoclonal antibodies to various epitopes. Each sequential binding interaction can be monitored for inhibition or enhancement from previously bound antibodies. One monoclonal antibody mapped to the carboxy terminal region of gp120 (MAb1, aa. 474-489) bound with similar reactivity to gp120 in the presence and absence of rCD4 implying binding to spatially separated epitopes. However, rgp120 captured by surface immobilized sCD4 or cellular CD4 resulted in a modulation of exposed and accessible binding epitopes such that MAb1 lost all binding to gp120. In addition, MAb1 when preincubated with conformationally unrestrained gp120 could no longer be detected bound to gp120 post CD4 binding implying a dissociation of MAb1 from gp120. These observations suggest a conformational change in gp120 post CD4 binding such that not only is this C-terminal region of gp120 no longer accessible to antibody binding but antibodies prebound to this region may be shed from gp120.

Q 154 **SIV/HIV Pseudotypes Demonstrate Envelope Mediated Host Range,** Kurt Zingler, Kathleen Page, and Dan Littman, Univ. of Calif. at San Francisco, Dep. of Micro & Immuno, San Francisco, CA, 94143

HIV-1 viral particles have been shown to pseudotype with a number of different retroviral envelopes. Using the HIV packaging system described by Page, et. al. *J. Virol.* **64**, p. 5270, we have created HIV-1 HXB2 particles (lacking HXB2 env.) bearing various SIV envelopes. Particles pseudotyped with SIVmac1A11 env are able to infect HUT.78 cells with titers similar to those of particles carrying the HXB2 env (TCID 50% of 10^3/ml). Unlike HXB2, the SIVmac1A11, particles are unable to infect HeLa-T4 cells. Similarly, SIVagmTYO1 and SIVmac239 pseudotyped particles are unable to infect HeLa-T4 cells. However preliminary results suggest $CD4^+$ fetal rhesus kidney line FRhK/4 and human glioblastoma U87.MG, which are resistant to HXB2 mediated infection, are susceptible to infection using these three SIV envelopes. Using the HUT. 78, FRhK/4, and U87.MG cell lies we are currently designing mutagenesis-based experiments to determine functionally important regions of these SIV envelopes.

Molecular Biology

Q 200 TAT TRANSACTIVATION OF LUCIFERASE - NOVEL ASSAY FOR REPLICATING HIV-1, Zvi Bentwich, Petra Lavate, Shoshana Israel, Alexander Honigman, Shimon Ulitsur, Ami Vonsover, Ruth Ben-Ari Institute of Clinical Immunology, Kaplan Hospital, Rehovot, Dept. of Molecular Genetics, Hebrew University Medical School, Virolum Ltd., and Central Viral Laboratory, Tel Hashomer, Israel

We have recently developed a highly sensitive and specific assay for active HIV. This is based on light emission by the luciferase enzyme stably transfected into human cells and specifically amplified by transactivation through the HIV Tat gene product. Such cells emit large amounts of detectable light upon exposure to extracts of HIV infected cells as well as upon infection with HIV and can also be followed continuously in culture conditions. We have used this system in studying the various stages of HIV infection both in experimental systems as well as during the natural course of infection and have compared it with other established methods for HIV detection such as viral culture, and PCR. The assay is highly sensitive and specific and is able to detect from 1-100 infected cells at various stages of the viral life cycle, both in vitro and in peripheral blood mononuclear cells and within 2-24 hrs following exposure of the detector cells to the viral infected cells. The importance of this assay is manifold. It will help develop and establish a much needed easy and fast assay for active and infectious HIV particles, shed light on the various phases of the natural course of HIV infection and facilitate the monitoring, screening and fast testing of various antiviral therapies.

Q 201 ***IN VITRO*** **TRANSCRIPTION FROM THE HIV-1 LONG TERMINAL REPEAT.**

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Detailed analysis of the mechanism of tat transactivation of HIV LTR-directed transcription will only be possible through developement of cell-free functional assays. Using run-on transcription in isolated nuclei, we found that provision of tat, from an exogenous source, resulted in the stimulation of transcription initiation, in that levels of hybridisation to different LTR fragments increased but the pattern of hybridisation did not change. We therefore looked at run-off transcription from the HIV-1 LTR using soluble extracts derived from nuclei of stimulated and unstimulated human carcinoma cells (HeLa) and human T-leukaemia cells (CEM). The level of transciption from the HIV-1 LTR, even in unstimulated cell nuclear extracts, was high. We have therefore used several methods to suppress transcription and thus derive levels of tat transactivation *in vitro* that approach the levels observed *in vivo* by transfection experiments.

Q 202 DEVELOPMENT OF A HUMAN IMMUNODEFICIENCY VIRUS-1 IN VITRO DNA SYNTHESIS SYSTEM TO STUDY REVERSE TRANSCRIPTASE INHIBITORS, Lawrence R. Boone, Katyna Borroto-Esoda, Division of Virology, Wellcome Research Laboratories, Research Triangle Park, NC 27709

An HIV-1 endogenous reverse transcriptase reaction was developed as an in vitro assay system to study the inhibition of reverse transcription by antiviral compounds. In alkaline agarose gels the maximum size reverse transcript migrated as a DNA molecule of approximately 9.5 kb, consistent with it being the (-) strand copy of the complete HIV-1 genome. A prominent subgenomic species of approximately 6 kb in size was consistently observed, and often represented the product of highest yield. The 6 kb reverse transcript contained sequences expected to be present only after copying the RNA genome to its 5′ end. The small size was therefore not due to a failure to copy the RNA template to the end, but rather a more complicated mechanism which appeared to involve template jumping to incorrect sites. Other minor bands of lower molecular weight were also frequently observed. Strand specific probes identified two discrete (+) strand species which were of the size expected for (+) strong stop DNA and DNA initiated from a secondary initiation site. No genomic length (+) strand DNA was observed, consistent with a model for replication involving a segmented (+) strand in the cytoplasmic DNA of HIV-1. AZT triphosphate was used to demonstrate the use of the system as an assay for inhibitors of reverse transcriptase. In the presence of 500 uM for each of the four natural dNTPs, synthesis of genomic length DNA was greater than 90 % inhibited by 0.8 uM AZT-TP.

Q 203 Activation of Tumor Necrosis Factors gene expression during HIV-1 infection of T cells is due to Tat.

L. Buonaguro, G. Barillari, H.K. Chang, V. Fiorelli, R.C. Gallo and B. Ensoli. Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Inflammatory cytokines, such as Interleukin 1 (IL-1), Interleukin 6 (IL-6) and Tumor Necrosis Factors α and β (TNF α and β), are increased in the sera of HIV-1 infected individuals. Among them, only TNFs are augmented during acute infection of T cells by HIV-1 and this is due to the viral transactivator gene product Tat. Transcriptional activation of the TNF β promoter requires only the I exon of the tat gene, but high levels of tat expression are necessary. However, cells chronically infected with pol-defective HIV-1 proviruses constitutively express Tat and produce TNF β. Induction of TNF β is increased by activation of infected cells with phorbol 12-myristate 13-acetate (PMA) which mimics antigenic stimulation, a frequent event in most groups at risk for AIDS. In turn, TNFs may increase the serum levels of IL-1 and IL-6 in HIV-1 infected individuals. As these inflammatory cytokines have been shown to enhance HIV-1 gene expression, the activation of TNFs by Tat may belong to a complex pathway in which HIV-1 uses viral products and host factors to increase its own expression and infectivity.

Q 204 BIOLOGICAL AND MOLECULAR PROPERTIES OF HIV-1 INTEGRASE DEFECTIVE MUTANTS. A. Cara, M. Robert-Guroff, R.C. Gallo, M.S. Reitz and F. Lori, LTCB, NCI, NIH, Bethesda MD 20892

Integration of viral DNA into the host cell genome is believed to be an obligatory step in the life cycle of most retroviruses. The pol gene product integrase seems to be necessary as well as sufficient for integration of retroviruses into the host cell genome. However, infection by some retroviruses, including visna lentiretroviruses and human immunodeficiency virus type 1 (HIV-1), is characterized by high levels of unintegrated DNA and a frequent lack of detectable integrated proviral DNA. This has raised the possibility that HIV-1 does not require integration (integrase) for replication. To address whether or not integrase is required for the replication of HIV-1, we introduced a stop codon in the middle of the reading frame for the integrase of two distinct molecular clones of HIV-1. Analysis of a biologically active plasmid containing a mutant HIV-1 genome confirmed that the p34 integrase was not produced, yet other viral proteins, including reverse transcriptase, were produced at levels comparable to those of the wild type. Transfection of this mutant genome into CD4+ Hela cells resulted in syncytia similar in size and number to those seen in the wild-type genome. However, neither of the mutant viruses were productively infectious for several cell lines, peripheral blood mononuclear cells or macrophages. These data indicate that the p34 integrase is required for HIV-1 replication in HIV-1 infected cells. Molecular aspects of this block of replication will be discussed.

Q 205 BIOLOGICAL CONTAINMENT OF HIV-1: A tat AND rev DEFICIENT VIRUS. Chen H, Malim M, Boyle TJ, Berend K, Cullen BR, and Lyerly HK, Departments of Surgery, Microbiology and Immunology, Medicine, and Pathology, and the Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710.

Laboratory exposure to HIV-1 has prompted the development of replication incompetent viruses for biosafety reasons. HIV-1 encodes at least 9 genes including trans-acting regulatory genes *tat* and *rev*. Deficiency of each gene independently renders HIV-1 replication incompetent. This feature was exploited to create a *tat* and *rev* deficient HIV-1 (HIV -tat/rev) and a recombinant T cell line (CEM/TART) which supports replication of HIV -tat/rev by providing *tat* and *rev* in trans.

HIV -tat/rev was constructed by engineering stop codons within the*tat* and*rev* genes of an HIV-1 molecular clone (HXB-3). A T cell line (CEM) was transformed with a retroviral vector (HCED10) containing*tat* and *rev* genes (CEM/TART). HIV -tat/rev virus, obtained from COS cells cotransfected with HIV -tat/rev provirus and pHCED10, was used to infect CEM/TART which were maintained in continuous culture, and the supernatants used to sequentially reinfect CEM/TART (x4). Standard p24 ELISA and reverse transcriptase assays were used to determine if the virus contained in supernatants could infect peripheral blood lymphocytes (PBL) or CEM. To demonstrate if known antiviral drugs inhibit HIV -tat/rev in a similar manner to HIV-1, infections were performed in the presence of standard concentrations of AZT. (see table)

VIRUS	p24 : pg/ml (avg.)		
	PBL	CEM	CEM/T
HIV -tat/rev: 8 weeks in continuous culture	0	0	180000
HIV -tat/rev: 4 sequential reinfections	0	0	53000
HIV-1 (HXB-3)	1719	2018	2018
HIV-1 + 5 uM AZT	--	0	0
HIV -tat/rev + 5 uM AZT	--	--	0

DNA from HIV -tat/rev infected CEM/TART maintained in continuous culture for 8 weeks was amplified by PCR and sequenced. No mutations in the engineered stop codons were found.

As shown by continuous culture, several reinfections, and DNA analysis, HIV -tat/rev is a stable, replication incompetent virus that is susceptible to inhibition by standard HIV-1 therapies, and whose early life cycle appears to parallel wildtype HIV-1. Therefore, this biologically contained HIV-1 replication system provides a safer method for the study of pharmalogical inhibition and immunologic neutralization of HIV-1 with a minimal risk of exposing workers to virulent, replication competent virus.

Q 206 INVOLVEMENT OF THE *c-myb* PROTO-ONCOGENE IN HIV-1 GENE EXPRESSION.

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Sequence analysis of the HIV-1 LTR has shown the position of cellular factor binding sites (NFKB - Garcia *et al*, 1987; Sp-1 - Jones *et al*, 1986). More recently it has been shown that the HIV-1 LTR contains one high affinity Myb-binding site, along with a number of low affinity binding sites (Dasgupta *et al*, 1990). Myb, the product of the proto-oncogene c-*myb*, is expressed in a number of cell types including hemopoietic cell lines and tissues where it is thought to be associated with the regulation of proliferation and differentiation.

The expression of c-*myb* in different CD4+ T-cell lines was examined in both cytoplasmic mRNA and nuclear protein. Levels of c-*myb* mRNA determined by Northern blot analysis were approximately 10 fold higher in MT2 cells (an HTLV-1 transformed line) compared with CEM cells, while unstimulated PBLs showed a level 1/10 that of CEM cells. Examination of Myb protein by Western blotting also showed the higher expression of c-*myb* in MT2 cells. Mobility shift assays were used to investigate the DNA binding activity of nuclear extract from unifected and HIV-1 infected MT2 and CEM cells. Using a synthetic Myb-binding template, we found that the protein levels observed by Western blotting correlate well with DNA binding activity using both core Myb responsive element (MBS-1) and HIV-1 LTR Myb responsive element (LTR-9) oligonucleotide sequence. These data suggest that the elevated levels of c-*myb* expression in MT2 cells is a requisite for the rapid kinetics of HIV-1 infection and virus production in these cells over other T-cell lines.

Garcia *et al*, EMBO J 1987, 6: 3761-3770.
Jones *et al*, Science 1986, 232: 755-759.
Dasgupta *et al*, PNAS 1990, 87: 8090-8094.

Q 207 COMPLEX SPLICING IN THE HTLV FAMILY OF RETROVIRUSES: NOVEL mRNAs AND PROTEINS PRODUCED BY HTLV-1.

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HTLV and HIV are complex retroviruses producing several additional proteins and regulating their expression via two essential regulatory factors. While HIV-1 produces more than 30 different mRNAs, HTLV-1 had been shown to produce three mRNAs. We investigated whether additional mRNAs can be produced by HTLV-1 by alternative splicing. A full length molecular clone of HTLV-1 generated from lymphocytes of an ATL patient, was transfected into HeLa cells. Cytoplasmic RNA was isolated, reverse transcribed, and PCR amplified using different pairs of primers. The resulting cDNAs were cloned and sequenced. Interestingly, in addition to the two known 3' splice sites (3'ss) used fot the env and tax/rex mRNAs, respectively, five additional 3'ss were identified. Three of these splice sites are in the "X" region at the 3' part of the genome and their utilization offers the possibility for the expression of two open reading frames known to exist within the proximal X region, pX-I and pX-II. Our data also demonstrate that HTLV-1 produces at least 7 different mRNAs; therefore, expression of HTLV-1 is more complex than previously thought.

The use of the splice sites in the proximale "X" region in combination with exon 2 generates mRNAs with the potential to encode two novel hybrid proteins: Rex-ORF1 (Rof) of 152 aa and Tax-ORF2 (Tof) of 241 aa. Like Rex, Tof accumulates in the nucleoli of transfected cells. The other viruses of the HTLV family such as HTLV-2 and bovine leukemia virus (BLV) also have a complex splicing pattern and are capable of producing additional proteins encoded in the proximal X region. These results suggest that HTLV-1 and other members of the HTLV family produce novel proteins, which may contribute to the biological properties of these viruses.

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Q 208 FUNCTIONAL ROLE OF THE *VPU* GENE IN HIV-1 INFECTION. Eric A. Cohen and Xiao-Jian Yao. Laboratoire de rétrovirologie humaine, Département de microbiologie et immunologie, Faculté de médecine, Université de Montréal, C.P. 6128, succ. A, Montréal, Canada H3C 3J7.

The human immunodeficiency virus type 1 encodes a gene designated *vpu* located immediately 5' to the envelope glycoprotein gene. The *vpu* protein is typically 81 amino acid long. The protein was shown to be an integral membrane protein and is phosphorylated at one or more serine.

Functional studies have shown that expression of *vpu* increased significantly the export of virus particles from infected $CD4^+$T- lymphocytes and reduced the accumulation of cell associated viral proteins. This reduction of cell-associated virion protein is correlated to a decrease in the rate of syncytium formation and cell killing in $CD4^+$ T cells. *Vpu* does not appear to be essential to HIV-1 replication at least in $CD4^+$T cell cultures. The phenotype of HIV-1 viruses carrying mutation in the *vpu* gene suggests that vpu increases the rate of virus export by facilitating the assembly or/and release of virus particles. Little is known about the mechanism by which *vpu* function. Recently, it has been shown that *env* and *vpu* proteins are expressed from the same mRNAs and are coordinately regulated by *rev*.

The objective of this study was to investigate the requirement of the *env* glycoproteins and CD4 receptor for the *vpu* function on virus export. Our results show that *vpu* increases significantly virus particles export in the absence of *env* glycoproteins and CD4 expression. In fact, our data suggest that *vpu* is critical for efficient virus particle formation and export in $CD4^-$ human cells in which spread of infection cannot occur.

Q 209 COEXPRESSION AND PURIFICATION OF TAT/TAR COMPLEXES UNDER NATIVE CONDITIONS FROM E.COLI. D.Cousens, T.Alnadaf and E.D'Souza. Department of Molecular Sciences, Wellcome Research Laboratories, Beckenham, Kent, U.K.

Structural studies on HIV Tat have in large been unsuccessful and impaired by the inability to obtain sufficient amounts of native protein. Purification of tat from heterologous expression systems has depended on denaturing conditions and subsequent refolding of the tat protein. It is not clear however whether such refolded tat is fully biologically active. We have previously shown that tat purified under non-denaturing conditions exhibits concentration dependant activity in a cellular transactivation assay and binds TAR RNA in a gel retardation assay. Tat purified under these conditions however was found to be associated with RNA presumably through non-specific association with the basic region of tat. We therefore chose to coexpress tat and TAR RNA in the same E.coli cells. The increased affinity of tat to TAR should therefore enable the purification of tat/TAR complexes which will be used in further structural studies.

Q 210 MOLECULAR CHARACTERIZATION OF GENE PRODUCTS ENCODED BY FELINE IMMUNODEFICIENCY VIRUS (FIV). J.H. Elder[1], P.A. Luciw[2], E.E. Sparger[2], B.L. Shacklett[2], D.C. Fontenot[3], R.C. Montelaro[3], T.R. Phillips[1], [1]The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037; [2]Department of Medical Pathology, University of California at Davis, CA 95616; [3]Department of Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA 15621. Feline immunodeficiency virus (FIV) is a lentivirus found associated with a debilitating immunodeficiency disease in the domestic cat. Analyses of several distinct isolates have revealed a molecular structure that shares many attributes of other lentiviruses, but yet is distinct. In particular, FIV encodes a deoxyuridine triphosphatase (DU) within pol. This feature is shared by other non-primate lentiviruses including Visna, CAEV, and EIAV as well as type B and type D retroviruses. The role of this enzyme in the virus life cycle is under investigation, but it is thought to play a role in reducing the inappropriate incorporation of dUTP during reverse transcription. FIV contains additional sORFs that might encode regulatory proteins. Starting from the 5' end of the genome, the first additional ORF is located at the end of pol, in the location of vif in HIV. The size of the predicted gene product is the same as for the primate virus, although no amino acid homology is apparent between the cat and human proteins. Site-directed antibodies to this putative FIV vif protein detect a 23 kd protein in virus-infected cells. The second sORF resides between pol and env and may encode a transcriptional activator protein. However, FIV contains no bonafide tat equivalent and no tar element is evident. The first 240 bases of the env open reading frame comprise the first coding exon of FIV Rev, similar to the organization of Visna virus. However, the second coding exon of Rev resides 3' to the end of the env coding region, rather than overlapping env as in other lentiviruses. The RRE is also shifted 3', near the end of env, rather than at the SU/TM junction. Studies using CAT reporter constructs containing the RRE indicate that Rev binds to the RRE and inhibits multiple splicing, similar to primate Rev. In spite of its unique features, FIV will make an excellent model for the development of intervention therapies for lentivirus infections.

Q 211 GENE EXPRESSION OF THE COMPLEX HUMAN SPUMA RETROVIRUS, Rolf M. Flügel, Martin Löchelt, Walter Muranyi, Jakob Weissenberger and Katrin Köhle, Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum, 6900 Heidelberg, FRG

Spuma or foamy viruses are a distinct subfamily of complex retroviruses, since regulatory genes are encoded 3' of *env*. Mutational analysis of an infectious human spumaretrovirus (HSRV) DNA clone with respect to the different *bel* genes was performed. The results showed that the *bel 1* gene is required for virus replication, localizes to the nucleus, and functions as transcriptional transactivator of its own LTR and to a lesser extent of that of HIV-1. Mutational analysis of *bel 2* and *bet* genes and their involvement into virus replication was monitored by measuring *gag* gene expression.

Transcriptional mapping of HSRV by PCR revealed that besides viral genomic RNA and several singly spliced env mRNAs, several *bel 1*, *bel 2*, and *bet*-specific transcripts were identified. These subgenomic HSRV *bel* mRNAs are either singly or multiply spliced and the corresponding gene products were found in virus-infected cells. *Gag* domains encompassing the capsid antigen protein and *env* domains containing surface and transmembrane proteins were produced by bacterial expression and used for raising polyclonal antisera. Processing of the *gag* precursors was studied in human embryonic lung cells by radioimmunoprecipitation and revealed a characteristic pattern. HSRV-specific ELISA and immunoblots based on *gag* and *env* domains were developed, and employed for screening and detecting HSRV-specific antibodies in human sera. HSRV antibody seroprevalence was studied by analyzing 3300 human sera with various diseases and from different countries. The potential association of HSRV with defined human diseases will be discussed.

Q 212 CHARACTERIZATION OF T-CELL KILLING DETERMINANTS OF AN EXTREMELY CYTOPATHIC HIV-1 ISOLATE. Sajal K. Ghosh, Yuexia Li, Beatrice H. Hahn and George M. Shaw. Department of Medicine, University of Alabama at Birmingham, AL 35294.

HIV-1 isolates recovered from patients at later stages of disease are usually more cytopathic, replicate faster, and show a wider range of tropism for different established T-cell lines. In infected individuals, the CD4+ T-cell count is an important indicator of disease state and individuals who experience rapid T-cell depletion are at high risk for AIDS. It is possible that the generation of more virulent T-cell tropic HIV-1 strains *in vivo* is an important step toward progression of the disease. In order to characterize the viral genetic determinant(s) for T-cell killing and whether such determinant(s) can be correlated with disease state, we molecularly cloned the predominant virus strain from an extremely cytopathic HIV-1 isolate, BC. This molecular clone(SG3) exhibited the same biological phenotype as the parental isolate. The envelope gene (gp120+gp41) of SG3, excluding the nucleotide sequences for first 11 amino acids, was then exchanged with the equivalent region of a macrophage tropic HIV-1 molecular clone(YU2) obtained from an uncultured brain specimen and with another weakly cytopathic HIV-1 molecular clone(SG9/3). Biological properties of these chimeric HIV-1 viruses were analyzed in H9, Jurkat, CEMx174, SUP-T1, and MOLT4/8 cells. The results showed that though the envelope gene of the cytopathic clone was sufficient to induce cell fusion and cell killing by otherwise noncytopathic clones, the extent of cytopathicity was lower in the chimeras. Moreover, cytopathicity induced by chimeras was not observed in SUP-T1 and MOLT4#8 cells. These results suggest that in addition to the envelope gene other portions of the viral genome may be involved in viral cytopathicity. Furthermore, cellular factors may also be involved in the full expression of viral cell-killing properties. Construction of additional chimeric viruses involving exchanges of other regions of the viral genome and characterization of their biological properties will be presented.

Q 213 AUTOINTERFERENCE BY NOVEL DEFECTIVE INTERFERING HIV-1 PROVIRAL DNAs

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The purpose of this study is to develop novel defective interfering (DI) HIV-1 particles which interfere with the replication of HIV-1. We have constructed five generations of prototype HIV-1 DI genomes which contain both HIV-1 LTRs, the interfering gene(s) together with a rev responsive element. Expression of the DI DNAs is dependent on the regulatory proteins of HIV-1. At the same time the structural proteins of HIV-1, except for env, are used for the replication of the DI particle. Within the context of this antiviral strategy, the DI genome encodes a chimeric CD4/env protein which arrests the transport of env to the cell surface and replaces it. Our goal is to generate DI particles which will be released from these cells, and which will be able to spread the interfering genes not only to other HIV-1 expressing cells but also to potential HIV host cells. This would allow to repeat the cycle. To favor the packaging of the DI genome at the expense of wildtype HIV-1, the DI genome also encodes a novel multitarget-ribozyme, which is targeted to specifically cleave HIV-1 RNA at ten different, relatively conserved sites within the gp120 region of env. In fact, the 4 Kb DI genomic RNA itself functions as a ribozyme.

The five generations of our prototype HIV-1 DI provirus DNAs were tested for their gene expression and their ability to interfere with HIV-1 replication. All prototype constructs as outlined above strongly interfered with HIV-1 replication as measured in a cotranfection assay with the infectious HIV-1 DNA clone pNL4-3 in HelaT4 cells. In all cases syncytia formation as well as the amount of p24 antigen in the cell supernatant decreased significantly, suggesting that released virus may be inefficient in reinfecting neighboring cells. Depending on the prototype DI construct used, interference was highly specific and a function of either CD4/env or multitarget-ribozyme expression, respectively. Some constructs contain both interfering genes and the selective packaging of these DI genomes and their ability to transfer the interfering genes to other HIV-1 expressing cells is currently being investigated..

Q 214 TAR-DEPENDENT TRANS-ACTIVATION OF HIV-1 & 2 IS HUMAN CHROMOSOME 12 SPECIFIC. Clyde Hart, Mark Westhafer, Judy Galphin, Chin-Yih Ou, and Gerald Schochetman. Division of HIV/AIDS, Centers for Disease Control, Atlanta, GA 30333.

The presence of chromosome 12 in human-hamster hybrid cells works in unison with the viral *tat* protein to greatly increase HIV-1 and HIV-2 LTR(LTR1 and LTR2)-directed gene expression. HIV-1 *tat* (tat_1) trans-activation of the LTR2 is not dependent on the presence of human chromosome 12, suggesting that multiple cellular pathways are involved in *tat*-induced trans-activation. To examine chromosome 12 dependent and independent pathways that support *tat* trans-activation, DNA plasmids containing wild type (wt) and TAR mutant LTR1- and LTR2-CAT constructs were transfected into cells. Cell lines containing human chromosome 12 had 0% to 6% the trans-activation levels when wt TAR was replaced with a mutant (ΔTAR) containing a 4 base substitution in the predicted RNA TAR loop. In chromosome 12-minus cells, ΔTAR supported 100% to 45% of the wt TAR trans-activation. LTR2 TAR mutants were insertion mutations in sequences predicted to form wt RNA loops +34 to +39 (ΔL1) or +68 to +73 (ΔL2). In chromosome 12-containing cells, ΔL1 supported trans-activation at 12%-13% the level of wt TAR; ΔL2 trans-activation varied from 25% to 77% of wt TAR. An LTR2 construct containing ΔL1 & ΔL2 (ΔL1+2) further reduced HIV-2 trans-activation to 1.5% to 4% of wt TAR. Chromosome 12-minus cells retained 80% to 33% of the wt TAR activities with ΔL1+2, except for a mink cell line than had a 6% activity versus wt TAR. Tat_1-induced LTR2-CAT expression was refractory to ΔL1 and ΔL2 in all cell lines tested. Our results indicate a major role for chromosome 12-encoded factors in TAR-dependent trans-activation. Although some non-human cells support significant levels of trans-activation, TAR-independent pathways appear to be involved and may not represent the the major human-host mechanism.

Q 215 ASSOCIATION OF cAMP WITH CYTOMEGALOVIRUS-INDUCED ENHANCEMENT OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) REPLICATION. M. Hassan, R. Pollard, and M. Nokta. The University of Texas Medical Branch, Galveston, Texas, USA.

Cytomegalovirus (CMV) infection constitutes a serious threat to patients with AIDS. Recently we reported that HIV infection of CD4 positive cells was associated with sustained elevation of cellular levels of cAMP. Moreover, cyclic nucleotide modulators enhanced HIV replication by increasing intracellular levels of cAMP. In this study the effect of CMV on HIV replication in CMV/HIV mixed infection and its relationship to cAMP was examined. MT-4 cells, CMV strain AD 169 and HIV strain 3b were used. Optimal enhancement (4.4 fold increase) of HIV replication was observed when MT-4 cells were infected with CMV at day 0 followed by HIV on day 4 postinfection (PI), as determined by reverse transcriptase activity on day 11 (PI). cAMP (measured by radioimmuno-assay) levels in cells infected with CMV alone, HIV alone or CMV/HIV together were 2, 3 and 5 fold above mock infected cells. CMV also enhanced the replication of HIV following UV-irradiated prior to infection, 4 fold and this was associated with a 2 fold increase in cAMP as well. These data suggest that CMV enhances HIV replication via a cAMP dependent pathway. These findings may have relevance to the identification of novel target sites for development of antiviral therapeutics.

Q 216 A RAPID, SENSITIVE METHOD FOR DETECTION OF HIV-1 SPECIFIC NUCLEIC ACID IN THE CULTURE SUPERNATANTS OF INFECTED CELLS, Hewlett, I.K., Bharat Joshi, Gary Riordan, Laurie Pollock and Jay S. Epstein, Lab. of Retrovirology, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892

Rapid, sensitive and specific PCR based methods have been developed for detection of HIV-1 specific nucleic acid in cell culture supernatants of virus infected cells. By these methods, viral DNA was detected in the supernatant of acutely infected H9 and U937 cells as early as 1 day post-infection; viral RNA was detected at day 2 in U937 cells and at day 5 in H9 cells, whereas significant amounts of p24 antigen in the supernatant were not detected until 3-4 days post-infection. Both DNA and RNA PCR on culture supernatant at 7 days post-infection allowed the detection of 25 pg of input viral p24 antigen. Supernatant from infected cells treated with AZT or ATA, both inhibitors of reverse transcriptase, had no detectable viral DNA or RNA suggesting that these markers may be of use in evaluating the antiviral activity of novel drugs. The increased sensitivity of PCR on supernatants, relative to tests for viral p24 antigen, makes these assays useful for analysis of cultures of peripheral blood mononuclear cells from infected individuals and patients on therapeutic regimens.

Q 217 SITE-SPECIFIC CLEAVAGE OF HIV TAR RNA USING A TAT-BASED CHEMICAL NUCLEASE,

Sumedha D. Jayasena and Brian H. Johnston, Cell and Molecular Biology Laboratory, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025

Although the conversion of DNA-binding molecules to site-specific chemical deoxyribonucleases has been an area of active investigation, efforts to achieve site-specific cleavage of RNA have been mainly limited to the use of ribozymes. Adapting other types of RNA-binding molecules for site-specific cleavage of RNA could prove useful for inactivating RNA viruses. Using the human immunodeficiency virus (HIV) as a viral model, we have demonstrated this approach by creating a site-specific chemical ribonuclease based on the HIV type 1 (HIV-1) Tat protein. Tat, an essential transactivator of gene expression in HIV, is believed to activate viral gene expression by binding to the transactivation response (TAR) site located at the 5' end of all viral mRNAs. The TAR element forms a stem-loop structure containing a 3-nucleotide (nt) bulge which is the site for Tat binding and is required for transactivation. Purified Tat and peptide fragments containing the Tat nuclear targeting domain bind to TAR *in vitro*. A 24-amino acid peptide consisting of this domain plus an additional C-terminal cysteine was chemically synthesized and covalently linked to 1,10-phenanthroline at the cysteine residue. The modified peptide binds to TAR sequences of both HIV-1 and HIV-2 and, in the presence of cupric ions and a reducing agent, cleaves these RNAs at specific sites. Cleavage sites on TAR sequences are consistent with peptide binding to the 3-nt bulge, and the relative displacement of cleavage sites on the two strands suggests peptide binding to the major groove of the RNA. These results, along with existing evidence of the rapid cellular uptake of Tat-derived peptides, suggest that chemical nucleases based on Tat may be useful for inactivating HIV mRNA *in vivo*.

Q 218 INTERACTIONS OF TAT AND NUCLEAR PROTEINS WITH TAR RNA, Janet Jeyapaul,

The Wistar Institute, 3601 Spruce Street, Philadelphia, PA19104.

Several studies have indicated that the binding of tat and nuclear proteins to HIV TAR RNA appears to be important for tat mediated transactivation. Thus the role of nuclear proteins in tat-TAR interaction was examined through evaluation of several synthetic peptides for ability to bind TAR RNA in vitro both in the presence and in the absence of Hela nuclear proteins. Three predominant complexes designated as C1 (the fastest moving complex), C2 and C3 were observed on incubation of TAR RNA with nuclear extract. C3 showed higher affinity of TAR RNA than C2 and C1. Incorporation of 50 -100 ng of tat 1-47 in the binding reaction containing nuclear protein and TAR RNA led to disappearance of bands C2 and C3 and concomitant release of the free probe.Tat 1-38 in which the acidic amino acids at position 2,5, and 9 were substituted with Ala did not dissociate TAR-NP as compared to wild type tat 1-38. This suggests importance of tat and TAR RNA binding nuclear protein interactions. Whether this interaction is due to direct binding of tat to TAR_RNA binding nuclear protein or via another nuclear protein is not known. The latter explanation may be likely due to the the concentration dependence of tat in dissociating the nuclear proteins off the TAR- RNA. It appears that as the concentration of tat increases to 400ng the dissociation is not evident and could be due to saturation of the intermediatary protein by excess tat and thus inhibiting its association with TAR -RNA binding proteins. As a first step , analysis of the nature of binding of proteins to TAR- RNA was done after heparin-agarose chromatography of nuclear extract and step-elution of the proteins with 0.1M to 0.54 salt concentrations. The results and significance of these findings will be presented.

Q 219 MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF SIVagm (SAB) FROM WEST AFRICAN GREEN MONKEYS,

Mojun J. Jin[1], Huxiong Hui[1], Jonathan S. Allen[2], George M. Shaw[1] and Beatrice H. Hahn[1], University of Alabama at Birmingham, Birmingham, Al 35294[1], Southwest Foundation for Biomedical Research, San Antonio, Texas 78228-0147[2]. Simian immunodeficiency viruses from African green monkeys (SIVagm) display a significantly greater genotypic variability than HIV-1, HIV-2, SIVmac and SIVsm viruses. To further study the nature and extent of SIVagm genetic diversity, we isolated four SIVagm(sab) viruses from wild-caught West African green monkeys and PCR amplified their complete LTR regions. Comparative sequence analysis revealed a close genetic relationship among West African SIVagm isolates (90% homology) but significant sequence divergence from East African SIVagm viruses (60% homology). In addition, West African SIVagm viruses contained a duplication of the TAR sequence, which so far has only been observed in HIV-2, SIVmac, and SIVsm viruses. To further analyse this group of viruses, we constructed a recombinant lambda phage library from one of these isolates and obtained four recombinant clones. Detailed restriction enzyme analysis demonstrated that one of these clones comprised a full-length provirus. To test its replication competence, we transfected Cos-1 cells and subsequently cocultured them with Molt4 clone8 cells. High RT activities were detected as early as 48 hours post transfection and virus induced syncytia formation was extensive. From these results we conclude that SIVagm from West African green monkeys comprise a distinct subgroup of SIVagm viruses. West African SIVagm viruses are highly divergent from East African isolates and exhibit features characteristic for HIV-2/SIVmac/SIVsm viruses. Sequence analysis of a complete SIVagm(sab) provirus is underway to ultimately determine its genomic organization and phylogenetic relationship to the other HIV/SIV viruses.

Q 220 BENZO(A)PYRENE INDUCES HIV-1 REPLICATION IN CHRONICALLY INFECTED PROMONOCYTIC CELLS, Joshi, B.H., Gary Riordan, Michael Norcross, Laurie Pollock, Jay S. Epstein and Indira K. Hewlett, Laboratory of Retrovirology, DTS, CBER, FDA, Bethesda, MD 20892

The chronically infected promonocytic cell line U1 is characterized by low constitutive production of HIV, inducible bt phorbol esters (TPA) and tumor necrosis factor-alpha. In order to investigate the effect of certain chemical carcinogens that may be environmental mutagens on the induction of a latent viral genome., U1 cells were treated with benzopyrene (BOP) and hexachlorohexane (HCH). At various time points after induction, culture supernatants were tested for the presence of viral p24 antigen. Viral particles were visualized by transmission electron microscopy (TEM) and HIV expression was evaluated by quantitative PCR using slot blots. HIV antigen in the supernatnt and viral RNA in cells was induced 20-fold, 6 days after treatment with BOP while aHCH treated cultures showed a 6-7 fold induction after 12 days. EM analysis revealed a 10-fold increase in virus production at day 12 in BOP treated cultures. Induction of antigen and RNA by TPA was comparable to that by BOP. Enhanced virus production by BOP was accompanied by an increase in NFkB binding activity as revealed by gel mobility shift assays. In conclusion, BOP, a potent chemical carcinogen, is an inducer of HIV gene expression. Since BOP is a constituent of cigarrette smoke and charbroiled products, our results may have implications for the pathogenesis of HIV infection in man.

Q 221 INTRACELLULAR TRANSPORT AND PACKAGING OF VPX IS MEDIATED BY AN HIV-2 SPECIFIC EVENT, John C. Kappes, Jeffrey S. Parkin, Joan A. Conway, George M. Shaw and Beatrice H. Hahn, University of Alabama at Birmingham, Birmingham, AL 35294 The genomes of HIV and SIV are complex and contain several accessory genes which modulate viral replication and pathogenicity. Of these accessory genes, vpx is unique to the HIV-2/SIV group of viruses and encodes a virion-associated protein which facilitates virus infectivity in natural target cells. Although important for the production of fully infectious and cytopathic HIV-2 virions, vpx is not required for virion assembly. To better understand the function of vpx and its significance as a component of the virion, we expressed this protein in a mammalian cell system and examined its intracellular transport and assembly requirements into mature virus particles. For eukaryotic expression, the entire vpx coding sequence was placed under the control of an SV40 early promoter and R/U5 elements of the HTLV-1 LTR (SR alpha promoter). Following transfection into Cos-1 cells, this construct (designated pSR-vpx) facilitated high-level transient expression of vpx as shown by Western blot analysis using monoclonal and polyclonal anti-vpx antibodies. Immunofluorescence microscopy of such transfected cells also revealed high levels of diffuse cytoplasmic and nuclear staining. When Cos-1 cells were transfected with a replication competent HIV-2 provirus, however, vpx was only detected on the cell surface. To investigate whether HIV-2 specific gene products and/or nucleic acids were required for vpx targeting, we coexpressed vpx with an HIV-2 vpx deletion mutant (pROD/XM7). Using double staining immunofluorescence analysis, we found colocalization of vpx and HIV-2 gag proteins similar to results obtained following transfection with a replication-competent HIV-2 clone. Using the same expression system, we also showed that vpx could be supplied "in trans" and packaged into a vpx-deficient provirus. In contrast, coexpression of vpx with HIV-1/HXB2 did not mediate packaging of vpx, nor did it facilitate targeting of vpx to the cell surface. These results suggest that vpx requires HIV-2 specific gene products and/or nucleic acids for membrane targeting and assembly into mature virion particles.

Q 222 A HIGHLY SENSITIVE NON-RADIOACTIVE MICROASSAY FOR HIV-1 REVERSE TRANSCRIPTASE, Bernhard König, Alfred Brunner, Gudrun Häusl and Hans Seidel, Department of Molecular Virology, Boehringer Mannheim GmbH, D-8122 Penzberg, FRG.

One of the key enzymes in the life cycle of HIV is the virus-encoded reverse transcriptase (RT) which represents a prime target for the development of compounds with anti-viral activity. To screen a large number of samples for their inhibitory effect on the RT we developed an ELISA-type Assay for RT activity using Biotin- and Digoxygenin-labeled substrates (deoxynucleoside triphosphates):

In our routine assay (100 µl volume) a mixture containing 50 mM Tris-HCl, pH 7.9, 50 mM KCl, 1 mM DTT, 0.02% Nonidet P40, 1 µM of dATP, dCTP and dGTP, 0.25 µM Digoxygenin-11-dUTP and 50 nM Biotin-16-dUTP is incubated with 20 ng HIV-1 RT (p66/p51, expressed in E. coli) in the presence of 1 µg HIV-1 template RNA (1080 nucleotides) and 20 ng of the corresponding 18-mer DNA primer in a streptavidin coated microtitration plate or tube. The polymerisation product is anchored to the solid surface via the Biotin/Strept-avidin interaction. RT activity is then measured by the activity of a peroxidase-anti<DIG>antibody conjugate bound to the digoxygenin labeled cDNA product. Under these assay conditions, IC_{50}-values for AZT-triphosphate, TIBO (R82150) and BI-RG 587 were 1 µM, 0.1 µM and 0.3 µM, respectively.

This assay is superior to the radioactive assay with regard to ease of handling (automation) and sensitivity. It is also suitable for the analysis of the pharmaco-kinetic profile of RT inhibitors in serum or plasma samples of experimental animals. Following p.o. administration of the non-nucleoside RT inhibitors BI-RG 587 and TIBO (R82150) to dogs at a dose of 50 mg/kg, our RT assay revealed clear-cut differences between the two compounds. BI-RG 587 reached peak plasma levels of 12.000 ng/ml within 30 minutes and had a $t_{1/2}$ of 3-5 h whereas TIBO plasma levels were below the detection limit of 300 ng/ml.

Q 223 FUNCTIONAL ROLE OF THE NUCLEOLAR TARGETING SIGNAL (NOS) OF HIV-1 Rev PROTEIN, Satoshi Kubota, Rika Furuta, Emiko Takano and Masakazu Hatanaka, Human Cancer Laboratory, Department of Molecular Virology, Institute for Virus Research, Kyoto University, Kyoto 606-01, Japan

The Rev protein of HIV-1,which regulates viral gene expression at post-transcriptional stages, is known to possess a nucleolar targeting signal (NOS) in its molecule. As previously reported by us, the NOS of Rev is characterized by extraordinary arginiene-rich motif and has an ability to accumulate various proteins into cell nucleoli when fused to their N-termini. We have been investigated the functional aspect of the NOS in Rev function by constructing the mutants of *rev* gene and analyzing them. One of these mutants, designated drev, expresses a Rev mutant (dRev) that contains a deletion of 7 amino acid residues in its NOS. This mutant displayed no Rev function and remained in cytoplasm out of nuclei when expressed in monkey kidney COS7 cells. Accordingly, the NOS turned out to be essential for function and nuclear/nucleolar localization of Rev. Furthermore, we have found that dRev acted as a potential inhibitor of Rev function in our assay system. Namely, when various amount of dRev-expression plasmid was co-introduced into COS7 cells with single amount of wild type *rev*-expressor, it inhibited the enhancement of gene expression caused by Rev in a dose-dependent manner. And interestingly, dRev interfered the nuclear/nucleolar localization of wild type Rev, while it inhibited Rev function strongly. Although many possibilities should be considered, it is reasonable to consider that this inhibitory effect may be due to Rev/dRev heteromer formation in cytoplasm, which may not be able to migrate into cell nuclei/nucleoli. Based on these findings, we propose a model of Rev oligomer formation in which the NOS acts as one of the "multimerizers".

Formerly, NOS has been thought to be the binding domain in Rev to its structured target RNA portion called RRE. However, the function built in NOS should be more pleiotropic than generally recognized. The other current data that may give insights into the entity of NOS function may be going to be presented as well.

Q 224 CHARACTERIZATION OF PROTEINS WHICH INTERACT WITH AN HIV-1 LTR NEGATIVE REGULATORY SEQUENCE. Georgina Lang[1], Kim Orchard[1], Mary Collins[1], David Latchman[2], Department of Cell and Molecular Biology[1], Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road, London, SW3 6JB, England, Medical Molecular Biology Unit[2], Department of Biochemistry, The Windeyer Building, University College and Middlesex School of Medicine, London, W1P 6DB, England.

Negative and positive regulatory elements, which exert effects upon transcription, have been identified within the HIV-1 LTR. One such region, the negative regulatory element (NRE), has been shown by DNAse I footprint analysis to bind nuclear proteins from human T cells. The cellular protein interacting with the major site within the NRE, site B (Orchard *et al* 1990), is of interest as a potential transcriptional repressor. Identification of the size of the nuclear protein which binds to site B has been determined by u.v. crosslinking studies and gel renaturation. Partial purification of site B binding protein, from Jurkat T cell nuclear extract, has been carried out using non-specific and specific DNA binding columns.

Orchard *et al.*, 1990, J. Virol. 64, 3234-3239.

Q 225 THE ANTI-PROLIFERATIVE EFFECT OF RECOMBINIANT gp120 AND ANTI-gp120 ANTIBODIES ON HIV NEGATIVE PERIPHERAL BLOOD LYMPHOCYTES, Teri J. Liegler and Daniel P. Stites, Department of Laboratory Medicine, University of California, San Francisco, CA, 94143.

HIV-1 env gp120 suppresses the proliferation of human peripheral blood cells and T cell lines to antigens or mitogens. This in vitro suppression may contribute to the reduced T cell proliferation responses to antigen noted in HIV infected individuals which can result in immunodeficiency independent of viral cytolysis. We measured the effects of CD4 binding and crosslinking agents on PBMC and highly enriched T lymphocytes from seronegatives whose cells are polyclonally stimulated by antibodies to the T cell receptor (TCR) αβ or CD3 chains. At saturating concentrations (10 μg/ml), recombinant gp120/HIV-SF2 (rgp120) or divalent anti-CD4 antibody (Leu3a) reduce the TCR-mediated stimulation of PBMC by 42% (+/-15) and 88% (+/-6), respectively. rgp120-mediated inhibition is further reduced by approximately 50% by adding rgp120-specific monoclonal antibodies, which suggests that additional CD4 crosslinking is necessary. Highly enriched (90-98% pure) CD4+ and/or CD8+ peripheral lymphocytes proliferate to TCR antibodies incubated with purified, irradiated autologous monocytes. Using these enriched lymohocytes, we find that proliferation of both CD4 T cells and a mixture of CD4 and CD8 T cells is substantially reduced in the presence of rgp120 regardless of the presence of anti-gp120. The anti-proliferative effect of rgp120 is consistently greater when both CD4 and CD8 cells are together. These data suggest a possible inhibitory mechanism involving CD4 and CD8 T lymphocyte interaction rather than simple downregulation mediated by CD4 epitope binding. We are further investigating this mechanism and relating these antiproliferative effects of rgp120 to changes in lymphocytes that lead to anergy or death. These data further emphasize the possible role of indirect mechanisms in promotion of immune attrition in HIV mediated diseases.

Q 226 EXTRACELLULAR HTLV-I TAX$_1$ PROTEIN INDUCTION OF NF-kB ACTIVITY AND STIMULATION OF TNF-β AND IMMUNOGLOBULIN KAPPA LIGHT CHAIN (Igk) GENE EXPRESSION IN LYMPHOID CELLS, Paul F. Lindholm, Robert L. Reid and John N. Brady, Laboratory of Molecular Virology, National Cancer Institute, Bethesda, Maryland 20892.

Human T-lymphotropic virus type I (HTLV-I) is the etiologic agent of adult T-cell leukemia (ATL). The transforming region of HTLV-I encodes two proteins, Tax$_1$ and Rex. In addition to its role in regulation of viral and cellular gene expression in the infected cell, Tax$_1$ may also function as an extracellular cytokine and influence the expression of genes in uninfected cells. We have analyzed the activation of NF-kB following exposure of cells to purified, recombinant Tax$_1$. NF-kB induction by Tax$_1$ protein occurred in the presence of cycloheximide. In addition, Tax$_1$ stimulation of lymphoid cells did not result in increased levels of NF-kB or c-rel RNA. These results indicate Tax$_1$ induction of NF-kB binding activity did not require *de novo* protein synthesis. Using the Igk NF-kB binding site as a probe, two distinct NF-kB gel shift complexes were induced by the Tax$_1$ protein. A slower migrating complex, C1, was inhibited by the addition of purified IkB. In contrast, the faster migrating C2 complex was not inhibited by IkB, but was increased by detergent treatment of cytoplasmic extracts, suggesting that its binding activity was also regulated by an inhibitor. Distinct NF-kB proteins interacted with the Igk and TNF-β promoter NF-kB binding sites. The Igk NF-kB binding site preferentially bound a 75 kD protein while the TNF-β NF-kB binding site associated predominantly with a 59 kD protein. The differences in the NF-kB proteins binding to these regulatory sequences may in part explain the differences in the kinetics of mRNA stimulation of these two genes by the Tax$_1$ protein and the functional differences between NF-kB promoter/enhancer elements.

Q 227 SUPPRESSION OF INTERLEUKIN 4 EXPRESSION BY HIV-1 TAT, Ralf Link[1], Min Li-Weber[1], Carlota Jäggle[2], and Peter H. Krammer[1], [1]Institute for Immunology and Genetics, German Cancer Research Center, Heidelberg, FRG, [2]Department of Immunopharmacology, E. Merck, Darmstadt, Federal Republic of Germany.

Deregulation of secretion of cytokines inducing T cell growth such as Interleukin 2 (IL-2) and Interleukin 4 (IL-4) may lead to dysfunction of T helper cells in AIDS. To test this hypothesis we cloned the IL-4 promotor in front of the CAT gene and used IL-4 expressing Jurkat T cells as a model system. We found that introduction of HIV-1 provirus into these cells downregulated IL-4 promoter activity. This effect was mediated by *Tat* and was dose dependent. Control experiments with HIV-1 LTR/CAT showed transactivation of HIV-1 expression by *Tat* as described. Endogenous IL-4 expression was also suppressed in stable Jurkat *Tat* transfectants, measured by IL-4 specific PCR. In addition, IL-4 promoter activity was suppressed in Jurkat cells cocultured with *Tat* transfectants and incubated with supernatant from such transfectants, respectively. *Tat* was also shown to inhibit IL-4 or anti-CD3 mediated growth of activated T cells. Thus, our data show that *Tat* downregulates IL-4 expression and T cell growth in *Tat* producing T cells and in neighbouring T cells that take up secreted *Tat*. Therefore, *Tat* may play an important role in the dysfunction of the immune system at an early stage of AIDS when relatively small numbers of T cells are infected by HIV-1.

Q 228 GENERATION OF A TEMPERATURE-SENSITIVE VIRUS BY INTRODUCTION OF A SINGLE AMINO ACID CHANGE IN THE PROTEASE DOMAIN OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1, Marianne Manchester[1,4], Daniel Loeb[2], Clyde Hutchison III[2] and Ronald Swanstrom[3,4], [1]Curriculum in Genetics, Departments of [2]Microbiology and Immunology and [3]Biochemistry and Biophysics, and [4]UNC Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, NC 27599

Human immunodeficiency virus type 1 (HIV-1) encodes a protease (PR) which cleaves the viral structural proteins and enzymes to their mature forms, and whose activity is required for production of infectious virus. A mutation in the protease flap region, valine 56 changed to glycine (V56G), resulted in temperature-dependent processing of the HIV-1 Pol polyprotein in a processing assay based on an *E.coli* expression system. The V56G PR mutation was introduced into the HIV-1 infectious clone HXB2, where it resulted in temperature-dependent processing of the viral Gag polyprotein *in vivo*. Additionally, a novel cleavage product of 27kD was generated at both the permissive and restrictive temperatures. Production of infectious virus particles at both temperatures was measured; less infectious virus was produced by the V56G mutant at the restrictive temperature.

The V56G mutation occurs in the flap region of PR, an area shown to undergo extensive movement during substrate contact. Computer modeling of PR indicated that the valine 56 sidechain lies in a hydrophobic pocket composed of the sidechains of Ile47, Ile54 and Pro79. Removal of the valine residue at this position could disrupt important hydrophobic contacts that contribute to stability of the flap at higher temperatures. Moreover, altering the position of the flap as a result of this substitution could explain the recognition of a novel processing site sequence in the viral Gag polyprotein by this mutant enzyme.

Q 229 **Regulation of Gene Expression of Simian Foamy Virus Type 1 and 3,** Ayalew Mergia, Rolf Renne and Paul A. Luciw, Department of Medical Pathology, University of California, Davis, CA 95616

Spumavirinae, or foamy viruses, are one of the three subfamilies of the retroviridae. These viruses have been found in non-human primates, cows, cats, hamsters, sea lions and man. A clear connection with any disease has not yet been demonstrated In the animal, spumaviruses appear to establish latency, and cytopathic effects are noted in tissue culture cells. We have molecularly cloned and sequenced the genomes of two simian foamy viruses (SFVs), isolates obtained from rhesus macaque (SFV-1) and african green (SFV-3) monkeys (Mergia and Luciw, Virology 184: 475-481, 1991; Renne et al., Virology In press). Sequence analysis reveals that SFV-1 and SFV-3 are 30% to 80% related to the human foamy virus (HFV) (Flugel et al. EMBO J. 6:2077-2084,1987; Maurer et al., J. Virol. 62:1590-1597,1988). In addition to *gag*, *pol* and *env* open reading frames (ORFs), both SFV-1 and SFV-3 contain two ORFs which extend from the end of the *env* gene into the 3'LTR; whereas the HFV genome encodes three ORFs in the corresponding region. The first ORF, designated *taf*, activates transcription directed by the homologous LTRs. The targets for transactivation have been mapped in the U3 domain of the LTRs. Current efforts are directed at elucidating the mechanism of transactivation in primate foamy viruses. Thus, members of each of the three retrovirus subfamilies (e.g., the oncoviruses , the lentiviruses, and the spumaviruses) encode transactivator genes that act through target elements in the LTR to regulate viral gene expression. These investigations provide a basis for comparing pathogenic and non-pathogenic primate retroviruses and for determining the molecular mechanisms that may account for retroviral latency and disease.

Q 230 EXPRESSION OF HIV-1 RNA WITH NEGATIVE-STRANDED POLARITY: CHARACTERIZATION OF NOVEL TRANSCRIPTS WITH COMPLEX SPLICING PATTERNS, Nelson L. Michael, Maryanne T. Vahey, George Chang, John Cooley, Lyn Gold, Jill Ruderman, and Robert R. Redfield, Department of Retroviral Research, WRAIR; Henry M. Jackson Foundation; Rockville, MD 20850.

We have studied the expression of HIV-1 RNA with negative-strand polarity in acute and chronic cell culture models as well as in infected patients using Northern blotting, quantitative RNA PCR, and cDNA sequencing. Negative-strand HIV-1 RNA is abundantly expressed in A3.01 cells and peripheral blood mononuclear cells acutely infected with the IIIb strain of HIV-1. The temporal pattern of negative-strand RNA expression mirrors that of positive strand RNA expression and virion production over three weeks of infection. H9 cells acutely infected with either MN, RF or IIIb also produce abundant amounts of negative polarity RNA. Conversely, the cell lines ACH-2 and U1, persistently infected with LAI, show a fall in the abundance of negative-strand viral RNA coincident with the rise in both positive-strand RNA and virion production. This suggests a regulatory role for negative-strand sequences in the maintenance of low-level viral gene expression in these systems. We have further shown expression of negative strand HIV-1 RNA in over 50 samples from patients with asymptomatic HIV-1 infection. The level of negative-strand RNA expression in the samples comprised a significant portion of the total viral RNA.

Negative polarity HIV-1 RNA exhibits complex splicing patterns that differ in position than those on the positive-strand. There are multiple splice donor and acceptor sites and polyadenylation signals encoded by the negative strand of HIV-1. Several open reading frames (ORFs) are also encoded on the negative-strand. The largest of these ORFs could encode a 189 amino acid protein. This ORF is conserved in many laboratory strains of HIV-1 as well as in strains from North America, Africa, and Thailand. The ubiquitous expression of negative-stranded HIV-1 RNA may serve regulatory roles (e.g. as negative sense RNAs) as well as providing for the production of novel HIV-1 proteins.

Q 231 DIFFERENTIAL REQUIREMENT OF TARGET SITES FOR TRANSCRIPTION FACTORS INVOLVED IN THE INDUCTION OF HIV IN PRIMARY MACROPHAGES, Ashlee V. Moses[1], Carlos E. Ibanez[1], Richard Gaynor[2], Jay A. Nelson[1], and Peter Ghazal[1], [1]Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037, and [2]Department of Hematology/Oncology, UCLA, Los Angeles, CA 90024.

Transcriptional regulation of the human immunodeficiency virus-1 long terminal repeat (HIV-1 LTR) is critical for controlling the replication state of the virus. HIV can be reactivated from the macrophages of asymptomatic individuals via a differentiation stimulus contributed by activated T cells. To explore the molecular mechanisms involved in this reactivation, primary macrophages from seronegative individuals were either stimulated (via activated T cell contact) or unstimulated and transiently transfected with an HIV-LTR fused to a CAT reporter construct. Macrophage stimulation resulted in a 20 fold transactivation of the LTR. To identify sequence elements responsive to macrophage stimulation, point mutations introduced into the LTR were tested in the expression system. Mutations in the TATA box and Sp1 sites were the most deleterious to induction of LTR activity, while a requirement for NFκB binding sites was also demonstrated. Mutations in sites corresponding to UBP/LBP binding allowed an upregulation of the LTR over that of the wild type. While the requirement for cellular factors in stimulated macrophages was far greater than that in unstimulated cells, the relative importance of the binding sites remained unchanged. The viral transactivator protein tat was introduced into the system. In unstimulated macrophages the requirement for TATA-binding factors remained the most critical for LTR activation, while mutations in TAR and in the NFκB sites significantly down-regulated LTR activity. A mutation in the NRE positively upregulated LTR activity. In stimulated macrophages in the presence of tat, the LTR displayed a relative independence from a requirement for cellular factor binding. In macrophages *in vivo*, a gradual release from either positive or negative regulation via cellular proteins may be an important mechanism whereby HIV is reactivated from latency and replicates to levels of pathogenic importance.

Q 232 **IDENTIFICATION OF INHIBITORY SEQUENCES IN THE ENV REGION OF HIV-1 AFFECTING mRNA STABILITY AND EXPRESSION**

Georgios Nasioulas, Christian P. Cunningham, Stefan Schwartz, George N. Pavlakis and Barbara K. Felber, Basic Research Program, National Cancer Institute-FCRDC, Frederick, MD 21702 USA

The expression of env of HIV-1 requires the presence of Rev responsive element (RRE) in cis and of the Rev protein in trans. Rev acts by binding directly to RRE. Rev is necessary for efficient transport and expression of the gag/pol, vif, vpr, and vpu/env mRNAs.

Rev dependence of env expression was proposed to require the presence of splice sites or, alternatively, the presence of instability sequences on the viral mRNA. We provide evidence that env expression of the authentic env mRNA is independent of functional splice sites. Interestingly, the presence of the RRE region had a strong inhibitory effect on env expression. Further analysis revealed the presence of an independent inhibitory sequence within this region. Therefore, HIV-1 mRNAs contain several distinct elements responsible for their stability. One element, INS-1, has been localized in the gag region (1). Another element lies in the pol region (2), while the RRE region within the env contains a third element. The mechanism of function of these negative elements is under investigation. Several experiments have suggested that additional cellular factors are necessary for Rev function. Biochemical fractionation has been used to identify cellular factors interacting specifically with Rev-RRE.

1. Schwartz, S., Felber, B.K. & Pavlakis, G.N. *J Virol* **66**, Jan. 1992.
2. Cochrane, A.W., *et al. J Virol* 65, 5305-5313 (1991).

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Q 233 SLOW KINETICS OF HIV-1 REVERSE TRANSCRIPTION IN MONONUCLEAR PHAGOCYTES CAN BE ACCELERATED BY ADDITION OF NUCLEOSIDE PRECURSORS, William A. O'Brien*, Ali Namazie†, Si-Hua Mao†, Mehran Mandegar†, Jerome A. Zack†, and Irvin S.Y. Chen†§, *Department of Medicine, West Los Angles VA Medical Center and UCLA School of Medicine, Los Angeles CA 90073; †Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024; §Department of Microbiology & Immunology, UCLA School of Medicine, Los Angeles CA 90024

Mononuclear phagocytes are important target cells for HIV-1 infection, and are associated with certain clinical manifestations. However, details of early events following infection of mononuclear phagocytes are not well characterized. Reverse transcription in activated peripheral blood lymphocytes (PBL) and T-cell lines is completed by six hours, but is arrested at an intermediate step in quiescent PBL. Because virus production appears relatively late in mononuclear phagocytes and these cells do not divide in culture, we compared the kinetics of reverse transcription following infection of activated PBL and mononuclear phagocytes obtained from the same donor. Completion of reverse transcription in mononuclear phagocytes occurs between 36-48 hours, and formation of full-length viral DNA can be blocked by AZT treatment, even if this is added to the cultures 24 hours after infection. To determine potential mechanisms of slow reverse transcription kinetics in mononuclear phagocytes, we increased the pool of nucleotides by treating the cultures with high concentrations of nucleotide precursors and found that this accelerated the kinetics of reverse transcription. Thus, the cellular milieu appears to play a role in determining the kinetics and the extent of reverse transcription in primary blood mononuclear cells.

Q 234 CELL-BASED FUNCTIONAL STUDIES USING HIV-1 rev PROTEIN OVEREXPRESSED IN *E. coli*, Michael J. Orsini, William W. Andrews, David M. Rekosh and Marie-Louise Hammarskjöld, Departments of Biochemistry, Microbiology and Oral Biology, State University of New York at Buffalo, Buffalo, NY 14214

The HIV-1 rev protein is required for expression of the viral structural genes *gag, pol* and *env*. We have overexpressed and purified rev protein from *E. coli*. The protein was demonstrated to be functional using a procedure which allowed uptake of rev into COS cells transfected with a rev-dependent construct. Our results demonstrated that the addition of purified rev protein to the cell culture medium of transfected cells, at concentrations ranging from 5-100 μg/ml, allowed the expression of gag and gag-pol proteins. Functional uptake was strictly dependent on the presence of 100 μM chloroquine in the cell culture medium.

Mutant rev proteins with amino acids 79 and 80 either substituted or deleted have also been expressed and purified. These proteins did not display rev activity by themselves, but displayed a transdominant phenotype in the activity assay when they were added to cells together with wild type rev protein or to cells transfected with a rev-expressing plasmid.

Studies which utilized Actinomycin D to block RNA synthesis demonstrated that rev could only act on newly synthesized RNA. This notion was supported by other experiments which demonstrated that rev could only act on mRNA synthesized close to the time of uptake.

Q 235 ANALYSIS OF CLUSTERED-SITE MUTATIONS WITHIN THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 LONG TERMINAL REPEAT. RESPONSES TO TRANS-ACTIVATION BY HUMAN CYTOMEGALOVIRUS, Primepares G. Pal and Deborah H. Spector, Department of Biology, University of California, San Diego, La Jolla, California 92093-0116.

We have studied the capacity of long terminal repeat (LTR) mutant constructs of the human immunodeficiency virus (HIV), provided by Dr. Peter E. Barry, to respond to transactivation by human cytomegalovirus strain AD169 (CMV) and the immediate-early (IE) gene products of CMV. These HIV LTR constructs were linked to the bacterial chloramphenicol acetyltransferase gene, and transient expression assays in transfected human foreskin fibroblast cells were used to measure transactivation of the LTR after either superinfection with CMV or cotransfection with a plasmid expressing the major CMV IE gene products IE1 and IE2. Analysis of these clustered-site mutations revealed that only the TATA box and upstream sequences from nucleotides -46 to -77 (relative to the transcription start site) corresponding to sites that bind the transcription factor Sp1 were critical for responsiveness to transactivation by CMV. Relative to wild-type HIV LTR, LTR constructs with double point mutations within Sp1-binding regions I, II, and III and base-pair substitutions within the TATA sequence were transactivated to only 10% and 5%, respectively. In accord with previously published results (Barry et al., J. Virol. 64:2932-2940, 1990), similar analysis revealed that in addition to the Sp1 binding sites and the TATA sequence, the region spanning nucleotides -6 to +18 was essential for maximal transactivation by CMV IE gene products. Mutations in this latter region, however, had no significant effect on the response of the HIV LTR to CMV infection.

These results suggest that expression of CMV IE gene products alone is not sufficient for maximal transactivation of the HIV LTR and that other CMV gene products or cellular factors induced by CMV infection may be critical components in the transactivation of the HIV LTR.

Q 236 HIV INFECTION IN MONOCYTES RESULTS IN cPKC INDEPENDENT NF-kB TRANSLOCATION AND SUBSEQUENT UPREGULATION OF NF-kB p50 SUBUNIT PROMOTER. Carlos V Paya, Rosa M Ten, Ronald T Hay, Jean L Virelizier, Laboratoire d'Immunologie Viral,Institut Pasteur, Paris; Dept Biochemistry, University of St Andrews, Scotland, UK.

The molecular mechanisms through which chronic HIV infection induces NF-kB DNA-binding activity in the monocytic cell line U937 were studied. The NF-kB complex found in the nuclei of infected U937 cells is a p50/p65 heterodimer as shown by the use of antibodies to p50 and c-rel, and competition with recombinant human IkB in band shift assays. In addition to the enhanced nuclear translocation, NF-kB levels were increased in both nuclear and cytosolic extracts of HIV-infected cells, suggesting an upregulated synthesis. Northern blot analysis showed a 2-4 fold increase of p50, but not p65 steady state mRNA in HIV-infected cells. This correlated with an increased activity of the p50 promoter transiently transfected in luciferase expression vectors in chronically infected cells. Deletion of the NF-kB binding motif of the p50 promoter abolished the HIV-induced activity. The increased p50 promoter activity is HIV-tat independent.A specific cPKC inhibitor abolished PMA- but not HIV-induced NF-kB translocation and increased p50 promoter activity. These data suggest that HIV activates NF-kB by inducing, through a cPKC-independent transduction pathway, nuclear translocation of p50/p65 complexes, with subsequent upregulation of p50 gene transcription. Increased production of p50 protein may lead to its accumulation in the cytoplasm in association with p65 and IkB, ready for further HIV infection-induced translocation in a permanent, self-perpetuated loop of amplification.

Q 237 IDENTIFICATION OF THE REV TRANSACTIVATION AND REV RESPONSIVE ELEMENTS OF FELINE IMMUNODEFICIENCY VIRUS. T.R. Phillips[1], C.Lamont[2], D.A.M. Konings[3], B.L. Shacklett[4], P.A. Luciw[4], J.H. Elder[2], [1]Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA; [2]Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA; [3]Department of Molecular, Cellular, and Developmental Biology, University of Colorado at Boulder, Boulder, CO; [4]Department of Medical Pathology, University of California, Davis, Davis, CA.

Spliced messages encoded by two distinct strains of FIV were identified. Two of the cDNA clones represented mRNAs with bicistronic capacity. The first coding exon contained a short open reading frame (orf) of unknown function, designated orf 2. After a translational stop, this exon contained the L region of the env orf. The L region resides 5' to the prdicted leader sequence of env. The second coding exon contained the H orf which began 3' to env and extended into the U_3 region of the LTR. The in-frame splicing of the L and H orfs created the FIV Rev gene. Site directed antibodies to the L orf recognized a 23 kd protein in infected cells. Immunofluorescence studies localized Rev to the nucleolus of infected cells. The RRE of FIV was initially identified by computer analysis. Three independent isolates of FIV were searched in their entirety for regions with unusual RNA folding (UFR) properties. An UFR region was not found at the Su-TM junction but instead was located at the end of env. Minimal-energy foldings of this region revealed a structure that was highly conserved among the three isolates. Transient expression assays demonstrated that both the Rev and RRE components of FIV were necessary for efficient reporter gene expression. Thus, multiple splicing events generated the FIV Rev message which encoded a protein that acted through a RRE to regulate gene expression.

Q 238 QUANTITATIVE COMPETITIVE POLYMERASE CHAIN REACTION (QC-PCR) FOR ACCURATE QUANTITATION OF HIV DNA AND RNA, **M. Piatak, *K.-C. Luk, **M. McGrath, ***B. Willaims, and *J. Lifson, *Genelabs Incorporated, Redwood City,CA; **University of California, San Francisco, and S.F. General Hospital, San Francisco, CA, ***Applied Imaging, Inc., Santa Clara,CA.*

Variations in reactions between replicate or sequence equivalent samples, including differential kinetics due to inhibitors and competition for primers due to amplification of background products, may result in non-uniform PCR amplification of target sequences. Without providing adequate internal controls for such effects, the basic PCR is thus not suitable for rigorous quantitative analysis. We have adapted a competitive PCR method (Gilliland, et al, PNAS, 87:2725, 1990) to achieve accurate quantitation of HIV DNA and RNA sequences. Varying known amounts of a deletant mutant competitive template, nearly identical to, but differentiable from the wild type target sequence, are added to replicate aliquots of a specimen containing an unknown amount of HIV-1 DNA or RNA, and PCR performed. The same primers amplify both the added competitive template and any wild type target sequences present; the added mutant template serves as a specific competitor for all aspects of the PCR, providing an internal control preferable to co-amplification of an unrelated target sequence. PCR products are quantitated by video image analysis of ethidium stained agarose gels. The amount of wild type HIV sequence present in the specimen is determined by comparison of the relative intensity of fluorescence for the wild type PCR product band from a given reaction with the band corresponding to a known amount of competitive template added to that reaction. When the fluorescence intensities are equal, the amount of wild type sequnce equals the known amount of added competitive template. The equivalence point may be determined by interpolation. For RNA-PCR, known amounts of a T7 transcript of the competitive template are added to aliquots of the test sample, providing an additional level of stringent control for the reverse transcription reaction that precedes the PCR. We have used this approach to detect and quantitate as few as 10 copies of HIV DNA or RNA. Determined HIV copy numbers in blood specimens from 23 infected patients at various clinical stages, receiving and not receiving AZT or ddI ranged from 8 to 2,542 HIV DNA copies per µg of total PBMC derived DNA and from 5 to >5,000 HIV RNA copies per ml of plasma. The procedure appears particularly promising for quantitative monitoring of virologic responses to therapeutic intervention in HIV infected patients, including evaluation of the effects of experimental agents on in vivo viral load.

Q 239 HUMAN IMMUNODEFICIENCY VIRUS-1 TAT PROTEIN INDUCES IMMUNOGLOBULIN SYNTHESIS IN VITRO BY NORMAL PERIPHERAL BLOOD MONONUCLEAR CELLS, Jukka Rautonen, Nina Rautonen, Natasha L. Martin, and Diane W. Wara, Department of Pediatrics, University of California, San Francisco, San Francisco, CA 94143

Hypergammaglobulinemia is one of the earliest and most common findings in patients with HIV infection. Its cause remains unknown. In the present study, we demonstrate that exogenous HIV-1 Tat protein induces Ig synthesis *in vitro* by normal PBMCs. The optimal concentrations of Tat of 100-300 ng/ml induced IgG and IgA synthesis by PBMCs from all six healthy children and six healthy adults studied, and IgM synthesis by PBMCs from all children and 4/6 adults (mean (±SE) increases in IgG, IgA, and IgM synthesis were to 199 (17), 255 (47), and 522 (115) % of baseline, respectively). However, even very low concentrations of 0.1-1 ng/ml of Tat induced Ig synthesis in most subjects. The observed induction of Ig synthesis could not be attributed to LPS, and was inhibited by monoclonal anti-Tat antibodies.

It has been previously reported that Tat can be released from HIV-infected cells and taken up by uninfected cells. We speculate that Tat might be able to imitate the effects of NF-κB, perhaps by inducing an NF-κB-like factor, and hypothesize that Tat, released by HIV infected cells and taken up by uninfected cells, may have a role in the pathogenesis of polyclonal B cell activation and hypergammaglobulinemia associated with HIV infection.

Q 240 REV-INDEPENDENT EXPRESSION OF HIV-1 GAG IN HUMAN CELLS

Stefan Schwartz[1, 2], Mel Campbell[1], Barbara K. Felber[1], and George N. Pavlakis[1]. Basic Research Program, National Cancer Institute-FCRDC, Frederick, MD 21702 USA. Department of Virology, Karolinska Institute, Stockholm, Sweden.

Expression of HIV-1 gag requires the presence of the Rev-responsive element (RRE) on the mRNA and the HIV-1 Rev protein in trans. Analysis of HIV-1 gag expression plasmids in human cells demonstrated that low levels of mRNA and protein were produced in the absence of Rev. This was caused by retention of the mRNA in the nucleus where it is either degraded or spliced. On the basis of these results it has been proposed that HIV-1 mRNAs contain inhibitory elements that prevent expression of gag in the absence of Rev (1, 2, 3). We have undertaken an analysis of inhibitory elements on HIV-1 mRNAs and we have identified one of these elements in detail. This element is 218 nucleotides in size and is located in the 5' part of the gag open reading frame. It acts by decreasing both mRNA and protein levels. The inhibitory effect could be overcome by Rev and RRE. Introduction of point mutations in this element resulted in Rev-independent Gag production. These results demonstrate that the identified inhibitory elements play an important role in the regulation of HIV-1 gag expression.

1. Schwartz, S., Felber, B.K. & Pavlakis, G.N. *J Virol* **66**, Jan. 1992.
2. Cochrane, A.W., *et al. J Virol* **65**, 5305-5313 (1991).
3. Maldarelli, F., *et al. J Virol* **65**, 5732-5743 (1991).

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Q 241 SEQUENCE HETEROGENEITY OF THE HIV-1 NEF GENE

Diane C. Shugars[1], Deborah Glueck[2], and Ronald Swanstrom[3], Departments of [1]Microbiology and Immunology, [2]Biostatistics, and [3]Biochemistry and Biophysics, and [3]UNC Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, NC 27599

The *nef* genes of HIV types 1 and 2 and the SIVs encode a protein (Nef) whose role in viral replication and cytopathicity remains unclear. The purpose of this study was to determine the extent of *nef* sequence variability *in vivo* and to assess proposed functions for Nef based on putative sequence similarities with other viral and cellular proteins. Human peripheral blood mononuclear cells (PBMCs) were isolated from thirteen HIV-1-infected individuals having low CD4 counts and clinical symptoms of AIDS or AIDS-related complex. Total DNA was prepared from the PBMCs without prior culturing. The viral *nef* genes were amplified from the purified DNAs using a nested-primer PCR amplification approach, cloned and sequenced.

Analysis of the nucleotide sequences revealed that all *nef* genes contain intact initiation codons and encode highly conserved myristylation signals. Specific premature termination codons at position 124 were detected in three of the 13 individuals and represented approximately 10% of all *nef* sequences. Pairwise analysis of deduced amino acid sequences showed extensive variation between sequences, ranging from 0.5% to 22% percent differences. Sequence variability resulted in amino acid insertions, deletions and/or duplications. A Nef consensus sequence was derived and used to identify numerous highly conserved stretches of amino acids primarily confined to the central region of the sequence. Query sequences derived from this region were used to search libraries for structurally similar proteins with known function. The results of these searches will be discussed. Further analysis of the consensus sequence revealed poor conservation of sequence elements identified in previous sequence similarity studies.

Q 242 DETECTION OF HIV BY CD4 CAPTURE AND RT-PCR,

Secondo Sonza*, John Mills*+, David J. Kemp+ and Suzanne M. Crowe *,Macfarlane Burnet Centre for Medical Research* and Walter and Eliza Hall Institute+, Melbourne, Victoria, Australia.

Current methods for quantitating viraemia in the plasma of HIV-infected persons by endpoint dilution in tissue culture assays are slow and laborious. We have developed an alternative method for detecting virus particles based on their capture by immobilised recombinant CD4 (rsCD4) and subsequent amplification of viral RNA by reverse transcription-polymerase chain reaction (RT-PCR). CD4 capture was used to ensure that only RNA from intact virions would be reverse transcribed and amplified.Standard 0.5ml PCR tubes were coated with 1ug rsCD4 (Ray Sweet, Smith Kline Beecham) in 0.1M carbonate buffer, pH9.6. To standardize the assay, supernatant from lymphoid cell culture of HIV-1 (DV) of known titre was serially diluted into coated tubes and allowed to adsorb to immobilised CD4. The tubes were then washed and the bound virus solubilized with 10ul 0.5% Triton X-100, heated to 65°C for 10min and cooled on ice. 10ul of RT reaction mix containing dNTPs, $pd(N)_6$ primer and M-MuLV RT was then added and cDNA synthesis allowed to proceed for 1hr at 37°C. Following the addition of SK38/39 *gag* primers and Taq polymerase and 30 cycles of amplification, a 115bp product could be detected on ethidium bromide-stained agarose gels from as few as 1000 $TCID_{50}$ of HIV-1. We have subsequently used the CAPTAGENE™-GCN4 assay (AMRAD Corporation Australia; Kemp *et al*, Gene 94: 1990, 222-228) for detection of amplified *gag* sequences. This assay uses nested primers and relies on the binding of amplified DNA via a sequence in one internal primer to the yeast DNA-binding protein, GCN4-fusion protein (AMRAD), coated onto microtitre plates. Biotin at the 5' end of the other internal primer is then detected by avidin-peroxidase and a chromogenic substrate. We are currently analysing plasma from HIV-infected asymptomatic, ARC and AIDS patients and correlating our findings with other measures of viraemia.

Q 243 LOCALIZATION OF TWO DISTINCT GLUCOCORTICOID RECEPTOR-BINDING SITES WITHIN THE HIV-1 GENOME

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Several retroviruses possess glucocorticoid responsive elements (GREs) which enable them to directly interface with the glucocorticoid receptor (GR) signal transduction pathway, resulting in an enhanced replication rate in presence of glucocorticoid hormones. Previous work in our laboratory has shown that glucocorticoid agonists dexamethasone (10^{-9}M) and cortisol (10^{-7}M) can stimulate HIV-1 replication in *de novo* infected lymphoid cell lines, and that the magnitude of this induction correlates with relative levels of GR present in these cell lines. Also, DNA sequence analysis has revealed the presence of a dozen discrete GRE-like patterns distributed throughout the HIV-1 genome. By using a mobility shift assay, we have demonstrated that the purified GR can specifically interact with one of these sequences, positioned within the HIV-1 LTR (NRE), and with a second one derived from the HIV-1 *vif* open reading frame. To assess the functional nature of these putative GREs, we subcloned them into a MMTV-LTR-luciferase construct from which endogenous GREs had been previously deleted. The activity of these recombinants was measured in a GR-negative cell line in presence and absence of co-transfected GR expression vector, and with or without glucocorticoid treatment. Our results show that the two HIV-1 sequences tested significantly enhanced baseline transcription (two-fold) and conferred mild glucocorticoid inducibility to the MMTV promoter. These data suggest that HIV-1 contains discrete functional GREs which might serve to modulate the rate of viral transcription and replication, or control some aspect of latency. This has important clinical significance since adjuvant corticosteroid therapy is currently gaining acceptance for the treatment of *p. carinii* pneumonia.

Q 244 ACTIVATION OF TRANSCRIPTION BY THE HIV-1 TAT PROTEIN, Christopher D. Southgate and Michael R. Green, Program in Molecular Medicine, University of Massachusetts Medical Center, 373 Plantation St., Worcester, MA 01605.

The Tat protein of the human immunodeficiency virus type 1 (HIV-1) activates transcription from the HIV-1 LTR promoter following binding to nascent TAR RNA downstream of the transcription initiation site. Because Tat functions when bound to RNA, and in a position-dependent manner, it has been proposed that Tat works by a novel mechanism. Here we perform a series of protein-fusion experiments, which reveal striking similarities between Tat and conventional cellular activators. Most significantly, we demonstrate that Tat can function when bound to upstream promoter DNA. This activity depends on a region within Tat, which is also required for Tat to function when bound to TAR RNA. In contrast, Tat's arginine-rich region, which is required for binding to TAR RNA, is dispensable for the function of DNA-bound Tat. When bound to either RNA or DNA Tat activity requires cooperation with promoter-bound cellular transcription factors. Finally, we show that Tat and a strong acidic activator stimulate transcription to comparable levels. Based upon these and other results we suggest that Tat and acidic activators act on a similar step in the transcription process. We are currently investigating Tat's mechanism of action using in vitro transcription assays and novel protein-fusion experiments.

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Q 245 REVERSE TRANSCRIPTION AND ESTABLISHMENT OF A REPLICATION-COMPETENT FORM OF HIV-1 DNA IN QUIESCENT PRIMARY CD4 LYMPHOCYTES. Celsa A. Spina, T. Jesse Kwoh and Douglas D. Richman. UCSD School of Medicine, VA Medical Center and Baxter Diagnostics, San Diego CA 92161

HIV-1 can enter quiescent CD4 lymphocytes and initiate a "silent" or non-productive infection. Subsequent induction of T cell activation and proliferation causes HIV to enter a complete, productive replication cycle. Little is understood about the interaction of viral and cellular elements to either maintain a latent viral state or initiate a competent viral replication cascade. To investigate this question, we have used an *in vitro* experimental system of purified, primary CD4 lymphocyte cultures from HIV-seronegative donors and acute infection with the LAV-1_{Bru} strain of HIV. PCR amplification techniques are used to detect viral DNA and spliced RNA products; productive virus replication is determined by elaboration of soluble p24 antigen.

Our prior studies demonstrated that HIV DNA is formed very early after infection of resting CD4 cells and slowly accumulates over days in culture. In the absence of cell stimulation, multiply-spliced RNA transcripts corresponding to *nef* are detected late, at ≥ 5 days. These data indicate that HIV can establish a non-productive but replication-competent form in such CD4 cells. Our current studies have addressed this point by using AZT to block reverse transcription at serial time points post-infection, and immediately prior to induction of complete replication with PHA + IL-2. The addition of AZT at 3 hrs., 1 day, or 3 days after infection of resting CD4 cells blocks the subsequent induction of soluble p24 by cell activation. However, at times ≥ 5 days, complete productive virus replication can be induced from infected cells in the presence of AZT. These results suggest that HIV DNA exists in an incomplete or replication incompetent form early after infection of quiescent CD4 cells, but transcription to full-length proviral DNA proceeds at a delayed or retarded pace in the absence of induced cell proliferation. PCR analysis, using a series of oligonucleotide primer pairs to different regions of HIV DNA, is now being applied to this system to elucidate the mechanism and control of delayed transcription in a quiescent cell state.

Q 246 ROLE OF TOPOISOMERASES IN THE REPLICATION OF HIV-1, Gilda Tachedjian, Darren K. Jardine, Stephen A. Locarnini* and Chris Birch*. Macfarlane Burnet Centre for Medical Research and Virology Department*, Fairfield Hospital. Yarra Bend Rd, Fairfield Australia 3078.

Topoisomerases are DNA modifying enzymes involved in DNA replication, transcription and recombination. Since various DNA topoisomers are found in HIV-infected T-lymphocyte lines we investigated whether inhibitors of eukaryotic topoisomerase type I and II and the prokaryotic DNA Gyrase inhibited HIV replication in infected MT-2 cells. Of the drugs tested, the DNA Gyrase inhibitor coumermycin A1 (CA1) was the only drug that inhibited at non-cytotoxic concentrations. Inhibitory activity was also observed in HIV-1 infected human peripheral blood leukocytes. CA1 did not inhibit the HIV-1 reverse transcriptase at concentrations that were inhibitory in cell culture. Consistent with a mechanism at or before integration, CA1 was unable to inhibit replication in chronically infected H9/IIIB cells. Whether CA1 is acting at the level of SC DNA or at integration is yet to be determined.

Contrary to recent reports# the topoisomerase type I inhibitor camptothecin did not inhibit HIV 1 in both acute and chronically infected cells at non-cytotoxic concentrations. Attempts to determine whether sucrose gradient purified virions possessed topoisomerase I activity revealed that SC DNA relaxing activity was present in both NP-40 lysed and unlysed preparations suggesting that cellular topoisomerase type 1 is co-purifiying with HIV-1. These data indicate that HIV-1 does not carry its own topoisomerase type I and that the role of eukaryotic topoisomerase type I in HIV 1 infected T-lymphocyte cell lines is unclear. The presence of topoisomerase type II and DNA Gyrase activity associated with HIV 1 will also be presented.

Priel et al. (1990) EMBO 12: 4167-4172.
Priel et al. (1991) AIDS Res Hum Retroviruses 7: 65-72.

Q 247 IDENTIFICATION AND STRUCTURAL ANALYSIS OF THE PRIMARY RNA BINDING SITE OF THE VISNA VIRUS REV PROTEIN. L.S. Tiley, and B.R. Cullen., Howard Hughes Medical Institute, Department of Microbiology and Immunology, Duke University Medical Center, Durham, N.C. 27710

Visna virus, a pathogenic lentivirus of ungulates, encodes a post-transcriptional regulatory protein functionally, mechanistically and topographically similar to the Rev transactivator of human immunodeficiency virus type 1 (HIV-1). However, these two proteins display discrete sequence specificities, ie. neither protein is able to function in conjunction with the response element of the other.

The RNA target sequence for the Rev protein of visna virus is located within the env gene and is predicted to have a high degree of secondary structure. The authenticity of the computer predicted secondary structure was confirmed using structure-specific ribonucleases. Gel-shift analysis and footprinting using E.coli-expressed visna Rev protein localized the specific binding site to a short stretch of RNA corresponding to a single hairpin-loop structure. The stem of this structure contains a purine-rich bulge reminiscent of the primary binding site of HIV-1 Rev. This suggests that although these two proteins have distinct sequence specificities, their binding sites possess certain structural features in common.

Q 248 ENZYMATIC ACTIVITY AND INFECTIOUS POTENTIAL OF HIV-1 REVERSE TRANSCRIPTASE MUTANTS IN THE HIGHLY CONSERVED YMDD AMINO ACID MOTIF, John K. Wakefield, Sandra A. Jablonski and Casey D. Morrow, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

The reverse transcriptase of HIV-1 contains a highly conserved YMDD amino acid motif believed to be important for enzyme function. To further explore the role of the YMDD motif in HIV-1 reverse transcriptase function, we have constructed a series of single amino acid substitutions. We have changed the methionine amino acid to a valine, alanine, serine, glycine or proline. The wild-type and mutant reverse transcriptases were expressed in E. coli, partially purified by phosphocellulose chromatography, and assayed for the capacity to polymerize TTP using a homopolymeric template poly(rA) with either a DNA (oligo dT) or an RNA (oligo U) primer. A change of the methionine (wild type) to valine (YVDD), serine (YSDD) or alanine (YADD) resulted in enzymes with similar in vitro (70-100%) activity as wild type using a poly (rA)-oligo(dT) template/primer combination. A methionine to glycine change (YGDD) resulted in a drastic reduction of the in vitro activity to 5-10% of that of the wild type enzyme, while the proline substitution (YPDD) resulted in a background enzyme level. The in vitro enzyme activity of the wild type and YVDD reverse transcriptase were similar using a poly(A)•oligo(U) template/primer combination. In contrast, the enzymes with YSDD or YADD mutations had 5-10% of the activity of the wild type enzyme, while the YGDD and YPDD mutant reverse transcriptases were inactive using this template/primer combination. Transfection of proviruses with the YVDD, YADD and YSDD mutations in the reverse transcriptase gene resulted in infectious virus within 7-10 days of culture, while the proviruses containing the YGDD and YPDD resulted in no infectious virus during this period. The viruses containing the valine mutation (YVDD) had similar replication kinetics as that of the wild type virus while those with YADD and YSDD mutations demonstrated slower kinetics of replication. Thus, the in vitro enzyme activity of the wild type, YVDD, YADD and YSDD mutant reverse transcriptases using the poly(rA)-oligo (U) template/primer correlated with the initial replication kinetics of viruses containing these mutations in the reverse transcriptase gene. These studies point to the critical role of the YMDD amino acid motif in the enzymatic activity of the HIV-1 reverse transcriptase as well as the subsequent replication potential of the virus.

Q 249 MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF A NEW, HIGHLY CYTOPATHIC HIV-2. Randy Talbott*, Gunter Kraus*, David Looney*, Leo Luznik*, William Morton#, Anne Schimidt#, Don Mosier**, Gabriele Bitterlich*, Joan Esnayra* and Flossie Wong-Staal*. *Departments of Medicine and Biology, UCSD School of Medicine, La Jolla, CA 92093; #Washington Regional Primate Center, Seattle, WA; **Medical Biology Institute, La Jolla, CA 92037

We have obtained an infectious molecular clone of HIV-2, designated KR, which mimics its parental virus (HIV-2/PE12) in its highly cytopathic and syncytia-inducing capabilities, particularly in the T-cell line Molt4/8. Nucleotide sequence of the genome showed that KR is related to and distinct from other HIV-2 viruses, and encodes non-truncated TM and Nef proteins, as well as tat, rev, vpr, vpx, and vif. In particular, an 11 base-pair deletion is found in the U3 region of the LTR. KR also differs from other HIV's in being able to infect unstimulated, TAC-negative peripheral blood lymphocytes. An LTR-CAT construct yields measurable basal activity, which is stimulated by Tat and by T-cell activation agents such as PMA. Studies with chimeric virus constructs utilizing regions of a slow replicating, low syncytia-forming virus ($HIV\text{-}2_{ISY}$) and KR suggest that the determinant for cytopathicity resides in the env gene. The KR virus also productively infected 5/5 *Hu-PBL-SCID* mice and 2/2 pig-tailed macaques. These infected animals will be followed for CD4/CD8 cell counts immune responses, divergency and biological properties of recovered viruses as well as other parameters. Those animals may provide useful systems for evaluating candidates for gene therapy directed at HIV.

Q 250 REGULATION OF THE HIV-1-LTR BY THYROID HORMONE RECEPTOR, *Vandana Yajnik* and Herbert H. Samuels, Departments of* Pathology*, Medicine, and Pharmacology, New York University Medical Center, 550 First Avenue, New York, N.Y. 10016

Members of the steroid/thyroid hormone receptor gene family have been reported to regulate transcription from the LTRs of a number of viruses. We have observed that thyroid hormone (T3) stimulates expression from human immunodeficiency virus type I (HIV-1)-LTR-CAT constructs. HeLa cells, which lack thyroid hormone receptor (T3R), were co-transfected with an HIV-1-LTR-CAT vector (pU3R-III-CAT(-453/+80)) and a vector expressing T3R. T3 stimulated CAT expression approximately 8-10 fold (T3/basal). Similar experiments were performed using glucocorticoid, retinoic acid, and vitamin D receptors. These receptors and their ligands did not stimulate the HIV-1-LTR. The sequences important for T3 stimultion of the HIV-1-LTR were mapped by coupling detailed functional analyses with receptor binding studies. Gel mobility shift assays were conducted to examine the binding of purified chick T3R-alpha to the -167/+80 fragment of the HIV-1-LTR. Without T3, the receptor formed gel shift complexes of low and high mobility. When T3 was added to the incubation there was a marked reduction in the abundance of the low mobility complex and an increase in the high mobility complex. We have noted two regions in the -167/+80 sequence of the LTR which bind T3R. The more distal element contains sequences identical to known thyroid hormone response elements (TREs). Introduction of mutations into these motifs completely abolishes stimulation of the HIV-1-LTR by T3R. When the wild type motifs were cloned upstream of a heterologous promoter, they mediated trans-activation by T3R in HeLa cells. Deletion of an essential cytosine residue in these TREs markedly reduces T3 stimulation. The other T3R binding site is localized closer to the TATA box (referred to as the proximal TRE). Although this element binds to purified chick T3R-alpha, it does not mediate trans-activation of the HIV-1-LTR when the more distal TREs are deleted. However, expression of the HIV-1 tat trans-activator converts the inactive proximal response element to one which now mediates strong T3 stimulation by T3R. These studies indicate that the HIV-1-LTR contains both tat dependent and independent TREs and provides a model for analysis of activation of T3R by other transcription factors.

Q 251 FUNCTIONAL ANALYSIS OF THE HIV LTR USING LINKER SUBSTITUTION MUTAGENESIS. *Steven L.* Zeichner[1], John Y.H. Kim[2], Gabor Hirka[3], Joseph Manupello[2], Peter Andrews[3], and James C. Alwine[2], [1]Pediatric Branch, NCI, NIH, Bld 10 Rm 13N240, Bethesda, MD 20892, [2]Department of Microbiology, School of Medicine, *Univ. of PA, Philadelphia, PA, and [3]The Wistar Institute, Philadelphia, PA

We have analyzed HIV LTR promoter function using linker substitution (LS) mutational analysis. 26 LS mutants were prepared across the U3 and the beginning of the R regions in an HIV LTR- CAT plasmid. Consecutive 18 bp regions of wild type LTR were replaced with an NDE I-Xho I-Sal I (NXS) polylinker. In unstimulated, TPA/PHA-stimulated and tat-expressing Jurkat cells transcriptional activity of many of the mutants was predictable, based on current knowledge of LTR regulatory elements. However, some of the mutants indicated additional regulatory sites. In addition, the fine structure analysis highlights differences in utilization of known regulatory regions, depending on the cellular conditions. The NF-kB sites are necessary for transcription under all cellular conditions; however, there is a marked difference in the requirement for one or two sites comparing stimulated Jurkat cells with unstimulated or tat-expressing Jurkat cells. Mutants on the 3'-side of the TATA element caused a more significant decrease in activity in tat-expressing Jurkat cells. Further upstream, a regulatory region between -183 and -130 was active in unstimluated and stimulated Jurkat cells. DNA mobility shift studies confirmed that altered promoter activity correlated with altered factor binding. Quite modest evidence for negative regulation appeared in the unstimulated and stimulated Jurkat cells. The LS mutants were also used to study regulatory sequences exhibiting differentiation dependence in human teratocarcinoma cells (NTERA-2). These cells follow distinct differentiation pathways after induction with retinoic acid or hexamethylene bis-acetamide. Differentiated cells of both pathways are susceptible to HIV infection, but our results how that cells of each pathway utilize a discrete set of LTR regulatory elements, some of which are in regions not active in lymphoid cells. Altered, differentiated cell NTERA-2 cell factor binding was also observed when these elements were mutated. Overall, our comparisons indicate significant cell-type, growth and differentiation differences in the transcriptional utilization of the LTR.

Q 252 **IN VIVO ANALYSIS OF THE HIV-1 LTR**

Alain ZIDER, Patricia FERGELOT, Muriel VERNET, Iman MAKEH, Gisèle GRIMBER, Pascale BRIAND, Catherine CAVARD.
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Transgenic mice carrying the HIV-1 LTR controlling the expression of the two reporter genes *cat* and *lacz* were generated for the analysis of the cellular and exogenous factors which may activate *in vivo* the viral sequences. The spontaneous expression in the skin and the eyes of these mice and the evidence of its activation in the skin by ultraviolet rays have been previously reported (J.Clin. Invest. 1990 86: 1369). To identify the cellular factors involved in the basal and the activated expression of the transgene, we have designed electrophoretic mobility shift assays showing that a NF-κB like binding activity was present in mouse epidermis and was increased after UV irradiation of this tissue. Since many proteins have been shown to interact with the κB sites, UV cross linking experiments are in progress to determine the molecular weight of the proteins present in the murine epidermis with or without activation. To test the biological relevance of these *in vitro* assays, transgenics carrying modified LTR linked to the *lacZ* gene have been produced. Their capacity to express the reporter activity will be discussed.

Pathogenesis

Q 300 INTERACTION OF HOST TRANSCRIPTIONAL FACTORS WITH HIV-1 LTR VARIES WITH VIRAL GENE EXPRESSION IN TRANSGENIC MICE, Scott H. Adler*, Leslie A. Bruggeman, Jeffrey B. Kopp, Abner L. Notkins and Paul E. Klotman, Molecular Medicine Section, LDB and LOM/NIDR, *the Howard Hughes Medical Institute-NIH Research Scholars Program, National Institutes of Health, Bethesda, MD 20892.

We have developed a transgenic mouse model carrying a replication-defective HIV-1 provirus (Kopp et al,*PNAS*; in press) which exhibits a variety of pathologic manifestations including skin lesions, renal disease, and a myopathy. The transgene included a 3.1kb deletion spanning the *gag* and *pol* genes. These transgenic animals were used to study the tissue-specific expression of the transgene in muscle, brain, heart, lung and kidney. The level of expression of the transgene, including 2, 4 and 7kb (full length) messages, varied in different tissues as determined by Northern hybridization of total cellular RNA. Maximal expression was seen in muscle and skin, with little or no expression seen in other organs. We are currently investigating the contribution of host transcriptional factors to this differential expression of viral mRNA. Gel mobility shift assays were performed using a 240 bp fragment of the pNL4-3 promoter (from -160 to +80), which contained the consensus sequences for the cellular transcriptional factors NF-κB, Sp1, CTF, TFIID and LBP, and nuclear extracts prepared from various organs of the heterozygote transgenic mice. Multiple gel shift patterns were observed indicating different interactions of host transcriptional factors with the LTR. The formation of the DNA/protein complex appeared to be dependent on the binding of NF-κB as determined by competition experiments with unlabeled oligonucleotides for the nuclear factor binding sites. We conclude that high levels of transgene expression in skin and muscle is associated with pathology in these tissues. Furthermore, the contribution of tissue-specific host factors to viral gene expression may be responsible for the sites at which pathology occurs.

Q 301 **NATURAL KILLER ACTIVITY AND PROTEIN KINASE C ACTIVATION IN AIDS PATIENTS**

Ignacio J. Ansotegui, Antonio Procopio, Anna R. Varani, Maurizio Carlesimo, Gabriella Scarlatti, Hans Wigzell, Fernando Aiuti, Magnus Gidlund and Maria Caterina Sirianni. Departments of Clinical Immunology and Experimental Medicine, University of Rome "La Sapienza", Rome, Italy and Department of Immunology, Karolinska Institute, Stockholm, Sweden.

The low affinity receptor for IgG (FcγRIII, CD16) when crosslinked results in a trigger signal for Natural Killer (NK) activity of peripheral blood lymphocytes (PBL). Tetradecanoyl phorbol acetate (TPA), a Protein Kinase C (PKC) activator, when used at nanomolar concentrations, induce cellular activation and release of CD16 from the NK cell surface.

Several groups have reported that patients with Acquired Immunodeficiency Syndrome (AIDS) have a normal number of CD16 positive cells, who maintain the binding to conventional targets but are not able to kill them.

The aim of our study was to determine if the NK killing deficiency of AIDS patients is mediated by a defect of PKC activation.

The measurement of CD16 release after stimulation with TPA was used as an indirect method to study PKC activity.

The release of CD16 was measured by FACS analysis. A specific inhibitor of PKC, staurosporine, was used as a control of CD16 release.

Our results indicate that release of CD16 is normal in both AIDS patients (CDC IV C1) and seronegative donors.

These results suggest that HIV-1 infection does not interfere with NK activation via PKC. Therefore the impaired NK killing does not involve a PKC pathway.

Q 302 BIOLOGIC AND MOLECULAR PROPERTIES OF AN INFECTIOUS MOLECULAR CLONE OF THE NONCYTOPATHIC HIV-2_{UC1}. Susan W. Barnett[1], Marguerita Quiroga[2], Albrecht Werner[1], Jay A. Levy[1], [1]University of California San Francisco, CA 94143-0128, [2]Chiron Corp., Emeryville, CA.

A full-length infectious proviral clone of the noncytopathic HIV-2_{UC1} strain grew well in peripheral blood mononuclear cells (PBMC), macrophages, and several T and B cell lines. As in the case of HIV-2_{UC1}, CD4+ T cell lines or PBMC infected with HIV-2_{UC1mc} showed little or no syncytia formation and no down-modulation of cell surface CD4 expression. In addition, both parental and cloned viruses exhibited reductions in *env* surface protein expression. DNA sequence analysis revealed substantial differences between HIV-2_{UC1mc} and other HIV-2 strains, especially in the *env* gene.

Comparison of UC1 *env* to other HIV-2's and SIV's

	%Identity	
Strain	Amino acid	Nucleotide
HIV-2 ST	71.1	75.0
HIV-2 GH	70.8	75.4
HIV-2 ROD	70.1	70.2
SIV mac142	69.9	75.3

Among these differences in *env*, the noncytopathic HIV-2_{UC1mc} appears to lack a cysteine residue in its signal peptide sequence. The presence of this cysteine is conserved in all other sequenced HIV-2 strains.

Q 303 MECHANISMS OF FELINE IMMUNODEFICIENCY VIRUS DISEASE POTENTIATION BY FELINE LEUKEMIA VIRUS

Amy M. Beebe, Tobie G. Gluckstern, E. Ellen Sparger, Michael Torten, Niels C. Pedersen, and Satya Dandekar, Departments of Internal Medicine and Veterinary Medicine, University of California, Davis, California.

Feline immunodeficiency virus (FIV) is a lentivirus that causes an AIDS-like syndrome in cats, providing an animal model for HIV infection. Like HIV infection in humans there is a transient initial phase of illness following exposure, and a long period of clinical normalcy between the initial and terminal AIDS-like phases of illness. What factors may contribute to differences in the rate of progression to the terminal stage of disease is unclear. The objective of the present study was to use the feline model to test the hypothesis that preexistent viral infections may potentiate HIV expression and accelerate disease progression.

Persistently viremic feline leukemia virus infected cats were experimentally infected with FIV. They became extremely ill and 5 of 10 died within 2-3 months. Using *in situ* hybridization on tissue sections, we detected very high levels of FIV RNA transcripts in mononuclear cells in peripheral blood, lymph node, bone marrow, brain, kidney, and gastrointestinal tissues of these cats. By contrast, we found only low levels of viral RNA in cats that remained asymptomatic. Cell tropism of FIV did not appear to be altered by coinfection with FeLV. To examine whether upregulation of FIV expression was due to FeLV induced changes in the cellular targets of FIV, we removed PBMC from FeLV carrier cats and infected them *in vitro* with FIV. No upregulation of antigen production was detected. Furthermore, FeLV did not transactivate an FIV LTR-CAT construct in a variety of cells tested.

These data provide no evidence for pseudotype formation or transactivation as mechanisms for the observed upregulation of FIV gene expression in FeLV infected cats. Since FeLV is known to be capable of immune suppression, its effects on subsequent FIV infection may be mediated through altering the host's ability to mount a sufficient immune response.

Q 304 EFFECTS OF HIV INFECTION IN HUMAN FETAL THYMUS IN THE SCID-hu MOUSE, Mark L. Bonyhadi, Hideto Kaneshima and Joseph M. McCune, SyStemix, Inc. Palo Alto, CA 94303

Individuals infected by the human immunodeficiency virus type 1 (HIV-1) exhibit progressive depletion and severely reduced function of peripheral CD4+ helper T lymphocytes. A variety of mechanisms have been proposed to account for the observed loss of CD4+ T cells, including virally mediated cytopathic killing of infected cells and deleterious infection of T cell precursors. The latter mechanism may be especially important in the setting of fetal and pediatric HIV infection, a time during thymic ontogeny when T lymphopoiesis is most active. We have previously shown that implants of human fetal thymus and fetal liver in the SCID-hu mouse form a conjoint organ which is functionally and structurally similar to normal human fetal thymus, that this "Thy/Liv" organ supports long-term human T lymphopoiesis *in vivo*, that the mature CD4+ and CD8+ T cells which emerge are phenotypically and functionally normal, and that the structure is permissive for HIV-1 infection. The intent of this study was to evaluate the effect of HIV-1 infection on T lymphopoiesis in the human fetal thymus, using the Thy/Liv implant as a surrogate *in vivo* model. The Thy/Liv implants were inoculated with primary patient isolates of HIV-1 or with infectious molecular clones of HIV-1. As a function of time after infection, replication of HIV-1 was assessed by p24 ELISA and by immuno-histochemistry. The effects of HIV-1 replication on T lymphopoiesis were observed by multiparameter flow cytometry. The results indicate that replication of primary HIV-1 isolates within the setting of human fetal thymus has profound effects on T cell development and are consistent with the hypothesis that regeneration of the peripheral T cell pool is precluded after HIV-1 infection in man. Data related to qualitative and quantitative features of these events as well as to potential mechanisms will be presented.

Q 305 EVOLUTION OF AZT CONFERRING MUTATIONS ASSESSED BY CLONAL ANALYSIS

Charles Boucher[1], Jolanda Tijnagel[1], Paul Kellam2, Brendan Larder[2]; [1] Department of Virology, Academic Medical Center and University of Amsterdam, the Netherlands.[2] Molecular Sciences Department, Wellcome Foundation Ltd, Langley Court, Beckenham, England.

During zidovudine (AZT) therapy of HIV 1 infected individuals AZT resistant isolates may appear. The development of AZT resistance is determined by amino acids changes at five amino acid residues (41, 67, 70, 215, 219) of the enzyme reverse transcriptase. Using the HeLa CD_4 plaque assay it is possible to observe a gradual decrease in AZT sensitivity of HIV isolates during treatment. Bulk analysis of viral populations for changes in the RT gene using differential PCR revealed an ordered appearance in a cohort of asymptomatic individuals. The first mutation appears at codon 70, after a period varying from 4-12 months, it disappears as the 215 and 41 mutations occur. Finally, the 70 mutation reappears although during the a-symptomatic disease stage no mutations at codon 67, 219 were detected. In two individuals with patterns as described above, multiple clones obtained from sequential isolates were analyzed. The RT gene of virus derived by PBMC cocultivation was amplified using PCR and the RT region containing the mutations was sequenced. This analysis of individual clones confirmed the temporal appearance of the 70 mutation. In addition, it showed that the 41 and 215 mutations can appear in separate viral populations, but eventually are present on the same viral genome. The presence of both a 41 and 215 mutation gives a clear selective advantage (20 fold increase in resistance) compared to the mutations alone. Additional isolates are currently being studied to investigate more closely the evolution of the mutations at codons 67 and 219.

Q 306 NUCLEAR TRANSPORT OF HIV-1 GENETIC MATERIAL: IMPLICATIONS FOR LATENCY AND PATHOGENESIS, Michael I. Bukrinsky, Natalia K. Sharova, and Mario Stevenson, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska 68198-5120

We have recently demonstrated that quiescent T cells are an inducible reservoir of latent HIV-1 in asymptomatic individuals (Bukrinsky et al., Science, 254:423-427, 1991). HIV-1 DNA is preserved in unintegrated state in these cells, but it is capable of integration upon cell activation. Here we report that block to integration in quiescent T cells is caused by inefficient HIV-1 DNA transport into the nucleus. Using HIV-1 1-LTR and 2-LTR circular DNAs (detected by PCR) as an indicator of successful nuclear transport, we have shown that the preintegration complex of HIV-1 is rapidly transported into the nucleus of the host cell by a process which requires ATP but which is independent of the cell cycle. A functional integrase protein is not necessary for the active nuclear transport of HIV-1 preintegration complexes. In the nucleus the HIV-1 DNA is found in two peaks after equilibrium density centrifugation: one with density 1.46 g/ml and another 1.36 g/ml. Reverse transcriptase activity was associated with the second peak. We suppose that the first peak represents mature preintegration complexes, while the second peak contains immature preintegration complexes with incomplete species of HIV-1 DNA.

These findings indicate that HIV-1 reverse transcription may proceed in the nucleus, as described for other lentiviruses. The preintegration complexes enter the nucleus by an active ATP-dependent mechanism. However, the nuclear transport is independent of cell cycle. These data are pertinent to our understanding of the mode of HIV-1 replication, as well as infection of terminally differentiated cells such as macrophages, dendritic and microglial cells.

Q 307 SIV EXPRESSION IN THE SPINAL CORD IS LOCALIZED TO THE MACROPHAGE AND ASSOCIATED WITH A SPECIFIC PATHOLOGIC FINDING. H. Burger[1], P. Campbell[2], A. Lackner[3], D. LaNeve[2], N. Peress[2], and B. Weiser[1]. Wadsworth Center for Research, Albany, NY[1], SUNY Stony Brook, NY[2], New Mexico Regional Primate Research Lab, Las Cruces, NM[3].

To evaluate the SIV-infected macaque as a model for AIDS-related neurologic disease, we studied spinal cords from SIV-infected animals. Previously, we studied a characteristic spinal cord finding in human AIDS patients called vacuolar myelopathy. We established that: 1) HIV-1 RNA is expressed in spinal cords with vacuolar myelopathy but not in control cords; 2) HIV-1 expression is localized to the macrophage; and 3) the level of HIV-1 RNA expression is directly correlated with the severity of clinical and pathological disease.

To extend our analysis to SIV and characterize the SIV-infected macaque as a model for HIV-1 neuropathogenesis, we continued to study the spinal cord. By using *in situ* hybridization, we analyzed spinal cords from 4 SIV-infected macaques with giant cell myelitis, an entity in SIV-infected macaques histologically resembling HIV-1 encephalitis in humans. In all 4 cords, we found high level SIV RNA expression. SIV RNA was localized primarily to the giant cell lesions, and to a lesser extent, to infiltrating inflammatory cells. Double-label analysis using combined *in situ* hybridization-immunohistochemistry, as well as immunohistochemistry alone identified both the multinucleated giant cells and mononuclear inflammatory cells to be monocyte/macrophage derived. As controls, we studied 9 infected animals with either normal spinal cords or myelitis due to documented opportunistic infection. The control cords showed minimal or undetectable levels of SIV expression, including 3 from animals with SAIDS who had CMV infection and macrophage infiltration of the spinal cord. These results parallel those in HIV-1 infection, where HIV-1 expression was detected only in cords with vacuolar myelopathy. They extend the previous studies demonstrating a role for immunodeficiency viruses in tissue pathogenesis and document in detail that the SIV-infected macaque is an excellent model to study the mechanisms of HIV-1 related neuropathogenesis *in vivo*.

Q 308 THE RELATIONSHIP BETWEEN HIV-1 VIRAL TITER AND VIRAL DNA COPY NUMBER IN PBMC OF CHILDREN WITH TRANSFUSION-ACQUIRED INFECTION. Chelyapov, N.V., Courville, T., Wittek, A.E., Brunell, P.A., Israele, V. Ahmanson Pediatric Center, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA 90048.

We have previously shown that progression of HIV infection in children was associated with an increase in the HIV-1 viral burden, as demonstrated by endpoint dilution culture techniques (Pediatrics 1991;87:921). For the same cohort of patients, we determined the HIV-1 viral DNA copy number in PBMC by quantitative PCR using end-labeled primers in the LTR-gag region of the viral genome. The mean increase in HIV-1 DNA copy number from PBMC of P1 (asymptomatic) to P2 (symptomatic) patients was smaller than the mean increase in viral titer. Patients with HIV-1 titers of 5 TCID/10^6 PBMC had a mean DNA copy number of 401/10^6 PBMC. In those with an HIV titer of 500 TCID/10^6 PBMC, the mean DNA copy number was found to be 9314/10^6 PBMC. Thus, a 100-fold increase in infectious virus titers was associated with only a 23-fold increase in the DNA copy number. The DNA to TCID ratio decreased from 80 in patients having a virus titer of 5 TCID/10^6 PBMC to 18 in patients with a virus titer of 500 TCID/10^6 PBMC. All observed differences were statistically significant. Fractionation of DNA from infected PBMC into high and low molecular weight fractions showed a predominance of extrachromosomal viral DNA for P1 patients and integrated DNA for P2 patients. These preliminary results provide some insight into the apparently greater efficiency of proviral DNA as HIV-1 disease progresses.

Q 309 V3 SEQUENCE ANALYSIS OF HIV-1 ISOLATES FROM BLOOD AND CSF INDICATES MARKERS FOR DISEASE PROGRESSION BUT DOES NOT IDENTIFY TISSUE-SPECIFIC DETERMINANTS. Francesca Chiodi, Barbara Keys, Bengt Fadell, Jenny Karis. Department of Virology, Karolinska Institute, Stockholm, Sweden.

The possibility exists that HIV-1 isolates infecting the brain undergo a process of adaptation in the tissue which select neurotropic variants of the virus. The HIV-1 V3 loop has been shown to be an important determinant for cell tropism. Accordingly, we have molecularly characterized isolates obtained in parallel from blood and cerebrospinal fluid (CSF) of 4 asymptomatic carriers, 2 patients with lymphoadenopathy and 4 AIDS patients. The first passage in PBMC was used for amplification by PCR with nested oligonucleotide primers which hybridize to conserved sequences flanking the V3 domain. PCR products (798 bp) were cloned into PGEM4z vector and an avarage of 4 clones from each isolate were sequenced. The resulting amino acid (aa) sequence from each clone consisted of 34 aa from the N-terminal flank, 35 aa from the V3 loop and 32 aa from the C-terminal flank.
The aa sequences of the clones from each virus were used to generate a consensus aa sequence. Blood and CSF isolates were compared to one another and to a consensus of U.S./European sequences. Based on this approach, we could not find clear evidence for tissue-specific signature sequences. Two aa residues (Asn 289 and His 308) however, appear to correlate with progression from early to advanced stage of HIV-1 infection. Experiments designed to establish the replicative capacity of the CSF and blood isolates in primary monocytes, T- and monocytoid cell lines are in progress.

Q 310 DIFFERENT FUNCTIONAL PATTERNS OF TH1-TH2 IN ASYMPTOMATIC, HIV-SEROPOSITIVE INDIVIDUALS. Mario Clerici, and Gene M. Shearer. Experimental Immunology Branch. National Cancer Institute, National Institute of Health,, Bethesda, MD 20892.

We measured the production of interleukin-2 (IL-2) and interleukin-4 (IL-4), cytokines generated by TH1 and TH2 T helper (TH) cells respectively, in peripheral blood leukocytes (PBL) of asymptomatic human immunodeficiency virus seropositive (HIV+) individuals, most of whom were without symptoms, to determine whether different profiles of dysfunction could be recognized in such patients. Antigen stimulated culture supernatants were tested using bioassays with either the IL-2 dependent CTLL or the IL-4 dependent cell line CT.h4S (kind gift of Dr. W. E. Paul and Ms. C. Kinzer, Laboratory of Immunology, NIAID, NIH). 45 HIV+ patients were studied in this way. Three different functional patterns were recognized : (1) high IL-2 and low IL-4 (n=16); (2) low IL-2 and high IL-4 (n=19) and (3) low IL-2 and low IL-4 (n=10). All the HIV- control (n=18) showed a type (1) profile.The different patterns were not dependent on the number of CD4+ TH or on the Walter Reed staging of the patients. The reciprocal patterns of lymphokine production could be shown as IL-2:IL-4 ratios, with the highest ratio in group (1) (2.12) and progressively decreasing ratios for groups (2) (0.08) and (3) (0.3). PBL from HIV- controls had an IL-2:IL-4 ratio of 5.54. IL-2 and IL-4 production from cryopreserved PBL of a donor followed longitudinally for more than three years demonstrated that the three profiles are sequential, such that (1) -->(2) -->(3). It is noteworthy that these TH1 and TH2 profiles were detected using primary cultures of PBL and did not require T cell cloning. Based on these data, we suggest a progressive change in TH function after HIV infection, such that a first stage in which TH1>TH2, is followed by a second stage in which TH2>TH1 and, finally by a reduction in both TH1 and TH2 activity. The mechanism responsible for this phenomenon is not known, but the role of interleukin-10, a TH2 product that down-regulates TH1 activity, is being investigated.

Q 311 SEQUENCE AND PROTEIN ANALYSIS OF A RAPIDLY-REPLICATING, NON-SYNCYTIUM-INDUCING HIV-1 ISOLATE, K.M. Coates Fryer, N.J. Deacon, and D.A. McPhee, Macfarlane Burnet Centre for Medical Research, Melbourne, Vic., Australia.

Variation of cytopathicity and tropism among clinical HIV-1 isolates often correlates with disease progression and replicative ability. Cytopathic variants, routinely isolated later in disease, are faster replicating and are capable of replication in T cell lines. We have characterized an Australian HIV-1 isolate (243925) from the csf of a patient with advanced AIDS which, despite rapid replication, does not induce syncytia and does not replicate in continuous cell lines (MT-2, CEM, U937, U138, and Jurkat-*tat*). This enabled the study of virus cytopathicity independently of replicative ability. This isolate was compared with a local rapidly replicating, syncytium-inducing HIV-1 isolate (228200). The isolates were biologically cloned, selected regions sequenced, and expression of related proteins in infected cells quantitated. The V3 domain of gp120 for 243925 corresponded to the macrophage tropic sequence reported by Hwang et. al. (1991), and that of 228200 to the T-cell tropic sequence. Neither virus infected macrophages, despite replication to titres of 10^5 and 10^4 in PBMCs, respectively. The gp120/gp41 cleavage site and gp41 fusion (C5) domains were highly conserved for both isolates, but there were a number of uncommon amino acids within *env* distinct from V3 and C5. Immunofluorescence and immunoblot analyses of infected PBMCs revealed no obvious production of *nef* by 243925 compared with 228200, despite production of other viral proteins. Sequencing *nef* directly from PCR products revealed that critical functional domains (Guy et al. 1990) were conserved for both isolates. Thus, apart from small sequence differences in *env* and observed differences in the production of *nef* there was no clear explanation for the differences in cytopathicity.

Guy et al., Virology 176:413, 1990 Hwang et al., Science 253:71, 1991

Q 312 QUANTITATION OF HIV-1 IN SEQUENTIAL BLOOD SAMPLES FROM PATIENTS WITH PRECIPITOUS DECLINE IN CD4 CELL COUNTS. Ruth I. Connor[1], Hiroshi Mohri[1], Yunzhen Cao[1], Guiling Gu[1], Emily Tsai[1], Pablo Rubinstein[2], Cladd Stevens[2] and David D. Ho[1,]1 The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY 10016, 2New York Blood Center, New York, NY 10023.

Levels of HIV-1 were quantified in sequential blood samples of selected individuals from a large cohort of homosexual men enrolled in a longitudinal study of HIV-1 infection. Six particularly interesting cases were chosen on the basis of divergent clinical courses. Four seroconverted during the study, followed by a 2-5 year period of clinical and immunological stability and a subsequent precipitous decline in CD4 cell counts. Two cases were characterized by a remarkable period of clinical and immunological stability, despite seroconversion in 1978-1979. Sequential aliquots of PBMC from each subject were retrieved from freezers and a portion of these cells used in quantitative cultures to determine the infectious titer of HIV-1. DNA extracted from the remaining cells was used in quantitative PCR to determine viral DNA copy numbers. Results indicate that temporal changes in the levels of HIV-1 correlate closely with fluctuations in CD4 cell counts during the course of infection. Sequential determinations of viral burden assessed by quantitative cultures agreed closely with results obtained using PCR methods. An exponential increase in the level of virus was directly associated with a sharp decline in CD4 cell counts in patients exhibiting a rapid, deteriorating clinical course. Conversely, persistent, low levels of virus were maintained for extended periods in the clinically and immunologically stable patients. These findings suggest that a burst of HIV-1 replication is responsible for the precipitous decline in CD4 lymphocytes. Studies are now underway to determine if the increased viral load is due to the emergence of a "virulent" strain of HIV-1 or the loss of specific immune responses.

Q 313 NEOPTERIN PRODUCTION IN HIV-INFECTED HUMAN MONOCYTES AND MACROPHAGES, Carol S.Dukes, J. Brice Weinberg, Duke and VA Med Ctrs, Durham, NC 27710.

Mononuclear phagocytes are known to produce neopterin *in vitro* after treatment with gamma interferon (IFN). Although serum neopterin levels are elevated in progressive HIV infection, it is not known if this is a direct effect of HIV on infected mononuclear phagocytes, or an indirect effect mediated *in vivo* by IFN production. Our objective was to compare neopterin production by HIV-infected and uninfected monocytes (Mo) and macrophages (Mac). Blood Mo and peritoneal Mac from healthy donors were infected *in vitro* with HIV-1_{BaL}. Negative controls included media alone, heat-inactivated HIV-1_{BaL}, and HIV-1_{IIIB}, a lymphocytotropic strain. Cells were cultured 10 days after inoculation, then incubated with 500 u/ml IFN for 72 hrs. Supernatants were then assayed for neopterin (by RIA) and reverse transcriptase. As expected, IFN treatment of Mo enhanced neopterin production (nmol/L/mg protein; mean of 3 experiments). In the absence of added IFN, HIV-1 inoculation did not significantly alter neopterin production. Likewise, there was no statistical difference between HIV-inoculated and control cells after IFN treatment; all had comparable enhancement of neopterin production. Mac also had increased neopterin production with IFN stimulation, though less than Mo, with no statistical difference between infected and uninfected cells.

	No IFN	+ IFN
No virus	10	432
HIV BaL	81	514
HIV IIIB	5	513
Heated HIV-BaL	20	635

In conclusion, IFN is a potent stimulator of neopterin production in human Mo and Mac. IFN stimulated neopterin production by Mo and Mac equally well, whether or not they were HIV-infected. There was a trend toward increased neopterin production in HIV-BaL-infected cells, especially in cells not treated with IFN, but this was not statistically significant. Our results suggest that elevated serum neopterin levels in HIV-infected patients are the result of *in vivo* IFN stimulation of Mo and Mac to produce neopterin rather than a direct result of HIV infection of mononuclear phagocytes.

Q 314 Cytokine mRNA Alterations in HIV infection.

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We have earlier shown that infection with HIV is followed by a strong immune activation and that the magnitude of this immune activation predicts subsequent development of AIDS. Increased serum immune activation molecules, e.g. neopterin, B2M, sIL-2R and cCD8, which are selectively produced in response to certain cytokines, indicate increased production of ctytokines. Many Cytokines are difficult to measure in serum because of rapid removal. We have, therefore, measured mRNA levels of various cytokines using quantitative PCR amplification in order to determine the patterns of cytokine production in subjects with established HIV infection. Messenger RNA for the three monokines, IL-1-alpha, IL-6 and TNF-alpha were found to be increased compared to mRNA levels in HIV seronegative subjects from the same cohort. This is consistent with an earlier report of increased IL-6 production in HIV infection (Martinez-Maza and colleagues) and reflect activation of monocytes. Activation of T lymphocytes was evident in increased mRNA for INF-gamma. Also serum INF-gamma was found to be increased. The findings support and substantiate that both monocytes and lymphocytes are activated in HIV infection.

Q 315 HTLV INFECTION OF MOUSE/HUMAN CHIMERAS

Gerold Feuer*, Jerome A. Zack*, W. Harrington†, Joseph D. Rosenblatt*, Willian Wachsman¥, and Irvin S.Y. Chen†§, *Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA; ¥UCSD School of Medicine, La Jolla CA; †Center for Blood Diseases, University of Miami, FL; §Department of Microbiology & Immunology, UCLA School of Medicine, Los Angeles CA

Severe combined immunodeficient (SCID) mice reconstituted with human peripheral blood leukocytes (PBL) serve as a useful small animal model system to study infection of human lymphoid cells with human retroviruses. Reconstitution of SCID mice with PBL from adult T-cell leukemia (ATL), HTLV-I-associated myelopathy (HAM), and HTLV-seropositive patients established HTLV-I infection in chimeric mice. Virus was detected by quantitative polymerase chain reaction (PCR) in cells recovered from the peritoneal cavity up to 20 weeks post-reconstitution. In some chimeric mice reconstituted with ATL or HAM PBL, the ratio of virus copies per human cell number increased from 0.1%, in the input PBL, to 100% in cells recovered from the peritoneal cavity. This suggests preferential proliferation of HTLV-infected lymphocytes in these mice. In one ATL case, five of five mice reconstituted with PBL developed a lymphoid tumor of human origin. These tumor cells were HTLV-I-infected, stained positively for the human CD4 marker, and could be engrafted into naive SCID mice. Engrafted tumor cells proliferated and eventually killed the host. These results are in stark contrast to HTLV-transformed cell lines, which fail to proliferate in the SCID mouse.

Finally, HTLV infection of SCID mice engrafted with human fetal thymus, liver and lymph node (SCID-hu) is also ongoing. The SCID-hu mouse potentially provides a small animal model system to examine HTLV infection, transformation and pathogenesis.

Q 316 CROSSLINKING CD4 BY HIV GP120 PRIMES T CELLS FOR ACTIVATION-INDUCED APOPTOSIS.

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During human immunodeficiency virus (HIV) infection there is a profound and selective decrease in the $CD4^+$ population of T lymphocytes. The mechanism of this depletion is not understood, as only a very small fraction of all $CD4^+$ cells appear to be productively infected with HIV-1 in seropositive individuals. Thus, it is unlikely that direct viral destruction accounts for the majority of $CD4^+$ T cell loss. Here we report that crosslinking of CD4 on human $CD4^+$ T cells followed by signalling through the T cell receptor for antigen results in activation dependent cell death by apoptosis. The induction of apoptosis was demonstrated by visualization of nucleosome-sized DNA multimers of 200 bp to form the characteristic "step ladder" appearance after size separation on agarose gels. Apoptosis was confirmed histologically by the characteristic changes of nuclear and cytoplasmic condensation, and was quantitated by assessing the proportion of fragmented DNA:chromatin DNA. Culture of T cells bearing the CD4 antigen with anti-CD4 antibodies under crosslinking conditions, in the absence of subsequent stimulation, or crosslinking of anti-TCR antibody, induced no fragmentation over background levels. In contrast, when cells were pretreated with anti-CD4 antibody and then incubated with anti-TCR antibody, DNA fragmentation increased significantly over background. In addition, induced DNA fragmentation was seen with cellular activation through TCR, after pretreatment with recombinant gp120 and polyclonal anti-gp120 (30.8±1.18% vs. 6.66±3.59% control, $p<.01$). Thus, some human peripheral $CD4^+$ T cells pretreated with gp120 die by apoptosis when activated through the TCR. Preliminary data suggest CD4 ligation also primes human thymocytes for activation-induced apoptosis. These results suggest a mechanism for the massive $CD4^+$ T cell depletion in acquired immune deficiency syndrome (AIDS), particularly in the face of concurrent infection and antigenic challenge with other organisms. In addition, these results suggest a mechanism for the abrogation of disease seen with injection of anti-CD4 antibodies in many animal models of human autoimmune disease. Finally, it is intriguing to speculate whether induced apoptosis of the $CD4^+CD8^+$ immature subset in thymus could account for depletion of both $CD4^+$ and $CD8^+$ cells seen in some patients with AIDS, with resultant autoimmunity due to loss of an immunomodulatory $CD8^+$ subset.

Q 317 TRANSIENT PEAKS IN PLASMA VIREMIA IN THE PROGRESSION TO AIDS,

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Objective: To investigate prospectively the use of plasma viremia as a prognostic indicator for the development of AIDS.

Methods: A total of 25 HIV infected, symptomatic patients enrolled in a variety of drug therapy trials were studied. Detailed questions concerning recent health and symptoms were asked at each visit. In addition, CD4 cell counts and quantitative HIV cell and plasma cultures were performed. Progression was defined as either the occurrence of an AIDS defining illness or death. Cox' bivariate and multivariate analyses were applied to the data.

Results: Seven of the 25 patients progressed and 3 were lost to follow-up. Eight patients had peak titers of plasma viremia of $\geq$1000 TCID/ml of plasma at some point in the study. Of these 6 occurred in patients who progressed. Progression of disease and high plasma viral titers were often associated with large decreases in CD4 cells of 25% or more within a 4-8 week period and with clinical complaints of fever and/or rash. Quantitative HIV cell cultures were much less predictive.

Conclusions: Our earlier results indicate that plasma viremia in HIV infected patients is caused by infectious immune complexes (IC). These IC may contribute to viral load by expanding the host cell range by use of alternate receptors. The IC may also explain some of the clinical symptoms observed in our patients. High plasma viral titers appear to be a better prognostic indicator than quantitative HIV cell culture, at least in symptomatic patients.

Q 318 ATTEMPTS TO DETECT NON-CULTIVABLE ORGANISMS IN THE BLOOD OF PATIENTS WITH ADVANCED HIV INFECTION USING PCR, Richard Frothingham, Rhonda B. Blitchington, Paul Chai, David H. Lee, and Kenneth H. Wilson, Infectious Diseases Section, Durham VA Medical Center, 508 Fulton St., Durham, NC 27705

Many patients with advanced HIV infection develop fever and weight loss. In some patients no infectious cause can be identified except HIV. We are studying such patients in an attempt to identify novel non-cultivable organisms in their blood. Systematic studies of microbial RNA or DNA from various ecosystems have shown that the majority of organisms were non-cultivable. We are using PCR primers directed at conserved regions of ribosomal DNA (rDNA). The primers amplify rDNA from bacteria, mycoplasmas, and rickettsias, but not from mammalian cells. We previously used this approach to identify the Whipple's associated bacillus, and a similar method has identified the agent of bacillary angiomatosis. PCR on blood samples has proved to be difficult as blood contains inhibitors of PCR. An extensive protocol was developed to purify microbial DNA from blood while removing inhibitors. Putative microbial DNA from a volume of 1.0 ml of blood could be amplified in a single PCR reaction, compared with a typical volume of 0.05 ml in previously reported protocols. Putative microbial DNA from blood from 4 patients with advanced HIV infection and 1 control was amplified. A PCR product amplified from 1 patient was sequenced and represented bacterial rDNA. The sequence was distantly related to that of known genera; the closest identified relative was *Hyphomicrobium vulgare*, with 84% of the bases identical and no gaps. The other 3 patients and control showed no PCR product. Positive PCR controls using *Escherichia coli* DNA showed that the inhibitors were successfully removed from 3 of the 4 patient blood samples and from the control. The positive result is very preliminary and may represent contamination. However, the ability to amplify microbial DNA from a relatively large volume of blood is an advance. PCR may be a feasible method for the detection of non-cultivable organisms in patients with advanced HIV infection.

Q 319 INHIBITION OF T CELL ANTIGEN RECEPTOR DEPENDENT PHOSPHORYLATION OF CD4 IN HIV-1 INFECTED CELLS. Glen N. Gaulton*, Lawrence F. Brass*, Danuta Kozbor+, Charles H. Pletcher*, and James A. Hoxie*, University of Pennsylvania, School of Medicine. Philadelphia, PA 19104, and +Thomas Jefferson University, Philadelphia, Pa 19107.

Inhibitory effects of HIV on T lymphocyte function have been linked to perturbation of signaling through the T cell antigen receptor/CD3 complex. Comparative biochemical analyses of signaling responses were performed in T cells that were either uninfected or chronically infected with the HIV-1/IIIB strain. Stimulation with antibodies to CD3 triggered both Ca^{2+} accumulation and phosphoinositide hydrolysis responses that were equivalent in uninfected and infected cells. Treatment with anti-CD3 or with phorbol diester also stimulated serine phosphorylation of CD4 molecules in uninfected T cells. However, phosphorylation of CD4 was not observed after anti-CD3 treatment in HIV-infected T cells despite normal phosphorylation responses to phorbol diester. Identical results were obtained using a T cell line that was infected with an *env* (gp160/120$^-$) HIV-1 defective variant. These studies indicate that infection with HIV-1 inhibits the activation of protein kinase associated with the T cell receptor/CD3 complex by a mechanism which is independent of viral *env* protein components.

Q 320 GP120 MEDIATED DISRUPTION OF T CELL ANTIGEN RECEPTOR SIGNALING. Frederick D. Goldman, Lynn Heasley, Gary Johnson, Nancy Haigwood and John C. Cambier, Division of Basic Sciences, Department of Pediatrics, National Jewish Center for Immunology, Denver, CO 80206

Qualitative T cell defects which occur in HIV infections have been proposed to result from CD4 receptor ligation by HIV envelope glycoprotein gp120 and secondary crosslinking host anti-gp120 antibodies. The tyrosine kinase (TK) p56lck is known to associate with CD4 at the cytoplasmic membrane surface and become phosphorylated (and presumably activated) upon receptor ligation. Recent evidence suggests that p56lck, as well as other TKs such as p59fyn, play important roles in signal transduction and T cell activation. However, the substrate specificity and mechanisms of regulation of these enzymes remains unclear. Studies described here were carried out to characterize p56lck tyrosine kinase activation following CD4 ligation and determine the role of this kinase in modulation of T cell antigen receptor (TCR) function. To assess this modulation, calcium flux in indo-1 loaded T cells was measured following TCR ligation. Prior ligation of CD4 (gp120 + anti-gp120) rendered cells hyporesponsive to subsequent stimulation by TCR ligation ($[120\ nM]_i$ vs $[600\ nM]_i$ in untreated control cells). Protein phosphorylation and immunoprecipitation experiments demonstrated that ligation of CD4 with monoclonal antibodies (mAb) or gp120/anti-gp120 resulted in an early transient phosphorylation of both CD4-associated and nonassociated p56lck. An in vitro kinase assay was developed employing putative physiologic peptide substrates derived from the zeta subunit of CD3, allowing quantitation of tyrosine kinase activity. Enzyme kinetic constants calculated for these peptides indicated that these enzymes (p56lck and p59fyn) exhibit distinct substrate specificity. Furthermore, specific kinase activity was preserved and could be measured in immunoprecipitated complexes of p56lck or p59fyn. Consistent with the kinetics of p56lck phosphorylation, induction of kinase activity following CD4 ligation with gp120 or mAb was demonstrated in immune complex kinase assays within 1 to 3 minutes following receptor ligation. Thus, the activation of p56lck following gp120 binding to CD4 and the ability of activated p56lck to phosphorylate specific CD3-zeta chain peptides in vitro suggest a possible mechanism whereby CD4 ligation by gp120 could attenuate cellular responses normally activated through the TCR.

Q 321 QUANTITATION OF HIV-1 SPECIFIC CYTOTOXIC T-LYMPHOCYTES AND CORRELATION WITH VIRAL LOAD, Thomas Greenough, Bruce Blaise, Kevin Byron, *Doreen Brettler, John L. Sullivan, Dept. of Pediatrics, Program in Molecular Medicine, University of Massachusetts Medical Center, *The Medical Center of Central Massachusetts-Memorial Hospital, Worcester, MA 01605. Applying limiting dilution analysis to quantitate activated cytotoxic T-lymphocytes (CTL) directed against GAG and ENV HIV-1 determinants, we have demonstrated frequencies between 1:7000 and 1:86000 in peripheral blood mononuclear cells (PBMC) from a population of HIV infected hemophilia patients. A series of 7 dilutions ranging between 1563-100000 PBMC/well as effectors were tested in replicates of 24 wells for lysis of chromium labelled target autologous B-lymphoblastoid cell lines (BLCL) infected with Vaccinia vectors expressing GAG- and ENV-specific proteins. CTL frequency was derived from the fraction of wells which failed to exhibit chromium release greater than 3 standard deviations above the mean of spontaneous release. HIV specific CTL frequency was correlated with measures of viral load. Quantitative blood, plasma and PBMC cultures were performed on the same samples from which CTL frequencies were calculated. Both plasma and PBMC titers ranged between 0 and 500 TCID/ml or TCID/10^6 cells respectively. Low plasma and PBMC viral titers appear to be associated with higher GAG-specific CTL frequencies. In conclusion: 1) HIV-specific CTL in HIV-1 infected hemophilic patients occur at a frequency of 1:7000 to 1:86000 PBMC and 2) High CTL frequency appears to correlate with low viral titers.

Q 322 HIV INDUCES AN IMMUNE ACTIVATION OF T LYMPHOCYTES THAT LEADS TO IMMUNE IMPAIRMENT. Bo Hofmann, Parunag Nishanian, Thang Nguyen, Praphaphone Insixengmay, John L. Fahey. CIRID, UCLA School of Medicine, Los Angeles, CA 90024-1747

We have earlier demonstrated that immune activation as well as cellular immune impairment were present in HIV infection. In this study, we show the joining mechanism behind these two findings; HIV proteins induce a partial immune activation that renders T lymphocytes refractory to subsequent activation.

We have demonstrated several steps in this mechanism. First, it was established that HIV proteins induced a signal into T lymphocytes, which led to activation of several Protein Tyrosine Kinases (PTK) in both CD4 and CD8 T cells. Next, by use of specific inhibitor (Tyrphostin 25), we showed that PTK activity was responsible for subsequent induction of Protein Kinase A (PKA). Induction of PKA was also accompanied by an increase in intracellular cAMP, which is necessary for activation of PKA. cAMP/PKA activity is known to inhibit processes leading to cell proliferation. Finally, the HIV-induced increased cAMP/PKA activity resulted in changes that impaired proliferation when cells were subsequently stimulated with PHA. These changes included the down regulation of membrane Protein Kinase C (PKC) activity, which is closely associated with cell proliferation. Evidence that HIV-induced cAMP/PKA activity was responsible for the down-regulation of PKC included the demonstration that a specific inhibitor of PKA (H89) resulted in increased PKC activity and that reagents which augmented intracellular cAMP (Bromo-cAMP, Cholera toxoid) resulted in decreased PKC activity. These studies show that HIV-induced decreased proliferative responses is linked to HIV-induced PTK activity which resulted in PTK-dependent augment tion of cAMP/PKA activity and the consequent down-regulation of the proliferation promoter-PKC.

Q 323 HIV-INDUCED PRODUCTION OF IL-6 BY THP-1 HUMAN MONOCYTE CELLS: ENHANCEMENT BY FACTORS PRODUCED BY HUMAN BRAIN CELLS. Milana Ivashchenko, Pari Nishanian, Jean Merrill, and Otoniel Martínez-Maza. Departments of Microbiology & Immunology, Neurology, and Obstetrics & Gynecology, UCLA School of Medicine, Los Angeles, CA 90024

In previous work, we have seen that the interaction of live or inactivated HIV-1 (endotoxin-free) with human monocytes can lead to cytokine production. Recently, we have seen that THP-1 cells (a human monocyte cell line) respond to inactivated HIV-1"HTLV-IIIb" (iHIV) with increased IL-6 production, when co-cultured with 1000 units/ml recombinant interferon-gamma (IFN-γ). Also, THP-1 cells, cultured in the absence of IFN-γ, were seen to respond to immobilized iHIV (β-propiolactone-treated HTLV-IIIb), recombinant HIV gp160, or anti-CD3 monoclonal antibody (mAb) with high levels (>10 units/ml) of IL-6 production, when cultured for 72 hours in medium supplemented with supernatants from the human U-251 astrocytoma cell line, or from primary human brain cell cultures (containing astrocytes and microglial cells). THP-1 monocyte cells cultured as described above did not produce elevated levels of IL-6 in response to recombinant HIV p24, anti-CD4 mAb, or an isotype control (IgG2a) mAb. IL-6 production was measured by ELISA. The concentration of endotoxin in all reagents was assessed by the *Limulus* assay: all reagents contained < 6 pg/ml endotoxin. U-251 cells or human brain cell primary cultures were seen to secrete detectable levels of IL-6 spontaneously at high cellular concentrations (1×10^6 cells/ml). However, the induction of IL-6 production by THP-1 cells, induced by iHIV or HIV gp160 plus factors present in the human brain cell cultures, did not appear to depend on the production of IL-6 in the brain cell cultures. These results suggest that some interaction between iHIV and a human monocyte cell line (THP-1) results in IL-6 production, and that brain cells produce factor(s) that can enhance the ability of THP-1 cells to respond to iHIV or HIV gp160 with cytokine production. The nature of these brain-cell produced factors is currently under investigation.

Q 324 "SILENT" HIV INFECTION AMONG FAMILY MEMBERS OF HIV SEROPOSITIVE ETHIOPIANS? Tamar Jehuda-Cohen, Ruth Miltchan, Ami Vonsover*, Zvi Bentwich, Ruth Ben Ari Institute of Clinical Immunology, Kaplan Hospital, Rehovot, *Central Virology Laboratories, Sheba Medical Center, Tel-Hashomer, Israel

The phenomena of HIV-1 infected individuals that remain seronegative is receiving increased attention and may probably be higher in Africa. Twenty thousand Ethiopian Jews have recently immigrated to Israel and have a 1.7% prevalence of HIV seropositivity. We have tried to identify exposed yet seronegative individuals in this population by testing the immediate family of the seropositive carriers. PBMC were cultured in the presence of pokeweed mitogen (PWM), and the supernatant fluids (SF) were tested for HIV specific antibodies, using ELISA and Western blot. Out of 44 people tested to date, 12 were known seropositive HIV-1 carriers. Of the 32 seronegative family members 6 were positive by the PWM test, and the SF gave all the specific bands on the Western blot. Four of them were children (>5 years old) to two seropositive mothers; the other two were a wife and a mother of seropositive males. PCR was positive on all of the positive samples using many different primers based on the new sequences of HIV reported in the Ethiopian population. These results suggest that a) There is a significant proportion of seronegative individuals that was previously exposed to HIV and may be silent carriers of the virus in this population. b) "Silent" infection may also represent a "better" way of dealing with HIV infection.

Q 325 HIV-1 PRESENTATION IN LYMPHOID FOLLICLES IN MAN AND IN CYNOMOLGUS MONKEYS. Piet Joling, Henk K Parmentier, Leendert Bakker, Lia Smit, Peter Biberfeld, Dik van Wichen, Henk-Jan Schuurman, Dept. Pathology and Microbiology, University Hospital, 3508 GA Utrecht, The Netherlands; Academic Medical Center, Amsterdam; Karolinska Institute/Hospital, Stockholm, Sweden

During persistent generalized lymphadenopathy (PGL) and following stages of HIV-1 infection, follicular dendritic cells (FDC's), the framework of lymphoid follicles, degenerate. We studied FDC in HIV-1 infection in man and SIV infection in cynomolgus monkey. In histology on human material, HIV-1 virions, antigens (presumably immune complexes) and mRNA is present in and around FDC. Thus FDC not only bind HIV-1 components, but can also be infected and actively produce virus. HIV-1 binding to FDC purified from normal tonsils was studied. Using FITC-labeled virus, binding was seen in the absence of anti-viral antibody. It was more extensive in the presence of fresh not-inactivated serum, indicative that HIV-1 binding to FDC not only occurs as immune complexes via Fc receptors (FcR), but also in a direct way, and with involvement of complement components. This was confirmed in experiments on blocking studies with anti-FcR and anti-C3R antibodies. Lymph nodes and spleens of SIV-infected cynomolgus monkeys, with and without azidothymidine (AZT) therapy, were studied. Lymphoid follicle abnormalities showed striking similarities those reported previously in human HIV-1 infection. In hyperplastic follicles of untreated animals, an abundance of virions was seen (electron microscopy) as well as SIV p28 antigen (immunohistochemistry). In animals on AZT therapy neither virus nor p28 was found. Thus, AZT treatment may result in the disappearance of HIV-1 from a site where they normally concentrate, e.g. lymphoid follicles. This is in line with the concept that virus presentation in follicles has a main pathogenetic effect on the clinical course of infection.

Q 326 INTERACTION OF THE HIV-1 TRANS-ACTIVATOR *TAT* WITH PRIMARY BRAIN CELLS, Dennis L. Kolson, Jeffrey Buchhalter, Christine Debouck, Francisco Gonzalez-Scarano, University of Pennsylvania Medical Center, Philadelphia, PA and SmithKline Beecham Pharmaceuticals, King of Prussia, PA

The HIV-1 transactivator protein, *tat*, is one potential mediator of central nervous system dysfunction in HIV-1 infection. It is released by lymphocytic cell lines in culture during the course of infection, and it has been shown to be toxic to rat neuroblastoma cells and invertebrate neurons *in vitro*. We studied the effects of *tat* in embryonic primary rat brain cell cultures, an important model system for *in vitro* studies of neuronal-glial interactions. Using recombinant *tat (IIIb)* we noted aggregation of neurons and astrocytes in these cultures. These aggregates formed over 48-72 hours of exposure, were seen with concentrations as low as 250-500 nM, and were maximal at a concentration of 1 μM. Other cell types present in the culture, including macrophages and microglia, appeared to be spared. Identical effects were seen in fresh (2-3 day) cultures prepared from embryonic neocortex as well as hippocampus, though no effects were seen in older (weeks) cultures. To demonstrate the specificity of this effect, we preincubated the *tat* with anti-*tat* antibodies, and completely inhibited the aggregation. Preliminary studies indicate that this effect maps to specific region(s) of the *tat* protein.

Q 327 **HIV BIOLOGICAL PHENOTYPE AS A PROGNOSTIC MARKER FOR AIDS**

Maarten Koot, Rene P.M. Keet*, Aster H.V. Vos, Ruud E.Y. De Goede, Marijke T.L. Roos, Peter T.A. Schellekens, Roel A. Coutinho*, Frank Miedema and Mathijs Tersmette. CLB, *GG & GD Amsterdam.

Objective: Previously we reported an strong association between syncytium inducing (SI) capacity of HIV isolates and progression to AIDS. To determine the prognostic value of HIV biological phenotyping we phenotyped sequential isolates of 178 symptom free seropositive individuals. **Results:** Longitudinal data from the period 1988-1990 were obtained for 178 persons. In the two year follow up 14 (66%) of the 21 persons displaying SI variants in 1988 progressed to AIDS, compared to 22 (14%) of the participants with NSI variants in 1988. Within two year a switch from a NSI to a SI isolate occurred in 23/157 individuals. Of the 45 persons with known moment of Phenotype conversion 23 (50%) progressed to AIDS Within 22 months after the emerge of SI HIV-1 variants. These 45 persons were compared with respect to CD4 decline, with a control group of participants from who never SI isolates were obtained, matched for age, length of seropositive period and serum HIV antigen status. Both the number of CD4 positive cells and the rate of CD4 decline until the moment of phenotype conversion did not differ from matched controls with only NSI viruses. However after the emerge of SI variants a significant increased CD4 decline was observed, resulting in CD4 counts below 0.2 within two years after phenotype conversion. **Conclusions:** These data indicate that SI HIV variants can emerge at low to normal CD4 counts during the asymptomatic period in HIV infection and that this SI phenotype is a valuable prognostic marker for the early identification of a subgroup of asymptomatic individuals with a high risk for progression to AIDS.

Q 328 THE CYTOPATHIC EFFECT OF HIV IS ASSOCIATED WITH APOPTOSIS,

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Large amounts of histones, H1, H2A, H2B, H3 and H4, were observed in total extracts of T4 lymphocytes and derived cell lines infected with the human immunodeficiency virus (HIV) type 1 or type 2. These histones were simply detectable by analysis of crude cellular extracts by polyacrylamide gel electrophoresis in SDS and staining the proteins with Coomassie-blue or by immunoblot assays using specific polyclonal antibodies. The histones were found to be localized in the nucleoplasm, bound to low molecular weight (LMW) DNA in the form of nucleosomes. The mechanism responsible for the accumulation of nucleosomes during HIV infection was found to be due to fragmentation of cellular DNA, a mechanism referred to as apoptosis or programmed cell death in which a nuclear endonuclease becomes activated and cleaves DNA at internucleosomal regions. Accordingly, the LMW DNA accumulated in the course of infection was found to have a characteristic pattern of nucleosomal ladder and its accumulation was reduced in the presence of zinc, a known inhibitor of the endonuclease. Routinely in acute HIV infections, the accumulation of nucleosomes was observed at least twenty four hours before lysis of infected cells and it was associated with the cytopathic effect (CPE) of HIV, characterized by vacuolization of cells and formation of syncytia. The mechanism whereby HIV infection induces CPE and apoptosis remains to be clarified. The detection of histones in the nucleoplasm can be used as a convenient marker for chromatin fragmentation during this process.

Q 329 **SELECTION FOR SPECIFIC V3 LOOP SEQUENCES ON TRANSMISSION OF HIV,** Andrew J. Leigh Brown, Lin Qi Zhang, Edward C. Holmes and Peter Simmonds[1], Division of Biological Sciences, [1]Department of Medical Microbiology, University of Edinburgh, EH9 3JN, Scotland.

Recent work from our laboratory has revealed that the pattern of variability within an infected patient detected directly by PCR-sequencing from peripheral blood samples is complex: there are significant differences between the plasma virus population and that of the cell-associated provirus from the same sample[1]. These results together with our analysis of the evolution of the plasma virus population[2], support the view that the high level of sequence variation in the hypervariable regions of *env* is due to selection for immunologically novel variants. However, we have observed within our patient population and among other isolates frequent examples of evolutionary convergence in the V3 region, implying significant constraints on the repertoire of viable sequences. We have now extended this analysis to the first stages of infection. Results from pre-seroconversion samples indicate that before an effective immune response is mounted, (1) there is no detectable sequence variability within a patient and (2) sequences from independently infected patients are all remarkably similar; grouping together in a maximum-likelihood phylogenetic analysis away from sequences obtained later in the infection of the same patients. These observations indicate strong selection at transmission for a specific sequence in this immunologically significant domain. Such a sequence would be the logical target for an *env*-based vaccine.

1. Simmonds, P. *et al.* J. Virol. 65: 6266-6276, 1991.
2. Holmes, E.C., *et al.* Proc. Nat. Acad. Sci., submitted.

Q 330 EFFECTS OF THE RECIPROCAL COMPLEMENTATION OF TWO DEFECTIVE HIV-1 MOLECULAR CLONES ON HIV-1 CELL TROPISM AND VIRULENCE, F.Lori, L.Hall, P.Lusso, P.Markham*, M.Popovic, R.C.Gallo and M.S.Reitz, LTCB, NCI, NIH, Bethesda MD 20892 and *Advanced Biosciences Lab., Kensington MD 20895

Human immunodeficiency virus type-1 (HIV-1) displays both interstrain and intrastrain genetic variability. Virus populations with extensive microheterogeneity have been defined as "swarms" or "quasi-species". Many of the genomes within HIV-1 swarms appear to be defective in one or more genes required for viral replication. It is unclear to what extent defective viruses play a role in the process of HIV-1 infection or in the pathogenesis of acquired immunodeficiency syndrome (AIDS). We have isolated two biologically active HIV-1 clones: 1) LW 12.3, which contains defects in the vif and vpr genes, and 2) MN ST.1, which has defects in the vpu gene and in the p6 portion of the gag gene. We demonstrate here that coinfection of peripheral blood mononuclear cells (PBMC) with two HIV-1 clones having different defects extends the cellular host range, significantly increases the replication rate of both viral genomes, and shortens the delay in production of the vpu-p6 defective MN ST.1. Coinfection renders the vif-vpr defective LW 12.3 competent for replication in PBMC. Coinfection of LW 12.3 and MN ST.1 also results in increased virulence and more dramatic cytophatic effects. Reciprocal complementation in trans seems to explain in part these results. This two component model represents a simplified version of the in vivo situation and illustrates one way in which interaction of defective viruses could increase the spread of infection and progression of disease.

Q 331 PATHOGENESIS STUDIES OF MOLECULAR CLONES OF SIMIAN IMMUNODEFICIENCY VIRUS (SIV) ANALYZED IN VIVO AND IN VITRO, Paul A. Luciw, Karen Shaw, Ronald E. Unger, Vicente Planelles, Michael Stout, Nancy Leung, Babak Banapour, Niels Pedersen, Martha Marthas, University of California, Davis, CA 95616

Two molecular clones of SIV have been extensively characterized in vitro and in vivo. SIVmac239 causes an AIDS-like disease in macaques, whereas SIVmac1A11 does not produce disease. SIVmac239 results in high virus load and persistent infection. SIVmac1A11 gives low virus load and appears not to persist in macaque PBMCs. In vitro, SIVmac1A11 grows in macrophages, and SIVmac239 is restricted for replication in macrophages. We have determined the complete nucleotide sequence of the genome of SIVmac1A11. Although both cloned viruses exhibit greater than 98% sequence homology, an number of significant differences are noted. SIVmac239 contains open reading frames (ORFs) for all viral genes except for a premature stop codon in *nef* which reverts upon infection in animals. A full frame for *nef* is encoded by SIVmac1A11; thus, *nef* is not the sole factor that determines virus load and pathogenesis in macaques. SIVmac1A11 contains a premature stop codon in the *vpr* gene and two premature stop codons in the TM region of the *env* gene. A cluster of 12 amino acids in variable region 1 (VI) of the SU domain of *env* also differ between SIVmac1A11 and SIVmac239. Scattered differences (single base changes) exist in the LTRs. To relate specific viral genes to biologic properties, we have constructed recombinant viruses which involve reciprocal exchanges of portions of the SIVmac1A11 and SIVmac239 genomes. Macrophage tropism in vitro is controlled, in part, by the *env* SU domain of SIVmac1A11. Studies in macaques infected with recombinant viruses reveal that 2 (or more) genes are responsible for persistent infection; candidates include *vpr* as well as *nef*. Analysis of additional recombinants will be required to elucidate viral gene function in SIV infection and pathogenesis.

Q 332 REDUCED VIRULENCE OF HIV-2 AS COMPARED TO HIV-1. Richard Marlink, Souleymane MBoup, Phyllis Kanki, Tidiane Siby, Ibou Thior, Karen Travers, Jim Hellinger Rachel Royce, Aissatou Gueye, Jean-Louis Sankalé, Dominique Ricard, Ibrahim Ndoye and Max Essex, Department of Cancer Biology, Harvard School of Public Health, MA, Boston 02115, USA.

Background. Despite the widespread prevalence of Human Immunodeficiency Virus Type 2 (HIV-2), the prevalence of AIDS cases in countries where HIV-2 predominates has not been as dramatic as the prevalence of AIDS in other regions of Africa where HIV-1 is more common. We hypnothesized that the rate of disease development may be less frequent with HIV-2 versus HIV-1 infection.

Methods. We undertook a prospective clinical study of a subset of HIV-2 and HIV-1 seropositive women and comparable seronegative women, from February 1985 through April 1991. Immunologic measurements of T-cell subset and skin testing to tuberculin antigen have been performed since the later part of 1988. A follow-up network of clinics and study physicians has been utilized to minimize lost subjects.

Results. Among 88 HIV-2 seropositive women followed for an average of 3.3 years, we observed only one AIDS case. Seven cases of HIV-related CDC IV disease, which were not AIDS-defining by either CDC or WHO criteria, were also observed. Among 24 HIV-1 seropositive women followed for an average of 2.9 years, we have observed 3 AIDS cases and 5 CDC IV disease cases which were not AIDS-defining. Rate ratios of these incidence rates of disease showed that AIDS and CDC IV disease were both significantly more likely to occur with HIV-1 infection than with HIV-2 infection. Immunologic parameters to date show that both HIV-2 and HIV-1 seropositive outpatients have significantly decreased median absolute T4 counts when compared to seronegatives, although T4 counts and T4/T8 T-cell ratios for HIV-2 seropositive women were less abnormal than HIV-1 seropositive women. In addition, anergy to skin testing was significantly more common in the HIV-1 versus the HIV-2 seropotitive group.

Conclusions. This natural history study uniquely demonstrates the differential rates of disease development between HIV-2 and HIV-1 infection. The immune parameters used to monitor HIV-1 infection may be abnormal in HIV-2 infection, but appear to be intermediate in range when compared to HIV-1 and seronegatives in an outpatient setting. Further studies are required to evaluate the full pathogenic potential of HIV-2 infection.

Q 333 ANALYSIS OF THE INTRACELLULAR INTERACTION BETWEEN HIV GP160 AND CD4 LEADING TO TRANSPORT BLOCK OF CD4, R.A. Martin, M.A. Jabbar, V. Subramaniam, and D.P. Nayak, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA. 90024.

Cell surface expression of CD4 is sharply down-regulated in CD4+ HIV-infected cells. We have examined the mechanism of CD4 down-regulation by coexpressing HIV envelope protein gp160 with CD4. Using a T7-vaccinia virus transient expression system, we found that CD4 expressed alone was efficiently transported to the cell surface, but when coexpressed with gp160 it is blocked within the endoplasmic reticulum. During transit through the membrane trafficking pathway, CD4 formed a specific intracellular complex with HIV gp160, which can be immunoprecipitated by antibodies against either gp160 or CD4(OKT4). The CD4-gp160 complex is localized to the endoplasmic reticulum and CD4 coexpressed with gp160 did not acquire endoH resistance. To further investigate the interaction between these proteins, we have made mutations at specific residues in both CD4 and gp160, which have been shown by others to abrogate binding in soluble assay systems. When CD4 mutants were expressed with wild-type gp160, results were similar to those seen when wild-type CD4 was used. CD4 surface expression continued to be suppressed, and immunoprecipitation data showed that intracellular binding to gp160 was decreased by only 20% when compared to wild-type. Similarly, coexpression of mutant gp160 with wild-type CD4 demonstrated that intracellular CD4-gp160 complex formation still takes place. Precipitation experiments showed that gp160 mutants bound CD4 at approximately 60% of wild-type values. Despite this decreased binding, CD4 and gp160 could clearly be coprecipitated from cell lysates, and CD4 surface expression was significantly suppressed . Thus, it appears that intracellular binding of CD4 to gp160 may not require the same sites as those found to be critical for binding in soluble assay systems. These studies are critical for defining the role of specific interactions between CD4 and gp160 during transport through the exocytic pathway, and thus may provide the basis for strategies designed to reverse CD4 down-regulation.

Q 334 TRANSPLACENTAL TRANSMISSION OF RETROVIRUS IN CATS AS A MODEL FOR FETAL INFECTION BY HUMAN IMMUNODEFICIENCY VIRUS, Lawrence E. Mathes, Jennifer L. Rojko and Kathleen A. Hayes, Center for Retrovirus Research, The Ohio State University, Columbus, OH, 43210

Pediatric human immunodeficiency virus (HIV) infection is perhaps the most tragic outcome of the AIDS epidemic. Transmission of HIV from mother to child has an incidence of roughly 30% and in most cases is thought to occur via the transplacental route. Animal models for transplacental transmission of lentiviruses have been poorly developed because of the low or negative incidence of fetal infection. However, pregnant cats infected with feline leukemia virus (FeLV) have a high incidence of transplacental transmission often resulting in fetal death and resorption. In this study, four 6- to 8-month old FeLV-positive female cats were mated with non-viremic males. Pregnancy was detected by palpation and hysterotomy was performed prior to term (full term is 63 days). From three of the four dams a total of 12 fetuses or fetal remnants in various stages of resorption were present at the time of hysterotomy (20 to 30 days). From the remaining dam, five ostensibly normal fetuses were collected at approximately 50-55 days of gestation. The direct mechanisms leading to fetal death and resorption are not known, but a preliminary study indicated elevated plasma levels of tumor necrosis factor alpha (TNFα) at the time of resorption. TNFα is the effector of endotoxin induced fetal death in a mouse model. Immunofluorescence staining of placental tissue demonstrated substantial FeLV antigen expression in maternal decidua and uterine milk gland epithelia as well as in placental cells resembling syncytiotrophoblasts. The high incidence of transplacental infection in the FeLV/cat model permits evaluation of antiviral therapies for the prevention of fetal infection by retroviruses.

Q 335 HIV-ASSOCIATED T CELL LYMPHOMA: EVIDENCE THAT HIV MAY DIRECTLY CAUSE T CELL TRANSFORMATION IN VIVO, Michael S. McGrath, Brian G. Herndier, Gregory Reyes* and Bruce T. Shiramizu, AIDS Program, Departments of Laboratory Medicine, Pathology, and Pediatrics, San Francisco General Hospital, University of California, San Francisco, CA 94143, and *Genelabs Incorporated, Redwood City, CA 94063

An HIV seropositive man died with a fulminant CD4 positive T-cell lymphoma. The tumor was pleomorphic and expressed HIV p24 antigen as well as IL-2 and IL-2 receptors (IL-2R). The tumor was monoclonal, HTLV-1, EBV, and HHV-6 negative, but contained a single clonally integrated copy of HIV-1. Using an inverse polymerase chain reaction (PCR) technique, the HIV integration site was mapped within the fur gene, just 5' to the fes/fps proto-oncogene on chromosome 15. Further PCR analysis showed that the 3' exon of fur and the fes/fps gene were expressed within tumor tissue but not within non-tumor tissue. These data suggest that HIV may have contributed to T-cell transformation through induction of an autocrine growth process (IL-2/IL-2R) and/or through insertional mutagenesis activation of the fes/fps oncogene. This is the first AIDS associated lymphoma where HIV has been shown to be directly implicated in the transformation process.

Q 336 **Evaluation of stability and detectability of HIV RNA by viral capture in serum and plasma.** William F. Mehaffey, Robert Coombs, Thomas C. Quinn and Denis R. Henrard.

Objective: Compare the levels of HIV RNA measured in serum versus plasma collected in the presence of various anticoagulants and evaluate the effect of multiple freeze-thaws on the detection of HIV RNA.

Methods: HIV RNA was assayed by RT/PCR after capturing intact viral particles with anti-envelope monoclonal antibodies covalently attached to latex microparticles. Eight fresh paired serum-heparinized plasmas and three paired citrated-heparinized plasmas were analyzed less than 24 hours after collection. Aliquots were frozen at -70 C, thawed 10 times and assayed by viral capture after 1, 3, 5, 7 and 9 thaws. Specimens were also tested after being left at room temperature for 2 and 6 days. All viral capture and RT/PCR reactions were done in duplicate.

Results: All 8 sera had significantly less detectable HIV RNA than their corresponding plasma. In contrast, no difference was observed between heparinized and citrated plasmas. The 2 specimens selected for freezing stability studies became negative for HIV RNA if diluted 100 and 1,000 fold, respectively. Up to 9 freeze-thaws did not measurably affect the detection of HIV RNA in these 2 samples, regardless of the anticoagulant.

Conclusion: Plasma is the material of choice for detecting HIV RNA by viral capture and the nature of anticoagulant does not appear to affect the sensitivity of the assay. In contrast to infectivity, which is known to decrease by about half a log upon freeze-thawing, virus-associated RNA appears suprisingly stable to multiple freeze-thaws, or to prolonged storage at room temperature.

Q 337 PROGRAMMED DEATH OF BOTH CD4+ AND CD8+ CELLS IN ASYMPTOMATIC HIV-INFECTION, Linde Meyaard, Sigrid A. Otto, Richard R. Jonker, M. Janneke Mijnster, René P.M. Keet and Frank Miedema.
Department of Clinical (Viro-)Immunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and the Laboratory for Clinical and Experimental Immunology of the University of Amsterdam, The Netherlands

The early stage of human immunodeficiency virus (HIV) infection is characterized by qualitative functional defects of antigen-reactive T cells. With only few cells infected, this can not be explained by direct viral infection. Refractoriness *in-vitro* of both CD4+ and CD8+ T cells to antigen-specific and polyclonal activation via the CD3/T-cell receptor complex (TCR) is not due to a failure to induce early signal-transduction events. We hypothesized that probably due to a systemic immune dysregulation, T-cells from HIV-infected individuals reach an anergic state which primes them for programmed cell death (PCD) upon antigenic stimulation.
After overnight culture, T cells from asymptomatic HIV-infected individuals showed condensed chromatin morphology and DNA-fragmentation characteristic for PCD. DNA fragmentation could be prevented by the addition of Zn^{2+}, known to inhibit endonuclease activity in PCD. Cell death was not restricted to CD4+ cells, the main target cells for HIV, but was observed in both CD4+ and CD8+ cells. In HIV-infected individuals the percentage of cells dying due to PCD was ± 8 % and could be increased by polyclonal stimulation via the CD3/T-cell receptor complex up to ± 23 %.
Our data imply that PCD contributes to the observed *in-vitro* T-cell dysfunction in asymptomatic individuals. Moreover, our findings offer a novel and systemic explanation for deletion of reactive T cells in HIV infection. PCD-mediated deletion of T cells after antigenic stimulation, *in vivo*, may result in a gradual loss of reactive T cells and thus disturb the balance between virus and host-defense.

Q 338 CYTOPATHIC PROPERTIES OF LENTIVIRUS ENVELOPE GLYCOPROTEINS. R.C. Montelaro, M.A. Miller, and T.A. Mietzner, Dept. Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

The intimate association of lentivirus envelope glycoproteins with a variety of membranes in infected cells can lead to cytopathic effects resulting from significant alterations in critical membrane properties and cellular functions. To date, however, there is little information available on the structural determinants or mechanisms of this envelope glycoprotein-mediated cytopathogenesis. During the course of developing general models for retrovirus glycoproteins, we have identified a unique peptide domain at the carboxyl terminus of lentivirus transmembrane (TM) proteins that is absent from oncovirus TM components. The peptide domain, designated lentivirus lytic peptide (LLP), consists of a highly positively charged arginine rich amphipathic helix that shares structural properties common to natural lytic peptides (magainins and cecropins) and to the binding domains of calmodulin regulatory proteins. In vitro functional assays using synthetic LLPs from HIV, SIV, and EIAV have demonstrated specific cytopathic and calmodulin binding activities in these TM peptide domains. These results suggest that the TM LLP may contribute to lentivirus cytopathicity by perturbations of membrane permeablility and by inhibition of calmodulin regulatory processes. Current experiments are in progress to examine LLP cytopathic mechanisms using peptide analogs and site directed mutants of this TM peptide component.

Q 339 B CELL ABNORMALITIES IN AIDS: CLONAL DOMINANCE OF ANTI-HIV-1 ANTIBODIES IN HEALTHY SEROPOSITIVE INDIVIDUALS. Sybille Müller*, Haitao Wang*, Gregg J. Silverman**, Greg Bramlet*, Nancy Haigwood† and Heinz Köhler*, San Diego Regional Cancer Center*, San Diego, CA 92121; Department of Medicine, University of California**, San Diego, CA 92093; Chiron Corporation†, Emeryville CA 94608

Using the criteria of light chain isotype expression by anti-p24, anti-gp120 and anti-reverse transcriptase antibodies we found evidence of frequent clonal restriction in healthy HIV-1 infected individuals. Antibodies to p24 and gp120 were purified from HIV-1 positive sera and analyzed by isoelectric focusing and immunoblot analysis. Isoelectric focusing of purified anti-p24 and anti-gp120 antibodies showed restricted banding patterns indicating oligoclonal antibodies. Analysis of the utilization of variable VH gene families in purified anti-p24 and anti-gp120 antibodies revealed a restricted and biased V gene usage. In contrast, the utilization of variable light chain families appeared to be random.

Furthermore, we used a monoclonal anti-idiotypic antibody to dissect the clonal nature of anti-HIV antibodies in seropositive healthy individuals. The analysis demonstrated that the 1F7 clonotypic marker is frequently shared by antibodies to three different HIV-1 antigens and are closely associated with oligoclonal or monoclonal antibody populations.

The existence of restricted and dominant anti-HIV-1 antibodies has implications for the pathogenesis of AIDS. Established B cell clones against neutralizing epitopes may prevent the recognition by primary B cells of variant viruses which can escape neutralization. Clonal B cell restriction also needs to be considered in the design and development of vaccines and immunotherapies.

Q 340 EXTENSIVE HIV-1 *ENV* SEQUENCE VARIATION IN THE C4-V4-C5 REGION, Shen Pang*, Eric S. Daar†, Harry V. Vinters¥, Tarsem Moudgil†, David D. HoΦ, and Irvin S.Y. Chen*§, *Department of Microbiology & Immunology, UCLA School of Medicine; †Cedars Sinai Medical Center, Los Angeles CA; ¥Department of Neuropathology, UCLA School of Medicine; ΦAaron Diamond AIDS Research Center, New York NY; §Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine

We investigated the C4-V4-C5 region of HIV-1 *env* gp120 in HIV-1-infected individuals at different stages of disease by PCR cloning and sequencing. Samples isolated from late disease stages showed that several distinct subtypes of HIV-1 co-exist in both infected blood and brain tissue. The silent mutation rates were much lower than the expected rate of random occurrence, and the frequency of subtypes differed between the brain and the blood, suggesting that selection pressures may play a role in the late stages of disease to favor amino acid sequence changes. We also studied variation early after infection in acute HIV-1 infection prior to seroconversion. Samples obtained shortly before or after seroconversion were analyzed, and extensive variation was also seen prior to seroconversion. Unlike the samples from late stages of disease, a dominant sequence was always maintained, and the silent mutation rates were similar to what would be expected based upon random occurrence.

Q 341 RAPID CHANGES IN UNINTEGRATED HIV-1 DNA LEVELS AFTER INITIATION OR CESSATION OF ANTIRETROVIRAL THERAPY, C. David Pauza, Parul Trivedi, Frank M. Graziano*, Department of Pathology and Laboratory Medicine and * Department of Medicine, University of Wisconsin, Madison, WI 53706

HIV-1 entry and replication generates three species of unintegrated viral DNA including circular forms with 1 or 2 LTR and a linear DNA that is the precursor to chromosomal integration. The larger circular molecule can be detected by specific polymerase chain reaction amplification of the junction sequence between the LTR. Importantly, the 2 LTR circular viral DNA is labile in peripheral blood lymphocytes that were infected in vitro or recovered from infected patients; the half-life of this molecule is 16 hours. Thus, the level of 2 LTR circular viral DNA is a direct indicator of the dynamics of viral infection in the peripheral blood. In a cohort of 82 seropositive patients, increasing levels of unintegrated viral DNA levels were highly correlated with decreasing CD4 cell counts. Other measures of total viral DNA load, p24 antigen levels or β2 microglobulin accumulation were not correlated with CD4 cell counts or with unintegrated viral DNA levels. All patients receiving antiretroviral therapy (AZT, ddI or ddC) had unintegrated viral DNA levels in mid to low range amounts. There were no examples of high unintegrated viral DNA in patients receiving nucleoside analog therapy. Additionally, patients beginning AZT therapy evidenced a rapid decrease in unintegrated viral DNA that was significant within 1 week after first exposure to drug. Similarly, patients ending their therapeutic regimen showed a remarkable increase in unintegrated viral DNA that was associated with rapid disease progression and a poor prognosis. Direct measurement of unintegrated viral DNA constitutes the most rapid indicator of therapeutic efficacy and may be used to evaluate drug effectiveness in individual cases or to explore the mode of action for candidate compounds.

Q 342 CONSTRUCTION AND CHARACTERIZATION OF A VIRULENT MOLECULAR CLONE OF EQUINE INFECTIOUS ANEMIA VIRUS, Susan Payne[1], Keith Rushlow[2], Maureen Flaherty[3], Stephanie Perry[3], Ronald Montelaro[2], and Frederick Fuller[3], Department of Molecular Biology and Microbiology, Case Western Reserve Univ. School of Medicine, Cleveland, OH 44106[1], Department of Molecular Genetics and Biochemistry, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261[2] and Department of Microbiology, Pathology, and Parasitology, North Carolina State Univ. College of Veterinary Medicine, Raleigh, NC 27606.

Equine infectious anemia virus (EIAV) is a member of the lentivirus subfamily of retroviruses, genetically and antigenically related to HIV-1. Ponies infected with virulent strains of EIAV develop an acute disease characterized by viremia, fever, and thrombocytopenia. We have recently generated a virulent molecular clone of EIAV. Virus from cells transfected with this provirus caused a severe febrile episode in an experimentally infected animal. This virulent clone will be an important tool for studying the molecular basis for EIAV disease as well as for production of defined virus stocks for studies of antigenic variation and vaccine development. The virulent clone was obtained by replacement of the LTR of an avirulent provirus with one containing a U3 LTR duplication of the sequence CAAT. The CAAT box duplication was previously noted in the LTRs of several virulent EIAV strains but was absent from three avirulent proviral clones. Thus LTR sequences appear to be one major determinant of EIAV virulence. The virulent virus replicates somewhat more slowly than the avirulent parent in cell culture, perhaps explaining the loss of the virulent phenotype of most EIAV strains upon passage in culture.

Q 343 INHIBITION OF CELLULAR AMINOPEPTIDASE ACTIVITY DECREASES IN VITRO HTLV III_B INFECTION OF HUT-78 CELLS. Pulido-Cejudo, G.*, Sherring, A., Campione-Piccardo, J. and Izaguirre, C.A. Laboratory Centre for Disease Control & Federal Centre for AIDS, Health & Welfare Canada.

Changes in aminopeptidase activities(APases) have been detected in various pathoimmunological processes where immunomodulation is thought to play an important role Using selective ß-naphthylamide substrates, we determined the activities of different APases following HTLVIII$_B$ infection of CD4+HUT-78 cells. Five days post-infection, both cellular and extracellular Arg,Leu and Pro aminopeptidases increased in association with cytopathicity and p24 levels. A two fold increase in aminopeptidase B(APase-B) and leucine aminopeptidase (LAP) was observed. Treatment of HUT-78 cells with 100 g/ml of UBENIMEX (a competitive inhibitor of APase-B and LAP) before and during infection, resulted in a ten fold decrease of extracellular p24 levels five days post-infection. In addition, UBENIMEX-treated cells showed lower cythopathic effect (30 syncytia/well) in comparison to infected cells alone (150 syncytia/well). Two hours post-infection, cell-associated virus in UBENIMEX-treated cells was three times lower than untreated cells (treated:p24 10pg/ml;infected alone:p24 31pg/ml). LAP, APase-B and virus-free-inactivated supernatants of HTLVIII$_B$ infected cells were coupled to cross-linked agarose and tested for their proteolytic activity using GP120 (V3 LOOP)HBX2 as substrate. Reverse phase HPLC revealed that GP120 V3 loop is cleaved into identical peptides both by immobilized cell supernatants and APase-B/LAP matrices. These results suggest that host APase activities may be involved in the processing of viral peptides and that competitive inhibition of APases decreases HTLVIII infection by blocking viral entry.

Q 344 BIOLOGICAL PHENOTYPE OF HIV-1 CLONES AT DIFFERENT STAGES OF INFECTION: PROGRESSION OF DISEASE IS ASSOCIATED WITH A SHIFT FROM MONOCYTOTROPIC TO T-CELL-TROPIC VIRUS POPULATIONS, Hanneke Schuitemaker , Maarten Koot , Neeltje A. Kootstra , Frank Miedema and Matthijs Tersmette , Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam

The composition of human immunodeficiency virus type 1 clonal populations at different stages of infection and in different compartments was analyzed. Biological HIV-1 clones were obtained by primary isolation from patient peripheral blood mononuclear cells under limiting dilution conditions, with either blood donor peripheral blood lymphocytes or monocyte-derived macrophages as target cells and the biological phenotype of the clones was analyzed. In asymptomatic individuals low frequencies of HIV-1 clones were observed. These clones were non-syncytium inducing and preferentially monocytotropic. In individuals progressing to disease, a hundred fold increase in frequencies of productively HIV-1 infected cells was observed due to a selective expansion of non-monocytotropic clones. In a person progressing to AIDS within 19 months after infection only SI clones were detected, shifting from MDM-tropic to non-MDM-tropic over time. From his virus donor, a patient with wasting syndrome, only SI clones, mostly non-MDM-tropic were recovered. Parallel clonal analysis of HIV-1 populations in cells present in broncho-alveolar lavage fluid and peripheral blood from an AIDS patient revealed a qualitatively and quantitatively more monocytotropic virus population in the lung compartment than in peripheral blood at the same time point. These findings indicate that monocytotropic HIV-1 clones, probably generated in the tissues, are responsible for the persistence of HIV-1 infection, and that progression of HIV-1 infection is associated with a selective increase of T-cell tropic, non-monocytotropic HIV-1 variants in peripheral blood.

Q 345 FELINE IMMUNODEFICIENCY VIRUS INFECTION OF SCID MICE ENGRAFTED WITH FELINE TISSUES: A MURINE MODEL FOR HIV DRUG TESTING, Dean W. Selleseth, Calvin M. Johnson, Tedd A. Childers, M.N. Ellis, Mary Tompkins, Wayne A.F. Tompkins; Division of Virology, Burroughs Wellcome Co., Research Triangle Park, North Carolina, USA, and North Carolina State University College of Veterinary Medicine, Raleigh, North Carolina, USA.

C.B-17 *scid/scid* mice (SCID mice) readily accept xenotransplants due to defective T and B lymphocyte function. When SCID mice are engrafted with sections of fetal feline thymus and/or lymph node, then given i.p. or i.v. injections of liver, bone marrow, peripheral blood lymphocytes, and/or spleen cells (SCID-fe mice), they are permissive for infection with feline immunodeficiency virus (FIV). Fetal feline lymph node and thymus tissues were surgically implanted under the mammary fat pads of anesthetized mice. Immediately after implantation, the mice were given an i.p. injection of a liver, bone marrow, and/or spleen cells. Two weeks after implantation, 27 SCID-fe mice were injected i.p. with 7×10^6 $NCSU_1$ FIV-infected feline peripheral blood mononuclear cells (PBMC) and 2 SCID-fe mice were given 3×10^7 uninfected feline PBMC. Ten of these mice were given a dose of 125 mg/kg/day Retrovir® (azidothymidine, AZT) in the drinking water beginning 24 hours prior to virus challenge and continuing until the end of the study. Two weeks post-infection, the mice were sacrificed and implants were analyzed for FIV proviral DNA by PCR amplification of a 782 base pair segment of the gag open reading frame. Specificity was confirmed by hyridization to a radiolabeled internal oligonucleotide. The number of mice positive for FIV by PCR (summarized below) indicate a lower frequency of detection of FIV provirus in AZT-treated animals as compared to untreated.

	Untreated	AZT treated	Uninfected controls
Thymus implant	11/17 (65%)	2/10 (20%)	0/2 (0%)
Lymph node implant	11/17 (65%)	4/10 (40%)	0/2 (0%)
Both implants	8/17 (47%)	0/10 (0%)	0/2 (0%)

Hybridization intensities of FIV-positive samples in which equal amounts of DNA were amplified by PCR were compared to determine relative levels of provirus in each sample. Comparison of 5 untreated mice with 5 AZT-treated mice showed a significant reduction in provirus burden associated with AZT treatment. The stronger hybridization signal seen in the untreated animals suggests viral replication in the feline tissues. Subsequent experiments have shown that the SCID-fe mice can also be infected with a cell-free FIV inocula. These data suggest that the FIV-infected SCID-fe mouse is a safe, realistic murine model for testing antiretroviral compounds.

Q 346 INTRACELLULAR LOCALIZATION OF HIV-1 RNA: POSSIBLE CORRELATION WITH CYTOPATHICITY, Mohan Somasundaran, Lars K. Beattie, Gary Bassell, Cornell Overbeeke, John L. Sullivan, Robert H. Singer, Dept. of Pediatrics & Cell Biology, Univ. of Massachusetts Medical School, Worcester, MA 01605. We have been investigating mechanism(s) of cytopathic effect of HIV-1 on CD4 T-cells. Peripheral blood lymphocytes and T-cells (c8166, H9, & CEM cells) were infected with HTLV-IIIb strain of HIV-1. A modified and novel in situ hybridization technique was used to detect HIV positive cells. Biotin-UTP labeled, nick translated whole HIV-1 genomic probe was hybridized to the viral RNA. At the post-hybridization step we used anti-biotin antibody followed by 1 nm gold-labeled anti-IgG and silver enhancement. This technique allowed us to process the stained cells for both light and electron microscopy analyses. We were able to observe the high resolution distribution of viral RNA intracellularly with respect to specific cytosolic organelles. This approach is particularly valuable for studying the localization of the viral RNA in acutely and chronically infected cells, and the effect of drugs that modulate viral replication on this process.

Q 347 IS THERE ANOTHER AGENT THAT CAUSES LOW CD4 COUNTS AND AIDS?, Thomas J. Spira and Bonnie M. Jones, Division of HIV/AIDS, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333. HIV-1 and -2 are known causes of low CD4 counts and AIDS. We have identified 6 persons with persistently low CD4 count (<250/cumm) who are repeatedly HIV-seronegative. Five are male and one is female. Ages range from 35 to 70 years. The clinical presentations include 1 Pneumocystis carinii pneumonia, 1 Cryptococcal meningitis, cryptococcoma and localized Herpes zoster infection, 1 disseminated Molluscum contagiosum, 1 oral and vaginal candidiasis, 1 Kaposi's sarcoma post renal transplantation, and 1 with multiple atypical mycobacterial pneumonias and idiopathic pulmonary fibrosis. Three have a history of blood transfusions (2, after the initiation of HIV screening); one of these also had a renal transplant. One has a history of intravenous drug use. One is a health care worker. Peripheral blood lymphocytes from them were tested by Western blot, viral culture and PCR for evidence of HIV infection. All of these tests were negative. Serology for HTLV-1 and -2 was also negative. CD4 lymphocyte counts ranged from 43 to 246/cumm with percentages ranging from 4 to 18. CD4/CD8 ratios ranged from 0.08 to 0.46. Several persons have had their low counts for several years. Preliminary data on 4 of these persons indicate that there is an elevated ratio of memory to naive CD4 lymphocytes when compared to HIV-infected persons with comparable CD4 lymphocyte counts. This is similar to what is observed in persons with HTLV-1 and -2 infection. In summary, we have identified 6 persons with low CD4 counts without evidence of HIV infection with clinical manifestations of opportunistic infections or tumors of varying severity. This suggests that HIV may not be the only infectious cause of immunosuppression in man.

Q 348 Desialylation of Peripheral Blood Mononuclear Cells Promotes Growth of HIV-1, Nicholas M. Stamatos, Peter J. Gomatos, Arnold Fowler, Nancy Dow, John Wohlhieter, Carolyn Deal and Alan S. Cross, Walter Reed Army Institute of Research, Wash. D. C. 20307-5100.

Activation of peripheral blood $CD4^+$ T lymphocytes *in vitro* establishes a permissive state for growth of HIV-1. Activated T lymphocytes express an increased amount of endogenous sialidase (neuraminidase) and are hyposialylated. We therefore determined if desialylation of quiescent PBMCs allowed growth of HIV-1. Peripheral blood mononuclear cells were treated with microbial neuraminidase or PHA prior to infection with HIV-1 at low multiplicity of infection. After 18 days in culture, large amounts of p24 Ag and reverse transcriptase were present in the medium of cells under both conditions. Neither viral component was found in the medium of untreated cells similarly infected through 21 days in culture. Promotion of viral growth in cells treated with neuraminidase was specific for that enzyme as viral growth was not detected when the enzyme was heat-inactivated or when the enzyme was preincubated before use with the specific, competitive inhibitors sialic acid or 2,3-dehydro-2-desoxy-N-acetyl-neuraminic acid. The maximal rate of HIV-1 production in neuraminidase-treated cells preceded or coincided with exponential growth of virus in PHA-activated cells and appeared to be independent of cell division. Immediately after neuraminidase treatment of PBMCs and seven days thereafter, there was no change in distribution of lymphocyte subsets nor in density of CD4 per cell. These data demonstrate that desialylation of PBMCs is sufficient to establish a permissive state for growth of HIV independent of cell division. These results also suggest that sialidase inhibitors may have therapeutic value in HIV-infected individuals.

Q 349 LONG TERM HIV INFECTION WITH NORMAL IMMUNE FUNCTION AND NEGATIVE VIRUS ISOLATION, John L. Sullivan, Mohan Somasundaran, Thomas Greenough, Ariane Alimenti, Ruth Hesselton, *Doreen Brettler, Dept. of Pediatrics, Program in Molecular Medicine, Univ. of Massachusetts Med. Ctr., *The Med. Center of Central Massachusetts-Memorial Hospital, Worcester, MA 01605. We have followed a 45 year old asymptomatic hemophilic male for eight years following exposure and infection with HIV-1 contaminated factor VIII concentrate. Eight years following infection he maintains 44% CD4 lymphocytes (absolute CD4 lymphocytes 1510) with 32% CD8 lymphocytes (absolute CD8 lymphocytes 1109). Strong antibody responses against HIV gp120, gp41, p55, p24 have persisted throughout the course of infection. ADCC antibodies directed against envelope glycoproteins measure $\geq 10^4$ in titer. Neutralizing antibodies directed against the RF, IIIb and other HIV-1 strains have been demonstrated. Circulating activated cytotoxic T cells directed against HIV-1 gag or env. proteins have been repeatedly absent. HIV-1 specific cytotoxic T cell responses can be generated in vitro following secondary stimulation with HIV-1 proteins. Repeated attempts to isolate HIV-1 using ACTG consensus methods, quantitative plasma and cell cultures, CD4 and monocyte enriched populations have been negative. Quantitative DNA PCR studies have been repeatedly negative, however, "boosted" PCR (detects 2 genome copies per 10^5 cells) has demonstrated the presence of HIV-1 DNA in peripheral blood mononuclear cells. In summary, we have identified a patient chronically infected with HIV who has developed HIV specific immune responses. Absence of viral replication is evidenced by negative viral cultures and the lack of circulating virus specific cytotoxic T cells. Efforts are currently underway to molecularly characterize the virus demonstrated by "boosted" PCR.

Q 350 COTRANSFECTION OF HIV-1 MOLECULAR CLONES WITH SLOW/LOW REPLICATIVE CAPACITY MAY YIELD PROGENY VIRUS WITH RAPID/HIGH PHENOTYPE. W. Tan, R. Fredriksson, Å. Björndal, P. Balfe and EM. Fenyö, Department of Virology, School of Medicine, Karolinska Institute, Stockholm S-105 21, Sweden, Department of Medical Microbiology, University College and Middlesex School of Medicine, London, UK.
In order to molecularly characterize rapidly and slowly replicating HIV-1 variants, molecular cloning of a rapid/high HIV-1 isolate 4803 was performed (Fredriksson et al. Virology. 1991; 181: 55 - 61). Seven infectious molecular clones were obtained and transfected into peripheral blood mononuclear cells (PBMC). Progeny virus obtained from six clones had slow/low phenotype, whereas one clone yielded rapid/high virus. Although separate transfection of each of the clones 12, 13 and 82 into PBMC gave rise to slow/low viruses, viruses recovered from cotransfection of the mixture of these clones exhibited rapid/high phenotype. To test whether recombination and/or complementation has taken place in the mixture of clones 12+13+82, the progeny virus was diluted to endpoint in 15 paralell series. All biological phenotypes, slow/low, rapid/high and intermediate were recovered. With the help of distinctive restriction enzyme markers, the original clones and recombinant viruses could be identified. The role of interactions between coexisting variant genomes in determining the biological phenotype of a viral population will be discussed.

Q 351 DIFFERENTIAL EFFECTS OF ZIDOVUDINE (ZDV) TREATMENT OF ASYMPTOMATIC INDIVIDUALS WITH DIFFERENT HIV-1 BIOLOGICAL PHENOTYPES
M. Tersmette, M. Koot, R.E.Y. de Goede, A.H.V. Vos, M.Th.L. Roos, P.Th.A. Schellekens, J.M.A. Lange[1], F. Miedema. Centr.Lab.Neth.Red Cross Blood Transf.Serv.& Lab.Clin.Exp.Immunol. Univ. Amsterdam.[1] Academic Medical Centre, Amsterdam, The Netherlands
Aim: To study the effect of ZDV on the natural course of infection in the presence of HIV-1 variants with different biological properties.
Methods: Clinical progression was studied of 51 asymptomatic persons with HIV-1 antigenemia and/or CD4+ cells <500/mm^3, participating in a double blind zidovudine trial. The ZDV-treated group (n=29) and the placebo group (n=22) were comparable for age, CD4+ cell numbers and HIV-1 antigenemia at start. The biological phenotype of sequential HIV-1 isolates from these persons was studied with the MT-2 cocultivation assay.
Results: At the start of the study syncytium-inducing (SI) variants were detected in 9/29 persons receiving ZDV vs. 4/22 controls. In the course of the study SI variants emerged in 6/20 ZDV-treated persons vs. 2/18 controls. For persons with SI isolates at start or developing SI isolates during the study, progression to AIDS was not significantly different between the treatment and control groups (7/14 vs. 2/6 respectively). In contrast, in the group without SI isolates, AIDS occurred in 0/14 treated persons vs. 4/16 controls.
Conclusions: ZDV treatment seems to prolong the asymptomatic period in persons without SI isolates. ZDV treatment, however, does not inhibit the emergence of SI isolates, nor AIDS progression in persons with SI isolates. Randomization for virus phenotype is advisable to improve the evaluation of novel antiviral drugs.

Q 352 CHARACTERIZATION OF HIV-1 ACCESSORY REGULATORY GENE EFFECTS UPON VIRUS REPLICATION IN PRIMARY CELL POPULATIONS

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Dramatic variations have been observed between different isolates of HIV-1 with regard to the types of cells they productively infect in vitro, and the kinetics of their subsequent replication. It has also been suggested that the virus undergoes a progressive selection in vivo within the individual infected host. Isolates derived from late stage AIDS patients have been reported to exhibit enhanced cytopathicity in vitro and an improved capability for replication in stable T cell lines, as compared with viruses taken from patients in earlier stages of the infection. We have recently derived a set of proviruses, beginning with clone HXB2, which exhibited dramatic alterations in their in vitro replication characteristics both in primary cells and in stable cell lines, as the multiple regulatory gene defects inherent in HXB2 were successively corrected. The changes introduced into HXB2 did not alter the predicted sequence of the exterior envelope glycoprotein, demonstrating that viral determinants in other parts of the genome can also exert powerful effects upon the characteristics of virus replication. For example, virus derived from one of these clones exhibits markedly enhanced replication characteristics in both primary T cells and macrophages, but despite this is diminished for growth in a panel of T cell lines compared with HXB2. Furthermore it appears that the vpr gene product is disproportionately important for vigorous replication of HIV-1 within primary macrophage populations. These effects were not predictable from earlier studies of the individual HIV-1 regulatory functions in simple replication experiments conducted in T cell lines.

Q 353 TRANSCELLULAR ACTIVATION OF THE HIV-LTR IN COCULTURED LYMPHOCYTES Ofra Weinberger, Adriana Marcuzzi, Christian A. Thomas, and Joshua Kosowsky, Department of Physiology and Cellular Biophysics, Columbia University, New York, N.Y. 10032
We have demonstrated transcellular activation of HIV-CAT in T cells following cocultivation with cells expressing HIV-1 *tat*. As a consequence of exposure to *tat*-expressing cells, the ability of T cells to respond to stimulatory signals (PHA, anti-T3) is compromised, as measured by the mobilization of Ca^{++} in cocultured cells. This transcellular activation is evident in as little as three hours of cocultivation, at ratios as low as 1:1,000 HIV expressing cells to target cells, and is dependent on the *tat* responsive element. We investigated the molecular requirements for transcellular activation of Jurkat cells. Coculture of Jurkat HIVCAT cells with cells expressing only *tat* did not result in activation of the LTR; subsequent analysis with deletion mutants and blocking antibodies demonstrated a requirement for env expression in addition to *tat* for transcellular activation to occur. The results suggest that the transient association of CD4 and gp120 in co-cultured cells is required for *tat* mediated transcellular activation. The events that follow CD4-gp120 binding in transactivation, however, do not require the gp120-neutralizing domain, in contrast to HIV-mediated fusion and infection. One of the unexplained aspects of the progression of AIDS is that immunological abnormalities are detectable before CD4+ T helper cell depletion occurs. The demonstration that a small number of HIV-expressing cells can affect a large number of uninfected bystander cells in a short period of time suggests a mechanism by which global immune dysfunction can precede the high prevalence of infected cells.

Q 354 EXPRESSION OF HUMAN IMMUNODEFICIENCY VIRUS RNA IN BONE MARROWS OF PATIENTS WITH A SPECTRUM OF HIV-1 DISEASE. B. Weiser[1], H. Burger[1], and J. Mladenovic[2], Wadsworth Center for Research, Albany, NY[1], SUNY Stony Brook, NY[2].

Hematologic abnormalities are a consistent finding during the course of infection with HIV-1. While cells known to inhabit the bone marrow can be infected with HIV-1 *in vitro*, *in vivo* studies examining the occurrence of virally infected cells in marrow are limited. In this study, *in situ* hybridization was used to detect HIV-1 RNA expression in bone marrows from patients with a broad range of HIV-1 related disease, CD4 counts and hematological abnormalities. Random diagnostic biopsy specimens were reviewed and categorized by morphological characteristics. Specimens from 40 patients were then selected for analysis by *in situ* hybridization with riboprobes (antisense and sense) spanning the majority of the HIV-1 genome. The results showed that 6/40 patients (15%) demonstrated HIV-1 expression in the marrow, 3 with many cells expressing high levels of HIV-1 RNA, and 3 with a few cells showing low level viral expression. These specimens were notable for hypercellularity and increased numbers of megakaryocytes and giant cells. Double-label analysis using *in situ* hybridization combined with immunohistochemistry for macrophages (marker: RCA, lysozyme, or KP1) and T lymphocytes (marker: CD3) localized HIV-1 expression to macrophages. Subsequent clinical correlation of patient characteristics with *in situ* hybridization data was made. HIV-1 RNA expression correlated with CD4 depletion and advanced HIV-1 infection rather than a specific hematologic or clinical diagnosis. This study demonstrated HIV-1 expression in the bone marrow of 15% of patients with a broad spectrum of HIV-1 disease. These *in vivo* data suggest that the frequency of HIV-1 infected cells in the bone marrow, like that of cells in the peripheral blood, increases with the progression of HIV infection. They also suggest that hematologic abnormalities in infected patients generally result from indirect effects of HIV-1 rather than direct infiltration of the bone marrow with HIV-1 expressing cells.

Q 355 SYNERGISM AMONG BOVINE RETROVIRUS INFECTIONS IN THE INDUCTION OF LYMPHOPROLIFERATION IN EXPERIMENTALLY INFECTED SHEEP. Cecelia A. Whetstone, Martin J. VanDerMaaten, Janice M. Miller, National Animal Disease Center, USDA/ARS, Ames, IA 50010

An association between proliferative responses and viruses in *Retroviridae* has long been recognized. Bovine leukemia virus (BLV), an oncovirus similar to human T-cell leukemia virus (HTLV-1), is the etiological agent of bovine lymphosarcoma. Bovine immunodeficiency-like virus (BIV), a lentivirus with structural, molecular and antigenic similarities to human immunodeficiency virus type 1 (HIV-1), causes lymphoproliferative response in experimentally infected animals. Bovine syntium virus (BSV), a spumavirus, is ubiquitous but not currently associated with any pathological condition. Recent reports regarding potential interaction, and enhancements of activity, due to coinfections with HIV-1 and other retroviruses stimulated our interest in determining whether similar interactions exist among bovine retroviruses. To test this hypothesis *in vivo*, we used sheep since they are susceptible to infection with all three bovine viruses and the marked oncogenic activity of BLV in sheep has long been recognized. Six sheep were inoculated intravenously (i.v.) with blood from BLV or BLV-BSV donor cows. Five to 7 months later, three of those sheep were inoculated i.v. with BIV tissue culture material. After inoculation, all sheep became seropositive to BLV, BIV inoculates seroconverted to BIV, and the five sheep that received BSV developed BSV antibodies. Between 7 and 10 months after inoculation, all three BIV-BLV-BSV sheep died with tumors. Two of those sheep developed a persistent lymphocytosis (PL) 2-3 months prior to death. One BLV inoculate also developed PL and died with tumors. The other two BLV-BSV inoculates remain clinically normal 27 months after inoculation. Development of PL and/or tumor appear independent of BSV, since the inoculate that received BLV only developed PL and tumors and the two BLV-BSV inoculates remain clinically normal. In a related study, sheep inoculated with only BIV remain clinically normal after 32 months. This preliminary study indicates that the sheep-bovine retroviral system may be a useful model to study oncogenesis and effects due to co-infections with retroviral agents.

Q 356 CHARACTERIZATION OF INCOMPLETE HIV-1 REVERSE TRANSCRIPTION IN QUIESCENT T-CELLS. Jerome A. Zack*, Allyson M. Haislip*, Paul Krogstad†, and Irvin S.Y. Chen†§, *Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024; †Division of Infectious Diseases, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA 90024; §Department of Microbiology & Immunology, UCLA School of Medicine, Los Angeles CA 90024

We have previously shown that HIV-1 can efficiently enter quiescent primary lymphocytes; however, the reverse transcription process is not completed in these cells. We further characterized the reverse transcription of HIV-1 in quiescent cells, and our results indicate that while initiation of reverse transcription occurs simultaneously in both activated and quiescent lymphocytes, this process proceeds more slowly in quiescent cells. We also performed experiments to address the role of partial reverse transcripts as intermediates in the viral life-cycle. We used AZT either before or after infection with HIV-1 to prevent formation of and further DNA synthesis by partial reverse transcripts, respectively. Our results indicated that partial reverse transcripts can contribute significantly to virus rescue from infected quiescent cells stimulated subsequent to infection. Furthermore, we established that mitogenic stimulation of infected quiescent cells induces re-initiation of DNA synthesis from partial reverse transcripts. However, the virus rescue is inefficient relative to the initial multiplicity of infection, and this is explained by inefficient completion of DNA synthesis from the partial reverse transcript. Thus, arrest of reverse transcription in quiescent cells may play an important role in HIV-1 pathogenesis by contributing to the inefficient infection of potential target cells in the peripheral blood of HIV-1-infected individuals.

Immune Responses and Vaccines

Q 400 IMMUNOGENIC TARGETING OF SYNTHETIC HIV-1 PEPTIDE VACCINES TO APCs BY CHIMERIC ANTI-MHC CLASS II AND ANTI-sIgD ANTIBODIES,

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Synthetic peptides encompassing pathogen-derived T plus B cell epitopes can function as complete immunogens that elicit neutralizing antibodies and native T cell memory. Their use is limited, however, because of the MHC-restricted nature of T cell responses and their inherently weak immunogenicity. To address these problems, we made use of recombinant DNA techniques to generate chimeras between HIV-1-derived immunogenic epitopes, primarily from the immunodominant "V3 loop" region of gp120, and antibody Fab fragments specific for molecules displayed on antigen-presenting cells (APCs), *e.g.*, MHC class II or surface IgD (sIgD). Hybrid oligonucleotide primers were used in recombinant PCR's to clone and express chimeric Fab fragments from several hybridomas producing anti-human or anti-murine MHC class II or sIgD antibodies, employing a recently described procedure for generating combinatorial, antigen-binding Fab libraries in the modified phagemid vector, pComb3. Molecular and immunochemical analysis indicated that the expected chimeric Fab fragments were correctly cloned and expressed in *E. coli*, and current efforts are targeted to purify sufficient quantities for determination of their biological activity. Such bacterially derived chimeric Fab fragments are expected to act not only as an inert carrier but, rather, as a specific targeting device that will focus the relevant HIV-1 epitopes at high density on the surface of APCs, thereby allowing a more efficient antigen presentation and, hence, vaccination.

Q 401 EFFECT OF IMMUNIZATION WITH GP-160 ON SPECTROTYPE OF HUMAN ANTI-GP120 ANTIBODIES,

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Over the course of HIV-1 disease, the protective function of the immune system deteriorates, probably including a decline in the number and/or function of B-cell clones recruited in the viral-antigen specific humoral response. One technique for measuring the number and activity of specific B-cell clones is to spectrotype; an individual's spectrotype against a given antigen is characteristic, an "immune fingerprint." The spectrotype can be obtained by separating antibodies in serum using iso-electric focusing, blotting, and reacting with labeled antigen (in this case, conformationally intact gp120). We have obtained the anti-HIV-1 (IIIB) gp120 spectrotype from 30 early stage, HIV-1 infected patients undergoing vaccine therapy with gp160 (IIIB, from baculovirus, MicroGeneSys). Of these patients, 25 displayed oligoclonal banding patterns. Of the reacting patients, 18 (72%) showed either an increase in band intensity or number of bands, or both, during the course of immunization. This is in sharp contrast with previous results showing only decreases in bands over the natural course of HIV-1 infection. Sera from 8 patients in a matched cohort, receiving a placebo vaccine, showed no such increase in number or intensity of bands over a one year period, consistent with the previous studies.

Q 402 HIV-2 TYPE AND STRAIN SPECIFIC NEUTRALIZATION MEDIATE BY HYPERIMMUNE GUINEA PIG SERA AGAINST HIV-2 GLYCOPROTEINS.

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The purpose of our study was to identify the target regions for type and strain specific neutralization in the HIV-2 envelope glycoproteins.
Seven peptides, representing five regions of the envelope proteins of the HIV-2 strain SBL6669 (Björling et al.PNAS 88, 6082, 1991) were used to immunize guinea pigs. The anti-peptide guinea pig sera were evaluated for their neutralizing activity (NA), as measured by inhibition of virus replication, against the homologous SBL6669 virus isolate and ten other heterologous HIV-2 isolates.
Neutralizing antibodies to the homologous virus were induced by peptides representing 3 regions in the gp 125 and 2 in the gp 36. Guinea pig sera against two overlapping peptides representing aminoacids (aa) 311-337 in the region corresponding to HIV-1 V3 showed a pronounced capacity to neutralize both homologous and the majority of heterologous HIV-2 isolates with titers ranging between 160 and 2560. Two additional target regions for homologous neutralization were found between aa 119-137 and 472-509; hyperimmune sera to both these peptides showed a low degree of cross NA. The transmembrane protein, gp 36 of HIV-2 also harbored two sites at aa 595-614 and aa 714-729. Both the regions are highly conserved among HIV-2 isolates and a significant NA was seen with several of the heterologous isolates.
We are now investigating to which extent the 311-337 region represents the principal neutralizing domain in HIV-2.

Q 403 ENHANCED IMMUNOGENICITY OF HIV PEPTIDES IN MICE AND MACAQUES BY IMMUNOTARGETING AND SYNTEX ADJUVANT.

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The immunogenicity of synthetic HIV peptides is substantially enhanced by "targeting" the peptides to cells that express MHC class II antigens by using class II-specific mAbs. In mice, we compared the enhancement obtained by immunotargeting to the adjuvant effect of aluminum phosphate. A synthetic HIV peptide, p24E-V3MN, consisting of the B-cell neutralization epitope from the V3 loop of the gp120 envelope glycoprotein of the MN isolate of HIV-1 and a T cell epitope from the p24 core protein, was used for this study. p24E-V3MN was conjugated to TIB92 mAb (anti-I-A^k specificity) and used to immunize (I-A^b x I-A^k) (C57Bl/6 x C3H/HeJ)F, mice. Two 1 ug doses of targeted p24E-V3MN induced anti-peptide ELISA reactivity that was more than 100-fold higher than the responses induced by 50 ug of free peptide in saline or in aluminum phosphate - a 5000-fold increase in immunogenicity. A similar enhancement in the immunogenicity of synthetic peptides as a result of targeting, compared to peptide in saline, was observed in 2 additional experiments with different peptides. In macaques, 100 ug doses of p24E-V3MN in Syntex SAF-M formulation induced substantial anti-p24E-V3MN and anti-V3MN reactivity in both of the immunized monkeys. None of the 4 macaques immunized with free peptide or peptide in aluminum phosphate had detectable anti-peptide reactivity. In a separate immunogenicity study in macaques, with an HIV peptide from the p24 core protein of HIV-1, significant anti-peptide and anti-p24 protein reactivity was induced in 1 of 2 macaques immunized with 10 ug doses of peptide conjugated to each of two mAbs that recognize non-polymorphic determinants on human HLA-DR antigens. No responses were observed in the 2 macaques immunized with peptide conjugated to a non-targeting isotype-matched control mAb. These results establish that both immunotargeting and SAF-M are valuable approaches to enhancing the immunogenicity of HIV synthetic peptides.

Q 404 LONGITUDINAL EVALUATION OF CD4 CELL IMMUNOPHENOTYPE AND FUNCTION IN HIV-1 SEROCONVERTERS, Chen-Cheng Chou, Lance E. Hultin, David Bockstoce, Vaheideh Gudeman, Mary Ann Hausner, Patricia M. Hultin, Jose Luis Matud, Parunag Nishanian, Roger Detels and Janis V. Giorgi, UCLA Schools of Medicine and Public Health, Los Angeles, CA 90024

It has recently been proposed that seroconversion is accompanied by a preferential decline in the CD4 cells that belong to the subset responsible for the proliferative response to soluble antigen. This concept is in conflict with our previously published report (J Immunol 138:3725, 1987) that indicates CD4 subsets defined by CD29 and CD45RA are not preferentially depleted in HIV infected persons at later stages of HIV infection. To determine whether a selective change in the $CD45RO^+$ or $CD29^+$ subsets of CD4 T cells occurs during the several months surrounding seroconversion, 16 seroconverters and their matched seronegative controls were studied for the 2-year period around the time of seroconversion. Eight of the men were rapid progressors and 8 were slow progressors. Five cryopreserved samples were studied from each person. Both the $CD45RO^+$ subset of CD4 T cells and that defined by $CD29^+$ were decreased selectively at the time of seroconversion in the rapid progressors but not in the slow progressors. Complexed and/or uncomplexed serum p24 was detected in 5 of the seroconverters, and all of these were rapid progressors. Presence of p24 was associated with rapid CD4 cell loss, as previously reported, but surprisingly not necessarily with loss of proliferative response to recall antigen. Although a selective decrease in CD4 memory cell number is a characteristic feature of CD4 cell decline in rapid progressors, it is not clear whether direct cytotoxic effects of HIV-1 or indirect anti-HIV-1 immunologic effects are responsible for the alterations in CD4 cell phenotypes.

Q 405 THE DEVELOPMENT OF CYTOTOXIC T-LYMPHOCYTES (CTL) IN SUBJECTS IMMUNIZED WITH HGP-30 A 30 AMINO ACID SUBUNIT OF HIV P17 CORE PROTEIN, Rebecca Coleman, Daniel Stites, James Scillian, Allan Goldstein, Paul Naylor, Prim Sarin, Vincent Simmon, and Peter Heseltine, James Kahn, Division of AIDS and Oncology, San Francisco General Hospital, Departments of Medicine and Laboratory Medicine, University of California San Francisco, San Francisco, CA 94110, University of Southern California, Department of Biochemistry, George Washington Medical Center and Viral Technologies Inc.

HGP-30 is a 30 amino acid synthetic peptide from an immunogenic region of the p17 HIV protein. p17 is detected on the surface of HIV and on the surface of HIV infected lymphocytes. p17 appears conserved from different isolates and p17 antibodies decline prior to the onset of AIDS and prior to the loss of other HIV directed antibodies. Antibodies directed to p17 cross-react with HGP-30. For purposes of vaccination, HGP-30 is bound to the carrier protein KLH and mixed with alum as an adjuvant. A phase 1 dose escalation study was initiated to evaluate the safety and immunologic *profile of HGP-30/KLH alum vaccine in non HIV-infected* volunteers. The dose level (number of subjects) were as follows: 10 μg/kg (6), 25 μg/kg (6), 50 μg/kg (6), and 100 μg/kg (3). All subjects were vaccinated intramuscularly on day 0, weeks 4 and 10. Local pain at the injection site was common but not clinically limiting. Clinical laboratory parameters were not affected by administration of the vaccine. There was no immunotoxicity and no effect on T-cell functional studies. Antibodies to the vaccine were transient and developed in a minority of subjects. T-cell proliferative responses are variable *and occurred in a minority of subjects. A positive (greater than* 50% increase above control) CTL response was observed in 4 of the first 11 subjects; and a near positive was observed in 3 volunteers. In general CTL positive responders also developed T-cell proliferative responses to HGP-30.

Q 406 SYNTHESIS OF HIV GP41 FUSION SEQUENCE N-TERMINAL TO THE HYBRID T1-SP10 PEPTIDE YIELDS PEPTIDES THAT FORM HIGH MOLECULAR WEIGHT COMPLEXES IN AQUEOUS SOLUTIONS. Douglas L. Cotsamire, Mary Kate Hart, Kent J. Weinhold, Thomas J. Matthews, Peter Cresswell, Richard M. Scearce, Dawn M. Jones, Alphonse J. Langlois, Thomas J. Palker, and Barton F. Haynes. Department of Medicine, Duke University Medical Center, Durham, NC 27710

The T1-SP10 HIV gp120 env peptides are comprised of the env T cell epitope T1 (aa428-443) and gp120 V3 loop sequence SP10 (aa 303-321). In mice, goats, and rhesus monkeys T1-SP10 peptides are potent inducers of T cell help and B cell neutralizing antibodies against native HIV gp120. When the V3 loop peptide SP10 was extended 5 to 7 aa (the A region) C-terminal to complete an HLA Class I-restricted CTL epitope, the resulting hybrid peptide, T1-SP10(A), was capable of inducing CD8+, HLA Class I restricted anti-HIV CTL in mice. In an effort to further improve the immunogenicity of T1-SP10 peptides, we synthesized the 12aa HIV env gp41 fusion (F) sequence (aa519-530) N-terminal to T1-SP10 and compared the immunogenicity and quaternary structure of T1-SP10(A) with F-T1-SP10(A) HIV env peptides. In mice, T1-SP10(A) and F-T1-SP10(A) peptides were equally potent as in vivo primers of anti-HIV CD8+, MHC Class I-restricted CTL. In goats and rhesus monkeys, T1-SP10(A) and F-T1-SP10(A) peptides both induced high serum titers of anti-HIV neutralizing antibodies. When run over a Sephadex G-75 molecular sizing column in TRIS/100mMKCl/5% glycerol, SP10(A) (MW 2891) eluted at 3 KDa, T1-SP10(A) peptide (MW 4770) eluted from 12 to 4 KDa, while F-T1-SP10(A) (MW 5930), eluted at 93 KDa. T1-SP10 peptide was capable of being incorporated into L-α dioleoyl lecithin/ cholesterol liposomes. We next added the bifunctional cross-linking *agent 3,3'-dithio-bis-propionic acid N-hydroxysuccinimide ester* (DSP) to SP10(A), T1-SP10(A) or F-T1-SP10(A) peptides in PBS followed by SDS-PAGE. Whereas SP10(A) and T1-SP10(A) did not form high MW complexes that could be cross-linked with DSP, F-T1-SP10(A) peptides did form multimeric complexes (up to 110 KDa). The ability of F-T1-SP10(A) peptide to form multimeric complexes and T1-SP10 peptides to interact with lipid membranes may be factors that contribute to their high degree of immunogenicity as carrier-free peptides.

Q 407 FOREIGN GENE EXPRESSION IN EBV-TRANSFORMED B-CELLS: POTENTIAL FOR THE DEVELOPMENT OF NOVEL CTL TARGET CELLS. Tyler J. Curiel[1], David J. Schoen[1], Daniel R. Kuritzkes[1], Elizabeth McFarland[1], Minoo Bakhtiari[1], David T. Curiel[2] and Ernst Wagner[3]. [1]The University of Colorado Health Sciences Center, Denver, CO, 80262; [2]The University of North Carolina, Chapel Hill, NC; and [3]the Research Institute for Molecular Pathology, Vienna, Austria.

Several HIV vaccines deliver immunogen via a vaccinia vector. Most current assays for the measurement of cytotoxic T lympohocyte killing employ infection of Epstein-Barr Virus transformed B-cells (LCLs) with recombinant vaccinia virus. High background (vaccinia) killing may hamper detection of HIV-specific responses with this system. The advantage of LCLs is their ease of production and maintenance in long-term culture. Unfortunately, stable foreign gene expression has proven difficult in these cells, and the efficiency of gene expression is low. We have devised a novel gene transfer method that allows transient expression of foreign genes in LCLs with high (up to 90%) efficiency. This method uses the transferrin (hTf) receptor as the vector binding site, and capitalizes on the ability of *adenovirus (Ad) to disrupt endosomes, which protects plasmid DNA from* rapid degradation. An anti-Ad antibody was conjugated to polylysine (pL), and separately hTf was also conjugated to pL. The plasmid pRSVL, which expresses a luciferase reporter gene (*luc*) under the control of an RSV promoter was bound to the anti-Ad/pL via the pL moiety, and this complex in turn bound to Ad. hTf/pL was added, and the resultant ternary complex incubated with LCLs. Incorporation of the EBV genes *EBNA-1* and *oriP* into plasmids (plasmid 220Luc) caused up to a 9-fold further increase in *luc* expression. LU = light units. A representative experiment is shown.

hTfpL/pRSVL	hTfpL/220Luc	AdpL/pRSVL	AdpL/hTfpL/220luc
320 LU	350 LU	1880 LU	16,800 LU

Gene expression can be further augmented in the following manner. Ad is biotinylated and conjugated to a pL-streptavidin carrier that has been complexed to the reporter plasmid. Using the previously described Ad/hTfpL/anti-Ad complex, 3700 LU *luc* activity was obtained with the 220Luc reporter, compared to 15,500 LU using the streptavidin/pL system. We are now making analagous HIV *gag*-containing plasmids to assess HIV-specific killing in this system. A further advantage of this system compared to vaccinia systems is that target cells stably expressing HIV genes may be obtained.

Q 408 EVALUATION OF MONOCLONAL ANTIBODIES TO SIV ISOLATES BY NEUTRALIZATION ASSAYS.

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Neutralizing antibodies are associated with protection from disease, clearance of virus and prevention of viral reinfection. Measurement of neutralizing responses may provide a basis for assessing the extent of protection afforded by immunization. Monoclonal antibodies (MAbs) may be used to identify epitopes in viral proteins that are important for neutralization. For HIV-1, the V3 loop is reported as the principal neutralizing determinant. Additional neutralization epitopes occur in the CD4 binding region and the V2 region of gp120 and in gp41.

Little is known about the neutralization epitopes in simian immunodeficiency virus (SIV) even though SIV infection of monkeys is the best animal model for AIDS. The SIV antibody project is a collaborative study organized by National Institute of Allergy and Infectious Diseases to characterize a panel of neutralizing monoclonal antibodies to envelope protein of SIV. The objectives of this study are: to determine neutralizing antibody titers of MAbs to SIV isolates; to compare neutralizing titers obtained with different assays; to compare the reactivity of MAbs with SIV isolates in serological assays; and to identify standards for SIV neutralizing studies.

Eleven MAbs to SIV envelope protein were supplied as purified immunoglobulin to collaborating laboratories. The antibodies were contributed by the National Institute for Biological Standards and Control, Hertfordshire, UK and SmithKline Beecham Biologicals, s.a., Rixensart, Belgium. Collaborating laboratories are characterizing the antibodies by neutralization and serological assays using a variety of SIV viral isolates.

Q 409 RESISTANCE OF HIV-1 RT TO TIBO DERIVATIVES INDUCED BY SITE-DIRECTED MUTAGENESIS, Karen De Vreese, Zeger Debyser, Rudi Pauwels, Jan Desmyter, Erik De Clercq and Jozef Anné, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

The tetrahydro-imidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)one and thione (TIBO) derivatives are potent antiviral agents which specifically inhibit HIV-1, but not HIV-2, reverse transcriptase (RT). The differential sensitivity of HIV-1 and HIV-2 RT to TIBO derivatives may be attributed to the amino acid sequence divergence between the two enzymes. To investigate this hypothesis, several amino acids in the conserved regions of HIV-1 RT were substituted by the corresponding amino acids of HIV-2 RT. The amino acid residues Y_{181} and Y_{188} proved crucial for the anti-HIV-1 RT activity of the TIBO derivatives. The amino acid substitutions $Y_{181}I$ and $Y_{188}L$ each annihilated the sensitivity of HIV-1 RT to the TIBO derivative R82150 without loss of enzymatic activity.

Q 410 CHARACTERIZATION OF ROMANIAN HIV VIRUS STRAINS, Ovidiu Dumitrescu, and Jay A. Levy, Cancer Research Institute, UC San Francisco, School of Medicine, San Francisco, CA 94143-0128

Twenty-six blood samples drawn from HIV-infected Romanian children were processed to isolate the human immunodeficiency virus by co-cultivation of the patients' peripheral blood mononuclear cells (PMC) with normal PMC prestimulated with PHA for 3 days. All the pediatric patients were born to seronegative mothers and were infected after birth, probably by blood transfusions or by injections with improperly sterilized needles. Fifteen viral isolates were obtained. All the strains replicate well in PMC and induced some cytopathicity. During the isolation procedures, reverse transcriptase (RT) levels were detected within the first week of culture in 3 cases; PMC cultures from 10 children became positive within the second week, and the remaining 2 cultures showed an elevated RT only in the third week. Viral isolates were observed to have different kinitics of replication in PMC. Studies are ongoing to determine viral tropism for macrophages and T-cell lines, to compare the biological properties of Romanian HIV isolates with HIV strains from other parts of the world, and to examine their sensitivity to serum neutralization as related to the sequences in the V3 loops of the viral envelope.

Q 411 SIMILARITIES OF THE AUTOLOGOUS NEUTRALIZING ANTIBODY RESPONSE IN HIV-1 INFECTED HUMANS AND SIV INFECTED MACAQUES. Fenyö, E.M.,[1] von Gegerfelt, A.,[1] Scarlatti, G.,[1] Öhman, P.,[1] Putkonen, P.,[2] Biberfeld, G.[2] and Albert, J.[3] [1]Department of Virology, Karolinska Institute, Departments of [2]Immunology and [3]Virology, National Bacteriological Laboratory, Stockholm, Sweden

Twenty seven serum samples from 19 individuals with varying severity of HIV-1 infection were tested for neutralization against sequential virus isolates. The kinetics of appearence of virus neutralizing antibodies during primary HIV-1 infection could be followed in four individuals. Five serum samples (of 27, 18%) neutralized the simultaneously collected autologous virus. Nine serum samples (of 13, 69%) contained neutralizing activity to autologous virus isolated 6-12 months earlier. The majority of samples with neutralizing activity were obtained from individuals with recently aquired HIV-1 infection.

Neutralizing antibodies to early reisolates could be demonstrated in four of seven Cynomolgus monkeys experimentally infected with SIV_{sm}. The neutralizing antibody response of a long survivor monkey showed a relatively broad specificity one year postinfection, whereas the neutralizing antibody response of the three immunodeficient monkeys remained isolate-specific and decayed over time.

The pattern of virus neutralization is thus similar in HIV-1 infected humans and SIV infected monkeys. In both cases, variant viruses resistent to neutralization by autologous sera emerge during the entire course of infection. The ability to produce neutralizing antibodies to autologous virus appears to correlate with the degree of immunodeficiency in the host.

Q 412 ROLE OF CD8+ IN AIDS AND IMMUNE MODULATION BY INTERFERON-ALPHA 2b, Milan Fiala and Jeffrey Gornbein, UCLA School of Medicine, Los Angeles, CA 90024

We have studied 70 patients to determine their immune compromise at different stages of AIDS. Whereas CD4+ deficiency below 200 cells x 10 /L inaugurated the initial opportunistic infection, patients usually did not suffer from severe morbidity due to disseminated cytomegalovirus (CMV) or Mycobacterium avium-intracellulare infections until CD8+ subset declined below 400 cells x 10 /L. Critical CD4+ deficiency did not by itself predispose to these infections. Zidovudine (ZDV) therapy alone stabilized mainly CD4+. Addition of interferon-alpha 2b (IFN-alpha) to ZDV increased, in a time dependent fashion, CD8+ in a majority of patients, whereas CD4+ were improved only in a minority. Beneficial clinical responses correlated with these peak CD8+ responses. CD8+ are important as cytotoxic antiviral cells. IFN-alpha may have beneficial effects in AIDS by modulating CD8+ to a greater extent than CD4+. Proportion of gamma-delta T cells appeared to increase after effective anti-mycobacterial therapy in patients with disseminated M. avium-intracellulare infection.

Q 413 DEFECTIVE T CELL RESPONSIVENESS TO RECALL ANTIGENS IN HIV-1 INFECTION IS NOT DUE TO SELECTIVE LOSS OF CD4 MEMORY CELLS AND IS NOT ABROGATED BY REVERSE TRANSCRIPTASE INHIBITORS *IN VIVO* OR *IN VITRO*, Janis V. Giorgi, Chen-Cheng Chou, Vaheideh Gudeman, Sheryl O'Rourke, Valentin Isacescu, Roger Detels and Ronald T. Mistuyasu, UCLA Schools of Medicine and Public Health, Los Angeles, CA 90024

In this study, we investigated two possible reasons for the poor proliferative response to recall antigens noted in HIV-1 seropositive people, i.e., 1) selective loss of CD4 memory cells and 2) immunosuppression by HIV-1 production *in vivo* or *in vitro*. In preliminary work using three-color immunofluorescence, we verified that the $CD45RO^+/CD45RA^-$ CD4 population is $CD29^+$. Thus, CD45 isoforms or CD29 can be used to define CD4 memory cells. However, CD29 is expressed at lower levels than the CD45 isoforms, and $CD29^+$ cells are not well resolved from those that are $CD29^-$. Consequently, $CD45RA^-$ CD4 cells were defined as memory cells. Proliferation was tested in response to tetanus toxoid, *Candida albicans* and influenza. Even when the proliferation of each person's lymphocytes to the recall antigens was corrected for the decreased number of $CD45RA^-$ CD4 memory cells in the culture, the response was still lower than that in healthy controls. With regard to anti-virals, *in vivo* use of zidovudine (AZT) did not restore this functional defect. Furthermore, AZT, dideoxycytidine and 3'-azido-2',3'-dideoxyuridine added to the *in vitro* cultures did not restore the response to recall antigens. These studies indicate that low proliferative responses of CD4 cells to recall antigens (1) cannot be accounted for by preferential depletion of CD4 memory T cells, and verify our previous report that CD4 memory cells are not preferentially lost during HIV-1 disease progression, and (2) cannot be reversed by anti-viral drugs *in vivo* or *in vitro*.

Q 414 CHARACTERIZATION OF DOMAINS IN SIV_{PBJ} GAG WHICH MAY INTERACT WITH THE ENV GLYCOPROTEIN, Silvia A. González and Arsène Burny, Department of Molecular Biology, University of Brussels (ULB), Belgium.

The mechanism by which gag proteins, present at the plasma membrane, incorporate surface-expressed envelope glycoprotein upon budding is unknown. Sequence analyses of HIV proteins suggest that a basic region on p17 gag could be involved in the formation of infectious particles by interaction with a negatively charged domain on gp41. To address this question, we generated a series of mutations within the homologous regions of the SIV_{PBJ} gag and env proteins and expressed them in vaccinia virus system. Analysis of the particulate material released by CV-1 cells coinfected by wild-type SIV_{PBJ} gag and env vaccinia recombinants showed that the budding particles incorporated both env gp120-gp41 complexes and gag proteins. However, when the SIV_{PBJ} env vaccinia recombinant was coexpressed with a gag mutant in which the basic region on p17 (residues 26-33) was mutated into a negatively charged region, the env glycoprotein was found to be incorporated into the recombinant-made particles to lower levels than those found for the wild-type recombinant. Studies as to determine whether the defect in these gag mutants can be abrogated by mutations within the acid region on gp41 are currently underway.

Q 415 INDUCTION OF HIGH TITER, CONFORMATION-DEPENDENT NEUTRALIZING ANTIBODIES EFFECTIVE AGAINST PRIMARY AND LABORATORY HIV-1 ISOLATES BY REPEATED IMMUNIZATION WITH $rgp120_{SF2}$, Nancy L. Haigwood[1], Jan McClure[2], and Kathelyn S. Steimer[1], [1]Chiron Corporation, Emeryville, CA 94608 and [2]Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

Immunogenicity studies in baboons and chimpanzees have shown that native, glycosylated recombinant gp120 from HIV-SF2 ($rgp120_{SF2}$) is effective in generating high titer neutralizing antibodies following multiple immunizations. These antibodies neutralize laboratory isolates from the United States, Europe, the Caribbean, and Africa (Haigwood *et al.*, 1992, J.Virol. **66**: 172). Recently, we have observed that sera from $rgp120_{SF2}$-immunized animals also have titers against primary field isolates in the range of titers observed in HIV-infected humans. Experiments were undertaken to localize the determinants to which these antibodies bind. Blocking studies of V3-specific antibodies induced by $rgp120_{SF2}$ immunization indicated that neutralization of isolates with conserved V3 core regions was due, in part, to better presentation of the principal neutralizing determinant by the native glycoprotein. Partitioning of the antibodies into linear and conformational fractions using $rgp120_{SF2}$ as an affinity reagent showed that cross-neutralization of isolates unrelated in the principal neutralizing determinant was associated with antibodies directed to conformational determinants in $rgp120_{SF2}$. As seen with the analogous antibody fraction from HIV antibody-positive human serum (Steimer *et al.*, 1991, Science **254**: 105), the conformation-dependent antibody fraction blocks the binding of HIV-SF2 gp120 to CD4. Native recombinant gp120 subunit immunization can induce cross-neutralizing antibodies directed both to the principal neutralizing determinant and to conformational determinants. These results indicate that a vaccine based upon $rgp120_{SF2}$ may be effective against a broad range of HIV-1 isolates.

Q 416 CONSTRUCTION OF SOLID MATRIX-ANTIBODY-ANTIGEN COMPLEXES CONTAINING SIV PROTEINS USING TAG-SPECIFIC MONOCLONAL ANTIBODY AND TAG-LINKED ANTIGENS. Tomas Hanke, Paul Szawlowski and Richard E Randall. Department of Biochemistry and Microbiology, University of St.Andrews, St.Andrews KY16 9AL, Scotland, UK.

We have previously shown that immunization with solid matrix-antigen-antibody (SMAA) complexes induces both vigorous humoral and cell-mediated immune responses and have suggested that this method of vaccination may be developed for use in humans, and potentially as a vaccine against AIDS. Here we demonstrate that a small oligopeptide can act as a tag for the construction of SMAA complexes using a tag-specific monoclonal antibody and tag-linked antigens. We show that a 14-amino acid oligopeptide, present in the P & V proteins of simian virus 5 (SV5), retains its antigenicity when attached to the C-terminus of 'foreign' proteins, in particular proteins of simian immunodeficiency virus (SIV), such that these proteins can be incorporated into SMAA complexes using a MAb that was originally raised against the native SV5 P & V proteins. Mice were immunized with SMAA complexes containing recombinant SIV proteins and monoclonal antibodies have been isolated that recognized their native counterparts. The murine tag-specific MAb is being currently humanized. The significance of these results in terms of the development of SMAA complexes as human vaccines is discussed.

Q 417 AUTOLOGOUS AND HETEROLOGOUS NEUTRALIZING ANTIBODY RESPONSES TO ISOLATES OF HIV-1, Carl Veith Hanson, Leta Crawford, Terri Wrin, Lynette Sawyer, Patricia Weber, Ryan Alfonso, Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, CA 94704

It has been observed in several laboratories that HIV isolates resist neutralization by autologous, contemporaneous sera. We therefore determined neutralizing antibody titers against autologous (homotypic) isolates and assessed the degree of antigenic relatedness between different patient isolates through cross-neutralization of heterologous sera and viral isolates. The temporal development of HIV neutralizing responses was also determined in longitudinal studies of individuals. In an intensively studied patient, the sera neutralized, after a several-week lag, prior autologous isolates with titers which steadily increased thereafter. Isolates obtained from the same patient subsequent to a particular serum were enhanced by that serum. Control mixing experiments ruled out a role for "blocking antibody". Other patients showed different patterns. We hypothesize a model in which neutralization-resistant variants continuously evolve at a rate faster than the humoral immune system can respond. The complex and largely unidirectional pattern of heterolgous cross-neutralizations can be qualitatively explained in this model, which hypothesizes approximately as many viral variants as there are subjects in the study. The data fail to establish classical "serotypes", but the model suggests that different subjects experience many of the same viral variants at some time in the course of disease and those with the broadest cross-reactivity may be at a more advanced stage of HIV disease.

Q 418 CD8 T CELL PHENOTYPES AT HIV-1 SEROCONVERSION, Hong-Nerng Ho, Lance E. Hultin, Chen-Cheng Chou, David Bockstoce and Janis V. Giorgi, UCLA School of Medicine, Los Angeles, CA 90024

An increase in CD8 T cell numbers occurs at the time of HIV seroconversion concomitant with CD4 cell decline. The elevated CD8 cell compartment contains primarily CD8 cells with activated and immature CD8 phenotypes, and effector cells for anti-HIV immunity have been described. Which subsets of CD8 cells mediate effective anti-HIV immunity and their relative representation during different stages of disease remain open questions. We have previously used three-color immunofluorescence analysis, using the monoclonal antibodies anti-CD38 and anti-HLA-DR, to enumerate four subsets of CD8 T cells. In the current work, we extend these studies using cryopreserved lymphocytes from homosexual men whose disease course is known. We demonstrate that alterations in CD8 subsets defined by these monoclonal antibodies begins at the time of or prior to seroconversion. The CD8 subset that is negative for both markers ($CD38^-$ $HLA\text{-}DR^-$) decreases dramatically at the time of seroconversion concomitant with CD4 decline. The increase in CD8 cells, which accompanies seroconversion, is in the activated CD8 subsets that express CD38 and/or HLA-DR. Of note is that those individuals who progressed rapidly had few cells with the $HLA\text{-}DR^+$ $CD38^-$ phenotype, the subset that we previously have identified to be associated with good prognosis in HIV seropositive people. These results indicate that the pattern of CD8 subset changes that occur at seroconversion may predict future disease outcome. We are sorting these cells to further elucidate their function in cytotoxicity and control of virus replication cultures.

Q 419 SINGLE AMINO ACID SUBSTITUTION IN HIV-1 p24 CONFERS REACTIVITY WITH AN HIV-2 SPECIFIC MONOCLONAL ANTIBODY, James A. Hoxie[1], Celia C. LaBranche[1], Patricia Vance[1], Jan McClure[2], Beatrice H. Hahn[3], Lester L. Gutshall[4], and Lucinda A. Ivanoff[4]; [1]Univ. of Penn., Phila, PA; [2]Bristol-Myers Squibb PRI, Seattle WA; [3]Univ. of AL, Birm., AL; [4]SmithKline Beecham, King of Prussia, PA.

We have evaluated a mutation in HIV-1 gag which conferred reactivity with an HIV-2 p24-specific monoclonal antibody (mAb). Sup-T1 cells chronically infected with HIV-2/ST were superinfected with cell-free virus from the HXB2D clone of HIV-1. Virus from these cells was passaged onto CEM cells, and infected cells cloned by limiting dilution. A cellular clone, termed E7D9, was obtained that reacted by immunofluorescence (IFA) with mAbs specific for HIV-2 and HIV-1 p24 gag proteins (3A8 and 25.4, respectively). Immunoprecipitation and immunoadsorption of radiolabeled viral proteins from the E7D9 cell line demonstrated a single p55 gag precursor and p24 that reacted with both 3A8 and 25.4. Southern blots demonstrated a single integrated provirus. Sequence analysis of a PCR-amplified fragment from the gag gene demonstrated three point mutations within p24 compared to HXB2D: one mutation was silent, one encoded a Val to Ile substitution at codon 11, and one changed a Thr to Ile at codon 200. Interestingly, at codon 200, all published sequences of HIV-1 contain Thr while the corresponding codon for all HIV-2's encodes Leu. Binding assays performed by ELISA on synthetic peptides showed that the Thr to Ile substitution in the HIV-1 p24 sequence was sufficient to confer reactivity with 3A8. In order to evaluate the biological effects of this substitution, a 420 bp fragment from E7D9 containing the Thr to Ile substitution was inserted into HXB2D, transfected into COS cells, and virus was passaged onto Sup-T1 cells. The p24 molecule produced by the infected cells reacted by IFA and Western blot with 3A8, confirming that Ile 200 is a component of the 3A8 epitope. Remarkably, during serial passage of infected cells, a progressive loss of 3A8 reactivity was observed. PCR and sequence analysis demonstrated that this loss resulted from point mutations in the ATT (Ile) codon to ACT (Thr) or GTT (Val). No other nucleotide changes outside this codon were observed. These findings demonstrate that a Thr to Ile substitution at codon 200 of HIV-1 p24 is sufficient to confer reactivity with an HIV-2 specific mAb. Although the structural consequences of this mutation are unknown, the exclusive presence of Thr at this position for HIV-1's and Leu for HIV-2's, as well as the spontaneous reversion of the Ile in our mutant HIV-1 to Thr or Val indicates strong selection pressures on gag gene products that are distinct for HIV-1 and HIV-2.

Q 420 STRUCTURAL ANALYSIS OF OVERLAPPING CYTOTOXIC T LYMPHOCYTE (CTL) EPITOPES IN A CONSERVED REGION OF THE HIV-1 ENVELOPE GLYCOPROTEIN, R. Paul Johnson, Alicja Trocha, Thomas Buchanan* and Bruce D. Walker, Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, 02114; and *Immunodiagnostics, Seattle, WA 98104.

Using CTL clones derived from HIV-1 seropositive subjects, we have identified a ten amino acid region in a conserved region of gp41 which contains overlapping epitopes presented by the HLA-B8 and B14 molecules. By analyzing the recognition of synthetic peptides with selected amino acid (aa) substitutions, we set out to define the effect of sequence variation in the context of two different HLA types and to determine the interaction of specific residues with either the HLA molecule or the T cell receptor. Peptides which contained both the B8- and B14-restricted gp41 CTL epitopes were synthesized containing aa substitutions either corresponding to natural sequence variation or containing serial single amino acid substitutions throughout the peptide. Analysis of variant peptides corresponding to natural sequence variation demonstrated that a single nonconservative aa substitution abolished recognition by both HLA-B8- and B14-restricted CTL. In contrast, a conservative substitution at this same position had little or no effect on recognition by CTL. Recognition of peptides with single serial aa substitutions depended in part on the presenting HLA molecule. Certain variants were recognized by HLA-B8-restricted CTL but not B14-restricted CTL and vice versa. Experiments are in progress to determine the ability of variant peptides to block sensitization by the index peptide, and these data should identify specific aa which interact either with the presenting HLA molecule or the T cell receptor. The results of these experiments emphasize the role that sequence variation in HIV-1 may play in generating variant viruses which escape immune recognition and may also aid in the identification of regions which may be useful in the design of a subunit vaccine.

Q 421 SAFETY AND ACTIVITY OF GLQ223 IN PATIENTS WITH AIDS OR ARC: REPORT OF A PHASE 1 STUDY, James Kahn, Rebecca Coleman, John Gambertoglio, Kenneth Gorelick, Paul Volberding and Roger Williams, Division of AIDS and Oncology, San Francisco General Hospital, Departments of Medicine and Pharmacy, University of California, San Francisco and San Francisco, CA 94110 and Genelabs Inc. 505 Penobscot Drive, Redwood City, CA 94063.

GLQ223 is a highly purified formulation of trichosanthin a 26-kDA single chain ribosome inactivating protein. In vitro exposure to GLQ223 (50 µg/L for 3 hours) inhibits HIV expression in chronically infected monocytes/macrophages. We conducted a phase 1B study to define the safety and pharmacokinetic profile of GLQ223 administered as a bolus with a prolonged steady state infusion. Escalating doses of GLQ223 were infused. Clinical laboratory, immunologic (CD3+, CD4+, and CD8+ lymphocytes and ß-2 microglobulin[ß-2M]) parameters were assessed. Twenty-one subjects participated in this study, 9 with AIDS and 12 with ARC. Dose levels (and number of subjects) were as follows: 8 µg/kg (3), 16 µg/kg (3), 24 µg/kg (6), 36 µg/kg (6) and 50 µg/kg (3). Pharmacokinetic evaluation based on the initial drug infusion was consistent with a biexponential model. GLQ223 plasma level of at least 50 µg/L were sustained for at least 3 hours at the two highest dose levels. No deaths or new or recurrent opportunistic infections occurred during the study. Recurrent HSV and HZV were noted. No CNS events were observed. A flu like syndrome characterized by muscle aches, throat pain, low grade fevers and chills associated with an increase in the CPK (MM fraction) developed in all subjects. It was easily controlled. All other clinical laboratory parameters were unchanged by GLQ223 administration. Immunologic parameters were assessed as change in parameter AUC from baseline. In this method, each value is plotted vs time, trapezoids are constructed and their area determined. A rectangle is plotted using the baseline determinations and its area subtracted from the sum of the trapezoids. The AUC's of CD3+ and CD8+ cells and ß-2M showed dose dependant increases. The CD4+ cells showed a non-significant tendency to increase with dose. GLQ223 has an acceptable safety profile and appears to show evidence of dose-dependent activity in HIV infected individuals.

Q 422 Diversification of HIV-1 strains after infection from a unique source

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In 1990 eight hemophilia B patients concurrently developed HIV-1 antibodies in spite of antibody screening of the plasma donors and of ß-propiolacton-UV inactivation of the clotting factor concentrate. All data pointed to one lot of clotting factor as the unique source of infection due to a contamination apparently originating from one seronegative, highly viremic donor (1). The accident is the only occasion known so far, where sequence data from a number of HIV-1 strains derived from different patients infected from a unique source could be analyzed immediately after infection.

Surprisingly, in spite of the high viremia of the donor and the early replicative phase of infection the recipients had passed through, the hypervariable regions V1 and V2 of the env gene derived from five patients immediately after seroconversion had 100 % homology. The strains of two other patients had two and five deviations, respectively, resulting in a homology of the seven strains between 100 % and 97.5 % (2).

To determine the degree of diversification of the prevalent viral genome, the patients were follwed up in the early 'latent' phase of HIV-1 infection. Again, V1 and V2 and a part of C2 of the env gene were amplified and direct sequencing of PCR products was carried out. The proviral DNA fragments of the patients still showed a very high degree of homology to the former consensus sequence. The sequence of the strain originally presenting two expressed substitutions displayed two more deviations nine months after the first investigation. The expressed mutations resulted in an alteration of the predicted secondary structure of the corresponding protein fragment. In addition, the virus which could originally not be cultivated, could now be cultivated as a low cytopathogenic agent.

These results suggest that
- the source of infection was unique
- the infecting virus was uniform
- the high genetic variability of HIV-1 cannot be demonstrated in the early replicative and the following 'latent' stage of infection
- a higher degree of diversification may become apparent in the later replicative stage of infection

(1) Kleim et al. (1990), Thrombosis and Haemostasis 64, 336-337
(2) Kleim et al. (1991), AIDS Res. Hum. Retrovir. 7, 417-427

Q 423 SERUM-CIDAL INACTIVATION OF HIV-1 AS A MECHANISM OF IRREVERSIBLE NEUTRALIZATION.

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Some sera from HIV-1-infected people irreversibly inactivate HIV-1, markedly reducing its infectivity titer for target cells. Inactivation is distinguished from conventional neutralization (which for these purposes, we define as the blocking activity of sera that occurs in the presence of target cells) in that it occurs before exposure to cells, occurs optimally at 37°C with kinetics that are considerably slower than binding kinetics, and does not occur at 4°C. Inactivation is mediated by the IgG fraction of serum and does not require a Ca^{++}-dependent or heat-labile cofactor. Several inactivation-sensitive epitopes in the carboxyterminal region of gp120 were identified using monospecific antisera and monoclonal antibodies. Neutralizing, conformation-dependent antibodies that block gp120 binding to CD4 did not mediate inactivation. This was surprising because soluble CD4 (which can be considered the optimal ligand for the CD4 binding site on gp120) does irreversibly inactivate HIV-1. Type-specific anti-V3 antibodies mediate inactivation in a type-restricted manner. However, the serum activity is broadly reactive in that diverse isolates are inactivated. A panel of 60 serial specimens from 15 HIV-1-infected men, 7 of whom went on to develop clinical AIDS and 8 of whom have remained stable, were tested in the inactivation assay and in a conventional neutralization assay. Serum-cidal activity, but not neutralization titer, was significantly lower in those who progressed compared to those who did not.

Q 424 LINEAR AND CONFORMATIONAL NEUTRALISING EPITOPES OF SIV ENVELOPE IDENTIFIED BY MONOCLONAL ANTIBODIES. Kent KA[1], Corcoran T[1], Rud E[2], Powell C[1], Thiriart C[3], Collignon C[3,4] and Stott EJ[1]. 1. National Institute for Biological Standards and Control, Potters Bar, Herts, UK. 2. Wellcome Research Laboratories, Langley Court, Beckenham, Kent, UK. 3. SmithKline Beecham Biologicals sa, B-1330 Rixensart, Belgium. 4. New England Regional Primate Centre, Southborough, MA, USA.

A panel of 28 monoclonal antibodies (MAbs) to SIV envelope glycoproteins have been generated and characterised. The MAbs identify at least 10 epitope groups by competition analysis and peptide mapping. Eight of the groups contain MAbs which recognise gp120 and the MAbs in the remaining two groups recognise gp32. Five MAbs neutralise the infectivity of SIVmac251 (KK5, 9, 10, 17 and 54) and these belong to three epitope groups. KK10 and 54, which show the weakest neutralising activity, map to amino acids 171-190 (the V2 region) of gp120. KK5 and 9 immunoprecipitate native gp120, they do not react in western blot (WB) and do not react with a series of overlapping peptides which span the whole of gp120. KK5 and 9 are in the same competition group. However, KK9 reacts only with SIVmac251 whereas KK5 is more broadly cross-reactive suggesting that the epitopes recognised are not identical. KK17 reacts well in WB, binds to an N-terminal fragment of gp120 expressed in E. coli (aa 8-303) but does not react with the overlapping peptides. The data suggests that MAbs KK5, 9 and 17 may recognise discontinuous or conformational epitopes. MAbs mapping to a region equivalent to the HIV-1 V3 loop (aa 321-340) did not show consistent neutralising activity.

Q 425 A simian mucosal model of induction of a dual peripheral mucosal and central systemic immunity to SIV antigens.
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Vaginal infection with the simian immunodeficiency virus (SIV) can cause simian AIDS in macaques. The objectives of this project were to elicit a mucosal immune response by an augmented oro-vaginal route of immunization in 17 macaques. The vaccine used was SIV gag p27 protein hybridized to the yeast retrotransposon virus-like particle (Ty-VLP). In order to elicit local cervico-vaginal and oral immune responses the hybrid vaccine was linked to cholera toxin B subunit (CTB). Sequential, 2 monthly oral immunizations of 500µg of p27 Ty-VLP linked to CTB and delivered in gelatin capsules, were followed by 3 vaginal immunizations of 200µg of the vaccine applied in solution to the mucosa. The sequence was reversed in another group of macaques, with 2 vaginal followed by 3 oral immunizations. Control animals were immunized with the Ty-VLP linked to CTB, p27 Ty-VLP without CTB or p27 linked to CTB. IgA and IgG antibodies were assayed in serum, saliva and vaginal washings by ELISA. Vaginal IgA and IgG antibody titres increased from 0 to 1:2-1:16 in all 4 macaques immunized by the vagino-oral route with p27 Ty-VLP linked to CTB, compared with titres of 0 to1:2-1:4 immunized by the oro-vaginal route. Rectal antibodies were detected only in 2/7 macaques but an increase in salivary IgA anti-p27 antibody titre (1:8-1:64) was found in all macaques. Significant increases in serum anti-p27 antibodies of 1:200-1:1600 were found in all macaques and the titres of IgA and IgG antibodies were similar, unlike the predominantly IgG antibodies found in systemic immunization. A significant increase in T cell proliferative response was also found in all macaques, when stimulated in vitro with p27 but not with Ty-VLP. These are circulating $CD4^+$ cells which can induce in vitro specific IgA and IgG anti-p27 antibody synthesis when reconstituted with B cells and macrophages. The results suggest that vagino-oral immunization with the hybrid p27 Ty-VLP linked to CTB elicits vaginal, salivary and serum IgA and IgG anti-p27 antibodies and circulating CD4 cells sensitized to p27. The vagino-oral route of immunization in macaques can now be used to investigate prevention of SIV infection by the vaginal route.

Q 426 GENERATION AND MAINTENANCE OF SUPPRESSION OF VIRAL REPLICATION IN SIV INFECTED MONKEYS, Barbara Lohman[1], Koen vanRompay[1], Ross Ramos[1], J.D. Kluge[1], Marta Marthas[1], and Niels Pedersen[2], [1]California Regional Primate Research Center, [2]Department of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616 CD8 mediated suppression of viral replication has been presented as a possible mechanism for controlling virus load in SIV infected monkeys. We developed an in vitro screen for suppression of SIV replication in SIV infected macaques. Briefly, washed whole blood from SIV infected monkeys was infected with cell free SIVmac1A11, washed twice, and resuspended in complete media supplemented with Interleukin 2 and Staphlococcus Enterotoxin A. Cultures were sampled twice weekly for detection of major core protein by antigen capture ELISA and maintained for 14 days. CD8 mediated suppression was measured by reconstitution of CD4 cells and CD8 cells isolated by immunomagnetic separation. The suppression of virus replication by whole blood was the same as observed for the CD8+CD4 subset reconstitution. Whole blood suppression was used to follow the responses of 40 monkeys infected with SIVmac239, SIVmac1A11, or 1 of 4 239/1A11 chimeras. All monkeys were screened prior to infection for levels of SIV production following in vitro infection. Five monkeys were low producers. Of the 5 low producers, 4 had maximal suppression after infection while 1 consistently had low levels of suppression and died with simian AIDS 8 months post infection. Suppression was associated with early infection and remained strong in monkeys with persistent or intermittent viremia. Even with persistent viremia there was variability among animals for ability to suppress SIV replication. Suppression was lowest in monkeys with transient viremia. Suppression of viral replication appears to be a consistent response to SIV infection, the strength of which is associated with the degree of viral load in peripheral blood.

Q 427 MAPPING THE HUMORAL RESPONSE IN HIV-1 INFECTED PATIENTS POST GP-160 IMMUNIZATION,
Lawrence D. Loomis[1], Felecia Mann[1], Robert R. Redfield[2], and Deborah L. Birx[2], [1]Retroviral Research Laboratory, Henry M. Jackson Foundation, and [2]Department of Retroviral Research, Walter Reed Army Institute of Research, Rockville, MD 20850

The entire sequence of HIV-1(LAI) gp160 was constructed using sets of 12-mer peptides overlapping by 8 amino acids. These peptides were used to Geysen map ("PEPSCAN") humoral responses in early stage, HIV-1 infected patients undergoing vaccine therapy with gp160 (IIIB, from baculovirus, MicroGeneSys). Two sets of matched peptides were used so that ELISA assays could be run on pre- and post-immunization serum simultaneously on the same Geysen block. Changes in antibody response throughout the envelope and transmembrane proteins were documented after immunization. The greatest responses were to the following regions: V3, N-terminus of gp 120, and the gp41 immunodominant region, in accordance with previous results on antibodies found during the natural course of disease progression. In addition to these, however, seroconversions to every region, whether constant or variable, were found. This included constant region 2, against which human antibodies have not previously been documented. Immunoblots employing fusion proteins of HIV-1 envelope sequences and end-point titrated peptide ELISA's were used to quantitate the magnitude of the new responses post immunization.

Q 428 **Activity of Cocktail V3-Peptide-Octamer Antisera.** David J. Looney[1,3], Chang Yi Wang[2], Ming Li[2], Alan Walfield[2], Barbara Hosein[2], Patricia Badel[3], Flossie Wong-Staal[3]. [1]San Diego VA Medical Center; [2]United Biomedical, Inc.; [3]Departments of Medicine and Biology, University of California San Diego.

Methods: A heptalysine-octamer multiple antigen peptide (MAP) system was used to present a 'cocktail' of HIV-1 (IIIB, MN, SC, RFII, WMJII,and SF2) V3-loop peptides using complete Freund's Adjuvant (CFA) or alum. Immunoreactivity was assayed using anti-peptide ELISA and western blotting. Neutralization activity (NA) of sera was assessed using syncytial focus and immunofluorescent cell focus assays, and the ADCC activity of sera was explored. **Results**: Octameric presentation of antigens produced higher and more persistent titers and elicited equivalent responses with either alum or CFA. Neutralization of homologous strains of HIV-1 rose to >1:19,000 after late boost (163 weeks). Cross neutralization of HIV-1 was found using monospecific and polyvalent octameric immunogens. Some sera displayed low titer (1:16-1:64) neutralizing activity against several wild-type isolates.
Comments: These observations suggest that V3-core cross-reactivity may be promoted by the use of the heptalysine-octamer MAP approach. Persistence of reactivity suggests that the fate of these synthetic immunogens *in vivo* may differ considerably from that of normal protein antigens presented with conventional adjuvants. V3-heptalysine octamer immunogens may represent one feasible approach to an HIV vaccine.

Q 429 **CD4-MIMICKING ANTIBODIES IN HIV-POSITIVE AND NORMAL HUMAN SERA.** Karin Lundin, Viveca Holmberg, Eric Sandström and Hans Wigzell. Departement of Immunology, Karolinska Institute, and Gay Mens Clinic, South Hospital, Stockholm, Sweden.

Most HIV+ human individuals make antibodies against the envelope protein gp120. Some of these antibodies may have an epitope that sterically resembles the CD4-molecule (the cellular receptor to which the virus bind via gp120) in the gp120-binding region. An earlier study (Lundin et.el. Scand.J.Immunol.27,1988) have shown that around 5% of HIV+ human sera contains such antibodies, seen as being anti-idiotypic to the anti-CD4 monoclonal antibody (mab) T4.2.

Here we will present the result from a study were sera from 208 HIV+ individuals and 204 healthy blood donors have been screened for the presence of this type of "CD4-mimicking" antibodies by using both the T4.2 mab as well as the OKT4A anti-CD4 mab. This study show the presence of two different CD4-like anti-gp120 antibodies reacting with either T4.2 or OKT4A. The frequency of anti-idiotypic antibodies to T4.2 was significantly higher in HIV-infected individuals compared to the control sera ($p<0.05$), but no correlation between the presence of this type of antibodies and clinical classification could be seen. Regression analysis of total CD4+ cell number and of % CD4+ cells will be done for each anti-idiotype positive individual as well as for "matched" controls of HIV-infected anti-idiotype negative individuals, to evaluate any prognostic value of this type of ab.

Q 430 ENHANCED PRESENTATION OF GP120 IN BI- OR TRI-MOLECULAR COMPLEXES TO SPECIFIC HUMAN T CLONES. Fabrizio Manca, Maria T.Valle, Daniela Fenoglio, Anna L. Kunkl, Susan Zolla-Pazner and Franco Celada. Dept. Immunology, Univ. of Genoa, 16132 Genoa, Italy; Dept. of Pathology, NYU, 10010 New York; Inst. Mol. Immunology, HJD-NYU, 10006 New York.

Recognition of HIV antigens by T cells is essential for antibody production and for CTL expansion. Procedures to enhance antigenicity of viral products for T helper cell expansion are desirable in a vaccine perspective. Since FcR on antigen presenting cells (APC) enhances uptake of antigens as immune complexes with potentiation of T cell activation, we constructed different gp120 containing immune complexes as bi- and tri-molecular structures.

Bi-molecular complexes contained gp120 and human Mabs. Tri-molecular complexes contained gp120 in association with gp41 or with CD4 plus anti gp41 human Mab or anti CD4 murine Mab respectively. APC were autologous PBMC and the responding gp120 specific T clones were obtained from a human T line specific for an immunodominant peptide of gp120 (aa 235-250).

A dose of gp120 that was ineffective for APC pulsing (<1 ug/ml) became antigenic in vitro when in complexed form. Facilitated uptake and T cell activation was detected also with murine Mab that binds CD4 without competing with gp120.

Antigenicity of gp120 for T cells can be enhanced by direct or indirect complexing with Mabs. This allows enhanced uptake of complexed antigen via FcR on APC, as well established in several experimental systems. It can be proposed that this physiological pathway may also be exploited in a vaccine perspective, in order to expand the specific T cell repertoire and to increase the chances of productive encounters between T helper and B cells specific for HIV antigens.

Q 431 ANTIBODY CLASS AND SUBCLASS RESPONSE TO HIV-1 INFECTION, Dean L. Mann,[1] Gina Hamlin-Greene,[2] Karen Banks,[2] and James J. Goedert[3]. [1]Laboratory of Viral Carcinogenesis, NCI-FCRDC, Frederick, MD, [2]BCDP, Program Resources, Inc./DynCorp, NCI-FCRDC, Frederick, MD, [3]Environmental Epidemiology Branch, NCI, Bethesda, MD 20894

This study was undertaken to assess the breadth of humeral immune response to HIV-1 by determining the immunoglobulin class and subclass antibodies in sera from infected individuals. HIV-1 proteins, gp160, gp120, p66, and p24, produced by recombinant technology, were individually bound to different sized polystyrene beads and incubated with dilutions of serum from 47 HIV-1 seropositive individuals obtained at a single time point and from 7 individuals at various times during the course of their disease. After washing, the beads were then incubated with fluoresceinated Ig class and subclass antibodies IgA_1, IgA_2, IgD, IgE, IgG_1, IgG_2, IgG_3, IgG_4, and IgM, the beads washed and analyzed by flow cytometry, (gating on the different bead sizes to discriminate the differential reactivity). In the total populations, all antibody classes and subclasses were represented to the various proteins with different combinations of class and subclass antibodies in the various individuals. The frequency of reactions in the total population (%) to each of the proteins is summarized as follows:

Protein	IgA_1	IgA_2	IgD	IgE	IgG_1	IgG_2	IgG_3	IgG_4	IgM
gp120	50	12	27	29	75	39	49	10	57
gp160	49	12	51	30	92	37	54	13	59
p66	24	9	38	9	49	9	24	9	NA
p24	20	6	53	0	48	35	29	21	NA

In the longitudinal study, a broadened class and subclass response appears to correlate with disease progression. A unique finding in this study is the presence of circulating IgD antibodies to viral proteins. These results have implication in understanding the nature of the immune response to HIV-1 infection since Ig class and subclass antibodies have different functions and tissue/fluid distribution. These are factors which may control virus spread in an individual and from individual to individual.

Q 432 **IMMUNOGENIC ANTIGEN-PHOSPHOLIPID COMPLEXES AGAINST IMMUNODEFICIENCY VIRUSES.** Raphael J. Mannino and Susan Gould-Fogerite, Department of Laboratory Medicine and Pathology, UMDNJ-New Jersey Medical School, 185 South Orange Avenue, Newark, New Jersey, 07103-2714. In the course of natural infection, the immune system oftentimes encounters antigenic determinants presented in the context of a lipid matrix. Hence, one approach to the formulation of prophylactic and therapeutic immunogens is to reconstitute important pathogen determinants into a lipid matrix so as to mimic the structural motifs that the immune system has evolved to recognize. Our laboratory has used this concept to construct a variety of HIV and SIV antigen-phospholipid complexes. i) "Mock" virus particles (MVP) can be prepared by disrupting the virion with detergent, removing the viral genetic information and reconstituting the viral proteins into a lipid bilayer. SIV-MVPs have been used to induce SIV specific antibodies in mice. ii) On the other extreme, small synthetic peptides representing HIV or SIV specific B cell, T helper cell and cytolytic T cell determinants have been formulated into phospholipid complexes which induce epitope-specific anitbody and cell-mediated immune responses. These peptide phospholipid complexes (PPC) are immunogenic in the absence of additional adjuvants or carrier proteins. iii) The envelope proteins of several viruses have been shown to exhibit strong adjuvant properties. Through combining aspects of MVPs and PPCs we have obtained simple, well defined and powerful immunogens.

Q 433 HIV-1 GAG- AND RT-SPECIFIC CYTOTOXIC T-CELL LINES DERIVED FROM A PERINATALLY INFECTED CHILD. E.J. McFarland, T.J. Curiel, D.J. Schoen, M.E. Rosandich, R.T. Schooley, D.R. Kuritzkes. Division of Infectious Diseases, University of Colorado Health Sciences Center and VA Medical Center, Denver, CO 80262.

Cytotoxic T-lymphocytes (CTL) specific for human immunodeficiency virus type 1 are thought to play an important role in controlling HIV-1 infection. Although HIV-1-specific CTL's are readily demonstrated in unstimulated peripheral blood mononuclear cells (PBMC's) of infected adults, they are less frequently detected in PBMC's from perinatally infected children. Using the CD3-specific monoclonal antibody 12F6 to expand T cells in the absence of specific antigen we have derived HIV-1-specific CTL lines from a long-term survivor of perinatally acquired HIV-1 infection. CTL activity was assessed using autologous EBV-transformed B-lymphoblastoid lines infected with recombinant vaccinia vectors that expressed relevant HIV genes as targets in a chromium-release assay. The specific cytotoxicity of three gag-specific lines and one reverse transcriptase (RT)-specific line studied in detail were as follows:

% Specific 51-Chromium release (E:T ratio 10:1)

	Targets		
Clone	vac/lac	vac/gag	vac/pol
BF2	0	55	ND
CC5	0	25	ND
DC12	0	39	ND
CB5	4	ND	43

Cytotoxicity was MHC Class I restricted and was blocked by antibody to the T-cell receptor complex. Analysis of cell surface markers by fluorescence-activated cell sorting demonstrated the CD3+CD8+CD4- phenotype of these cells. Of note, HIV-specific cytotoxicity of unfractionated, unstimulated PBMC's from this patient was less than 5% when tested against HIV-1 env, gag, or RT-expressing targets. Our results suggest that children with perinatally acquired HIV-1 infection, like adults, can develop HIV-specific CTL responses, even though this CTL activity may not be detectable in unstimulated PBMC's. Further studies of the HIV-specific CTL response in perinatally exposed children may provide insights into the mechanism of perinatal transmission, the interaction of HIV with the immature immune system, and the role of cellular immunity in the progression of HIV disease in children.

Q 434 HETEROGENEITY OF THE V3 LOOP OF THE HIV-1 ENVELOPE SEQUENTIALLY DERIVED FROM FOUR ADULTS. T. McNearney*, Z. Hornickova*, A. Birdwell*, M. Arens*, R. Markham#, and L. Ratner*, *Depts of Medicine, Molecular Microbiology, and Pediatrics, Washington University School of Medicine, St. Louis, Mo. and #School of Hygiene and Public Health, Johns Hopkins School of Medicine, Baltimore, Md.

To assess the role of naturally occurring envelope divergence, DNA was isolated from peripheral blood lymphocytes derived from four unrelated adults and the sequences encoding the V3 region of envelope (nucleotides 6578-6917), were amplified and sequenced. Blood samples reflected a gradual dimunition in the CD4 count over 3-4 years, with values that ranged 1000 to less than 200. Comparison of the various clones with HXB2 revealed an overall amino acid sequence divergence of 11- 24% among representative samples from all four patients. Sequence analysis among the clones derived from the same patient demonstrated predicted amino acid divergences of 0-18%. Among clones from the same blood sample, sequence divergence derived from the early and late period samples ranged between 0-.011% and 0-15%, respectively. This increase in sequence heterogeneity was correlative with the decreasing CD4 counts noted in the later blood samples. In several clones, predicted amino acid changes altered residues of the V3 loop previously shown to influence in vitro biological properties. The V3 loop sequences GPGRAF were well conserved in the majority of clones. Cysteine positions were strictly conserved in all clones. Several clones demonstrated a loss or gain of predicted N-glycosylation sites over time. Comparison between the V3 region and NEF sequences demonstrated a greater divergence in the V3 loop. The wide range of V3 loop variation in naturally occurring isolates suggests that this region can tolerate a large amount of heterogeneity and this sequence diversity may include nonrandom changes important for disease progression.

Q 435 GENERATION OF PRIMARY, HIV-1-SPECIFIC T CELL RESPONSES IN VITRO, Anita Mehta, Sergiusz Markowicz and Edgar G. Engleman, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

Little is known about the interaction between antigen presenting cells and HIV-1 epitopes that activate antigen-specific T lymphocytes in previously uninfected individuals. We seek to address this problem through the development of a method for generating and analyzing primary human T cell responses to foreign antigens, in vitro. Recently, we reported that blood derived dendritic cells (DC) are extremely potent stimulators in the autologous and allogeneic mixed lymphocyte reactions (J. Clin. Invest. 85:955-961, 1990). On the basis of these findings, we have been exploring the ability of antigen-pulsed DC to generate primary T cell responses to foreign antigens. In initial studies DC obtained from healthy volunteers were pulsed with two model antigens, keyhole limpet hemocyanin (KLH) or sperm whale myoglobin (SWM), and co-cultured with fresh autologous T cells obtained from individuals not previously sensitized to these antigens. Using this approach, both KLH and SWM specific T cell lines were generated. In contrast, purified monocytes from the same individuals pulsed with the same antigens failed to generate antigen-specific lines. In our most recent studies, blood DC from healthy HIV-seronegative volunteers were pulsed with synthetic peptides based on sequences derived from immunogenic HIV-1 gag regions, and co-cultured with autologous CD8+ T cells for a period of 6-8 weeks. Thereafter, the expanded cells were harvested and examined for their ability to lyse autologous, HIV-1 peptide coated macrophages or DC. T cell lines generated in this manner demonstrated potent HIV-1 specific, HLA class I restricted killing. On the basis of these findings, we believe that a detailed analysis of the primary human T cell response to a variety of foreign antigens, including HIV proteins, is possible. Such information should prove useful in the development of an AIDS vaccine.

Q 436 HIV-1 MAJOR NEUTRALIZING DETERMINANT EXPOSED ON HBsAG PARTICLES IS IMMUNOGENIC IN PRIMATES Michel Marie-Louise*, Schlienger Katia*, Mancini Maryline*, Dormont Dominique**; Rivière Yves*** and Tiollais Pierre*.*Unité de Recombinaison et Expression Génétique and ***Unité de Virologie et Immunologie Cellulaire, Institut Pasteur, Paris, **Ministère de la Défense, CRSSA, CEA, Fontenay aux Roses, France

Objective: The HIV-1 major neutralizing determinant is contained within a disulfide loop located in the envelope gp120 third variable domain (V3 loop). To increase its immunogenic potential, we have exposed this epitope (HIV-1 Bru strain) at the surface of a highly antigenic structure, the hepatitis B surface antigen (HBsAg).

Methods: Six rhesus monkeys divided in 2 groups received 2 intradermic injections at 1 month interval and 1 boost 8 months later of either V3/HBsAg or non hybrid recombinant particles, i.e. HBsAg (1 control animal per group). Immunization was potentiated with alum in one group and Syntex adjuvant in the other one.

Results: Immunized monkeys have developed persistent antibodies and T cell proliferative response against both part of the fusion protein (HBsAg and HIV-1). Antibodies neutralizing both virus infectivity and virus-induced cell fusion were detectable in 2 macaques after boosting. Moreover, HIV-1 specific cytotoxicity was found against autologous target cells expressing the HIV-1 env gp160 at their surface.

Conclusions: Since there is an overlap between populations at risk from hepatitis B virus (HBV) and HIV, HBsAg recombinant particles may be relevant carriers for HIV-1 major epitopes and could offer a new approach to the development of and AIDS vaccine.

Q 437 ***Mycobacterium avium intracellulare*** **INFECTION OF HIV-1 INFECTED HUMAN MACROPHAGES RESULTS IN DECREASED MONOKINE PRODUCTION AND CELL DEATH,** Gale W. Newman, Hui-Xian Gan, Theresa G. Kelley, Osama Kandil and Heinz G. Remold, Department of Rheumatology & Immunology, Harvard Medical School and Brigham & Women's Hospital, Boston, MA 02115.

Human peripheral blood derived macrophages were infected with HIV-1, BaL strain, for 14 days then infected with *Mycobacterium avium intracellulare (MAI)*, serovar 4, or treated with LPS. At 4, 24, 48 and 72 hrs post *MAI* or LPS treatment, Mφs were harvested for mRNA preparation and supernatants collected for cytokine protein analysis, p24 and reverse transcriptase (RT) activity. LPS induced a rapid (4 hr) production of steady state mRNA for TNF-α, IL-1ß and IL-6 in both HIV-1 infected and noninfected Mφs. In contrast, coinfections of HIV-1 and *MAI* had a 24 hr delayed induction of TNF-α, IL-1ß or IL-6 mRNA transcripts as well as protein when compared to HIV-1/LPS stimulated and LPS stimulated only Mφs. In addition, mRNA and protein production of the cytokines remained higher, up to 72 hr, in the HIV-1/*MAI* infected cells than in HIV-1/LPS, HIV-1 alone, *MAI* alone or LPS only treated Mφs. No differences were found in p24 or RT activity between HIV-1/MAI or HIV-1 infected Mφs. *MAI* infection also caused decreased viability of the HIV-1 infected cells with lysis occurring at 96 hr post *MAI* infection. No cell death was noted with HIV-1/LPS, LPS, *MAI* infected or control cells at this time point. We have found in previous experiments that survival of human Mφs infected with *MAI* correlates with rapid and increased production of TNF-α and IL-6. HIV-1 infection of human Mφs appears to retard cytokine induction in cells coinfected with *MAI* and this reduction correlates with decreased viability of the cells. This dysregulation of one or several monokines and subsequent cell death in HIV-1 infected Mφs coinfected with *MAI* may thus further increase the pathogenesis of AIDS. Supported by NIH grants HL43510 and AI31006.

Q 438 EFFECTS OF MEASLES IMMUNIZATION ON T CELL SUBSETS IN HIV-INFECTED PERSONS, Janet K.A. Nicholson, Mary Ann Sprauer, Laurie E. Markowitz, Robert C. Holman, and Bonnie M. Jones, Centers for Disease Control, Atlanta, GA 30333

During a recent measles outbreak in an institution, measles vaccine (live measles-mumps vaccine) was offered. Since several of people who where potentially exposed to measles were infected with HIV, T cell subsets were monitored to determine what effect immunization had on these subsets. Thirty-nine HIV-infected and 19 control subjects consented to be immunized and donate blood for the study. Blood was drawn immediately preceding and 3 weeks after the immunization, separated, and frozen. Specimens were paired and analyzed for T cell subsets. In each run, at least one control subject was included. In both control and HIV-infected subjects there was a decrease in percent CD3+ and CD8+ cells after immunization, though the decrease was similar in both groups. There was an increase in CD20+ cells in both groups, but again, this change was similar in both groups. The only subpopulations in which the changes were different between the groups was in the CD8+DR+ and the dull CD8+CD11b+ populations (natural killer cells). In the HIV-infected subjects, there was a decrease in CD8+DR+ cells, whereas in the control subjects, there was little change in this parameter. The decrease in CD8+ cells in the control subjects was due to a decrease in the CD8+DR- cells. Vaccination to measles did not result in any profound changes in T cell subsets in either the HIV-infected subjects or the controls, and therefore was determined not to be deleterious to the immune status of HIV-infected subjects.

Q 439 EXPRESSION OF DISTINCT PHENOTYPES OF HIV-1(MN) BY CLONED, VIRUS-INFECTED CELL LINES. Stephen M. Nigida, Jr., Carole H. Smith, and Larry O. Arthur. AIDS Vaccine Program, PRI/DynCorp, National Cancer Institute--Frederick Cancer Research & Development Center, Frederick, Maryland 21702.

Due to inaccurate reverse transcription and to somatic mutations, continuous propagation of HIV-1 in cell lines *in vitro* can result in the generation of multiple virus genotypes and in the accumulation of diverse virus phenotypes. In attempts to obtain cell lines producing genetically and phenotypically homogenous virus, HIV-1(MN)-producing H9 cells were cloned by limiting dilution and assayed for the expression of HIV-1. One hundred twenty-four cloned cell lines were established; eight virus-producing clones were identified and propagated *in vitro*. Examination by electron microscopy revealed mature HIV virions, in varying quantity, in the seven clones examined. All eight clones produced HIV-1 antigens as detected by indirect immunofluorescence assays and excreted HIV-1 *gag*-product (p24) into the supernatant fluid as detected by enzymeimmunoassays: quantities of p24 produced varied between clones from 2 to 4,800 ng/ml at 24 hours' incubation and from 4 to 7,000 ng/ml at 72 hours' incubation. Only six of eight clones generated detectable levels of reverse transcriptase (RT) in the supernatant fluid: from 8.0×10^4 to 5.7×10^6 CPM/ml and from 1.2×10^5 to 4.9×10^6 CPM/ml at 48 and 72 hours' incubation, respectively. Undiluted supernatant fluids were assayed for their ability to infect cell lines and human peripheral blood mononuclear cells (PBMC) in vitro. Supernatant fluids from four of seven clones tested induced synthesis of RT and *gag*-product (p24) in H9, CEM-SS, and THP-1 cell lines, but only one induced synthesis of RT and p24 in PBMC through 32 days in culture. Further studies are being performed to examine the nature of the genetic information in and the virus proteins produced and processed by these cloned cell lines. HIV-producing cell cultures thus contain and express multiple virus genotypes/phenotypes which are evident upon examining individual clones of virus-producing cells.

Q 440 HIV SPECIFIC CTL INHIBIT HIV REPLICATION IN INFECTED MACROPHAGES, Douglas F. Nixon, Matt Collin, Sarah Rowland-Jones, Peter Illei, Siamon Gordon and Andrew J. McMichael, Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DU, U.K. and Sir William Dunn school of Pathology, Oxford, U.K.

HIV can infect mononuclear phagocytes, compromising their antigen presenting function, as well as forming an important reservoir of infected cells. Cell mediated immune mechanisms may be especially important for anti-viral immunity against HIV infected macrophages where virus is present in a transcriptionally latent form and where cell-cell infection probably occurs. In order to see if CTL could restrict the growth of HIV in macrophages, (as CTL have previously been shown to express anti-HIV activity against HIV infected T cells in vitro), macrophages from seronegative donors were infected with HIV and then incubated with CTL. Macrophages were isolated from HLA typed seronegative donor PBMC by adherence and cultured at approximately 5 X 10^5/well. HIV-BAL was added at day 6 (M.O.I. 5 X 10^{-3}) and CTL added on day 7. HIV specific CTL were generated by stimulation with mitogen activated autologous peripheral blood mononuclear cells (PBM) and restimulated on peptide pulsed-irradiated autologous B lymphoblastoid cell lines. Influenza specific CTL were used as control CTL. Both HLA matched and mis-matched CTL were used in these experiments. HIV production was monitored by serial p24 antigen measurements. Preliminary results show that HIV specific CTL, but not influenza specific CTL, can inhibit HIV replication in infected macrophages.

Q 441 HOMOLOGOUS NEUTRALIZING DOMAINS OF HTLV-I AND HTLV-II gp46 ENVELOPE GLYCOPROTEINS, Thomas Palker, Emily Riggs, Derry Spragion, Andrew Muir, Aine McKnight, Paul Clapham, Robin Weiss and Barton Haynes, Duke University Medical Center, Durham, NC and The Institute of Cancer Research, Chester Beatty Laboratories, London, U.K. Twelve synthetic peptides (SP-1,2,3,4,4A,5,6,7,8,9,10,11) containing hydrophilic amino acid sequences of human T-cell lymphotropic virus type I (HTLV-I) envelope glycoprotein were coupled to tetanus toxoid and used to raise epitope specific antisera in goat and rabbits. All anti-peptide antisera specifically bound to immunizing peptides in RIA and ELISA, while 10 of 12 sera bound either native or denatured forms of HTLV-I envelope in RIPA and Western blot assays. Low neutralizing antibody titers raised to peptides SP-2 (amino acids 86-107), SP-3 (176-189) and SP-4A (190-209) as well as to combined peptide SP-3/4A (196-209) were detected in the VSV/HTLV-I pseudotype assay. To elicit higher titered neutralizing antibody responses, two goats (#20,21) were immunized with a combined inoculum containing peptides SP-2, 3, and 4A linked to tetanus toxoid. Neutralizing antibody titers in these 2 animals ranged from 1/40 - 1/640 in the pseudotype assay and 1/20 - 1/320 in the syncytium inhibition assay. Neutralizing antibodies could be absorbed with peptide SP-2 (a.a. 86-107) as well as a truncated peptide containing envelope amino acids 88-98, but not with equimolar amounts of peptides SP-3/4A or SP-7. To map critical amino acids that contributed to virus neutralization within amino acids 88-98, each amino acid was sequentially replaced by alanine. The resulting 11 synthetic peptides with single amino acid substitution were then used to absorb 3 neutralizing goat anti-peptide antisera (#20,21, and 128). Asparagines at positions 94 and 96 were required for absorption of neutralizing antibodies from all 3 sera; the lysine at position 90 (sera #21, 128) and the proline at position 92 (serum #20) were also found to be important. The homologous region of HTLV-II envelope glycoprotein contained within amino acids 82-97 (PHWIKKPNRQGLGYYS) elicited anti-peptide, neutralizing antibodies to HTLV-II that were type-specific. Thus, amino terminal regions of HTLV-I (a.a. 88-98) and II (a.a. 82-97) gp46 env protein both contain linear, neutralizing determinants.

Q 442 A RETROSPECTIVE STUDY OF T CELL SURFACE PHENOTYPE IN CHILDREN WITH AN INITIAL CLASSIFICATION OF P-0, Susan Plaeger-Marshall, Patricia Hultin, Jeanne Bertolli, Sheryl O'Rourke, Janis V. Giorgi, E. Richard Stiehm, and Yvonne J. Bryson, Departments of Pediatrics, Medicine, and Biomathematics, UCLA School of Medicine, Los Angeles, CA 90024

The early diagnosis of HIV infection in at-risk infants born to HIV seropositive mothers (i.e., CDC classification P-0) is critical to timely therapeutic intervention. In order to identify lymphocyte phenotypes with potential usefulness as surrogate markers for infection, we performed two-color immunofluorescent analysis of T cell surface antigens on peripheral blood samples from 23 P-0 children ranging in age from <1 to 11 months at time of testing. The results were compared retrospectively when the subjects' HIV status became known (5 infected and 18 uninfected). The antigens assessed included the differentiation marker CD45RA on CD4 and CD8 cells, the Leu 8 homing receptor on CD4 cells, and the activation markers CD38 and HLA-DR on CD8 cells. Our results indicate that prior to diagnosis, the infected children had *1)* decreased CD4 cell percents (45 vs. 53%), with a decreased proportion of $CD45RA^-$ cells within the CD4 population (12 vs. 16%) *2)* a decrease in Leu 8^+ CD4 cells (88 vs. 93%), *3)* increased CD8 cell percents (26% vs. 19%) reflected primarily in the $CD45RA^-$ subset (13 vs. 9%), *4)* a disproportional expansion of CD8 cells expressing CD38 (92 vs. 81%), and *5)* an increase in the proportion of $HLA\text{-}DR^+CD8$ cells (11 vs. 9%). We conclude that CD4 memory T cells are decreased and activated CD8 T cells, in particular $CD38^+$, are increased in HIV infection and are present prior to diagnosis in infected at-risk infants. Further study of these markers as surrogates for early diagnosis is warranted.

Q 443 HUMAN MONOCLONAL ANTIBODIES TO THE V3 LOOP OF GP120 MEDIATE VARIABLE AND DISTINCT EFFECTS ON BINDING AND VIRAL NEUTRALIZATION BY A HUMAN MONOCLONAL ANTIBODY TO THE CD4 BINDING SITE. Marshall Posner, Lisa Cavacini, Charlotte Emes, Jennifer Power, Miroslaw Gorny* and Susan Zolla-Pazner*, Department of Medicine, New England Deaconess Hospital and Harvard Medical School, Boston, MA 02215 and *Department of Pathology, New York University Medical Center, New York, NY 10016.

Interactive effects between human monoclonal antibodies specific for the V3 loop (V3-1 and V3-2) and a discontinuous epitope within the CD4 binding site (F105, IgG_1) of HIV-1 gp120 were evaluated using flow cytometry of HIV infected cells and neutralization of cell free virus. Cell surface binding was measured by preincubating cells with first antibody, washing and adding labelled test antibody at 50% maximal binding for flow cytometry. Simultaneous addition of 25% maximal neutralization titer of first antibody with titered quantities of test antibody were used in MT-2 cytotoxicity assays. Preincubation of RF infected cells with either V3 loop antibody, significantly enhanced the binding of F105 to these cells. No enhancement was observed by flow cytometry when F105 was added prior to the addition of V3 loop antibody. Neutralization of RF was most enhanced by the combination of F105 and V3-2. A slight additive effect was seen with the F105 and V3-1 pair. In contrast, no enhancement in antibody binding to MN infected cells was observed by flow cytometry regardless of antibody pairs examined. Similarly, no enhancement was observed in neutralization of this virus with these antibody pairs, although each antibody alone had significant neutralizing activity. Both neutralization results correlated with flow cytometry. Similar results have been observed with the SF2 strain. These data support the notion that either a conformational change occurs with binding of V3 loop antibodies which enhances the binding and neutralizing activity of antibodies directed to the CD4 binding site of gp120 or new antigenic sites are exposed by the V3 loop antibodies on cell surfaces and virions. In addition, flow cytometry results appear to predict neutralization effects in this system.

Q 444 SYNERGISTIC INHIBITION OF HIV-1, B.J. Potts, K. Field, Y. Wu, M. Posner*, L. Cavacini*, and M. White-Scharf. Repligen Corporation, Cambridge, MA 02139 and *Division of Hematology, New England Deaconess Hospital, Boston, MA

Three MN-V3 loop directed mMAb (59.1, 50.1, 83.1) with different epitopes, binding affinities and neutralization potencies were evaluated for their ability to synergize at varying ratios with rSCD4 or with a huMAb (F105) directed to the CD4 binding domain of gp120. Synergy was measured by 90% and 100% neutralization (SN) endpoints using the lab adapted HIV strain-MN grown in CEM-SS cells, an MN like monocyte isolate (AD-87) and two field isolates assayed in PBL's. Reduction in virus replication determined by the RT assay was analyzed by the dose-effect analysis under conditions where there was exclusive and non exclusive synergy, by determining fold increase in 90% endpoints of the combined reagents and by determining the reduction in $TCID_{50}$ of a characterized viral stock in the presence of the individual and combined reagents. Mechanisms of the resulting synergy was mathematically analyzed by determining the median effect plot, biochemically by using a whole virus ELISA and CD4 ELISA and biologically by FACS analysis of MN infected H9 cells.

Data from these studies suggest that 59.1 and 83.1, which bind to the tip of the V3 loop, when combined at ratios of 1:1 or 2:1 with F105 or rSCD4 resulted in a synergistic increase in SN of MN. The MAb 50.1 which binds to the left side of the V3 loop did not synergize with F105 at any ratio tested. These studies also demonstrated that both the V3 and non V3 reagents must neutralize individually at least weakly for synergy. In addition, the synergistic increase in SN is evident with field isolates in PBL's. The median-effect plot of the SN data and the FACS analysis suggest that the interaction between the V3 and non V3 reagents are mutually non exclusive which is consistent with the hypothesis that HIV-1 entry into a cell is a two step event. Results from the ELISA and virology studies suggest that F105 and rSCD4 are acting by a similar mechanism to facilitate an increase in the SN endpoint of the combined reagents. This is consistent with previous studies that report overlapping binding sites on the gp120 for both F105 and rSCD4. The SN potential of a V3 antibody when combined with rSCD4 or F105 can be increased by 10 to 1000 fold and will prove to be a very valuable HIV directed therapeutic.

Q 445 HIV IMMUNE GLOBULIN: EVALUATION OF PRE- AND POST-EXPOSURE EFFICACY IN CHIMPANZEE AND SCID-HU-PBL MODELS, Linda Andrus, Krishna K. Murthy and Alfred M. Prince, Laboratory of Virology and Parasitology, the Lindsley F. Kimball Research Institute of The New York Blood Center, New York, NY 10021 and the Southwest Foundation for Biomedical Research, San Antonio TX 78284

We have reported previously that an HIV immune globulin preparation (HIVIG) with a high titer of virus neutralizing antibodies protected chimpanzees against a challenge with about 100 TCID50 of HIV IIIb (Vaccines '91, Cold Spring Harbor; AIDS RESEARCH and HUMAN RETROVIRUSES, In Press).

To determine whether such a preparation might be effective in post-exposure prophylaxis of HIV infection, chimpanzees were inoculated intravenously with 100 TCID50 of HIV IIIb and then treated with 10 mL/Kg of HIVIG, or a control IVIG without anti-HIV antibodies, 1 or 4 hours later. Five month follow up results of this experiment will be reported. The results of analogous experiments carried out in vitro, and in the SCID-Hu-PBL model will also be reported.

Q 446 THREE-COLOR CYTOFLUOROMETRIC ANALYSIS OF CD8 CELL COEXPRESSION OF DR, CD38, AND CD57 IN HIV-1 INFECTION, Harry E. Prince and Eric R. Jensen, American Red Cross Blood Services, Los Angeles, CA 90006.

Published studies performed using 2-color flow cytometry indicate that DR+CD8, CD38+CD8, and CD57+CD8 cell subsets are increased in HIV-1 infection. It is unclear, however, to what extent these CD8 cell subsets overlap. We thus employed 3-color flow cytometry to assess DR, CD38, and CD57 coexpression by CD8 cells in cryopreserved mononuclear cells from uninfected controls and HIV-1-infected former blood donors in CDC classes II, III, and IV (N=12/group). These studies utilized PerCP-anti-CD8, either PE-anti-DR or FITC-anti-DR, PE-anti-CD38, and FITC-anti-CD57. When DR+CD8 cell subsets were assessed, both the CD57+ and CD57- subsets of DR+CD8 cells were increased in all 3 HIV groups, whereas only the CD38+, but not the CD38-, subset of DR+CD8 cells was increased. When analyzing changes in CD38+CD8 cell subsets, only the DR+ and CD57+ subsets, but not the DR- and CD57- subsets, were increased. Similarly, only the DR+ and CD38+ subsets of CD57+CD8 cells were increased. Taken together, these findings suggest considerable, but not total, overlap of the DR+CD8, CD38+CD8, and CD57+CD8 cell subsets often elevated in HIV-1 infection. To further characterize this overlap, 3-color cytofluorometric studies were recently initiated to analyze coexpression of DR, CD38, and CD57 by purified CD8 cells in the 4 study groups. These studies utilize PerCP-anti-DR, PE-anti-CD38, and FITC-anti-CD57. Our preliminary data confirm our earlier results, revealing an increased percentage of DR+CD38+CD57+ CD8 cells in HIV-1 infection. In addition, these studies reveal a marked increase in the DR-CD38+CD57+CD8 cell population, even though the total percentage of CD38+CD8 cells or CD57+CD8 cells that are DR- is decreased. Thus, it appears that two distinct subsets of CD38+CD57+CD8 cells, one DR+ and one DR-, are increased in HIV-1 infection. These findings may prove pertinent to studies characterizing CD8 cell subsets important in controlling HIV-1 infection.

Q 447 AUTOMATION OF AN HIV NEUTRALIZATION ASSAY USING PATIENT VIRUS, PBMC AND PCR DERIVED MOLECULAR ENDPOINTS. Merlin L. Robb, Victoria R. Polonis, Maryanne Vahey, John M. Wages,Jr., My Ngoc Tran, Arnold K. Fowler, Nelson L. Michael, Suzanne Gartner and Robert R. Redfield. Walter Reed Army Institute of Research, 13 Taft Ct., Rockville, MD. 20850.

The presence of serum antibodies which inhibit HIV infection in vitro has not been consistently associated with a favorable clinical outcome. Most neutralization assays have utilized prototype, laboratory adapted viral strains and neoplastic cell lines as targets. Neither the viral agents nor the target cells, therefore, are representative of natural infection. In addition, the endpoint derived from these assays, syncytia formation and p24 antigen, does not permit a quantitative comparison of several isolates against a particular antibody or serum. We have developed a neutralization assay which utilizes viral isolates derived from patients and peripheral blood mononuclear targets (PBMC). The endpoint of the assay is the number of infectious events as defined by the formation of full length genomic HIV DNA. Titered patient viral stocks are incubated with dilutions of serum prior to infecting PBMC. The infected PBMC are washed twice and seeded onto 48 well microtiter plates. 72 hours post-infection the cells are split and half of the cells are digested in lysis buffer and proteinase K to provide a DNA lysate for PCR. Primers from the gag region are employed in an automated, microtiter PCR format optimized to provide a linear relationship between DNA template and PCR product through a range of 30 to 10,000 copies of template. PCR product is quantified using automated HPLC. Copy numbers of HIV DNA are derived from standard curves which are generated within each PCR run by plotting the log peak area versus log cloned HIV DNA. Neutralization is expressed as percent reduction of infectious events in 10,000 PBMC compared to a normal human serum control.Equivalence of amplifiable, cellular DNA in each lysate is confirmed by amplification of a ß-globin sequence. The assay has been compared to other neutralization assays using serum and virus from patients in vaccine therapy trials and maternal-infant transmission pairs.

Q 448 ANTIBODY-DEPENDENT ENHANCEMENT OF SIV INFECTION: DOMAIN MAPPING.

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Complement-mediated, antibody-dependent enhancement (C'-ADE) of SIV infection has been described previously (Montefiori et al, J. Virol. 1990). The mechanism of C'-ADE for SIV infection has not been well-described; however, it is known that it involves antibodies to SIV and proteins of the complement system. For the analogous virus in humans, HIV, antibodies to two immunodominant domains of gp41 and proteins of the alternative complement pathway are required. For both HIV and SIV, cells bearing both complement receptors and CD4 are necessary. The SIV proteins that are involved in C'-ADE of SIV infection are not known; however, serum from macaques immunized with whole-inactivated SIV (Montefiori et al J. Virol. 1991) can mediate C'-ADE in vitro. In this study we demonstrate that animals immunized with either whole inactivated SIV or recombinant SIV envelope proteins have antibodies in their sera that mediate C'-ADE of SIV infection in vitro. In further studies, four monkeys previously infected with a nonpathogenic molecular clone of SIV, SmH4, were immunized with a synthetic SIV peptide homologous to the HIV primary enhancing domain, AA586-610. These animals developed a boost in their C'-ADE titers demonstrating that this is an enhancing domain for SIV. Furthermore, these sera can mediate C'-ADE of HIV infection suggesting a high degree of cross-reactivity between the HIV and SIV enhancing domains. These data reinforce the utility of the SIV-rhesus macaque model for studying immunopathogenesis of SIV and HIV in vivo. Further experiments in rhesus macaques to test the in vivo role of enhancing antibodies are currently underway.

Q 449 DISTINCT ANTIGENIC SITES ON HIV GP120 IDENTIFIED BY A PANEL OF HUMAN MONOCLONAL ANTIBODIES

James E. Robinson[1], Hironori Yoshiyama[2], Debra Holton[1], Steven Elliott[1], David D. Ho[2], 1.Departments of Pediatrics and Microbiology, LSU Medical Center, New Orleans, LA 70112; 2. Aaron Diamond AIDS Reseach Center and NYU School of Medicine, New York, NY 10016. Thirteen human monoclonal antibodies (HMabs) to HIV-1 gp120 were characterized with respect to their competitive binding patterns with each other or with soluble CD4. Seven distinct patterns which tentatively represent different antigenic sites on gp120 were observed and grouped as follows: I - 15E, 2.1H, 10E, F91; II - 1.7B, 4.8D; III - A32, 2.11C; IV - C11; V - 2.3B, 2.12A; VI -2.3A; and VII - 1.9B. HMabs underlined were derived from the same HIV infected patient, N70.

Only HMabs in group I compete strongly with soluble rCD4. Group II HMabs compete with group I HMabs but less well with CD4; in fact their binding to gp120 was enhanced by CD4. The binding of F91 (group I) was unusual in being also inhibited by both 2.11C and A32 (group III) but not vice versa. All HMabs in groups I - V bind to reduction-sensitive, glycosylation dependent conformational epitopes, whereas 2.3A (VI) and 1.9B (VII) bind to linear epitopes, located in the C-terminus and V3 region, respectively. HMabs 2.3B and 2.12A (group IV) bind to highly variant epitopes and 1.9B (group VII) binds only to strains containing MN-like V3 sequences. HMabs in all other groups bind to mostly conserved epitopes, although minor strain variation was observed with individual HMabs. Of the HMabs which have been tested for neutralizing activity, 1.5e, 2.1H, 1.7B, 4.8D (groups I and II) HMabs neutralize divergent strains of HIV, 1.9b (VII) neutralizes strains with MN-like V3 regions, 2.3A (VI) has poor neutralizing activity. The remaining HMabs are currently being evaluated for neutralizing activity.

Q 450 Use of bisbecific antibodies to systematically evaluate the role of FcγR and various myeloid cell-surface molecules in the process of monocyte and macrophage HIV-1 infection

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As with other lentiviruses, monocytes and macrophages are known to be targets for HIV-1 infection, and to serve as reservoirs and possible vehicles for viral dissemination. The primary cell surface receptor for HIV-1 is CD4, which is present on a subclass of T cells, and to a lesser extent, on monocytes and macrophages. Both Fc receptors and complement (C') receptors have been thought to facilitate viral entry and infection of monocytes by antibody-HIV-1 complexes, and by C'-coated HIV-1, respectively. In order to selectively link HIV-1 to different molecules at the surface of the monocyte, we constructed bispecific antibodies (BsAb) composed of two antibody Fab fragments covalently linked together, one Fab fragment was directed toward FcγRI, or FCγRII, or FcγRIII, or CD33, or HLA class 1, the second Fab fragment was directed to the gp120 or gp41 glycoprotein on the HIV-1 envelope. Two different HIV-1 isolates, HIV-1 IIIB and the monotropic HIV-1 JR-FL were used to infect monocytes and macrophages from seronegative donors. Pretreatment of monocytes with an anti-CD4 monoclonal antibody, Leu 3a, was performed in some cases to prevent the binding of virus to CD4.

Our findings indicate that interaction with FCγRI and FcγRII on monocytes, and FcγRI, FcγRII and FcγRIII on macrophages decreases infection with HIV-1 under condition of high antibody opsonization. However, interaction with HLA class I or CD33 did not decrease infection with HIV-1 compared to controls (no BsAb). Moreover, infection was completly blocked in all cases by pretreatment of the cells with Leu 3a, indicating that viral interaction with CD4 is requierd for infectivity, even under conditions of antibody-mediated binding of HIV-1 to FcγR. These preliminary studies show that BsAb can be used to specifically target viral particles to FcγR on monocytes and macrophages, and result in viral neutralization. If FcγR-mediated neutralization involves endocytosis and intracellular degradation of virus, such an approach could be used to target antigenic viral component to the macrophage, and may improve viral processing for antigenic presentation.

Q 451 Identification and characterization of linear immunodominant domains in the SIV_{sm} envelope glycoproteins.

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Macaques infected with SIVsm represent a valuable model to design immunoprophylactic intervention of AIDS. In the last few years our group has focused on the possibility of developing a vaccine partially based on synthetic peptides. In connection to this, we have synthesized 36 peptides of variable length, representing regions of the SIVsm envelope glycoproteins, 34 from gp120 and 2 from the transmembrane protein gp32. The peptides are now used in ELISA to assay reactivity of sera obtained from ten experimentally SIVsm infected macaques. The macaque sera reacted consistently with three of the peptides representing aa 170-196, aa 313-346 and aa 514-537 of the outer envelope glycoprotein gp120. With the purpose of developing monospecific reagents and of studying linear neutralizing domains in the SIVsm envelope, we have immunized guinea-pigs with the entire set of peptides. The capacity of these hyperimmune sera to react with native protein and to mediate neutralization in a homologous system is presently being evaluated in our laboratory.

Q 452 LIMITING DILUTION ANALYSIS OF T HELPER CELL PRECURSOR FREQUENCY TO INFLUENZA AND TO ALLOANTIGENS IN HIV POSITIVE INDIVIDUALS

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We have previously tested the T helper cell (Th) function of asymptomatic, HIV seropositve (HIV+) patients, using an in vitro bioassay for IL-2 production. Peripheral blood leukocytes (PBL) from HIV+ patients and HIV- control donors were tested for Th function when stimulated with influenza A virus (FLU) or HLA alloantigens (ALLO). Three distinct response patterns were seen including: responsiveness to both FLU and ALLO; responsiveness to ALLO but not to FLU; and unresponsiveness to both stimuli. We demonstrated a time-dependent progression from a state of responsiveness to both stimuli progressing to a state of unresponsiveness to both stimuli, progressing in the order outlined above. The earliest Th defect is the loss of response to FLU indicating a selective defect in CD4+ MHC self-restricted Th function. The later loss of ALLO IL-2 response represents a more severe Th dysfunction involving both CD4+ and CD8+ T cells. We have recently adapted a limiting dilution analysis technique developed by Adams and Orosz to quantify the Th cell frequencies specific for FLU and ALLO in HIV+ patients and HIV- control donors. We observed that the loss of Th cell function in HIV+ individuals to FLU and ALLO as tested previously in bulk culture is correlated with a reduction in Th precursor frequencies to these two antigens. Based on these data, we hypothesize that the progressive loss of the function first to recall antigens then to HLA alloantigens is due (at least in part) to a decrease in the Th precursor frequency specific for these antigens.

Q 453 COMPLEMENT ACTIVATION BY HUMAN MONOCLONAL ANTIBODIES TO HIV-1, Gregory T. Spear, Brenda L. Sullivan, Alan L. Landay, Daniel M. Takefman and Susan Zolla-Pazner, Dept Immunology/Microbiology, Rush Medical School, Chicago, IL 60612 and Dept of Pathology, New York School of Medicine, New York, NY 10016.

A panel of anti-HIV-1 human monoclonal antibodies (mAb) were tested for their ability to activate human complement (C) on infected cells. Activation was assessed by flow cytometric detection of cell surface C3 deposition. While mAb to gp41 activated C, mAb to the V3 loop activated C 5-10 fold more efficiently. Some mAb to V3 activated C comparably to a pool of antibody from patients. Complement activation correlated with the amount of mAb bound to the cells. Activation of C by anti-gp41 mAb was increased by pretreatment of cells with soluble CD4 (sCD4). This was due to increased exposure of the gp41 epitopes since more mAb binding was detected. Conversely, sCD4 pretreatment decreased C activation by anti-V3 mAb which was associated with decreased binding of anti-V3 mAb. The effects of sCD4 were more pronounced when using HIV-IIIB than when using HIV-MN infected cells which is consistent with the observation that gp120 can be dissociated more readily from HIV-IIIB. These results show that HIV-1 V3 epitopes are present at sufficient density to efficiently activate C via mAb and suggest that C activating antibody in patients may be directed against this region.

Q 454 SYNERGISTIC NEUTRALIZATION OF HIV-1 BY COMBINATIONS OF ANTIBODIES SPECIFIC FOR DIFFERENT EPITOPE CLUSTERS OF gp120, Shermaine A. Tilley*, William J. Honnen*, Sujata Warrier*, Mary Racho*, Ting-Chao Chou+ and Abraham Pinter*, *Public Health Research Institute, New York, NY 10016 and +Memorial Sloan-Kettering Cancer Center, New York, NY 10021. We have previously reported that human monoclonal antibodies (HuMAbs) against the CD4 binding site and V3 loop of gp120 synergistically neutralize HIV-1, such that 10- to 100-fold lower doses of each HuMAb is required when combined at a 1:1 ratio than when used alone to achieve the same level of neutralization (VIIth Intl. Conf. AIDS, Florence, Abs. M.A.70, 1991; Retroviruses of Human AIDS and Related Animal Diseases, Sixième Colloque Des Cent Gardes, Paris, 1991, in press). We also observed unidirectionally enhanced binding of an anti-CD4 binding site HuMAb, 1125H, to recombinant gp120 in the presence of an anti-V3 loop HuMAb, 4117C, that could account for the synergism between these HuMAbs in neutralization. More recently, we have extended these studies to include a variety of different HIV-1 strains as well as other monoclonal and affinity-purified antibodies specific for the two epitope clusters discussed above. Thus far, we have documented synergistic neutralization by pairs of anti-CD4 binding site and anti-V3 loop antibodies specific for two different CD4 binding site epitopes and three different V3 loop epitopes, and synergistic neutralization of three different HIV-1 strains has been observed. Studies are in progress to assess effects of each of these antibodies on binding of the others to gp120 in order to compare these results with the degree of synergism observed between given pairs of antibodies. We have also recently isolated a potent neutralizing chimpanzee monoclonal antibody (ChMAb) against HIV-1 whose binding to gp120 is inhibited by mouse mAbs against the V2 region but not by mAbs against the V3 loop or CD4 binding site. Results of studies assessing the ability of this ChMAb to synergistically neutralize HIV-1 in combination with anti-V3 loop and/or anti-CD4 binding site antibodies will be presented.

Q 455 HIV PROTEIN SPECIFIC CYTOTOXIC EFFECTOR CELLS CAN BE EXPANDED IN VITRO USING AUTOLOGOUS B CELL LINES EXPRESSING GAG, ENV OR NEF AS ANTIGEN PRESENTING CELLS, Cécile A.C.M. van Els*, Carel A. van Baalen*, Michèl R. Klein@, Anna Maria Geretti*, René I.P.M. Keet*, Frank Miedema@, Albert D.M.E. Osterhaus*, *Laboratory of Immunobiology, Natl. Institute of Public Health and Env. Protection, Bilthoven, *Municipial Health Service, Amsterdam, @Department of Clinical Viro-Immunology, Central Laboratory of the Netherlands Blood Transfusion Service, Amsterdam, The Netherlands.

Infection by HIV leads to the generation of HIV protein specific cytotoxic T lymphocytes (CTL) in humans. These can be demonstrated in fresh or in cultured peripheral blood mononuclear cells (PBMC) of seropositive individuals. CTL may play a crucial role in the immune response against HIV by recognising and destroying infected cells which express HIV peptides in the context of MHC class I surface molecules. Although high frequencies of HIV specific CTL have been described in some patients, in general the anti-HIV CTL reactivity and overall specificity reported after infection is low. Most *in vitro* stimulation strategies used have been based on nonspecific polyclonal activation. In this study we introduce a novel approach to specifically expand HIV directed CTL using autologous B lymphoblastoid cell lines (BLCL) infected with recombinant vaccinia virus (rVV) expressing HIV proteins as antigen presenting cells. Thus PBMC from a series of healthy seropositive individuals were stimulated with gag, env, or nef. Subsequently the cultures were analysed for lytic activity on ^{51}Cr labeled autologous or mismatched uninfected BLCL, BLCL infected with the relevant rVV or control rVV, or BLCL pulsed with relevant peptides, as target cells. High MHC restricted responses were found for gag in 2 out of 3, for env in 2 out of 4, and for nef in 1 out of 2 cultures. Furthermore we observed expansion of virus specific effector cells with as yet unknown fine specificity. These cells are now being characterised as to their phenotype, fine specificity and MHC restriction requirements.

In conclusion, we show that stimulation of PBMC with the individual HIV proteins is a useful tool to study HIV directed cytotoxicity. This method may be of special interest for the design of candidate AIDS vaccines and follow up studies in vaccinated individuals.

Q 456 STABLE CELL LINES EXPRESSING GP160 OF HIV-1, Carol D.Weiss and Judith M. White. Dept. of Pharmacology, University of California, San Francisco, CA 94143-0450.

We established three stable CHO cell lines that express wild-type or truncated forms of the HIV-1 (HXB-2 clone) envelope glycoprotein. The wild-type cell line expresses gp160 and gp120 at the surface and readily forms large syncytia when co-cultivated with CD4+ cells. Another cell line secretes the ectodomain of gp160. Supernatants and cell lysates of this cell line show 140 kD and 120 kD molecules. The third cell line expresses the gp160 ectodomain that is membrane-anchored by a phosphatidyl-inositol (PI) glycan tail. This molecule is expressed at the cell surface and can be released from the cell surface by PI-specific phospholipase C. Syncytia are not seen when these cells are co-cultivated with CD4+ cells. These cell lines, which produce wild-type or water soluble forms of gp160, should be valuable reagents for our studies of gp160 structure and function.

Q 457 STRUCTURAL CHARACTERIZATION OF THE V3 DOMAIN OF SEVERAL HIV-1 ISOLATES, Carl Wild and Thomas Matthews, Department of Surgery, Duke University Medical Center, Durham, NC 27710, Laszlo Otvos, The Wistar Institute, Philadelphia, PA 19104.

The third variable domain of the HIV-1 envelope gp120 has been referred to as the principal neutralizing determinant of the virus[1]. The assignment has been made not because the V3 region is the only target of neutralizing antibody but rather because it is the principal target noted in experimentally induced polyclonal sera. Significant levels of neutralizing antibody recognizing other more conserved sites has been considerably more difficult to induce. In contrast, multiple forms of envelope based immunogens including native and denatured gp120 and gp160 and short synthetic peptides have been found to induce effective V3 antibodies. In order to take advantage of the ease with which the V3 domain can be targeted some effort has gone into typing virus isolates on the basis of V3 sequence[2]. The results indicate extensive heterogeneity on the basis of primary sequence but a more limited diversity based on secondary structure predictions. If the latter predictions are accurate it might be possible to use that information to reduce the numerous HIV-1 isolates into a more manageable number of neutralization sensitive V3 families. As an approach to that end we have synthesized peptides corresponding to the V3 loop region of the prototypic IIIB, B_{A-L}, and MN isolates and have used nuclear magnetic resonance (NMR) spectroscopy and circular dichroism (CD) to study these systems for evidence of secondary structure. The results of those studies as well as serological properties of these cyclized loop peptides will be presented.

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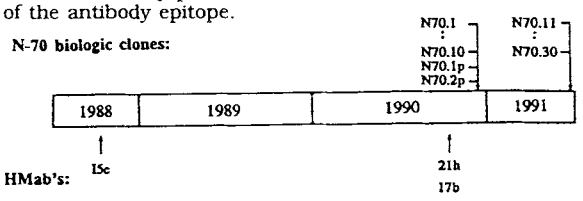
Q 458 **HIV-1 ISOLATES AND BIOLOGIC CLONES THAT HAVE ESCAPED IN VIVO FROM SEQUENTIAL HUMAN MONOCLONAL ANTIBODIES (HMab) TO THE CD4-BINDING SITE**, Hironori Yoshiyama[1], James E. Robinson[2], Hiroshi Mohri[1], Tuofu Zhu[1], Yunzhen Cao[1], David D. Ho[1], 1. The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY 10016, 2. LSU School of Medicine, New Orleans, LA 70112.

HMab against a cluster of conformational epitopes in or near the CD4-binding site of HIV-1 gp120 have been shown to have broad HIV-1 neutralizing activity. Three such HMab (15e, 17b and 21h) were sequentially isolated from an HIV-1 infected person, N-70, as shown in the figure. These HMab block gp120-CD4 interaction, neutralize divergent HIV-1 isolates, and recognize distinct but overlapping epitopes. Two bulk viral isolates, as well as a total of 32 biologic clones of HIV-1, have also been obtained from N-70 as shown. The susceptibility of the 1990 isolate and clones #1 through #10 to neutralization by 15e has been determined. The 1991 isolate and additional biologic clones are currently being tested for neutralization by 15e. Similar studies using 21h and 17b are also in progress. This type of analysis should permit us to study in detail the development of neutralization escape mutants in vivo. Furthermore, the comparison of the gp120 nucleotide sequences of biologic clones that differ in neutralization susceptibility to one of these HMab may provide information on the determinants of the antibody epitope.



Therapy

Q 500 HIV-1 PROTEASE IS ACTIVE IN ACUTELY INFECTED A3-01 CELLS, Lonnie D. Adams*, Alfredo G. Tomasselli*, Paul Robbins+, Bernard Moss+ and Robert L. Heinrikson*, *Biochemistry Dept, Upjohn Laboratories, Kalamazoo, MI 49001, +National Institutes of Health, Bethesda, MD

Actin and possibly other cellular proteins are hydrolyzed by HIV-1 protease during acute infection of A3-01 cells in culture. Previous work has shown that cellular proteins such as actin, troponin C, Alzheimer amyloid precursor protein, pro-interleukin 1β (1) and calcium-free calmodulin (2) can serve *in vitro* as substrates for HIV-1 protease. Here we demonstrate that 4 new polypeptides seen in 2-D gels of A3-01 cells acutely infected with HIV comigrate with actin fragments produced by HIV-1 protease hydrolysis of purified actin. None of these actin fragments are produced by endogenous cellular proteases. While *in vitro* incubation of A3-01 cell sonicate with HIV-1 protease shows that many cellular proteins can serve as substrate, actin appears to be preferentially cleaved in infected cells. The significance of actin cleavage is not clear at this time, however, hydrolysis of physiologically important cellular proteins by HIV protease during infection may have important consequences relative to viral pathology.

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Q 501 **CHANGES IN HIV-1 DNA AND RNA QUASISPECIES DURING AND AFTER AZIDOTHYMIDINE TREATMENT**

Jan Albert[1], Johan Wahlberg[2], Joakim Lundeberg[2], Susan Cox[1], Erik Sandström[3], Britta Wahren[1], Mathias Uhlén[2] [1]Dept. of Virology, National Bacteriological Laboratory, [2]Dept. of Biochemistry and Biotechnology, Royal Institute of Technology, and [3]Venhälsan, Södersjukhuset, Stockholm, Sweden

Development of resistance to antiviral compounds, such AZT and ddI, is an increasingly recognized problem in the treatment of HIV-1 infected patients. We have developed biological and molecular methods to rapidly assess such resistance, which were used to study various aspects of AZT resistance.

Methods: Sequential virus isolates from four patients, who had been AZT treated for up to 27 months, were tested for resistance to AZT, ddI, FLT and fosfonoformic acid on fresh lymphocytes in a microtiter-based assay. We also studied four patients who discontinued long-term AZT treatment. The reverse transcriptase gene from proviral DNA in uncultured patient cells and viral RNA in serum and plasma was amplified by nested PCR and directly sequenced by a new automated DNA sequencing method. Sequence heterogeneity within the individual samples was roughly quantified.

Results: All AZT treated patients gradually accumulated multiple mutations, some of which have been reported to confer AZT resistance. The mutations were surprisingly stable after discontinuation of AZT treatment. One patient reverted from AZT resistance after nine months, whereas three other remained resistant after four, nine and twelwe months, respectively. There was no major difference between sequences obtained from plasma or cells. Analysis of ddI treated patients is under way

Conclusions: We have established rapid and reliable methods to determine antiviral sensitivity and sequence of HIV-1 in clincal specimens. We have identified two not previously reported mutations, which may be of importance for development of AZT resistance. Reversion from AZT resistance was infrequent and slow.

Q 502 THE ISOLATION, CHARACTERIZATION AND EFFECTS OF AZT-CHAIN TERMINATED SINGLE-STRANDED HIV-1 DNA FRAGMENTS, Eric J. Arts and Mark Wainberg, McGill AIDS Centre and the Department of Microbiology and Immunology, Montreal, Quebec, Canada, H3T 1E2

The viral enzyme, reverse transcriptase (RT) is thought to incorporate AZT triphosphate over thymidine triphosphate into the replicating strand of viral DNA, thus causing chain termination. Although chain termination is the hypothesized mechanism of nucleoside analog antiviral activity, direct evidence in support of this activity is lacking. Specific oligonucleotide primers were used in a quantitative version of the polymerase chain reaction (PCR) technique to identify and isolate these rare and small 3' nucleoside analog single-stranded (ss) chain terminated viral DNA fragments (ssV-DNA). One primer pair (A13-S1) amplifies the initally reverse transcibed piece of HIV-1 DNA in the U3 region of the 5'LTR while another other primer pair (SG4-AG4) amplifies one of the last replicated pieces of HIV-1 ss DNA found at 5' end of the gag gene. The ratio of the amplified products of the first to the last reverse transcribed piece of HIV-1 ss DNA indicates the relative amount of ssV-DNA present in Hirt extracts of HIV-1 infected, nucleoside analog treated Jurkat cells. The nucleoside analogs, 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxyinosine (ddI), and 2'-deoxy-3'-thiacytidine (BCH-189), at concentrations of 2, 200 and 50uM respectively caused nearly a 10:1 ratio of chain termination in the reverse transcription of HIV-1 RNA in Jurkat cells. We also showed that increasing the concentration of nucleoside analogs in HIV-1 Jurkat cell infections increases the amount of ssV-DNA. Interestingly, nucleoside analogs at high concentrations inhibited viral production yet did not prevent the integration of proviral DNA into the host genome. A time course of HIV-1 infected, AZT-treated Jurkat cells showed that ssV-DNA can survive at least 72 hours in the host cell. We hope to show, through ***in vitro*** reverse transcription assays and translation assays, that these ssV-DNA, antisense to 5' HIV-1 RNA, may inhibit future replication of new infectious virus and the translation of HIV-1 RNA produced from integrated proviral DNA.

Q 503 *IN VIVO* ANTIVIRAL ACTIVITY OF BRL 47923 AGAINST RAUSCHER MURINE LEUKAEMIA VIRUS (RMLV)

R.J. Ashton[1], J.J. Harvey[2], S.E. Macatonia[2], A.J. Edwards[2], S.C. Knight[2], D.N. Planterose[1], A.G. Brown[1], and R.M. Perkins[1]. SmithKline Beecham Pharmaceuticals, Great Burgh, UK[1], and Clinical Research Centre, Harrow, UK[2].

9-[2-(Phosphonomethoxy)ethoxy]adenine, BRL 47923, is a novel acyclic nucleotide analogue with selective antiviral activity against HIV, FIV, and visnavirus (see poster by Perkins et al.) Here we demonstrate the utility of this compound for *in vivo* treatment of a retroviral infection using the RMLV model. *In vitro* BRL 47923 is a selective inhibitor of RMLV replication with a 50% inhibitory concentration of 1.1μg/ml and a 50% cytotoxic concentration of >100μg/ml (cf. AZT, 0.001 and 3.7μg/ml respectively). Balb/c mice, infected intravenously with $7x10^4$ RMLV focus forming units/mouse, were dosed subcutaneously with 0.4mmols/kg of BRL 47923 or AZT, b.i.d., for up to 14 days. BRL 47923 was shown to be an effective inhibitor of virus-induced splenomegaly (86%, inhibition) and virus replication in the spleen. AZT treatment resulted in a similar inhibition in splenomegaly (84%) but gave a greater inhibition of virus expression in splenocytes. Both compounds effectively inhibited viraemia (plasma RT), and virus-induced effects on erythrocyte and white blood cell counts. Investigation of the immunosuppressive effects of RMLV by FACS analysis of spleen cells demonstrated a reversal of helper/suppressor T cell ratios. Significantly, an impairment in the antigen presenting capacity of dendritic cells was also observed, similar to that reported in AIDS patients. As measured by these indicators, RMLV-induced immunosuppression was effectively inhibited by treatment with BRL 47923 or AZT. No gross toxicity was observed with either compound. Further tests will be necessary in this and other model systems, to assess whether BRL 47923 could play a role in the antiviral treatment in AIDS.

Q 504 ZIDOVUDINE EFFECTS IN HIV INFECTION: PRELIMINARY STUDY OF CHANGES IN 11 LYMPHOID MARKERS, Bass H.Z.*, Hardy D.**, Mitsuyasu R.T.**, Wang Y.X.+, Cumberland W.+, Fahey, J.L.*, *Center for Interdisciplinary Research in Immunology Disease (CIRID) and Jonsson Comprehensive Cancer Center, **AIDS Clinical Research Center, +Department of Biostatistics, University of California, Los Angeles, CA 90024

HIV infection induces substantial changes in the expression of many lymphocyte phenotypic antigens, and increased serum levels of activation products as well as depletion of CD4 lymphocyte numbers. The current study was undertaken to determine whether 7 lymphocyte phenotypic changes associated with HIV infection are altered by zidovudine administration. This study also investigated how zidovudine-induced phenotypic changes compare to serum activation marker changes in neopterin and beta-2 microglobulin. Levels of the 4 major lymphoid subsets were also measured. Elevated pretreatment CD38 expression was reduced significantly at 2 weeks and CD71 (transferrin receptor) was decreased at 2 to 8 weeks of zidovudine treatment. These markers returned to pre-treatment ranges at different rates. The kinetics of CD38 reduction and return to pre-treatment levels appeared to be similar to serum neopterin and ß2M changes. CD4 lymphocytes showed a transient increase, most evident at 8 weeks of treatment. Lymphoid phenotypes that did not show significant changes after zidovudine therapy included CD57, CD11b, CD45RA and leu 8 markers as well as CD8 T cells, CD20 B cells and CD56 NK cells. The fact that some lymphocyte phenotypic markers change and others do not indicate that more than one mechanism of immune perturbation is associated with HIV infection. Several phenotypic markers (CD38, CD71 and, possibly, HLA-DR) that are susceptible to zidovudine effects (but differ from CD4 T cell changes) are candidate surrogate markers for evaluation of anti-HIV treatment.

Q 505 **CD4-*PSEUDOMONAS* EXOTOXIN: POTENT ACTIVITY AGAINST CELLS EXPRESSING HIV AND SIV ENVELOPE GLYCOPROTEINS WITH WIDELY DIFFERING AFFINITIES FOR CD4, AND ANTI-VIRAL EFFECTS AGAINST PRIMARY HIV ISOLATES,** Edward A. Berger, Per Ashorn, and Bernard Moss, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892

CD4(178)-PE40 is a genetically engineered hybrid toxin containing a portion of human CD4 linked to the effector domains of *Pseudomonas* exotoxin A. We previously showed that the molecule selectively kills cells expressing the envelope glycoproteins of HIV or SIV, and inhibits HIV-1 spread in T-cell and macrophage cultures. Here we report the activity of the hybrid toxin against cells expressing diverse forms of the HIV-1, HIV-2, and SIV_{mac} envelope glycoproteins, encoded by vaccinia vectors. Interestingly, the potency of CD4(178)-PE40 was unaffected by wide variations in CD4 binding affinities of these envelope glycoproteins. The hybrid toxin was also active against mutant envelope glycoproteins lacking either the gp120/gp41 cleavage site or the gp41 cytoplasmic tail. These results with envelope glycoproteins of laboratory-adapted viral strains suggest that the potency of CD4(178)-PE40 may be relatively insensitive to variations in envelope glycoprotein structure occurring in HIV-infected individuals. In preliminary experiments, the hybrid toxin potently inhibited spreading infection by primary HIV-1 isolates known to be refractory to neutralization by soluble CD4. Such information is particularly critical in view of the recent initiation of Phase I clinical trials of CD4(178)-PE40.

Q 506 **A MURINE AIDS MODEL FOR THE ANTIVIRAL ACTIVITY *IN VIVO* OF HIV PROTEASE INHIBITORS, Paul L. Black[1,2], Mary Beth Downs[3], Mark G. Lewis[2,4], Ronald C. Bell[3], John Baldoni[5], Paul DalMonte[5], Peter DeMarsh[5], Geoffrey B. Dreyer[5], Dennis M. Lambert[5], Stephen R. Petteway, Jr.[5], and Michael A. Ussery[1]; [1]Food and Drug Administration, Rockville, MD 20857; [2]Southern Research Institute-Frederick Research Center, Frederick, MD; [3]USAMRIID, Ft. Detrick, Frederick, MD; [4]Henry M. Jackson Foundation, Rockville, MD; [5]SmithKline Beecham, King of Prussia, PA.**

We examined whether rationally designed synthetic inhibitors of HIV protease would be active *in vitro* against other retroviruses with highly conserved aspartic proteases and whether sufficient concentration of inhibitors could be maintained *in vivo* to affect the disease course in infected mice. Seven rationally designed protease inhibitors with varying degrees of activity against HIV were tested *in vitro* in blinded fashion for their ability to suppress the replication of Rauscher murine leukemia virus (RMuLV) and simian immunodeficiency virus ($SIV_{sm/pbj}$) by plaque or syncytia reduction and p27 level in supernatant. The same 3 compounds were active against RMuLV, SIV, and HIV, with IC_{50} values in the range of 100 to 1000 nM and with little or no cytotoxicity at much higher concentrations (≥0.1 mM). The active compounds inhibited the processing of gag polyproteins of all 3 viruses. In order to test the antiviral activity of HIV protease inhibitors *in vivo*, RMuLV-infected mice were treated with either an active or an inactive compound for 14 days (bid, IP). The active peptide reduced viremia in a dose-dependent manner, compared with the excipient control, while the control peptide was inactive. Pharmacokinetic studies suggested that IP administration is not optimal for this compound, and further studies will attempt to identify optimal routes, doses, and schedules of administration. Thus RMuLV should provide a good model for the preclinical evaluation of this class of therapeutic agents for HIV.

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Q 507 ANTIVIRAL ACTIVITY OF AZT IN COMBINATION WITH OTHER ANTI-HIV AGENTS, Robert W. Buckheit, Jr., J. Germany-Decker, E.L. White, L. Ross, L.B. Allen, and W.M. Shannon, Southern Research Institute, Birmingham, AL, USA

The toxicity of 3'-azido-3'-deoxythymidine (AZT) and the appearance of drug resistant virus isolates emphasizes the importance of the development of alternative strategies for the successful therapy of AIDS patients. Combination antiviral chemotherapy provides an attractive therapeutic strategy since the dose of the individual agents may be lowered to reduce toxicity and limit the development of drug- resistant mutants. It is generally accepted that combinations of two or more drugs may be required to efficiently inhibit the spread of HIV in an infected individual and to prevent the pathogenic effects of the virus. We have been examining combinations of both AZT and ddI with other antiviral agents, including nucleoside and non-nucleoside reverse transcriptase inhibitors and compounds which act at alternative steps of retroviral replication. The analysis of these drug combinations was performed by the three dimensional model of Pritchard and Shipman. Antiviral data for the drugs alone and in combination is obtained through a primary screening assay utilizing the XTT method for evaluating the ability of the compound to inhibit HIV-1 induced cell killing. Confirmation of these results is obtained by analysis of reverse transcriptase activity in each sample. Classical biochemical analysis of combinations of reverse transcriptase inhibitors is also obtained and compared with cell culture data. We have identified compounds exhibiting significant synergy when utilized in combination with AZT or ddI, which may be therapeutically useful in the treatment of AIDS.

Q 508 DEVELOPMENT OF A CELLULAR ASSAY SYSTEM TO EVALUATING THERAPEUTICS FOR AN ABILITY TO BLOCK HIV-1 ACTIVATION FROM LATENCY.

Salvatore T. Butera,[1] Paul Feorino,[2] Raymond F. Schinazi[2] and Thomas M. Folks[1]. [1]Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, Georgia, 30333 and [2]Laboratory of Biochemical Pharmacology, Emory University School of Medicine/Veterans Affairs Medical Center, Atlanta, Georgia 30322.

If novel therapeutic compounds or strategies can be developed to prolong the clinically latent period prior to AIDS, some hope may be afforded the estimated 1 million HIV-1$^+$ but asymptomatic individuals. During clinical latency, HIV-1 resides in a reservoir population of CD4$^+$ cells, the majority of which do not express viral RNA. Activation of dormant HIV-1 in these cells may well contribute to clinical progression in AIDS.

Recently, we have described a novel promyelocytic model of chronic infection, OM-10.1. OM-10.1 cells clonally harbor a single proviral copy and remain CD4$^+$ while constitutively expressing low levels of HIV-1. However, OM-10.1 cells rapidly respond to treatment with TNF-α by down modulating CD4 and dramatically increasing HIV-1 expression. We have used the OM-10.1 model, which mimics the CD4$^+$ *in vivo* latent viral reservoir, to screen therapeutic compounds for an ability to prevent HIV-1 activation.

Compounds were evaluated by pretreatment with OM-10.1 cells for 4 hr followed by TNF-α induction for 24 hr. Evidence of HIV-1 activation was monitored by cellular CD4 down modulation and culture supernatant RT activity. Of the > 50 compounds initially tested, 3 (FLT, INF-γ, and desferoxamine) were found to be modest inhibitors (20-40% inhibition) of viral activation. Enhanced (> 80%) inhibitory activity was achieved when these compounds were tested in combination.

In conclusion, the OM-10.1 system permits a rapid and reliable means of evaluating therapeutic compounds as well as intracellular signaling inhibitors for an ability to prevent HIV-1 activation from latency.

Q 509 INHIBITION OF HIV-1 REPLICATION BY BLOCKING TAT EXPRESSION AND ACTIVITY WITH ANTISENSE RNA EXPRESSING PLASMIDS AND MULTIPLE TAR CONSTRUCT. H. -K. Chang, J. Lisziewicz, V. Fiorelli, R. C. Gallo and B. Ensoli. Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Antisense nucleic acids have been used to inhibit HIV-1 gene expression and replication. An alternative approach is to introduce antisense RNA expressing constructs (protective gene) into cells. HIV-1 Tat is a regulatory gene product essential for viral gene expression and replication. Tat also mediates cellular activities (vascular cell growth, activation of TNF gene expression) which may play a role in AIDS-associated disorders. Thus, Tat was chosen as target to inhibit HIV-1 infection. Two antisense expressing plasmids of Tat (ASTATI and ASTATII) were constructed under the control of Adenovirus major late gene promoter and tested by transient cotransfection with plasmid containing HIV-1 LTR-driven CAT gene and Tat expressing plasmids into human T lymphoid cell line (Jurkat). Both antisense constructs inhibited the expression of Tat up to 70%. These constructs also blocked the rescue effect of Tat in HeLa CD4+ cells containing tat-defective proviruses. A dramatic HIV-1 inhibitory effect was obtained with the antisense constructs in acutely infected T cells. To maximize the blocking effect, a multiple-TAR construct was cotransfected with the tat-antisense plasmids. The association resulted in 90% of inhibition of Tat expression. This suggests that the combination of two protective genes directed against the same target but with different mechanism of action is optimal to block HIV-1 infection in vitro and may be required in vivo.

Q 510 MAPPING THE REGIONS OF HIV REVERSE TRANSCRIPTASE RESPONSIBLE FOR RESISTANCE TO SEVERAL NONNUCLEOSIDE INHIBITORS, Jon H. Condra, Emilio A. Emini, Leah Gotlib, Donald J. Graham, Donald W. Lineberger, William J. Long, Abner J. Schlabach, Jill A. Wolfgang, and Vinod V. Sardana. Department of Virus and Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Inhibition of the Human Immunodeficiency Virus (HIV) reverse transcriptase (RT) has been a major focus in anti-AIDS drug development. This enzyme is required for viral infectivity, and is the target of the presently available nucleoside drugs for AIDS therapy, 3'-Azido-2',3'dideoxythymidine (AZT) and 2',3'-dideoxyinosine (ddI). A separate pharmacologic class of structurally distinct nonnucleoside inhibitors has been recently described. These include R82150, BI-RG-587, and the pyridinone derivative L-697,639.

The nonnucleoside compounds effectively inhibit the RT from HIV type 1 (RT1) but not the corresponding type 2 enzyme (RT2). To address the structural basis for this differential drug sensitivity, we have expressed a series of molecular RT1/RT2 chimeras and site-directed RT mutants in *E. coli* and correlated drug sensitivity of the purified enzymes with the presence of specific amino acid residues from the parent RTs. RT2 was completely sensitized to these drugs by substituting RT1 residues 101-106 and 155-217. Within these regions, residues 176-190, and to a lesser extent, 181 with 188, were the principal determinants of RT2's resistance to all these compounds relative to RT1.

Mutations K103N/Y181C were previously shown to render HIV-1 in culture 1000-fold resistant to L,697,639 (Nunberg *et al.*, J. Virol. 65:4887-4892 (1991). We examined the effects of these and other specific RT1 amino acid substitutions on drug sensitivity of the purified enzyme *in vitro*. K103N alone conferred 17- to 100-fold resistance to the nonnucleoside inhibitors, while Y181C yielded 30- to 600-fold resistance. Both mutations together yielded >1000-fold resistance, consistent with virus inhibition data. Mutants Y181F and Y188F were fully drug-sensitive, showing that the tyrosine -OH groups are not required for drug interaction. Of all single mutants examined, the Y181I mutation (as in RT2) conferred the highest degree of drug resistance. These results suggest that clinical resistance to any of these nonnucleoside inhibitors will probably extend to other members of this same pharmacologic class.

Q 511 DITIOCARB STIMULATES HIV-1 IN VITRO. B. Conway (1), D. Ko (1), N. Hawley-Foss (1), L. Filion (1), S. Baruchel (2). (1) Division of Infectious Diseases, Ottawa General Hospital, Ottawa, Canada K1H 8L6 (2) Hematology Service, Montreal Children's Hospital, Montreal, Canada H3H 1P3

The objective of this study was to evaluate in vitro susceptibility of clinical strains of HIV-1 to ditiocarb, an antioxidant thought to modulate viral replication. Peripheral blood mononuclear cells (PBMCs) were isolated from 6 asymptomatic HIV-1-infected individuals (CD4 lymphocyte count 200-500 cells/µl) who had previously received antiretroviral therapy. After stimulation with PHA-P and IL-2, the PBMCs were resuspended in the presence of 0, 2 or 20 uM ditiobarb for 72 hours, then harvested for PCR. The change (%) in viral load in the presence of ditiocarb was taken as a measure of drug effect. Under similar experimental conditions, 5/6 isolates had been shown to exhibit a significant reduction in viral load in the presence of AZT and/or ddI.

	Change in Viral Load			
	2 uM ditiocarb	20 uM ditiocarb	1 mM ddI	10 uM AZT
Patient 1	+ 119%	+ 67%	- 49%	- 30%
Patient 2	+ 50%	+ 33%	- 58%	- 12%
Patient 3	+ 100%	+ 14%	- 55%	- 75%
Patient 4	- 14%	+ 43%	- 17%	+ 14%
Patient 5	+ 21%	+ 47%	+ 0%	+ 0%
Patient 6	+ 49%	- 14%	- 39%	- 52%

In most cases, at physiologic concentrations, ditiocarb appears to enhance viral replication in stimulated infected PBMCs. The mechanism of this effect is currently under study.

Q 512 RELATIONSHIP BETWEEN THE IN VITRO HIV-1 INHIBITORY DOSE OF rCD4-IgG AND THE EX VIVO PLASMA-ASSOCIATED HIV-1 TITER. Robert W. Coombs, A.C. Collier, J.W. Gibson, K.E. Nelson, K. Chaloupka, A. Ammann, L. Corey. University of Washington, Seattle, WA, and Genentech, Inc., San Francisco, CA

The purpose of this study was to determine if the administration of rCD4-IgG (ACTG protocol 121) to patients with CDC class IV HIV-1 infection resulted in a decreased plasma-associated HIV-1 titer.

Twelve patients at the University of Washington received from 30 to 1000 micrograms/Kg/dose of rCD4-IgG by IV bolus injection one to three times per week for 12 weeks. Plasma HIV-1 titers (TCID50/mL of plasma) were determined at the start and end of therapy. The inhibitory dose (ID) 50 and 90 percent for rCD4-IgG was determined by titering rCD4-IgG against dilutions of the HIV-1-infected plasma.

The median (25th-75th percentiles) plasma HIV-1 titers at baseline and week twelve of therapy were 410 (139-699; range 0-3495) and 139 (84-699; range 0-3495) TCID50/mL, respectively (P=NS, Wilcoxon signed-rank). The maximum plasma levels of rCD4-IgG were approximately 21 mcg/mL. For plasma HIV-1 isolates, the median ID50 was 58 mcg/mL (14-250) and ID90 was 484 mcg/mL (130-2540); the ID50/90 did not change over the twelve weeks of rCD4-IgG therapy.

In conclusion, the ID50/90 in plasma of clinical HIV-1 isolates was higher than the concentration of rCD4-IgG achieved following the 1000 mcg/Kg/dose; the trend towards a lower plasma HIV-1-titer suggests that higher plasma levels of rCD4-IgG may be needed for an antiviral effect.

Q 513 ALLOSTERIC INHIBITION OF ENDOGENOUS HIV-1 REVERSE TRANSCRIPTION BY TIBO DERIVATIVES. Zeger Debyser, Anne-Mieke Vandamme, Rudi Pauwels, Jan Desmyter and Erik De Clercq, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

TIBO and HEPT compounds have been shown to interact with the reverse transcriptase (RT) of HIV-1 in a way different from classical RT inhibitors. In all enzymatic studies carried out with artificial exogenous template/primers, RT inhibition by these compounds was found to be template-dependent. We have now established an endogenous RT assay, whereby the viral RNA serves as the natural template, to investigate kinetics and characteristics of inhibition.

HIV-1 RT inhibition by TIBO was dependent on which of the four substrates was present in limited concentration. TIBO preferentially inhibited the RNA-dependent DNA polymerization function of the enzyme, and kinetics of inhibition was compatible with an allosteric mode of inhibition. Positive cooperativity was noted for the natural substrate (dGTP), which confirms earlier findings with exogenous RT assays. Our observations are indicative of RT activity regulation by varying substrate concentration and interference of TIBO with this process.

Q 514 LIPOSOME TARGETING TO HIV-INFECTED CELLS VIA RECOMBINANT SOLUBLE CD4 AND CD4-IgG (IMMUNOADHESIN). Nejat Düzgüneş[a,b], Diana Flasher[a], Steven Chamow[d], Avi Ashkenazi[d], Paul Dazin[c] and Krystyna Konopka[a], [a]Department of Microbiology, University of the Pacific School of Dentistry, San Francisco, CA 94115; [b]Department of Pharmaceutical Chemistry and [c]Howard Hughes Medical Institute, University of California, San Francisco, CA 94143; [d]Genentech, Inc., South San Francisco, CA 94080

HIV-infected cells producing virions express the viral envelope glycoprotein gp120/gp41 on their surface. Recombinant soluble CD4 (rsCD4, the ectodomain of CD4) binds gp120 with high affinity and can inhibit the infectivity of laboratory strains of HIV-1. We have investigated whether liposomes could specifically bind to HIV-infected cells, using rsCD4 or CD4-IgG (immunoadhesin) as targeting ligands. RsCD4 was chemically coupled by 2 different methods to liposomes containing rhodamine-phosphatidylethanolamine in their membrane as a fluorescent marker. One method involved thiolating the rsCD4 and coupling to the liposomes via a maleimide-derivatised phospholipid. In the other method, the oligosaccharides on rsCD4 were coupled to a sulfhydryl-derivatised phospholipid, utilizing a novel bifunctional reagent. The liposomes were incubated for 1 h at 37°C with either H9 cells chronically infected with HIV-1 (H9/HTLV-III$_B$ cells), or uninfected H9 cells. The cells were washed 4 times, fixed in paraformaldehyde, and analyzed by flow cytometry. CD4-coupled liposomes bound specifically to H9/HTLV-III$_B$ cells and not to H9 cells. CD4-coupled liposomes also bound specifically to monocytic THP-1 cells chronically infected with HIV-1(HTLV-III$_B$). Control liposomes without coupled CD4 did not bind significantly to any of the cells, while rsCD4 could competitively inhibit binding of the CD4-coupled liposomes to both H9/HTLV-III$_B$ and infected THP-1 cells. CD4-IgG could also be used as a ligand to target liposomes with covalently coupled Protein A (which binds the Fc region of the CD4-IgG) to H9/HTLV-III$_B$ cells. The CD4-liposomes inhibited the infectivity of HIV-1 in A3.01 cells, and also bound rgp120. Our results suggest that liposomes containing antiviral or cytotoxic agents may be targeted specifically to HIV-infected cells.

This work was supported by NIH Grant AI-25534 and a grant from Liposome Technology, Inc.

Q 515 DECREASED DIDANOSINE (ddI) SUSCEPTIBILITY OF HIV-1 CLINICAL ISOLATES AND MOLECULAR CLONES ASSAYED ON PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC). Eron JJ, Chow Y-K, Videler J, Bechtel LB, Cooley, TC*, Hirsch MS, and RT D'Aquila. Massachusetts General Hospital/Harvard Medical School and*Boston City Hospital, Boston, MA.

We assayed the in vitro ddI susceptibility of HIV-1 clinical isolates (obtained prior to and during ddI monotherapy) and HIV-1 molecular clones. Two drug susceptibility methods were used: 1) PCR quantitation of HIV-1 DNA in cells infected in vitro (Eron,et al.,PNAS,in press) and 2) p24 antigen measurement in the supernatant fluids of in vitro cultures. We used only PHA-stimulated, HIV-1 seronegative PBMC for all virus isolation, propagation, titration, and drug testing. Twelve subjects on long term ddI, initially as part of a phase I clinical trial, underwent virologic study. Clinical data were available from these subjects and others in the cohort who had been on ddI for > 4 months. DNA sequencing directly from PCR-amplified HIV-1 DNA was focused on portions of the reverse transcriptase (RT) gene. Molecular clones were constructed from wild-type HIV-1 (HXB2) using in vitro site-directed mutagenesis.

Clinical isolates obtained on ddI therapy showed small, reproducible decreases in susceptibility compared to pre-ddI isolates. The ave. increase in IC_{50} was 2 to 11-fold. The ave. IC_{50} of each of the pre-ddI isolates ranged from 0.4μM to 1.2μM and the ave. IC_{50} of isolates obtained on therapy ranged from 1.6 μM to 4.9 μM. DNA sequencing identified a mutation at RT codon 74 (encoding a leu->val substitution) in the post-ddI isolates, either alone (subjects prior to the trial received no AZT or < 4 mo. of AZT) or in combination with a persistent AZT resistance mutation at codon 215 (subjects had previously received >4 mo.of AZT).

Preliminary susceptibility testing of viruses derived from molecular clones suggests that a combination of mutations at codons 74, 215, and 219 confers a greater decrease in ddI susceptibility than the codon 74 mutation alone. The combination clone-derived viruses had a 2-4 fold higher IC_{50} than clone-derived viruses with only the 74 mutation which was slightly higher than wild-type. The absolute IC_{50} values are less and the relative changes in susceptibility with the mutations are less than were seen with molecular construct-derived viruses studied by a CD4+ HeLa plaque assay after MT-2 cell passage(St. Clair, et al., Science, 1991). This difference may be due to our exclusive use of seronegative donor PBMC in all HIV-1 cultures. In this small sample, disease progressed more rapidly on ddI therapy in subjects receiving ddI after >4 mos. prior AZT than in subjects on initial ddI.

Q 516 A NEW DRUG FOR AIDS - EXPERIMENT RESEARCH OF EFFECT OF AI AN NING ON IMMUNE FUNCTION OF SMALL MICE AND ITS FUNCTION OF ANTIVIRUS AND ANTIBACTEREA, ASSOCIATE PROFESSOR: Min Fanzhong, Xuan Ruisheng, Guangxi Traditional Chinese Medical College.

AIDS is caused by invasion of HIV. Which damages the function of human immune system, offers a chance of invasion to other causatire agents such as bacteria and virus and forms multifarious opportunity infection. So many kinds of diseases may occur on AIDS patients. Accordingly, choosing chinese herbs for treating AIDS should bace on the following four aspects: (1) the herbs with function of anit-HIV. (2) the herbs with features of wide-spectrums or specialization antivirotic and antibiotic. (3) the herbs with function of adjusting immunity. (4) the herbs with function of symptom therapy to AIDS.

AI AN NING is a kind of drug of TACH (short for Treating AIDS Chinese Herbs). The production of TACH was ratified through appraisal of experts' meeting organized by Shanghai health bureau in August, 1990.

The formula of AI AN NING was made according to the four aspects above. which has function of elimnating evils (antivirotic and antibiotic) and enforcing body's resistance (enforcing immune function), can be applied to prevention of high incidence and treatment of early-stage AIDS.

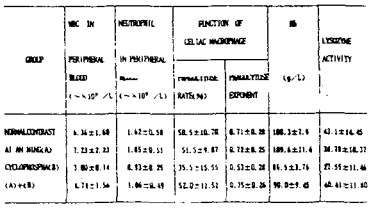
The result of experimental research of effect of *AI AN NING* on immune function of small mice and its function of anitvirus and antibacteria are reported as follows:

1. EFFECT ON IMMUNITY OF SMALL MICE

(1) DETERMINE OF FUNCTION OF CELIAC MACROPHAGE OF SMALL MICE (see scheme 1)

EFFECT ON THE FUNCTION OF NONSPECIFIC IMMUNITY OF SMALL MICE

(scheme 1)



(2) EFFECT ON PLAQUNE FORMING CELL (PFC IN SPLEEN AND ON SEROLOGICAL SPECIFICAL ANTIBODY OF SMALL MICE

Q 517 Comparison of two novel anti-viral drugs against primary HIV-1 infected monocytes Filion, Lionel, G[*†], Logan, Diane[@†], Izaguirre, Carlos[#*], & Conway, Brian[*†]. Department of Microbiology & Immunology[*], Ontario Cancer Treatment and Research Foundation[@], Department of Medicine[†], University of Ottawa, and Federal Centre for Aids[#], Ottawa, Canada, K1H 8M5

HIV-1 infects not only T cells but also cells of the mononuclear phagocytic system (MCPS). These cells are comprised of monocytes, Langerhans cells, dendritic cells, microglial cells. We have developed a system in our laboratory to test anti-viral drugs against HIV-1 infected MCPS cells. Doxorubicin and etoposide are employed with efficacy in the treatment of infectious Kaposi's sarcoma. The mechanism of anti-tumour activity is unknown and possibly involves an indirect effect via antiviral activity. We have previously demonstrated that a 24 hour treatment of daunorubicin or doxorubicin could effectively control HIV-1 replication in U937 cells for at least 20 days. This drug was more toxic to T cells and had a marginal effect in controlling HIV-1 replication in infected T cells which had been infected for 24 hours prior to treatment. We have performed experiments with acutely (24 hrs) or chronically (96 hrs) HIV-1 infected monocytes and treated these cells with doxorubucin (0 to 100 ng/ml) or etoposide (VP-16) (0 to 100 ug/ml) for 24 hrs. The cultures were washed and recultured. Cell viability, morphology and p24 Ag levels were assessed every 3 to 4 days for 3 weeks. Doxorubicin had a more toxic effect than etoposide but was effective in controlling p24 Ag expression from acutely infected cells. However etoposide was less toxic to the monocytes and p24 antigen expression was reduced in both acutely and chronically infected cells over the whole time period. The conclusion from our experiments is that some anti-cancer drugs may be effective against HIV-1 replication in some cells types but not in others. The anti-topoisomerase activity of etoposide may be the mechanism by which this drug inhibits HIV-1 replication.

Q 518 POTENTIATION OF THE ANTIRETROVIRAL ACTIVITY AND ANABOLISM OF PURINE-2',3'-DIDEOXYNUCLEOSIDE ANALOGS BY INHIBITORS OF IMP DEHYDROGENASE, Laurence L. Bondoc, Jr., Gurpreet S. Ahluwalia, Neal R. Hartman, David A. Cooney, Hiroaki Mitsuya, David G. Johns, and Arnold Fridland. Department of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis, TN 38101 and LMC, DTP and COP, DCT, NCI, NIH, Bethesda, MD 20892

Phase I clinical trials have demonstrated a role for the purine nucleoside analog 2',3'-dideoxyinosine in the treatment of patients with severe HIV infection. We have found that the phosphorylation of ddI and its analogs 2',3'-dideoxyguanosine (ddG) can be increased up to 20-fold by inhibitors of the enzyme IMP dehydrogenase (ribavirin, tiazofurin or mycophenolic acid) in a number of cultured human T lymphoid cells. Concomitant with this increase in anabolite accumulation from ddNs was a significant increase in the anti-HIV activity of ddI and ddG when combined with ribavirin in the ATH-8 or with tiazofurin in the MOLT-4 assay system. Treatment with the IMPD inhibitors resulted in marked intracellular elevation of IMP as a consequence of IMPD inhibition and a decrease in GTP and dGTP levels and no change in dATP. A strong correlation was established between the accumulation of IMP and the potentiation in ddN triphosphate formation. Fold increase in dideoxynucleotide accumulation, however, varied for the various ddNs and among the various T cell lines studied. These results support the hypothesis that the potentiation of the ddNs when combined with ribavirin or other IMPD inhibitors is a consequence of increased intracellular activation of the relevant phosphotransferase by the increased concentration of IMP, the major phosphate donor for the initial phosphorylation in the anabolism of these drugs in human T cells. (This work was supported in part by grants AI27652 from NIH, CORE P30 CA21765 and American Lebanese Syrian Associated Charities.)

Q 519 SUSTAINED ZIDOVUDINE TREATMENT ON HEMATOPOIESIS IN IMMUNODEFICIENT MICE.

Vincent S. Gallicchio, Nedda K. Hughes and Kam Fai-Tse, Departments of Clinical Sciences and Medicine, University of Kentucky and Veterans Administration Medical Centers, Lexington, KY. 40536-0084

Zidovudine has been the drug of choice in the treatment of human AIDS. However, associated with the use of zidovudine has been the development of hematopoietic toxicity, the mechanism of which is still not clearly defined. We report here studies designed to evaluate dose-escalation of zidovudine, i.e., 0.1 and 1.0 mg/ml placed in the drinking water on hematopoiesis in C57BL/6 normal and LP-BM5 immunodeficiency virus infected mice. Over a six-week evaluation period, compared to normal controls, murine immunodeficiency disease (MAIDS) infection was associated with reduced hematopoietic progenitors, i.e., CFU-E, BFU-E, CFU-GM, and CFU-Meg from marrow and spleen. Following zidovudine treatment, further suppression of progenitors was observed, notably from the marrow, while increased progenitors were observed from the spleen. Spleen derived erythroid progenitors CFU-E were increased 950% from MAIDS infected mice receiving 1.0 mg/ml drug after 4-weeks exposure, compared to non-drug treated MAIDS control animals. Splenic BFU-E were increased 564% after 6-weeks exposure compared to non-drug treated MAIDS infected control animals. This study suggests the bone marrow is particularly sensitive to zidovudine toxicity, which is, at least early in exposure, appears to be compensated by splenic derived progenitors. Overt toxicity develops when, at least in this immunodeficiency model, the spleen is unable to provide progenitor cells in response to zidovudine exposure *in vivo*.

Q 520 INHIBITION OF HIV-1 PRODUCTION BUT NOT VIRAL DNA SYNTHESIS IN MICROGLIAL CELLS TREATED WITH TWO NUCLEOSIDE ANALOGS : AZT AND BCH189, Romas Geleziunas, Francois Boulerice, Hy Goldman and Mark A. Wainberg, McGill AIDS Centre, McGill University, Montreal, Quebec, Canada, H3T 1E2

Human Immunodeficiency virus type 1 (HIV-1) infection of the central nervous system generally leads to encephalopathy and progressive dementia. To study the effect of nucleoside analogs on the replication of HIV-1 in the human brain, we established cell cultures derived from human fetal brain. Primary cultures of neuroglial cells, derived from trypsinised brain tissue, were susceptible to a low level productive infection using virus produced by a cellular clone (UHC-1) derived from a HIV-IIIb chronically infected U-937 cell line (Boulerice *et al.* 1990 J. Virol. 64: 1745-1755). Northern blot analysis revealed that such cells contained each of the 9.2 kb unspliced genomic, 4.3 kb singly spliced and 2kb multiply spliced species of HIV-1 mRNA. Cell cultures enriched for macrophages (microglial cells), astrocytes and oligodendrocytes were established and examined for susceptibility to infection. Neither astrocyte nor oligodendrocyte enriched cultures could be productively infected as judged by p24 antigen release into culture supernatants. However, enriched cultures of macrophages supported productive infection by both UHC-1 and JR-FL viruses but not by SF162 or Ada-M isolates. Generally 1-2% of such cells expressed p24 antigen as judged by indirect immunofluorescence. While pretreatment of such macrophage enriched populations with 20 nM AZT failed to block infection, pretreatment with 1μM AZT, followed by removal of drug after 6 days successfully prevented production of progeny virus until 15 days. However, addition of 1 μM AZT, 24 hours after viral inoculation, did not prevent productive infection. Pretreating brain-derived macrophage-enriched cultures with a novel nucleoside analog BCH189, at 4.3 μM or adding this drug simultaneously with the viral inoculum, inhibited both viral p24 production in the culture supernatant and HIV-1 specific mRNA synthesis until 18 days post-infection. However, HIV-1 DNA could be detected by PCR, using primer pairs located in the env gene.

Q 521 DEVELOPMENT OF HAMMERHEAD RIBOZYMES AS INHIBITORS OF HIV-1 REPLICATION. John C. Guatelli and Douglas D. Richman, San Diego Department of Veterans Affairs Medical Center and the UCSD School of Medicine, San Diego CA 92161.

Two hammerhead ribozymes have been tested in several formats for activity against HIV-1 RNA. Ribozyme1 was designed to cleave a sequence within the first coding exons of *tat* and *rev*. Ribozyme2 was designed to cleave the junction sequence of exon1 and exon4 (a *tat* splice junction). These ribozymes have been tested in cell free reactions with synthetic targets, in transfections when placed in *cis* on the HIV genome, and for ribozyme1, by challenge with virus of lymphoblastoid cell lines (CEM) that express constitutively ribozyme RNA.

Ribozyme1 and 2 cleaved specifically their RNA targets in cell free reactions. Ribozyme2 failed to cleave RNA containing the junction sequence of exon1 and exon5 (a *nef* or *vpu/env* splice junction) and failed to cleave RNA containing the genomic exon4 splice acceptor sequence. Thus, ribozyme2 appeared specific for *tat* mRNA.

When ribozyme1 or ribozyme2 was inserted into the 3' end of a full length HIV-1 genome (pNL43), the resulting ribozyme insertion mutants remained biologically competent but exhibited reduced levels of replication after transfection into CEM cells as assayed by p24 antigen production. This reduction was evident only 7-14 days after transfection.

CEM cells were stably transfected with an expression vector containing ribozyme1. The resulting cell lines failed to show a reduction in p24 antigen after challenge with HIV-1 at high multiplicity when compared to the parental line.

A working hypothesis is that the expression level or localization of ribozymes are factors important in achieving efficacy within cells. Ribozymes inserted in *cis* within the HIV-1 genome may show effects because they are replicated to high levels on viral RNAs. Such *cis*-ribozyme experiments may be useful to screen potential target sites in the HIV-1 genome for impact on viral replication.

Q 522 CONSTRUCTION OF RETROVIRAL VECTORS ENCODING HIV-1-REGULATED DIPHTHERIA TOXIN A CHAIN: AN APPROACH TO GENE THERAPY FOR AIDS, Gail S. Harrison, Ian H. Maxwell, Françoise Maxwell and Cynthia J. Long, Division of Medical Oncology, University of Colorado Health Sciences Center, Denver, CO. 80262.

Retroviral vectors were constructed, based on LNSX (Miller and Rosman, BioTechniques 7: 980-990, 1989), containing HIV-regulated diphtheria toxin A chain sequences. These sequences were derived from pTHA43 (Harrison et.al., Human Gene Therapy 2: 53-60, 1990) which has low basal expression and requires both Tat and Rev for maximal trans-activated expression. The HIV-1 sequences included in the retroviral proviral constructs were -167 to +80 of the LTR (including the enhancer and TAR region) and 5925-8490 of the env region (including the Rev Response Element, and Cis-acting Repressive Sequences to reduce basal expression). Three retroviral constructs were generated, two of which contained wild type DT-A sequences, and differed from each other in the orientation of HIV LTR and DT-A sequences relative to viral transcription. The third construct contained an attenuated DT-A chain, tox 176, (Maxwell et.al., Mol and Cell. Biol. 7: 1576-1579, 1989). The proviral constructs showed similar basal and trans-activated levels of expression to the parental plasmids in transient co-transfection assays. Amphotropic retroviruses were generated, using either a "ping-pong" procedure or a standard transfection/infection procedure. Both procedures resulted in production of amphotropic retrovirus, at titers ≤22,000 CFUs/ml. Packaging clones containing HIV LTR and DT-A sequences were scored positive on the basis of DNA-based PCR (using cell extracts) and RNA-based PCR (using reverse transcribed cDNA from cell supernatants, after Morgan et.al., Human Gene Therapy 1: 135-149, 1990). Preliminary results suggest that H9 cells infected with the DT-A containing retroviruses showed an impaired ability to produce HIV (details to be presented). The data presented here, and earlier data showing that stable cell lines could be derived with plasmid-based regulated DT-A sequences (Harrison et.al., AIDS Res. and Human Retrov., in press), show that very low basal expression of DT-A may be achieved both in plasmid and retroviral constructs, and support the ultimate feasibility of toxin gene therapy for AIDS.

Q 523 SUSCEPTIBILITY OF HIV-1 TO AZT IN PATIENTS RECEIVING ddI. Nanci Hawley-Foss, D.W. Cameron, D. Ko, B. Conway. Division of Infectious Diseases, Ottawa General Hospital, Ottawa, Canada K1H 8L6.

The objective of this study was to evaluate in vitro susceptibility of HIV-1 to AZT in patients currently receiving ddI. We identified 10 AIDS patients in whom antiretroviral therapy was changed from AZT to ddI following progression of immune disease while on AZT. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque sedimentation. After stimulation with PHA-P and IL-2, the PBMCs were resuspended in the presence of 0 or 10 μM AZT and incubated for an additional 72 hours. The cells were then harvested and processed for PCR. Product detection was performed using a newly developed quantitative microtiter plate assay. The reduction (%) in viral load in the presence of AZT was taken as a measure of antiviral susceptibility.

In 4 patients, no reduction in viral load was observed in the presence of AZT, indicating persistent high-grade resistance. In 6 patients, 25-77 (51.7 ± 17.6)% reduction was observed, indicating susceptibility to AZT. In one case, previous high-grade AZT resistance had been documented. Thus, we conclude that susceptibility to AZT can be observed in patients on ddI following clinical failure of AZT. This lends support to alternative therapeutic strategies such as sequential or alternating use of AZT and ddI.

Q 524 PROPHYLACTIC AZT THERAPY PREVENTS EARLY CD4-LYMPHOCYTE DECLINE BUT NOT PRIMARY VIRUS INFECTION IN FELINE IMMUNODEFICIENCY VIRUS INOCULATED CATS, Kathleen A. Hayes, and Louis J. Lafrado, and Lawrence E. Mathes, Center for Retrovirus Research, The Ohio State University, Columbus, OH, 43210.

In this study the efficacy of prophylactic AZT therapy was evaluated in cats inoculated with the Mount Airy isolate of feline immunodeficiency virus (FIV-MA). Weanling cats were given AZT by continuous subcutaneous infusion (30 mg/kg/day) beginning 48 hrs before FIV-MA inoculation and continuing for 28 days. Virus challenge controls consisted of age-matched, untreated cats inoculated with FIV-MA. Parameters evaluated were CD4 and CD8 lymphocytes counts, CD4/CD8 ratios, FIV seroconversion and antibody titers, and provirus levels in peripheral blood leukocytes (PBL) as determined by polymerase-chain-reaction (PCR). The CD4 counts and CD4/CD8 ratios of FIV inoculation controls dropped significantly between 4 and 6 wks post inoculation (PI) and continued to be suppressed beyond 24 wks PI. The AZT treated cats, however, had normal CD4 counts and CD4/CD8 ratios throughout the first 9 wks although these values declined in some animals by 24 wks PI. The antibody responses for the AZT-treated, FIV inoculated cats were virtually identical to the FIV inoculation controls and were evident by 2 wks PI. Although PBL samples collected in the first 7 wk were negative for provirus by PCR using nested primer pairs for *gag*, by 24 wks PI all animals from both AZT-treated and untreated groups were PCR positive. The results suggest that prophylactic AZT therapy protects lymphocytes from virus mediated decline during the treatment period. However, the rapid seroconversion and the PCR results indicate that primary FIV infection was not prevented even during the AZT treatment period.

Q 525 STRUCTURE AND FUNCTION OF NUCLEOCAPSID (NC) PROTEIN: A TARGET FOR DRUG DEVELOPMENT, Louis E. Henderson[1], Robert J. Gorelick[1], Patricia J. Powell[1], Raymond C. Sowder, II[1], Julian W. Bess, Jr[1]., Larry O. Arthur[1] Michael F. Summers[2], Terri L. South[2], Paul R. Blake[2], Gabriela Perez-Alvarado[2], Mark R. Chance[3], Irit Sagi[3], [1]AIDS Vaccine Program, PRI/DynCorp, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702. [2]Dept. of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, MD 21228, [3]Dept. of Chemistry, Georgetown University, Washington, D.C. 20057.

All retroviral nucleocapsid (NC) proteins and Gag precursors contain one or two copies of an amino acid sequence (-Cys-$(X)_2$-Cys-$(X)_4$-His-$(X)_4$-Cys-) (CCHC array). Mutations altering conserved residues in CCHC arrays of HIV-1 or SIV render the mutants noninfectious and deficient in genomic RNA packaging. Electron micrographs of mutants show immature particles but "western" blot analysis of purified mutants shows near normal proteolytic processing of Gag proteins. The structure and function was further elucidated by showing that CCHC arrays bind zinc in the mature virus and purified NC protein and form peptide structures that interact directly with nucleic acids. Retroviruses contain approximately one gram atom of zinc per mole of CCHC array and zinc-edge extended X-ray absorption fine structure analysis of intact retroviruses shows the zinc is coordinated to three sulfurs and one nitrogen. HIV-1 and SIV NC proteins contain two CCHC arrays and solution state NMR of the HIV-1 NC protein-zinc complex has provided a three dimensional model for the protein in solution. Coordination to zinc imposes peptide conformations with aromatic residues extended into solution. In HIV-1, these aromatic residues are phenylalanine in the first CCHC array and tryptophan in the second; the SIV protein has tryptophan in both CCHC arrays. The intrinsic fluorescence of tryptophan residues is highly sensitive to zinc coordination and nucleic acid binding. The fluorescence quenching suggest that the aromatic residues interact directly with single-stranded nucleic acids and may intercalate between bases and help provide apparent specificity of binding.

Q 526 A DECREASE IN VIRUS LOAD MEASURED BY RNA AND DNA PCR AND CELL CULTURE IN ASYMPTOMATIC SUBJECTS TREATED WITH COMBINATION THERAPY. Mark Holodniy, David Katzenstein, Mark Winters, Jose Montoya, Robert Shafer, Michael Kozal, Margaret Ragni, and Thomas C. Merigan, Center for AIDS Research, Stanford University Medical Center, Stanford, CA. 94305

Quantification of viral load in HIV disease has become increasingly important as a marker of antiviral efficacy. Assays are available to measure HIV nucleic acid and infectious virus. We have applied these techniques in vivo to assess antiretroviral activity of combination therapy. Five HIV infected, drug naive subjects were administered combination therapy with ZDV and ddI. Plasma and PBMC were obtained twice at baseline and then at one, two, six and 9 months after the initiation of therapy. Plasma HIV RNA and PBMC proviral DNA were extracted, and then with RNA, reverse transcribed to cDNA. The resulting DNA was amplified and then PCR product detected and quantitated using a nonisotopic microplate method. PBMC were also cultured by a micro culture, cell dilution method. Finally, PBMC DNA was analyzed genotypically for pol gene mutations using a nested PCR strategy. Results show that plasma HIV RNA copy number fell from 2170 $\pm$ 660/ml to undetectable at 1 month, with continued suppression at 9 months. HIV proviral DNA copy number decreased from 2260 to 491/10^6 CD4 cells at 9 months. Cell dilution cultures were positive in 4 of 5 subjects at baseline and in only 1 of 5 after 6 and 9 months. CD4 count increased from 390 $\pm$ 30/mm^3 pretherapy, to 439 $\pm$ 66/mm^3 after 9 months of therapy. None of the subjects were p24 antigenemic throughout study. No mutations were detected from PBMC DNA for codon 215 and 70 in the HIV pol gene. These findings suggest that plasma RNA , cellular DNA or cell dilution culture can quantitate changes in viral load after combination antiretroviral therapy and that suppression can be maintained for 9 months. The lack of genotypic changes which have been associated with ZDV resistance is encouraging.

Q 527 POTENTIAL OF STERICALLY STABILIZED (STEALTH™) LIPOSOMES AS A DRUG DELIVERY SYSTEM AGAINST KAPOSI'S SARCOMA; S.K. Huang[1], F.J. Martin[3], G. Jay[4], J. Vogel[4], D. Papahadjopoulos[1] and D.S. Friend[2]; [1]Cancer Res. Inst., [2]Dept. of Pathol., University of California, San Francisco, CA 94143, [3]Liposome Technology, Inc., Menlo Park, CA 94025, [4]Laboratory of Virology, Jerome H. Holland Laboratory, Rockville, MD 20855.

Transgenic mice bearing the HIV tat gene which induces dermal lesions resembling Kaposi's sarcoma (KS), a common malignant tumor in AIDS (J. Vogel et al., Nature, 335, 606-611, 1988), were used to investigate the distribution of sterically stabilized liposomes. These animals were injected with sterically stabilized liposomes (between 80-100 nm in diameter) composed of PC/C/PEG-DSPE (10:5:0.8) encapsulating colloidal gold. To detect the distribution of liposomes in the KS lesions, samples were fixed at 24 hr after i.v. injection and processed for light and electron microscopy. Silver enhancement of the liposome-entrapped colloidal gold was employed as a light microscopic marker for the liposomes in thick sections. The silver marker was visible predominantly in the dermis surrounding the KS lesions (either earlier or mature) which are characterized by a proliferation of fibroblast-like spindle cells and abnormal blood vessels close to the epidermis. Dense, silver-enhanced colloidal gold often surrounded vascular channels and scattered erythrocytes. Some spindle cells and macrophages were heavily labeled. The distribution of colloidal gold after injection of sterically stabilized liposomes was much higher in the KS lesions than in the adjacent normal skin, where they also crossed postcapillary venules. By electron microscopy, intact liposomes with colloidal gold were found in vascular channels around erythrocytes. The accumulated gold particles were observed in the endosomes and lysosomes of macrophages, pericytes, and some spindle-shaped cells in the KS lesions. We conclude that sterically stabilized liposomes with prolonged circulation time in blood are able to traverse the irregular and discontinuous endothelium of Kaposi's sarcoma, and extravasate extensively into the lesion. Due to the properties of high accumulation exclusively in KS lesions and direct uptake by some tumor cells, sterically stabilized liposomes appear highly attractive as carriers of chemotherapeutic agents for this neoplasm.

Q 528 GENE INHIBITION OF HIV-1 REPLICATION: A COMPARATIVE AND MECHANISTIC STUDY, ***William James and Paul Crisell,*** *Sir William Dunn School of Pathology, University of Oxford, U.K.*

We have analysed the ability of a number of antisense RNAs (ARs), ribozymes and decoy RNAs to inhibit the replication of HIV-1 using retroviral transduction of inhibitory RNA-encoding genes. The majority of AR genes tested were non-inhibitory but a minority gave rise to up to 80% inhibition of virus replication (Rhodes & James, *J Gen Virol* **71**: 1965-74, 1990; Rhodes & James, *AIDS* **5**: 145-51, 1991; James, *Antivir Chem Chemother* **2**:in press). An examination of these results shows that they are inconsistent with the commonly held beliefs about antisense action and have led us to propose that it is the local folding potential of the complementary RNAs, rather than the information content of the target RNA, that determines the inhibitory potential of the antisense molecule. In this paper we describe a series of experiments designed to provide a rigorous proof of this hypothesis with the aim of providing a sound rational basis for the design of inhibitory genes for intracellular immunization. In addition, we show that the inhibitory effects are not the result of the activation of generalized dsRNA-dependent systems of cellular resistance to virus infection. We also tested the popular contention that antisense methodologies are too sensitive to strain-dependent sequence variation to be effective. We found that a) an inhibitory antisense RNA against strain IIIB also inhibited strains MN, RF and SF2 with indistinguishable potency and b) that virus emerging from challenged, antisense-expressing cultures was still equally sensitive to antisense inhibition in a second challenge experiment as was the parental virus. In parallel studies, using identical vector and cell systems, we have examined the effectiveness of two other methods of gene-inhibition: ribozymes and decoy RNA. With one ribozyme, constructed by the inclusion of the hammerhead motif into a non-inhibitory antisense gene, 95% inhibition of HIV-1 replication was observed for extended periods, protecting the cells from cytopathic effects exhibited by the unprotected, control cultures. The most effective inhibitory construct, however, encoded a multimeric TAR decoy RNA. In our system, this produced over 99% inhibition of HIV-1 replication. Since each of these classes of inhibitory gene can be delivered by identical vectors but act by different mechanisms, we are exploring the potential of a combinatorial approach, including inhibitory antisense RNA, ribozyme and decoy RNA-encoding genes in a single vector.

Q 529 SUPPRESSION OF INDUCTION OF HIV EXPRESSION IN CHRONICALLY INFECTED PROMONOCYTIC CELLS BY AN ORGANIC THIOPHOSPHATE WR151327, Thea Kalebic, Philip S. Schein[1], Philip A. Pizzo, Pediatric Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD [1]U.S. Bioscience, West Conshohocken, PA 19428

An increasing body of evidence suggests that reducing agents may interfere with both the acute HIV infection and the activation of latent HIV in chronically infected cells. In a previous study, we demonstrated that glutathione (GSH), glutathione ester (GSE), and N-acetyl cysteine (NAC) suppress the induction of HIV expression in chronically infected promonocytic U1 cells stimulated with the viral inducers TNFα, IL-6, and PMA. The purpose of the present study was to investigate whether another thiol containing agent, the organic thiophosphate WR151327 (1-Propanethiol, 3-[[3-(methylamino) propyl] amino], dihydrogen phosphate (ester) may also inhibit the activation of expression of latent HIV. As a cellular model of HIV latency, chronically infected promonocytic U1 cells which harbor in their genome two copies of proviral DNA was used. To monitor HIV activity, the level of reverse transcriptase was measured in the supernatants. We found that WR151327 (4mM) suppressed approximately 60% expression of HIV in U1 cells stimulated with both TNFα and PMA. No cytotoxic effect on U1 cells due to WR 151327 was detected at that concentration. The suppressive effect lasted for eight days following a single treatment of cells with WR151327. Interestingly, the HIV-inducing ability of TNFα was not affected by 6 hours incubation with WR151327, suggesting that this drug acts preferentially at the intracellular level. Compared to GSH, GSE, and NAC, WR151327 at the same concentration more effectively suppressed the expression of HIV in chronically infected cells stimulated with viral inducers PMA and TNFα. Potentially, WR151327 may have a similar effect in vivo.

Q 530 DIETHYLCARBAMAZINE: POTENTIAL ADJUNCT TREATMENT FOR HIV INFECTION? Lynn W. Kitchen, M.D., Jaime E. Hernandez, M.D., James A. Ross, Marshall University School of Medicine, Huntington, WV.

We have found previously that oral administration of diethylcarbamazine (N,N-diethyl-4-methyl-1-piperazine carboxamide [DEC]), a biological response modifier agent long used in the prevention and treatment of the filariases, was associated with (1) prolonged survival in newborn mice inoculated with murine leukemia virus (a trend toward less severe brain and splenic pathologic lesions consistent with untreated Cas-Br-M infection was also noted [JCLI, in press]); and (2) reduced serum viral infectivity and FeLV serum antigen levels in chronically FeLV-infected cats. DEC treatment of 2 kittens shortly after evidence of infection with FeLV significantly reduced the rate of decline of circulating lymphocytes in comparison to 2 untreated littermates (Kitchen LW, Mather FJ, and Cotter SM, JCLI 27:179-181, 1988). Because DEC is an inexpensive, orally bioavailable, and relatively nontoxic drug, and all available data indicate that it can be given safely during pregnancy, we investigated its effect on AIDS-related opportunistic bacterial/fungal infections using mouse models. DEC treatment was associated with (1) higher serum antibody levels to the inoculated organism 1 month following i.p. inoculation of live bacteria (Streptococcus pneumoniae, Haemophilus influenzae); (2) decreased levels of brain S. pneumoniae when administered with pneumococcal vaccine followed by lethal S. pneumoniae challenge; (3) reduced brain levels of Cryptococcus neoformans, Candida albicans, and Aspergillus fumigatus; and (4) reduced brain weights (swelling) in C. neoformans-inoculated mice.

Q 531 ANTIVIRAL THERAPY DIRECTED AGAINST THE REV RESPONSIVE ELEMENT BLOCKS REV ACTIVITY AND HIV INFECTION IN VITRO. Mary E. Klotman, Ge Li, Juliana Lisziewicz, Simon Daefler, Daisy Sun, Gerald Zon, Flossie Wong-Staal and Robert C. Gallo. Laboratory of Tumor Cell Biology, NIH, Bethesda, Md. 20892.

The Rev protein is an essential gene of HIV-1 that acts post-transcriptionally to increase the expression of unspliced or singly spliced RNA. Recombinant purified Rev protein binds directly to its target sequence in the viral messages, the Rev responsive element (RRE). Further mapping studies have localized this binding site to the 5'end of the RRE. Since Rev activity is required for viral infectivity, interference with the Rev-RRE interaction should be an effective antiviral approach. We synthesized a series of phosphorothioate and methylphosphonate-modified oligonucleotides directed against the RRE including the Rev binding site and tested their ability to block Rev function in a transient transfection assay and their ability to block acute infection in vitro. In a transient transfection assay when chloramphenicol acetyl transferase activity (CAT) is dependent on Rev expression, antisense oligonucleotides directed against stem I and stem loop II of the RRE blocked Rev activity as indicated by a decrease in CAT activity when compared to random hexamer or a homopolymer of 28 deoxycytidines. The extent of blockade was dose dependent. While an antisense oligonucleotide of 15 bases in length directed against the 3' end of the RRE did not block Rev activity, a twenty-eight base sequence against the same region did. Stem I and stem loop II oligonucleotides inhibited acute HIV-1 infection of H9 cells as determined by a decrease in HIV RNA, reverse transcriptase, syncytial formation, p17 and p24. These results suggest that interference of the Rev-RRE interaction might be a target for antiviral therapy.

Q 532 EFFECT OF CYTOKINES ON THE ANTICRYPTOCOCCAL ACTIVITY OF HUMAN BRONCHOALVEOLAR MACROPHAGES (BAM)

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Background: Meningitis caused by Cryptococcus neoformans (CN) remains a leading cause of morbidity and mortality in patients with AIDS. The alveolar macrophage is the first line defense against the inital pulmonary innoculation and subsequent dissemination of the infection. We present data which suggests a role for recombinant cytokines in the treatment of cryptococcal infections. Becuase it is unclear if all elements necessary for appropriate opsonization of CN are present in the human alveolus, we performed these studies using opsonized and unopsonized organisms.

Methods: Bronchoalveolar lavage was performed on normal human voluteers. Cells were cultured with media or with tumor necrosis factor (TNF), macrophage colony stimulating factor (MCSF), interleukin-2 (IL-2), or interferon gamma (IFN) for 24 hours. Time course studies were performed with 6, 24 and 48 hour incubations with MCSF. After incubation, BAMs were cocultured with 40,000 colony forming units of CN for 24 hours. Growth inhibition was determined by comparing the colony forming units from cultures containing cells with those from cultures of CN alone.

Results: Results of the 48 hour incubations were as follows.

	Media	TNF	IFN	MCSF	IL2
% Inhibition +PHS	41.2	38.8	50.0	49.0	40.0
% Inhibition -PHS	21.4	32.1	37.0	27.8	28.0

The enhancement of anticryptococcal acitivity for MCSF was calculated by comparing the CFU in groups of BAM alone with those containing BAM and MCSF.

	6 hours	24 hours	48 hours
% Enhancement	56.1	35.2	18.9

Conclusions: Human BAM have greater anitcryptococcal activity against serum opsonized CN. Although all of the cytokines tested improved the anitcryptococcal activity of BAM against unopsonized CN, only IFN and MCSF were able to augment acitity against opsonized CN. This suggests the different receptors required for the binding and internalization of CN by BAM are under the control of different activation pathways and can be augmented uprequlated by different cytokines. The rapid response of BAM to MCSF suggests that new receptors or proteins are not required for this effect. These studies provide support for the investigation of recombinant cytokines in human cryptococcosis.

Q 533 STUDIES ON THE BIOCHEMICAL BASIS FOR HIV RESISTANCE TO AZT, Simon F. Lacey, John E. Reardon, Eric S. Furfine, Sharon D. Kemp and Brendan A. Larder, Department of Molecular Sciences, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS U.K. and Department of Experimental Therapy, Burroughs Wellcome, Research Triangle Park, North Carolina 27709, USA.

Five mutations have been identified to date in the HIV reverse transcriptase (RT) gene as capable of conferring resistance to 3'-azidothymidine (AZT, Zidovudine). The biochemical basis for this resistance has so far remained obscure. Here we describe a quantitative PCR study on HXB-2 (sensitive) and RTM-C (recombinant mutant with 4 amino acid changes conferring 100-200 - fold higher AZT-resistance) infection timecourses in the presence of AZT. We have been able to show a >10 fold difference between the two strains in the level of full length linear genome synthesis in the presence of 20 micromolar AZT. This agrees with the data from plaque reduction assays which give a 100-200 fold increase in resistance but contrasts with the data from our kinetic studies on recombinant RTs purified from E.coli. We have done steady-state kinetics on the enzymes corresponding to HXB-2 and RTM-C, using several template-primers and AZTTP, d4TTP, and NH_2TTP as inhibitors under a range of assay conditions. We found only moderate (2-fold) differences between the K_i values for the two enzymes. The RNase H activities associated with the two enzymes were qualitatively and quantitively similar. Possible explanations for these paradoxical results will be considered.

Q 534 HIV-1 PROTEASE INHIBITOR (SKF 108922) INTERACTION WITH AZT IN ACUTE AND CHRONIC HIV-1 INFECTIONS in vitro, D. M. Lambert, H. Bartus, G. B. Dreyer, T. D. Meek, B. W. Metcalf and S. R. Petteway, Jr., Depts. of Antiinfectives, and Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406-0939

Synthetic peptide analog inhibitors of HIV-1 protease inhibit the spread of infectious virus in acutely infected T-cells and production of infectious virions in chronically infected cells. These inhibitors work predominantly late in the infectious cycle while AZT works early. To investigate whether they exhibited additive or synergistic drug interaction effects, SKF 108922, an HIV-1 protease inhibitor, was compared with AZT. Antiviral activity of these compounds was evaluated in three separate in vitro assays. (1) In acute infection of Molt-4 cells with HIV-1 strain IIIB, co-treatment with these compounds demonstrated additivity affording a 2-3-fold enhancement of activity of both compounds. (2) In co-cultivation experiments with Molt-4 cells and chronically infected H9 cells, SKF 108922 demonstrated potent synergy with AZT. Whereas, with a chronically infected CEM cell line, the interaction was additive. (3) AZT treatment of the H9/IIIB chronic cell line alone demonstrated no inhibitory effect, but in comparison SKF 108922 was potently inhibitory. Co-treatment of H9/IIIB chronically infected cultures with both SKF 108922 and AZT showed activity similar to SKF 108922 alone. These data suggest that the antiviral effect of AZT is primarily manifest on acute infection. In contrast, HIV-1 protease inhibitors exert a potent antiviral effect on both acute and chronic infections. Since AZT and SKF 108922 have different viral targets and inhibit different stages of infection, it is likely that co-treatment with AZT and a protease inhibitor could have an additive or synergistic effect in vivo. The in vitro data to be presented support this concept.

Q 535 "IN VITRO" GENERATION OF ZIDOVUDINE RESISTANCE IN AN HIV-1 ISOLATE.

Sally A. Land[1], Nicholas J. Deacon[2], David J. Hooker[2] and Chris J. Birch[1]. [1]Virology Department and [2]Macfarlane Burnet Centre for Medical Research, Fairfield Hospital, Yarra Bend Road, Fairfield 3078, Victoria, Australia.

HIV-1 isolates from individuals receiving longterm zidovudine therapy are usually resistant to high levels of the drug (5µg/ml, 18.7µM). However, it has been difficult to generate resistant strains "in vitro". We attempted this procedure to enable comparison of the virological properties of resistant isolates induced "in vitro" and "in vivo".

A zidovudine-sensitive isolate (ID_{100} ⩽0.05µg/ml) was serially passaged in peripheral blood mononuclear leucocytes for 7 months in increasing concentrations of the drug, resulting in the production of an isolate 20-fold less sensitive to zidovudine. This resistant isolate produced high levels of HIV antigen, virion-associated reverse transcriptase (RT) activity, and infectious virions in the culture supernates. Classical giant multinucleated cells could also be observed in infected cultures. Infection of a continuous lymphoblastoid cell line (MT-2) with extracellular virus in the presence and absence of zidovudine induced syncytia formation accompanied by RT activity, although this activity was less in cultures grown in the presence of the drug. Limited DNA sequencing showed a change at amino acid 70 (from lysine to argine) was associated with the decreased susceptibility to zidovudine. No changes from wild type were seen at 67,215 and 219. We conclude that it is possible to select for a zidovudine-resistant HIV-1 isolate by continued exposure of a drug-sensitive virus to increasing concentrations of the drug.

Q 536 SKF 86002, AN INHIBITOR OF TNFα AND IL-1 SYNTHESIS, SIGNIFICANTLY REDUCES THE PRODUCTION OF HIV-STIMULATING ACTIVITY BY ACTIVATED MONOCYTES. J. Leary, P. Clark, U. Prabhakar, D. Lipshutz and K. Esser. SmithKline Beecham Pharmaceuticals, King of Prussia, PA.

It has been proposed that activation of HIV in chronically or latently-infected T cells plays a role in AIDS pathogenesis. Cytokines, most notably TNFα, can activate virus production from chronic or latent infections of T-cells in vitro with activation apparently occurring through TNFα induction of NFκB and subsequent transactivation of HIV transcription (Folks et al., 1989). Sensitivity to TNFα activation was a common phenotype in H9 cells chronically infected with HIV strain IIIB, with 8 of 10 cloned cell lines producing elevated virion RT levels in response to recombinant TNFα. Similar virus induction was observed in response to supernatant medium from human monocytes stimulated with lipopolysaccharide (LPS) in vitro. The virus stimulating activity produced by these monocytes was neutralized by monoclonal antibody against TNFα. Supernatants from cultured human monocytes stimulated with LPS in the presence of 10 μM SK&F 86002 were significantly less active in inducing HIV reverse transcriptase production by chronically-infected H9 cells. In contrast, AZT did not inhibit either production of TNF by monocytes or production of HIV by chronically-infected H9 (T-cells) induced with TNF. SKF 86002 is an inhibitor of arachidonic acid metabolism and also blocks monocyte production of TNFα and IL-1β (Lee et al., 1989). These results indicate that pharmacological regulation of TNF synthesis is a potential strategy for AIDS therapy.

Lee, et al., 1989. Agents and Actions 27:277-279.

Q 537 **CARDIOTOXICITY OF DIDEOXYNUCLEOSIDES IN RATS INCLUDES MORPHOLOGICALLY DISTINCT AND SELECTIVE MYOCARDIAL LESIONS.** William Lewis, Thomas Papoian, and Phillip Kennedy. Department of Pathology and Laboratory Medicine, UCLA School of Medicine, Los Angeles, CA 90024-1732.

FDDA (2′-Fluoro-2′,3′-dideoxyarabinosyl adenosine), a fluorinated dideoxynucleoside (ddN), may be a potent antiretroviral agent in AIDS. Some ddNs exhibit cardiotoxicity at high doses in rats (Lewis et al., 1991) and similar changes may occur in humans treated with ddNs (Willoughby et al., Circulation 84:II3, 1991 [abstract]). To evaluate cardiac toxicity of some ddNs in the rodent model, adult female Sprague Dawley rats were treated with 75-300 mg/kg FDDA using a single intraperitoneal injection. After 1, 3 or 6 days, rats were anesthetized and subjected to perfusion fixation with 10% neutral buffered formalin. FDDA caused isolated zones of cardiac hyaline and eosinophilic myocyte change which were visible by light microscopy. Cardiac lesions appeared localized, particularly to the left ventricle apex, and appeared focally subendocardial. The cardiotoxic threshold was approximately 75 mg/kg FDDA. Myocardial lesions were microscopically discernable 3 days after FDDA treatment. No comparable FDDA-induced skeletal myotoxic changes were found in deltoid or quadriceps muscle.

In parallel, rats were exposed to didanosine (dideoxyinosine, DDI) in chow (doses: 50 and 500 mg/kg/day). After 35 days, rats were subjected to perfusion fixation with 2% glutaraldehyde and hearts were examined by transmission electron microscopy (TEM). DDI (50 or 500 mg/kg/day) induced no cardiac mitochondrial changes on TEM. A positive control for the system used rats treated with zidovudine (azidothymidine, AZT) at approximately 100 mg/kg/day. Hearts from AZT treated rats yielded characteristic mitochondrial changes including swelling and cristae disruption after 35 days which were similar to those previously reported (Lewis et al., 1991). Results show that ddNs exhibit selective cardiac toxicities. ddN toxic targets in cardiac myocytes may include mitochondria.

Q 538 DIFFERENCES IN THE EFFECTS OF ANTIVIRUS AGENTS AND ADMINISTRATION SCHEDULES ON HIV-INDUCED IMMUNE SYSTEM ACTIVATION. Min Liu*, G. Skowron#, T. Merigan,+ N. Aziz* and J.L. Fahey*.
*UCLA, School of Medicine, Los Angeles, CA 90024; #Brown University, Providence RI 02908; +Stanford University, Stanford, CA 94305; and the ACTG 047 Research Group.

Zidovudine (AZT) and dideoxycytidine (ddC) increase CD4 T cell levels and AZT increases survival in HIV infection. These drugs were administered separately or alternately in ACTG protocol 047. We have compared the effect of these two drugs on the immune activation induced by HIV infection. Serum samples including baseline and serial samples for 24-48 weeks of treatment were studied in 87 subjects with ARC or AIDS and CD4 levels from 9 to 319/mm^3 (mean level 170/mm^3). AZT administered daily (200mg, q4h) caused progressive reduction in elevated ß2M levels. (Mean reduction of 45% toward the normal mean level at 6 months). Intermittent AZT (given in alternate weeks) however, showed a saw-tooth effect, e.g. substantial reduction at end of weeks on AZT, and returned to baseline at end of weeks off. Intermittent ddC (0.01 mg or 0.03 mg/kg, q4h), in contrast, had lesser one-week effects but had a delayed and cumulative suppressive effects on HIV-induced immune activation. AZT and ddC, given alternately by week or month, had complementary effects. ß2M is produced by B and T cells. Serum neopterin, which is produced largely in macrophages, was also changed by AZT and ddC. These serum measurement provide quantitative and convenient methods to demonstrate and compare the effects of AZT and ddC on HIV-induced immune pathology.

Q 539 *TRANS*-DOMINANT REV MUTANTS CAN PROTECT T CELL LINES FROM HIV-1 INFECTION WITHOUT DISTURBING NORMAL FUNCTION, Michael H. Malim, William W. Freithmuth*, Jingsong Liu*, Terence J. Boyle, H. Kim Lyerly, Bryan R. Cullen and Gary J. Nabel*, Departments of Microbiology and Immunology, Medicine and Surgery, Duke University Medical Center, Durham, NC 27710 and *Departments of Internal Medicine and Biological Chemistry, University of Michigan Medical Center, Ann Arbor, MI 48109-0650.

The specific inhibition of HIV-1 replication by certain mutant forms of virally encoded proteins offers the possibility for both therapeutic and preventative strategies in the control of HIV-1 infection and/or AIDS. Certain mutant forms of the essential nuclear *trans*-activator, Rev, are known to be effective inhibitors of wild type Rev function, and therefore HIV-1 replication. To date these proteins, termed *trans*-dominant negative mutants, have only been shown to function in transient transfection assays. In an effort to explore these inhibitory phenomena more fully, the cDNA encoding one such mutant (M10) was used to construct an amphotropic murine retroviral vector. This, as well as control vector expressing ß-galactosidase, was then used to stably transduce the T cell leukemia lines CEM, Jurkat and EL-4. Following subcloning, several of the CEM derived lines were shown to express detectable levels of M10 mRNA and protein. The resistance of these lines to viral replication was monitored after challenge with a range of virus titers generated from either a molecular clone (HXB-3/NL4-3 chimera) or the HIV-IIIB pool. Strikingly, a number of the M10 expressing, but not control, lines displayed resistance to acute infection over a period of four weeks. Importantly, T cell function was not detectably impaired by constitutive expression of the M10 protein since the CEM lines had retained levels of CD4 positivity and mitogen (PMA) sensitivity comparable to those of the control lines. In addition, the secretion of interleukin-2 subsequent to mitogen stimulation was unaffected by M10 expression in either the Jurkat or EL-4 lines. Taken together these data indicate that stable expression of a *trans*-dominant Rev mutant in a T cell suppresses HIV-1 replication without compromising lymphocyte function and suggest that this approach may have potential in the control of this important human pathogen.

Q 540 BIOCHEMICAL CHARACTERIZATION OF HIV-1 REVERSE TRANSCRIPTASE WITH MUTATIONS ASSOCIATED WITH DDI RESISTANCE AND COLLATERAL AZT SENSITIVITY. J. Louise Martin[1], Phillip A. Furman[1], Brendan A. Larder[2], and Jeanne E. Wilson[1]; [1]Division of Virology, Burroughs Wellcome Co., Research Triangle Park, NC USA, [2]Division of Molecular Sciences, Wellcome Research Laboratories, Beckenham, Kent, England

Genomic analysis of isolates obtained from AIDS patients treated with ddI following long-term AZT treatment revealed a mutation at position 74 associated with decreased sensitivity to ddI. Molecular clones were constructed by site-directed mutagenesis and subcloned from the M13 vector into an over-expression vector, pKK233. The protein was purified by means of immunoaffinity chromatography and biochemical and kinetic characterizations were done. Reverse transcriptase L74V catalyzed substrate dNTP's as efficiently as wild type with no change in K_m. Inhibitor analysis revealed that there is an increased K_i for ddNTP's but no change in the K_i for AZTTP. The mutation at position 74 is also known to supress the effect of the AZT resistance mutation at position 215. Molecular clones were constructed by site-directed mutagenesis to contain both of these mutations. Kinetic analysis with substrates and inhibitors for this mutant will be discussed.

Q 541 NOVEL ANTI-HIV COMPOUNDS FROM NATURAL PRODUCTS. James B. McMahon, Robert J. Gulakowski, Kirk R. Gustafson, Kirk P. Manfredi, John H. Cardellina II, and Michael R. Boyd. Laboratory of Drug Discovery Research and Development, DTP, DCT, NCI, Frederick, MD 21702-1201

The National Cancer Institute has recently initiated a new screening program to support the identification of new synthetic and natural products with anti-HIV properties. In the natural products effort, crude extracts from plant, marine and microbial sources are tested initially for anti-HIV activity. The antiviral screen measures the ability of compounds to interfere with cell killing induced by HIV. This assay has been used successfully in bioassay-directed purification of a number of new chemotypes which possess potent anti-HIV activity. Several of these compounds have been studied in detail using a variety of in vitro antiviral assay systems. Sulfolipids, isolated from cyanobacteria, inhibited HIV-1 induced cell killing with an EC_{50} of 20μM. The purified sulfolipids protected $T4^+$ lymphocyte cells over a concentration range of 35-125 μM; cytotoxicity was observed at approximately 250 μM. A second class of anti-HIV natural products was isolated from the tropical tree Homalanthus accuminatus. Bioassay-guided purification led to prostratin, a member of the phorbol ester class of diterpenes. At concentrations which strongly inhibited HIV replication, prostratin exhibited a reversible cytostatic effect on cellular proliferation. Interestingly prostratin inhibited HIV-replication in both normal and immortal cells derived from human monocyte/macrophage cultures. Recently a series of dimeric alkaloids have been isolated from the tropical vine Ancistrocladus abbreviatus. Two compounds, named michellamines A and B, at concentrations 30-100μM produced complete protection from HIV-1 cytotoxicity with an EC_{50} of 20μM. Furthermore, michellamine B showed potent antiviral activity against the cytopathic effects of HIV-2 as well as HIV-1, a property not frequently observed for known natural or synthetic compounds. These ongoing studies reinforce the validity of empirical screening and bioassay-guided purification as a fruitful strategy for the discovery of new active anti-HIV leads for possible drug development compounds.

Q 542 QUANTIFICATION OF HIV IN SPECIMENS FROM PATIENTS WITH HIV INFECTION Shizuko Aoki-Sei*, Robert Yarchoan*, David E. Kleiner**, Seiiji Kageyama*, James Pluda*, Kathleen M. Wyvill*, Samuel Broder*, and Hiroaki Mitsuya*. *National Cancer Institute, and **Clinical Center, National Institues of Health, Bethesda, MD.

We attempted to quantify HIV-1 DNA and RNA in various specimens from HIV-1-infected individuals by using polymerase chain reaction (PCR). The quantitative PCR assay we established could detect as few as 10 HIV-1 proviral DNA copies per 10^6 PBM. We subsequently found that the numbers of proviral DNA copies in PBM from patients (n=12) substantially decreased ($p < 0.02$) following therapy with dideoxyinosine (ddI or didanosine) for 8-14 weeks. However, the decrease in the proviral DNA content observed was all within one log. We then quantified HIV-1 genomes in various organ tissues obtained at autopsy. In 10 autopsy cases studied, lymphoid organs (in particular lymph nodes) were found to contain the highest numbers of HIV-1 proviral DNA copies among various tissues examined. Furthermore, much higher numbers of HIV-1 mRNA copy were found in biopsied lymph node tissues as compared to HIV-1 proviral DNA. These data suggested that (i) lymphoid organs represented major sites of HIV-1 replication *in vivo*; (ii) PBM is unlikely to be a proper source for assessing the viral load. We then undertook quantification of circulating HIV particles (as RNA) using PCR following reverse transcription (RNA-PCR). Our RNA-PCR methodology employed ultracentrifugation of plasma samples followed by RNA extraction, reverse transcription, PCR, liquid hybridization, autoradiography, and densitometry. This methodology detected as few as 10 to 30 HIV-1 particles/ml and could detect the virus in plasmas from 76 of 77 (98.7%) infected individuals tested, while ELISA failed to detect p24 antigen in 43 of 72 (59.7%) plasmas ($p<0.00001$). A significant decrease in the levels of plasma HIV-1 particles was also observed in 10 of 10 patients treated with ddI for 8 to 14 weeks ($p=0.0051$).

Taken together, the current data suggest that the quantitation of HIV-1 viral DNA, RNA, and virus particles by PCR is feasible and may theoretically contribute to the elucidation of mechanisms of the pathogenesis of HIV-1 infection, and in particular, our methodology for quantification of HIV-1 in plasma may be useful for an overall monitoring of patients receiving antiretroviral therapy. However, it should be noted that larger studies are required to determine its sensitivity, specificity, and usefulness.

Q 543 GENE TRANSFER INTO PRIMARY CULTURE PBL CAN PROTECT CELLS FROM HIV INFECTION

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Several protocols designed to treat patients with genetically engineered cells (gene therapy) are in progress. In viral infections (which can be thought of as acquired genetic diseases) it may be possible to apply the technology of gene therapy to deliver anti-viral agents directly to infected cells. We have developed several approaches that use retroviral vectors to genetically modify lymphocytes such that they now manifest anti-viral activity. It is within reason to consider that the appropriate genetic engineering of lymphocytes could be of therapeutic benefit to individuals suffering with life threatening viral infections (eg. HIV-1). Cells may be modified so that they either directly possess anti-viral activities, or that they can indirectly stimulate anti-viral activity. Different strategies for anti-HIV gene therapy approaches will be described. Retroviral vectors have been constructed that express one or more of the following gene products: sCD4 (or a sCD4 derivative), transdominant HIV rev mutants, a tat and rev regulated human α2-interferon, and an HIV inducible diphtheria toxin. Analysis of these AIDS gene therapy systems has demonstrated protection from HIV-1 infection in primary culture human lymphocytes.

Q 544 INHIBITION OF HIV-1 ENTRY BY NOCODAZOLE.

Quoc V. Nguyen, Mohan Somasundaran, Richard A. Koup and John L. Sullivan. Department of Pediatrics, Univ. Mass. Med. Center, Worcester, MA 01655 and Aaron Diamond AIDS Research Center, New York, NY 10016.

We have studied the effects of nocodazole, a microtubule polymerization blocker, on entry and replication of human immunodeficiency virus type 1 (HIV-1) in human T cells undergoing acute or chronic infection. The presence of nocodazole during acute infection was associated with a dose related and reversible reduction of p24 and reverse transcriptase activity. Nocodazole, however, did not inhibit viral replication or cellular protein synthesis after the establishment of infection. In experiments where ^{125}I-gp120 was incubated with human T cells in the presence of nocodazole, anti-CD4 immunoprecipitates of intracellular proteins did not co-immunoprecipitate gp120. Flow cytometric studies of HIV-1 infected peripheral blood mononuclear cells revealed that cells treated with nocodazole expressed increased levels of both CD4 and gp120 on the cell surface, while amounts of surface MHC class I and class II were unchanged or less compared to untreated controls. We conclude that the mechanism for nocodazole inhibition of HIV-1 replication in acute infections is due to its blockage of CD4 receptor-mediated cell entry.

Q 545 PHOTOINACTIVATION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) BY BENZOPORPHYRIN DERIVATIVE, Jan North, *Robert Coombs and Julia Levy. Quadra Logic Technologies Inc., Vancouver, British Columbia, Canada and *Department of Laboratory Medicine, University of Washington, Seattle, WA 98195.

Benzoporphyrin derivative (BPD), a photosensitizer molecule, shows considerable promise as an agent for the elimination of viral contaminants from blood. The absorption properties of BPD (it is optimally activated by light at 688 nm) makes it suitable for this purpose, since at this wavelength light penetrates blood and plasma with little or no interference from hemoglobin.

In a previous study using the feline leukemia virus (FeLV) as our retrovirus model we demonstrated that 4 μg/ml of BPD, when activated by narrow band red light, effectively eliminated infectious cells from artificially spiked blood and whole blood drawn from viremic cats experimentally infected with the virus.

Based on the promising results with FeLV, we have used BPD to investigate its potential use in the eradication of the human immunodeficiency virus. Preliminary results show that BPD has a significant effect on reducing infectious HIV from artificially spiked blood products.

Q 546 VIRAL RESISTANCE TO HIV-1 SPECIFIC PYRIDINONE REVERSE TRANSCRIPTASE INHIBITORS. Jack H Nunberg*, William A Schleif, Evelyn J Boots, Julie A O'Brien, Julio C Quintero, Jacob M Hoffman, Mark E Goldman, and Emilio A Emini; Merck Sharp and Dohme Research Laboratories, West Point, PA 19486, *present affiliation: Genentech, Inc., South San Francisco, CA 94080.

We have recently described a new class of reverse transcriptase (RT) inhibitors - pyridinone-containing compounds which specifically inhibit HIV-1 RT and which prevent the spread of HIV-1 infection in cell culture. As one approach to study the mechanism of action of these non-nucleoside analog inhibitors, we isolated resistant mutants of HIV-1 in cell culture. Serial passage in the presence of inhibitor yielded virus which was 1000-fold resistant to compounds of this class. Bacterially expressed RTs molecularly cloned from these viruses were also resistant. The resistant RT genes encoded two amino acid changes, K103 to N and Y181 to C, each of which contributed partial resistance. The resistant viruses were sensitive to nucleoside analog inhibitors such as AZT and ddI, but were cross-resistant to the structurally unrelated non-nucleoside analog inhibitors TIBO R82150 and BI-RG-587. Thus, these non-nucleoside analog RT inhibitors may share a common binding site on RT and may all comprise a single pharmacologic class of RT inhibitor. Ongoing clinical studies using the pyridinone RT inhibitor L-697,661 will examine the question of viral resistance in infected individuals.

Q 547 ZIDOVUDINE INDUCES DOSE-DEPENDENT ALTERATIONS IN RAT SKELETAL MUSCLE MITOCHONDRIA, Thomas Papoian, Basilio Gonzalez, Giulia d'Amati, Marc Futermick, William Tsai, and William Lewis, Department of Pathology and Laboratory Medicine, UCLA School of Medicine, Los Angeles, CA 90024-1732

Zidovudine (azidothymidine, AZT) inhibits human immunodeficiency virus replication and reduces AIDS severity. Amongst its limiting side effects is myopathy. We determined AZT-induced molecular, biochemical, and ultrastructural changes in a rat model of AZT myopathy. Rats were given drinking water with or without AZT at 0.2 mg/kg for 20 weeks (ave. 29 mg/kg/day; low dose) and 1.0 mg/kg for 5 weeks (ave. 102 mg/kg/day; high dose). Quadriceps and gastrocnemius muscle samples were subjected to the following: 1) Northern analysis of total RNA and Southern analysis of total and mitochondrial (mt) DNA using cDNA probes for cytochrome b (Cyt-b), cytochrome c oxidase subunit-I (CO-I), and beta actin coding region; and 2) citrate synthase and cytochrome c oxidase enzymatic activities of muscle homogenates. For ultrastructural examination, rats in parallel were perfusion-fixed with 3% glutaraldehyde and skeletal muscle samples processed for electron microscopy. Results from Northern analysis showed decreased abundance of selected mtRNAs in muscle samples from rats treated with low and high doses of AZT. Southern analysis of total muscle DNA revealed no change in abundance of mtDNA relative to actin DNA. However, purified mtDNA revealed AZT-induced alterations in the various electrophoretic forms of mtDNA when compared to controls. Ultrastructural examination revealed mitochondrial alterations consisting of swelling and cristae disruption in rats treated with high dose but not low dose AZT. In the low dose group, citrate synthase and cytochrome c oxidase enzymatic activities showed no decreases in activity. These results suggest that AZT induces molecular and ultrastructural alterations in rat muscle mitochondria that are AZT dose-dependent. Decreases in mtRNAs occurred with both high and low doses, while ultrastructural alterations were observed only at the higher dose. Longer treatment durations may be necessary to determine if decreased mtRNA abundance at the lower AZT doses may lead to altered mitochondrial function and structure. This experimental approach may be useful to examine AZT-induced mitochondrial or toxic myopathies.

Q 548 9-[2-(PHOSPHONOMETHOXY)ETHOXY]ADENINE, BRL 47923, A POTENT AND SELECTIVE INHIBITOR OF HIV REPLICATION. R.M. Perkins[1], C. Patience[2], R.J. Ashton[1], S. Love[1], K. Barnes[1], O. Jenkins[1], G. Donker[1], K. Reynolds[1], L.M. Elphick[1], D.N. Planterose[1], M.D. Kenig[1], S.J. Darlison[1], R.A. Vere Hodge[1], D.M. Duckworth[1], H.T. Serafinowska[1], M.R. Harnden[1], R.A. Weiss[2], and A.G. Brown[1]. SmithKline Beecham Pharmaceuticals, Gt. Burgh, UK[1], and Chester Beatty Laboratories, UK[2].

9-[2-(Phosphonomethoxy)ethoxy]adenine, BRL 47923, is a member of a novel series of acyclic nucleotide analogues in which a phosphonic acid bearing moiety is attached to the N-9 of a purine via an N-O bond. It was selected for further evaluation based on its selective antiviral activity against HIV-1 replication in human peripheral blood lymphocytes (PBLs), 50% inhibitory concentration (IC_{50}) 0.24μM (cf. 0.006μM for AZT). Using an MTT-based cytotoxicity assay in replicating PBLs the 50% cytotoxic concentration was >100μM. This clearly demonstrates that BRL 47923 represents a potent and selective inhibitor of HIV-1 replication in cell culture. AZT-resistant HIV-1 was not cross-resistant to BRL 47923. This compound also displays antiviral activity against the animal lentiviruses, FIV and visnavirus as well as the murine retrovirus, Rauscher MLV. These activities will facilitate the further evaluation of this compound *in vivo*. No antiviral activity was found against members of the herpesvirus family. Biochemical studies with the diphosphate of BRL 47923 indicate that the likely mode of action of this compound is via inhibition of the viral reverse transcriptase (IC_{50} = 1.5μM). Also, inhibition of DNA polymerases from MRC-5 cells was observed (IC_{50} for α/δ, β and γ were 54, 0.035 and 0.054μM respectively). A major limitation of AZT use in the treatment of AIDS is its bone marrow suppressive effects. In human bone marrow colony stimulating assays the concentration (160μM) of BRL 47923 required for 50% inhibition of erythroid colony formation was over 1500-fold more than that for AZT (<0.11μM). Granulocyte - macrophage colony formation was not strongly inhibited by either compound. In addition BRL 47923 had no detectable effect on mitochondrial DNA synthesis in human cells at 10μM while ddC was inhibitory at 0.3μM. This potent and selective activity in cell culture combined with its reduced effects on bone marrow colony formation makes BRL 47923 a promising new anti-HIV agent.

Q 549 A UNIFIED GENE THERAPY APPROACH TO THE TREATMENT OF AIDS, Jack A. Ragheb, Richard A. Morgan, Anthony Ridgway*, Larry Couture, and W. French Anderson, Molecular Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, 20892, and *Blood Products Division, Bureau of Biologics, Ottawa, Ontario, K1A OL2, Canada

Using retroviral mediated gene transfer, we have developed a multipronged gene therapy approach aimed at disrupting the life cycle of the AIDS virus. To block entry of the virus into cells, lymphocytes can be genetically engineered to secrete a truncated form of CD4, the receptor for the AIDS virus. This soluble form of the receptor acts as a molecular decoy, which binds the virus and prevents it from entering the cell. This approach not only protects the genetically modified cells, but their unmodified neighbors as well, thus preventing the initiation of the viral life cycle. To abort the life cycle of the virus in those cells that have already been infected, we have created novel trans- dominant mutants of the Rev regulatory protein and developed two independent assays to measure their activity. In one assay, we used an HIV LTR driven CAT reporter gene flanked by intron and RRE sequences (in this arrangement CAT expression is stringently dependent on both Tat and Rev). The second assay is based on the ability of CD4 bearing HeLa cells to form synciata when the HIV envelope is expressed in these cells. In a transient expression assay, we have been able to inhibit synciata formation by 80% in the presence of one of our retroviral vector based Rev trans-dominant mutants. By blocking the expression of envelope in an infected cell, these Rev trans-dominant mutants would be expected to prevent the production of mature HIV virions and may also reduce the cytopathicity that has been attributed to the expression of HIV envelope on the surface of infected cells. In order to destroy cells that harbor a dormant copy of the virus, we have constructed so called suicide vectors. These vectors carry the gene for diphtheria toxin in the same construct as described above for the CAT reporter gene. As for CAT, the expression of the diphtheria toxin gene is stringently dependent on the expression of Tat and Rev. Thus, only cells that had been genetically modified with this construct and that also express HIV would be destroyed by the toxin. Finally, these independent points of attack on the HIV life cycle have been packaged together into double and triple gene retroviral vectors. This has been accomplished by incorporating the internal ribosome entry sites found in picornoviruses into our murine based retroviral constructs. A cell modified with these polycistronic vectors, can now benefit from the protection of multiple defense mechanisms against the virus.

Q 550 ANTI-HUMAN RETROVIRAL PROPERTIES OF COMPOUNDS PURIFIED FROM CHINESE MEDICINAL HERBS, [1]F.W. Ruscetti, [1]T. Fu, [2]B-Q. Li, [2]N.W. Baylor and [1]H-F. Kung. [1]BRMP-LMI, FCRDC, [2]BCDP, PRI/DYNCORP, Frederick, MD 21702.

Recently, several Chinese medicinal herbs or the compounds purified from these plants such as GLQ223 and glycyrrhizin have been shown to block the replication of human immunodeficiency virus (HIV-1). The herbs, Scutellaria baicalensis Georgi and Phyllanthus Niruri (PHY) have been used to treat infectious diseases such as hepatitis. Baicalin (BA), 7-D-glucuronic acid-5,6-dihydroxy-flavone, purified from Scutellaria and partially purified PHY were able to inhibit HIV-1 infection in vitro in a dose responsive manner. BA and PHY inhibited HIV-1 infectivity as measured by a focal syncytium forming assay and release of HIV p24 from HIV-infected peripheral blood mononuclear cells (PBMC). Furthermore, the enzymatic activity of purified recombinant HIV-1 reverse transcriptase (RT) was inhibited by both compounds. In addition, both compounds produced concentration-dependent inhibition of human T cell leukemia virus-I (HTLV-I) replication in chronically infected T and B cell lines as determined by release of HTLV-1 p19. Resistance to HTLV-1 infection and viral-mediated transformation was seen in PMNC from cord blood pretreated with BA prior to co-cultivation with lethally irradiated HTLV-1 producing cells. Both compounds were able to inhibit RT activity in HTLV-1 infected cells. Neither cytotoxic nor cytostatic effects of the drug were seen at optimal anti-viral concentrations on control or infected cells. These results suggest that BA and PHY have therapeutic potential against human retroviral infections.

Q 551 CORRELATION OF ZIDOVUDINE PHARMACOKINETICS AND RESPONSE OF SURROGATE PARAMETERS OF EFFICACY IN HIV. Jan Sahai, K. Gallicano, B. Conway, G. Garber, N. Foss, and D. William Cameron. Department of Medicine, University of Ottawa, and Ottawa General Hospital Regional Comprehensive Care AIDS Clinic, Ottawa, Canada K1H 8L6.

An association has been reported between zidovudine (ZDV) dosage, kinetics and development of hematologic toxicity. Thirteen HIV infected men participated in a prospective 4 week study to seek correlation of ZDV pharmacokinetics and anti-viral efficacy measured by change in CD4 T cell counts and B_2 microglobulin (B-2M) levels as surrogate parameters. All patients received an induction dose of ZDV 200 mg Q4H for 4 weeks. Blood was obtained for ZDV levels on day 28 at 0, 0.25, 0.5, 0.75, 1, 1.5, 2.0, 2.5, 3, 4 h. Serum B-2 M and % CD4 T cells were measured on days 1 and 28. Mean values ± S.D. for ZDV area under the concentration time curves (AUC 0-4h) were 57.8 ± 12.9 mcg*min/mL. A significant correlation was found between ZDV AUC 0-4h and (i) decrease in B-2 M ($r = 0.75$, $p < 0.01$); (ii) increase in % CD4 ($r = 0.58$, $p=0.05$). This correlation of ZDV AUC and response of surrogate parameters may represent a drug concentration or metabolism related effect upon the rate and/or degree of immune restitution after initiation of ZDV therapy.

Q 552 DEVELOPMENT AND ANALYSIS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 RESISTANT TO HIV-1 SPECIFIC PYRIDINONE REVERSE TRANSCRIPTASE INHIBITORS, William A. Schleif, Emilio A. Emini, Audrey Rhodes, Donna L. Titus, Leah Gotlib, Jon H. Condra, and Vera W. Byrnes, Department of Virus and Cell Biology, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486

Human immunodeficiency virus type 1 (HIV-1)-specific pyridinone reverse transcriptase (RT) inhibitors prevent HIV-1 replication in cell culture (Goldman et.al. Proc. Natl. Acad. Sci. USA 88:6863-6867, 1991). These compounds act directly to inhibit RT via a mechanism that is noncompetitive with respect to deoxynucleoside triphosphates. By serially passaging HIV-1 in the presence of increasing inhibitor concentrations (Nunberg et.al. J. Virology 65:4887-4892, 1991) up to 250nM we selected virus (P4) resistant to compound L-697,661. The concentration of compound L-697,661 required to inhibit virus growth by 95% (CIC95) in MT-4 cells is 50-100nM. When the CIC95 was determined using P4 resistant virus, inhibitory concentrations ranged from 800 to 1600nM (approximately 10 fold resistant verses wild type). Two additional passages of this virus in the presence of 1000nM L-697,661 yielded virus (P6) that was 1000 fold resistant.

To determine the mutations that conferred drug resistance we cloned and expressed the reverse transcriptase (RT) gene from L-697,661 resistant virus isolates. Clones were screened using a modification of the *in situ* RT assay (Prasad and Goff, J. Biol. Chem. 264:16698-16693, 1989), in the presence of L-697,661. To date, sequence analysis of P4 isolates indicated that clone A3C4 contained amino acid substitutions at 100 (Leu→Ile) and 188 (Tyr→His), whereas clone A1C11 contained 100 (Leu→Ile), 108 (Val→Ile) and 221 (His→Tyr). Mutagenesis studies of recombinant RT confirmed that the Tyr→His mutation at residue 188 was important in conferring resistance to the non-nucleoside inhibitors (re: J. Condra et.al. poster). Further analyses of these and other RT clones will give a better understanding of the amino acid residues conferring drug resistance.

Q 553 INHIBITION OF HIV-1 REPLICATION BY NOVEL MULTITARGET-RIBOZYMES UNDER HIV-1 CONTROL

Manfred Schubert, Akhil C. Banerjea, Karl Haglund, George G. Harmison and Chang-Jie Chen, Laboratory of Viral and Molecular Pathogenesis, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

Problems with the potential therapeutical use of ribozymes targeted against HIV-1 are: 1. the high nucleotide sequence variabilty between different virus isolates, 2. the high mutation rate of HIV-1, 3. the site selection for efficient cleavage in vivo and 4. the necessary level of ribozyme expression. The purpose of this study is to design novel ribozymes which address these problems.

We have designed and constructed a collection of mono-, di-, penta- and nonaribozymes of the hammerhead type, which are, in the context of our antiviral strategy, targeted to cleave at 10 different but relatively conserved sites within the gp120 region of the HIV-1 env gene. There were great differences in the efficiency of cleavage at these sites in vitro. Unexpectedly, one out of 10 sites was not cleaved at all. At equal molar ratios, multitarget-ribozymes, designed to cleave at multiple sites were more efficient in cleaving a 1.35 Kb env transcript than mono- or diribozymes. Cleavage of the target sequence at least once was most efficient using a 400 bp nonaribozyme which was directed to cleave at nine different sites within env. The nonaribozyme, when part of a 3.5 Kb RNA transcript, also cleaved the 1.35 Kb substrate RNA, demonstrating that functional ribozymes can be part of a large RNA transcript.

We have inserted the nonaribozyme into the proviral DNA genome of our prototype defective interfering HIV. With this construct, expression of the nonaribozyme was under control of the HIV-1 LTR and it was, therefore, dependent on the regulatory proteins of HIV, which is suicidal for HIV-1. Cotransfection of this DNA with the infectious HIV-1 DNA clone into HelaT4 cells resulted in a drastic inhibition of syncytia formation. Cotransfection of the same DNA containing only a single monoribozyme did not inhibit either HIV replication nor syncytia formation. These data demonstrate that multitarget-ribozymes may be able to overcome many of the problems listed above. They are very promising for their use in a highly specific antiviral strategy.

Q 554 DRUG SUSCEPTIBILITIES OF HIV-1 ISOLATES FROM ASYMPTOMATIC HIV-1 INFECTED PATIENTS (PTS).

Shafer RW, Katzenstein D, Kozal M, *Katzman M, *Fiscus S, *Myer W, *Gupta P, *Coombs R, *Rasheed S, *Ragni MV, Merigan TC. Center for AIDS Research, Stanford University Medical Center and *ACTG 143 Protocol Virology Group

OBJECTIVE: To determine in-vitro zidovudine (ZDV) and didanosine (DDI) susceptibilities of HIV-1 isolates from asymptomatic HIV-1 infected patients early in the course of therapy with ZDV.

METHODS: 80 consecutive asymptomatic HIV-infected pts with CD4 counts between 200-500 were enrolled in a multicenter ACTG protocol. One half were ZDV-naive; one half had received ZDV for ≤ 13 months. None had received DDI. At study entry, 3 million PBMCs from each pt were cultured in a quantitative microculture assay. A mixture of cells and supernatant from this assay was frozen on day 21. This mixture was subsequently thawed and recultured to generate a cell-free virus stock. Susceptibility testing was performed by measuring P24 antigen inhibition after culturing 10-100 $TCID_{50}$ of viral stock for 7 days with donor PBMCs which were either drug-free or which were pre-incubated with ZDV (0.0005,0.005,0.05,0.5,5 uM) or DDI (0.08,0.4,2,10 uM). The concentration of drug which resulted in 90% reduction of P24 antigen as compared to the control was defined as the IC_{90}.

RESULTS: Cell-free virus stocks of sufficient titer (≥100 $TCID_{50}$/ml) for performing susceptibility tests were generated from 65 pts. Among 35 ZDV-naive patients, the mean log IC_{90} was -2.3±0.3 uM ZDV. Among 14 patients treated with ZDV 12-13 months, the mean log IC_{90} was -1.5±0.6 uM ZDV ($p<0.001$). The 95% confidence interval for the difference between the log IC_{90} of the 2 groups was .5 - 1.2 log. The mean log IC_{90} for DDI did not differ between the two groups of pts (-0.2 ± 0.4 uM DDI).

CONCLUSIONS: Drug susceptibility testing could be performed on greater than 80% (65/80) of isolates from asymptomatic pts. Low-passage clinical isolates from pts who had been treated with ZDV for 12-13 months were significantly more resistant to ZDV than similar isolates from pts who were ZDV-naive. None of the ZDV-treated patients had decreased susceptibility to DDI.

Q 555 DEVELOPMENT OF DRUG RESISTANT HIV-1 VARIANTS. Takuma Shirasaka, Robert Yarchoan, Mary C. O'Brien, Robert Husson, Takashi Shimada, Kathy M. Wyvill, Barry Anderson, Samuel Broder, and Hiroaki Mitsuya. National Cancer Institute, National Heart, Lung, and Blood Institute, Bethesda, Maryland.

We studied whether patients with AIDS or ARC receiving therapy with azidothymidine (AZT or zidovudine), dideoxycytidine (ddC), and/or dideoxyinosine (ddI or didanosine) could develop drug resistance. Pairs of pre- and post-therapy HIV-1 strains were isolated from adult patients before and after antiviral therapy and were compared for phenotypic and genotypic changes.

HIV-1 strains were obtained from 4 patients who received an alternating regimen of AZT and ddC (AZT/ddC) for ≥15 months. 3 strains were found to be highly resistant to AZT but still sensitive to ddC and ddI *in vitro*. Nucleotide sequence analysis of the reverse transcriptase-encoding region revealed ≥1 of four AZT-related genotypic changes reported by Larder *et al.* An HIV-1 strain isolated from a patient who received 41 months of AZT/ddC therapy showed a decrease in sensitivity to ddC in addition to a high level of AZT resistance. This isolate did not have any of the reported AZT-related mutations but had 8 amino acid substitutions. HIV-1 strains were also isolated from 5 patients who received ddI for ≥18 months. Changes in sensitivity to ddI were not appreciable in any of the post-therapy HIV-1 strains, while certain common genotypic changes were identified in ≥1 strains. Such genotypic changes included a Leu^{74} -> Val substitution. 3 of the 5 patients had received AZT before ddI therapy. These 3 patients had HIV-1 strains that were resistant to AZT (although all the strains remained sensitive to ddI). The AZT resistance declined when AZT was withheld, while some of the AZT-related mutations persisted in PBM for up to 2 years.

Our data suggest that (i) HIV-1 develops resistance to AZT more readily than to ddC and ddI; (ii) AZT resistance may decrease after switching to ddI therapy; and (iii) AZT/ddC combination therapy does not block the emergence of AZT resistant variants, while the possibility that ddC delays the development of AZT resistance remains to be determined. It should be stressed that the current data do not provide a basis for concluding that AZT/ddC or ddI are inferior, equivalent, or superior to AZT as therapy of AIDS. More research is needed to make clinical correlations between *in vitro* drug sensitivities, genotypic changes of HIV-1, and clinical outcome in HIV infection.

Q 556 SPECIFIC INHIBITION OF α-GLUCOSIDASE I OF THE GLYCOPROTEIN PROCESSING ENZYMES IN HIV INFECTED CELLS BY 6-O-BUTANOYL CASTANOSPERMINE (MDL 28574). Debra Taylor*, Tara Brennan*, Mohinder Kang+, Gordon Bridges*, Prasad Sunkara+ and Stanley Tyms*, Marion Merrell Dow Research Institute,*MRC Collaborative Centre Laboratory, Mill Hill, London NW7 1AD and +Cincinnati, Ohio, USA.

Inhibitors of α-glucosidase 1 or α-mannosidase I modulated the synthesis of HIV glycoproteins in chronically infected cells in different ways observed by SDS-PAGE although the production of infectious virus was reduced in both cases. Castanospermine and its 6-O-butanoyl derivative, both inhibitors of α-glucosidase I purified from pig-kidney, caused the accumulation of high glucose glycopeptides in these cells; these were absent after treatment with deoxymannojirimycin, an inhibitor of α-mannosidase I. This is consistent with the order of hydrolysis of glucose and mannose residues on glycans during glycoprotein processing. Treatment with castanospermine or 6-O-butanoyl castanospermine produced a dose related accummulation of high glucose oligosaccharides with IC50 values lower by about 20-fold in favour of the acyl derivative: this correlated with it's greater antiviral potency. Confirmation of the specificity of inhibition by castanospermine or 6-O-butanoyl castanospermine was determined by analysis of glycans released from treated chronically infected cells after exposure to purified α-glucosidase I. The proportion of radiolabelled glucose released approximated 33% corresponding to the removal of the outer α1-2 linked glucose and was evidence for the specificity of the octahydoindollizines for α-glucosidase I of the host-cell. Even so, the drug candidate 6-O-butanoyl castanospermine had no effect on the growth of a range of T-cell lines or PBL's. Neither were there deleterious effects on concanavalin A induced lymphocyte blastogenesis or the expression of a range of surface markers including CD25, the IL 2 receptor. This is evidence of the selectivity of 6-O-butanoyl castanospermine for α-glucosidase I, with the growth of HIV being more critically dependent on the enzyme activity than the growth of the host-cell or expression of its surface receptors.

Q 557 DEVELOPMENT AND IMPLEMENTATION OF AN HIV PROTEASE ASSAY UTILIZING A FLUORESCENT SUBSTRATE TO SCREEN FOR PROTEASE INHIBITORS FROM NATURAL PRODUCTS AND SYNTHETIC COMPOUNDS, Paul K. Tomich, Michael J. Bohanon, and Janet C. Lynn, Chemical and Biological Screening, The Upjohn Company, Kalamazoo, Mi 49001.

HIV-1 protease provides an ideal target for therapeutic treatment of AIDS because of its absolute requirement in viral maturation and replication, the low spontaneous mutation rate for its encoding gene, and the great homology between other immunodeficiency viruses. We have developed an assay utilizing a peptide modeled on the HIV-1 *gag* polypeptide, residues 128 to 137. The peptide was biotinylated at the amino terminal end and fluorescently labeled at the carboxyl terminal end. This material can be collected on polystyrene beads. Upon cleavage with HIV-1 protease, a decrease in fluorescence occurs. Utilizing this assay, we have screened a variety of microbial sources as well as multiple chemical templates for possible inhibitors of this enzyme. We have obtained several classes of compounds from both sources. One low molecular weight compound from our chemical inventory exhibits competitive kinetics with a Ki of 0.5 ± 0.06 μM. A search of our database yielded ca. 570 compounds in the same family of which some exhibited inhibition. A chemical synthetic effort has commenced to make improved derivatives of this agent. We shall present data that describe the assay in detail.

Q 558 POTENT ANTIVIRAL ACTIVITY OF A PROTEASE INHIBITOR (A-77003) ON FIELD ISOLATES OF HIV-1 EX VIVO AND IN VITRO, Takuo Toyoshima[1], Hiroshi Mohri[1], Dale J. Kempf[2], Daniel Norbeck[2], Yunzhen Cao[1], Mindell Seidlin[1] and David D. Ho[1], 1. The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY 10016, 2. Abbott Laboratories, Abbott Park, IL 60064.

HIV-1 gag and pol products are processed by a virally encoded protease before they are packaged into mature virions. Protease inhibitors can block this cleavage and prevent the maturation of infectious HIV. We evaluated the anti-HIV-1 activity of A-77003, a C2 symmetric inhibitor, on samples of plasma and peripheral blood mononuclear cells (PBMC) from ten patients with different stages of disease. Each plasma or PBMC sample was assayed for the titer of infectious HIV-1 using end-point-dilution cultures in the presence of 0, 0.1, 0.5, 1 or 10μM of A-77003. In these ex vivo assays of both plasma and PBMC, no virus was detectable in most cases at or above the concentration of 0.5μM of A-77003. In addition, in standard in vitro testing of five clinical isolates, we found that the 50% inhibitory doses (ID50) of A-77003 for these viruses were quite low and ranged from 0.02 to 0.4 μM. Therefore, A-77003 has shown potent anti-HIV activity not only against primary isolates in vitro but also against viruses contained in patients' plasma or PBMC ex vivo. These promising preclinical findings suggest that the protease inhibitor A-77003 is an outstanding antiviral agent for clinical development.

Q 559 EFFECTIVE INHIBITION OF IMMUNODEFICIENCY VIRUS REPLICATION IN LYMPHOCYTES BY RETROVIRAL MEDIATED GENE TRANSFER Frank Y.T. Tung Department of Pathology College of Medicine, University of Florida, Gainesville, Florida 32610-0275.

To test the feasibility of gene therapy approach for AIDS patients, an antiviral gene derived from Simian Immunodeficiency Virus(SIV), was efficiently targeted into CD4+ lymphocytes through retrovirus-mediated gene transfer. A 5.6 Kbp DNA fragment of SIVmac239 (NarI-SphI) encompassing the primer binding site, gag-pol, vif and 5'env and a 3.5 kbp DNA fragment of SIVmac239 (SacI-SacI) containing vif,vpx,vpr,rev,tat and env were separately subcloned into the BamHI site of the expression vector pZIPneoSV(x) in both sense and antisense orientations. These expression constructs were transfected into the amphotropic packaging cell line GpenvAm12, and cell lines producing high titers of recombinant virus with neo-resistant markers were isolated. Hut-78 cells were transduced by these packaged recombinant virus and G418 resistant cells were selected. G418-resistant cells were then infected with uncloned SIVmac, HIV-I and HIV-2. The viral activity in the challenged cells was monitored by reverse transcriptase activity in the supernatant and PCR from isolated cellular DNA. The results indicated that the transduced Hut-78 were not only effectively protected from SIVmac infection but also from infection with HIV-I and HIV-2. No viral activity was detectable in some protected cells even after long-term culture. This observation may lead to the development of somatic gene therapy treatment for AIDS patients.

Q 560 THE SAFETY, PHARMACOKINETICS, AND ANTIVIRAL ACTIVITY OF N-ACETYLCYSTEINE IN HIV-INFECTED INDIVIDUALS, Robert E. Walker, H. Clifford Lane, Christine M. Boenning, and A.S. Fauci, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

HIV infection is associated with reduced serum and intracellular thiol levels. N-acetylcysteine (NAC), a "pro-drug" of cysteine, the rate-limiting precursor for glutathione synthesis, may serve to replenish thiol stores. NAC has been shown in vitro to inhibit HIV LTR-directed gene expression as well as basal levels of HIV replication and cytokine-induced stimulation of HIV in lymphocytic and monocytic cell lines and PBMC. In follow-up of these observations, we initiated an open-label Phase I/II study in HIV-infected individuals with CD4<500 to evaluate the safety and pharmacokinetics of repeated administration of NAC and to assess changes in immunologic, virologic, and biochemical markers of HIV disease over a 14 week study period. Patients were assigned to 1 of 4 dose level groups ranging from 3.7 to 100 mg/kg IV tiw and from 600 to 4800 mg PO qd. To date, 9 patients have been enrolled. No serious toxicities related to NAC have occurred thus far. The C_{max} with IV administration was 10μM for the 3.7 mg/kg dose and 55μM for the 11 mg/kg group. A $T_{1/2}$ of <30 min. has been observed. With oral dosing at the first two levels, no plasma NAC has been detected. A preliminary analysis of the first group of patients revealed no significant changes in CD4 counts, p24 antigenemia, plasma viremia, or plasma cysteine levels over the study period. The data generated at the higher dose levels should allow us to determine the potential efficacy of NAC in patients with HIV infection.

Q 561 THERAPY OF AIDS OR SYMPTOMATIC HIV INFECTION WITH ALTERNATING OR SIMULTANEOUS AZT/DDI REGIMENS: INTERIM ANALYSIS OF A RANDOMIZED PROTOCOL, Robert Yarchoan, Jill A. Lietzau, Otis Brawley, Bach-Yen Nguyen, James M. Pluda, Kathleen M. Wyvill, and Samuel Broder, Medicine Branch, National Cancer Institute, Bethesda MD 20892

Combination therapy for human immunodeficiency virus (HIV) infection offers a number of theoretical advantages over single-agent therapy. There are arguments to support either alternating or simultaneous regimens: alternating therapy may reduce long-term toxicity by providing "drug holidays", while simultaneous therapy may yield drug synergy or delayed development of resistance. However, there are little data on which is the best approach. We initiated a study of 3'-azido-2',3'-dideoxythymidine (AZT, zidovudine) and 2',3'-dideoxyinosine (ddI, didanosine) in 40 patients (pts) with AIDS or symptomatic HIV infection who had <350 CD4 cells/mm^3 and little or no prior antiretroviral therapy. Pts were randomized to receive alternating AZT/ddI (AZT 600 mg/day for 3 wks alternating with ddI 500 mg/day for 3 wks) or simultaneous AZT/ddI (AZT 300 mg/day plus ddI 250 mg/day). Pts have now been followed for up to 49 wks. One pt on the alternating regimen elected to discontinue the study, and one pt on the simultaneous regimen had the AZT held for 4 wks because of anemia; otherwise, both regimens have been well tolerated with relatively little toxicity. The mean CD4 counts remained above baseline on both regimens for up to 36 wks. At wk 18, the mean change in CD4 cells was +33 cells/mm^3 on the alternating regimen and +99 cells/mm^3 on the simultaneous regimen (P_2=.11, N.S. for the difference between the regimens). At wk 27, the mean increases were +29 and +80 cells/mm^3 respectively (P_2 = 0.27, N.S.). Each of 12 pts (3 on alternating and 9 on simultaneous therapy) with detectable serum HIV p24 antigen at entry had decreases which were sustained for up to 36 wks. Pts on both regimens reported increased energy and appetite and experienced weight gain. These preliminary results indicate that both alternating and simultaneous therapy with AZT and ddI can have anti-HIV activity lasting for at least 36 wks; continuation of this study may provide data to judge the relative merits of each regimen.

Late Abstracts

HIV-1 VIRUS PARTICLE FORMATION: MECHANISMS INVOLVED IN THE ASSEMBLY OF VIRUS CORE AND ENVELOPE PROTEINS, Christopher Aiken, Jin-Ping Song, and Didier Trono, Infectious Disease Laboratory, The Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA 92037

Enveloped viruses effectively exclude cellular glycoproteins from their surfaces. However, infection of cells by more than one enveloped virus often results in the generation of viral pseudotypes—hybrid viruses consisting of the viral core of one virus and the envelope glycoprotein of another. Such phenotypic mixing often leads to expanded viral tropisms. On this basis, it has been hypothesized that viral envelope glycoproteins share common signals crucial for recognition by viral matrix components, and that these signals are not present in cellular glycoproteins.

We and others have previously shown that infectious HIV-1(MuLV) pseudotypes are readily formed. However, attempts to generate functional MuLV(HIV-1) pseudotypes by co-transfection of various MuLV and HIV-1 expression vectors into COS cells have so far been unsuccessful. Infectivity was assayed using a β-galactosidase indicator cell line expressing human CD4. Our preliminary analysis indicates that the failure to generate functional MuLV(HIV-1) pseudotypes results from the exclusion of HIV-1 envelope glycoproteins from the surface of MuLV particles. This suggests that specific signals regulate the interaction between the HIV-1 core and envelope proteins. In addition, we have recently discovered a new processing event targeting HIV-1 Env that appears to coincide with HIV-1 maturation. We are currently analyzing its role in HIV-1 infectivity. These results and their implications for HIV-1 assembly and transmission will be discussed.

EXPRESSION OF HUMAN Fab FRAGMENTS SPECIFIC FOR HUMAN IMMUNODEFICIENCY TYPE 1 USING *ESCHERICHIA COLI* Jennifer S. Andris*, Evan Hersh**, E. Sally Ward*, and J. Donald Capra* *Department of Microbiology and Graduate Program in Immunology, U.T. Southwestern Medical Center, Dallas, TX 75235-9048 and **Arizona Cancer Center, Tucson, AZ 85721. Early studies demonstrated that HIV immune sera from infected individuals did not prevent HIV infection in chimpanzees that were challenged with the virus. However, in contrast to these early studies, more recent data has demonstrated that hyperimmune human immunoglobulin preparations, as well as polyclonal chimpanzee IgG with neutralizing activity, protect against challenge with HTLV-IIIB. Since some HIV antibodies have been shown to enhance the infection *in vitro*, rather than neutralize the virus, these conflicting results may be explained, in part, by the heterogeneous nature of the immunoglobulin preparations used--i.e., protection may depend on the absence of enhancing antibodies. We have characterized a number of human anti-HIV neutralizing antibodies at the nucleotide level and are expressing these antibodies in an *E. coli* expression system. This method will provide us with the ability to generate and screen a large number of mutant molecules for reactivity to HIV. By using random mutagenesis we hope to develop anti-HIV antibodies that have higher affinities and increased neutralizing activity when compared to the parental molecule. Production of such homogeneous preparations may provide a means of improved passive immunity to HIV.

PET-OWNERSHIP AMONG PARTICIPANTS IN THE MULTICENTER AIDS COHORT STUDY (MACS), Frederick J. Angulo, Roger Detels, Judith M. Siegel, Janice P. Dudley, and the MACS, Department of Epidemiology, School of Public Health, UCLA, Los Angeles, CA 90024

Background: An estimated 30-40 percent of the HIV-infected people in the US own pets and, although no published research compares the potential risks and/or benefits of pet ownership for HIV-infected persons, many HIV-infected individuals have been advised to find new homes for their pets. We present baseline data of a sub-study within the MACS (a cohort of homosexual men in Baltimore, Chicago, and Los Angeles) begun in April 1991 to address these issues.

Methods: Between April-September 1991, a self-administered questionnaire was completed by 2,440 individuals in the MACS (of whom about 45 percent were HIV-infected).

Results: Forty-five percent of participants currently own pets (no difference between serostatus) and an additional fifteen percent had a pet in the last 5 years. Sixty-three percent of HIV-infected individuals had heard that HIV-infected persons should not own a pet (usually through the media, from a friend, or from their physician). The majority cited cats (85%) and birds (55%) as the greatest risks. Few HIV-infected persons had given away their pets, although many participants expressed concerns about possible zoonotic infections from their pets.

Conclusion: The percent pet ownership among HIV-infected individuals in the MACS remains high despite concerns of possible health risks. We are conducting a 2 year follow-up study to assess the health risks versus the psychological benefits of pet ownership among HIV-infected persons.

THREE-DIMENSIONAL STRUCTURAL STUDIES OF A COMPLEX OF HIV-1 REVERSE TRANSCRIPTASE WITH A MONOCLONAL ANTIBODY Fab FRAGMENT, BOTH IN THE PRESENCE AND ABSENCE OF A BOUND dsDNA PRIMER-TEMPLATE MIMIC. E. Arnold, A. Jacobo-Molina, R. G. Nanni, A. D. Clark, Jr., R. L. Williams, X. Lu, J. Ding, A. Zhang, A. L. Ferris[1], P. Clark[1], and S.H. Hughes[1]. Center for Advanced Biotechnology and Medicine (CABM) and Department of Chemistry, Rutgers University, 679 Hoes Lane, Piscataway, NJ 08854-5638; 1-National Cancer Institute-Frederick Cancer Research Facility, P.O. Box B, Frederick, MD 21701.

Using recombinant HIV-1 RT p66/p51 heterodimer expressed in and purified from *E. coli*, we have obtained crystals of the heterodimer itself, and in complex with dsDNA primer-template mimics and with monoclonal antibody Fab fragments. We are pursuing structure determination of crystals of a ternary complex of HIV-1 RT p66/p51 heterodimer, the Fab fragment of a monoclonal antibody (previously designated 28), and dsDNA oligomers whose sequence corresponds to the HIV-1 primer-binding site [Jacobo-Molina et al. (1991) PNAS 88:10895-10899]. These crystals diffract X-rays to 4 Å resolution without DNA present, but diffract to 3.1 Å resolution when either cocrystallized with or soaked with dsDNA oligomers.

We have recently obtained a structure of HIV-1 RT independently for both forms of the crystals (with and without bound 18:19 base-paired dsDNA) at 7 Å resolution. We have measured X-ray diffraction datasets to 3.5 Å resolution at the Cornell High Energy Synchrotron Source and are pursuing the structure determination at high resolution. The double-stranded nucleic acid substrate binds in a groove on the surface of the enzyme, and the RNase H domain can be identified near one end of the dsDNA. These crystals of HIV-1 RT should be especially relevant for visualization of antiviral agent complexes with the enzyme. Knowledge gained from experimental determination of complexes with different classes of RT inhibitors may contribute to the development of better treatments for AIDS.

DETERMINANTS OF MACROPHAGE TROPISM FOR SIMIAN IMMUNODEFICIENCY VIRUS (SIVmac)

Babak Banapour, Marta L. Marthas, Ross A. Ramos, Murray B. Gardner, Neils C. Pedersen, Paul A. Luciw; University of California, Davis CA 95616

Two highly related molecular clones of SIVmac, SIVmac239 and SIVmac1A11, demonstrate differential tropism for macrophages (Banapour et al. Virology 18:12-19; 1991). To map viral determinants of macrophage tropism, reciprocal recombinant genomes were constructed between these two clones. Infectious recombinant viruses were rescued by transfection of cloned viral genomes into the permissive lymphoid cell line, CEMX174. Analysis of one pair of reciprocal recombinants revealed that an internal 6.2 kb DNA fragment of SIVmac1A11 was necessary and sufficient for both syncytium formation and efficient replication in macrophages. This region includes the codong sequences for a portion of the gag gene, all of pol, vif, vpx, vpr, the first coding exons of tat and rev, and the external env glycoprotein (gp130). Thus, the transmembrane glycoprotein of env, the nef gene, the second coding exons of tat and rev, and the long terminal repeats are not essential for in vitro macrophage tropism. Analysis of additional recombinants revealed that syncytium formation, but not virus production, was controlled by a 1.4 kb viral DNA fragment in SIVmac1A11 encoding only the external env glycoprotein, gp130. Thus, gp130 env of SIVmac1A11 is necessary for entry of virus into macrophages but not sufficient for a complete replication cycle in this cell type. We conclude that gp130 and one or more genetic elements (exclusive of the LTR, transmembrane env glycoprotein, second coding exons of tat and rev, and nef) are essential for a complete replication cycle of SIVmac in rhesus macrophages. Implications of in vitro analysis of these viruses for viral pathogenesis will be discussed.

INDUCTION OF MULTINUCLEATED GIANT CELL FORMATION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS BY THE VIRUS SIVsmm PBJ 1.9. Padmavathi Baskar, Opendra Narayan and James E.K. Hildreth, Departments of Pharmacology and Molecular Sciences and Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205

SIVsmm PBJ1.9 is an extremely virulent clone of SIVsmm PBJ14 and causes an acute lethal disease in pig tailed macaques, with death occuring six to eight days after infection. The disease is characterized by bloody mucoid diarrhea, lymphoid hyperplasia and giant cell pneumonia. Here we have developed an in vitro model for the production of multinucleated giant cell (MGC). Peripheral blood mononuclear cells (PBMC) from normal healthy human subjects when cultured in the presence of anti-class II MHC monoclonal antibody and PBJ, not either alone, resulted in a significant increase in the formation of MBC within four days. Experiments using transwell chambers indicated that formation of MGC from purified monocytes was not a result of T cell syncitia, but dependent on cytokines elaborated by the PBMC in the presence of PBJ and addition of anti-class II MHC antibody to monocytes. While PBJ grown in PBMC was a potent inducer of MGC in the presence of anti-class II MHC antibody, PBJ grown in CEM174 failed to do so. Based on these results, we discuss the mechanism of MCG formation and its possible involvement in the acute pathogenesis of SIV infection.

PATHOGENESIS OF AIDS-LYMPHOMAS IN A MONKEY MODEL, P. Biberfeld[1], H. Feichtinger[1,3], Su-ling Li[1], E.E. Kaaya[1], P. Putkonen[2], I. Ernberg[2], D.V. Ablashi[4], R.C. Gallo[4], G. Biberfeld[2], [1]Immunopathology Lab., Karolinska Institute, [2]National Bacteriological Laboratory, Stockholm, Sweden, [3]Department of Pathology, University of Innsbruck, Austria, [4]Lab. of Tumor Cell Biology, NCI, NIH, Bethesda, USA.

AIDS patients are at a highly increased risk to develop non-Hodgkin's lymphomas (NHL), which mostly have histopathological features of high grade malignancy and a very poor prognosis. Different subsets of AIDS-NHL have been defined with regard to clonality, association with EBV, c-myc involvement, histology and anatomical distribution. Recently we have observed that cynomolgus monkeys with an AIDS-like disease after infection with SIVsm (strain smm3, from Dr. P. Fultz and H. McClure, Yerkes Primate Res. Center, USA) also developed malignant lymphomas in a very high frequency (13/33 - 39%). All of the monkey tumors were high grade NHL with lymphoblastic, immunoblastic or pleomorphic histology. The NHL development appeared related to the time and degree of immunodeficiency but independent of time of infection, virus dose, age and sex of the monkey. From immunohistochemistry and Ig-gene rearrangement (Southern blot) studies, all lymphomas were B-cell derived clonal proliferations with a CD-marker profile of follicular-stage, mature B-cells. By electron microscopy EB-like virus was found in 2/3 lymphoma derived cell lines. Immunocytochemistry with human reference sera and Mab's showed expression of EBNA-like reactivity in all lymphomas to a varying degree. A similar reactivity was also found with some of the tumor bearing monkey sera. Also antibodies to EBV-VCA were found in tumor bearing animals before and after SIV infection. At the DNA level all lymphomas contained sequences crosshybridizing with a 3,1 kb BAM HIW probe to the LIR of human EBV; no SIV sequences were found in tumor cells. Our observations indicate a marked similarity of SIV associated lymphomas in cynomolgus monkeys with a major subtype of human AIDS lymphomas, which is EBV associated. This monkey model thus appears useful for studies of the importance of immunosuppressive and oncogenic factors in HIV lymphomagenesis and offers possibilities for studies of specific prevention.

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TAT-DEPENDENT MODIFICATION OF TAR RNA IN XENOPUS OOCYTES; MUTATIONAL AND CHEMICAL ANALYSES, Andrew D.Blanchard, Robert Powell, Martin Braddock, Alan Kingsman and Sue Kingsman, Virus Molecular Biology Group, Dept. of Biochemistry, South Parks Road, Oxford, OX1 3QU.

Tat regulates HIV-1 gene expression via its interaction with the TAR sequence present as a stem loop structure at the 5' end of all viral transcripts. Activation by Tat is mainly transcriptional but in Xenopus oocytes Tat activates translation of TAR containing RNA. TAR RNA is however, only translated in oocytes after injection into the nucleus with Tat. As a consequence of the Tat/TAR interaction the TAR containing RNA is covalently modified and is translated in a Tat independent manner on re-injection into fresh oocytes . The modification could involve alterations to the primary sequence and/or the secondary structure of TAR. To determine whether perturbation of the secondary structure of TAR influences modification by Tat, we have analysed in vivo the effect of several mutations in and around the TAR bulge. A deletion of the bulge, that has no effect on pairing of the stem, abolishes Tat activation and modification. Conversely alterations to the TAR secondary structure, e.g. mutating residue A27 to a G, have little effect on activation and the RNA is still modified. These results indicate that secondary structure is not correlated with Tat responsiveness. Chemical and genetic analyses are being used to determine whether sequence specific or general modifications are mediated by Tat.

NEONATAL DISEASE INDUCED BY SIV INFECTION OF THE RHESUS MONKEY, R. Bohm, B. Davison-Fairburn, G. Baskin, M. Murphey-Corb, Tulane Primate Center, Covington, LA. 70433.

Pediatric HIV infection manifests clinical, pathologic, and immunologic differences when compared to adult infection. To evaluate whether these differences exist in the SIV-infected rhesus monkey, we compared the effects of SIV infection of neonates to those previously determined for monkeys >1 year of age. Sixteen infants were separated from their mothers within 48 hours of birth and nursery-reared. Seven newborns were inoculated with 10 I.D.50 of SIV/DeltaB670; 9 infants served as uninfected controls. Neonates were followed sequentially by physical examinations, serology, (p27) antigenemia, complete blood counts, and lymphocyte subset analysis. A complete necropsy was performed at death. Five of 7 infected animals had persistent antigenemia and died within a mean of 31 days (range 26-41 days) p.i., an interval considerably shorter than seen for older animals (mean=210 days). The CD4+CD29+ helper-inducer lymphocyte population increased in all neonates during the first 7-21 days after birth and then gradually declined, but, unlike SIV-infected juvenile rhesus monkeys, did not undergo selective depletion prior to death. Likewise, no changes in total percentages of CD4+, CD8+, or B lymphocytes were observed between control and infected infants. Clinical signs of infection and necropsy findings were similar to those observed in older animals, but recurrent bacterial infections, primarily of the GI and respiratory tracts, were perhaps more common. These findings will prove useful in diagnosing neonatal infections which occur transplacentally in the SIV: monkey model.

Rev-Dependent Transactivation of gp160 Expression in *Drosophila melanogaster* S2 cells, David W. Brighty and Martin Rosenberg, Department of Gene Expression Sciences Smithkline Beecham Pharmaceuticals, King of Prussia, Pa 19406

In mammalian cells the temporal regulation of HIV-1 gene expression is achieved by a posttranscriptional mechanism that is dependent upon the viral *rev* gene product. Rev enhances the expression of viral structural genes during the late stages of viral infection by relieving the nuclear sequestration of partially spliced or unspliced viral transcripts. We have examined the Rev-dependent expression of gp160 in stably transfected Drosophila S2 cells and employed these cells as a model system for the functional dissection of the Rev regulatory pathway. We find that in Drosophila cells expression of gp160 has an absolute requirement for coexpression of the viral Rev protein. Subcellular fractionation of RNA reveals that in the absence of Rev gp160 transcripts are retained within the nucleus while cytoplasmic accumulation of gp160 mRNA occurs only in cells which coexpress Rev. Immunoprecipitation of Rev from cotransfected Drosophila cells demonstrates that Rev is tightly associated with gp160 transcripts *in vivo* and this association is dependent upon the presence of the rev responsive element (RRE). Furthermore, a deletion analysis of gp160 suggests that information within the gp41 coding region is sufficient to confer Rev responsiveness in Drosophila cells and that a cis-acting nuclear retention signal is present within the gp41 coding sequences of gp160. This nuclear retention signal is responsible for the selective nuclear retention of gp160 transcripts in the absence of Rev. Our studies demonstrate the exquisite versatility of the Drosophila system as a tool for further dissection of the Rev regulatory pathway.

LACK OF AUTOLOGOUS NEUTRALIZING ANTIBODIES IN THE CEREBROSPINAL FLUID OF HIV-1 INFECTED INDIVIDUALS

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Objective: To evaluate the presence of autologous and heterologous neutralization in the cerebrospinal fluid (CSF) of HIV-1 infected individuals.
Material and Methods: Virus isolations were conducted on blood and CSF and collected at the same day as the CSF samples from 12 HIV-1 infected indivivuals at different clinical stages. Neutralization assays using PBMC as target cells were run simultaneously with ID-50 titrations of the virus and the neutralizing titer was estimated at TCID 10-50.
Results: None of the CSF samples and only one of seven serum samples could neutralize the autologous CSF isolate. CSF samples collected one to two years later from the same patients also lacked autologous neutralizing antibodies against these isolates. However, some CSF samples were able to neutralize heterologous CSF isolates albeit in low titers. HIV-antibody positive control sera could readily neutralize all of the CSF isolates demonstrating that these isolates were not resistant to neutralization *per se*. IgG antibodies against the HIV-1 envelope protein and the proposed major neutralizing domain (the V3 region of HIV-1 gp120) were present in some CSF samples although they lacked neutrafizing activity.
Conclusion: This study clearly shows that there is a lack of autologous neutralizing antibodies to HIV-1 in CSF and only a low frequency of heterologous neutralization of CSF-derived HIV-1 isolates.

ROLE OF ADCC AND NEUTRALIZATION IN VERTICAL TRANSMISSION OF HIV-1

Per A. Broliden, Kristina Broliden, Eric Sievers and Paolo Rossi. Department of Virology, National Bacteriological Laboratory, Department of Virology and Immunology, Karolinska Institute, Stockholm, Sweden; Children's Hospital and Medical Center, Seattle, USA

Objective: The prognostic and protective role of antibodies mediating cellular cytotoxicity (ADCC) and neutralization was evaluated in sera of HIV-1 infected mothers and their consecutively followed children.
Material and Methods: Serum samples were available from a total of 80 children (0-2 years of age) and the functional antibody activities were analyzed against two different HIV-1 strains, IIIB and RF. Presence and titers of these antibodies were correlated with clinical outcome at various stages of follow up. Detection of viral DNA by PCR or seropositivity by ELISA and WB at 15 months of age were used to define an infected child.
Results: The presence and titers of ADCC mediating and/or neutralizing antibodies in maternal sera were not predictive for the outcome of HIV-1 infection in the children in individual cases. No significant difference was seen when comparing the presence of neutralizing antibodies between the uninfected and infected children. When dividing the infected group according to clinical status differences could be seen. only one of 20 AIDS patients had a high neutralizing titer (≥ 1:640) against IIIB. Four patients had a low titer (1:20) and the remaining had no detectable neutralizing antibodies at all. In contrast, 10 of 16 infected non-AIDS children had neutralizing antibodies ($p<0.05$). Similarly, no significant difference was seen when comparing the presence of ADCC mediating antibodies between the uninfected and the infected group of children. However, a significantly higher frequency of ADCC was seen in the non-AIDS children compared with the AIDS children ($p<0.05$).
Conclusion: The factors influencing the differences in clinical outcome of perinatal HIV-infection still remain unclear. However, this study clearly show that the presence of antibodies mediating ADCC and neutralization is associated with a better clinical status in the infected children.

A SECOND ORIGIN OF DNA PLUS-STRAND SYNTHESIS IS REQUIRED FOR OPTIMAL HIV REPLICATION

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Replication of retroviruses requires reverse transcription of an RNA genome into double-stranded DNA. Synthesis of each strand of retroviral DNA is initiated at a distinct site by a specific primer, which will define each end of the linear DNA molecule. The origin of retroviral DNA plus-strand synthesis is determined by a polypurine tract (PPT), which defines the 5' limit of the long terminal repeat (LTR). A specific feature of HIV and other lentiviruses is the presence of a second copy of the PPT located near the center of the genome. We recently reported that this central PPT is used as a second origin for the plus-strand of viral DNA, since it determines a discontinuity (or "gap") in that strand, yielding two discrete plus-strand segments on unintegrated linear DNA. Mutations replacing purines by pyrimidines in the HIV-1 central PPT, which do not change the amino-acid sequence, are able to significantly slow down viral growth as they reduce plus-strand origin at the center of the genome. One of these mutants (mutant 225), carrying 4 pyrimidines in the central PPT, grows 2 weeks later than wild-type virus in CEM cells and in PBLs, and has no detectable central gap. The introduction in this mutant of a wild-type copy of the PPT at a different site creates a new gap at that site and appears to improve replication. Titration of each mutant on CD4 +Hela cells carrying an HIV1 LTR LacZ construct shows a six fold difference in titer between wild type virus and 225 mutant .Our findings confirm the role of PPTs as initiation sites for the synthesis of retroviral DNA plus-strand and demonstrate the importance of a second such origin for HIV replication in vitro.

HIV-1 LTR DIRECTS EXPRESSION IN THE CNS NEURONS OF TRANSGENIC MICE

John R. Corboy, Jeanine Buzy, Lynn Lindstrom and Janice E. Clements, Department of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205,

The human immunodeficiency virus (HIV) is the cause of AIDS and the AIDS dementia complex (ADC), but the pathogenesis of ADC remains uncertain. Although in situ hybridization studies rarely find signs of infection in CNS neurons, recent quantitative studies suggest significant neuronal dropout in brains of patients with ADC. Also, many in vitro experiments have found that multiple CNS cells, mostly negative for CD4, can be infected with various strains of HIV-1. Transgenic mice provide an animal model to examine the role of individual HIV genes in the pathogenesis of AIDS and ADC. The HIV-1 long terminal repeat (LTR) contains all of the viral transcriptional elements, and could be important for differential expression of HIV-1 in various tissues. Previous reports of transgenic mice made with HIV-1_{IIIB} LTR's showed no expression in the CNS. We have constructed transgenic mice utilizing the LTR's of HIV-1_{IIIB}, as well as those isolated from the CNS of an ADC patient, HIV-$1_{JR\text{-}CSF}$ and HIV-$1_{JR\text{-}FL}$, attached to the bacterial B-galactosidase (lac Z) as a reporter gene. Expression in 6-12 week old heterozygous F_1 mice was examined by X-gal analysis. All three LTR's expressed to high levels in the thymus, but only the CNS-derived LTR's expressed in brain. HIV-$1_{JR\text{-}CSF}$ expressed strongly in the cerebrum, thalamus, and hypothalamus, and less so in hippocampal neurons, cerebellar purkinje cells, and retinal ganglion cells. HIV-$1_{JR\text{-}FL}$ expressed strongly in the septal nuclei, superior colliculus, optic tract, pontine nuclei and discretely within the CA3, CA4 regions of the hippocampus. The significance of these findings will be discussed in light of current theories of the pathogenesis of ADC.

EVALUATION OF MATERNAL-FETAL TRANSMISSION OF 2 SIV ISOLATES WHICH VARY IN VIRULENCE. B Davison-Fairburn, GB Baskin, R Bohm, LN Martin, M Murphey-Corb, Tulane Regional Primate Research Center, Tulane University, Covington, LA 70433.

Four rhesus monkeys (2 of Indian origin, 2 of Chinese origin) were inoculated with a highly pathogenic ($SIV_{DeltaB670}$) and 4 with a minimally pathogenic ($SIV_{macBK28}$) SIV isolate during the 2nd or 3rd trimester of pregnancy. Infants were delivered vaginally but separated from their mothers and not allowed to nurse.

All 4 $SIV_{DeltaB670}$ inoculated dams showed peaks of antigenemia 2 weeks after inoculation, and both Indian origin monkeys became immunosuppressed and had clinical signs typical of simian AIDS. The 4 $SIV_{macBK28}$ inoculated dams had low levels of antigenemia and remained immunocompetent. Colostrum from one $SIV_{DeltaB670}$ inoculated dam was positive for SIV by culture. Six months after delivery, none of the infants is unambiguously infected, although recurrent positive PCR signals in PBL suggest possible low levels of infection in some infants. Placental lesions including infarcts, placentitis, and deciduitis were observed with an increased incidence in $SIV_{DeltaB670}$ inoculated animals.

Virus positive milk shows that breast-feeding is a possible mode of maternal-fetal transmission of SIV. Immunosuppression may be a cause of increased placental damage leading to a breakdown of the placental barrier which would allow infected maternal blood and inflammatory cells to reach the fetal circulation. A feasable model for this complicated phenomenon may be developed by optimizing the macaque species, the virus isolate, the timing of infection relative to the stage of pregnancy, the stage of maternal disease, and by manipulating contributing factors such as placental damage.

CELLULAR INTERACTIONS AND MOLECULES INVOLVED IN HIV INDUCTION FROM MONONUCLEAR CELLS OF SEROPOSITIVE HUMANS, Michael Diegel, Patricia Moran , Jeffrey Ledbetter, Nitin Damle, Peter Linsley, and Joyce Zarling, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle WA 98121

Monocytes and CD4+ T cells are known reservoirs of HIV. We found that when peripheral blood lymphocytes/monocytes (PBLMC) from HIV seropositive donors were activated with soluble anti-CD3, which is Fc receptor (FcR) -dependent stimulation, there was up to 1000 times more p24 produced than when activated with immobilized anti-CD3 (FcR independent stimulation). However immobilized anti-CD3 induced much higher levels of T cell proliferation. To study whether Fc dependent signaling by soluble antibody was important for induction of HIV, FcR blocking antibodies were added to soluble CD3 (G19-4, FcRII binding) activated PBLMC. Blocking antibody to FcRII inhibited soluble G19-4 induced p24 production by at least 95%. The cellular interactions between T cells and monocytes were investigated because of the large differences in p24 production induced by soluble anti-CD3 versus immobilized anti-CD3. Blocking antibodies to T cell and monocyte adhesion molecules were incubated with PBLMC (depleted of CD8+, NK and B cells) in the presence of soluble anti-CD3 (OKT3 or G19-4). Soluble anti-CD2 blocked anti-CD3-induced p24 production by at least 99% in the presence or absence of exogenous IL-2. Furthermore, addition of soluble antibodies to LFA III (the ligand for CD2), ICAM-1, and LFA I (the ligand for ICAM-1) inhibited p24 production by at least 96%. Finally, CTLA-4 (a ligand for B7 on antigen presenting cells) inhibited p24 production (50-90%), but was less effective in the presence of exogenous IL-2. These results indicate that i) there is a dissociation between HIV induction and T cell proliferation ii) primary signaling through the T cell receptor is required to induce latent HIV iii) secondary signaling necessary for p24 production is produced by adhesion molecules found on monocytes and T cells.

COMBINED EFFECTS OF UVB RADIATION AND ANTI-HIV DRUGS ON THE HIV-1 PROMOTER. E. Gajewski*, S. Rasheed*, J. Z. Beer**, A. G.Strickland**, K. M. Olvey**, and B. Z. Zmudzka**, (*) Laboratory of Viral Oncology and AIDS Research, University of Southern California School of Medicine, Los Angeles, CA 90032, and (**) Center for Devices and Radiological Health, FDA, Rockville, MD 20857.

Ultraviolet (UV) radiation in the UVB range (280-315 nm) activates HIV and is considered as a possible factor in enhancing virus replication in vivo. Evidence suggests that HIV promoter activation by UV is related to DNA damage. The level of such damage may,in turn, be affected by compounds that interfere with DNA repair such as 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxynucleosides that are precursors of inhibitors of repair polymerase B. To determine how activation of the HIV promoter by UVB is affected by some anti-HIV drugs, we examined the combined effects of UVB and AZT or 2',3'-dideoxycytidine (ddC) on the HIV promoter. The experiments were conducted using HeLa cells stably transfected with the HIV-1 promoter and the chloramphenicol acetyl transferase (CAT) gene as the reporter gene (HIVcat/HeLa A5 cells). The HIV promoter activity increased with increasing UVB fluence (0-1.2kJ/m^2), reaching a maximum at approximately 0.6 kJ/m^2 and then decreasing as the fluence was further increased. Cells were exposed to UVB (at 0.2-1.5 kJ/m^2) in the presence or absence of AZT or ddC at non-cytotoxic concentrationS (1-10uM) and CAT activity was measured at post-exposure time intervals ranging from 16 to 28 h. Our results indicate that AZT or ddC alone did not activate the HIV promoter, and HIV promoter activation by UVB in the presence of AZT was negligible. In contrast, a 10-fold increase in HIV promoter activity was observed 21 h after UVB exposure in the presence of ddC while a comparable level of promoter activity was observed at 27 h in the samples irradiated without ddC. These data suggest that ddC may accelerate activation of UVB-induced HIV-promoter activity.

Functional analysis of natural variation in the long terminal repeat (LTR) of human immunodeficiency virus type 1. J.L.M.C. Geelen[1], S.E.C. Koken[2], J.L.B. van Wamel[2], B. Berkhout[2] and J. Goudsmit[2]. [1]Department of Medical Microbiology, Academic Hospital Maastricht, Maastricht, The Netherlands. [2]Department of Virology, University of Amsterdam, AMC, Amsterdam, The Netherlands. The HIV-LTR is a highly compressed promoter which contains multiple regulatory segments (eg 3 Sp-1 sites and 2 NF-kB sites). Variation within these elements may result in different replication rates, different responses to T-cell activation and different pathogenicity. Therefore, we decided to screen for natural variants in the LTR and to measure the promoter activity. DNA was extracted from either direct patient material or from cocultivated cells and screened for length polymorphism in the NF-kB and Sp-1 region of the LTR after amplification by the polymerase chain reaction. If length differences were found compared to the standard LTR, the complete LTR region was amplified, cloned and sequenced. Two types of length polymorphism were detected: an additional Sp-1 site or a duplication of a region 5' to the NF-kB sites. The effect of these duplications on the promoter/enhancer efficiency was tested by the chloramphenicol acetyl transferase assay after transfection into a number of different cell lines. The natural occurring four Sp-1 site variant proved to be the most efficient. This was confirmed by measuring the replication rate of viruses in which the variant LTRs were introduced. Based on coinfection experiments the duplication of the region 5' to the NF-kB sites resulted in a decreased replication rate. Gel mobility shift assays were performed to test whether specific protein binding sites reside within this new DNA motif.

ZIDOVUDINE THERAPY IN HIV-POSITIVE WOMEN DURING PREGNANCY - A CASE STUDY:

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Zidovudine has become a standard therapy in treatment of advanced HIV-infection. Very few experiences have been reported on the use of Zidovudine during pregnancy. Apart from possible pediatric indications for Zidovudine we have found remarkable benefit in Zidovudine-therapy when applied for maternal reasons. We report on 4 pregnancies of HIV-positive patients visiting our outpatient-department. In these pregnant women we used Zidovudine therapy as a last resort under unfavourable conditions. As indication for the therapy we took opportunistic infections. thrombocytopenia, low CD4-counts, a low CD4/CD8 ratio, as well as positive p24 antigen results and repeated virus isolation from the maternal blood. We treated the patients with Zidovudine during the third trimester of their pregnancy. Monitoring of the maternal Zidovudine blood-level showed no drug accumulation compared with non pregnant patients. In all cases, we found immediate positive effects on lab parameters, as well as on the personal well-being of the patients. Apparently, there were no negative side effects on the development of the fetus. We report on the follow-up of our patients and their children which are not between 15 and 24 months old. As comparision we consider the courses of pregnancies of 4 other HIV positive patients not treated with Zidovudine. In critical events we found less positive courses of those pregnancies particularly concerning the fetal development and postpartal state.

VERTICAL TRANSMISSION OF HIV INFECTION IN UGANDA

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A prospective study of HIV infection in Ugandan women and their infants is underway in Kampala Uganda in collaboration with the Ugandan AIDS Control Programme. HIV seropositive and a control group of HIV seronegative women were followed through pregnancy and delivery. Infants delivered were followed closely in a study clinic with careful growth monitoring, history taking, and physical exams. HIV serology was obtained at 18 months

Over 600 infants have been followed, 269 who would be 18 mos. or older; 193 born to HIV seropos. mothers and 76 to seroneg. mothers. 220 infants have complete followup to 18 mos. 27(14%) of these infants born to seropos. mothers have died compared to 2(2%) infants born to seroneg. mothers. All of these 27 infants were less than 18 mos. old at the time of death; 6(23%) had no symptoms of HIV infection, 13(50%) had symptoms suggestive of HIV infection, and 7(27%) had clinical AIDS by the WHO or CDC clinical criteria. Of the 132 infants born to seropos. mothers who are still alive at 18 mos., 25 are HIV infected by serology (18.9%). No infants born to seroneg. mothers are infected. Of these 25 infected infants, 20% are well, 28% have clinical AIDS, and the remaining 52% have symptoms associated with HIV infection but not yet AIDS. Estimates of vertical transmission range from 21% to 31% when deaths are included.

Prolonged diarrhea, hepatomegaly, splenomegaly, repeated skin infections, generalized lymphadenopathy, and parotitis were all significantly associated with HIV infection in the infants. Developmental delay, failure to thrive, and neurologic abnormalities were also associated with HIV infection in the infants.

PROTECTION FROM VIREMIA AND DISEASE PROGRESSION BY INFECTION WITH ATTENUATED SIV,

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A variety of isolates of the HIV-2/SIV_{sm} family of primate lentiviruses have been characterized at the genetic level, but after propagation in human T-cell lines. SIV_{BK28} is a full length molecular clone derived from SIV_{mac251} infected Hut-78 cells which we have found to be relatively non-pathogenic in macaques. The objective of this study was to determine if prior infection with an attenuated strain influences disease progression after subsequent challenge with highly virulent strains. Rhesus macaques infected with SIV_{BK28} for over 2 years, asymptomatic with no evidence of disease progression were challenged with 100 MID_{50} of the 11-88 stock of $SIV_{mac251/32H}$ or the highly pathogenic macaque *in vivo* passaged strain SIV_{t8980}. Prior infection with SIV_{BK28} protected three out of four animals from viremia and terminal disease while unchallenged SIV_{BK28} infected controls remained disease-free. These data support observations that viremia is an important factor in disease progression and secondly demonstrates that prior infection with a minimally pathogenic isolate may permit aquisition of sufficient protective immunity to limit subsequent infection/challenge by more virulent strains.

Hence, these findings suggest that the pathogenic or virulence properties of the initial inoculum may influence establishment of subsequent HIV strains and greatly influence the course of disease.

INVESTIGATION OF THE ABILITY OF MONOCLONAL ANTIBODIES THAT BIND TO CELL SURFACE FORMS OF $p17^{gag}$ TO MEDIATE ADCC REACTIONS,

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We have characterized the ability of two monoclonal antibodies (Mabs) against $p17^{gag}$ to bind to viral antigens displayed on the surface of live HIV-1 infected cells. Cell surface binding was demonstrated by flow cytometry and quantitated by a radiolabeled antibody binding assay. In order to investigate whether these antibodies can mediate antibody-dependent cell cytotoxicity (ADCC), we examined the cell surface expression of *gag* in appropriate ADCC target cells infected with either HIV-1 or vDK, a vaccinia vector encoding HIV-1 *gag-pol*. CEM.NKR and AA-2 cells infected with HIV-1 and RH-LCL cells infected with vDK were analyzed by flow cytometry to quantitate binding of anti-p17 Mabs G11g1 and G11h3 (Shang et al., J. Virol. 65:4798, 1991). For the RH-LCL/vDK system, specific binding by these Mabs was observed in 25-35% of the cells, compared to 65-95% binding for an anti-vaccinia polyclonal serum. Only 1-2% of control cells infected with wild-type vaccinia virus bound the anti-p17 Mabs. The cell surface p17-containing antigen was detected on 25% of AA2 cells infected with the IIIB or MN strain of HIV-1, whereas 35% of these cells were stained with an anti-gp160 serum. It thus appears that these cell lines can provide appropriate targets for ADCC. Since the anti-p17 Mabs are of rat origin, ADCC is first being measured with rat splenocytes and peripheral lymphocytes as effector cells. The ability of human PBLs to function as effector cells will also be investigated.

HIV V3 SEQUENCE ANALYSIS OF THE DENTAL CLADE AND SURROUNDING COMMUNITY, Martin D. Hill[1], Lawrence G. Abele[2], and Lionel Resnick[1]. [1]Retrovirology Laboratory, Mt. Sinai Medical Center, Miami Beach, FL 33140 and [2]School of Arts and Sciences, Florida State University, Tallahassee, FL 32306.

In a singular case, a Florida dentist with AIDS was determined by the Centers for Disease Control (CDC) and Los Alamos National Laboratories (LANL) to have probably transmitted HIV to five of his patients. Three criteria were used by LANL to determine epidemiological linkage: 1) a 95% or greater nucleotide sequence homology; 2) the sharing of a common node in a phylogenetic analysis; and, 3) the sharing of more than four "signature" amino acids with the dentist. To reexamine this case, we obtained blood samples from the five infected dental patients and from over thirty HIV infected individuals from the surrounding community. Viral DNA was isolated from lymphocytes, the V3 env region was amplified by PCR, cloned and then the nucleotides were sequenced. Our sequences were compared to viral sequences determined by the CDC and examined for phylogenetic relationships. The results of our studies will be presented.

INDUCTION OF DIFFERENTIATION AND NF-κB ACTIVITY IN HIV-1 INFECTED MYELOID CELLS

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The effects of HIV-1 infection on myeloid differentiation, induction of NF-κB DNA binding proteins and NF-κB regulated cytokine expression were investigated in a new model of HIV-1 infection. PLB-985 cells represent a myelomonoblastic cell population capable of granulocytic or monocytic differentiation following induction with different agents. PLB-985 cells were infected with HIV-1 strain IIIB; selection of chronically infected PLB-IIIB cells generated a population with a more monocytic phenotype as determined by differential staining, expression of monocyte specific surface markers and increased transcription of the *c-fms* proto-oncogene.Chronic HIV-1 infection of PLB cells did not lead to constitutive cytokine production; however, stimulation of PLB-IIIB cells by PMA or TNF resulted in high levels of IL-1ß and TNF-α RNA and protein. To investigate the molecular basis of monocytic differentiation and gene expression in HIV infected cells, regulation of NF-κB activity was examined. Electrophoretic mobility shift analysis using an NF-κB binding site from the IFN-β PRDII domain (P2) revealed distinct protein-DNA complexes in extracts from PLB-IIIB cells compared to PLB-985 cells. U.V. cross-linking analysis demonstrated that in PLB-985 cells NF-κB p65-p50 complexes were induced by PMA or TNFα treatment and binding could be inhibited by recombinant IκB (rIκB). In PLB-IIIB cells, a 70kD protein was TNFα and PMA inducible but other P2 DNA binding proteins of 90, 100, and 105kD were constitutively present. This "novel" complex in PLB-IIIB cells had higher affinity for the P2 NF-κB site than for the HIV enhancer and its binding activity was not inhibited in the presence of rIκB. A similar complex was also induced in PLB-985 cells by Sendai virus infection. HIV-1 infection of myeloblasts may alter the type and/or stoichiometry of NF-κB protein-DNA interactions, thus contributing to differential NF-κB mediated gene expression.

PRODUCTION OF SIV AND HIV-1 ENVELOPE PROTEINS IN CHO CELLS,

Gillian Hutchinson, Andrew Rhodes, Mariangela Spitali, Gary Clements, Geoffrey Yarranton and Paul Stephens, Celltech Ltd., Slough, UK.

Recombinant gp120 proteins from the SIVmac251 and the GB8 strain of HIV-1 have been expressed in Chinese Hamster ovary cells using the glutamine synthetase selection system developed at Celltech. Permanent cell lines have been established and expression of recombinant protein has been demonstrated by reactivity of culture supernatants with specific SIV and HIV-1 monoclonal antibodies. Purified gp120 has been obtained by immunoaffinity chromatography methods and an enzyme-linked immunosorbent assay for SIVgp120 is being developed. In addition, mutagenesis has been carried out on the HIV-1 GB8 env gene to remove the transmembrane domain in order that a soluble form of GB8gp160 can be expressed in CHO cells. Data from several laboratories suggest that the envelope protein of HIV assembles into a dimeric or tetrameric structure (Berman et al., 1989, Earl et al., 1989, Pinter et al., 1989, Weiss et al., 1990). Since the oligomeric forms may be more structurally and immunologically relevant than the monomeric forms, our studies will investigate the oligomeric nature of the recombinant sgp160. Latest results will be presented.

Berman et al (1989) J.Virol. 63: 3489-3498
Earl et al (1989) Proc.Natl.Acad.Sci. USA 87: 648-650
Pinter et al (1989) J.Virol. 63: 2674-2679
Weiss et al (1990) J.Virol. 64: 5674-5677

EFFECT OF HUMAN CYTOMEGALOVIRUS ON HIV REPLICATION IN HUMAN BRAIN-DERIVED CELL LINES. Francoise Jault, Stephen A. Spector,* and Deborah H. Spector. Departments of Biology and Pediatrics,* University of California, San Diego, La Jolla, CA 92093

Human cytomegalovirus (HCMV) is commonly found associated with HIV in neuronal lesions in patients with AIDS and may act as a cofactor in the development or progression of the AIDS associated dementia. To analyze the molecular interactions between these two viruses, we have previously studied their replication in human fibroblasts and in U373 MG astrocytoma/glioblastoma cells, both of which are fully permissive for the growth of each virus. In these cells, dual infection with HCMV and with HIV pseudotyped with amphotropic retrovirus glycoproteins results in a significant inhibition of HIV RNA and protein synthesis. This repression appears to occur early in the HCMV life cycle and requires HCMV gene expression but not viral DNA replication. Recently, we have extended these studies to five additional human brain-derived cell lines (of neuroblastoma, astrocytoma/glioblastoma, and undifferentiated glioblastoma origin) which differ in their ability to support HCMV replication. The use of HIV (amphotropic retrovirus) pseudotypes allowed us to bypass the restriction at the adsorption/penetration step in all of these CD4 negative cell lines, with HIV synthesis increased up to 10,000 fold relative to infection of these cells with non-pseudotyped HIV. The level of HIV produced did vary in the different cell lines, further confirming the notion that cellular factors also influence the infection. In contrast to our previous results, dual infection of these five additional cell lines with HCMV and HIV pseudotypes had no effect on HIV replication. The only exception was one neuroblastoma cell line in which a transient stimulation of HIV production was observed. Studies are currently in progress to characterize fully the extent of HCMV gene expression in these cells and to determine whether additional cellular factors influence the interaction between these two viruses in the coinfected cell.

Norman Human Neonates' Leukocytes are Deficient in Antibody-Dependent Cellular Cytotoxicity (ADCC) of Human Immunodeficiency Virus-Infected Cells

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Vertical transmission of HIV requires that the virus circumvent fetal and neonatal immune defenses. We hypothesized that normal human neonates' leukocytes would be less efficient than normal adults' in mediating ADCC and/or natural killer cytotoxicity (NKC) of HIV infected cells. Mononuclear cells (MC) from the umbilical cords of 8 healthy neonates and 8 adults were purified on Ficoll hypaque and tested as matched pairs. Effector cells were added to ^{51}-chromium labeled H9 cells, chronically infected with HIV III_B at effector to target ratios (E:T) of 25:1 to 100:1. Normal human serum or serum from HIV infected persons was added at a predetermined optimal final dilution 1/200. NKC and ADCC were measured after 4 hours and the two groups compared by Wilcoxson signed rank test. Neonatal MC were comparable to adults' in NKC (20.3±2.6 (mean ± SEM) vs 19.2 ± 1.7% lysis at E:T 25:1; 36.0 ± 3.5 vs 41.7 ± 1.9% lysis at 100:1). Neonatal MC were significantly less effective in ADCC (9.4 ± 3.8 vs 19.3 ± 3.2% lysis at E:T 25:1; 14.2 ± 5.3 vs 27.7 ± 5.4% lysis at 100:1 ($p < 0.02$ for each)). Deficient ADCC lysis of HIV infected cells may be an important contributor to vertical HIV transmission.

INHIBITION OF HIV-1 REPLICATION BY PHOSPHONOFORMATE ESTERS OF 3'-AZIDO-3'-DEOXYTHYMIDINE, John A. Koch, Fatemeh Fazely, Dorothy Trites, Andre Rosowsky and Ruth M. Ruprecht, Department of Biological Chemistry and Molecular Pharmacology and Department of Medicine, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts 02115.

Studies have been conducted to investigate the efficacy of 5'-(O-alkoxycarbonylphosphinyl; ACP) derivatives of AZT against HIV-1 replication as measured by reverse transcriptase activity (RT) in culture supernatants. A monoethyl ester of the ACP derivative, 3'-Azido-3'-deoxy-5'-O-[P-(ethoxycarbonyl)-P-hydroxyphosphinyl]thymidine (ECP-AZT), was chosen for further investigation since the length of the side chain of this compound is approximately equivalent to the triphosphorylated moiety of AZT. ECP-AZT was tested for toxicity and its ability to inhibit HIV-1 replication in human CD4+ T-lymphocytes when added 4 hours post infection. Growth was arrested by less than 10% at drug concentrations up to approximately 10^{-3}M. In contrast, a 50% inhibition of RT activity relative to non-treated HIV-1 infected cells was noted at concentrations from 10^{-7} to 10^{-6}M. When ECP-AZT was added 4 hours prior to infection the IC_{50} for viral replication was approximately 10^{-6}M. Complete inhibition and no toxicity were noted at a concentration of 10^{-4}M. The effect of ECP-AZT on RT acivity in a cell-free assay was conducted to determine whether the antiviral activity could be attributed solely to the interaction with RT. An IC_{50} value for RT inhibition (10^{-4}M) was found to be approximately 100 fold greater relative to the IC_{50} for viral replication suggesting that either metabolism of ECP-AZT is required for activity against RT or that ECP-AZT or its metabolites are interacting with other viral targets. ECP-AZT was rapidly degraded by mouse sera but remained stable when exposed to human sera. ECP-AZT may be regarded as a novel 5' blocked antiviral dideoxynucleoside which has a broad therapeutic window.

DEVELOPMENT OF SENSITIVE ELISA ASSAYS FOR HIV PROTEINS FOR USE IN STUDIES OF VIRUS NEUTRALIZATION. Suman Laal, Aby Buchbinder and Susan Zolla-Pazner, Department of Veterans Affairs Medical Center, 423 East 23rd Street, New York, NY 10010.

Several human monoclonal antibodies to gp120 of HIV have been developed that neutralize viral infectivity in vitro. However, the mechanism of neutralization is not known. Shedding of envelope glycoprotein gp120 by the viral particle has been proposed as a possible mechanism of neutralization and studies are underway to determine if anti-HIV monoclonal antibodies neutralize via the above mechanism. Simple sensitive ELISA's for quantitation of picogram amounts of individual viral proteins gp160, gp120, gp41, and p24 have been established for these studies. The assays have been calibrated with purified, recombinant forms of these antigens and have sensitivity limits of 30-50 pg. The gp160 and gp120 assays are based on glycoprotein immobilization by Con A, making it feasible to use them for diverse viral strains. The sensitivity of the assays allows us to monitor the ability of monoclonal antibodies to neutralize infectious virus, thus providing a functional assay for mass screening of hybridomas. The assays, and the results obtained by their use for screening and neutralization studies will be presented.

POLYCLONAL AND MONOCLONAL HIV-1 NEUTRALIZING ANTIBODIES

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Polyclonal (PAb) and monoclonal antibodies (MAb) directed to pre-selected epitopes of HIV-1 are useful reagents to characterise structure-function relationships of viral proteins. Such antibodies find a major application in the delineation of epitopes that can be included in candidate vaccines for the induction of (cross)-neutralizing antibodies. In addition, the role of different variable sites in determining the biological phenotype of HIV-1 variants can be investigated using these antibodies.

We used synthetic peptides homologous to the third variable domain (V3 or Principal Neutralizing Determinant) of different HIV-1 variants (i.e. IIIB and MN) to generate specific PAb and MAb. These antibodies have been extensively characterized by ELISA, immunocytochemistry, FACS-analysis, Pepscan-analysis and biological assays (cell-free virus neutralization and syncytium formation inhibition). MAb directed against the apex of the V3-loop of IIIB neutralize the homologous virus but not MN, as the presence of glutamin (Q) and arginin (R) residues is essential for binding. MAb directed against the lower left side and right side of the IIIB V3-loop do not neutralize IIIB. PAb directed against the apex of the MN V3-loop neutralize IIIB and in addition four field isolates.

CLONING OF THE CATALASE-PEROXIDASE GENE OF MYCOBACTERIUM TUBERCULOSIS.

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Tuberculosis is a major opportunistic infection in AIDS patients and the emergence of resistance to the most commonly used drug, isoniazid (INH), is aggravating the problem. Resistance of M tuberculosis to INH has been associated with the loss of catalase-peroxidase activity. However, the exact relationship between these 2 phenomena is not known. Cloning of the catalase-peroxidase gene of M tuberculosis was undertaken as an initial approach to determine whether a mutation in the structural gene or an abnormality of its regulation may explain the association between the loss of catalase-peroxidase activity and resistance to INH.

The genomic DNA from M tuberculosis H37Ra strain sensitive to INH (ATCC #25177) was digested with EcoRI-Sau3AI and cloned into the EcoRI-BamHI restriction sites of the phagemid vector pBluescript. The recombinant vectors were then electroporated into an E coli K12 derivative strain, UM2 (KatE2, KatG15), lacking catalase-peroxidase activity. The transformants were screened for clones expressing catalase and peroxidase. Five clones were identified by their ability to produce O_2 in the presence of H_2O_2 (catalase activity) and to react with diaminobenzidine (peroxidase activity). The DNA insert size in the recombinant vectors varied from 800 base-pairs (bp) to about 2,500 bp.

The clone UM2 (pZL) containing the 800 bp DNA fragment was selected for further studies. A probe constructed from the insert by random priming hybridized with only M tuberculosis (sensitive and resistant to INH) and M bovis DNA but not with any other mycobacterial species. The sequence analysis of this insert is currently being completed. The corresponding DNA segment from a M tuberculosis resistant to INH will be sequenced to identify possible mutations.

These studies may help to understand the possible relationships between INH-resistance and catalase-peroxidase activity in M tuberculosis.

NEUTRALIZING ANTIBODY TO AUTOLOGOUS HIV VIRUS ISOLATES IN ASYMPTOMATIC MOTHERS,

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The presence of high titers of neutralizing antibodies (N AB) to HIV 1 has been associated with asymptomatic stages of HIV-1 infection suggesting a protective role against disease progression. High maternal AB titers to the gp 120 V3 hyper variable loop were also correlated with a decrease in perinatal transmission; however results are inconsistent. Due to the heterogeneity of HIV even within an individual, studies using lab virus strains such as HIV III B or MN to assay N AB activity may not be clinically relevant. We therefore developed an assay to test the capability of sera from HIV infected mothers to neutralize their own and their infant's viral isolates and correlated this with perinatal transmission.

Methods: We biologically cloned HIV isolates from 7 asymptomatic HIV infected mothers by limiting dilutions of co-cultured PBM cells. Virus stock was grown in PBMCs from the last positive well. We tested, two fold dils of each maternal serum (1-/10-1/320) against her respective virus and measured infectivity end points in PBMCs by P24 AG in culture sups. N AB titers were determined by the serum dil that showed $\geq$50% inhibition of P24AG compared to controls.

Results: Of the 7 women only 3 had detectable N AB against autologous isolates at 1/160->320. Of these 3 mothers, one transmitted infection to her infant. This infant however; was two months premature and may not have had sufficient passage of maternal AB. Two mothers without detectable N AB transmitted HIV to their infants. Virus load was similar in these mothers as measured by quantitative co-culture (1.6×10^3 - 4×10^4). These preliminary results suggest that this assay may be useful in assessing the importance of neutralizing AB for protection against perinatal infection and may more clearly represent the role of these ABs in vivo.

THE INTERLEUKIN BINDING FACTOR, ILF, BINDS SPECIFICALLY TO PURINE-RICH MOTIFS IN HIV LTR AND INTERLEUKIN-2 PROMOTER AND ACTIVATES THEIR GENE EXPRESSION,

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ILF gene encodes a 60 Kd protein which binds specifically to purine-rich motifs in the negative regulatory element region of human immunodeficiency virus-1 long terminal repeat (HIV LTR) and the NFAT (nuclear factor of activated T cells) site in interleukin-2 (IL-2) promoter. ILF RNA contains two spliced products which diverge in the 3'-end and these RNA are expressed constitutively in a variety of both lymphoid and non-lymphoid tissues. Sequence analysis reveals that the DNA binding domain of ILF has strong homology to the *fork head* DNA binding domain which is also found in the Drosophila homeotic protein, *fork head*, the hepatocyte-specific factors, HNF-3, and a human T cell leukemia virus-1 LTR binding factor, HTLF. Other potential regulatory domains found in ILF include a nucleotide binding site, an N-glycosylation motif, a signal for ubiquitin-mediated degradation, and a nuclear location signal. Gel retardation analysis revealed that the fork head binding region of ILF was sufficient for confering DNA binding to a number of related purine-rich sequences. Transient expression of the ILF gene in HeLa and Jurkat cells activated gene expression directed by HIV and IL-2 promoters. These results suggest that ILF is an activator of HIV and IL-2 gene expression.

SPECIFIC COMBINATIONS of NF-κB SUBUNITS ACT IN SYNERGY WITH TAT-I TO STIMULATE THE HIV ENHANCER,

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NF-κB is a protein complex which can interact with the *cis*-acting regulatory regions of many viral and cellular genes. This transcription factor is composed of at least two subunits derived from a family with similarity to the *c-rel* oncogene. Previous studies have shown synergistic activation by NF-κB and the tat-I gene product on the HIV enhancer. To determine whether specific members of the NF-κB family contribute to this effect, we have examined the ability of different NF-κB subunits to interact with tat-I to activate transcription of HIV in Jurkat T leukemia cells. We have found that the product of p49/p100 DNA binding subunit, together with p65, can act in synergy with tat-I to stimulate the expression of HIV-CAT. Despite this effect, it is unlikely that there is a direct interaction between NF-κB subunits and Tat, because similar similar stimulation was observed after co-transfection with chimeric activators of HIV enhancer derived by fusion of the acidic domain of herpesvirus VP16 to the amino terminal conserved region of p105 and *c-rel*. This synergistic effect is therefore likely to be the result of an increase in transcriptional initiation complexes which can interact with Tat. Little synergy is observed with a 50 kd form of p105 NF-κB or *rel* in combination with p65 or full-length *c-rel*, which do not stimulate the HIV enhancer in these cells. These findings suggest that the combination of p49/p100 and p65 NF-κB can act in synergy with the tat-I gene product to stimulate the synthesis of HIV RNA.

ANTIBODIES TO rgp120 IN rgp120-IMMUNIZED ANIMALS AND IN HIV-1 SEROPOSITIVE INDIVIDUALS, C. Lucas, M. Peterson, A. Shahzamani, A. Ammann and P. Berman, Departments of Medicinal and Analytical Chemistry, Medical Affairs and Immunobiology, Genentech Inc., South San Francisco, CA 94080.

In preparation for HIV-1 vaccine trials with recombinant gp120 in humans, we have developed radioimmunoprecipitation (RIP) and ELISA assays to examine the antibody reactivity to either $rgp120_{IIIB}$ or $rgp120_{MN}$, or fragments of these molecules, in the serum of immunized animals and of HIV-1 seropositive individuals. To this end, the serum from rgp120 immunized animals and samples from 6 HIV-1 seronegative and 6 HIV-1 seropositive individuals were examined in various immunoassays. In HIV-1 seropositive individuals, serum reactivity by RIP or by ELISA was strong to both $rgp120_{IIIB}$ and $rgp120_{MN}$, with titers ranging from 3.2 to 4.5. Because of the high anti-gp120 antibody levels already present in these individuals, differences in reactivity to either $rgp120_{IIIB}$ or $rgp120_{MN}$ were not easily demonstrated with either type of assay. On the other hand, reactivity of antibodies to the V3 region of $gp120_{IIIB}$ or $gp120_{MN}$ was distinguished by ELISA, where seropositive individuals are generally reactive to $V3_{MN}$ (titers of 3.2 to 3.5), but not to $V3_{IIIB}$. In $rgp120_{IIIB}$-immunized animals, serum reactivity to $gp120_{IIIB}$ and to $gp120_{MN}$ was easy to differentiate by RIP and by ELISA. Developing antibodies were first reactive to $gp120_{IIIB}$, and when titers reached 3.0 by RIP, cross-reactivity to $gp120_{MN}$ became measurable. Subtle differences in reactivity to either $gp120_{IIIB}$ or $gp120_{MN}$ can be demonstrated in different immunoassay formats, and serotype-specific immunoassays are required to quantitate antibodies to gp120, to the V3 loop region of gp120, or to the CD4 binding region of the molecule.

GP120 BINDING INDUCED CONFORMATIONAL CHANGES IN THE V1 DOMAIN OF THE CD4 PROTEIN. Ch. Manzoni, L. Dirckx, J. Mous
PRTB Hoffmann-La Roche Ltd., Basel,Switzerland

A C-terminal extended V_1 domain of human CD4 was expressed in E. coli and purified by chromatography on a Ni-chelate coloumn via its N-terminal 6-His label. This mutant,V_1^*, was not recognized by m'Abs known to bind to epitopes present on the first domain (V_1) of native human CD4. Furthermore, in contrast to other recombinant V_1 constructs, the mutant V_1^*, was unable to bind to the envelope protein gp120 of HIV-1. Whereas recombinant authentic V_1 inhibited HIV-infection in vitro, mutant V_1^* enhanced the spread of HIV-infection as demonstrated by increased formation of syncitia. This enhancement could not be blocked by the m'Abs Leu3A and OKT4A which otherwise inhibit gp120/CD4 interaction.

Immunological studies employing different CD4 expressing cell-lines show that after binding of gp120 to CD4, latter receptor protein changes its conformation and assumes a structure similar to V_1^*.

Preliminary results indicate that binding of V_1^* itself to various cell lines correlates with syncitia formation after HIV-infection.

We postulate that V_1^* and a similar structure induced in CD4 by gp120, interact with a cell surface component essential for membrane fusion.

HUMAN PHARMACOKINETICS OF STEALTH® LIPOSOMES CONTAINING DOXORUBICIN, Francis Martin, Liposome Technology, Inc., Menlo Park, CA 94590 and Alberto Gabizon, Hadassah University Hospital, Jerusalem, Israel.

Preclinical studies show that "Stealth" liposomes containing a polyethylene glycol lipid (PEG-PE) circulate for prolonged periods, accumulate in experimental tumors and improve the activity of encapsulated anthracyclines. Here we examine the pharmacokinetics (PK) of doxorubicin encapsulated in PEG-PE liposomes (DOXIL™) in 10 cancer patients refractory to conventional chemotherapy. The objectives were to compare the PK of free doxorubicin (F-DOX) and DOXIL in the same patients and to measure the PK of DOXIL at two dose levels. One cohort of 4 patients received F-DOX at 25 mg/m^2, followed 3 weeks later by a course of DOXIL at the same dose. The rest of the patients received two successive courses of DOXIL, 25 mg/m^2 (1st course) and 50 mg/m^2 (2nd course), with a three week interval. All treatments were given by IV bolus injection (5-15 min) at a drug concentration of 1.5-2.0 mg/ml. DOXIL was well tolerated at both dose levels. DOXIL was cleared very slowly from plasma with a distribution half-life of 21 to 54 hrs. compared with rapid clearance of the free drug ($t_{\alpha} \sim 5$ min), resulting in differences greater than 100-fold in AUC. With DOXIL, essentially 100% of the injected dose was recovered in the estimated plasma volume shortly after injection, and nearly all the drug measured in plasma remained encapsulated within the liposomes at all time points up to 7 days following injection. The apparent volume of distribution of DOXIL was only slightly greater than the estimated plasma volume, indicating that the distribution of the liposomal drug is initially restricted to the intravascular compartment. A linear increase in plasma drug levels was seen as the dose of DOXIL was raised from 25 to 50 mg/m^2, with no major effects on PK parameters. These results are consistent with preclinical observations and suggest that DOXIL clearance and distribution are controlled by the liposome carrier, thus providing a means of substantially altering the kinetics and distribution of the drug. DOXIL uptake in human tumors is currently under study.

MODULATION OF VIRUS EXPRESSION IN SIV INFECTED MACROPHAGE CULTURES BY NEUTRALIZING ANTIBODIES AND AZT, M.F. McEntee, M.C. Zink, M.G. Anderson, H. Farazadegan, C. Flexner, O. Narayan, Departments of Comparative Medicine and Pharmacology, The Johns Hopkins University School of Medicine and School of Public Health, Baltimore, MD 21205

Primary macaque macrophages were inoculated with $SIV_{mac}251$. Twentyfour hours later immune macaque serum, containing neutralizing antibodies to $SIV_{mac}251$, or AZT (final concentration = 10uM) were added to determine whether these reagents would modulate virus replication in previously infected cells. The virus replicated productively and cytopathically in the absence of both reagents but not after preincubation with neutralizing serum or following pretreatment of the macrophages with AZT. Addition of immune serum 24 hours after virus inoculation resulted in a sustained, non-cytopathic infection in these cultures characterized by low levels of viral DNA, RNA and protein, in comparison to untreated controls, and the inability to form syncytia following cocultivation with uninfected indicator cells. In situ hybridization and immunocytochemistry showed that 1) antibodies prevented the spread of virus between cells and 2) the individual cells produced less viral RNA and protein than those maintained in non-immune serum. The removal of antibodies from treated cultures resulted in resurgence of full scale virus replication. Addition of the serum 3,4,5 and 6 days after virus inoculation resulted in a gradual loss of the ability to limit p24 production and an inability to prevent cytopathic expression of the virus infection. This modulation of virus expression in individual cells by neutralizing antibodies may be due to the ability of the antibodies to prevent reinfection by progeny particles. Experimental data will also be presented showing that a comparable situation exists when the infected cultures are treated with AZT 24 hours after inoculation.

TARGETED DISRUPTION OF CD43 GENE IN CEM CELLS, Manjunath Narasimhaswamy and Blair Ardman, Departments of Geographic Medicine/Infectious Disease and Hematology/ Oncology, New England Medical Center, Boston, MA 02111.

We have detected, in sera from HIV+ subjects, IgG autoantibodies that bind specifically to CD43 (leukosialin or sialophorin), a leukocyte membrane sialoglycoprotein. These autoantibodies bind to a partially sialylated form of CD43 expressed by normal human thymocytes. Others have shown that CD43 expression by mature T lymphocytes is defective in the Wiskott-Aldrich Syndrome, an X-linked, severe immunodeficiency syndrome. Whether autoantibodies to CD43 or its defective expression affect T cell function is unknown. Moreover, the actual role of CD43 in immune physiology is poorly understood. Recent studies suggest that CD43 contributes to T cell activation during the process of antigen recognition. To directly address the function of CD43 on T-lymphocytes, we disrupted one CD43 allele in CEM cell line by gene targeting. The targeting vector contains a 2.7 kb (nt497-3290) Pst1 fragment of genomic CD43 sequence interrupted at the Sph 1 site (nt 1297), by a neomycin resistance gene cassette lacking internal poly A site (derived from pMC1 Neo). Following transfection, the cells were seeded in 96 well tissue culture trays and selected in G418. A novel approach was used to screen the G418 resistant colonies. On the basis of gene dosage effect, the functional loss of an allele should result in a 50% decrease expression of the protein. The G418 resistant clones were stained with anti-Leu 22 (an anti-CD43 monoclonal) and examined by FACS. A reduced expression of CD43 was seen in 30/300 clones tested. 15 such clones were subjected to Southern analysis and 2 clones were confirmed to have been successfully targeted. The functional properties of these CD43-heterozygote cells and techniques used to render them CD43-deficient will be presented.

PREVALENCE OF HIV-I AND HIV-2 INFECTIONS IN NIGERIA, AFRICA

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We have evaluated 3854 sera collected from in various geographical locations in Nigeria between 1985 and 1990. All samples were screened for the presence or absence of HIV-1 and HIV-2 antibodies using two different EIA and immunoblot confirmatory tests. Overall, 78 samples (2%) were reactive for the presence of HIV-1 antibodies and 49 samples (1.3%) were positive for HIV-2 antibodies. Among the 6 groups of individuals tested, the prostitutes (n=60) had the highestprevalence of HIV-1 infection, with 10% testing positive. The prevalence of HIV-1 among individuals with sexually transmitted diseases (STD) (n=610) was 4.1%, among tuberculosis (TB) patients (n=140) was 1.6%, among blood donors (n=151) was 1.3% among other patients (n=1253) was 1.6%, and among health care workers was 1.3%. The incidence of HIV-2 in the same population as screened for HIV-1 was 6.7% in the prostitutes compared with 3.4% in the STD patients, 1.4% in the TB patients, 0.6% in other patients and 0.9% in blood donors. No health care worker tested positive for HIV-2 infection and only 5 individuals tested positive for both HIV-1 and HIV-2. Males and females were equally likely to test positive for HIV-1 (2%) or for HIV-2 (1.3%). However, the prevalence for HIV-1 (3.3%), HIV-2 (2.2%) or both infections was greatest among individuals who were 20-29 years old.

HTLV-I TAX, T CELL MITOGENS AND IL-2 INDUCE IL-2 RECEPTOR-BETA (IL-2Rß) GENE EXPRESSION

Leslie J. Parent, Marion R. Fung, Jan A. Hoffman, and Warner C. Greene, HHMI, Department of Medicine, Duke Univ. Med. Center, Durham, NC 27710

The interleukin-2 receptor alpha (IL-2Rα, Tac, p55) and IL-2Rß (p70/75) subunits combine to form the functional high affinity IL-2 receptor complex present on activated human T cells. While IL-2Rα gene expression is known to be inducibly regulated at the level of transcription, the constitutive versus inducible nature of IL-2Rß gene expression and its level of regulation has remained controversial. Using sensitive S1 nuclease protection analyses of RNA isolated from highly purified populations of primary T cells, we now demonstrate that the IL-2Rß gene is constitutively expressed at low levels in both CD4+ and CD8+ resting T cells and at higher levels in unstimulated natural killer cells. We further show that activation of these cells with various mitogens including OKT3, PHA, Con A and IL-2 leads not only to *de novo* IL-2Rα gene expression but also heightened IL-2Rß mRNA expression. Nuclear run-on assays indicate that these stimulatory effects reflect transcriptional activation of the IL-2Rα gene but post-transcriptional changes in IL-2Rß mRNA expression. In contrast, the HTLV-I *tax* gene does stimulate IL-2Rß gene transcription as evidenced by its activation of IL-2Rß promoter-CAT reporter plasmids in Jurkat T cells. Tax activation of IL-2Rß appears to involve both the NF-κB and CREB families of enhancer binding proteins based on the partial phenotypes of Tax mutants that activate one but not both pathways. Together, these studies emphasize the complex nature of IL-2Rß gene expression involving both constitutive and inducible components regulated at transcriptional and post-transcriptional levels.

NUCLEOTIDE SEQUENCE VARIATION IN THE V3 REGION OF UGANDAN HIV-1 PROVIRUSES, Gary Pestano and William M.O. Boto, Department of Biology, The City College, The City University of New York, NY 10031

The third hypervariable (V3) region and several other determinants in the envelope glycoprotein gp120 of HIV-1 are prime candidates for the modeling of a virus neutralizing subunit vaccine(s). Nevertheless, there is wide-spread concern that the variability in the cDNA sequence encoding these immunodominant epitopes will compromise the efficacy of the candidate vaccines currently under development. Such reservation is particularly strong in the case of the principal virus neutralizing V3 epitope. A comprehensive analysis of the genetic and immunogenic composition of the indigenous HIV-1 isolates that cause infection in distinct communities is thus central to the design of a broadly-acting immunoprophylactic therapy. We have sequenced nucleotide residues 6871-7177 in the ENV gene which encode the V3 loop in HIV-1 isolates amplified from blood specimens of symptomatic and asymptomatic cohorts in Uganda. The proviral DNA present in 20 µl of proteinase K-digested lysate of whole blood or ficoll hypaque-purified leukocytes, was subjected to PCR amplification using nested primers. The 306 bp PCR products were either directly cycle-sequenced or cloned into Kan^R pCR-1000 vector. Recombinant plasmid purified from bacterial transformants was sequenced by the dideoxinucleotide chain termination method using (α-^{35}S)thio-dATP and Sequenase. In this report, we show that the Ugandan proviral DNA clones UP-2a, UP-2b, UP-2c and UP-7a are about 85% homologous to the predominant North American isolate MN, in the entire region sequenced. These preliminary findings clearly bear significant implications for the design of an effective subunit vaccine(s) for protection against those indigenous HIV-1 isolates that afflict the population in Uganda and possibly other endemic regions of Africa.

MUTATION ANALYSIS OF THE CD4 BINDING REGION OF SIV ENVELOPE, V. Planelles*, †N, Leung, †R. Unger, §N. Haigwood, §C. Scandella, §L. Misher, †M. Gardner, †P. Luciw, *Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles CA; †Department of Medical Pathology, University of California, Davis; §Chiron Corporation, Emeryville CA

The simian immunodeficiency virus (SIV) envelope glycoprotein consists of two major domains, the extracellular domain (gp130) and the transmembrane domain (gp30). gp130 is the major viral element responsible for attachment to the cellular receptor, CD4.

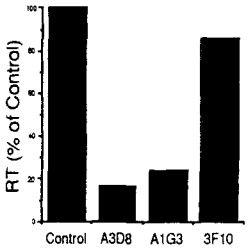
SIVmac1A11 is an infectious molecular clone from a rhesus macaque. A transient expression system for SIVmac1A11 gp130 in COS cells was developed, which yields glycosylated recombinant gp130 that is conformationally native, as determined by binding to soluble CD4. A panel of SIV envelope mutants was created by site-directed mutagenesis, and the corresponding constructs were transfected into COS cells. Envelope glycoproteins from these transfections were characterized by envelope-capture ELISA, RIPA and CD4 binding. Two of three mutations near the carboxy terminus of gp130 reduced or abolished binding.

Restriction fragments containing wild-type and mutant envelope sequences were used to construct full-length proviruses that were transfected into mammalian T-cell lines to generate virus stocks. Infectivity of these virus stocks was assessed by infection of CEMX174, reverse transcriptase assay and assessment of syncytium-inducing ability. One biologically active mutant virus was identified, 1A11-70, which was reconstructed with an envelope gene that did not detectably bind CD4. The resulting virus was still able to infect CEMX174 cells and induce syncytia in a fashion that was indistinguishable from wild-type virus.

Additional experiments will be designed to quantitatively compare the syncytium-inducing ability of 1A11-70 and wild-type 1A11, and to determine whether there is a correlation between CD4-binding affinity and fusogenic potential.

HIV-1 INFECTION OF HUMAN MONOCYTES: THE ROLE OF THE HYALURONATE RECEPTOR (CD44). E.D. Rivadeneira, T. J. Matthews, B. F. Haynes, J. B. Weinberg. VA and Duke University Medical Centers, Durham, NC 27705

The interaction of HIV-1 gp120 and the cellular CD4 is critical for viral entry into susceptible cells. Other cell surface molecules may be important during HIV-1 infection of human mononuclear phagocytes. To study this, we examined the ability of various murine monoclonal antibodies, directed toward different membrane antigens, to influence HIV-1 infection of human blood monocytes and peritoneal macrophages *in vitro*. Cells from normal donors were isolated by density gradients, adherence, and washing, and then inoculated with HIV-1$_{BaL}$ after treatment with antibodies. Cultures were examined for cytopathology, and supernatants were collected weekly for reverse transcriptase (RT) assay. The anti-CD44 antibodies A1G3 and A3D8 decreased HIV-1-induced cytopathology and supernatant RT levels in both monocytes and macrophages, whereas an anti-Class I MHC antibody (3F10) had no effect when compared to untreated controls (see graph). With A3D8, this protective effect was dose-related and disappeared with increasing dilutions of antibody (1:100 to 1:400). The inhibitory effects were seen with ascites and purified immunoglobulin. Monocytes treated with the CD44 ligand, hyaluronic acid (1-125 ug/ml), had diminished morphological changes and RT expression (ID50 ≈ 5 ug/ml). Chondroitin sulfate, a polyanion which does not bind to CD44, had no effect. These results suggest that the monocyte hyaluronate receptor (CD44), in addition to CD4, may serve as a membrane molecule important in HIV-1 infection.



RAPID INDUCTION OF HIV-SPECIFIC CYTOTOXIC T LYMPHOCYTES BY AN UNMODIFIED FREE PEPTIDE FROM THE V3 LOOP OF gp120 J. Sastry, P. Nehete, V. Sharma, J. Morkowski, C. Platsoucas[1] and R. Arlinghaus, Departments of Molecular Pathology and Immunology[1], The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

Efforts to generate a vaccine to prevent infection by human immunodeficiency virus (HIV) have focused on inducing neutralizing antibodies. However, cytotoxic T-lymphocyte (CTL) responses are a major immune defense mechanism required for recovery from many different virus infections. Since CTL epitopes can be defined by short synthetic peptides, we searched for HIV peptides that elicit a viral specific CTL response in mice. We have developed a new method for screening CTL-inducing peptides involving a single injection into the foot-pad of mice to prime CTLs in the draining popliteal lymph node of mice within 10 days. Our results demonstrate that a 15 amino acid peptide (aa 315-329) derived from the V3 loop of HIV gp120 caused a rapid induction of peptide-specific and gp160-specific CD8-positive CTLs. Lysis of targets is specific since cells preincubated with unrelated peptides are resistant to lysis as are cells of a different MHC haplotype pretreated with the cognate peptide. Pretreatment of restimulated node cells with complement plus anti-CD8 but not anti-CD4 removed the lytic activity. We also successfully induced *in vivo* CTL activity with an unmodified synthetic peptide from the influenza nucleoprotein indicating general applicability of our method for rapid screening of CTL epitopes. Because HIV replication has been reported by several labs to occur mainly in lymph nodes of infected patients, the rapid induction of HIV-specific CTLs in proximal lymph nodes by unmodified peptides emphasizes the physiological significance of our findings. Based on these results, we propose that a cocktail of CTL-inducing peptides from the V-3 loop and other genes of HIV will form the basis of a prototype vaccine for HIV.

CLINICAL APPLICATION OF EX-VIVO CELLULAR THERAPY OF HIV-1 INFECTION, Mark W. Sebastian, Paul A. Ahearne, John A. Bartlett, Paul Simon, Cynthia Place, Dani P. Bolognesi, Kent J. Weinhold, Duke University Medical Center, Durham, NC 27710.

The eradication of cellular resevoirs of infectious HIV-1 remains a formidable obstacle in the successful treatment of HIV-1 infection. Strategies aimed at utilizing lymphokine activated killer cell (LAK) activation of HIV-1 seropositive patient peripheral blood mononuclear cells (PBMC) were explored in large scale (leukapheresis, n=2) and small scale (100ml blood donation, n=17). These studies involoving ex-vivo Interleukin-2 (IL-2) activation of HIV-1 seropositive patients' PBMC in the presence of Zidovudine (AZT) has culminated in the implementation of a Phase I clinical trial of intravenous IL-2 and LAK cell infusion. Findings in the pre-clinical studies that led to this clinical trial include: 1) Levels of LAK activation in infected patient PBMC cultures comparable to those seen in normal donor PBMC, 2) Significant reduction of viral burden in cells cultured for HIV-1 after LAK activation, including HIV-1 cultures devoid of detectable virus, 3) Significant levels of LAK activity, augmented NK activity, and increased HIV-1 specific cytotoxicity as documented by standard ^{51}Cr release assays, and 4) Augmented major histocompatibility complex (MHC) restricted anti-HIV-1 activity against envelope and core determinants. The patients who have undergone leukapheresis with reinfusion of LAK cells have manifested minimal systemic toxicity, high levels of activation, and augmented HIV-1 specific anti-gp120 cellular cytotoxic reactivities that show persistence in vivo. Ongoing monitoring of anti-HIV-1 cellular cytotoxicity, HIV-1 culture, and Fluorescence Activated Cell Sorting analysis in these patients will demonstrate whether attractive theoretical and promising in-vitro data will translate into therapeutic efficacy in the treatment of HIV-1 infection.

IN VITRO ENHANCEMENT OF HIV-1-SPECIFIC CYTOTOXIC T LYMPHOCYTES (CTL), Premlata Shankar, Jessica Fabry, Laurel Smith, Paul R. Skolnik and Judy Lieberman, Divisions of Hematology-Oncology and Geographic Medicine and Infectious Diseases, New England Medical Center, Boston, MA 02111 and Harvard School of Public Health, Boston, MA 02115

The CTL response to HIV-1 may be a significant factor in blocking disease progression. HIV-specific cytotoxicity of T cell lines generated from seropositive individuals by PHA and rIL-2 stimulation is dominated by the recognition of a small number of peptide epitopes encoded by HIV-1_{IIIB} envelope and reverse transcriptase. T cell lines from asymptomatic individuals grow exponentially without further stimulation for approximately 3 weeks, multiplying approximately 10^5-fold. We have examined 2 possible approaches to select in vitro for HIV-specific CTL. HIV-specific cytotoxicity is greatly enhanced by selection with immunodominant HIV-1 peptides. Limiting dilution analysis reveals a high frequency (8%) of gp160-specific T cells in an unselected T cell line from an HIV-1 seropositive individual. After exposure to peptide-APC, approximately one third to one half of the T cells are peptide-specific; after 2 cycles of peptide-stimulation, virtually every cell in the culture recognizes the stimulating peptide. Gp160-specific cytotoxicity of peptide-selected T cells is comparable to that of gp160-specific clones. To bypass the need to determine immunodominant epitopes, we have developed a method to select HIV-specific CTL by incubation with autologous B cells, infected with HIV-vaccinia recombinants and γ- and uv-irradiated to inactivate virus. In 3 lines from HIV-seropositive subjects, 3/3 are enhanced for gp160-specific cytotoxicity, 2/2 for gag-specific CTL and 1/2 for RT specificity. The degree of enhancement is comparable to that obtained with a single exposure to peptide-APC. Because T cell lines enhanced for HIV-1 specificity are highly cytotoxic, are free of detectable virus and can be grown to virtually unlimited numbers, they may be useful for immunotherapy to treat HIV-related disease.

QUANTITATION OF HIV-1 IN GENITAL TRACT SECRETIONS, Opendra K. Sharma, Gregory Milman, *David D. Ho and **Deborah J. Anderson. National Institute of Allergy and Infectious Diseases, Bethesda, MD; *Aaron Diamond Res. Ctr., New York, NY; and **Harvard Medical School, Boston, MA.

Although heterosexual transmission is a major mode of HIV infection, little is known about the quantity, infectivity, and tropism of HIV or infected cells in genital tract secretions. Currently, assays of HIV from genital tract secretions are problematic because of the limited volume of specimens, presence of toxic and inhibitory factors, variable stability of HIV, and frequent contamination of HIV cultures by bacteria and yeast. The prevalence of HIV in genital secretions may also be affected by clinical and biological variables such as the frequency of sample collection and processing of specimens, disease stage, inflammation, presence of HIV antibodies, and therapeutic intervention. In recently published reports, detection of infectious HIV in semen by co-culture varied from 10% to 57%, and by PCR from 4% to 74% for HIV DNA, and 37% to 65% for HIV RNA. Due to limited sample volumes and the instability of HIV on storage it is not possible to determine whether the variability in detection is inherent in genital secretions or is due to the differences in experimental protocols.

A workshop was convened by the National Institute of Allergy and Infectious Diseases to establish a collaborative study for evaluating standard panels of HIV in semen, cervical/vaginal secretions and breast milk. Standardized cell-free and cell associated HIV stocks, seminal plasma from seronegative semen donors, and HIV-spiked specimens will be provided to laboratories with established interest and expertise in this area. These materials will be used to optimize methodology, and to compare sensitivity and specificity of methods used by different laboratories.

SEQUENTIAL ANALYSES OF BLOOD FROM AN INDIVIDUAL ACCIDENTALLY EXPOSED TO HIV

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Five sequential blood samples from an individual who was pierced by an HIV-contaminated needle, were analyzed at 1 (?) 2,5,8 and 10 months post-exposure. Although the exact date of exposure is unclear, our data indicate that the first and second serum samples showed antibodies reactive with HIV p24, p18 and p55. It was indicated that this individual had started post-exposure prophylactic treatment with AZT a few days after the needle-stick. However, the dose, regimen or the date of starting AZT was not revealed. At 5 and 8 months, the serum samples did not show any reactive band in the Western blot or HIV-genes by PCR. The blood sample at 10 month however, was repeatedly reactive by PCR although remained distinctly negative for HIV antibodies. The presence of HIV proviral DNA was confirmed by using radiolabeled and nonisotopic (Gen-Probe) detection systems. All samples were also cocultivated with pHA-stimulated peripheral blood mononuclear cells, but none yielded HIV in vitro. We conclude that this individual started to produce antibodies upon initial exposure to HIV. However, because the replication of HIV was suppressed for several months by AZT or other factors in the body, additional antibodies were not made and the latent infection was detected by PCR after several months' incubation period.

STUDIES OF MATERNAL CLINICAL AND IMMUNOLOGICAL FACTORS WHICH RELATE TO PERINATAL HIV-1 TRANSMISSION. Sison, A.V.*, Sever, J.L.**+, Brandt, C.D.**+, Rakusan, T.A.**+, Chan, M.M.**+, Campos, J.M.**+, Fuccillo, D.A.++, Dhanireddy, R.+++, *Dept Ob/Gyn, Georgetown Univ Med Center, Wash D.C., **Dept Peds, George Wash Univ Sch Med, Wash D.C., +Children's National Med Center, Wash D.C., ++SRA Technologies, Rockville, MD, +++Dept Peds, Georgetown Univ Med Center, Wash D.C., 20007.

We studied several maternal clinical and immunological factors which may influence mother-to-child transmission of HIV-1 in 25 HIV-infected pregnant women up to delivery and their 25 corresponding newborns. **METHODS:** Mothers were tested throughout pregnancy and at delivery for HIV AB by ELISA/WB, titer of HIV-specific IgG AB, HIV p24, HIV DNA by the polymerase chain reaction (PCR), and virus by culture (VC). End-dilution titers were then performed on PCR+ maternal specimens and data on days to positivity on VC+ samples were collected. Cord blood at delivery and newborn blood were also tested for the above. **RESULTS:** Mean maternal age = 28 yrs. Maternal risk factors: 44% IV drug abuser, 52% heterosexual, 4% bld transfusion. All but one mother were asymptomatic (one mother had AIDS while pregnant). All newborns appeared healthy at birth; five were premature (< 37 wks). Mean maternal CD4 at delivery = 645. Maternal viral/immunological results:

	PCR+	VC+	p24+	IgG titer(mean)	Days to VC+ (mean)
MOTHERS OF INFECTED INFANTS (n=5/0 AIDS)	5	4	4	1:3,000	14 days
MOTHERS OF INFANTS, INFECTION STATUS STILL INDETERMINATE (n=20/1 AIDS)	17	8	10	1:4,070	11.4
INFANTS(n=25)	5	3	7	1:3,043	14

Mothers of infected infants had PCR end-dilution titers ranging from 1:25 to 1:1,000, which were not significantly different from titers of mothers whose infants remain HIV PCR and VC negative. **CONCLUSIONS:** 1] We found no correlation between infection status in the infant and maternal titer of HIV IgG, titer of HIV DNA by PCR, days to positivity of VC, or gestational age at delivery, 2] Very high maternal HIV IgG is common, probably explains the long persistence of this antibody in infants of seropositive mothers, but does not seem protective in infants from infection.

Preferential depletion of CD4+ T cells in transgenic mice that express the HIV-1 nef gene. Jacek Skowronski, Roberto Mariani and Larry Usher. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Development of AIDS is likely to be a result of HIV gene expression in target cells, however, it is not well understood which viral genes can perturb the normal function of infected T cells *in vivo*. We demonstrate here that expression of the HIV-1 *nef* gene *per se* in transgenic mice results in significant and preferential depletion of the CD4+ T cells. The surface CD4 antigen expression on transgenic thymocytes is low and thymic development of CD4+ T is perturbed. *Nef* gene expression is associated with augmented mitogenic response of thymic T cells. This suggests that *nef* may promote viral replication in infected T cells. It appears that *nef* targets an evolutionary conserved cellular pathway(s) and transgenic mice are likely to provide valid small animal models to study the HIV-1 *nef* gene.

REGULATION OF GENE EXPRESSION DIRECTED BY THE LONG TERMINAL REPEAT OF THE FELINE IMMUNODEFICIENCY VIRUS, Ellen E. Sparger[1], Barbara L. Shacklett[1], Lisa Renshaw-Gegg[1], Neils C. Pedersen,[1] John H. Elder[2], and Paul A. Luciw[1]; [1]University of California, Davis, CA 95616 and [2]Research Foundation of Scripps Clinic, La Jolla, CA 92037

The long terminal repeat (LTR) of a retrovirus contains sequence elements which controll viral gene expression in infected cells. We have examined regulation of LTR-directed gene expression in feline immunodeficiency virus (FIV), a T-lymphocytopathic lentivirus associated with a fatal AIDS-like disease in domestic cats. Two independent virus isolates, designated FIV-Petaluma and FIV-PPR, have been molecularly cloned. Both clones (termed pF34 and pPPR) produce infectious virus after transfection of permissive feline cells. Basal promoter activity of the LTRs was measured in various cell lines in transient expression assays using plasmids containing the viral LTR linked to the bacterial chloramphenicol acetyltransferase (CAT) gene. Both LTRs were strong promoters in several cell lines. Mutational analysis of the FIV LTR revealed *cis*-acting elements which are putative binding sites for cellular transcription factors and a negative regulatory element (NRE). Cotransfection of FIV LTRs with either pF34 or pPPR proviral DNA or with FIV subgenomic clones containing various viral open reading frames resulted in low-level or no transactivation. Analysis of site-specific mutants showed that a potential AP-1 site in the U3 domain of the LTR was required for enhanced FIV promoter activity in human T lymphoid cells (Jurkat) treated with phytohemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA). Promoter function of both FIV LTRs was also enhanced in cells treated with either forskolin, an inducer of intracellular cyclic-AMP (c-AMP) or dibutyryl c-AMP. A putative ATF site was the target for c-AMP-induced responses mediated by protein kinase A. These studies revealed that cellular transcription factors play a significant role in regulation of FIV gene expression.

STABILIZATION OF CD4 LYMPHOCYTE LEVELS IN HIV-INFECTED INDIVIDUALS RECEIVING MISMATCHED, DOUBLE-STRANDED RNA PLUS AZT. D. R. Strayer, I. Brodsky, E. Pequignot, D. Shapiro, and W. A. Carter. Hahnemann University, Philadelphia, Pennsylvania, 19102; HEM Pharmaceuticals Corporation, Philadelphia, Pennsylvania, 19103.

Mismatched, double-stranded RNA, Ampligen®, is a *multifunctional drug with both immunomodulatory and antiviral* activities. Ampligen® inhibits an early step in HIV replication, unlike nucleoside analogues, which block at later steps. In vitro studies have shown that the combination of Ampligen® plus AZT acts synergistically to inhibit HIV replication. Accordingly, we evaluated 11 HIV-infected individuals (without AIDS) with absolute CD4 lymphocyte levels > 100 cells/mm^3 (*median* CD4 level = 201) and a prior history of Ampligen® administration who received Ampligen® (400 - 3,500 mg/week) combined with AZT (200 - 800 mg/day). The combination was well tolerated with no evidence of toxicities over that expected for AZT alone. Analysis of CD4 lymphocyte levels demonstrated a long-term (> 12 months) increase in CD4 cell levels above a 90 day baseline period *prior to the addition of AZT. The increase* (10 - 20%) in CD4 cell levels was significant ($p<0.05$, one sided) during three 90 day intervals following 12 months of combined therapy. The duration of the increase is longer than expected for AZT alone, but is consistent with known synergy between AZT and Ampligen®. A controlled clinical trial which is currently under way will further evaluate the safety and activity of Ampligen® *plus AZT in HIV-infected individuals.*

ANTIGENIC SITES INVOLVED IN PROTECTION OF VACCINATED MONKEYS AND THE ROLE OF A NEW NEUTRALIZATION EPITOPE IN THE ENVELOPE GLYCOPROTEIN OF SIV. José V. Torres, David E. Anderson, Babak Banapour, Eli Benjamini and Murray Gardner, University of California, School of Medicine, Davis, CA 95616.

Development of safe and effective anti-retroviral vaccines requires the use of an immunogen which does not include the virus genome but that elicits a strong, broad and lasting immunological response. The simian immunodeficiency virus (SIV) *infection of macaques is the best model for the study* of immunodeficiency-causing retroviruses which infect humans. Various groups have been able to protect monkeys against low titer cell-free live virus challenge. However, since the *immunogens consist of inactivated virus which still contains* the genome, direct application of these preparations to human AIDS is confronted with serious questions about safety.

We have worked with sera from monkeys protected against challenge by immunization with inactivated SIV. Using synthetic peptides we have tried to identify the areas in the envelope glycoprotein (gp130) which are the targets for protective antibodies present in vaccinated monkeys. This study includes antibody binding assays using synthetic peptides and recombinant proteins as well as neutralization assays using molecular clones and biological isolates.

We have identified a neutralization epitope in gp130 of SIV_{MAC}. Antibodies specific for this epitope are able to neutralize the *in vitro* infectivity of SIV_{MAC}. Monkeys infected with SIV_{MAC} as well as vaccinated with inactivated cell-free virus develop neutralizing antibodies which are directed to this region of the envelope glycoprotein. Our results indicate which regions of the envelope glycoprotein are necessary for the induction of a protective immune response. This information should be useful in the design and development of successful retroviral vaccines.

COMPARISON OF RT GENE SEQUENCES FROM HIV-1 STRAINS WITH DIFFERENT SENSITIVITY TO TIBO DERIVATIVES. Anne-Mieke Vandamme[1], Zeger Debyser[1], Koen Andries[2], Paul A.J. Janssen[2], Rudi Pauwels[1], Jan Desmyter[1] and Erik De Clercq[1], Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium[1] and Janssen Research Foundation, B-2340 Beerse, Belgium[2]

The inhibitory effects of TIBO derivatives on the HIV-1 reverse transcriptase (RT) varies from one HIV-1 strain to another [Pauwels *et al.*, Nature 343, 470-474 (1990)], probably due to differences in the amino acid composition of the RT. We have now amplified and sequenced the relevant parts of the HIV-1 RT gene. MT-4 cells were infected with different laboratory and clinical HIV-1 strains, cells were cultured until cytopathicity was observed, and from the cell lysates RT sequences were amplified, using the P5 and P7 primer pair [Fitzgibbon *et al.*, AIDS Res. Hum. Retrovir. 7, 265-269 (1991)]. These PCR fragments were analysed and purified on agarose gel. Direct sequencing was performed according to the cycle sequencing protocol (Life Technologies), involving dideoxy sequencing with [^{32}P]-labeled primers, Taq polymerase, and a cycling protocol which allows sequencing from 50 fmol (or less) of amplified template. Mutations found in this way were interpreted as originating from the virus strains since direct sequencing levels out the occasional errors made by Taq polymerase. Correlation between RT gene sequence and sensitivity to TIBO derivatives will be discussed.

ANTI-HIV ACTIVITY OF THE TAT INHIBITOR Ro5-3335 IN DIFFERENT CELL CULTURES. Myriam Witvrouw, Anne-Mieke Vandamme, Dominique Schols, Rudi Pauwels, Jan Desmyter and Erik De Clercq, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

The TAT gene product of HIV is required for viral replication. Originally described as a transcriptional activator protein, TAT is now assumed to control gene expression at both the transcriptional and post-transcriptional level. TAT may be considered as a suitable target for the antiviral therapy of HIV infection, since an inhibitor of TAT may have the potential to keep the virus in its dormant state. We evaluated the *in vitro* anti-HIV-1 and anti-HIV-2 activity and cytotoxicity of the TAT inhibitor Ro5-3335 in MT-4, CEM, MOLT-4 and PBL cells as well as persistently HIV-1-infected HUT-78 (HUT-78/HTLV-III$_B$) cells. Only in PBL cells was Ro5-3335 able to inhibit HIV-1 replication at a concentration that was well below (50-fold lower) than the cytotoxic concentration. In the other cell systems Ro5-3335 inhibited HIV-1 replication at a concentration that was equal to or at most 5-fold lower than the cytotoxic concentration. Possible reasons for the cytotoxicity of Ro5-3335 will be discussed.

COMPARISON OF ELISAs FOR FELINE IMMUNODEFICIENCY VIRUS (FIV) SEROLOGY, BASED ON RECOMBINANT VIRAL PROTEINS AND FIV SPECIFIC MONOCLONAL ANTIBODIES. Kees Weijer, Guus Rimmelzwaan, Kees Siebelink, George Reid, Os Jarrett, Bernard Moss, Albert Osterhaus. The Netherlands Cancer Institute, Amsterdam, The Netherlands; The National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands; MRC Retrovirus Research Laboratory, University of Glasgow, Glasgow, Scotland; Laboratory of viral diseases, National Institute of Health, Bethesda, USA.
FIV seropositive cats may suffer from a variety of non-specific clinical symptoms and therefore specific methods for the diagnosis of FIV infections is desired. In addition, identification of FIV carrier cats is essential in controlling the spread of infection among cat populations. Five different ELISAs are compared in this study, using serum samples of naturally and experimentally infected cats: a p24 complex trapping blocking ELISA; an anti-p24 monoclonal antibody based indirect ELISA; a recombinant viral p17/p24 ELISA; a recombinant viral gp120/41 ELISA and a FIV whole virus ELISA. In general a good correlation was observed in the serological responses of cats experimentally infected with the Petaluma strain of FIV, and of a dutch isolate (Amsterdam 19) of virus derived from an infectious molecular clone of FIV(Siebelink et al,J.Virol.in press), measured in the respective ELISAs. Preliminary results show that following FIV infection gp120/41 specific antibodies first can be detected, followed by anti p17/24 antibody serum response. Serum samples of one cat which was infected with the Petaluma strain of FIV scored negative in the p24 ELISAs but positive in the env specific ELISAs early after infection. Furthermore, some naturally infected cats were shown to be seropositive for gp120/41, but seronegative for p24, which may reflect the phenomenon found in HIV-infected men in which disease is progressing to AIDS. Therefore the mutual screening on antibodies specific for both gag and env encoded proteins is desired.