# HUMAN RETROVIRUSES, CANCER AND AIDS: APPROACHES TO PREVENTION AND THERAPY

Dani Bolognesi, Organizer

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# Human Retroviruses, Cancer and Aids: Approaches to Prevention and Therapy

## *Keynote Address*

**P 001** EVOLUTION OF RETROVIRUSES AND OTHER RETROSEQUENCES, Howard M. Temin, McArdle Laboratory, 450 N. Randall Ave., University of Wisconsin, Madison, Wisconsin 53706.
People are always interested in origins. In particular, many people are asking now what was the origin of HIV and AIDS. Did the virus exist for a long time? Did it recently evolve from a monkey virus? Did it have another origin? Similarly, there has been speculation about the origin of retroviruses in general. Evolutionary theory attempts to discern origins by tracing patterns of common descent. This tracing requires knowledge of relatives and precursors. It also requires distinguishing between homologous and analogous characters. Retroviruses have been known since the early 1900's. Retrovirus replication has certain distinctive features which result in particular patterns of nucleotide sequences. In addition, retrovirus replication involves an unusual amount of genetic variation (mutation). Thus, retroviruses evolve rapidly. Roughly twenty per cent of the vertebrate genome is made up of the products of reverse transcription. The different neucleotide sequences indicate that different mechanisms were involved in their formation. However, there appears to be an evolutionary homology among many of them. Can we conclude anything about retrovirus and HIV origins?

## *New Retroviruses in Man and Sub-human Primates*

**P 002** THE PATHOBIOLOGY OF SIMIAN T-LYMPHOTROPIC RETROVIRUSES AND RELATED HUMAN VIRUSES, Phyllis J. Kanki[1], Souleyman M'boup[2], Francis Barin[3] and Myron Essex[1], [1]Department of Cancer Biology, Harvard University School of Public Health, Boston, MA; [2]Laboratory of Bacteriology, University of Dakar, Dakar, SENEGAL; [3]Virology Laboratory, CHRU Bretonneau and UER Pharmaceutical Sciences, Tours, FRANCE.
It has been recently recognized that the T-lymphotropic virus family also includes closely related agents that infect certain primate species. Simian T-Lymphotropic Viruses Type III (STLV-III) have been described in healthy African Green monkeys (Cercopithecus aethiops), healthy mangabeys (Cercocebus sp.) and captive immunodeficient rhesus macaques (Macaca mulatta). STLV-III demonstrates T4 tropism, in vitro growth characteristics and ultrastructural morphology similar to HTLV-III/LAV, the etiologic agent of human AIDS. Most importantly, the STLV-III viral antigens have been determined as gp120/160, gp32, p64, p55, p53, p31, p24 and p15 with only minor variation from the analogous env, gag, pol and 3'orf products of HTLV-III/LAV. $STLV\text{-}III_{AGM}$ has recently been cloned and genetic analysis again demonstrates the close relationship of this simian retrovirus to HTLV-III/LAV.

STLV-III is known to infect from 30-70% of African Green monkeys, all seropositive animals have been healthy. In contrast STLV-III has been closely linked with an immunodeficiency syndrome in Asian macaques. STLV-III induced disease in the rhesus macaque demonstrates many clinical and pathologic similarities with that of human AIDS. Thus, representing an important animal model for the testing of vaccine approaches that could be useful in HTLV-III/LAV prevention. Further understanding of the biology of STLV-III in African primates such as the African green monkey may help us better understand the specific viral alterations or viral-host interactions that are involved in the pathogenicity of this broad class of viruses.

We have recently described a new human T-lymphotropic virus closely related to $STLV\text{-}III_{AGM}$ in healthy people of West Africa, termed HTLV-4. Both HTLV-IV and STLV-III have similar viral protein profiles and demonstrate bidirectional cross-reactivity with the major antigens of HTLV-III/LAV. HTLV-4 does not appear to be cytolytic to T4 lymphocytes although it does induce multi-nucleated synctial cells. Extensive seroepidemiologic studies have shown a 1-20% seroprevalence rate of HTLV-4 in West Africa, in the absence of any association with AIDS. The further characterization of conserved cross-reactive epitopes between these viruses may represent a unique opportunity to identify relevant candidates for an effective subunit AIDS vaccine. Further study of viruses in this broad family with differing pathogencity may additionally provide new clues as to how the unique pathogenicity of the AIDS virus can be prevented.

**P 003** BIOLOGICAL AND IMMUNOLOGIC FEATURES OF INFECTION WITH THE HUMAN IMMUNODEFICIENCY VIRUS (HIV). Jay A. Levy, Cancer Research Institute, Department of Medicine, University of California, School of Medicine, San Francisco, CA, 94143.

Our laboratory has isolated over 400 different HIV from peripheral mononuclear cells, serum, plasma, urine, tears, saliva, semen and vaginal fluids. We have demonstrated a biologic heterogeneity of HIV as reflected by their ability to infect a variety of different human cells, including T cells, B cells, macrophages, cultured brain cells and other established human cell lines. Differences in their capacity to replicate in normal human peripheral mononuclear cells from ten different individuals also have been denibstrated. Variations can be observed as well in the induction of cytopathology in infected cells, extent of antigen expression, and kinetics of virus replication. The viruses can also be distinguished by their sensitivity to neutralization. Some HIV appear to have similar type-specific envelopes since they are neutralized by the same sera, whereas others (e.g., Haitian and African isolates) have different envelope epitopes; they can only be neutralized by selective sera.

Cellular immunologic responses against the virus indicate that the T suppressor cell is an important regulator of virus replication. Most likely a lymphokine from this cell inhibits virus replication in some individuals. This activity appears to be HLA restricted and may determine the progression of disease. These biologic and immunologic features of HIV infection can affect development of AIDS.

## *The HTLV Genomes and Their Products*

**P 004** THE SOR, TAT AND 3'ORF GENES OF HTLV-III/LAV(AIDS VIRUS). Suresh K. Arya Laboratory of Tumor Cell Biology, National Cancer Institute, National Institute of Health, Bethesda, MD 20892

HTLV-III/LAV genome contains four novel genes termed here sor, tat, 3-orf and art/trs. The putative sor and 3-orf genes were predicted from the nucleotide sequence analysis and the tat and art/trs genes were discovered by functional analyses. We cloned and characterized the functional analogs of these novel genes by way of cDNA cloning. The analyses of the clones showed that the tat and 3-orf clones and presumably also the sor clone consist of three exons, bringing together sequences from the 5'end (R and leader), middle and 3'-LTR (U3 and R). Contrary to previous predictions, the tat coding sequence is located in the second exon between the pol and env genes and in part overlapping the 3'-portion of the env gene but using reading frame distinct from the pol and env genes. The 3'orf coding sequence is located in the third exon extending into 3'-LTR and reading frame in common with the gag and sor genes. The sor coding sequence is located between the pol and tat genes with 5'portion of the sor gene sharing sequences with 3'-portion of the pol gene but using different reading reading frame.

By employing an in vitro transcription-translation procedure, we have shown that the sor, tat, and 3-orf open reading frames are in fact genes that function in vivo. The products of these genes are immunogenic in the natural host thus identifying three novel antigens. We are now mapping the immune-reactive domains of these genes by analysis of the deletion mutated genes. While the gene products of all the deletion mutants of the 3'orf and sor genes tested thus far immune react with serum of rabbit immunized with bacterially produced antigens, only the products of the genes with deletion towards the 5'end immune react with human AIDS sera. This shows that (i) not all of the potential immunogenic epitopes are exposed in vivo, (ii) The dominant inmunogenic epitopes of the 3-orf and sor gene products are located towards the carboxy end, and (iii) the immune-reactivity and immunogenicity of these gene products is structurally and/or conformationally dependent. We are now exploring the in vitro procedure further to see if it can be used to determine the numbers and sizes of the open reading frames in any given cloned DNA.

**P 005** THE TRANSACTIVATOR GENE OF HTLV-I ACTIVATES EXPRESSION OF THE CELLULAR PROMOTERS REGULATING INTERLEUKIN-2 AND INTERLEUKIN-2 RECEPTOR GENE EXPRESSION

Warner C. Greene, Mark Feinberg, Niki Holbrook, Flossie Wong-Staal, Robert C. Gallo, and Miriam Siekevitz, NIH, Bethesda, MD 20892.

HTLV-I infection of human $T4^+$ T lymphocytes may lead to the development of the Adult T Cell Leukemia (ATL). HTLV-I infected leukemic T cell lines uniformly display abnormally large numbers of high and low affinity membrane receptors for interleukin-2(IL-2) and some of these cells also produce IL-2. HTLV-I is a type C retrovirus which, in addition to containing tandem long terminal repeats (LTRs), gag, pol, and env genes, also contains a transactivator (tat-I) gene whose 42 kd protein product activates in trans the transcription of viral genes controlled by the LTR. These findings raised the possibility that the tat-I gene product might also alter the expression of certain cellular genes including IL-2 and the IL-2 receptor. To test this possibility, expression plasmids capable of producing high levels of functional tat-I protein were prepared and used for cotransfection studies with plasmids containing the the 5' regulatory regions of the IL-2 and IL-2 receptor genes linked to chloramphenicol acetyl transferase (CAT) gene. Jurkat T cells were used for transfection as these require only one stimulus (PHA or PMA) to express IL-2 receptors while two stimuli (PHA + PMA) are required to produce IL-2. Transfection of the functional tat-I plasmid, in the absence of other stimuli, produced a 5-10 fold increase in IL-2 receptor promoter activity. In contrast, tat-I alone had only minimal effects on IL-2 promoter activity but synergized (15-20 fold) with PMA which alone was ineffective. These findings suggest that the tat-I gene product is capable of partially activating the IL-2 promoter. Cyclosporin A completely blocked PHA and PMA induced activation of the IL-2 promoter. In contrast, the tat-I gene product removed cyclosporin A sensitivity of IL-2 promoter activation. These findings suggest that the tat-I gene product alters IL-2 expression at a point distal to site of cyclosprin A inhibition. A variety of of other cellular and viral promoters were unaffected by the tat-I gene product. The specific effects of the tat-I gene on IL-2 receptor and IL-2 gene expression suggest that an autocrine or paracrine mechanism of growth may occur at an early stage of HTLV-I induced T cell transformation.

**P 006** HUMORAL IMMUNE RESPONSE TO RETROVIRAL INFECTIONS IN HUMANS, Tun-Hou Lee, Jonathan Allan, Min-ji Chou, Xiaofang Yu, Mary F. McLane, Robert Redfield, Donald Burke, and Max Essex Department of Cancer Biology, Harvard University School of Public Health, Boston, MA 02115 and Deptartment of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC 20307-5100

The family of human T-lymphotropic viruses have four known members: HTLV-1, HTLV-2, HTLV-3, and HTLV-4. HTLV-1 is etiologically associated with adult T-cell leukemia/lymphoma. HTLV-2 was originally isolated from a patient with a variant form of hairy cell leukemia. Isolation of a few more HTLV-2 strains has been reported. However, the disease association between HTLV-2 and any disease, if any, remains undetermined. HTLV-3 (or variously called LAV, ARV, HIV) is the etiologic agent of AIDS. HTLV-4 was originally isolated from Western African prostitutes. By the degree of the serologic crossreactivity, HTLV-4 is the closest relative of HTLV-3.

Sequences coding for structural proteins and non-structural proteins have been identified in the genomes of HTLV-1, HTLV-2 and HTLV-3. Generally, structural proteins are more immunogenic than non-structural proteins. Among the structural proteins it is known that envenlope proteins elicit the strongest humoral response, thus are the best antigens for the screening of prior exposure to this family of retroviruses. Certain humoral responses to HTLV-1 antigens and HTLV-3 antigens are also known to be associated with disease manifestations. Such an association, if proven to be causal, could provide some insights on how the pathogenic processes of HTLV-1 and HTLV-3 infections can be intervened.

**P 007** RECEPTOR-BINDING AND FUSION DOMAINS OF THE HTLV-III ENVELOPE, Joseph Sodroski, Wei Chun Goh, Craig Rosen, Ernest Terwilliger, Andrew Dayton, Joseph Potz, Richard Fisher*, and William Haseltine; Dana Farber Cancer Institute, 44 Binney Street, Boston, MA 02115; Harvard Medical School, 25 Shattuck Street, Boston, MA 02115; and *Biogen, 14 Cambridge Center, Cambridge, MA 02142.
Membrane fusion mediated by the HTLV-III envelope glycoprotein is an important process in both virus entry into and killing of T4+ cells by HTLV-III. The ability of animal antisera raised to synthetic peptides corresponding to the primary amino acid sequence of the HTLV-III envelope to inhibit syncytium formation was examined. Domains potentially important to the function of the HTLV-III envelope were further defined by mutagenesis. The mutant envelope genes were examined for the ability to encode proteins capable of supporting virus replication and promoting host cell membrane fusion.

## *Mechanism of Virus Pathogenesis*

**P 008** MOLECULAR ASPECTS OF REPLICATION AND PATHOGENESIS OF THE HUMAN LEUKEMIA AND AIDS RETROVIRUSES, William A. Haseltine, Joseph Sodroski, Craig Rosen, Wei Chun Goh, Ernest Terwilliger, Andrew Dayton, Roberto Patarca, Laboratory of Biochemical Pharmacology, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115
Retroviruses have been shown to be the etiologic agents of human leukemias and lymphomas and the acquired immune deficiency syndrome and related disorders. These viruses are unusual amongst retroviruses both with respect to virus genomic structure and replication cycle. The human leukemia virus is poorly transmissable and poorly infectious. It has the capacity, upon co-cultivation with infected cells, to transform primary T4+ lymphocytes *in vitro*. The virus contains a 1,500 region located between the envelope gene and the 3' LTR. This region encodes several proteins, one of which is an activator of LTR transcription. This gene product also induces - directly or indirectly, genes associated with T cell proliferation. The role of X-region encoded genes in virus replication and transformation will be discussed. The AIDS virus encodes at least four genes (*sor*, 3' *orf*, *art*, and *tat*) in addition to *gag*, *pol*, and *env* genes common to all retroviruses. Mutation analysis reveals that the 3' *orf* gene is not required for virus growth. The viruses lacking the *sor* gene can replicate slowly. The *tat* and *art* genes are absolutely required for viral growth. Both the *tat* and *art* genes appear to be post transcriptional regulators of expression of virus structural proteins. The mechanism of action of these genes will be discussed. The envelope gene protein of the AIDS virus has been shown to be cytotoxic to T4+ cells. The mechanism of the AIDS virus T4+ cell killing will be discussed.

**P 009** TRANSACTIVATIONS OF GENE EXPRESSION DIRECTED BY THE LONG TERMINAL REPEAT (LTR) OF HUMAN IMMUNODEFICIENCY VIRUS (HIV): SEQUENCE REQUIREMENTS, MECHANISM, AND T-CELL ACTIVATION, Paul A. Luciw[1], Sandra Tong[2], Michael Walker[2], and B. Matija Peterlin[2]. 1 University of California, Davis, Ca. 95616 and 2 University of California, San Francisco, Ca. 94143.

Transactivation of HIV gene expression is mediated through the action of a virally encoded transactivator (TAT) gene on a transacting response element (TAR) in the HIV LTR. We have used site-directed mutagenesis procedures to determine which sequences in the TAT gene are required for transactivation. Also, the precise sequences in the LTR which constitute TAR have been identified by site-directed mutagenesis. Transient expression assay systems have revealed that elevated levels of mRNA can account for TAT-mediated transactivation. Thus, both transcriptional and post-transcriptional events appear to play a role. The regulation of gene expression directed by the HIV LTR has been studied in a human T-cell line (Jurkat). Stimulation of Jurkat cells with phytohemagglutinin and phorbol myristate leads to increases of interleukin-2 (Il-2) and Il-2 receptor gene expression. We noted 5-6X increases of HIV LTR directed gene expression when stimulated Jurkat cells were compared to unstimulatedcells. These results show that signals which activate T-cells also act on the LTR to stimulate HIV gene expression. Thus, the onset of AIDS and efficient replication of HIV may require a concomittant viral infection or stimulus which leads to T-cell activation.

## *Expression of Viral Genes in Bacteria, Yeast and Mammalian Cells*

**P 010** EXPRESSION OF HIV GENES IN YEAST, R.A. Kramer, E. Premkumar Reddy*, K. Fargnoli and M. Schaber, Department of Molecular Genetics, Roche Research Center and *Department of Molecular Oncology, Roche Institute of Molecular Biology, Hoffmann-La Roche, Nutley, NJ 07110. We have engineered various parts of the gag/pol and env coding regions from a cloned HIV provirus ( HXB-3, Shaw et al, Science 226: 1165 [1984]) for expression in Saccharomyces cerevisiae. The gag/pol expression resulted in synthesis of the gag precursor and gag protease with processing similar to that in infected cells occurring in the yeasts. Thus the frameshifting required for protease synthesis takes place in yeast as well. The env gene was engineered for yeast expression with or without its signal peptide and with varying amounts of env coding region. In addition, several construcrts contained part of the gag p24 gene fused downstream of the env region. In all cases, the presence of the signal peptide resulted in large increase in molecular weight that was due to glycosylation. The presence of the gag sequences seemed to facilitate the immunological detection of the glycosylated material suggesting that it may be a poor antigen as a result of the carbohydrate addition.

**P 011** BIOLOGICAL ASPECTS OF THE INTERACTION BETWEEN THE T4 ANTIGEN AND THE GP120 GLYCOPROTEIN OF THE AIDS RETROVIRUS, HIV, Laurence A. Lasky, Department of Molecular Biology, Genentech, Inc., 460 Pt. San Bruno Blvd., So. San Francisco, CA. 94080

The most important initial interaction in the process of infection by the AIDS retrovirus, HIV, is undoubtedly that between the viruses external glycoprotein, gP120 and its receptor, the T4 antigen. We have begun to investigate this interaction using several methods. A soluble form of the envelope glycoprotein has been produced in large quantities and purified to a high degree. This antigen has been iodinated and has been found to interact specifically with a recombinanat T4 antigen expressed on the surface of chinese hamster ovary cells. This interaction can be competed with OKT4 monoclonal antibodies which are know to inhibit virus infection. Competition experiments have demonstrated that the virus envelope protein binds to the T4 antigen with a high binding constant. This binding can be inhibited by human and animal antibodies known to neutralize virus infection. These results suggest that a soluble form of the viral envelope antigen can interact with high specificity with the T4 antigen. In order to investigate potential regions of interaction, various deletion and point mutations have been made in the HIV envelope protein. These mutant proteins have been analyzed for their ability to bind to the T4 receptor. This approach has pinpointed several potential areas of gP120-T4 interaction, and may provide insights into the constant regions of the gP120 viral protein which interact with the T4 surface antigen.

**Abstract Withdrawn**

**P 013** HTLV-III NEUTRALIZING ANTIBODIES AND CELLULAR IMMUNITY ELICITED BY RECOMBINANT ENVELOPE PROTEINS, Scott Putney[1], James Rusche[1], Thomas Matthews[2], Kai Krohn[3], Debra Lynn[1], Jennifer Jackson[1], W. Gerard Robey[3], Annamari Ranki[3], Marjorie Robert-Guroff[4], Dani Bolognesi[2], Flossie Wong-Staal[4], Robert Gallo[4], [1]Repligen Corporation, One Kendall Square, Building 700, Cambridge, MA 02139, [2]Department of Surgery, Duke University Medical School, Durham, NC 27710, [3]National Cancer Research Institute, Frederick Cancer Research Facility, Frederick, MD 21701, [4]Laboratory of Tumor Cell Biology, National Cancer Research Institute, National Institutes of Health, Bethesda, MD 20892, The HTLV-III envelope primary translation product (gp160) was expressed in insect cells using a baculovirus expression vector encoding the $HTLV\text{-}III_B$ envelope. This protein is glycosated and has the same apparent molecular weight as gp160 from virus infected cells. Antibodies from animals immunized with purified protein neutralize the $HTLV\text{-}III_B$ virus and the neutralization titer of this antisera is approximately five-fold higher than that of antisera to native gp120 isolated from the virus. In addition to eliciting a humoral immune response gp160 elicits cellular immunity.

In addition, we have expressed the carboxyl terminal half of gp120 in E. coli and immunization with this protein elicits neutralizing antibody titers equivalent to those elicited by fully glycosated gp120. This means that glycosation is not required for an HTLV-III neutralizing response and localizes neutralizing epitopes to this half of gp120. Fragments of this molecule have been expressed and purified in an attempt to further localize neutralizing epitopes. Several HTLV-III variants have been expressed and the cross-neutralization patterns of antisera to these variants are being studied.

## *Progress in Development of Anti-viral Vaccines*

**P 014** DEFINITION OF HIV DETERMINANTS WITH VACCINE POTENTIAL Gordon R. Dreesman, Patrick Kanda, Tran C. Chanh, Jorg W. Eichberg, and Ronald C. Kennedy, Southwest Foundation for Biomedical Research, San Antonio, TX 78284
The complexity of the genetic potential of HIV strongly suggests that methods used to prepare conventional viral vaccines may not be applicable. Thus we have elected to define individual regions or epitopes associated with the HIV envelope glycoproteins (gp120 and gp41) which have potential to induce neutralizing or blocking activity. Once defined, these regions could then be included in the design of an effective vaccine. Our initial approval has included the characterization of synthetic peptide analogous of gp120 and gp41. Murine monoclonals to defined peptides of gp120 and gp41 respectively, reacted with HIV-infected lymphocyte surfaces. Rabbit antisera to each peptide reacted with native viral antigens as determined by ELISA, radioimmunoprecipitation and western-blot analysis. More importantly these anti-peptide sera neutralized HIV infectivity in vitro. The gp41 peptide has been used to ascertain its potential to induce protection in chimpanzees. An additional region which has potential for consideration in the final design of an HIV vaccine is the structure of the virus which reacts with T-cell surface CD4 determinants. We have generated a monoclonal anti-idiotypic antibody to a CD4 specific monoclonal preparation (Leu3a) which: 1) reacts with HIV antigens by ELISA; 2) reacts with HIV-infected lymphocytes; 3) reacts with a 110-120 KD viral subunit and 4) partially neutralizes HIV infectivity in vitro. This reagent is used to identify the site associated with gp120 which reacts with the CD4 receptor.

## Human Retroviruses, Cancer and Aids: Approaches to Prevention and Therapy

**P 015** PERSPECTIVES IN THE QUEST FOR A VACCINE AGAINST AIDS, Maurice R. Hilleman, Merck Institute for Therapeutic Research, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486. The development of a vaccine against AIDS presents unusual theoretical difficulties because of hypervariability in the antigenic composition of the principal surface glycoprotein and because of transfer of the virus in infected cells to cells of the immune system and to privileged sites beyond the blood-brain barrier. The genome of human immunodeficiency virus (HIV) is complex and encodes a variety of polypeptides that do not appear on the surface of the virus particle, but that may be present on the outer membrane of infected cells rendering them susceptible to immunologic identification and destruction. An analogy is presented in hepatitis B virus infection in which antibody against core and e antigen may provide at least a partial immunoprotective effect. Epitopes that are relevant to immunoprotection against HIV are being sought and these might be presented to the immune system as killed, subunit, synthetic, attenuated live virus, or live vector systems, some of which may present serious problems of safety or capability to immunize. Hepatitis B surface antigen vaccine, produced by recombinant technology in yeast and now licensed for general use, has established precedents for safety and protective efficacy of this form of recombinant product that might find application to a vaccine against AIDS. Technical and practical considerations relevant to the development of a vaccine against AIDS will be presented.

**P 016** EARLY TRANSCRIPTION BY PARTIALLY PURIFIED ENZYMES FROM VACCINIA VIRIONS: ISOLATION OF A TRANS-ACTING TRANSCRIPTION TERMINATION FACTOR. Stewart Shuman, Steven Broyles and Bernard Moss, Laboratory of Viral Disease, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892. An RNA polymerase fraction which transcribes vaccinia virus early genes has been partially purified from virus cores by deoxycholate extraction, removal of endogenous DNA, and chromatography on DEAE-cellulose. Accurately initiated and terminated RNAs are synthesized by this enzyme in the presence of a linear duplex DNA template. A molar excess of RNA product to template DNA is observed. Transcription is abolished in the presence of 2 g/ml heparin. Glycerol gradient sedimentation resolves the transcription system into two components: (I) a rapidly sedimenting DNA-dependent RNA polymerase fraction capable of initiation at the early promoter with elongation of RNA beyond the site of *in vito* termination to yield a runoff transcript, and (II) a more slowly sedimenting fraction, itself devoid of RNA polymerase, which restores efficient termination when added back to fraction (I). Termination by this trans-acting factor does not occur via endonucleolytic processing of RNA. Termination factor is heat-labile and resistant to N-ethyl maleimide. Termination by the factor requires specific sequence information upstream of the site of termination. RNA transcripts synthesized by the reconstituted system do not remain associated with the DNA template.

**P 017** FUNCTIONAL ANALYSES OF MUTANT HIV GENOMES. Ron Willey, Akio Adachi, Howard Gendelman, Daryl Daugherty, Rosamond Rutledge, Klaus Strebel, and Malcolm Martin, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.

The contributions of individual human immunodeficiency virus (HIV) genes during productive cytotoxic infection have been investigated by analyzing the phenotypes of a series of mutant viral genomes. Rapid assays to monitor: 1) the assembly of virus particles or 2) virus infectivity have been used to evaluate mutants involving the pol, env, and A genes of HIV.

Nucleotide substitutions affecting highly conserved potential N-linked glycosylation sites in gp120 coding sequences were inserted into an infectious molecular clone of HIV. One of 4 env mutants with single amino acid substitutions was unable to infect $CD4^+$ lymphocytes although virus production assays indicated the assembly of particles expressing reverse transcriptase and containing gp120. In a separate study, a 625 bp deletion was introduced within the "A" (sor) gene resulting in an HIV mutant which can establish a chronic infection of $CD4^+$ cells. Experiments describing revertants of non-infectious HIV mutants will be described.

**P 018** HIV binding to the CD4 molecule: conformation dependence, antibody inhibition, epitope mapping, and potential for idiotypic mimicry. J.S. McDougal, J.K.A Nicholson, G.D. Cross, S.P. Cort, M.S. Kennedy, and A. Mawle. Immunology Branch, Division of Host Factors, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333.

HIV binds to $CD4^+$ T cells via a complex of the viral envelope glycoprotein gp110 and the CD4 molecule. We treated virus with a variety of physical, chemical, and enzymic agents to determine their effect on the capacity of HIV to bind to $CD4^+$ T cells. Reduction and alkylation (but not alkylation alone) and trypsin digestion (but not glycolytic enzyme digestions) of HIV destroyed its capacity to bind. Taken together, these results indicate that the CD4 binding site of gp110 requires a proper tertiary protein conformation that is dependent on covalent disulfide bonds. The effect is likely due to intra-chain disulfide bonds since there is no evidence that gp110 forms intermolecular disulfide bonds with itself or with other viral proteins. If the tertiary structure conferred by disulfide bonding is not disrupted, the proper tertiary and secondary conformation dependent on noncovalent forces appear to be thermodynamically favored because treatment of virus with denaturants followed by their removal did not effect the capacity to bind.

We considered the possibility that the binding site (idiotope) of certain anti-CD4 monoclonal antibodies might mimic the molecular conformation of the CD4 binding site on the gp110 molecule. To this end, we screened a panel of anti-CD4 monoclonal antibodies for inhibition of HIV binding. Four anti-CD4 monoclonal antibodies were potent inhibitors of virus binding (OKT4A, OKT4D, OKT4F, and Leu3a), and we prepared rabbit anti-idiotypic sera to these. None of the anti-idiotypic reagents reacted with virus or inhibited virus binding. Further search or different approaches may yet yield an idiotope that is an "internal image" of gp110.

## *Progress in Design of Anti-viral Agents*

**P 019** LABORATORY AND CLINICAL EXPERIENCE WITH AZIDOTHYMIDINE,
David W. Barry, Sandra Nusinoff Lehrman, Wellcome Research Laboratories, Research Triangle Park, NC 27709

Azidothymidine (AZT) is a potent inhibitor of the replication of Human Immunodeficiency Virus (HIV) and other mammalian retroviruses in vitro. The 50% inhibitory dose ($ID_{50}$) for HIV measured by determining the decrease in reverse transcriptase (RT) activity in treated cultures of H9 cells is .013 µg/ml. Ninety percent protection from HIV-induced cytopathic effects occurs at AZT concentrations of 0.13 µg/ml. AZT triphosphate is a selective inhibitor of HIV RT. The virus-coded enzyme is about 100-fold more sensitive to inhibition by AZT triphosphate than normal cellular alpha DNA polymerase. Incorporation of AZT into new DNA results in chain termination. AZT is converted to its active triphosphate form by cellular kinases. Complex changes in intracellular nucleotide pools may occur as a result of AZT anabolism in vitro. Depletion of some intracellular nucleotide pools may be associated with the dose limiting toxicity observed in clinical trials.

AZT was administered to 33 patients in a Phase I trial designed to determine the pharmacokinetics, safety and tolerance of the agent in clinical practice. AZT was well tolerated after both intravenous and oral administration. Almost complete absorption was documented following oral dosing. Peak plasma levels achieved with oral drug are approximately 70% those attained with the same dose by the intravenous route. Cerebrospinal fluid levels of AZT averaged 50% of plasma levels. Extensive conversion of AZT to its 5' glucuronide metabolite was documented. Bone marrow suppression, manifested by anemia, neutropenia and leukopenia, was the dose limiting toxicity. Platelets and lymphocytes were relatively spared.

A 282 patient placebo-controlled trial of AZT in certain patients with AIDS and advanced ARC was begun early in 1986. This trial was terminated in September of 1986 when interim analysis of data showed excess mortality and morbidity in those patients who had been randomized to receive placebo. At the time the study was terminated 16 placebo patients and 1 AZT recipient had died ($p<0.001$). When compared to placebo recipients, AZT patients had fewer opportunistic infections and those that did occur appeared less severe. The drug treated patients also had objective evidence of improvement in immune function such as statistically significant increases in $T_4$ lymphocyte counts and total lymphocyte counts from baseline values and development of delayed type hypersensitivity to a battery of skin test antigens. Clinical improvements were noted more frequently in the AZT treated group compared to placebo recipients.

**P 020** INHIBITION OF THE IN VITRO INFECTIVITY AND CYTOPATHIC EFFECT OF HTLV-III/LAV BY PURINES AND PYRIMIDINES WITH THE RIBOSE MOIETY IN A 2',3'-DIDEOXY-CONFIGURATION,
Samuel Broder, M.D., National Cancer Institute, National Institutes of Health, Bldg. 10, Room 12N214, Bethesda, MD 20892.

Although a number of anti-viral agents are now being considered for the experimental therapy of AIDS, to date no therapy has been shown to cure HTLV-III/LAV infection or restore the underlying immunodeficiency. Moreover, the chronicity of infection with HTLV-III/LAV and the propensity of the virus to infect the brain make it necessary to explore new classes of drugs which have the potential for oral administration and penetration across the blood/brain barrier. In the current study, we tested the capacity of purine and pyrimidine nucleoside derivatives to inhibit the infectivity and cytopathic effect of HTLV-III/LAV in vitro. We have focused our efforts on 2',3'-dideoxynucleosides, which as triphosphates are known to be chain terminators of DNA synthesis, and there are data to suggest that the viral DNA polymerase (reverse transcriptase) is much more susceptible to the chain-terminating activity than mammalian DNA polymerase alpha. We have found that with the ribose moiety of the molecule in a 2',3'-dideoxy-configuration, almost every purine or pyrimidine suppresses HTLV-III replication in vitro, however, dideoxythymidine had less activity in our system than the others. Interestingly, the substitution of an azido group at the 3'-carbon in place of a hydrogen (3'-azido-3'-deoxythymidine) significantly restored the anti-retroviral effect of the dideoxythymidine derivative. An analysis of five adenosine congeners, which differed only in the sugar moiety, revealed that reduction (an absence of the hydroxyl groups) at both the 2' and 3' carbons of the ribose was necessary for an antiviral effect, and an additional reduction at the 5'-carbon (the site of phosphorylation after entry into the target cells) nullified the antiviral activity. Recent studies suggest that nucleosides which are in a 2',3'-dideoxy-configuration may have the capacity to inhibit diverse retroviruses (both human and animal) in vitro. The key determinant of anti-retroviral effect seems to be the capacity of the target cell to anabolically phosphorylate the nucleoside analogue; a lack of effective anabolic phosphorylation will make a retrovirus appear to be drug resistant. Clinical trials with 3'-azido-3'-deoxythymidine (AZT) have shown good oral bioavailability and penetration across the blood/brain barrier in patients with AIDS. A multi-center controlled trial has shown that AZT can confer a significant survival advantage to patients with AIDS compared to placebo. This observation serves as a stimulus for further clinical research with new dideoxynucleoside analogues.

**P 021** Virologic Endpoints in Antiretroviral Chemotherapy Trails

WADE P. PARKS*, E.S. PARKS*, M. FISCHL*, R. MAKUCH**, M. LEUTHER**, J.P. ALLAIN***, *University of Miami School of Medicine, Miami, FL, **Yale University School of Medicine, New Haven, CT, ***Abbott Laboratories, North Chicago, IL.

Virologic measures may provide useful adjuncts to clinical or immunologic endpoints to assess the efficacy of chemotherapeutic agents in antiretroviral trials. Surrogate laboratory endpoints may be especially important in asymptomatic or mildly symptomatic patients where clinical endpoints are infrequent or will require prolonged observation. Two independent virologic measures, virus recovery from peripheral blood leukocytes (PBL) and p24 antigen detection in plasma or serum, have now been evaluated in placebo-controlled trials of 3'-Azido-3'-deoxythymidine (AZT) and Ribavirin which involved a total of 72 patients. Virus recovery was positive 92% of the total sample; detection of virus in cultures of PBL's by a supernatant p24 antigen radioimmunoassay (RIA) varied inversely with the absolute T4 lymphocyte count. Higher levels of T4 lymphocytes were associated with longer time required for detection of p24 in supernatant fluids. Direct testing of p24 antigen in serum was positive in 15/34 (44%) LAS patients with lymphadenopathy, 10/26 (69%) ARC patients and 10/12 (63%) AIDS patients suggesting a correlation of p24 antigen positively with both clinical state and T4 counts. In addition p24 antigen was inversely correlated to the level of p24 antibody measured either by a competitive ELISA assay using rDNA antigen or $^{125}$I-virion p24 RIA. Treatment with AZT produced significant diminution in virus recovery within one month of treatment and there was a concomitant and significant decline in p24 antigen levels in patients receiving AZT, but not placebo patients. The correlation of these laboratory endpoints with clinical endpoints suggests that they will be useful laboratory endpoints for efficacy studies in future antiretroviral drug trials.

**P 022** INHIBITION OF HTLV III/LAV VIRUS IN TISSUE CULTURE BY OLIGODEOXYNUCLEOTIDE HYBRIDIZATION ARREST, John Goodchild and Paul Zamecnik, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, Robert Letsinger, Northwestern University, Evanston, ILL. 60201, and Prem Sarin, National Cancer Institute, Bethesda, MD 20892.

Oligodeoxynucleotides (12-26mers) complementary to critical conserved regions of the primary structure of HTLV III/LAV were synthesized and tested for capacity to inhibit replication and expression of this virus in tissue cultures of H9 cells. Inhibition of viral replication, as measured by reverse transcriptase, and of P15 and P24 protein expression was found (1). Degree of inhibition was related to choice of hybridization site, with splice donor, acceptor, and primer binding regions being most effective. Unmodified oligodeoxynucleotides appeared to enter cells, and uptake was linear with time up to ten minutes at 37°, with $10^{-6}$ molar external concentration being taken up to approximately a 5 percent level internally ($\sim 5\times10^{-8}$ molar). Uptake was inhibited by $5\times10^{-4}$M dinitrophenol, indicating an endergonic transport process. Modification of a 12mer oligomer by addition of two internucleotide trichloromethyldimethylethyl phosphotriester groups (2) resulted in enhancement of survival of the oligomer extracellularly and intracellularly in a tissue culture system.

(1) Zamecnik, P. C., Goodchild, J., Taguchi, Y., and Sarin, P. S. Proc. Natl. Acad. Sci. USA 83, 4143-4146 (1986).
(2) Letsinger, R. L., Groody, E. P., and Tanaka, T. J. Am. Chem. Soc. 104, 6805-6806 (1982).

## *Clinical Trials*

**P 023** AIDS AND THE BONE MARROW: BIOLOGY AND CLINICAL TRIALS, Jermone E. Groopman, M.D., Division of Hematology and Oncology, New England Deaconess Hospital, Harvard Medical School, Boston, MA 02215.

Hematologic abnormalities are commonly observed in association with human immunodeficiency virus (HIV) infection in man. We have studied the pathophysiology of such hematologic abnormalities, particulary leukopenia and anemia. Bone marrow progenitors of myeloid and erythroid lineage were grown with recombinant human GM-CSF and recombinant erythropoietin in the presence and absence of purified immunoglobulin from HIV seropositive individuals. There was a dose response in terms of suppression of in vitro hematopoiesis by HIV seropositive immunoglobulin. This effect was reproduced using heteroantisera from animals immunized with recombinant HIV envelope protein gp130. It appears that there may be antibody mediated suppression of hematopoiesis which contributes to leukopenia and anemia following infection with HIV. Futhermore, data indicate that HIV infects important bone marrow accessory cells such as monocyte-macrophages and may directly infect progenitors as well. Despite such in vitro observations, there appears to be a normal dose response of myeloid and erythroid progenitors to recombinant human CSF in the absence of suppressive antibody. Clinical trials of recombinant human GM-CSF in AIDS patients are currently underway.

# Human Retroviruses, Cancer and Aids: Approaches to Prevention and Therapy

## *New Retroviruses; HTLV Genomes*

**P 100** INFECTION BY HTLV-III AND CYTOPATHOLOGY OCCUR AT DIFFERENT LEVELS OF EXPRESSION OF THE T4 GENE, Anna Aldovini, Mark B. Feinberg, Robert C. Gallo and Flossie Wong-Staal, Laboratory of Tumor Cell Biology, NIH, NCI, Bethesda MD 20892.
The depletion of T4 lymphocytes is the central phenomenon of the HTLV-III-induced pathology and how the HTLV-III infection leads to this picture is, at the moment, one of the most interesting and debated topics. Evidences that the interaction of the CD4 molecule with the envelope protein of the virus is one of the crucial events in the cytopathic infection by HTLV-III are now accumulating. Although it is known that the CD4 molecule is also part of the receptor for the virus, the levels of expression required for infection and susceptibility to the cytopathic effect seem not to correlate. A clonal T-cell line 67-I, immortalized by HTLV-I and showing OKT-8 phenotype, is susceptible to infection but not to cell-killing by HTLV-III. We have used this cell-line to demonstrate that, despite the OKT-8 phenotype, the infection can be clocked by treatment with appropriate antibody recognizing the CD4 molecule, indicating that very low level of T4 expression is sufficient to allow HTLV-III infection. The range of cell types expressing equivalently low levels of CD4 in vivo has yet to be rigorously defined. In vivo infection of these cells, which potentially are all target for HTLV-III, may provide a long-lived reservoir for virus production. Furthermore, the T4 expression of the 67-I cell line has been modulated using different retroviral vectors carrying the T4 gene. Results of HTLV-III infection in the modified 67-I cell line with specific regard to the cytopathic effect will be presented.

**P 101** REVERSE TRANSCRIPTASE ACTIVITY (RTA) IN PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC) CULTURES (CXS) FROM PATIENTS (PTS) WITH KAWASAKI DISEASE (KD). Jane C. Burns, Raif S. Geha, Jane W. Newburger, Fred S. Rosen, Mayr M. Walsh, Amy L. Reinhart, Alice S. Huang, Donald Y.M. Leung. Harvard Medical School, Children's Hospital, Dept. of Medicine, Boston, MA 02115.

Polymerase activity has been reported in PBMC cxs of pts with KD (Nature 323:814,1986). To further define its template specificity and persistence after clinical recovery and gammaglobulin (GG) therapy, we studied 89 cxs from 33 KD pts and 33 controls. Of the KD pts 23/33 (70%) showed RTA above the mean + 2 SD for control pts (5.6 picomoles dTMP incorporated). The mean peak RTA for KD PBMC cx supernatants was 8.2 pmoles ± 1.8 vs. 3.7 pmoles ± 0.5 for the controls ($p < .05$). GG therapy did not prevent expression of RTA which was detected 3-8 weeks post-treatment in cxs from 4/6 KD pts. RTA was detected in PBMC from pts as late as 9 years following acute KD. Co-cultivation of PBMC or cell-free supernatants from PBMC cxs with the HUT-78 cell line yielded RTA $>$ control mean +2 SD (3.1 pmoles) in 14/18 (78%) attempts. Analysis of template/ primer specificity showed no increase in dTMP incorporation when assay conditions were altered to favor DNA pol I or terminal transferase. Duplicate assays to compare polyrA:oligodT vs. polyrC:oligodG showed comparable activity for the KD cxs. We conclude that the polymerase activity associated with the particulate fraction of supernatants from KD PBMC is consistent with RTA and can be detected late after recovery which suggests a retrovirus as the causative agent of KD.

**P 102** IDENTIFICATION OF CYTOPATHIC DETERMINANTS OF FELINE AIDS VIRUSES. P.R. Donahue, E.A. Hoover*, J.M. Overbaugh, S.L. Quackenbush*, C.M.C. de Noronha, and J.I. Mullins. Harvard School of Public Health, Boston, MA; *Colorado State University, Fort Collins, CO.

We recently identified a strain of feline leukemia virus (FeLV) which reproducibly induces an immunodeficiency syndrome (feline AIDS) in cats which is very similar to human AIDS. Two forms of feline AIDS virus genomes are distinguishable by restriction site differences: an early form viral genome, evident in bone marrow cells at onset of viremia; and disease-specific variant virus genomes which appear after an age-dependent prodromal period prior to disease onset. Molecular clones of both viral forms were obtained from tissues of cats with AIDS. Disease-specific viral clones were replication defective *in vitro*, but could be rescued from feline fibroblasts by early form virus to generate a mixture which was lymphocytopathic *in vitro* and which induced feline AIDS *in vivo*. In contrast, cloned early form virus was replication competent, noncytopathic *in vitro*, and apathogenic in vivo. The complete nucleotide sequence of the early form as well as the env and LTR regions of 2 disease-specific clones was determined. Blocks of significant sequence divergence were observed only in the gp70 gene.

To identify viral determinants of pathogenicity, recombinant viruses were constructed between disease-specific and early form virus clones and tested for lymphocytopathicity in vitro. One recombinant virus, containing early form gag and pol sequences and disease-specific env-3'LTR sequences, was profoundly lymphocytopathic. Further analyses of this region, via recombinant viruses containing disease-specific viral gp70, p15E, or LTR regions, or containing individual blocks of gp70 sequence divergence, will be reported.

## Human Retroviruses, Cancer and Aids: Approaches to Prevention and Therapy

**P 103** HTLV-I AND HIV COINFECTION IN AIDS ASSOCIATED HIGH GRADE NON-HODGKIN'S LYMPHOMA,
Ellen G. Feigal, Michael S. McGrath, University of California, San Francisco General Hospital, San Francisco, CA, 94110
Seroprevalence of HTLV-I and human immunodeficiency virus (HIV) were examined by ELISA and Western blot in 3 patient groups to determine the relationship of these retroviruses to the development of B cell non-Hodgkin's lymphoma. Twenty patients with AIDS, excluding lymphoma; 19 pateints with high grade non-Hodgkin's lymphoma with AIDS or at risk for AIDS, and 100 healthy asymptomatic HIV seropositive gay men were studied at San Francisco General Hospital from November of 1985 through July of 1986. All of the high grade non-Hodgkin's lymphoma patients were seropositive for HTLV-I and HIV, contrasted to a 5% and 14% HTLV-I seroprevallence rate in patients with AIDS, but without lymphoma and healthy HIV seropositive gay men, respectively (p,<.0001). Cytofluorographic analysis revealed a statisically significant decrease in the number of T4 lymphocytes in the lymphoma group compared to the other 2 groups. Dot blot analyses of lymphoma tissue derived DNA detected HTLV-I envelope gene sequences in 5 out of 6 samples. Only 2 of these 6 lymphomas contained significant amounts of Epstein-Barr virus. Monoclonal antibodies to the envelope glycoprotein of HTLV-I (gp61/68) identified infected cells (0.2-1%) within all lymphoma tissue sections. These data suggest a significant but indirect role for HTLV-I in the genesis of AIDS associated non-Hodgkin's lymphoma. Mechanisms by which HTLV-I may induce antigen specific malignant B cell proliferation will be presented.

**P 104** THE ROLE OF THE SOR GENE IN THE BIOLOGY OF HTLV-III, Amanda Fisher[1] Barbara Ensoli[1], Lucinda Ivanoff[2], Stephen Petteway[2], Mark Chamberlain[2], Lee Ratner[3], Robert Gallo[1] & Flossie Wong-Staal[1]. [1] Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, Bethesda MD 20205, [2] E.I. Dupont, Willminton, Delaware, [3] Division of Hematology and Oncology Washington University, St. Louis, MI 63110.
The sor gene (for short open reading frame), lies between between the pol and tat genes of HTLV-III, overlapping at its 5' end with the former. The product of the sor gene is a protein of apparent molecular size 23,000 daltons (Lee et al. 1986). The role of the sor gene in viral replicaton and pathology is unclear although initial reports suggest that the sor gene is not crucial for either property (Sodroski et al. 1986). To investigate in more detail the possible function of this gene a series of variants of HTLV-III were generated which contained mutations in sor. These mutants were designed to disrupt the sor open reading frame (without affecting tat and pol), by inserting translational stop codons. Three such mutants and a deletion mutant which lacked the entire sor gene were all vastly reduced in their ability to generate infectious HTLV-III virions when transfected into a variety of permissive cells. Analysis of tat function has shown that this deficiency does not result from an impairment of trans-activation potential. These data argue that sor like trs/art and tat has a primary role in controlling virus production by infected cells. The probable mechanism by which sor enhances viral replication will be discussed.

**P 105** CHARACTERIZATION OF THE HUMAN SPUMA (FOAMY) RETROVIRUS. R.M. Flügel, A. Rethwilm, B. Maurer, H. Bannert, and G. Darai, Institute for Virus Research and Medical Virology of the University, German Cancer Center, 69 Heidelberg, FRG.
A retrovirus that was isolated from a nasopharynx carcinoma of a human patient by Achong and Epstein in 1971 was molecularly cloned into lambda and plasmid vectors. Recombinant clones that represent the genome of the human spuma-retrovirus (HSRV) and that were established from both viral DNA and cDNA were characterized by Southern blot hybridizations and nucleotide sequence analysis. The long terminal repeat is 963 bp long. Its 5'-boundary is defined by an 18 bp sequence complementary to the mammalian lysine-specific transfer RNA.
The long terminal repeat of HSRV was analysed and can be subdivided into a U3, R, and U5 region of 616, 193, and 155 bp. It contains one poly(A)-addition signal that perfectly matches the canomical consensus sequence. The start site of viral transcription was determined by S1 nuclease protection analysis and found to be preceded by a perfect TATA-box at -25 bp. The 5'-LTR of HSRV is preceded by a 17 bp polypurine tract.
The genomic organization of HSRV resembles that of the lentiviruses, e.g. the env of HSRV is comparable in size to the env gene of the human AIDS virus. However, the gag gene organization seems to be distinct and different from that of lentiviruses. A novel gene, provisionally termed y, was found to be located between the env gene and the 5'-LTR of HSRV. The deduced amino acid sequence of the corresponding gene product py encodes a protein of 364 amino acid residues and does not show homology to sequences of other retroviruses including human T-cell lymphotropic viruses. The molecular clones of the HSRV genome were used to screen DNA from different groups of human patients. The results will be discussed.

**P 106** COMPARATIVE ANALYSIS OF ANTIBODY RESPONSES OF NONHUMAN PRIMATES TO INFECTION BY HUMAN AND SIMIAN T-LYMPHOTROPIC RETROVIRUSES, Patricia N. Fultz, William Switzer, Neile Greene, Rebecca Mullally and Harold McClure[1], AIDS Program, Centers for Disease Control, Atlanta, Georgia 30333; [1]Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia 30322.

Antibody responses to infection by T-lymphotropic retroviruses were characterized in several animal model systems: chimpanzees infected with HIV strains LAV-1, ARV-2, or LAV-II; mangabeys infected with SIV/SMM; and rhesus macaques infected with SIV/SMM or LAV-II. Antibody reactivity to specific viral proteins was detected by immunoblot and radioimmunoprecipitation. Following acute infection of chimpanzees with LAV-1 or ARV-2 or macaques with SIV/SMM, antibodies to gag and env gene products were detected in serum during the first and second months after infection whereas antibodies to pol gene products were usually detected at later times. In contrast, only antibodies to env gene products gp34 and gp140 were detected in serum from macaques and a chimpanzee infected with LAV-II. Interestingly, one macaque that developed antibodies only to env gene products, and none to gag gene products, following inoculation with SIV/SMM, also developed persistent thrombocytopenia, diarrhea, lymphadenopathy, and greater than 20% weight loss. Serum from animals in all groups also is being tested for neutralizing activity, the first appearance of which was variable in chimpanzees infected with HIV. Little or no neutralizing activity was detected in serum from mangabeys or macaques infected with SIV/SMM.

**P 107** THE 3-DIMENSIONAL TRAJECTORY OF DNA IN ENHANCERS AND ORIGINS CAN BE CONTROLLED BY PROTEIN BINDING, BY SPECIFIC SEQUENCES, AND BY DIVALENT CATIONS. Jack D. Griffith, Caroline Laundon, and Cheng-Hsilin Hsieh Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, N.C. 27514.

Studies on the 3-dimensional trajectory of DNA have demonstrated that unique sequence arrangements can have profound effects on the bending of DNA. Using a highly bent trypanosome DNA we have discovered that sequence-directed bends have the properties of a molecular switch in that they can flip between a straight and bent form in a highly cooperative manner. This transition is stimulated by various metal ions at concentrations which for some are in the physiologic range. Because sequence directed bends are being found as common elements of many enhancers and origins, we propose that they function by pre-determining the folding (looping) of DNA by specific proteins. We will describe the straight to bent transition of these switching elements, the looping of DNA by lambda repressor, and present a high resolution bending map of the SV40 enhancer-origin region. Initial studies with the HTLV-3 LTR will be reviewed.

**P 108** AIDS AND ARC PATIENTS WITH HIV/LAV2 INFECTION : CLINICAL AND VIROLOGICAL STUDIES. Martine Harzic, Marie-Anne Rey, Christine Katlama, Pierre-Marie Girard, Dominique Roulot, Patrick Yeni, François Clavel, Françoise Brun-Vezinet et al. Hopital Claude Bernard, France.

We report here the isolation and characterization of the newly described HIV/LAV2 in 3 patients with AIDS and 1 patient with ARC, hospitalized in France. The clinical picture is similar to that described in patients infected by HIV/LAV1, but a difference in pathogenicity between the two viruses might be a possibility. Three patients originated from West Africa and one from Portugal.
Viral isolates were characterized by hybridization with HIV/LAV1 and LAV2 DNA probes. In all the patients , LAV2 isolation from the cerebrospinal fluid and/or LAV2 IgG antibodies intrathecal synthesis demonstrate the neurotropism of this virus. HIV/LAV1 and LAV2 serological studies were performed by Elisa, Western Blot and radioimmmunoprecipitation assay. The sera from the four patients are negative by HIV/LAV1 Elisa. This report demonstrates that LAV2 is pathogenic, present in Europe and West Africa and shows that LAV2 specific assay is needed to perform LAV2 infection diagnosis.

**P 109** GENOME ORGANIZATION AND SEQUENCE HOMOLOGY BETWEEN HUMAN IMMUNODEFICIENCY VIRUS AND TYPE 3 SIMIAN T-LYMPHOTROPIC VIRUS, Vanessa Hirsch, Norbert Riedel, Hardy Kornfeld and James I. Mullins, Harvard School of Public Health, Boston, MA 02115.

Simian T-lymphotropic virus type-3(STLV-3) shares many biologic characteristics with the human immunodeficiency virus (HIV), utilization of the T4 molecule as a receptor, destruction of T4-lymphocytes similar ultrastructural morphology, Mg +2 dependent reverse transcriptase and major gag-, env-, pol-, and 3′ orf-encoded viral proteins of similar size and immunologically cross-reactive. We recently described the molecular cloning of STLV-3 from African Green monkeys by virtue of weak nucleic acid cross-reactivity with HIV. Now nucleic acid sequence analysis of the 3′ 4Kb of STLV-3 reveals that HIV and STLV-3 share a similar genome structure. Coding regions previously found to be unique to HIV and shown to encode either regulatory or presently unknown functions are preserved in STLV-3. This analysis also reveals a high degree of conservation of predicted protein sequence within a recently recognized short open reading frame of HIV (referred to as R). Predicted amino acid sequences of the envelope gene of STLV-3 and HIV share 50-55% homology in regions identified as constant domains of the HIV env gene and analogous positioning of 19 of 19 cysteine residues. Conserved positioning of the 3′ orf, tat-3, and art open reading frames as well as regions of highly conserved amino acid sequences within open reading frames were also detected. This analysis suggests candidate structural features of the envelope protein important to interaction with the T4 receptor on T-lymphocytes, and suggests that the 3′ orf protein, previously thought to be nonessential, plays an important role in the life cycle of each virus.

**P 110** **PATHOGENESIS OF FELINE RETROVIRUS-INDUCED AIDS,** Edward A. Hoover[1], James I. Mullins[2], Sandra L. Quackenbush[1], Julie M. Overbaugh[2], and Peter R. Donahue[2]. [1]Department of Pathology, Colorado State University, Fort Collins, CO, 80523, and [2]Department of Cancer Biology, Harvard School of Public Health, Boston, MA, 02115.

Feline leukemia virus (FeLV), a naturally occurring, contagiously transmitted retrovirus of cats, provides long standing precedent for the retroviral etiology and pathogenesis of analogous human cytopathic lympho-hemopoietic diseases. It is now clear that specific retrovirus-associated anti-proliferative diseases, most notably feline acquired immunodeficiency syndrome (AIDS) and aplastic anemia (AA), are caused by specific variant feline leukemia virus genomes. We have shown that molecularly cloned FeLV variants possess cell-lineage-specific cytopathicity *in vitro* and rapidly reproduce either AIDS or AA *in vivo* upon inoculation into pathogen-free cats. The feline AIDS FeLV variant exhibits target-tissue-specific replication, is often present as unintegrated viral DNA, and its appearance prefigures the onset of immunodeficiency syndrome in cats. Moreover, nucleotide sequence analysis of the genomes of feline AIDS-inducing variant and its minimally pathogenic parent virus reveals only two regions of substantial alteration concentrated in two hypervariable regions of the gp70 gene. These and other studies identifying target-cell-specific cytopathic effects of cloned FeLV's provide insight into the viral genetic determinants of immunodeficiency disease and other cytopathic retroviral diseases.

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**P 111** MOLECULAR ANALYSIS OF THE GENOMIC ORGANIZATION AND REPLICATION OF MASON-PFIZER MONKEY VIRUS: AN IMMUNOSUPPRESSIVE D-TYPE RETROVIRUS. E. Hunter, S. Rhee, and X. Hui. Dept. Microbiology, University of Alabama at Birmingham, B'ham, AL 35294

Mason-Pfizer monkey virus (M-PMV) is the prototype virus of the D-type retroviruses; a group that has recently been closely linked with a natural acquired immunodeficiency syndrome (SAIDS) in macaque monkeys. Experiments have shown that both newborn and juvenile rhesus monkeys inoculated with M-PMV can suffer a pronounced immunosuppression that may result in extensive lymphoid depletion and death from opportunistic infections.

In order to obtain a better understanding of the molecular organization of M-PMV, the phylogeny of D-type retroviruses and a possible basis for simian AIDS, we have molecularly cloned an infectious M-PMV provirus and have determined the complete nucleotide sequence of the clone. While the gag, pro and pol genes show regions of significant homology with mammalian B-type and intracisternal A-type retroviruses, the envelope glycoprotein gene, env, is highly homologous to the avian C-type virus, reticuloendotheliosis virus. The different phylogenies of the pol and env genes indicate that the D-type family originated from recombination between two different virus families.

Molecular genetics approaches have been used to investigate the process of infectious viral assembly. These studies show that while fatty acid modification of the gag gene precursor protein is not required for intracytoplasmic assembly of capsids, it is absolutely essential for transporting the preformed capsids to the membrane. Thus non-myristylated capsids accumulate in virus 'factories' in the cytoplasm. On the other hand, virus assembly and release does not require biosynthesis of envelope glycoproteins, since a mutation that prevents synthesis of the env gene products does not affect virion release. However, the glycoprotein(-) particles released from such mutant virus-infected cells are completely non-infectious. Studies in this potentially important system will provide valuable information on the relative roles of viral and cellular genes in retrovirus replication.

**P 112** IMMUNOLOGIC AND GENETIC COMPARISON OF LAV-2 AND STLV-III, Myra Jennings[1], Francoise Barre'-Sinoussi[2], J.C. Chermann[2], Francoise Rey[2], Linda Lowenstine[3], Preston Marx[3], Nick Lerche[3], Paul Luciw[1], Murray Gardner[1], University of California Davis, Department of Medical Pathology[1], Pasteur Institute, Paris France[2], California Primate Research Center[3].
We have begun a collaborative study of human and simian T-lymphotropic lentiviruses. LAV-2 isolated from humans with AIDS in West Africa is being compared with STLV-III isolated from healthy sooty mangabeys (SM) in US primate facilities and zoos. Preliminary serologic results suggest that human LAV-2 antisera react more specifically than LAV-1 antisera with the core and envelope antigens of STLV-III/SM and STLV-III/SM antisera react more specifically with the core and envelope antigen of LAV-2 than with LAV-1. Further immunologic studies and genomic comparisons of LAV-2 and STLV-III by restriction mapping and nucleotide sequencing are in progress to determine the extent of relatedness.

**P 113** MOLECULAR CLONING AND BIOLOGICAL ACTIVITY OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE-4. Hardy Kornfeld, Norbert Riedel, Gregory Viglianti, Vanessa Hirsch, and James I. Mullins. Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115
Human T-cell lymphotropic virus type-4 (HTLV-4) was identified in healthy individuals in West Africa by virute of serologic cross-reactrivity with simain T-cell lymphotropic virus type-3 (STLV-3), the closest known relative of the Human Immunodeficiency Virus (HIV). Using molecularly-cloned STLV-3 DNA as probe, we identified sequences nearly identical to STLV-3 in the genomic DNA of 3 HTLV-4-infected Hut-78 cell lines and obtained 4 distinct full-length molecular clones of HTLV-4 proviral DNA from one of these lines (PK289). Thrity of 31 restriction endonuclease sites present in the consensus map of STLV-3 are conserved in the HTLV-4 consensus site map. A single unique PvuII site present in 6 different STLV-3-infected cell lines and 2 HTLV-4-infected cell lines was absent in the PK289 HTLV-4-infected cell line and all 4 HTLV-4 clones. Transfection of Hut-78 cells with 2 of 4 HTLV-4 clones produced transmissable virus which caused formation of giant multinucleated cells but with minimal cytolysis, indicating that HTLV-4 induces some but not all of the cytopathic effects attributed to HIV. Infection of Hut-78 cells with HTLV-4 was inhibited by pre-treating cells with anti-CD4 monoclonal antibodies (OKT4, OKT4A-F) but not with monoclonal anti-HLA-Dr, suggesting that HTLV-4, like HIV, and STLV-3 uses CD4 as a receptor.

**P 114** HIV-1 AND-2 RELATED RETROVIRUSES IN AFRICAN GREEN MONKEYS AND OTHER PRIMATES, Reinhard Kurth[1], Albrecht Werner[1], Peter Centner[1], Ute Mikschy[1], Franz-Josef Ferdinand[1], Michael Baier[1], and Wally Becker[2]. [1]Paul-Ehrlich-Institute, 6000 Frankfurt 70, West Germany; [2]University of Stellenbosch, Tygerberg Hospital, Cape Town, Republic of South Africa. There is as yet no satisfying animal model for AIDS. During the course of our studies of HIV-1 related retroviruses in higher primates we realized that African Green Monkeys and at least one Baboon species harbour retroviruses that are more closely related to HIV-2 than to HIV-1. Serological cross-reactions between the $SIV_{AGM}$ and $SIV_{Pap}$ and HIV-2 were established by Elisa, Western blot, immunofluorescence, peroxidase staining and RIPA. As the SIV replicate poorly, RIPAs are primarily employed to define the size of the virus analysis of restriction enzyme patterns and nucleotide sequencing. Seropositive members of the natural host remain healthy, although virus could repeatedly be isolated from them. Cross-challenge experiments to transmit the viruses to new species have been started to attempt to establish an animal model for AIDS.

# Human Retroviruses, Cancer and Aids: Approaches to Prevention and Therapy

**P 115** ESTABLISHMENT OF A LYMPHOID CELL LINE AND ISOLATION OF HUMAN IMMUNODEFICIENCY VIRUS IN TAIWAN.
Wu-Tse Liu. National Yang-Ming Medical College and Clinical Virology Laboratory, Veterans General Hospital, Taipei, R.O.C.

A lymphoid cell line was established _in vitro_ by long-term cultures of peripheral lymphocytes from a local case of adult T-cell leukemia. The culture cells revealed atypical lymphocytes in stain. The growth cycle of the cultured cells was less than 24 hrs. The cloned cells showed 68-73% of T4 or T8 markers and Tac positive. The chromosome analysis indicated abnormal in number (50-108) and also in constitution.
This cell line was used for virus isolation from the first native case of acquired immune deficiency syndrome (AIDS) in Taiwan by co-cultivation of the lymphocytes. Typical type D particles with a cylindrical core in morphology were observed in thin-sections of the infected lymphocytes. Type C particles were also seen in some cells. Further analysis of the virus particles by kits of enzyme-linked immunosorbent assay (ELISA) revealed that both HTLV I and HTLV III were detected in the supernatant of the co-culture.

**P 116** HIV SEROPOSITIVE SERA CONTAIN ANTIBODIES MIMICKING CD4 MOLECULES.
Lundin K.Nygren A.Flodby P. and Wigzell H.Department of immunology Karolinska institute 10401 Stockholm Sweden.
The tropism of HIV towards CD4+ cells seems predominantly caused by the select binding of Gp120 to the CD4 molecules.We have developed a sensitive radioassay to determine the degree of this binding.We have also designed a radioimmunoassay measuring the binding of iodinated anti-CD4 antibodies(Abs) to CD4 using a battery of monoclonals (mAbs).Of these, T4.2 is the best in blocking binding of Gp120 to CD4, at levels below 1 ug/ml.On screening antisera from HIV-seropositive humans for their ability to block Gp120 to CD4 we have not found any serum failing to block in this assay.We reasoned that some anti-Gp120 Abs may be so sterically similar to CD4 that they may inhibit anti-CD4 binding to the antigen. We have found this to be true since, out of 20 HIV-seropositive sera 1 displayed a high titer in blocking T4.2 binding and 2 had significant but lower inhibitory titers.The specificity of inhibition was confirmed by the failure of the same sera to block other anti-CD4 mAbs as well as anti-T3 Abs from binding.The select inhibition of T4.2 binding was not affected by removal of the Fc region..We conclude that those infected with HIV may produce anti-Gp120 Abs with molecular mimicry to CD4 molecules, i.e. antiidiotypic for anti-CD4 Abs.The capacity of such Abs to interfere with normal immunefunctions involving CD4+ T cells will be discussed.

**P 117** POLYMERASE CHAIN REACTION ON RNA: A RAPID, SENSITIVE METHOD FOR HIV DETECTION,
George J. Murakawa[1], R. Bruce Wallace[2], John Zaia[2], and John J. Rossi[2].
University of California, Los Angeles, CA 90024[1] and City of Hope, Duarte, CA 91010[2].

We have developed a technique for the rapid amplification and detection of cellular HIV RNA. Using a method similar to the Polymerase Chain Reaction Amplification [Saiki, _et al._, _Science_ 230: 1350 (1985)], we have amplified a region within the 3' ORF coding region. Two converging synthetic deoxyribonucleotide primers were used for multiple cycles of denaturation, primer annealing, and primer extension with reverse transcriptase (and DNA polymerase I). The reactions are electrophoresed in agarose, blotted onto a charged nylon membrane, and probed with a radioactive oligonucleotide. As few as 100 RNA molecules of specific RNA are required for amplification and detection. Moreover, 10 ng of total cellular RNA extracted from HIV-infected cells can be amplified and positively detected in less than one day. Finally, we have tested HIV-infected cells treated with inhibitory deoxyribonucleoside methyl phosphonated oligonucleotides and have ascertained an oligonulceotide complementary to the first _tat_ mRNA acceptor site for RNA splicing inhibited viral proliferation about 1000 fold. Amplification of RNA using the Polymerase Chain Reaction should be applicable to clinical detection of HIV-infected patients as well as have general application for detecting specific RNA transcripts.

**P 118** MAPPING OF IMMUNOGENIC AND IMMUNODOMINANT EPITOPES ON HUMAN IMMUNODEFICIENCY VIRUS (HIV) gp120 USING ENV-ENCODED SYNTHETIC PEPTIDES, Thomas J. Palker, Thomas J. Matthews, Michael E. Clark, Alphonse Langlois, George J. Cianciolo, Richard Randall, Gilbert White, Bijan Safai, Ralph Snyderman, Dani P. Bolognesi and Barton F. Haynes, Duke University, Durham, NC 27710, Memorial Sloan Kettering, New York, NY and University of North Carolina, Chapel Hill, NC 27514.
Seven synthetic peptides (SP-1, 10, 10A, 11, 14, 15 and 22) derived from hypoglycosylated, hydrophilic amino acid sequences of HIV gp120 envelope glycoprotein were used to map the host antibody response to gp120. Five of the seven peptides from gp120 (SP-10, 10A, 14, 15, and 22) were recognized by antibodies in HIV+ patient sera, and one of these peptides (SP-22), derived from a conserved region at the carboxy- terminus of gp120 was found to be immunodominant. Peptide SP-22 absorbed up to 100% of anti-gp120 antibody reactivity from some HIV+ patient sera in Western blot assays and up to 79% of anti gp120 antibody reactivity in competition radioimmunoassay (RIA). Affinity purified anti-SP-22 patient antibodies reacted with HTLV gp120 in Western blot assay and RIA but did not neutralize HIV _in vitro_ nor react with the surface of HIV- infected cells in immunofluorescence (IF) assays. In Western blot assays anti-SP-22 antibodies reacted with gp120 from divergent strains of HIV, including HTLV-IIIB, LAV and HTLV-IIIRF. HIV+ patient antibodies that were affinity purified with peptide SP-10 derived from the central portion of gp120 reacted both with gp120 in RIA and with the surface of HIV-infected cells in IF assay. The ability of anti-SP-10 affinity purified human antibodies and a panel of anti-SP-10 monoclonal antibodies to neutralize HIV _in vitro_ is under study.

**P 119** Detection of Serum Antibody to Human Immunodeficiency Virus Using a Synthetic Peptide from a Conserved Sequence of the gp 41 Envelope Protein. Elliot Parks, Alice Whalley and Richard S. Smith. Johnson & Johnson Biotechnology Center, Inc., La Jolla, California and Ortho Diagnostic Systems, Inc., Raritan, New Jersey

A synthetic peptide from a conserved region of the gp 41 envelope protein of Human Immunodeficiency Virus has been utilized to capture antibody in an experimental ELISA assay. This assay has been evaluated with over 2500 sera from normal blood donors, diagnosed AIDS patients, individuals at risk for AIDS and patients with unrelated illnesses. Results indicate that this synthetic peptide assay is both specific and sensitive for the detection of antibody to HIV. Analysis of serial bleeds from individuals at risk for AIDS demonstrates that this peptide assay is capable of detecting antibody as early as commercial viral lysate assays. Furthermore, this peptide can detect antibody to gp 41 envelope protein in cases where immunoblot analysis indicate that the sera are positive for antibody to p 24 core protein but unreactive for antibody to gp 41 antigens. The absence of extraneous material in this synthetic antigen preparation contributes to a low false positive rate and a high signal to noise ratio. These characteristics indicate that this synthetic HIV envelope peptide may have utility in future diagnostic assays.

**P 120** HIV/LAV2 INFECTION : SEROLOGICAL STUDIES. Marie-Anne Rey, Martine Harzic, Marie Christine Dazza, Eric Delaporte, Stéphane Gadelle, Jean-Jacques Madjar, Françoise Brun-Vezinet et al. Hopital Claude Bernard, France.

LAV2 is a human immunodeficiency virus different from the prototype HIV/LAV1/HTLV-III. LAV2 has been first isolated from 2 West African patients presenting AIDS. Then, LAV2 infection has been diagnosed in symptomatic and asymptomatic european et West African people. We tested sera collected in West African areas for the presence of LAV2 antibodies. IgG antibodies to LAV2 were detected by Elisa and Western Blot using purified supernatants from LAV2 infected CEM. Results show that about a third of the LAV2 positive sera, from symptomatic and asymptomatic subjects, were completely negative by HIV/LAV1 Elisa. Specific LAV2 serological assays are needed to perform HIV seroepidemiological studies as to screen blood donations.
Comparative LAV1 and LAV2 Western Blot analysis of the sera were performed using the two viruses electrophoresed in the same running assay. HIV/LAV1 and LAV2 core proteins share common epitopes but there is no cross-reactivity between the envelope glycoproteins of the two viruses. Only the presence of antibodies to the envelope glycoproteins allows to distinguish between the two infections. Double infection with HIV/LAV1 and LAV2 was diagnosed in some sera from West African countries.

**P 121** VIRAL DETERMINANTS OF PERSISTENT UNINTEGRATED VIRAL DNA (P-UVD) AND ITS ROLE IN CYTOPATHIC FeLV INFECTIONS. Norbert Riedel, Julie Overbaugh, Edward A. Hoover* and James I. Mullins. Harvard University School of Public Health, Boston, MA., and *Colorado State University, Ft. Collins, CO.

Transient production of unintegrated viral DNA (UVD) is normally observed in cells for the first few days following infection with a retrovirus. A characteristic feature of HIV infections *in vitro* and feline AIDS virus infections *in vivo* and *in vitro* is the persistence of often high levels of UVD (P-UVD). Previous studies have correlated transient production of high levels of UVD with cytopathic avain retrovirus infection. It has therefore been suggested that P-UVD may play a role in AIDS virus cytopathicity.

To determine the viral genetic determinants of P-UVD production and its role in cytopathic retrovirus infections, we constructed a number of chimeric FeLVs by exchanging complete genes or gene fragments using conserved restriction sites. Our findings indicate that a variety of perturbations, involving multiple genes and multiple intragenic regions of the viral genome, result in cell-type specific production of P-UVD. Very small changes in the viral genome, involving as few as 10 amino acid substitutions in the extracellular glycoprotein, are sufficient to result in the production of high levels (>10 copies/cell) of P-UVD. Moreover, production of high levels of P-UVD can occur in the absence of viral cytopathic effects. However, high levels of virus replication, and P-UVD, result in marked viral genome instability demonstrable by restriction site variablity *in vitro*.

**P 122** TROPICAL SPASTIC PARAPARESIS--THE POSSIBLE AETIOLOGICAL ROLE OF THE HUMAN T-CELL LEUKEMIA/LYMPHOMA VIRUS HTLV-I. Pamela Rodgers Johnson, Vladimir Zaninovic, Owen StC. Morgan, Robinson Biojó, Carlos Mora, Cesar Arango, Ralph M. Garruto, Clarence J. Gibbs, Jr., D. Carleton Gajdusek, National Institutes of Health, Bethesda, MD 20892; Universidad del Valle, Cali, Colombia; University of the West Indies, Kingston, Jamaica. Endemic tropical spastic paraparesis (TSP) is the commonest chronic neurological condition seen in many equatorial regions of the world. The etiology has not to date been determined and TSP has previously been regarded as multifactorial in origin, with toxins (especially cyanide), undernutrition, treponematosis, parasitic infections, viral agents and genetics all being implicated as causative or predisposing factors. We evaluated the prevalence of IgG antibodies to HTLV-I in Jamaican patients with TSP and report that 77% of patients were seropositive and 67% had antibodies in the cerebrospinal fluid (CSF)—a higher prevalence than that reported for non-Hodgkin's lymphoma (55%) in the adult Jamaican population. Previous studies have shown the overall seropositivity rate for the Jamaican population over 20 years to be 8.1%. Colombian TSP patients had an even higher degree of positivity: 96% in serum and 92% in CSF. Colombian patients, however, had a higher degree of parasitic infections which could be due not only to endemic parasitism but also to immunosuppression caused by HTLV-I. A high percentage of TSP patients with IgG antibodies to HTLV-I has also been found in Martinique, the Seychelles and Trinidad and represents the first consistent abnormality found in TSP patients from various regions of the world. Japan has also reported an HTLV-I associated myelopathy. These results suggest that HTLV-I or other antigenically related retroviruses may have neurotropic properties in addition to the ability to alter the host immune response. The clinical uniformity of TSP among patients in high-incidence foci and the high positivity for IgG antibodies to HTLV-I in serum and CSF of TSP patients lessens the likelihood that cases are of a heterogenous aetiology.

**P 123** REVERSE TRANSCRIPTASE (RT) ACTIVITY, RETROVIRAL SEROLOGY, AND SERUM INTERFERON (IFN) LEVELS IN KAWASAKI DISEASE (KD). A.H. Rowley, B.J. Poiesz, J.L. Sullivan, O.T. Preble, and S.T. Shulman. Children's Memorial Hosp., Chgo., IL 60614, SUNY, Syracuse, NY 13210, U. Mass., Worcester, MA 01605, and the Uniformed Services U., Bethesda, MD 20205.

We recently reported evidence that KD may be etiologically related to a retrovirus. (Lancet, Sept. 6, 1986). We performed co-cultivation of acute KD patient peripheral blood mononuclear cells (PBMC's) with the human lymphoblastoid cell lines MOLT-3, MOLT-4, HUT-78, and CEM in RPMI 1640 media containing PHA, IL-2, and in some cases, polybrene, anti-$\alpha$ interferon, and PHA- stimulated normal adult or cord blood PBMC's. PBMC's from healthy adult and febrile child controls were co-cultivated in an identical way. Culture supernatants were assayed weekly for $Mg^{++}$-dependent RT activity: aliquots were centrifuged and the pellet resuspended in tris-HCl with KCl, DTT, $MgCl_2$, Triton X-100, poly(rA)oligo(dT) and $^3$H-TTP. After incubation, duplicate aliquots were spotted onto glass fiber filters, washed, dried, and counted. RT activity was detected in 16/341 KD co-cultivation flask supernatants but in only 2/278 supernatants from control flasks ($p<0.01$). None of 4 convalescent KD sera had antibody to gp120 or gp41, envelope glycoproteins of HIV, by immunoblot analysis. None of 8 convalescent KD sera had IgG antibody to HTLV-I, and none of 7 had IgM antibody to HTLV-I or IgG antibody to HTLV-II by ELISAs utilizing purified whole disrupted virus. 18/20 acute KD sera but only 2/7 childhood controls had circulating IFN levels >4 IU/ml ($p<0.005$). These findings support the possibility that KD is etiologically related to a retrovirus, but serologic studies thus far do not show cross-reactive antibodies to HTLV-I, HTLV-II, or HIV in patients with KD.

**P 124** THE REGULATION OF IMMUNE CELL FUNCTION IN VITRO BY RECOMBINANT ENVELOPE GLYCOPROTEIN OF HUMAN IMMUNODEFICIENCY VIRUS, R. Shalaby, P. Berman, and A. J. Ammann. Genentech, Inc., So. San Francisco.
The effect of human immunodeficiency virus, (HIV) recombinant envelope glycoprotein 120 (rgp120) on the functions of peripheral blood mononuclear cells (PBMC) in vitro was investigated. rgp120 (1-5 ug/ml) inhibited concanavalin A (ConA) induced PBMC proliferative response in a dose related manner as determined by $^3$H-thymidine incorporation. Glycoproteins of an unrelated virus failed to cause a similar inhibition. Tetanus toxoid-induced PBMC response was also inhibited by rgp120 (5 ug/ml) in five of six experiments. rgp120 also interfered with ConA-induced expansion of T-cells, particulary the cytotoxic T8+ population, as determined by staining with fluorescein conjugated monoclonal antibodies. In addition, the presence of rgp120 in pokeweed mitogen stimulated PBMC cultures caused significant reduction in IgG secreting cells enumerated in a reverse hemolytic plaque assay. Finally, exposure of PBMC to rgp120 resulted in a selective blocking of the T4A epitope of the T-helper T4 membrane surface marker. These results suggest that rgp120 can interfere with helper cell function through the interaction with membrance surface structures. These data also suggest that rgp120 in in vitro studies may be of help in understanding the mechanisms of virus-induced immunodeficiency in patients with HIV infection and in clarifying the role of T4 receptors in helper cell activities.

## *Pathogenesis; Viral Gene Expression in Exogenous Cells*

**P 200** ROLE OF ANTIBODY-COMPLEMENT COMPLEXES IN THE PROCESS OF DESTRUCTION OF LYMPHATIC TISSUES IN AIDS. Djordje Ajdukovic, Simon Garzon, Merwyn Gornitsky and Drasko D. Pekovic, Institut Armand-Frappier, University of Montreal and Jewish General Hospital, Montreal, Canada.
Employing direct immunofluorescence [IF] and immunogold electron microscopy [IEM] we have recently demonstrated the presence of HIV antigens in peripheral blood lymphocytes [PBL], salivary lymphocytes [SL] as well as in lymphatic tissues obtained from HIV-positive patients. Similarly, the HIV particles have been found in various stages of development in PBL and SL using IEM. A small proportion of the infected cells were morphologically multinucleated giant cells and showed signs of cytolysis. Although small number of cells are infected by the virus and the percentage of the transformed cells is usually even lower the cytolytic and cytopathic reaction should be considered in the destruction of lymphatic tissues of HIV-infected individuals. Using IEM we also found, that the sera from AIDS and ARC patients contain Ab reacting with the cell membrane and some intracellular structures of PBL from normal subjects. Almost all infected cells and high percentage of uninfected cells of HIV positive patients displayed antibody-complement [Ab-C3] complexes on their membrane suggesting that an Ab-C3 complex-mediated cytolysis may be a major mechanism responsible for the rapid reduction in number and function of lymphatic tissues and cells in HIV-positive patients.

**P 201** STUDIES OF THE CNS IN CHIMPANZEES PERSISTENTLY INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS. David M. Asher, Richard Yanagihara, Axel Wolff, Clarence J. Gibbs, Jr., Jaap Goudsmit, Leon Epstein, Peter Ruczicka, Prem Sarin, Daisy Sun, Lisa Marselle, and D. Carleton Gajdusek. National Institute of Neurological and Communicative Disorders and Stroke and National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, University of Amsterdam, The Netherlands 1105AZ, and University of Medicine and Dentistry of New Jersey, Newark, NJ 07103.
Chimpanzees can be regularly infected with human immunodeficiency virus (HIV) but have not yet developed overt AIDS. In HIV-infected humans CNS involvement may precede other signs of AIDS. In such patients virus and viral antigens, as well as locally-synthesized antibodies, are usually detected in the CSF. CSF was collected from eight chimpanzees persistently infected with HIV. studied CSF of four animals contained ELISA antibodies in titers as high as 1:10. In no CSF was there convincing evidence of intrathecally-synthesized antibody nor were oligoclonal bands of globulin present. CSF contained no detectable viral antigens nor was virus isolated. A brain biopsy was performed on one chimpanzee with CSF antibodies. The tissue was histologically normal, no viral RNA was detected by in-situ hybridization, and no virus was isolated (although virus was present in blood obtained at the same time). We have not yet obtained clear evidence that the brain is involved in persistent HIV infection of chimpanzees. However low levels of antibodies to HIV in CSF of half the animals may indicate early noninflammatory invasion of the CNS.

**P 202** INCREASED INTERLEUKIN 1 (IL-1) AND IL-1 INHIBITOR PRODUCTION IN ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS). Monique A. Berman, Christy I. Sandborg, Brian S. Andrews, and George J. Friou. California College of Medicine, University of California Irvine, Irvine, CA 92717.
Abnormal monocyte functions, including low interleukin 1 (IL-1) production, have been reported in AIDS. We have fractionated culture supernatants from unstimulated peripheral blood mononuclear cells (PBMC) to determine whether the low IL-1 activity in AIDS was due to the presence of IL-1 inhibitors. The results demonstrate that PBMC from patients with AIDS produce greatly increased amounts of IL-1 activity compared with controls. The increase in IL-1 activity (up to 10 fold) was highest in the IL-1 fractions of 30-35 K molecular weight (MW), rather than 17 K MW IL-1 fractions. The functional significance of the shift to more high MW IL-1 activity in AIDS is not clear. To assay for the presence of IL-1 inhibitors, PBMC supernatant fractions (Sephacryl S-200, chromatofocusing) were tested against 100 U of IL-1 activity in the thymocyte proliferation assay. A 20 fold increase ($p<0.01$) in inhibitory activity in the 6-9 K MW weight range was found in the AIDS patient group. The greater increase in this inhibitor compared with IL-1 masks the IL-1 activity present in PBMC supernatants resulting in low apparent IL-1. The activity of the 6-9 K MW IL-1 inhibitor appears to be specific against IL-1 dependent T lymphocyte maturation, while other IL-1 mediated effects (i.e. fever, B cell activation) are not inhibited by this factor. The increased production of this inhibitor may play an important role in the immune deficiency in AIDS by interfering with T cell maturation and with regeneration of the T cell pool.

**P 203** SCREENING FOR HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN CORNEA DONORS.
Alexander A. Bialasiewicz and Gerhard J. Jahn, Dpts. of Ophthalmology and Clinical Virology, University of Erlangen-Nürnberg, West Germany

Recent reports have indicated that HIV may be found in the cornea and conjunctiva of patients with the acquired immunodeficiency syndrome (AIDS). Furthermore, storage solutions do not interact with the viability of the virus.Thus, there is a potential risk for cornea recipients to acquire a HIV infection.without appropriate testing.

We have evaluated 152 cornea donors from 10/85 - 12/86 in the area of Erlangen-Nürnberg, a mixed rural/urban population. Mean age was 62.5 yrs. (range:13-90) indicating a majority of "natural" deaths. Serum was obtained post mortem and processed for HIV in ELISA (Abbott) and Western Blots.

In the population studied, 4 were HIV-ELISA positive (ages:20,25,68 and 74), 3 of them had a positive Western Blot (ages:20, 68 and 74). The 20 yr. old donor had been killed in an automobile accident and had a history of homosexual contacts, although clinically he had appeared healthy. The 68 and 74 yr. old donors had died from "natural" death causes without signs of AIDS or the AIDS-related complex. Retrospectively, multiple blood transfusions could be determined during the course of major abdominal surgery in 1982 and 1983 in smaller community hospitals, when routine screening of HIV in blood donors had not yet been introduced.

From our study we conclude that history-taking of cornea donors is not sufficient for the estimation of a potential HIV risk. Although HIV-positive patients are known to be accident-prone because of their neurological abnormalities, it is not solely this population that may represent a possible health hazard, but also the older "natural" deaths with previous unscreened blood transfusions.

**P 204** HERPESVIRUSES - POSSIBLE COFACTORS IN AIDS PATHOGENESIS, Cindy A. Bohan, Robert F. Rando, Philip Pellett, Paul Luciw* and A. Srinivasan, Centers for Disease Control, Division of Viral Diseases, Atlanta, Georgia 30333; *Department of Medical Pathology, University of California, Davis, California 95616. Both the mechanism of pathogenesis of AIDS in individuals infected by the human T lymphotropic virus (HTLV III, HIV, LAV) and the proportion of infected individuals who develop the immunodeficiency syndrome are unknown. Epidemiological studies indicate that wide variation is noted from time of exposure to HIV to the onset of AIDS. A role for host susceptibility factors or cofactors in the progression of disease has been proposed. Since 50% to 90% of individuals infected with HIV are exposed to a variety of bacterial, viral, fungal, and parasitic disease agents, the possibility of these agents acting as cofactors in the manifestation of AIDS is an important hypothesis to consider. Utilizing the LTR sequences derived from a HIV molecular clone coupled to the bacterial gene coding for chloramphenicol acetyltransferase, we examined the effect of herpesviruses on HIV-LTR directed gene expression. Both HeLa and HLF cells, transfected with HIV-LTRCAT (pLTR-CAT), showed low, but detectable levels, of CAT activity; however, when transfected cells were infected with HSV-1, HSV-2, CMV, and VZV, the level of CAT activity increased relative to pLTR-CAT transfected alone. The transactivation of HIV- LTR directed expression by herpesviruses seems to be specific as the SV40 early promoter and the LTR of BLV did not show transactivation effects. The viral gene product(s) responsible for transactivation can be provided by infecting cells with whole virus or by transfecting cells with subgenomic viral DNA fragments.

**P 205** SUPERINFECTION OF HTLV-III/LAV-INFECTED HUMAN T LYMPHOCYTES WITH CMV RESULTS IN A HIGH RATE OF CELL LYSIS. D. Casareale. M Fiala, L.A. Cone and C.M. Chang. Eisenhower Medical Center, Rancho Mirage, California and Scripps Clinics and Research Foundation, La Jolla, CA.
The effect of superinfection with CMV on T cell lysis was studied using a lymphocytotropic strain of CMV and a T cell line (CR-10/NIT) persistently infected with HTLV-III/LAV. Superinfection of CR-10/NIT cells with CMV at a M.O.I. of $6x10^5$ PFU/ml resulted in induction of pre-early, early and late antigens in the CR-10/NIT cells within 8 to 10 days. Electron microscopy studies revealed cell lysis and the presence of both CMV and HTLV-III/LAV particles in 60% of lysing cells 10 days after superinfection with CMV. Studies on the kinetics of cell lysis after superinfection of CR-10/NIT cells with CMV revealed onset of lysis 24 hours after superinfection with CMV culminating in the destruction of the cell culture between day 9 and 10. These data suggest that CMV may convert a mildly cytopathic infection with HTLV-III/LAV into a highly lytic process.

**P 206** PATHOGENESIS OF AIDS: QUANTITATION OF HIV INFECTION OF MACROPHAGES BY CYTOFLUOROGRAPHIC ANALYSIS, Suzanne Crowe, John Mills, Michael McGrath, University of California, San Francisco & San Francisco General Hospital, San Francisco, CA 94110
HIV infection of CD-4 bearing lymphocytes alone cannot explain the immune dysfunction of AIDS. Macrophages infected with HIV may contribute to the pathogenesis of AIDS by serving as a virus reservoir and by functioning abnormally. Quantitative analysis of surface antigen expression and phagocytic function of monocyte-macrophages, and measurement of viral antigens in infected cells, has been difficult using the conventional approach of immunofluorescent staining of cells on slides. We have devised a tissue-culture system which permits maintenance and differentiation of human peripheral blood monocyte-macrophages in suspension culture for prolonged periods of time. This method permits correlation of CD-4 and/or HIV antigen density on infected and uninfected macrophages with phagocytic function by two color immunofluorescent cytofluorographic analysis. To date these studies have shown: (1) CD-4 antigen is present on monocytes, with antigen density showing marked donor variability (28-85% of cells). CD-4 antigen expression increases 10-fold during the first 1-2 weeks of culture. (2) Monocyte-macrophages between 2 hours and 57 days of age can be infected with HIV, and the p24 antigen of HIV can be detected in up to 62% of cells with monoclonal antibody staining.
Tissue culture methodology, cytofluorographic analyses and data relating to the functional capacity of infected macrophages will be presented.

**P 207** ANTIGENIC DETERMINANTS IN THE HTLV-III$_{RF}$ ENV GENE RECOMBINANT ANTIGENS EXPRESSED IN ESCHERICHIA COLI. Sylvia Crush-Stanton and Michael L. Berman, Bionetics Research, Inc., Rockville, MD.

The env gene from the HTLV-III$_{RF}$ strain, proviral clone λHAT-3, was engineered as a env-lacZ gene fusion in an E. coli expression vector. Various 5' and 3' deletions of the env sequences were isolated. The hybrid proteins synthesized by 11 separate fusions were characterized by Western blot analysis and ELISA tests using sera from infected patients. The results show that there are three immuno-dominant regions in these clones. One of these lies within codons 54-123 of the gp41 gene sequences. The two others map to the amino terminus and the carboxy terminus of the bacterially expressed gp120 gene sequences. The results with the gp120 recombinants show that hybrids carrying amino acids 30-182 or 483-520 are immunoreactive, while a hybrid with amino acids 182-462 is unreactive.

The systematic analysis of these types of families of hybrid proteins can help localize regions of immunological importance. This approach allows precise mapping of areas that may be important for clinical diagnostics or for vaccine development.

**P 208** SUPPRESSION OF IN VITRO HEMATOPOIESIS FOLLOWING HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION. R.E. Donahue, M.M. Johnson, L.I. Zon, S.C. Clark, and J.E. Groopman. Genetics Institute, Inc., Cambridge, MA and Division of Hematology/Oncology, New England Deaconess Hospital, Boston, MA.
Abnormalities including leukopenia, anemia, and thrombocytopenia are commonly observed in patients with the acquired immunodeficiency syndrome (AIDS) or the AIDS-related complex (ARC). We examined the effects of two recombinant hematopoietins, human granulocyte/macrophage-colony stimulating factor (rGM-CSF) and recombinant erythropoietin (epo), on *in vitro* growth of bone marrow progenitor cells from untreated AIDS or ARC patients. Bone marrow progenitor cells from all 8 patients in the study were responsive to rGM-CSF and epo when cultured in the presence or absence of normal human serum. Sera or purified immunoglobulin from AIDS or ARC patients, however, suppressed colony formation by bone marrow cells from AIDS or ARC patients but not from healthy individuals. Purified rabbit immunoglobulin to recombinant HIV envelope protein gp 120 reproduced the suppressive effects of *in vitro* hematopoiesis observed with immunoglobulin from HIV seropositive subjects. HIV was recovered from pooled bone marrow progenitors from AIDS marrow when these progenitors were co-cultivated with normal peripheral blood mononuclear cells. Thus, it appears that antibody in the serum of individuals after infection with HIV may contribute to immune-mediated suppression of hematopoiesis in AIDS or ARC patients. In the absence of antibody, bone marrow progenitors may be infected with HIV but they respond normally *in vitro* to rGM-CSF and epo.

**P 209** ISOLATION OF HIV FROM PLASMA OF HIV-INFECTED INDIVIDUALS. Larry Falk, Alan Landay, Mark Knigge, Mark Kennedy, Michael Leuther, Debbie Paul, Bernard Blauuw, Michael Bice, Joel Spear and Harold Kessler. Abbott Laboratories, North Chicago, Il. 60064 and Rush-Presbyterian-St. Luke's Medical Center, Chicago, Il. 60612.
Our previous efforts to recover HIV from plasma of HIV-infected individuals were unsuccessful. Recently we have cultivated HIV from plasma that was relatively fresh - cultures were inoculated within 24 hours of the blood being drawn. Plasma was filtered (0.45 um) and 1 ml was inoculated into 3-4 day old cultures of PHA-stimulated, human mononuclear leukocytes growing in RPMI-1640 medium supplemented with interlukin-2, polybrene and anti-α-interferon. Lymphocytes from the same patients were also cultured for HIV isolation. Plasma and lymphocyte isolation cultures were monitored for virus replication by an antigen (Ag) capture EIA specific for HIV p24 Ag. HIV was isolated from plasma (P) and/or lymphocytes (L) from patients in these categories: AIDS (n=10) P=7 and L=10; ARC (n=7) P=2 and L=3; asymptomatic (n=6) P=1 and L=4; acute HIV infection (n=2) P=1 and L=2. Viral isolates were confirmed as HIV. Infectivity titrations were performed with 2 negative and 4 HIV positive plasma; HIV was detected in undilute - $10^{-2}$ concentrations of plasma. These studies showed that: i) good correlation existed between detection of HIV Ag in sera (9/29) *vs.* biologic infectivity of plasma in cell culture (11/29), ii) in at least 4 patients HIV infectivity was demonstrated for plasma although the level of HIV Ag in serum was below detection by HIV Ag EIA and iii) infectivity titrations showed concentrations of infectious HIV were low. These assays will be useful for monitoring HIV in patients on antiviral therapy.

**P 210** ROLE OF CYTOMEGALOVIRUS IN PROGRESSION AND TREATMENT OF AIDS, Milan Fiala, Domenic Casareale and Lawrence A. Cone, Eisenhower Medical Center, Rancho Mirage, CA 92270.
In patients infected with HIV, CMV viremia is detectable in subgroup IV-A, and its intensity increases in subgroups IV-B-C, and -D. CMV causes retinitis, pneumonia, mucosal ulcers and contributes to the neurological complications. CMV role is supported by viral cultures of blood and tissues, cytochemical and immunological studies, and by results of 9,(1,3 dihydroxy-2-propoxymethyl)guanine therapy. Complicating bacterial infections in patients with CMV viremia respond to ceftazidime. In 2 patients CMV viremia and M. avium bacteremia were suppressed by successive treatments with DHPG, and ansamycin with clofazimine. Since CMV is immunosuppressive we have studied its cofactor role in AIDS in vitro and in vivo. In T4+ lymphoblastoid line infected with HIV (CR-10/NIT), CMV productively infects the lymphoblasts and greatly enhances their lysis as reported elsewhere. In patients CMV circulates in polymorphonuclear (PMN) and mononuclear (PBM) leukocytes. It is present at a higher titer in PBM of AIDS patients ($10^{-4}$ FFU/cell) compared to PBM of renal allograft recipients ($10^{-5}$ FFU/cell or less). Genomic analysis revealed differences between the matched PMN and PBM isolates in two AIDS patients suggestive that specific CMV strains are adapted to lymhocytes.

**P 211** DIRECT DETECTION OF HTLV-III ANTIGENS ON LYMPHOCYTES FROM PATIENTS WITH AIDS AND AIDS RELATED COMPLEX, Andrew Goldstein, Mark Loveless, Patricia Watson Martin, Randy Hodges, Sue Caouette, Paul Yoshihara, and Denis Burger, Epitope, Inc., Portland, OR 97006.
Monoclonal antibodies were produced against HTLV-III, the etiologic agent of AIDS and ARC. One of these monoclonal antibodies, designated 3D8, was reactive with the viral core protein (gag). Antibody 3D8 was used to detect expression of HTLV-III antigens on the H9 cell line following viral infection as well as on lymphocytes from seropositive patients. Forty-five Western Blot-positive patients representing AIDS, ARC and an asymptomatic group were studied using flow cytometry. A significant percentage of T4-positive lymphocytes from individual patients were stained with antibody 3D8. The highest percentage of HTLV-III positive cells came from patients with AIDS (up to 50% staining of T4 cells) although patients with ARC and patients without symptoms also demonstrated significant expression of HTLV-III antigens. Moreover, there was a correlation between the number of patients expressing HTLV-III antigens on T4 cells and clinical grouping. These data suggest that a higher percentage of T4-lymphocytes express HTLV-III antigens than has been predicted by detection of intracellular HTLV-III mRNA.

**P 212** EXTRACELLULAR HIV ANTIGEN IN PERIPHERAL BLOOD AND LYMPH NODES AND TRANSITION TO AIDS, Jaap Goudsmit, Frank de Wolf, Joep Lange, Deborah Paul, Thea Vroom, Roel Coutinho, Jan van der Noordaa, Virology Department, University of Amsterdam, Abbott Laboratories, North Chicago, Ill, Slotervaart Hospital, Amsterdam, Municipal Health Service, Amsterdam, The Netherlands.
Human immunodeficiency virus antigen (HIV-Ag) in serum is associated with AIDS or transition to AIDS and frequently accompanied by low or declining levels of antibodies to the major core protein p24. Between Oct 1984 and April 1985, 927 homosexual men entered a prospective study on the incidence of HIV infection and development of AIDS; 228 of the men were seropositive for HIV antibodies (HIV-Ab) at entry of the study. 57 of 699 initially seronegative men developed HIV-Ab within a period of 2 years. 11 (19%) of these men had HIV-Ag prior to seroconversion, transiently; none of these 11 developed AIDS as yet. Another 11 (19%) of these men showed persistence of circulating HIV-Ag following acute infection; 4 of these developed AIDS or ARC to date. Of the 228 Ab seropositive men 36 (16%) were HIV-Ag seropositive at intake, another 25 (11%) seroconverted for HIV-Ag. In all cases antigenemia persisted. AIDS was diagnosed in 16 (26%) of 61 HIV-Ag seropositive and in 2 (1%) of 167 HIV-Ag negative men. In 35 (92%) of 38 HIV-Ag pos/Ab pos sera tested HIV-Ag circulated as free antigen and in 17 (45%) of these sera HIV-Ag was detected in immune complexes as well. 8 (33%) of 24 HIV-Ag neg/Ab pos sera were shown to contain HIV-Ag in complexed form. Lymph nodes of some of these men contain HIV-Ag in the extracellular network of immune complexes around follicular dendritic cells.

**P 213** Heterogeneous susceptibility to UV-HIV induced T4 cell cytopathology in the presence of PHA by normal PBL and immunosuppression of PHA response by UV-HIV in the absence of cytopathology. Lee A. Henderson, Nasar Quershi and Robert F. Garry, Department of Microbiology and Immunology, Tulane Medical School, New Orleans, LA 70112.
PBL from normal volunteers were treated with ultraviolet- inactivated human immunodeficiency virus (UV-HIV) in the presence and absence of PHA or with PHA alone. Cells were examined by flow cytometry using monoclonal antibodies specific to a variety of T cell subsets. Three of seven subjects exhibited a 25-30 % decrease in Leu 3a positive cells and a variable, but consistent, increase in Leu 2a positive cells cultured with UV-HIV and PHA as compared to PHA alone. T helper/T suppressor cell ratio also decreased profoundly. Several subjects were refractory to UV-HIV induced cytopathology even after preactivation of PBL in the presence of PHA and subsequent reculture with PHA and UV-HIV. In contrast, UV-HIV completely suppressed PHA stimulated proliferation of donor PBL at all concentrations of PHA and all times examined. Recovery of PHA responses will be discussed. Our results suggest that PHA or, perhaps, antigen is required for selective killing of Leu 3a positive cells. Not all individuals are susceptible, however, indicating predisposing factors as yet unidentified. Even in the absence of Leu 3a killing and alteration of helper/suppressor ratio, immunosuppression is observed, suggesting that different proteins may mediate cell killing and immunosuppression. Alternatively, the same protein may serve several functions, namely immunosuppression and cytopathology, but there is heterogeneity in the normal population with respect to susceptibility.

**P 214** COMPARISON OF A NOVEL RIPA/SDS-PAGE TO IMMUNOBLOTTING AND VIRUSCULTURE
J.G.Huisman,M.Tersmette,N.Lelie,+C.v.d.Poel,++J.M.A.Lange,+H.Reesink,++J.Goudsmit and F.Miedema, Central Lab. Neth. Red Cross BT Service, incorporating Lab. of Exp. and Clin.Imm.Univ.of Amsterdam,+ Red Cross BB Amsterdam,++Dept.of Virol.Univ.of Amsterdam.
A novel developed RIPA using $^{125}$I-labeled HIV antigens, enriched for gp120/41$^{env}$ (GRIPA) was compared to immunoblotting (IB) for sensitivity and specificity for HIV antibodies. Sequential sera from 10 seroconverted homosexuals were tested. In all cases antibodies to gp120/41 and more prominent to p24$^{gag}$, were detected. In 2 cases these antibodies were detected earlier than by IB. It appeared that the GRIPA was 100 times more sensitive for anti-p24 in serial dilutions of 8 serumsamples, taken at seroconversion, while the detection of anti-gp120/41 was similar in IB. In one of 78 randomly chosen EIA-negative sera from homosexuals, antibodies to p24 could be detected. This early seroconversion was confirmed 3 months later by IB. The specificity of the GRIPA was demonstrated by analysing EIA-negative sera from homosexuals taken with a 3 months interval during 2 years. All sera were found negative in the GRIPA and the persons revealed no signs of HIV infection. Six blood donors reactive for p24 in IB for 2 years but negative in the GRIPA, were also studied. Virusculture was attempted with 6 seropositive asymptomatics as a positive control group. The six p24 IB positive persons were culture negative, while a 100% correlation existed between GRIPA and virusculture. It is concluded that reactivity in IB to p24 may be false positive, whereas reactivity in the GRIPA to p24 only is highly suspicious. This feature in addition to the high sensitivity for gp120/41 makes the GRIPA an useful confirmatory assay in sera with conflicting results in other HIV antibody assays.

**P 215** High-level bacterial expression of HIV gag and env polypeptides and their use as diagnostic reagents in immunoblots. Gerhard Jahn, Sylvia Harzenetter, Otto Erlwein, Angelika Lenz, Michael Bröker* and Bernhard Fleckenstein, Institut für Klinische Virologie der Universität Erlangen-Nürnberg, 8520 Erlangen, Loschgestr. 7, W-Germany. *Research Laboratories of Behringwerke, Marburg.

To obtain diagnostic reagents for the detection of acquired immunodeficiency virus (HIV) exposure, we have expressed viral antigens representing gag and env proteins. The vectors preferentially used had been constructed for expression of large quantities of fused proteins in E. coli (Gene Anal. Techn. 1986, 3:53-57). The plasmid carries the E. coli lac promotor and portions of the E. coli lac Z gene. Stable fusion proteins were taken for electrophoretic transfer blot assays and compared to reactive bands in conventional immunoblots carried out with proteins of gradient purified virions; the results of Western blotting with those fusion proteins (containing epitopes of gag p15, p17, p24, env gp41) were in general comparable with conventional ELISA, immunofluorescence, immunoblot, and commercially available ELISA-tests based on recombinant proteins. Some examples with critical sera emphasized the reliability of Western blots with bacterially expressed proteins.

**P 216** MECHANISM OF HTLV-III INFECTIONS AND ACQUIRED IMMUNEDEFICIENCY SYNDROME (AIDS),
Surinder K. Kad, S.U.N.Y, Health Sciences Center, Syracuse, N.Y. 13210.
Human T lymphotropic virus, Type III (HTLV-III) is a retrovirus which contains several viral genes for replication: 1)gag gene codes for viral core proteins of p15(carboxy terminal), p19(aminoterminal) and p24(major viral core protein) 2)pol gene for reverse transcriptase 3)env gene(envelope) 4)pX gene5)lor gene6)tat gene(critical for viral replication) 7)Long terminal repeat(LTR) sequences contain RNA polymerase binding site & regulate expression of viral genes. HTLV-III has remarkable lymphotropism particularly for OKT4+ lymphocyte. Molecular basis of this resides partly in binding of viral env protein to T4 molecule. Persistent lymphadenopathy is due to cytotoxic response against infected T4 cells. Virus penetrates cell at this receptor site. HTLV-III carries within its core an enzyme that synthesizes DNA, reverse transcriptase. This enzyme converts viral RNA into double-stranded DNA.It moves from cytoplasm to nucleus where it integrates into host cell DNA.Once infection occurs, it is likely to stay for lifetime. Retroviruses may hasten normal differentiation of helper T cells. Antibodies are formed to viral envelope. Antibodies produce autoimmune disease by cross-reacting with normal cellular proteins. Viral envelope proteins are highly immunosuppressive. HPA-23, Ribavirin and 3-azido-3-deoxythymidine are promising drugs for treatment of AIDS as these drugs bind with DNA and interfere with DNA replication and transcription of RNA and this ultimately results in disruption of nucleic acid function. These drugs may not be very effective early in disease process until after HTLV-III has integrated into host cell DNA. These drugs may not penetrate infected T4 cells when AIDS is too far advanced as the binding receptor sites on the cells may have been lost.

**P 217** EXPRESSION OF A HUMAN ENDOGENOUS PROVIRUS, ERV3, Nobuyuki Kato[1], Erik Larsson[2], and Maurice Cohen[1]. 1. BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701. 2. University of Uppsala, Uppsala, Sweden.
Endogenous proviruses are a potential source of new infectious retroviruses as a result of recombination with exogenous retroviruses, and have the ability to activate expression of other cellular genes with the proviral promoter. ERV3 is a full length human endogenous provirus that is single copy in human DNA and has a long open reading frame in the env gene. In the screening of ERV3 RNA expression in humans, one normal tissue, placental chorion, revealed reproducibly high levels of ERV3 specific mRNA equal to about 0.1% of the total mRNA. ERV3 mRNA expression in most normal and malignant tissues was at a level of 2-8% of that in placental chorion; however, several normal tissues including gallbladder, testis, breast, and several malignant tissues including stomach (polyp), glioma and Wilm's tumor revealed a higher transcript level equal to 10-30% of that in placental chorion. In contrast to normal chorion, expression of ERV3 proviral RNA was almost completely supressed in all examined choriocarcinoma cell lines. The basis for this difference is under investigation. The structure of three poly (A) + ERV3 RNAs (9 kb, 7.3 kb, and 3.5 kb) that are expressed in placental chorion were analyzed by Northern blot hybridization and S1 mapping. Results showed that all three RNAs are spliced env mRNAs that lack the gag gene and most of the pol gene. The 3.5 kb RNA is a typical subgenomic transcript whereas the 9 kb and 7.3 kb RNAs extend through the 3' LTR and are spliced again at a site 370 bp downstream of the 3' LTR. This is the first observation that endogenous proviral RNAs contain some cellular sequences. Research sponsored by NCI under contract NO. N01-CO-23909 with Bionetics Research, Inc.

**P 218** HIV SPECIFIC T CELL RESPONSE AND NEUTRALIZING ANTIBODIES IN GOATS AND CHIMPANZEES IMMUNIZED WITH NATIVE gp120 OR WITH RECOMBINANT ENVELOPE PROTEINS, Kai J.E. Krohn, W. Gerard Robey, Scott Putney, Thomas Panavalil, Robert C. Gallo, Peter J. Fischinger and Annamari Ranki, LTCB, NCI,Bethesda, MD 20892, Office of the Director, FCRF, Frederick, MD and Repligen Corporation, Cambridge, MASS.
HIV specific T cell response and neutralizing antibodies were studied in animals immunized either with native gp120, purified from HTLV-IIIB infected H9 cells or with recombinant proteins representing various parts of the HIV ENV genome. Goats and chimpanzees immunized with gp120 had type specific neutralizing antibodies but a group specific T cell response to whole HIV virions and gp 120, assayd by $^{3}$HTdR incorporation and by IL-2 or LIF production. Early bleed from goats immunized with non-glycosylated envelope proteins produced in E. Coli had neutralizing antibodies, but no T cell response. In contrast, goats immunized with glycosylated recombinant gp160, produced in insect cells and representing both the external as well as transmembrane part of the ENV, showed a group specific T cell response and neutralizing antibodies. The demonstration of a group specific cellular immune response to HIV even when vaccine candidates representing only one isolate is used raises hope for the development of a vaccine for HIV infection.

**P 219** ENHANCEMENT OF NEUTROPHIL OXIDATIVE METABOLISM BY HTLV-I RELATED ANTIGENS FROM MT-2 CELLS. Louis J. Lafrado, James R. Blakeslee, Jr., Mark G. Lewis, Charlene S. Dezzutti, and Richard G. Olsen.

This investigation addresses the characterization of HTLV-I or related proteins from MT-2 cells (HTLV-I positive human T-cell line). MT-2 cells were cultured in RPMI 1640 medium supplemented with HB101 or HB102. Protein characterization was performed using SDS-PAGE; immunoreactivity was tested with rabbit anti-HTLV-I sera and human ATL (adult T-cell leukemia) sera by Western blot analysis. Molecular weights of MT-2-derived proteins were determined to be around 68 kd, 46 kd and 20 kd. Neutrophils from pig-tail macaques were exposed to titrated volumes of MT-2 proteins to determine their effect on oxidative metabolism. Neutrophil activation was assessed by the light release index measured in millivolts (mV). Neutrophil killing is dependent on oxidative metabolism. Oxidative metabolism was induced using latex beads and the phorbol ester, TPA. TPA directly activates protein kinase C (PKC) which is critical to neutrophil functional capacity. Exposure of macaque neutrophils to HTLV-I proteins resulted in enhanced oxidative metabolism in response to the general stimulus latex beads and PKC stimulation by TPA. The light release index for TPA stimulation after exposure to HTLV-I proteins was increased to 419 mV from 147 mV ($p<0.05$) while the light release index for latex beads was increased to 694 mV from 399 mV (p0.01). These data suggest the potential of MT-2-derived HTLV-I proteins to stimulate immune response mechanisms in challenged macaques.

**P 220** ANTIBODIES TO P18 AND GP120 EPITOPES MARK ASYMPTOMATIC HIV INFECTION,
Joep Lange, Livea Giurgea, Willy Krone, Lia Smit, Frank de Wolf, Claudine Bruck, Jaap Goudsmit, Virology Department, University of Amsterdam, The Netherlands, Institut Pasteur du Brabant, Brussels, and Smith Kline-RIT, Rixensart, Belgium.
Using conventional immunoblot techniques it has been shown in longitudinal studies that transition to AIDS is preceded by a decline in antibody titer to HIV gag gene encoded proteins, concomitant with appearance of HIV antigen (Ag) in serum. With a modification of the immunoblot procedure, in a 2 year follow-up study of 30 HIV antibody (Ab) seropositive men (10 of whom developed AIDS), a different pattern emerged: a) IgG reactivity to HIV envelope (gp120) epitopes was clearly present in 80% of asymptomatic Ag negative subjects, whereas it was less often found in asymptomatic Ag subjects and AIDS patients; b) Sera from Ag negative asymptomatic subjects showed persistent strong reactivity to the p18 band, whereas in Ag positive asymptomatic subjects and AIDS patients this reactivity was either absent or disappeared; c) IgG reactivity to p24gag did not differ as clearly among groups. Titers of neutralizing Ab in a syncytium inhibition assay and in an infectivity inhibition assay tended to parallel reactivity to p18 and gp120 epitopes.

**Abstract Withdrawn**

**P 222** HLA-DR IS INVOLVED IN THE RECEPTOR SITE FOR HLV, Dean L. Mann, Larry O. Arthur, William Blattner, and Mikulas Popovic, NCI, NIH, Bethesda MD 20892.
Binding of HLV to various cell types is mediated by interaction of the viral large envelope protein (gp 120) and the cell surface CD4 molecule. The possibility that major histocompatibility (MHC) complex class II gene products might be involved in virus-cell interaction was suggested in an earlier study which reported inhibition of HIV infection with some monoclonal antibodies reacting with HLA D region products. We investigated this possibility in the following experiments. HIV or immunopurified gp 120 was incubated with PHA stimulated peripheral blood lymphocytes (PBL) for varying periods of time and then exposed to monoclonal antibodies detecting HLA-DR, DP, DQ, T4, T4a, HIV gp 120 and analyzed for reaction by flow cytometry. After 15 min of exposure to whole virus, significant decrease in the T4a and HLA-DR antibody binding occurred. After 120 min, HLA-DR reactivity returned to levels observed on unexposed cells while reactions with T4a antibody remained decreased. In contrast to reaction with HLA-DR, antibody binding to HLA-DQ increased and HLA-DP remained stationary. When gp 120 was used, HLA-DR increased to levels 2X that observed in unexposed PBL. HLA-DQ and DP expression was unchanged. These results demonstrate the interaction of HIV with the CD4 molecule and HLA-DR but not HLA-DP or DQ. The return of HLA-DR expression after 120 min of HIV exposure is explained by interaction of isolated gp 120 with CD4 which increased (in 15 min) the ability to detect HLA-DR. The interaction of HLA-DR with CD4 in the binding site for HIV has important implications in the biology of HIV as HLA-DR is functionally important in immune regulation and response.

**P 223** The generation of a secondary CTL response to EBV in vitro is not impaired by the presence of HIV in the culture. Alison C. Mawle, Judy M. Scheppler and J. Steven McDougal. Immunology Branch, Division of Host Factors, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333.

We have used the in vitro generation of CTL to EBV transformed B cell lines to assess the effects of HIV on secondary CTL generation. EBV-specific CTL were generated by stimulating fresh PBL from healthy, EBV-seropositive individuals with irradiated autologous EBV-transformed B cell lines with or without HIV present in the culture. Cultures assayed for EBV-specific CTL showed equivalent killing whether or not HIV was present in the culture. HIV could be added to PBL up to 7 days before stimulation with autologous EBV lines without affecting CTL generation. This time period is sufficient to render cultures negative for T4 positive cells. The inability to demonstrate a cytotoxic response to HIV in vitro in individuals infected with the virus suggests that there may by a defect in the generation of these cells, possibly stemming from a deficiency in T4 help. These data indicate that at least in vitro an adequate secondary CTL response can be mounted in the presence of HIV.

**P 224** HUMAN IMMUNODEFICIENCY VIRUS (HIV) INDUCES GENE EXPRESSION FOR INTERLEUKIN-2 (IL-2) RECEPTORS ON HUMAN MONOCYTES, Nancy L. McCartney-Francis, Diane E. Mizel, Janice B. Allen, Larry M. Wahl, Phillip D. Smith, Thomas M. Folks, and Sharon M.Wahl, NIDR, NIH, Bethesda, MD 20892.
Exposure of human monocytes to agents such as bacterial lipopolysaccharide (LPS) or γinterferon (γIFN) induces functional and phenotypic changes including IL-2 receptor expression. In the present study we investigated the role of IL-2 receptor expression in monocyte activation in vitro and then examined the effect of HIV infection on IL-2 receptor expression on monocytes in patients with acquired immunodeficiency syndrome (AIDS) and in vitro. Purified monocytes, isolated by countercurrent centrifugal elutriation and activated with LPS or γIFN, expressed receptors for IL-2 as detected by flow microfluorometry. The expression of the IL-2 receptors on the monocytes correlated with cell maturation as indicated by increased HLA-DR expression. The addition of recombinant IL-2 to suboptimally activated IL-2 receptor-positive monocytes regulated interleukin-1 (IL-1) production and enhanced both the production of reactive oxygen intermediates and cytotoxic activity. Since peripheral blood monocytes from patients with AIDS expressed increased levels of IL-2 receptors, HLA-DR, and IL-1 mRNA, we evaluated the role of in vitro HIV infection of monocytes on these phenotypic and functional changes. Normal monocytes cocultured with HIV in vitro expressed increased surface receptors for IL-2. Furthermore, the addition of recombinant IL-2 to these cells caused an increase in reverse transcriptase activity, suggesting that IL-2:IL-2 receptors may play a regulatory role in the infective process.

**P 225** RECEPTOR MEDIATED LEUKEMOGENESIS: HTLV-I ASSOCIATED B CELL TRANSFORMATION INVOLVES HTLV-I ENVELOPE GP61:CELL SURFACE IgM INTERACTIONS, Michael S. McGrath, Valerie Ng, Connie Rainer, University of California, San Francisco General Hospital, San Francisco CA 94110.
The high rate of coinfection with HTLV-I and HIV in AIDS patients with B-cell lymphoma (Feigal, E & Mcgrath, MS, this conference) led us to investigate an in vitro model of HTLV-I associated B-cell transformation. We characterized five immortalized human B cell lines which arose in vitro after cocultivation with an irradiated, HTLV-I immortalized T-lymphoma line. All five lines bound fluoresceinated HTLV-I, had clonal immunoglobulin gene rearrangements, contained clonally integrated HTLV-I, and synthesized and secreted HTLV-I envelope glycoprotein (gp61/68). All five cell lines secreted IgM that immunoprecipitated HTLV-I gp61/68. HTLV-I gp61/68 could not be detected on the surface of these cell lines, but was present cytoplasmically and in a secreted form complexed with IgM. Addition of purified HTLV-I to the $DL_1$ B cell line stimulated in vitro growth as did the addition of anti-$DL_1$ idiotypic monoclonal antibodies. These studies are consistent with the receptor mediated leukemogenesis hypothesis which predicts that continous stimulation of cell surface immmunospecific receptors with an endogenously produced viral antigen plays an active role in the lymphocyte transformation process.

**P 226** HIV INFECTION OF PROMONOCYTIC U937 CELLS DOWN REGULATES CLASS-II EXPRESSION, DIMINISHES ACCESSORY CELL FUNCTIONS AND INDUCES DIFFERENTIATION-LIKE PHENOTYPIC CHANGES, F. Miedema, A.J.C. Petit, M. Tersmette, F.G. Terpstra and R.E.Y. de Goede, Central Lab. Netherlands Red Cross Blood Transfusion Service, incorporating the Lab. of Exp. and Clin. Immunology of the Univ. of Amsterdam, Amsterdam, The Netherlands

Surface marker analysis and functional studies were performed with promonocytic U937 cells that had been infected with HIV and persistently produced virus as detected by supernatant reverse transcriptase activity. Accessory-cell function of U937/HIV cells on anti-T3 Mab- and Con A- induced T-cell proliferation was decreased to 20-60% compared to that of non-infected U937 cells. Similar accessory-cell defects were demonstrated in the monocytes of asymptomatic seropositive homosexuals, with normal CD4 T-cell numbers and ARC and AIDS patients. The accessory-cell defect could be partially restored with r-IL-2. Addition of $\geq 10^4$ TCID50 HIV did not affect T-cell proliferation under these culture conditions. Expression of MHC class-II antigens on U937/HIV cells was 3-10 fold decreased compared to non-infected U937 cells. HIV infection induced expression of CD11 (C3bi receptor) and p150/95 adhesion molecules and induced enhanced expression of LFA-1 $\alpha$ and $\beta$ chains. Expression of these adhesion molecules resulted in strongly enhanced PMA-induced aggregation of U937/HIV compared to non-infected U937 cells. In addition U937/HIV cells, contrary to U937, intensely stained for cytoplasmic non-specific esterase activity. The phenotypic changes strikingly resemble the effects of differentiation-inducing agents (i.e. PMA, DMSO) on the U937 phenotype. The loss of accessory-cell function and decreased class-II expression by infected monocytic cells suggest that monocytes (APC) may be important target cells through which the immune system is affected by HIV.

**P 227** ZINC STATUS IN HIV INFECTION, Millard, M.C., Shoemaker, J.D., Johnson, P.B., University of Illinois College of Medicine at Urbana-Champaign

The relationship between $T_4/T_8$ ratio and serum, red blood cells and leukocyte zinc (Zn) in ten HIV-infected homosexual men and two uninfected homosexual men was examined. The $T_4/T_8$ ratios ranged from 0.04-1.2. Seven-day dietary diaries reveled that most of the men self-supplemented with Zn and other nutrients. Serum Zn was 0.93-0.96 μg/dl in three subjects who had $T_4/T_8$ ratios of 0.08-0.3 who were clinically well. In three subjects with $T_4/T_8$ ratios of 0.04-0.1 who had current opportunistic infections, serum Zn was 0.66-0.72 μg/dl, despite self-supplementation with Zn. Serum Zn of the other subjects, who had $T_4/_8$ in the range 0.6-1.2 covered the range encountered in the first two groups: 0.69-0.99 g/dl. Erythrocyte and leukocyte Zn did not correlate with serum Zn, $T_4/T_8$ ratios, absolute $T_4$ number or clinical condition. In leukocytes, Zn ranged from 150-530 and cooper 8.7-37 pM/$10^6$ cells. Neither correlated with any patient parameter studied. Zinc/copper ratios in leukocytes were in the range 6.4-26.8 but did not correlate with HIV infection or stage of illness. HIV infection alone did not appear to lower zinc in any tissue studied in this series of patients.

**P 228** DEVELOPMENT OF A NOVEL DIAGNOSTIC REAGENT AND SYNTHETIC VACCINE FOR AIDS USING A p17 GAG ANALOGUE (HGP-30).

P.H. Naylor, C.W. Naylor, M. Badamchian, S.Wada, A.L. Goldstein, D.K. Sun, A.H. Thornton, P.S. Sarin GWU Med. Center, Washington, D.C. 20037 and Nat'l Cancer Inst., Bethesda, Md. 20892.

An antiserum prepared against thymosin $\alpha_1$ (which shares a region of homology with the p17 gag protein of the AIDS associated virus (HTLV III/LAV)) neutralzes the AIDS virus and prevents its replication in H9 cells (Sarin et.al. Science 232:1135, 1986). Using HPLC and Western blot analysis we have identified an immunoreactive protein with a molecular weight of 17,000 daltons in a HTLV III/LAV retroviral extracts. In contrast, no immunoreactivity was found in retroviral extracts from a number of non-human species. Heterologous antiserum prepared against a 30 amino acid synthetic gag peptide (termed HGP-30 and containing the homologous thymosin $\alpha_1$ epitope) cross-reacts specifically with the p17 protein of the AIDS virus and effectively neutralizes _in vitro_ viral infectivity. The demonstration that this synthetic analogue is immunogenic and that antibodies to HGP-30 cross-react not only with the synthetic peptide but also with the HTLV IIIB p17 viral protein provides 1) a new and potentially more specific candidate for development of a synthetic peptide vaccine for AIDS, 2) a unique diagnostic reagent to detect HTLV III/LAV infection in seronegative individuals and 3) confirms a connection between AIDS and thymus.
(Supported by NCI Grant CA24974 and Alphal 1 Biomedicals, Inc).

# Human Retroviruses, Cancer and Aids: Approaches to Prevention and Therapy

**P 229** CHARACTERIZATION OF HIV ANTIGEN (Ag) FROM HUMAN SERUM, Deborah A. Paul and David G. Mack, Abbott Laboratories, North Chicago, Il. 60064.
HIV Ag has been detected in serum of infected individuals using a sandwich EIA with human and rabbitt anti-HIV on the solid-phase and as probe Ab, and with a second Ab label. Whether this represents whole viral particles or free viral proteins was investigated. The assay primarily detects p24 core Ag due to the rabbit anti-HIV used, which has 90% of its anti-HIV activity inhibited by HIV core Ag, and only 8% of its activity inhibited by env protein. Two lines of evidence, immunological and biochemical, indicate that the core Ag detected is free and not associated with viral particles. Immunologically, Ag activity in serum can be neutralized 100% by pre-incubating the Ag + sample with anti-p24 from a guinea pig immunized with HIV p24 purified on SDS-PAGE. Anti-p24 monoclonal Ab is also able to neutralize Ag activity, 50% to 65%. If Ag activity was associated with whole virus, then anti-env alone should also be able to neutralize the activity . However, human anti-HIV with no p24 activity but with high env activity does not neutralize Ag activity. Biochemically, on sucrose density gradients, the serum Ag activity remains near the top of the gradient ($\rho = 1.081$) and is not pelletable. When Ag + serum is affinity purified using the rabbit anti-HIV from the assay, then sized on Sephacryl S-200 (in 4M Guanidine), Ag activity is seen at a mol wt of 25000. This purified protein remains to be sequenced after enough material has been obtained. Thus both immunologic and preliminary biochemical evidence indicate that the Ag detected in serum is not virus associated, but represents free viral core protein. This does not mean that infectious virus may not also be present, since cell-free virus can be isolated from some Ag + (and some Ag neg.) serum (L. Falk, this symposium).

**P 230** ROLE OF MONOCYTES IN HIV INFECTION AND PATHOGENESIS, C. David Pauza, The Salk Institute, P.O. Box 85800, San Diego California
The unique properties of monocytes allow them to play a significant role in the establishment of chronic viral infections. Of particular interest are the relationships between monocyte differentiation, susceptibility to infection, and virus production. I have used the human monocyte cell lines HL-60 and U937, and the HIV isolate LAV, to explore the consequences of monocyte differentiation for virus infection. The cells were infected with LAV and simultaneously induced to differentiate by the addition of the phorbol ester tetradecaneoyl phorbol acetate (TPA). At 2.5 days after this treatment, the differentiated cells release fifty-fold fewer viral particles than the undifferentiated controls. These data were supported by analysis of the virus-specific RNA content of total cytoplasmic RNA preparations. Surprisingly, the disparate virus production was due to a specific defect in viral uptake; differentiated monocyte lines are deficient in viral uncoating. Virus preparations, with the RNA labeled by $H_3^{32}PO_4$ or $^3$H-uridine incorporation, were used to infect T-lymphoid (CEM or MOLT-3), HL-60, and U937 cells. At 24 hours post infection, the cells were analyzed to determine the total number of cell-associated counts, the fraction of counts resistant to trypsin treatment (virus particles internalized), and the percentage of trypsin resistant counts which were RNase-sensitive (uncoated viral particles). Differentiated HL-60 and U937 cells uncoat HIV at a rate at least three-fold lower than control cultures. I am also examining the role of anti-viral antibody in mediating monocyte destruction of virally infected T-cells (by the Antibody Dependent Cellular Cytotoxicity mechanisms) and the potential for antibody to enhance the uptake of HIV into differentiated and undifferentiated monocytes.

**P 231** PHENOTYPIC AND HELPER-INDUCER FUNCTION STUDIES IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) POSITIVE AND NEGATIVE HEMOPHILIACS. L. Perelmutter, C.A. Izaguirre, and J. Drouin, Laboratory Centre for Disease Control and University of Ottawa, Ottawa, CANADA.
The HIV virus infects selectively the CD4 subset of T cells which decreases markedly as the disease evolves. Our objective was to determine if HIV infection of CD4 cells affects their intrinsic helper-inducer function. The helper-inducer subset is recognized by the antibodies anti-CD4 and 4B4 (Coulter). The supressor-inducer is recognized by the antibodies anti-CD4 and 2H4 (Coulter). We studied 12 hemophiliac patients, 6 were negative and 6 were positive for HIV antibodies. Blood cells were obtained and separated by density gradient centrifugation, rosseting with sheep erythrocytes and sequential panning with monoclonal antibodies anti-CD4 and 2H4. The following fractions were obtained: B cells, T cells, CD4 cells, CD4/4B4 cells and CD4/2H4 cells. The T cells and subsets were cocultured with B cells in the presence of pokeweed mitogen and Immunoglobulin production was measured after 7 days in culture. Cells were also phenotyped for CD4,CD8,CD3,CD19 and monocytes. We found: 1. Two of the HIV negative patients had CD4:CD8 ratios $<1$, however, both patients have not received factor therapy for at least 4 years. The helper-inducer function of purified CD4/4B4 cells was normal in all HIV negative patients. 2. All HIV positive patients had CD4:CD8 ratios of $<1$; the helper function was undetectable in T cells, however, the purified CD4/4B4 cells showed helper function in 3 patients but it was absent or markedly reduced in the other 3 patients.. In conclusion, there is intrinsic deterioration of helper function in some HIV positive patients while others maintain such function, however the helper function in such patients is suppressed due to other cellular or humoral influences.

**P 232** CHARACTERIZATION OF THE LATENT PERIOD AND THE DEVELOPMENT OF NEUTRALIZING ANTIBODIES IN EARLY SEXUALLY TRANSMITTED HIV INFECTION, Annamari Ranki, Jaakko Antonen, Sirkka-Liisa Valle, Jean-Pierre Allain, and Kai J.E. Krohn, LTCB, NCI, Bethesda, MD 20892; Inst. Biomed. Sci., Univ. of Tampere, Finland, and Abbott Lab., North Chicago, IL .
We have prospectively followed immunological and virological events in 15 Finnish men who contracted HIV infection through sex and seroconverted. A common finding was a latent period, lasting for 4 to 18 months, when only viral antigen (sandwich-EIA) or anti-gag antibodies alone (Western blot and CIA-RA) were seen. Clinically, no symptoms except for onset of seborrheic dermatitis in some were recorded. During this time, $T_H$ cell numbers were normal but a defect was seen in cell mediated immunity to soluble recall antigens in half of the cases, and in none of the cases neutralizing antibodies could be detected with the sensitive ATH-8 cell microassay. Using RNA in situ hybridization, occasional positive cells of monocyte - dendritic cell lineage were seen. In the majority of the cases a full blown anti-viral antibody response developed first after a verified DNA virus (EBV, CMV, HBV) infection whereafter $T_H$ cells started to diminish. It is possible that these viruses enhance HIV replication by transactivation. Neutralizing antibodies started to appear first after the full blown antibody response, and they reached the highest titers along with the development of lymphadenopathy.

**P 233** DUAL INFECTION OF HTLV-I AND HIV IN PATIENTS WITH AIDS AND B-CELL LYMPHOMA, Rasheed, S., Su, S., Norman, G.L., Gill, P.S., and A.M. Levine, Departments of Pathology and Medicine, University of Southern California School of Medicine, Los Angeles, CA. 90032. The Acquired Immune Deficiency Syndrome (AIDS) and the adult T-cell leukemia have been etiologically associated with infections by the Human Immunodeficiency Virus (HIV) and the Human T-cell lymphotropic virus type I (HTLV-I) respectively. Although HIV and HTLV-I are structurally distinct, the mechanisms by which these viruses induce leukemia or AIDS is not clear. We have tested several thousand human sera by highly sensitive immunoblot techniques and observed that although HIV or HTLV-I infection is common among individuals at risk to develop AIDS and in patients with adult T-cell leukemia respectively, about 15% of the sera from patients with AIDS-related B-cell lymphoma were reactive for antibodies to both HTLV-I and HIV. Furthermore, retroviruses isolated from two patients, who were positive for antibodies to both viruses, exhibited variations from both HTLV-I and HIV prototype retroviruses.

**P 234** TRANS-ACTIVATION OF HIV LTR BY HERPESVIRUS IE PROTEINS, Robin A. Robinson[1], Jay Nelson[2], and Flossie Wong-Staal[3], University of Texas Health Science Center, Dallas, Texas 75235[1], Scripps Clinic and Research Foundation, La Jolla, CA 92037[2], and National Institutes of Health, Bethesda, Maryland 20205[3]

Genomic expression among human T-cell lymphotropic viruses (HTLV) and various DNA tumor viruses is regulated by viral encoded proteins capable of trans-acting transcriptional activities. Stimulation of expression directed by the HTLV-I and -III long terminal repeats (LTR) was noted in human epithelial, fibroblast, B-lymphoid, and T-lymphoid cell types infected with herpes simplex viruses (HSV) types I and II and human cytomegalovirus (HCMV) or transfected with HSV immediate early protein, ICP4, and HCMV immediate early protein, 72 kd polypeptide. No specific activation of the HTLV LTR was observed in these human cells by the large T antigens of SV40 and polyoma viruses or by the HSV immediate early protein, ICPO. Unlike the HTLV-III tat gene product, the activation of HTLV-III directed expression by the HSV ICP4 and HCMV 72 kd polypeptides was localized to a region between -117 and -65 nucleotides upstream of the LTR 5' initiation site for transcription. These results suggest a putative role of these herpesviruses in the pathogenesis of HTLV-III in certain cell types.

**P 235** FUNCTIONAL MAPPING OF THE ENVELOPE OF HTLV-III, Bruno Starcich, Amanda Fisher, Robert Gallo & Flossie Wong-Staal. Laboratory Tumor Cell Biology, National Cancer Institute, NIH, Bethesda, MD 20205. Recently much attention has focused on the envelope protein of HTLV-III. This entity is implicated in T4 cell killing and syncitia formation *in vitro* and the probable induction of neutralising antibody *in vivo*. Genetic analyses of the envelope gene of different HTLV-III isolates show it to comprise of highly conserved and divergent regions within the extracellular and transmembrane domains (Starcich et al. 1986; Hahn et al. 1986). To locate and map biologically important regions within the envelope protein, we have been systematically producing envelope mutants of the biologically active molecular clone of HTLV-III (pHXB2-D) using a primer directed mutagenesis approach. Mutations have been made in the first and second conserved regions (amino acid positions 50-52, 119-121, 244-246, 274-276), and the forth and fifth variable regions (396-414, 459-469) as well in the transmembrane domain; locations that we predict to be functionally critical in processes of cell killing, T4 binding, syncitia formation and neutralisation. The biological capacities of these mutated genomes and virus produced from such in currently under study, and will be presented.

**P 236** REPLICATIVE PROPERTIES OF TRANSSECTIONAL AND LONGITUDINAL HIV ISOLATES FROM SERUM HIV-ANTIGEN POSITIVE AND NEGATIVE INDIVIDUALS, M. Tersmette, R.E.Y. de Goede, I. Winkel, J.M.A. Lange*, J. Goudsmit*, F. de Wolf**, F. Miedema and J.G. Huisman, Central Lab. Netherl. Red Cross Blood Transfusion Service, incorporating the Lab. of Exp. and Clin. Immunol. of the Univ. of Amsterdam, *Dept. of Virol. of the Univ. of Amsterdam, **Municipal Health Service, Amsterdam, The Netherlands
Virus replication in co-cultures of lymphocytes of seropositive individuals and selected seronegative donor lymphocytes, was quantitated by RT activity and by a HIV-p24 antigen capture assay, using Sepharose-coupled rabbit anti-HIV ab and 125-I-labeled anti-p24 Mab. In most cases virus replication was detected by the ag-capture assay within 2 weeks, even in cultures late ( 3 weeks) positive for RT. HIV was detected in lymphocyte cultures of 8/8 AIDS patients, 9/10 ARC patients and 10/14 asymptomatic persons. On average virus was first detected in AIDS/ARC on day 10 and in asymptomatics on day 14. Syncytia were seen more often in AIDS/ARC cultures (6/12) compared to asymptomatics (2/14). Seropositive asymptomatics were tested for serum HIV antigen in the Abbott antigen test. HIV was isolated from all antigenemic but from only 67% of HIV-antigen negative asymptomatics. No correlation existed between serum HIV-antigen titer and replication rates. Studied longitudinally over a 2-year follow-up period, sometimes persons that were antigenemic throughout, remained asymptomatic although rapidly replicating virus that did not induce syncytia was detected. Interestingly, in one antigenemic person all but the first of six samples collected over a 18 month period prior to development of AIDS, rapidly yielded virus isolates that did induce syncytia. Investigation of the relationship of HIV isolate characteristics to clinical course in larger groups are underway.

**P 237** IMMUNOLOGICAL ANALYSIS OF HTLV-III p15, F. diMarzo Veronese[1], R. Rahman[1], R.C. Gallo[2], M.G. Sarngadharan[1], [1]Bionetics Research, Inc., Rockville, MD 20850; [2]Lab of Tumor Cell Biology, NCI, Bethesda, MD. 20205. The first open reading frame of $HTLV-III_B$ genome has been identified as the *gag* gene. The proteins encoded by this gene are p17 as the amino terminal protein, p24 as the middle peptide and p15 as the carboxy terminal end. A moncolonal antibody recognizing p15, designated M35/2F8 has been developed and used to further characterize this protein. p15 was purified from an extract of H9 cells producing $HTLV-III_B$ by an immunoaffinity procedure employing immobilized purified M35/2F8 IgG. In addition to p15, M35/2F8 purified the precursor of *gag* proteins (p53), another smaller precursor p39 and a very small peptide of approximate 9Kd. H9 cells producing $HTLV-III_B$ were then labeled with [$^{35}$S]-cysteine and [$^{3}$H]-leucine, immunoprecipitated with M35/2F8 and analyzed by SDS-PAGE. No immunoprecipitation of p9 has been observed when the cells were labeled with [$^{35}$S]-cysteine. However, p9 was distinctly immunoprecipitated when [$^{3}$H]-leucine labeled cells were analyzed. Taken together these results demonstrated that *gag* p15 is indeed processed into two smaller proteins p7 and p9 as anticipated and that M35/2F8 recognizes an epitope on the cysteine-free residue p9.

**P 238** HUMORAL AND CELLULAR RESPONSES TO HIV AND POLYPEPTIDES IN A MODEL SYSTEM.
B. Wahren[1], E.M. Fenyö[2], F. Chiodi[2], R. Kurth[3], J. Ghrayeb[4], S. Putney[5], R. Gallo[6] and D. Bolognesi[7], National Bacteriological Laboratory[1] and Karolinska Institute[2], S-105 21 Stockholm, Sweden; Paul Ehrlich Institut[3], D-6000 Frankfurt, FRG; Centocor[4], Malvern, PA 19355; Repligen[5], Cambridge, Mass.; National Cancer Institute[6], Bethesda, MD 20892; and Duke University[7], Durham, NC 27710.
A model system was established for studies of humoral and cellular immunity to HIV antigens in primary and reactivated infection and after vaccination. Macaques (Macaca fascicularis) were immunized with purified HIV, a cell extract rich in gp120 or polypeptides of cloned genes for parts of p24, gp41 and gp120 (pE3). Western blots best showed the appearance of antibodies to nucleocapsid protein while antibodies to higher molecular weight envelope glycoproteins were better demonstrated by radioimmunoprecipitation. With whole HIV, antibodies to p24 appeared first, and sometimes were the only ones to be demonstrable. Several immunizations with HIV were required to obtain antibodies to gp120, and the response was weak. (g)p41 also had a poor immunizing effect. IgG synthesis from B-cells in vitro was well demonstrable to whole HIV, and generally paralleled the antibody titers of sera after multiple immunizations. The HIV-specific lymphocyte proliferation response as measured by DNA synthesis was best seen with p24, followed by (g)p41, pE3, gp120 and whole HIV.

**P 239** HIGH LEVEL PRODUCTION OF HIV PROTEINS IN A EUCARYOTIC EXPRESSION VECTOR SYSTEM,
Anthony B. West and Thomas M. Roberts, Dana-Farber Cancer Institute, Boston, MA 02115.
A Baculovirus expression vector system is being used in our laboratory to over produce components of the human immunodeficiency virus (HIV). This system, developed in the laboratory of Max Summers, utilizes the capacity of the Baculovirus to replicate as a recombinant in cultured insect cells and synthesize large quantaties of proteins of interest. A recombinant Baculovirus strain was made with the pol sequence (minus the gag portion) from a biologically active clone of HIV. Extracts from infected cells show a high degree of reverse transriptase activity and exhibit the template and cation requirements charactersitic of the HIV enzyme. In addition, there is evidence of different sized proteolyic fragments of the peptide the enzymatic properties of which are being determined. A cDNA copy of the tat gene was also inserted in the Baculovirus and a peptide of approximatley 14 kD is being synthesized. Both proteins are being made at a level of aproximately 40-60mg/liter of infected suspension cells and are currently being purified. In vitro systems will be developed to study the enzymatic features of wild type and mutant forms of both proteins.

**P 240** THERAPEUTIC CONTROL OF HIV ANTIGENIC EXPRESSION IN NEURAL CELLS ISOLATED FROM HUMAN FETUS NERVOUS SYSTEM,
Brian Wigdahl[1], Erik De Clercq[2], and Prem S. Sarin[3], The Department of Microbiology, The Pennsylvania State University College Medicine, Hershey, PA, 17033[1], Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium[2], Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD, 20892

Human immunodeficiency virus (HIV), the primary etiological agent of acquired immunodeficiency syndrome (AIDS), has been implicated in the causation AIDS-associated neurological dysfunction and may be responsible for an increasing number of neonatal immunologic and neurologic disorders. However, as yet there is no model system available to investigate the interaction of HIV with the developing human nervous system *in vitro*. To examine the intracellular events associated with HIV infection of the human fetus nervous system we infected cells obtained by enzymatic dissociation of aborted human fetus dorsal root ganglia and their attached spinal roots and nerves. The expression of the HIV *gag* gene protein products (p17 and p24) was detected in a subpopulation of cells with a non-neuronal morphology, reaching a maximum within 3 days. Although 70% of the non-neuronal neural cells were p17- and p24-positive 3 days after infection, a majority of the cell population survived acute HIV infection, with the expression of p17 and p24 decreasing below the limit of detection by 12 days postinfection. Additional studies have demonstrated that treatment of HIV-infected human fetal neural cells with recombinant human leukocyte interferon alone or in combination with 3'-azido-2', 3'-dideoxythymidine effectively reduced the number of HIV p17/p24-positive non-neuronal neural cells at 3 days postinfection. This system may prove useful for examining the neuropathogenesis of HIV infection of the developing human nervous system and the therapeutic modification of HIV antigenic expression in human fetal neural cells.

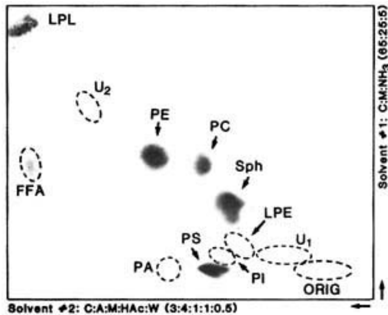
**P 241** FUSION AS A MEDIATOR OF CYTOLYSIS IN MIXTURES OF UNINFECTED CD4+ LYMPHOCYTES AND CELLS INFECTED BY HUMAN IMMUNODEFICIENCY VIRUS, B. Yoffe, D. Lewis, B. Petrie, C. Noonan, J. Melnick and F. Hollinger, Depts. Virology, Micro. & Immunol., Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030.
Acquired immune deficiency syndrome (AIDS) is caused by a retrovirus, human immunodeficiency virus (HIV), which is tropic for $CD4^+$ cells. The envelope protein, GP 120, of HIV binds to the CD4 molecule and allows entry of the virus. There is, however, a discrepancy between the numbers of infected lymphocytes found in vivo and the severe depletion of $CD4^+$ lymphocytes that occurs. We have developed an in vitro cytotoxicity assay which examines cellular interactions between uninfected lymphocytes and cells persistently infected by HIV (H9). We found that addition of $CD4^+$ but not $CD8^+$ or $CD16^+$ cells to chromium labeled H9-HIV cells resulted in lysis of the H9-HIV cells. Addition of antibody reactive to the 3a epitope but not the OKT4 epitope of the CD4 molecule was capable of blocking the lysis of H9-HIV cells. Light microscopic and autoradiographic analysis showed that prior to cytolysis of H9-HIV cells, multinucleated giant cells were formed from fusions between H9-HIV cells and large numbers of uninfected $CD4^+$ cells. Not only did the H9-HIV cells die but $CD4^+$ cells in the syncytia died as well. These data may explain the paradox that exists in vivo in which a dramatic depletion of $CD4^+$ lymphocytes occurs in the presence of a small number of HIV-infected $CD4^+$ cells. Our experiments suggest that some of the pathogenesis in HIV infection may be mediated by syncytia formation in vivo.

**P 242** CHARACTERIZATION OF AN ACTIVATION ANTIGEN EXPRESSED ON T CELLS FROM ARC AND AIDS PATIENTS, Paul Yoshihara, Andrew Goldstein, Mark Loveless, and Denis Burger, Epitope, Inc., Portland, OR 97006.
Utilizing an H9-derived HTLV-III viral extract as immunogen, we have produced a monoclonal antibody (3G12) which is reactive with peripheral T cells from sero-positive patients. Antibody 3G12 stains from 20-60% of T4-positive lymphocytes from Western Blot-positive AIDS, ARC, and asymptomatic patients as compared to less than 15% for normal Western Blot-negative controls. 3G12 monoclonal antibody is reactive with H9 cells, mitogen-activated T cells, macrophages, and B cells, but not resting T cells, or two other T lymphoma cell lines (CEM and HTL). Western Blot analysis with 3G12 showed reactivity with two bands (24 and 55 Kdal) using H9-derived viral lysates, but only one band (24 Kdal) when CEM-derived lysates were used. In addition, 3G12 antibody immunoprecipitated molecular weight structures of 20,28,33, and 55 Kdal from uninfected H9 cells. Comparision of 3G12 and anti-DR antibodies showed identical reactivity patterns against the previously mentioned cell types. However when anti-DR antibody was reacted on Western Blot against H9-derived viral lysates, only the 55 Kdal band was developed. Thus 3G12 monoclonal antibody is reactive with a structure (cellular or viral) which is not recognized by anti-DR antibodies, and which may be involved or associated with HTLV-III infection.

**P 243** T CELL-MEDIATED IMMUNITY TO HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN CHIMPANZEES IMMUNIZED WITH A RECOMBINANT VACCINIA VIRUS EXPRESSING HIV ENVELOPE GLYCOPROTEINS Joyce M. Zarling,* Jorg W. Eichberg,** Patricia A. Moran,* Jan McClure+ and Shiu-Lok Hu* *Oncogen; Seattle, WA, 98121; **Southwest Foundation for Biomedical Research; San Antonio, TX,78284; +Genetic Systems Corp.; Seattle, WA 98121.
We previously reported that a recombinant vaccinia virus, v-env5, expressing HIV envelope (env) glycoproteins, induces antibodies to these glycoproteins in several species and induces helper T cells in macaques that recognize env glycoproteins. In this study, immunization of chimpanzees with v-env5 (but not with a recombinant vaccinia virus that expresses a herpes simplex virus glycoprotein), resulted in the generation of T cells that proliferate and produce interleukin-2 in response to stimulation with HIV or with purified env glycoproteins. In addition, cytotoxic T cell (CTL) clones were isolated from PBL of v-env5 immunized chimpanzees following stimulation with env glycoproteins. The CTL clones were found to lyse autologous target cells infected with v-env5 but not with parental vaccinia virus. These results indicate that immunization of chimpanzees with a recombinant vaccinia virus that expresses env glycoproteins results in the generation of HIV specific helper T cells and CTL, and also that HIV env glycoproteins serve as target antigens for cytotoxic T cells.

## Anti-viral Strategies and Clinical Trials

**P 300** LIPID COMPOSITION STUDIES OF HIV (HTLV-III/LAV) MEMBRANES. R. C. Aloia*[1], F. C. Jensen*[2], C. C. Curtain*[3], P. W. Mobley*[4] and L. M. Gordon*[5]. *[1] J. L. Pettis V.A. Hospital & Loma Linda University, Loma Linda, CA.; *[2] Cytotech, San Diego, CA.; *[3] CSIRO, Australia; *[4] Calif. State Polytechnic University, Pomona, CA.; *[5] Rees-Stealy Research Foundation, San Diego, CA.




Although much information on HIV (HTLV-III/LAV) has accumulated, little attention has been paid to the lipid envelope. We have thus examined the lipid composition of HIV grown on Hut-78 cells. Lipid extracts were analyzed for total phosphorous and cholesterol and the phospholipid classes were separated and identified by 2-D thin layer chromatography (Figure). The cholesterol / phospholipid ratio was 0.84 and the lipid/protein ratio (mg/mg) was 0.22. The composition of the major phospholipids (mole %) was: phosphatidylcholine (PC) = 23.4%; phosphatidylethanolamine (PE) = 26.3%; sphingomyelin (Sph) = 27.5%; phosphatidylserine (PS) = 16.2%; phosphatidylinositol (PI) = 1.9%; phosphatidic acid (PA) = 0.6%. The C/P ratio and the phospholipid class distribution are similar to those of the human erythrocyte.

**P 301** IMMUNE RESPONSE IN CHIMPANZEES INOCULATED WITH AN HTLV III GP120 SUBUNIT VACCINE. L. O. Arthur[1], W. G. Robey[2], S. W. Pyle[1], J. W. Bess, Jr.[1], P. L. Nara[2], R. V. Gilden[1], T. J. Matthews[3], D. P. Bolognesi[3], and P. J. Fischinger[2]. [1]Program Resources, Inc., NCI-Frederick Cancer Research Facility (FCRF)), Frederick, MD 21701; [2]Virus Control Unit, Office of the Director, NCI-FCRF, Frederick, MD 21701; [3]Duke University Medical Center, Durham, NC 27710.
The chimpanzee is the only animal found to be reproducibly infectable with HTLV III/LAV, also known as Human Immunodeficiency Virus (HIV), and as such will be the animal model for HIV vaccine studies. We have prepared a prototype subunit vaccine consisting of the 120,000 dalton external outer envelope glycoprotein (gp 120) of HIV. This gp120 outer envelope glycoprotein was purified from membranes of HTLV-IIIb-infected H9 cells by immunoaffinity chromatography as previously described (Robey, et al., PNAS 83:7023, 1986). The purified gp120 gave a single band on SDS-PAGE and the major internal HIV antigen, p24, was not detected in the vaccine preparations when examined in p24 competition radioimmunoassays. Chimpanzees which received three inoculations (50 ug each) of gp120 prototype vaccine developed antibodies which precipitated the gp120 and neutralized HIV in *in vitro* infectivity assays. Antibody to the major core antigen, p24, was not detected in the vaccinated chimpanzees. HIV viral stocks have been prepared and titered *in vitro*, and after *in vivo* infectivity titration in chimpanzees, will be used to challenge the vaccinated animals. Vaccination and challenge with the homologous virus would complete the first step in evaluating HIV vaccination effectiveness. If successful, the ability of vaccinated animals to resist challenge with a heterologous HIV will be assessed.

**P 302** FAILURE TO DETECT ANTILYMPHOCYTIC ANTIBODIES (ALA) IN AIDS. G.N. Beall, S. Lal, O. Sattentau, I. Weller and P.C.L. Beverley, Harbor-UCLA Med Ctr, Torrance CA 90509, The Middlesex Hospital Medical School, and ICRF, School of Medicine, University College, London.
Several studies have produced evidence for ALA in AIDS. To further study ALA, we attempted to demonstrate them by immunofluorescent (IF) flow cytometry. Normal human peripheral blood mononuclear cells (PBMC) and the T cell line, CEM, were incubated with sera from 21 patients with AIDS and 10-HIV seronegative blood donors. ALA were not detected in the AIDS sera with IF-rabbit $F(ab)_2$ anti-human gamma, intact anti-mu, or anti-alpha. A small number of CEM cells, 2%, fluoresced, with either AIDS or normal serum. Also, PBMC did not discriminate between AIDS and normal serum although a larger proportion of PBMC were IF. We were able to detect ALA from patients with SLE with both CEM and PBMC. In contrast, incubation of either CEM or PBMC with AIDS sera, and to a lesser degree, normal sera, enhanced the binding of IF intact rabbit anti-gamma. Anti-gamma was not bound by CEM cells unexposed to human serum. The binding was blocked by rabbit Ig, demonstrable in CEM fixed in 1% formalin, unrelated to the density of CD4 on CEM cells, and could not be blocked with monoclonal anti-CD4. Conclusion: We have failed to demonstrate ALA in AIDS sera with reagents that identify ALA in SLE. AIDS sera promote non-specific binding of anti-gamma to CEM and PBMC.

**P 303** HEMATOLOGIC TOXICITY OF AZIDOTHYMIDINE (AZT),
S. Bozzette, R. Fleck, C. Stayboldt, C. Kennedy, J.A. McCutchan, S. Spector, and D. Richman, University of California, San Diego, CA 92103

To study the hematologic toxicity of AZT, 5/5 AIDS and 1/11 ARC patients receiving 1500 mg/d AZT in a controlled trial underwent a total of 8 bone marrow examinations (AZT-BM). 7 AZT-BM were performed for anemia and 1 for thrombocytopenia. There was a mean 12 week interval (range 6-18 weeks) between the initiation of therapy and bone marrow examination. In this same period we observed ↓ mean Hgb (10.8→6.5), ↑ mean MCV (88→108), ↓ mean WBC (3100→2200), ↓ mean absolute neutrophil count (1900→1000), and ↑ mean platelets (119,000→165,000). Bone marrow biopsy, clot, and aspirate were examined by 2 blinded and independent observers. 8 AZT-BM were compared to control bone marrows (CON-BM) from 8 AIDS or ARC patients not receiving AZT. AZT-BM in comparison to CON-BM revealed marrow aplasia (1 vs. 0), erythroid hypoplasia (5 vs. 2), megaloblastic erythropoiesis but not myelopoiesis (4 vs. 1), plasma cell hyperplasia (7 vs. 4), eosinophilic hyperplasia (5 vs. 2), granulomas (0 vs. 3), acid fast bacteria (0 vs. 3), and lymphoid aggregates (0 vs. 2). In summary, an increased incidence of anemia and leukopenia in the peripheral blood and megaloblastic erythroid hypoplasia in the bone marrow are the most striking hematologic findings in patients on AZT.

**P 304** MODIFICATION OF HIV ENVELOPE LIPIDS, PROTEIN STRUCTURE AND INFECTIVITY BY AL721, A UNIQUE LIPID MIXTURE,
F.T. Crews, J. Laurence, D.I. Scheer, M.E. Weksler, P. Sarin, C. Klepner and A.S. Lippa, Univ. of Florida Medical School, Gainesville, FL, Cornell Univ. Medical School, New York, NY, Yale University, and Praxis Pharm. Inc.

Envelope virions are known to be surrounded by a lipid membrane scavenged from the host cell during budding. Viral attachment proteins are embedded within this viral membrane. Analysis of sucrose gradient-purified HIV (human immunodeficiency virus) demonstrated that the virus was approximately 50% lipid by mass. The major lipids were cholesterol (chol) and phospholipids (PL) in a molar ratio of approximately 0.6. AL721, a unique mixture of lipids designed to modify lipid membrane properties, alters HIV lipid composition, reducing the chol:PL molar ratio to 0.37. Fluorescent polarization measurements indicate that AL treatment markedly reduces the apparent microviscosity of the HIV membrane. Spectra of endogenous tryptophan residues in HIV proteins indicate that AL treatment modifies viral protein structure. Taken together, these studies suggest that AL alters HIV envelope lipids, resulting in an altered envelope environment for the HIV cell attachment protein. Since the membrane environment is known to alter protein conformation/orientation it is possible that the change in the HIV membrane has disrupted the structure of the HIV cell attachment protein.

When AL721 was incubated in the presence of both HIV and host cells, infectivity of both mitogen-stimulated human peripheral blood lymphocytes and H9 cells was inhibited with an $ED_{50}$ of approximately 0.1 mg/ml. Repeated administration of AL721 for several days following an initial infection of cells resulted in a complete loss of reverse transcriptase activity from the infected cultures. AL721 added to the reverse transcriptase assay had no effect on activity. We suggest that AL721 may block both virion-cell and cell-cell infection. It is hypothesized that the 110-120kD cell attachment protein of HIV requires a membrane environment which is disrupted by AL721 such that infectivity is prevented.

**P 305** RIBAVIRIN TREATMENT OF AIDS AND ARC SUPPRESSES HIV AND ENHANCES LYMPHOCYTE PROLIFERATION.
C. Crumpacker[1], W. Heagy[2], C. Andrews[3] G. Bubley[1], J. Munroe[3], B. Finberg[2], M. McLean[4], S. Hussey[1], C. Mulder[3], M. Essex[4], Dept. of Medicine Harvard Medical School, Beth Israel Hospital[1], Dana-Farber Cancer Institute[2], U. Mass. Medical Center[3], Harvard School of Public Health[4].

Oral Ribavirin (RBV) in a single dose regimen was given for eight weeks. to 10 patients with AIDS and 5 patients with ARC in a Phase 1 Study. Drug was well tolerated and all but one patient completed the 8 week study. Two patients required transfusions for anemia during drug treatment. In 7 of 9 patients, initially positive for HIV in blood at start of treatment as detected by co-cultivation with normal lymphocytes and induction of RT and a rigorous RNA-DNA hybridization assay, virus was not detected after 2 weeks of RBV treatment. Virus remained absent in blood during 8 weeks of RBV treatment but returned in 5 of 7 on stopping drug. Lymphocyte proliferation assays to PHA or Con A improved in all patients with RBV treatment and T4 levels transiently increased with RBV but returned to baseline on stopping drug. Skin test reactions returned in 3 of 15 patients.

During RBV treatment and follow-up AIDS patients had fewer opportunistic infections, especially PCP infections but 2 of 5 ARC patients progressed to AIDS. This phase one study indicates RBV is safe and well tolerated for AIDS and ARC patients. RBV treatment is associated with suppression of HIV and enhancement of lymphocyte proliferation.

**P 306** *HIV (HTLV-III/LAV) STABILITY: ROLES FOR LIPID COMPOSITION AND FLUIDITY.*

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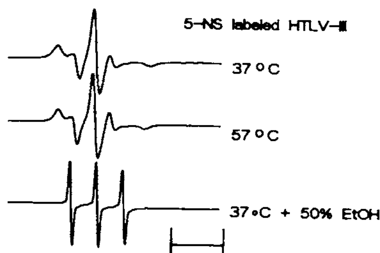
The fluidity of the HIV lipid envelope was studied with Electron Spin Resonance (ESR) techniques. Labeling of intact virions with 5-nitroxide stearate (5-NS) and 16-nitroxide stearate suggests that the lipids are arranged in a bilayer, with the envelope interior more fluid than the surface. ESR spectra of 5-NS labeled HIV show that the fatty acid probe rotates about its long axis, with limited flexibility (Fig.; horiz. bar = 30 G). The fluidity of 5-NS labeled HIV is low at 37°C, similar to other retroviruses and red cells, and is probably due to the high C/P. Ethanol completely disrupts the envelope, contributing to its rapid inactivation of HIV. Contrarily, heating to 57°C causes much less fluidization, and this may play a role in the slower viral inactivation seen at high temperatures. There may be a critical "minimum" ordering due to high C/P that is necessary for viral stability. This supports the view [Sarin et al. (1985) N Engl J Med 313, 12898] that the liposome AL 721 reduces HIV infectivity by fluidizing the envelope through cholesterol extraction.




**P 307** METABOLISM AND ANTI-HIV ACTIVITY OF 2-HALO-2',-3'-DIDEOXYADENOSINES,

T. Haertle, C.J. Carrera, J.S. McDougal, D.D. Richman and D.A. Carson, Scripps Clinic and Research Foundation, La Jolla, CA 92037; Department of Medicine, University of California San Diego, La Jolla, CA 92037; Center for Disease Control, Atlanta, GA 30333

The normal human peripheral blood T lymphocytes that are potential targets of HIV infection have minimal thymidine kinase activity, but abundant deoxycytidine (CdR) kinase activity. Deoxyadenosine and related compounds are substrates for CdR kinase, and are selectively phosphorylated by human T cells. Mitsuya and Broder showed that 2',3'-dideoxyadenosine (ddA) has potent anti-HIV activity. However, ddA is degraded rapidly by adenosine deaminase (ADA). We have synthesized the 2-Fl, 2-Cl, and 2-Br derivatives of ddA, and have analyzed their metabolism and anti-HIV activity in T cells. Unlike ddA, the halogenated derivatives are resistant to ADA. Cultured human CEM T lymphoblasts converted the ddA congeners to the respective 5'-triphosphate derivatives. Experiments with a CdR kinase deficient mutant T cell line showed that phosphorylation was catalyzed exclusively by this enzyme. At concentrations from 3-20 μM, the 2-halo-ddA derivatives inhibited HIV-induced cytopathology in MT-2 T lymphoblasts. The deoxynucleosides also blocked infection of peripheral blood T cells with HIV, as detected by the release of viral antigens. These results demonstrate that the 2-halo-2',-3'-ddA derivatives are metabolically stable and have *in vitro* anti-HIV activity.

**P 308** RAPID PHOTOCHEMICAL INACTIVATION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV),

Carl Veith Hanson, California Department of Health Services, Berkeley CA 94704.

HIV-infected cells and intact HIV virions are potentially hazardous to laboratory personnel who process such materials and use them as antigens in immunoassay or immunization procedures. Traditional virus inactivation techniques unfortunately alter viral proteins and other surface components to varying extents -- thus compromising the immunoreactivity or other desired properties of the material. We have found that HIV, on the other hand, is exceptionally sensitive to inactivation by photoreaction with 4'-aminomethyl-4,5',8-trimethylpsoralen ("AMT"). A $10^5$-fold inactivation is achieved in 30 seconds or less. AMT readily passes through viral coats, and in the presence of longwave (not germicidal) ultraviolet light forms covalent adducts with nucleic acid inside intact virions. Since antigenicity, enzyme activities and other non-nucleic acid properties are not detectably modified by the photoreaction, large safety margins may be created by treatments vastly in excess of that required to eliminate detectable infectivity. Even crude cultures may be successfully inactivated prior to processing, and the kinetics of inactivation are the same for cell-free and cell-associated infectivity. We routinely use the technique for the safe preparation of HIV antigen for use in EIA, Western blot, monoclonal antibody development and the preparation of immunofluorescence slides containing inactivated infected cells.

**P 309** CHARACTERIZATION OF A NEW HTLV-I INFECTED CELL LINE WHICH IS SUCEPTIBLE TO HTLV-III AND ITS APPLICATION. Toshio Hattori, Atsushi Koito, Kenji Shirono, Shuzo Matsushita, Masao Matsuoka, Norio Asou, Kiyoshi Takatsuki. Kumamoto University Medical School, Kumamoto Japan '
Interleukin-2 independent HTLV-I infected cell line (SKT-1B) was established from a pateint with adult T cell leukemia (ATL). HTLV-III (100 fold concentrates of culture supernatants of H9/HTLV-III) caused a marked cytopathic effect (100% at day 6) and a rapid <u>gag</u> antigen expression (10% at day 3) on this cell line and these effects of HTLV-III were more prominent than another HTLV-I carrying cell line (MT-2) and HTLV-I non infected cell line (H9). The pattern of T cell receptor β chain gene rearrangemet of SKT-1B was the same as that of fresh ATL cells, confirming that SKT-1B is derived from ATL cells. Surface phenotypic analysis showed that SKT-1B had four fold expression of CD4 antigen than that of H9 and the expression of HTLV-I related antigens examined by a serum from a patient with ATL and a human monoclonal antibody against envelope protein of HTLV-I (0.5α) was lower than that of MT-2. Lyophilized HTLV-III was heated with lyophilized factor VIII concentrates and residual activities of HTLV-III was assessed. The results showed that HTLV-III retained its infectivities even after heating for two hours at 65°C. Thus it was implicated that long-time heating is necessary to inactivate HTLV-III in lyophilized states.

**P 310** THE EFFECTS OF RIBAVIRIN and rIFN-γ ON THE PROLIFERATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS, Wyrta Heagy, Clyde Crumpacker, Phillip Toltzis and Robert Finberg, Laboratory of Infectious Diseases, Dana Farber Cancer Institute; Division of Infectious Diseases, Beth Israel Hospital; and Depts. of Medicine, Pediatrics, and Pathology, Harvard Medical School.

The combined effects of interferon-γ (rIFN-γ) and ribavirin (RBV) on cultures of lectin-stimulated peripheral blood mononuclear cells (PBMC) were investigated. RBV has been shown to inhibit HTLV-III infection of human peripheral blood lymphocytes and to decrease viral protein synthesis in infected T cells. The addition of RBV to lectin-stimulated cultures of PBMC isolated from AIDS and normal donors inhibited lymphocyte proliferation. Cellular DNA content, measured by propidium iodide staining and cytofluorometry, and the incorporation of $^3$H-Thymidine and $^{14}$C-Leucine were reduced in cells incubated with the antiviral agent. The effects of RBV were dose dependent; the incorporation of $^3$H-Thymidine and $^{14}$C-Leucine was inhibited $\geq$ 50% in lymphocytes which received 10-40 µg/ml of the antiviral agent. The addition of *E. coli* derived human rIFN-γ to cultures of PBMC reversed the lymphocyte inhibition in low concentrations (0.1-10 µg/ml) of RBV. In concentrations of the antiviral agent $\geq$ 10 µg/ml lymphocyte responsiveness to mitogens was only partially restored by rIFN-γ.

<u>The Effects of RBV & rIFN-γ on the Incorporation of $^3$H-Thymidine (cpm x $10^3$) in Cultures of PBMC</u>

| | | Con A | RBV + Con A | rINF-γ + Con A | RBV + rIFNγ + Con A |
|---|---|---|---|---|---|
| *Conc* | | (10 µg/ml) | (1 µg/ml) (10 µg/ml) | (500 U/ml) (10 µg/ml) | (1 µg/ml) (500 U/ml) (10 µg/ml) |
| <u>Subjects</u> | | | | | |
| *Normals*: | (a) | 30.7 ± 5.7 | 17.4 ± 0.7 | 27.4 ± 3.2 | 30.6 ± 2.7 |
| | (b) | 34.4 ± 1.8 | 18.6 ± 1.1 | 44.2± 1.1 | 32.0 ± 2.5 |
| *AIDS:* | (a) | 12.8 ± 1.4 | 6.1 ± 0.5 | 19.7 ± 1.5 | 15.4 ± 2.7 |
| | (b) | 17.2 ± 2.8 | 4.6 ± 1.6 | 25.1± 3.3 | 10.8 ± 4.1 |

These preliminary data suggest that optimal therapy for HTLV-III infections may require combinations of antivirals and immunomodulating agents.

**P 311** DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIGEN DURING ACUTE AND CHRONIC INFECTION, Bjarne Lindhardt*, Edgar Lauritzen£, Kay Ulrich*, and Bo Hofmann$, *The Fibiger Institute, £Statens Seruminstitut, and $Rigshospitalet, Copenhagen, Denmark.
To estimate the frequency of HIV antigenemia during acute and chronic infection, we used a sandwich enzyme linked immunosorbent assay (ELISA) to detect HIV antigen in serum from 25 persons seroconverting, 10 patients with AIDS, 10 ARC patients, 40 seropositive healthy homosexual males, and 10 seropositive blood donors. Among the seroconverters, 8 patients had more than 4 months separating the latest antibody negative and the first antibody positive serum, and none of these had HIV antigenemia. In the remaining 18 patients, HIV antigen was detected in 9 (50%). In 7 of these antigen was detected within 120 days before the first antibody positive serum, which in all were antigen negative. Two patients had antigenemia 25 and 30 days before and during seroconversion. In one of these, the antigen disappeared during the first week after seroconversion. The other patient was lost for follow up. Accordingly, HIV antigen was detected in 80% of AIDS patients, in 60% of ARC patients, in 10% of the asymptomatic seropositive males, and in none of 10 blood donors identified at regular blood bank screening. These findings illustrate a high degree of HIV antigenemia in acute and symptomatic HIV infection, findings having important implications regarding seronegative high risk individuals and the blood donor screening.

**P 312** EVALUATION OF DIDEOXYCYTIDINE AS AN ANTIVIRAL DRUG FOR PREVENTING FELINE LEUKEMIA VIRUS INFECTION IN CATS, Lawrence E. Mathes, Phyllis Polas, Gary Kociba, Cheryl Swenson, Richard Sams, Richard Olsen, Ohio State University, Columbus, OH 43210.

In this study the pharmacokinetic, toxicity and in vitro and in vivo prophylactic effects of dideoxycytidine (DDC) were evaluated in the feline leukemia virus (FeLV)/cat animal model. In in vitro studies DDC inhibited FeLV infection of 3201 cells at drug concentration of 0.4 µg/ml medium. The pharmacokinetic values for DDC were determined in 8 week old cats. The mean clearance and half-life for DDC was 5.8 ml/min/Kg and 54.6 min respectively. Using these values a continuous infusion regimen was designed to establish steady state plasma levels of between 0.5 and 1 µg/ml. DDC was delivered using implanted osmotic pumps (Alza, Palo Alto, CA) at a rate of 2.5 µl/hr. DDC was mildly toxic at plasma concentrations of 0.5 to 2 µg/ml, inducing regenerative anemia in 2 of 4 cats during the first week after pump implantation. This condition resolved within the following week. One week after DDC treatment began, 3 of 4 cats were challenged with infectious FeLV by I.P. route. Challenged cats had DDC plasma levels between 0.4 and 0.9 µg/ml. In the face of these DDC levels all challenged cats developed chronic FeLV viremia. These results indicate that DDC at plasma steady state concentrations of between 0.9 µg/ml does not prevent the establishment of FeLV disease.

**P 313** PHOSPHOROTHIOATE ANALOGS OF OLIGODEOXYNUCLEOTIDES : NOVEL INHIBITORS OF REPLICATION AND CYTOPATHIC EFFECTS OF HTLV-III/LAV (HUMAN IMMUNODEFICIENCY VIRUS)
Makoto Matsukura, Kazuo Shinozuka, Gerald Zon, Hiroaki Mitsuya, Jack S. Cohen, and Samuel Broder, National Cancer Institute and Food and Drug Administration, Bethesda, MD 20892
Nuclease-resistant phosphorothioate analogs of several oligodeoxynucleotides have been synthesized, and tested in vitro for anti-viral activity against HTLV-III/LAV on human T-cells. Two anti-sense sequences (14-mers) complementary to the HTLV-III/LAV genome, a sense sequence, a random sequence, and homo-oligomers of dA and dC of two lengths (14 and 28-mers) exhibited a significant inhibitory effect upon viral replication and cytopathogenicity under conditions of viral excess. The anti-viral activity was strikingly linear with GC content for the 14-mers, the longer phosphorothioate analogs were more effective than the shorter ones. None of homologous sequences of unmodified normal oligomers, methylphosphonate analog, and N-methylthymine-containing phosphorothioate analog showed any anti-viral effects. These results suggest that the anti-viral effect of phosphorothioate analogs of deoxynucleotides is brought about by binding to certain viral component(s), possibly viral nucleotide-sequences. We have also observed that 14-mer homol-oligomer of deoxycytidine phosphorothioate synergistically enhanced the anti-viral effect of 2',3'-dideoxyadenosine. These data suggest that phosphorothioate analog of oligodeoxynucleotide could be a novel class of therapeutic agent against acquired immunodeficiency syndrome(AIDS) and related diseases.

**P 314** HTLV-REGULATED EXPRESSION OF A TRANSFECTED DIPHTHERIA TOXIN GENE, Ian H. Maxwell, L. Michael Glode and Françoise Maxwell, University of Colorado Health Sciences Center, Denver, Colorado 80262.
Transfection of a diphtheria toxin A chain gene (DT-A), linked with appropriate transcriptional regulatory sequences, may enable selective killing of specific cell types (Maxwell, Maxwell and Glode: Cancer Res. 46:4660 (1986)). We are exploring the use of HTLV trans-activation systems for directing toxin expression to HTLV infected cells. In initial experiments we have employed HTLV II regulatory elements to take advantage of the low basal expression level observed from the HTLV II LTR (Sodroski et al.: Science 225:381 (1984)). An expression plasmid, pTH9, containing DT-A attached to this LTR was transfected into Raji cells expressing or not expressing the HTLV II tat product (Rosen et al.: J. Virol. 57:379 (1986)). The presence of this trans-activator resulted in several fold increased toxin expression from pTH9, as indicated by a transient assay (inhibition of gene expression from a co-transfected plasmid). However, the observed differential in expression of DT-A due to the tat II product was much less than that of CAT activity from an HTLV II LTR driven cat gene (pU3-II; Sodroski et al.). Transcript mapping experiments are in progress with a view to explaining this difference and to increasing the tat II dependence of DT-A expression. Trans-activator-controlled toxin gene expression might eventually provide a novel therapy for HTLV-induced diseases by enabling the elimination of malignant cells or of virus-infected cells prior to viral replication.

**P 315** 2',3'-DIDEOXYNUCLEOSIDES: BROAD SPECTRUM ANTIRETROVIRAL ACTIVITY AND MECHANISM OF ACTION, Hiroaki Mitsuya, Shuzo Matsushita, John E. Dahlberg, Ruth F. Jarrett, Makoto Matsukura, Michael J. Currens, Stuart A. Aaronson, Marvin S. Reitz, and Samuel Broder, National Cancer Institute, Bethesda, MD 20892.
Pyrimidine and purine dideoxynucleoside analogues can significantly inhibit the in vitro replication and/or cytopathogenicity of a wide range of human and animal retroviruses at concentrations that do not inhibit the growth and functions of target cells. Dideoxynucleoside analogues block the infection of HTLV-III/LAV in vitro against T-cells and one such analogue, 3'-azido-3'-deoxythymidine (AZT) has been shown to be effective in patients with AIDS. We have learned that dideoxynucleoside analogues including AZT can block the infectivity of HTLV-I as well as HTLV-IV against helper/inducer T-cells in vitro. These analogues also inhibit the infectivity of animal lentiviruses (caprine arthritis and encephalitis virus, equine infectious anemia virus) by more than five orders of magnitude. Dideoxynucleoside analogues appear to block reverse transcription from viral RNA to viral DNA by acting as DNA chain-terminators. Under certain conditions, dideoxynucleosides can block the viral DNA synthesis and viral mRNA expression in cells exposed to HTLV-III/LAV. Both purine and pyrimidine dideoxynucleoside-5'-triphosphates serve as substrates for the HTLV-III/LAV reverse transcriptase to elongate a DNA chain by one residue, after which the chain is terminated. These analogues strongly inhibit HTLV-III/LAV reverse transcriptase activity but much less mammalian DNA polymerase alpha. These results suggest that dideoxynucleoside analogues could be promising agents for further studies in the treatment of patients with human retrovirus-related diseases.

**P 316** CONTROL OF VARIOUS VIRALLY INDUCED DISEASES BY IMMUNE STIMULATING PROPERTIES OF UKRAIN, J.W. Nowicky, M. Greif, F. Hamler, W. Hiesmayr, W. Staub, Ukrainian Anti- Cancer Institute, Laimgrubengasse 19/5, A - 1060 Vienna, Austria.
Antiviral properties of the immune stimulating agent Ukrain were first noticed during clinical studies on oncological patients. In such patients, no matter whether they are chemotherapeutically treated or not, intercurrent viral infections are frequent. The reason for this lies in natural and/or artificially induced immunological disturbances. Beyond simple influenca-like infections viral diseases, mainly caused by the herpes group, may rise severe problems in adjuvant therapy of immune deficient patients. Whenever Ukrain therapy was applied, the signs of viral infection diminished rapidly. A secundary antibiotic effect was also noticed in bacterial and mycotic infections. One dog treated with Ukrain because of leukemia possibly caused by retroviruses showed normal leucocyte values after Ukrain therapy. One HTLV 3 patient with obvious signs of AIDS was treated seven times with small quantities of Ukrain. Compared with the first monoclonal evaluation of the T-lymphocyte subpopulations there was an increase by ten percent in T-Helpers about one month after Ukrain therapy.

**P 317** THYMOSTIMULIN TREATMENT IN ARC PATIENTS, Lucia Palmisano, Instituto di Ricerca C. Serono, Rome, Italy; Massimo Galli, Adriano Lazzarin amd Mauro Moroni, Dept. of Infectious Diseases, University of Milan, Milan, Italy; Francesco M. Gritti and Enzo Raise, Dept. of Infectious Diseases and Teodoro Chisesi, Dept of Heamatology, Ospedale Maggiore, Vicenza, Italy; Fernando Aiuti, Dept. of Allergy and Clinical Immunology, University "La Sapienza", Rome, Italy.
Fifty-four Italian patients suffering from ARC were included in an open, comparative trial; 34 of them were given thymostimulin (TP-1), 1 mg/Kg/die for two weeks, then twice weekly up to 6 months. The remaining 24 patients served as controls, receiving no immunoactive treatment. Follow-up lasted one year. Results: a significant difference between the 2 groups was seen regarding total leukocytes and absolute number of lymphocytes ($p=0.008$ and $0.015$), whereas borderline signifcancies were seen for OKT3 and OKT4 values. Delayed hypersensitivity skin tests (Multitest) showed a highly significant ($p=0.0001$) difference in favour of thymostimulin group, after 12 months. Clinically a significant improvement was observed among treated patients as far as lymphadenopathy, weight loss and fatigue are concerned. These results, though preliminary, induced us to start a multicenter, randomized trial in ARC. An interim analysis on 100 patients, evaluated after 6 months of follow-up, will be presented.

**P 318** LOW MOLECULAR WEIGHT PEPTIDES WHICH BIND TO THE CD4 RECEPTOR AND INHIBIT HIV BINDING AND INFECTIVITY,

C.B. Pert*, M.R. Ruff**, J.M. Hill*, R.M. Berman*, W.G. Robey[†], L.O. Arthur[††], F.W. Ruscetti[†] and W.L. Farrar[†], *Clinical Neuroscience Branch, NIMH, **Laboratory of Microbiology and Immunology, NIDR, NIH, Bethesda, Maryland 20892, [†]National Cancer Institute, NIH and [††]Program Resources, Inc., FCRC, Frederick, Maryland 21701-1240.

We find that a 60 kD protein, immunoprecipitable by Mab OKT4, is present on membranes from human brain as well as human T cells. Furthermore, the HIV envelope glycoprotein ($^{125}$I-gp120) can be specifically covalently affixed to a molecule present on rat, monkey and human brain membranes to yield a complex which is indistinguishable from that formed on human T cells. T4 antigen has been studied on unfixed squirrel monkey, rat and human brain sections by autoradiography using the Mab OKT4. A highly conserved neuroanatomical pattern has been demonstrated suggesting an analogous organization in these three mammalian brains. Furthermore, the localization of $^{125}$I-gp120 receptor binding appears similar to that of T4 and is highly reminiscent of patterns for many previously characterized neuropeptide receptors.
A computer-assisted analysis of the viral envelope glycoprotein (gp120) suggested that a previously unremarkable octapeptide sequence within the gp120 protein, which we have synthesized and termed "peptide T", may play an important role in HIV attachment. Thus, peptide T and three rationally designed peptide analogs, each with a systematic amino acid substitution, potently inhibit specific $^{125}$I-gp120 binding to brain membranes. Additionally, when tested in a viral infectivity assay these peptides show the same rank order and similar absolute potency to block HIV infection of human T cells. Peptide T may thus provide a useful pharmacological or immunological basis for the control and treatment of AIDS.

**P 319** IMMUNOASSAYS FOR THE DETECTION OF HIV ANTIGEN AND ANTIBODY IN PATIENT SAMPLES.

K.J. Reagan, J.E. Neumann, C.M. Crane, K.R. Huskins, S.M. Wos, S.B. Lambert, and S.R. Harris, Medical Products Dept., DuPont Co., Wilmington DE 19898

A sensitive microplate ELISA to measure p24 core antigen has been developed. The assay was originally validated for detection of viral antigen in cultures of infected leukocytes as a replacement for the reverse transcriptase assay. With minor protocol changes, the ELISA functions to detect p24 in serum/plasma. The sensitivity for purified p24 in plasma was determined to be 0.03 ng/ml (6 pg/sample). This ELISA will detect antigen in specimens that are negative for anti-p24 as well as in those which possess anti-p24 but are in antigen excess. Results will be presented which demonstrate the performance with serum/plasma from low and high risk individuals. The presence of anti-p24 in serum/plasma causes inhibition of the ELISA presumably via immune complex formation. Among specimens confirmed positive for anti-p24 by western blot, complete recovery of spiked antigen required sample dilution ranging up to 1:10E+6. Therefore, application of this or similar assays to screen donated blood is both incomplete and misleading without defining the HIV antibody status.
Adaptation of HIV enzyme amplified immunoassays to membrane surfaces designed to function without automation is underway. HIV antibodies were monitored using recombinant proteins, one specific for a portion of the viral envelope and another from the gag region. The proteins were adsorbed onto separate areas of a nitrocellulose strip. HIV antigen was similarly monitored by sandwich immunoassays on nylon membranes. Two assays were devised, one to detect the major core protein, p24, and the other to detect envelope glycoproteins, gp120 and gp41. Measurement of p24 has been the more sensitive method.

# Human Retroviruses, Cancer and Aids: Approaches to Prevention and Therapy

**P 320** ANTIBODIES TO X- AND POL-RELATED PROTEINS OF HUMAN HEPATITIS B VIRUS,
Marietta Stemler[+o], Xiao-Huan Liang[o], Julia Hess*, Rüdiger Braun*, Hans Will[+], and Claus H. Schröder[o], [o]Institut für Virusforschung, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg, FRG, *Institut für Medizinische Virologie der Univ. Heidelberg, Im Neuenheimer Feld 324, 6900 Heidelberg, FRG, and [+]Max Planck-Institut für Biochemie, Am Klopferspitz, 8033 Martinsried, FRG.
In vitro synthesized Hepatitis B Virus proteins have successfully been used for the identification of antibodies in human sera which are directed against the gene product of open reading frame X (ORF X). It has been speculated that X-specific antibodies may represent a marker common to HCC patients. Using the same methodology we examined samples of human serum for the presence of antibodies directed against gene products of ORF X or of ORF P the latter of which is assumed to encode the endogenous DNA polymerase of HBV. Different parts of the reading frames were cloned in two vektor systems that allow the expression of cloned sequences as part of MS2- or ßgal-fusion proteins respectively. Antibodies reacting with these fusion proteins were screened for in immunoblot analyses. Competition experiments in which sera were preincubated with individual fusion proteins were carried out in order to demonstrate the specificity of signals obtained. Three different sets of human sera were analysed: i) sera of 60 patients with markers of a prior HBV infection but negative for HBsAg ii) sera of 20 acutely or chronically infected patients and iii) sera of 22 HBsAg positive HCC patients. The fraction of anti-HBx positive sera in the first two groups did not exceed 25%. Among the 22 sera of HCC patients only two displayed a specificity for X fusion proteins. Based on these findings X-specific antibodies do not appear to be serum markers common to HCC patients. Two of the sera with markers of a prior infection and one serum of a chronically infected patient reacted in immunoblot analyses with a protein synthesized in E. coli that contains the middle part of the predicted pol protein. Other E. coli fusion proteins containing overlapping or different regions of ORF P were used in order to localize roughly pol epitopes. The serum of the chronically infected patient displayed a reactivity pattern indicating that domains including the C-terminus of the predicted pol protein are immunogenic whereas the other two sera reacted with a restricted part only. Our findings are further indirect evidence for the in vivo expression of pol frame related proteins.

**P 321** EFFECT OF AZIDOTHYMIDINE (BW A5090) ON HIV INFECTION OF H9 CELLS IN CULTURE.
Marilyn S. Smith, E. Leigh Brian, and Joseph S. Pagano, University of North Carolina, Chapel Hill, NC, 27514
The new anti-retroviral agent, AZT (BW A509U), is currently the most successful drug in clinical use for AIDS patients. The effect of this thymidine analogue, 3'-azido-3'-deoxythymidine, on the replication of the LAV strain of the HIV was evaluated using susceptible H9 cells. Cells were pretreated with concentrations of drug ranging from 0.1 to 100 uM, infected and maintained in medium containing drug. Virus production was assayed by reverse transcriptase assays, and virus-specific DNA was analyzed by Southern blots probed with cloned LAV sequences. By 4 days post infection, the infected cells without drug reached a peak of RT activity that was sustained. Increasing concentrations of AZT caused increasing delays in virus production; however, all replicate cultures at non-toxic levels of the drug (up to 25 uM) eventually produced as much virus as the non-drug-treated infected cells despite the continued presence of drug. Levels of intracellular unintegrated viral-specific DNA paralleled the reverse transcriptase levels. Virus-caused cytopathic effect was likewise delayed in drug-treated cultures. We are currently examining the virus produced at the higher levels of drug to determine whether resistance to AZT has developed.

**P 322** CLINICAL IMPROVEMENT OF PATIENTS WITH HIV-RELATED IMMUNE DYSFUNCTION ON MISMATCHED dsRNA (AMPLIGEN) THERAPY.
D.R. Strayer, W.A. Carter, I. Brodsky, L. Einck, G.L. Simon, R.S. Schulof, and H.F. Henriques. Dept. of Neoplastic Diseases, Hahnemann Univ., Phila., PA 19102; HEM Research, Inc., Rockville, MD 20852; and Dept. of Medicine, George Washington Univ., Wash., D.C.
Seven ARC/pre ARC and three AIDS patients were treated with 200-250 mg IV infusions of mismatched dsRNA, poly(I)-poly($C_{12}$,U), twice weekly for 8-16 weeks. All ten patients demonstrated restoration of delayed-type hypersensitivity (DHR) within 2-7 weeks. Two patients became virus negative as measured by lymphocyte co-culture. Seven patients had quantitative reductions in HIV polymerase gene expression as measured by nucleic acid hybridization. 2/5 patients showed quantitative decreases in viral p24 antigen in serum (Abbott Labs) during treatment. Decreases in viral parameters by one or more methods were demonstrated in 8/10 patients. 2/2 patients demonstrated 5 fold increases in HIV neutralizing antibody titers. T4 cells were stable or increased in 10/10 patients. 7/8 patients reported symptomatic improvement and increases in activity levels. Reductions of lymphadenopathy were noted in 3/5 patients. Complete reductions of enlarged parotid, spleen, liver and lymph nodes were documented in one patient. Another patient cleared a chronic oral yeast infection. The only opportunistic infection occurred in a patient (AIDS-post PCP) who died following a second PCP. No side effects or drug toxicity were observed. Significant clinical improvements, return of DHR, reductions in HIV viral parameters, and the absence of side effects/drug toxicity, suggest mismatched dsRNA is a candidate for enlarged clinical trials in HIV infected individuals.

**P 323** AIDS IN HOMOSEXUALS WITH SPINDLE-ENDOTHELIAL CELL ABNORMALITIES AND WITH MELIOIDOSIS, Deja Tanphaichitra, S. Sahaphong, Mahidol-Ramathibodi University. Medical Center, Bangkok 10400, ThailandePOBox 4-217, Bangkok Thailande

AIDS is clinically characterised by fatal opportunistic infections such as Pneumocystis carinii, Cryptosporidium and/or malignancies, notably Kaposi s sarcoma. Here we described the first case of AIDS with early Kaposi's sarcoma like lesions in homosexual male drug addict and have compared the clinical and laboratory findings with those of another homosexual male having melioidosis due to Pseudomonas pseudomallei Both cases had positive anti-HTLV-III/LAV or HIV antibodies and reversed T-helper/T-suppressor subset ratios. Both had positive antibodies to CMV and EBV.

| | Case1 | Case2 |
|---|---|---|
| Abnormal Chest X-Rays | Rt. basilar lesion | Interstitial pneumonitis |
| Lung biopsy | Not done | Intersitial pneumonitis |
| Pathogens | Candida, Cryptosporidium | Candida, CMV, EBV. |
| T-cell subset ratio | 0.3 | 0.34 |
| Skin lesion biopsy and rare infection | Extravasation of RBC Endothelial cells with Nuclear pockets, Tubular Inclusion bodies | Pseudomonas pseudomallei |

**P 324** HIV TRANSMISSION BY TRANSFUSION EXAMINED BY DONOR SCREENING AND RECIPIENT TRACING, June Whaun, Shirley Coffey, Donald Burke, Walter Reed Army Inst. Res., Wash, DC

Acquired immune deficiency syndrome (AIDS) has no defined curative treatment or vaccine as yet. An antiviral strategy instituted has been the screening of donor blood and exclusion of positive donors to safeguard the nation's blood supply. This strategy has been facilitated by the development of tests for the detection of antibodies to human immunodeficiency virus (HIV). From July 1985 to March 1986, a 9-month period, over 70,000 Army donors were screened by enzyme-lined immunoassay (EIA) by local post blood banks; 657 donors were excluded. Of these 657 donors, 95% were service members, young (av. age 26.5 yr.), and 88% male. One hundred fifty-eight of these were confirmed positive by EIA by our laboratory and only 57 were positive by the definitive western blot test. A donor history was obtained by 54%; 45% of these had donated blood in the past. To date, one donor traced gave blood three times. Of the 7 recipients, 2 died of their primary disease, 1 is currently being traced and tested. One hundred percent of the 4 remaining recipients fully traced developed HIV infection. Our studies of donor screening and recipient tracing show virus transmissibility in blood is very efficient with far-reaching and potentially disastrous consequences for the general population. Furthermore, these results mandate data-sharing by blood bank, plasma collecting agencies and transfusion centers in order to limit viral transmission.

**P 325** RESPONSE OF HTLV-III/LAV(HUMAN IMMUNODEFICIENCY VIRUS)-ASSOCIATED NEUROLOGICAL DISEASE TO THE ADMINISTRATION OF 3'-AZIDO-3'-DEOXYTHYMIDINE, Robert Yarchoan, Gary Berg, Arturo Brunetti, Margaret A. Fischl, Rose V. Thomas, Steven M. Larson, Charles E. Myers, and Samuel Broder, National Cancer Institute and NIH Dept. of Nuclear Medicine, Bethesda, MD 20892 and the University of Miami, Miami, FL 33101.

Neurological manifestations of HTLV-III infection are increasingly being recognized as an important clinical problem and, as has been shown by Drs. Shaw, Gallo, and co-workers, the brain is a major site of replication of HTLV-III. Based on these findings, we have administered 3'-azido-3'-deoxythymidine (AZT), a dideoxythymidine analogue with potent in vitro activity against HTLV-III and which has been shown to cross the blood brain barrier, to four patients with HTLV-III-associated neurological disease. Three of these four patients (two with dementia and one with dementia and peripheral neuropathy) had substantial impovement in their neurological symptoms as assessed by clinical examination, neuropsychiatric testing, and nerve conduction studies. One of the patients whose dementia improved was studied by positron emission tomography (PET). At the start of therapy, he had decreased glucose metabolism in the occipital, posterior temporal, and thalamic regions; a repeat PET scan after 13 weeks on AZT had become more normal. The fourth patient, who presented with a T10 paraplegia, had no significant change upon being given AZT. These results support the hypothesis that certain AIDS virus-associated neurological abnormalities are reversible following the administration of anti-retroviral chemotherapy and they provide a rationale for a larger controlled study in patients with this disorder.

**P 326** INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) USING AN OLIGONUCLEOSIDE METHYLPHOSPHONATE, John A. Zaia, Edouard Cantin, John J. Rossi, Patricia A. Spallone, George Murakawa, Ramon Erijta, Bruce Kaplan, and R. Bruce Wallace, City of Hope, Duarte, CA 91010.

Antisense oligonucleotides have been synthesized which inhibit HIV replication and selected viral protein synthesis (P Zamecnik et al, PNAS 83: 3143, 1986). We have studied the antiviral effect of two oligodeoxynucleoside methylphosphonates (OMPs) which were synthesized in an antisense (OMP-A, 3' TCTTAACC 5') or a sense (OMP-S, 3' AGAATTGG 5') configuration based on the RNA sequence of the first splice acceptor site of the tat III gene (5'...AGAAUUGG...3'). OMPs were synthesized from methylphosphonamidites using a solid phase reaction and were purified by ion exchange chromatography.

H9 cells were treated with varying concentrations of OMP, washed, and infected with HIV [strain HTLV-$III_B$, 0.01 reverse transcriptase (RT) units per $10^6$ cells] or mock infected. The development of multinucleated cells (CPE), RT, p24, and viral RNA was inhibited by a single exposure to OMP-A but not by OMP-S. In chronically HIV-infected H9 cells, OMP-A treatment inhibited RT activity. These antiviral effects were transitory, and viral CPE or RT returned to control levels approximately 11-14 days after OMP treatment. OMPs induced no cellular toxicity in uninfected H9 cells. Selective targeting of the tat III gene with antisense OMP appears to have potent antiHIV activity and might have a role in the treatment of AIDS.

# *LATE ADDITIONS*

**P 400** NON-CYTOPATHIC LATENT INFECTION OF HUMAN CELLS OF NERVOUS SYSTEM (NS) ORIGIN BY HUMAN IMMUNODEFICIENCY VIRUS. Stephen Dewhurst, Koji Sakai, Mario Stevenson, Joel Bresser[1], and David J. Volsky, University of Nebraska Medical Center, Omaha, Nebraska 68105 and [1]M.D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas 77030.
The hypothesized neurotropism of HIV was studied using four well-characterized human cell lines of nervous system (NS) origin. Three of these expressed high levels of the astrocytic marker, glial fibrillary acidic protein (GFAP), and none exhibited characteristics of hematopoeitic cells. All NS cells were found to be fully permissive to HIV replication after transfection with biologically active HIV clones. Intriguingly, these lines also contained low levels of gene transcripts encoding the T4/HIV receptor, suggesting that they might be susceptible to infection by HIV. This was verified by the detection of viral transcripts and polypeptides in NS cells at 2 days after exposure to HIV. Interestingly, Northern blot analysis indicated that aberrant splicing of viral RNAs occurs in infected glial cells. This observation may be related to the minimal virus replication and lack of cytopathic effects in NS cells, in spite of the persistence of viral gene expression. It is hypothesized that low T4 expression and restricted viral gene activity may contribute to the maintenance of HIV in a non-cytopathic, latent state.

**P 401** IDENTIFICATION OF FUNCTIONAL REGIONS IN THE HIV REVERSE TRANSCRIPTASE BY SITE-DIRECTED MUTAGENESIS. Brendan A.Larder, Dorothy J.M.Purifoy, Kenneth L.Powell and Graham Darby. The Wellcome Research Laboratories, Beckenham, Kent, U.K.
We are currently studying the reverse transcriptase (RT) of HIV expressed in E.coli, with the aim of identifying those regions and specific amino acid residues involved in the catalytic activity of the enzyme. Initially, a large DNA fragment comprising most of the HIV pol gene, including the protease and RT sequences, was sub-cloned into an M13 expression vector which contains the strong inducible "tac" promoter. Infection of E.coli with this recombinant bacteriophage (mpRT1) resulted in significant levels of RT activity being induced. Through a series of manipulations, including oligonucleotide-directed deletion mutagenesis, the protease and endonuclease sequences flanking the RT coding region have been removed, giving a construct (mpRT4) which expresses large amounts of the native enzyme. The RT expressed by mpRT4 exhibits similar properties to the "authentic" enzyme found in HIV-infected cells, including sensitivity to PFA and AZT-TP, and apparent $M_r$ (~66 kDa).
A number of regions of the RT which share homology with other polymerases have been probed by site-directed mutagenesis and using this apporach, we have identified two small areas of the RT polypeptide which are likely to be involved in enzyme function, one of these being centred around two Asp residues (positions 185 and 186). It is hoped that studies of this nature will provide information about the interaction of HIV RT with its substrates and may facilitate the more rational design of chemotherapeutic agents.

**P 402** ACTIVATION OF LATENT EPSTEIN-BARR VIRUS BY INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) by X. Li, P.K. Lai, F. Sinangil and D.J. Volsky. Molecular Biology Laboratory, Dept of Path. and Microbiol, Univ. of Nebraska Medical Center, Omaha, NE68105.

Elevated antibody levels to EBV antigens and increased frequency of EBV-infected B cells are often found in blood from subjects infected by HIV. To investigate whether HIV might have a direct effect on EBV life cycle, 10 lymphoblastoid cell lines (LCL-EB) and 3 EBV-positive Burkitt lymphoma cell lines were infected with HIV. Staining of the infected cells by monoclonal antibodies to the diffused and restricted forms of early antigens (EA-D, EA-R) and virus capsid antigen (VCA) showed significant increase in the proportion of $EA^+$ cells in all LCL-EB and 2 of the tumor cell lines, P3HR1 and Raji, but not in EBV-converted Ramos cell line. P3HR1 and 5 LCL-EB lines also showed significant increase in $VCA^+$ cells. In contrast, no such increase was observed in Raji and 2 LCL-EB lines. No HIV-related cytopathic effects were observed in any of the infected cell lines. Kinetic studies showed that the proportion of EBV-antigen positive cells increased 2 days after HIV infection and peaked at 3 days. It declined thereafter to background level on day 7. These results suggest that HIV infection transiently activates latent EBV-infected cells to produce virus. The possibility of direct molecular interaction between HIV and EBV genomes is currently under investigation. Supported by Grants CA37465 and CA43464.

**P 403** "TYPE SPECIFIC BINDING OF SURFACE EXPRESSED GP120 BY ANTI-GP120 SERUM" Lyerly, H.K., Weinhold, K.J., Matthews, T.J., Putney, S., Rusche, J., Langlois, A.J., and Bolognesi, D.P.

Anti-gp120 antibodies mediate virus neutralization and infected cell lysis. Broadly reactive gp120 neutralizing antibodies have been demonstrated in patients infected with HIV, however anti-gp120 serum produced in experimental animals contain only type specific neutralizing antibodies. Because anti-gp120 antibodies have recently been shown to mediate ADCC, antibodies generated against purified gp120, gp-160, a recombinant protein representing the midportion of gp-120, PB1 and a murine monoclonal anti-gp120 were analyzed for binding to cell surface expressed gp120 on cells infected with the heterologous virus isolates $HTLV\text{-}III_B$, $HTLV\text{-}III_{RF2}$, and $HTLV\text{-}III_{MN}$ and to cells bearing purified gp120 from $HTLV\text{-}III_B$ and $HTLV\text{-}III_{RF2}$. Surface expression of gp-120 was determined by flow cytometry using high titered human serum demonstrating all infected cell lines and all cells bearing gp120 had high levels of surface expressed viral protein. Similar levels of staining were achieved with specific anti-gp-120 against $HTLV\text{-}III_B$ infected cells and $HTLV\text{-}III_B$ gp120 bearing cells. However, these antibodies did not bind to cells infected with $HTLV\text{-}III_{RF2}$ or $HTLV\text{-}III_{MN}$ or bearing $HTLV\text{-}III_{RF2}$ gp120. Analysis of heterologous gp120 surface expression by flow cytometry may be a sensitive indicator of broadly reactive antibodies generated against purified or recombinant subunits of viral proteins.

**P 404** EXPRESSION OF LARGE AMOUNTS OF NATIVE AND MUTATED FORMS OF THE HIV ENVELOPE PROTEINS USING A SV40 LATE REPLACEMENT VECTOR, David Rekosh*, Anders Nygren***, Ewa Lindstrom***, Hans Wigzell*** and Marie-Louise Hammarskjold**, *Departments of Biochemistry and **Microbiology, SUNY at Buffalo, N.Y. and ***Department of Immunology, Karolinska Institute, Stockholm, Sweden.

An eukaryotic expression vector producing large amounts of the HIV envelope proteins (gp 160/120) has been constructed by introducing the Sal I/Xho I fragment from the BH10 isolate into a SV40 late replacement vector. The vector is a shuttle vector that replicates to high copy numbers in both E. coli and eukaryotic cells permissive for SV40 replication.

Transfection of the HIV recombinant into CV1 monkey cells gave high levels of expression of gp 160 and gp 120 in approximately 30% of the transfected cells. By several criteria the proteins were indistinguishable from those produced during infection. The proteins were localized to both the cytoplasm and the plasma membrane and some of the gp 120 was shed into the culture medium. Approximately 0.5 ug of envelope protein could be extracted from $10^6$ cells. Thus this transient vector system provides an abundant source of native envelope protein for purification and characterization.

In addition several recombinants designed to express mutated forms of the envelope proteins have been created.

**P 405** SELECTIVE *IN VITRO* INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) REPLICATION BY 3'-AZIDO-2',3'-DIDEOXYURIDINE (CS-87).
R. F. Schinazi[1,4*], C. K. Chu [2], M. -K. Ahn [2], and J. -P. Sommadossi [3], and H. McClure [4]. Veterans Admin. Med. Ctr./Emory University, Atlanta, Ga[1]; Univ. of Georgia, Athens, Ga [2]; Univ. of Alabama, Birmingham, Al [3]; and Yerkes Primate Center/Emory University, Atanta, Ga[4].

Among the compounds synthesized as analogs of 3'-azido-3'-deoxythymidine (AZT), 3'-azido-2',3'-dideoxyuridine (CS-87) was found to have significant anti-HIV activity in human peripheral blood mononuclear (PBM) cells ($ED_{50}$ = 0.2 μM). The antiviral activity of CS-87 was determined by: (1) a reverse transcriptase assay of disrupted concentrated virus; (2) an HIV p24 radioimmunoassay of Triton-X100 treated cell supernatant; and (3) quantitation of HIV-induced proteins by western blot analysis of solubilized cells that had been exposed to virus and drug. The $ED_{50}$ of CS-87 in a murine retrovirus UV-XC assay in SC1 cells was about 65-fold less than in human cells.

CS-87 was not toxic to uninfected human PBM cells when tested up to 200 μM; the therapeutic index appears to be close to 1,000. The compound was less toxic ($ID_{50}$ = 21 μM) to human granulocyte-macrophage precursor cells than either AZT or its 5-ethyl analog (CS-85). When administered to 6-week old BALB/c mice (intraperitoneally 60 mg/kg per day for 7 days) CS-87 exhibited no untoward effects; its intraperitoneal $LD_{50}$ in mice is greater than 800 mg/kg. In rhesus monkeys a single intravenous dose of 60 mg/kg resulted in a transient elevation of liver enzymes; mutiple intramuscular injections at a dose of 10 mg/kg for one week did not produce any apparent toxicity.

The antiviral activity of CS-87 in PBM cells was prevented by 2'-deoxyuridine and 2'-deoxycytidine, but not by thymidine, uridine or cytidine. Similar results were obtained in infected CEM cells. Preliminary HPLC studies of extracts from PBM cells treated with CS-87 indicate that CS-87-5'-monophosphate is the major metabolite.

The high *in vitro* therapeutic index and low toxicity of CS-87 in animals suggest that this compound merits additional studies in experimental models for AIDS and, hopefully, in the therapy of HIV infections in humans.

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**P 406** POST-TRANSCRIPTIONAL MODULATION OF CELLULAR IL-2 RECEPTORS DURING INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV),
M. Stevenson, C. Meier, X. Zhang, D. Volsky. University of Nebraska Medical Center, Omaha, Nebraska 68105.

Infection of Human T-lymphocytic cell lines resulted in a gradual loss of several cell surface receptors. Infection of CR-10 cells (a cytopathicity resistant subclone of CEM cells) and HBD-1 cells resulted in a gradual loss of cell surface receptors for OKT4/OKT4A (HIV receptor) OKT3, OKT8 OKT11 but not of OKT9 (transferrin receptor). Infection of the HBD line, which consitutively expresses the IL-2 receptor, resulted in a dramatic down modulation of IL-2 receptor molecules. Surface receptor decline was accompanied by a rapid increase in the expression of HIV antigens and mRNA.

Despite the down modulation of cell surface receptors, HIV infected and non-infected cells contained similar steady state levels of messenger RNAs for T4, T8, T3 and IL-2 receptor genes. Analysis of T cell receptor gene rearrangement revealed that no distinct cell subpopulations were selected out upon infection with HIV. We conclude that HIV infection induces post-transcriptional down modulation of several T cell receptors. Such a down modulation, expecially of T4 and the IL-2 receptor, may have important implications for HIV pathogenesis.

**P 407** USE OF 7H13 BACTEC BROTH FRO THE RECOVERY OF MYCOBACTERIA FROM AIDS BLOOD, M. Salfinger, D. Piot, E. Stook, L. Heifets, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO, and Park Plaza Hospital, Houston, TX.

Mycobacterial infections are significant complications in patients with acquired immunodeficiency syndrome (AIDS). From July 1985 to December 1986 at the Park Plaza Hospital in Houston, Texas, 195 patients with AIDS were screened for mycobacteria in blood. Forty of the 195 patients (21%) had positive blood cultures for mycobacteria (M. avium complex, 31 patients; M. fortuitum, 2 patients; M. chelonae, 1 patient; M. tuberculosis, 1 patient; M. avium complex mixed with M. kansasii, 1 patient; identification pending, 4 patients). The aim of this study was to compare the efficacy of three different media for recovery of mycobacteria from blood: 7H11 agar, standard 7H12 BACTEC broth (containing antimicrobial supplement PANTA), and new 7H13 broth (30.0 ml per vial, contains sodium polyanethol sulfonate and Tween 80), supplemented with bovine serum albumin.

Beginning in August 1986, blood from each AIDS patient was collected in two vacutainer tubes containing anticoagulant. The blood of one tube was mixed with 30 ml of sodium desoxycholate (0.3%) and incubated for 10 minutes at room temperature. After centrifugation for 25 minutes at 3.500 x g, the pellet was resuspended in 0.2% bovine albumin solution to achieve a total volume of 4.0 ml. Two media, 7H11 agar plate and 7H12 vial, were inoculated with this concentrated specimen, 0.5 ml per each. Blood from the second tube, 5.0 ml, was transferred directly to the new 7H13 broth vial.

Out of 119 processed specimens, 17 were positive for mycobacterial growth: 15 were recovered from all three media, one was missed in the 7H13 vial, and another failed to grow in both liquid media. These preliminary data show that the three media have the same recovery rate. The new 7H13 broth vial allows a larger volume of blood to be inoculated directly into medium, thus eliminating the time-consuming and potentially hazardous processing step of concentrating the blood specimen.

NOTES