VIRUSES AND HUMAN CANCER

Robert C. Gallo, William Haseltine, George Klein and Harald zur Hausen, Organizers

February 2 — February 9, 1986

Keynote Address

D1 A TURNING POINT IN TUMOR VIRUS RESEARCH. Renato Dulbecco, The Salk Institute, La Jolla, CA 92037

Research in tumor virology has had an enormous impact on the understanding of cancer. The discovery of oncogenes has made it possible to explain the various modalities by which cancers arise. Cancers however progress through a series of stages to reach the fully developed malignant phenotype; this is true of all cancers, including those initiated by viruses. There is good reason for believing that progression results from changes in cellular genes. The mechanism of these changes and their connection to the initial events, therefore, became important questions in cancer research. Some relevant hypotheses will be discussed. An important point is that in order to understand the mechanism of progression, more must be known about the cellular genome. It is suggested that in order to gain this knowledge, a cellular genome must be completely sequenced. For a variety of reasons it should be the human genome.

Biology of Human T Lymphotrophic Retroviruses

D2 IMMUNOPATHOGENESIS OF HTLV-III INFECTION, Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

The acquired immunodeficiency syndrome (AIDS) is caused by the human retrovirus termed human T lymphotrophic virus (HTLV)-III or lymphadenopathy associated virus (LAV). This virus has the extraordinary property of selective tropism for a specific subset of T lymphocytes termed helper or inducer T cells defined by the T4 or Leu 3 phenotypic marker. Infection of the T4 subset results in a cytopathic effect on the target cell. Since this subset is responsible for the induction of a wide range of immunologic functions, a broad range of immunologic abnormalities ensues. The immunologic defect is manifested by both quantitative and qualitative abnormalities in T4 cell functions. Of note is the fact that that there is a selective and early defect in the subset of T4 cells which is responsible for antigen recognition. Furthermore, abnormalities in antigen-presenting cells (monocyte/macrophages) compounds the defect in response to specific antigens. The presence of additional abnormalities of monocytes likely reflects the infection of these cells with the HTLV-III/LAV. B cells are also abnormal in that they are polyclonally activated and defective in their response to de novo antigens. Abnormalities of natural killer cells and cytotoxic T cells likely reflect a defect in inductive signals delivered by the T4 cell. Certain of the monocyte and cytotoxic cell functional abnormalities can be corrected in vitro by T4-derived soluble mediators such as interleukin-2 and interferon gamma, further implicating the lack of an inductive T4 signal as the cause of these abnormalities.

Following infection of suspensions of T4 cells with the AIDS retrovirus, a small proportion of cells survive and latently harbor virus in the absence of spontaneous virus secretion. Of note is the fact that these cells can be induced to secrete virus under appropriate conditions, representing important implications for the study of latent infection in the host. Studies on the differential effects of virus on various cloned retroviral populations of T4 cells as well as the direct stimulatory effect of the virus on subpopulations of B cells will be discussed.

D3 AN ANIMAL MODEL OF HTLV-I INFECTION: SERIAL TRANSMISSION OF HTLV-I BY BLOOD TRANSFUSION IN RABBITS, I. Miyoshi, S. Yoshimoto, M. Fujishita, K. Yamato, S. Kotani, M. Yamashita, and Y. Ohtsuki, Departments of Medicine and Pathology, Kochi Medical School, Kochi 781-51, Japan

Human T-cell leukemia virus type I (HTLV-I) is the causative agent of adult T-cell leukemia/lymphoma. The virus has a unique property to transform not only human lymphocytes but also lymphocytes from various species of animals (monkey, cat, rabbit, and rat). Seroepidemiological data suggest that HTLV-I can be transmitted by blood transfusion and by close family contact. In search for an animal model, we have successfully infected monkeys and rabbits with HTLV-I. This paper is concerned with serial transmission of the virus by blood transfusion in rabbits. A virus-producing cell line (Ra-1) was established from male rabbit lymphocytes by co-cultivation with a lethally irradiated human T-cell line (MT-2) persistently infected with HTLV-I. For virus transmission, a rabbit was infected with HTLV-I by intravenous inoculation of mitomycin C-treated Ra-1 cells and was used to initiate serial blood transfusion. Recipients of blood, after seroconversion, served as donors for the next transfusion, with donor and recipient being of the opposite sex. HTLV-I could be transmitted from rabbit to rabbit with 20 ml of whole blood or washed blood cell suspension (fresh or stored for 1-2 weeks at 4°C) but not with cell-free plasma. Seroconversion occurred 3-4 weeks after blood transfusion and the highest anti-HTLV-I titers ranged from 1:20 to 1:640 by indirect fluorescence assay on MT-2 cells. The virus is now in the fifth passage through rabbits. From recipients of the first to fourth transfusion, virus-producing cell lines, Ra-2 to Ra-5, were established by cultivating lymphocytes in the presence of TCGF. Three of the four cell lines became TCGF-independent after two months to one year of continuous culture. Chromosome analysis of each cell line showed a normal rabbit karyotype with sex chromosomes of the recipient. We then investigated the effect of X-irradiation (6,000 rad) on donor blood. Seroconversion occurred in rabbits transfused with blood that had been irradiated immediately before transfusion but not in rabbits transfused with blood that had been irradiated and stored for 1-2 weeks at 4°C. Thus, our rabbit model has demonstrated that HTLV-I can be readily transmitted by blood transfusion and that this may be preventable by prior irradiation of blood.

D4 INTERACTION OF HUMAN T-CELL LYMPHOTROPIC RETROVIRUSES (HTLV) WITH TARGET CELLS, Mikulas Popovic, Poul Markovics, Robert C. Gallo, Suzane Gartner, LTCB, NCI, NIH, Bethesda, MD. 20892.

HTLV comprise three well-defined types termed Human T-cell Lymphoma/Leukemia Virus type I, type II, (HTLV-I & HTLV-II) and Human T-cell Lymphotropic Retrovirus type III (HTLV-III). HTLV-I closely associated with adult T-cell leukemia and HTLV-III, also called lymphadenopathy associated virus (LAV), is the primary cause of the acquired immunodefficiency syndrome (AIDS). HTLV-II was isolated from a T-cell variant of hairy cell leukemia and its role as an etiological agent is not known.

It was earlier demonstrated that OKT4+/Leu3a+ T cells are preferentially infected with HTLV, however, the targets in vivo for HTLV-I are T cells with suppressor function and for HTLV-III are T-cells with helper function. Although both transforming retroviruses, HTLV-I and HTLV-II, can infect various cell types, they immortalize only T-cells. Moreover, helper or cytotoxic functions of the mature T-cells can be altered upon infection with these viruses.

To extend previous observations that different subsets of T-cells can be infected and immortalized by HTLV-I, cord and peripheral blood T-cells were separated according to the cell surface markers into populations expressing only T4 (OKT4+), T8 (OKT8+) or were negative for both antigens. All three T-cell populations predefined by cell surface markers were susceptable to HTLV-I infection. Positivity for the viral core protein HTLV-Ip19 was in the range of 42% to 59% and these cells exhibited a capacity for indefinite growth in vitro. Their T-cell markers were preserved following virus infection. Similarly, four different T-cell fractions separated by cell size using Percoll gradients were susceptable to HTLV-I infection and immortalization. However, attempts to infect and immortalize large granular lymphocytes (LGL) gave consistently negative results. Thus, different subsets of T-cells distinguished by cell surface markers or size can be infected and immortalized by HTLV-I with exception of LGL cells.

Unlike HTLV-I or II, HTLV-III/LAV does not immortalize T-cells, is strongly cytopathic and exhibits a restricted host range in various human cells. It has been shown that some B and monocyte-macrophage cells can be infected in vitro by the virus. To determine whether cells other than T lymphocytes can harbor the virus in vivo, attempts to identify HTLV-III/LAV positive cells in brain tissue from an AIDS patient were carried out. Cultures were established from brain specimens and the cell types and their virus expression were studied. Growth, morphological and cytochemical characteristics of HTLV-III/LAV positive cells in primary cultures exhibited properties of microglial origin. These cell cultures continuously produced virus over 3 months. The interaction between these virus-producing cells and T-cells will be discussed.

Molecular Biology of Human T Lymphotrophic Viruses

D5 MOLECULAR GENETIC ANALYSIS OF HUMAN T-CELL LEUKEMIA VIRUSES, Irvin S.Y. Chen, Allan J. Cann, Richard B. Gaynor, Joseph D. Rosenblatt, Dennis J. Slamon, William Wachsman, Division of Hematology-Oncology, UCLA School of Medicine, Los Angeles, CA 90024

The HTLV/BLV class of retroviruses is associated with specific lymphoid malignancies. HTLV-I is the likely etiologic agent for adult T-cell leukemia endemic to regions of Japan, the Caribbean, and Africa, and bovine leukemia virus is the cause of bovine leukosis in cattle. HTLV-II was associated with a single case of T-cell variant hairy-cell leukemia and a definitive etiologic role had not previously been determined. A second isolate of HTLV-II was recently identified in another patient with atypical hairy-cell leukemia. Molecular analysis of the virus and its association with the patient provides strong evidence for an etiologic role of the virus in the malignancy.

The mechanism of transformation by HTLV is unknown; however, the BLV/HTLV class of retroviruses has a fourth gene known as the x gene (or x-lor gene) not found in other retroviruses. This gene encodes proteins of 40 kd and 37 kd in HTLV-I and HTLV-II, respectively. The x proteins are essential for HTLV replication and act by inducing transcription by the HTLV LTRs. These proteins are localized to the nucleus of infected cells and have a relatively short half-life, consistent with the function as a transcriptional regulatory protein. We find that the x proteins have distinct properties in regards to their ability to activate different promoters, and we have investigated these properties by constructing recombinants between the proteins and by in vitro mutagenesis.

The mechanism of transformation by HTLV has been proposed to involve induction of abnormal cellular transcription by the HTLV x proteins. We provide support for this hypothesis by demonstrating that the HTLV x protein is functionally related to the adenovirus E1A protein. Both proteins will activate adenovirus promoters which are normally inducible by E1A. These observations suggest common mechanisms of action for the HTLV x and adenovirus E1A proteins and demonstrate that the HTLV x protein has the capacity to activate the expression of genes other than those of HTLV. We have investigated the functional relation between HTLV x and adenovirus E1A proteins by examining the sequence requirements for induction of promoters. Comparison of the mechanism of action of the two transcriptional regulatory proteins will provide further insights into the mechanism of pathogenesis by HTLV.

D6 ADULT T-CELL LEUKEMIA AND HTLV-I, M. Miwa, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan

Human T-cell leukemia virus type I (HTLV-I) genome has been known to be clonally integrated in the leukemic cells of patients with adult T-cell leukemia(ATL) and the nucleotide sequence of this genome has been reported. We determined the nucleotide sequence of an infectious clone of another type of human T-cell leukemia virus(HTLV-II), which also transforms normal T-lymphocytes as HTLV-I.

In HTLV-II, in addition to gag, pol, env and X genes, there is an open reading frame for protease gene covering 3' end of gag gene and 5' end of pol gene, which can code for a sequence of 178 amino acids. The comparison of the nucleotide sequence of HTLV-II to HTLV-I disclosed significant homologies for gag, pol, env and X genes. Both nonsynonymous (amino acid-altering) and synonymous substitutions of nucleotides were the smallest in X gene. We identified pX-IV and Xc gene products in HTLV-I and HTLV-II infected cells, as reported also by other investigators. Recently we identified a pX product, which has a molecular weight of 41 KD, also from cell lines of monkey infected with a retrovirus similar to HTLV-I. Although HTLV-I gene products including pX product were not found at a significant level in the peripheral leukemic cells of ATL patients, it may be important for the early stage of leukemogenesis in ATL.

A unique cell line, ATL-1K, has an integrated HTLV-I provirus, but it does not express the HTLV-I antigens. The DNA of provirus of this cell line is heavily methylated. 5-Azacytidine treatment caused partial demethylation, but it did not cause HTLV-I expression. ATL-1K cells were cocultured with a HTLV-I producer cell line, MT-2, to obtain various sublines of ATL-1K cells which possess HTLV-I provirus in addition to the original provirus. But only a minority of the sublines expressed HTLV-I antigens at low levels.

Although it has been claimed that HTLV-I is integrated in the leukemic cells of patients with ATL in the endemic area, we found 5 cases of clinically typical ATL from non-endemic area in which apparently there is no detectable HTLV-I provirus in the leukemic cells by Southern blot hybridization. Antibody against HTLV-I is also negative in the sera of these five patients. This suggests that there may be some factor(s) other than HTLV-I in leukemogenesis of certain cases of ATL.

Human Papilloma Viruses

D7 MORPHOLOGICAL AND CLINICAL CORRELATES OF GENITAL PAPILLOMAVIRUS INFECTION

Christopher P. Crum and Saul J. Silverstein, Departments of Pathology and Microbiology and the Cancer Research Center, Columbia University College of Physicians and Surgeons, N.Y., N.Y. 10032

Current studies indicate a strong correlation between specific morphological changes and certain HPV strains in precancerous squamous epithelium of the cervix, vulva and vagina (1,2,3). HPV type 16 is the most commonly detected HPV in cervical lesions in our experience, and 90% of these exhibit some morphological features associated with CIN(3,4). However, over 50% of CIN lesions containing HPV 16 exhibit in addition, well differentiated epithelium characteristic of conventional condyloma. DNA-DNA in-situ hybridization analysis of CIN lesions containing HPV 16 has localized virus DNA sequences to areas that resemble both condyloma and CIN. The greatest concentration of sequences occurred in nuclei of cells with koilocytotic atypia. Furthermore, in 10% of lesions containing HPV 16, capsid antigens, indicative of late gene expression and virus maturation, are present. Clinical and colposcopic studies indicate considerable overlap in gross morphologic patterns between lesions containing HPV 16 and other HPV's. Thus, well differentiated CIN lesions containing this virus may at times be indistinguishable from conventional condylomata, both histologically and clinically. Furthermore, HPV°s associated with both condylomata and intraepithelial neoplasia have been isolated from normal appearing squamous epithelium, demonstrating that HPV sequences exist beyond the treatment area and their presence influences subsequent recurrences(5).

1) Glssman L et al. Proc Natl Acad Sci USA 80: 560, 1983;2) Durst M et al. Proc Natl Acad Sci USA 80: 3812, 1983;3) Crum CP et al. N Engl J Med 310:880, 1984; 4)Crum CP et al. J Virol 54: 675, 1985;5) Ferenczy A et al. N Engl J Med 313: 784, 1985.

D8 HUMAN PAPILLOMAVIRUSES AND NEOPLASIA OF THE SKIN, Gérard Orth, Unité INSERM 190, Unité des Papillomavirus, Institut Pasteur, Paris, France.

The first evidence for the oncogenic potential of human papillomaviruses (HPVs) was obtained through the study of epidermodysplasia verruciformis (EV). This rare skin disease is characterized by disseminated, refractory, flat wart-like and macular skin lesions, and by the development of skin carcinomas in about 30% of the patients. EV is a multifactorial disease involving genetic, immunological and extrinsic (actinic) factors, in addition to infection with specific HPV types. A number of HPVs (at least 15 types) have been characterized in benign EV lesions. HPV DNA sequences are regularly detected in EV carcinomas but, in contrast to benign lesions, the types associated with cancers are usually restricted to HPV5. Some other EV-specific HPV types (HPV8, HPV14, HPV17) have only seldomly been encountered. HPV5 genomes are usually found as free monomeric or oligomeric DNA molecules in EV carcinomas. This is in contrast with HPV DNA sequences in genital cancers, which are usually integrated into the host DNA. Evidence for the transcription of HPV5 genomes in primary and metastatic EV carcinomas has recently been obtained. The available data indicate that HPV5 has an oncogenic potential and play a role in the malignant transformation of EV lesions. Infection by these HPVs must be considered as a major risk factor for the development of cancers in EV patients.

HPV DNA sequences have only rarely been detected in premalignant or malignant lesions of the skin in the general population. Two HPV types, HPV34, and an HPV5-related type, HPV36, have been characterized from a case of Bowen's disease of the skin and a case of actinic keratosis, respectively. Their genomes have not been detected in any of the other neoplastic skin lesions studied so far. This further stresses the role of genetic, immunological and extrinsic factors in the abnormal susceptibility of EV patients to a set of specific HPV types, and, more generally, in the expression of the oncogenic potential of HPVs.

ORTH G (1986). Epidermodysplasia verruciformis : a model for understanding the oncogenicity of human papillomaviruses. In Papillomaviruses, Ciba Foundation Symposium 120, Pitman, London, in press.

Human Hepatitis Virus

D9 CHROMOSOMAL DNA REARRANGEMENTS MEDIATED BY HBV INTEGRATION IN HCC AND ACTIVATION OF INSULIN-LIKE GROWTH FACTOR II TRANSCRIPTION, Charles E. Rogler, Okio Hino*, and Chun-Yeh Su, Albert Einstein College of Medicine, Liver Research Center, Bronx, NY 10461 and *Cancer Institute, Tokyo Japan

Hepatitis B virus (HBV), a virus with known carcinogenic potential, integrates into cellular DNA during long term persistent infection in man, and is nearly always found integrated into hepatocellular carcinomas (HCC). Chromosomal abberations including deletions and translocations of cellular DNA are associated with several types of carcinomas. To determine if HBV integration may cause specific chromosomal abberrations which may lead to HCC we have cloned several HBV integrations from single integration HCC and studied the arrangement of cellular sequences flanking those integrations. Unique cellular flanking sequences from cloned HBV integrations were used to probe Southern blots of tumor and normal genomic DNAs digested with various restriction endonucleases. This allowed construction of restriction endonuclease maps of normal and tumor cellular sequences flanking HBV integrations. Rearrangement of cellular sequences at the site of HBV integration was observed in all four HCC's thus far studied. Three cases involved deletion of cellular sequences. The deletions were from 0.3 to 13.5 Kb in length and occurred on chromosomes 6, 9 and 11. In one of these cases we have directly demonstrated that the deleted DNA is lost from the tumor cell and not translocated to another location or amplified autonomously in the tumor cells. The fourth case involved a DNA translocation between chromsomes 17 and 18 at the site of HBV integration. The chromosomal location of HBV integrations was determined by hybridizing flanking probes to DNA's from mouse-human somatic cell hybrids containing defined human chromosomes. HBV DNA integration was also shown to preferentially occur in the cohesive ends of the virion DNA. These results show that HBV acts as an agent causing chromosomal abberrations and that this property may in part, account for the oncogenic potential of this virus.

An HBV induced deletion in the short arm of chromosome 11 was localized to position 11p13 which is in the region of the Wilms tumor locus.[1] Analysis of RFLP's in HCC DNA using Insulin and IGF II probes from chromosome 11p indicate that in some tumors a switch from the heterozygous to the homozygous condition may occur for chromosome 11. Activated transcription of the IGF II but not the Insulin gene is also observed in most HCC's when compared to normal adult liver. The significance of the correlation between enhanced IGF II transcription and chromosome changes in 11p are being investigated.

[1]Rogler, E.C. et al (1985) Deletion in chromosome 11p associated with a hepatitis B integration site in hepatocellular carcinoma. Science 230:319-322.

D10 INTEGRATION OF HEPATITIS B VIRUS DNA IN HEPATOCELLULAR CARCINOMA.

Anne Dejean & Pierre TIOLLAIS.

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world and hepatitis B virus (HBV) infection is responsible for at least 80 % of cases world-wide.
Structure of HBV DNA in hepatitis B surface positive HCC has been studied by Southern blotting. In all cases HBV DNA integrated into the host genome has been detected. In addition, occasionally, free HBV DNA was observed in the early stage of tumor growth. Integrated HBV sequences are not indicative of tumorous tissue since they were also observed in the non-tumorous part of livers as well as in chronic hepatitis or cirrhosis without HCC.
The genetic organization of integrated HBV DNA was determined by molecular cloning. Viral sequences can be either complete genomes or subgenomic fragments that are often rearranged. In two independant cases the host-virus junction was mapped within either DR1 or DR2, the two copies of the direct repeat localized at both sides of the cohesive ends of the HBV genome. HBV DNA can therefore integrate via a specific viral DNA sequence. No evidence for a specific cellular integration site has been reported to date.
The structure of HBV integration site was studied in an early tumor. Seven overlapping clones representing 3.2 kb of cellular DNA at the unoccupied site were isolated from the non-tumorous liver DNA. This region of the normal allele was extremely rich in unique sequences. Comparison of the restriction map of the unoccupied site isolated from the non-tumorous part of the liver with that of the integrated site cloned from the tumorous part showed a complete homology in the host sequences. This shows that the viral integration was not accompanied by large deletion, amplification and/or translocation. To assign the integration site to a specific chromosome, a panel of 17 mouse-human and chinese hamster-human somatic cell hybrids were analysed by Southern blotting. The results clearly localized the integration site to chromosome three. HBV integration was present in only one of the two chromosomes. The normal allele was sequenced at the exact site of integration. Results showed that HBV integration occured with a micro-deletion of 7 to 12 bp. The analysis of the nucleotide sequence of the normal allele over a long distance was determined. The HBV insertion occured in the middle of an open reading frame (orf) or 519 bp. Presence of two acceptor and donnor like sequences were localized respectively at the 5' and 3' end of the orf, suggesting that this orf is an exon. Moreover HBV integration generates a new orf made of hybrid viral-host sequences.
The role of HBV in HCC is not understood at the molecular level. HBV may act as an initiator and further characterization of integrated viral DNA and their flanking sequences might allow us to find a common element between the different integration sites and hence clarify the process of HBV oncogenesis.

Epstein-Barr Virus

D11 THE ROLE OF EPSTEIN-BARR VIRUS IN CELL TRANSFORMATION, Kevin Hennessy, David Whang, David Liebowitz, Timothy Dambaugh, Jeffrey Sample, Mary Hummel, Ellen Woodland and Elliott Kieff, Division of Biological Sciences, University of Chicago, Chicago, ILL 60637

Epstein-Barr Virus (EBV) infection of B lymphocytes results in sustained cell proliferation. The entire virus genome persists in the proliferating lymphocyte as an episome or as a complete viral genome integrated into cell DNA. Some defective viral DNA molecules are also frequently present. Despite the presence of complete viral genomes, most virus infected lymphoblastoid cell lines are only partially permissive for virus replication. Some lymphoblastoid cell lines are only latently infected. These have been particularly useful for analysis of EBV gene expression in latency and cell growth transformation. Four viral messenger RNAs encode three nuclear proteins and a membrane protein in latently infected cells. The coding region for each of these genes is known, revealing the sequence of the four proteins.

The effects of each of these genes is being studied in primary and continuous human and rodent cell lines. Results to date indicate that EBNA1 and EBNA2 have little effect alone on the growth of primary and continuous rodent cell lines. Expression of the latent membrane protein gene results in an increased resistance of NIH3T3 and Rat-1 cells to the deleterious effects of low serum on cell growth. In Rat-1 cells, LMP expression also causes loss of contact inhibition, anchorage independent growth in soft agar and increased tumorgenicity in nude mice.

D12 EBV CELLULAR INTERACTIONS. Joseph S. Pagano, M.D., Director, Lineberger Cancer Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27514

EBV infects both epithelial cells and B-lymphocytes producing several types of virus-cell relations. Dissection of the molecular features of the latent state of infection from the oncogenic relation has been elusive. In vivo, lymphocytes remain latently infected whereas epithelial cells produce virus that is excreted in the oropharyngx. Although EBV alone produces polyclonal proliferation and immortalization of B-lymphocytes, oncogenic transformation as in Burkitt's lymphoma (BL) seems to require a second step, namely, specific chromosomal translocations with activation of c-myc.

In addition to nasopharyngeal carcinoma (NPC) other varieties of epithelial malignancies are being associated with EBV infection. In these cases there is generally a longer latent period during which time EBV presumably initiates the transformation process. The steps in this process are quite undefined, and in vitro models of epithelial cell transformation are as yet unavailable. Transcription of the latent EBV genome in NPC does not resemble what little is known about EBV transcription in BL, but does resemble transformation in latently infected BL cell lines. However an activated transcriptional state has also been detected in NPC which has so far not been related to the distinctive antibody responses found in patients with NPC. Nor do the EBV proteins expressed in NPC tissues necessarily correspond to the antibody responses, whose origin and significance remain obscure.

Finally, infection or transformation of B-lymphocytes with EBV appears to have effects, previously unsuspected, on lymphocyte surface markers with, for example, induction of leu 3 in at least some EBV-infected or transfected lymphocyte lines. This and other EBV-induced changes may confer infectibility on B-lymphocytes by HTLV-3. Whether some such cooperativity at the cellular level between HTLV-3 and EBV might contribute to the high incidence of B-cell lymphomas in AIDS is a possibility that presents a new set of EBV-cell interactions for investigation.

D13 CIS- AND TRANS-ACTING TRANSCRIPTIONAL REGULATION ASSOCIATED WITH AN EPSTEIN-BARR VIRUS-DERIVED PLASMID REPLICON. J. Yates[1], D. Reisman[2], and B. Sugden[2]. [1]Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York 14263. [2]McArdle Laboratory, University of Wisconsin, Madison, Wisconsin 53706.

Epstein-Barr virus (EBV) is a human herpesvirus that infects and transforms B-cells into immortalized, proliferating blasts. The viral genome of 172 kbp is maintained as a multi-copy plasmid in almost all transformed lines examined. Two genetic elements of EBV act together to permit plasmid maintenance: oriP, a cis-acting element, allows plasmids carrying it to replicate, and the gene encoding EBV nuclear antigen EBNA1 is required in trans for oriP to function. We have introduced deletions into both oriP and the EBNA1 gene to identify the minimal sequences required by each element.

OriP contains two essential cis-acting components: a 20-member family of 30-bp repeats and a 65-bp region of dyad symmetry about 970 bp away. The deletion analysis revealed that the family of 30-bp repeats alone, while incapable of supporting plasmid replication, could increase the expression of linked drug resistance markers up to 100-fold. The two components of oriP were then assayed for their capacity to enhance transcription. Assays were performed using EBV-positive cell lines (Raji, a Burkitt's line and 721, an in vitro transformant), EBV-negative cell lines (Wilson, an American Burkitt's line and 143, an osteosarcoma line), and clones derived from Wilson and 143 that stably express introduced copies of the EBNA1 gene. While the region of dyad symmetry showed no enhancing activity, the family of 30-bp repeats, independent of their orientation, enhanced transient expression of chloramphenicol acteyl transferase expression from the SV40 early promoter or the HSV tk promoter up to 80-fold relative to the control plasmid lacking the 30-bp repeats. The level of enhancement found with the tk promoter was generally at least 8-fold higher than that found with the SV40 early promoter. This enhancement was observed with the EBV-positive lines and with the EBNA1-positive derivatives of Wilson and 143 cell lines but not with EBNA1-negative Wilson and 143 cell lines, demonstrating dependence of the enhancing activity of the 30-bp repeats on trans-activation by EBNA1.

Deletions within the EBNA1 coding sequence indicated that both C-terminal and N-terminal regions of the protein are required for both replication and transcriptional enhancement. The C-terminal 190 amino acids of EBNA1 are able to bind to both the 30-bp repeats and to the region of dyad symmetry of oriP (D. Rawlins, et al., Cell, in press). Taken together, these results indicate that binding to the 30-bp repeats by EBNA1 alone is not sufficient for transcriptional enhancement. As was found previously for replication, the repetitive Gly-Ala domain of EBNA1 is dispensable for trans-activation of transcriptional enhancement.

Prevention of Transmission of Human Cancer Viruses

D14 STRATEGIES FOR VACCINE DEVELOPMENT AGAINST THE AIDS RETROVIRUSES, Dani P. Bolognesi, Department of Surgery, Duke University Medical Center, Durham, NC 27710

While functional vaccines have been developed for animal retroviruses, the obstacles to be overcome for successful vaccinations against HTLV-III are formidable. Thus strategies for development of preventive vaccines against the AIDS retrovirus will have to consider issues such as genomic diversity, the multiple mechanisms and routes of infectious virus transmission and the role of the extensive layer of carbohydrate on the exterior glycoprotein, among others. These questions will be discussed and possible strategies to overcome them will be outlined.

D15 ANTI-IDIOTYPE IMMUNIZATION AS UNCONVENTIONAL VACCINES, Hilary Koprowski, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104

In spite of the enormous progress in the field of molecular biology, concepts of vaccine production today are not very different than those envisaged by Pasteur 100 years ago. There are, however, developments in research to produce more modern vaccines which may lead to drastic changes in our approaches to deriving more efficient vaccines. Better presentation of antigen is provided by the use of micelle preparations (ISCOM) to which the antigen in question is adsorbed. The use of viral components for immunization instead of complete virions has been tried in many viral infections but has not been very successful. Expression of viral antigen by either bacteria or yeast carrying the viral genome may lead in isolated cases to the production of a vaccine. Insertion of a viral genome into a vaccinia virus has led to the successful immunization of animal hosts with the vaccinia virus against a variety of viral diseases, and this method holds good prospects for the future. Once the modulation of an immune response to peptide antigens is better understood, synthetic peptides may find their place in vaccination against some viral infections.

Vaccination with anti-idiotype is based on the premise that it carries an internal image of the antigen and induces immunity similar to that obtained using an inactivated virion preparation or a live attenuated virus. One of the more important aspects of these studies is the ability of anti-idiotype to induce an antibody (Ab_3) after immunization of different animal species. This Ab_3 must inhibit the binding of anti-idiotype to the animal antibody (Ab1), inhibit binding of Ab1 to theviral antigen, and express characteristics similar to, if not identical with, those of Ab1. Only then can one assume that the anti-idiotype carries the internal image of the antigen and is potentially useful for vaccination purposes.

The obvious advantages in using anti-idiotype for vaccination are its complete inocuousness and the ability to immunize neonates who are often unable to respond directly to viral or bacterial antigen.

D16 USE OF VACCINIA VIRUS FOR THE PRODUCTION OF LIVE RECOMBINANT VACCINES. Bernard Moss, Laboratory of Viral Diseases,NIAID, Bethesda, MD 20892

Vaccinia virus was used extensively for the immunoprophylaxis of smallpox until that disease was totally eradicated. Advances in genetic engineering and the molecular biology of poxviruses, however, have made it possible to use vaccinia virus as a vector for the expression of genes from other pathogenic agents. Such recombinant viruses have been shown to synthesize and correctly process a variety of foreign proteins in tissue culture cells and stimulate humoral and cell mediated immunity in experimental animals. Although the recombinant viruses retain infectivity and thus can be administered by intradermal inoculation, their virulence has been markedly reduced. Attenuation has been achieved by inactivation of the thymidine kinase or other genes not essential for replication. At this time, vaccinia virus recombinants have been shown to protect animals against diseases caused by influenza virus, herpes simplex virus, hepatitis B virus, rabies virus, vesicular stomatitis virus, respiratory syncytial virus, and Friend murine leukemia virus. The usefulness of a recombinant vaccinia virus that correctly expresses the envelope gene of HTLV-III/LAV is under evaluation.

T Lymphotrophic Retroviruses

D17 **TRANS-ACTIVATION OF VIRAL AND CELLULAR GENE EXPRESSION BY SV40 T ANTIGEN.** James C. Alwine, Gregory Gallo, Jane Picardi and Janis M. Keller. Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Trans-activation of gene expression is a prevalent and important mechanism for gene expression control in both viral and cellular systems. We have studied the mechanism by which Simian virus 40 (SV40) T antigen activates the viral late promoter as well as cellular promoters. We have defined an element within the viral late promoter which is responsible for trans-activated promoter activity. This element is orientation dependent and functions in the absence of the viral origin of replication, thus it is activated by neither replication nor T antigen binding at the origin. In addition, this same element is needed for the trans-activation of the late promoter by the pseudorabies virus immediate early protein. This utilization of the same element by a very different viral trans-acting protein suggests that the viral proteins do not mediate trans-activation directly. Instead, our data suggest that the viral proteins activate, or induce, one or more cellular factors which are directly responsible for promoter activation. This mechanism is supported by work we have done in cells containing no SV40 T antigen. The data also suggest that T antigen mediated activation of cellular genes may involve the same activated, or induced, cellular factors used for the activation of the late promoter. Thus the production of a viral trans-acting protein, like T antigen, appears to result in the activation of cellular factors which, in turn, directly activate both viral and cellular genes by a similar mechanism. Under conditions where the virus persists, this continued abnormal production of cellular gene products can account, at least in part, for disease states.

D18 CHARACTERIZATION OF A LYMPHOTROPIC LENTIVIRUS ISOLATED FROM A PIG- TAILED MACAQUE WITH LYMPHOMA, R. Benveniste[1], L. Arthur[2], C-C. Tsai[3], R. Sowder[4], L. Henderson[4] and S. Oroszlan[4] [1] National Cancer Institute, Frederick, MD 21701, [2] Program Resources, Inc., Frederick, MD 21701, [3] University of Washington Primate Center, Seattle, WA 98195, [4] Litton Bionetics, Inc., Frederick, MD 21701

A retrovirus has been isolated on the human T cell line HuT 78 after cocultivation of explants of a lymph node from a pig-tailed macaque (M. nemestrina) that had died with malignant lymphoma in 1982 at the University of Washington Primate Center. Electron microscopy revealed the characteristic morphology of a lentivirus. This isolate, designated MnLLV (M. nemestrina lymphotropic lentivirus), replicates to high titers in various lymphocyte lines of human and primate origin but not in several monolayer lines. SDS-PAGE of purified MnLLV reveals bands of structural proteins at 28K, 16K, and a doublet at 14K that do not comigrate with the viral proteins of an HTLV-III isolated on HuT 78 cells, or with those of EIAV or a macaque type D retrovirus. The antigenic relatedness of MnLLV to other viruses was studied in specific radioimmunoassays (RIA) for the major core protein of HTLV-III, HTLV-I, MPMV (a type D virus), as well as in a broad RIA for the type C major core antigen. Lysed virus pellets of MnLLV competed partially only in the HTLV-III p24 assay. The slopes of the competition curves indicate that these two lentiviruses are distantly related to each other. Immunoblots of some human AIDS sera that contain antibodies that react with HTLV-III p24 also recognize the 28K protein of MnLLV. Transmission studies have been initiated at the University of Washington in order to investigate the pathogenicity of MnLLV in different primate species.

D19 **TRANSCRIPTIONAL PROPERTIES OF HTLV** by Alan J. Cann, William Wachsman, Richard B. Gaynor, Jan Williams and Irvin S.Y. Chen. Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

A rapid and quantitative transient transfection assay for transcriptional regulatory properties of the HTLV χ gene has been developed. We have used this system to demonstrate that the HTLV χ gene is responsible for the transcriptional regulatory properties of HTLV in infected and transformed cells and that no other genes of HTLV are involved. The transcriptional regulatory properties of the HTLV-I and HTLV-II χ proteins was examined and shown to be different with respect to their specificities. These differences have been investigated quantitatively using high efficiency expression vectors for the effects of the χ genes upon different promoters. We have also demonstrated that the HTLV-II χ gene product is capable of activating transcription from adenovirus early promoters, normally inducible by the adenovirus E1A gene product in infected cells, supporting the hypothesis that the χ gene is the transforming gene of HTLV. These results are the first demonstration that the χ protein is capable of activating transcriptional promoters other than the HTLV LTRs and provide direct support for the hypothesis that the HTLV χ gene is capable of activating cellular genes. We will further investigate the role of HTLV χ in activation of heterologous promoters by analysing the sequences required for activation by the HTLV χ of adenovirus early promoters. These studies may lead to predictions as to the cellular genes which may be activated by the HTLV χ gene in T cells, ultimately leading to transformation of the cell.

D20 A T LYMPHOBLASTOID CELL LINE FROM A PATIENT WITH AIDS-RELATED COMPLEX(ARC), D. Casareale & D.J. Volsky, Univ. of Nebraska Medical Center, Omaha, NE, USA

We report on the establishment of a T lymphoblastoid cell line, which is 100% susceptible to HTLV-III/LAV infection. Lymphocytes from peripheral blood were cultured in RPMI 1640 medium supplemented with 10% FCS and IL-2. A rapid growth of IL-2-independent cells resulted within two months after initiation of culture. The cell line was designated DC-10. Cell surface marker analysis revealed the following: 100% of the cells carried the OKT-4 marker, 66% OKT-11, 59% OKT-8. None expressed the M-1 or B-1 markers. The DC-10 cells were found not to express HTLV-III/LAV or HTLV-I antigens. Since 100% of the DC-10 cells were positive for the OKT-4 surface marker, which is believed to be the receptor for HTLV-III/LAV, they were tested for infectability with cell-free virus supernatant from a HTLV-III/LAV producer line. Three days after infection, virtually 100% of the cells became positive for HTLV-III/LAV antigens as determined by immunofluorescence staining. To test whether or not the DC-10 cells released infective virus the supernatant from a 2 week-old culture of viral antigen-positive DC-10 cells was tested for reverse transcriptase or used to infect CEM cells or uninfected DC-10 cells. Both the RT and the infection assays were positive, indicating that the DC-10 cells produce infective virus. In conclusion, we report on the establishment of an IL-2-independent OKT-4-positive T cell line from a patient with ARC. Cells of this line are negative for HTLV-III/LAV viral antigens, but are susceptable to infection by the virus. The usefulness of the DC-10 cells for HTLV-III/LAV detection assays and for virus replication studies is being investigated.

D21 ANALYSIS OF THE BIOCHEMICAL AND TRANSFORMATION PROPERTIES OF SV40 DELETION MUTANTS. C.N. Cole[1], M.J. Tevethia[2], and J.C. Alwine[3], [1]Department of Biochemistry, Dartmouth Medical School, Hanover, NH, [2]Department of Microbiology, Pennsylvania State University, Hershey, PA, and [3]Department of Microbiology, University of Pennsylvania, Philadelphia, PA.

Biochemical and transformation properties have been determined for a large set of SV40 mutants with deletions and other alterations affecting the large T antigen. These studies indicate that sequences in the vicinity of amino acid 145 are required for binding to the SV40 origin of DNA replication. Sequences in the vicinity of amino acids 500-600 are required for ATPase activity. Sequences between amino acids 683 and 708 (the carboxy terminus) are required for adenovirus helper function and also define a function required late during infection of monkey cells by SV40. Mutants with small deletions affecting each of these three properties retain the ability to immortalize primary mouse embryo fibroblasts at wildtype frequencies, indicating that these functions of large T are not required for immortalization. In contrast, mutants whose deletions result in a reading frame shift, causing production of truncated T antigens containing the amino terminal 138, 508 or 585 residues of large T are unable to immortalize cells. A mutant with the amino terminal 626 residues transforms at wildtype frequency. Thus, the carboxy-terminal 82 amino acids of large T are not required for immortalization. Experiments are in progress to examine the ability of mutant T antigens to trans-activate the SV40 late promoter, and to determine the roles played by portions of the SV40 large T antigen not studied using this set of deletion mutants. Additional mutants are being prepared and characterized with deletions at these sites.

D22 AN HTLV-III/LAV-RELATED VIRUS FROM MACAQUES, R.C. Desrosiers, N.L. Letvin, M.D. Daniel, L.O. Arthur[1], R.D. Hunt and N.W. King, New England Regional Primate Research Center, Harvard Medical School, Southboro, MA 01772, and [1]Frederick Cancer Research Facility, Frederick, MD 21701.

A T-cell tropic retrovirus with morphology and growth characteristics similar to the human AIDS virus (HTLV-III/LAV) has been isolated from four diseased macaques. Three of the macaques had an immunodeficiency syndrome and the fourth had malignant lymphoma. We refer to this virus as simian T-lymphotropic virus type III (STLV-III). Antigenic relatedness between STLV-III and HTLV-III/LAV has been demonstrated by radioimmune competition assay and by radioimmune precipitation. Lysed STLV-III specifically competed with binding of HTLV-III/LAV p24 to goat HTLV-III/LAV antisera. ^{35}S-labeled proteins from STLV-III infected cells could be specifically immunoprecipitated not only by positive macaque sera but also by selected HTLV-III/LAV positive human AIDS sera (Science 228:1201). Southern blot hybridization of DNA from STLV-III infected cells using a full length cloned HTLV-III/LAV DNA probe did not reveal significant levels of hybridization even at low stringency; the overall degree of sequence conservation between STLV-III and HTLV-III/LAV thus appears to be less than 75%. Following inoculation with STLV-III, four of six rhesus macaques died within 160 days with a wasting syndrome, opportunistic infections, a primary retroviral encephalitis and immunologic abnormalities. The fifth animal died similarly at 340 days post-inoculation but lacked the brain lesions. The sixth animal remains alive and healthy despite our continued ability to isolate virus from peripheral blood lymphocytes; this animal had the strongest antibody response of of the six. (Contract NO1-CO-23910).

D23 A REGION OF THE HERPESVIRUS SAIMIRI GENOME REQUIRED FOR ONCOGENICITY, Ronald C. Desrosiers, New England Regional Primate Research Center, Harvard Medical School, Southboro, MA 01772.

Herpesvirus saimiri naturally infects squirrel monkeys, producing no signs of disease in this species. Infection of other New World primates such as marmosets and owl monkeys results in a rapidly progressing T-cell lymphoma. L-DNA sequences between 0.0 and 4.0 map units (4.5 kilobasepairs) are not required for replication of the virus but these sequences are required for the lymphoma inducing capacity. Two replication-competent mutants with deletions in this region, 11att (-0.5 to +1.6 map units) and S4 (+0.4 to +4.1 map units), have lost their ability to induce lymphomas and do not immortalize common marmoset lymphocytes in an *in vitro* assay. Restoration of the DNA sequences restored the lymphoma-inducing and immortalizing capacity. Another constructed deletion mutant called KH (+0.9 to +1.35 map units) retains its ability to immortalize common marmoset lymphocytes. These results indicate that sequences immediately leftward from 0.9 map units or immediately rightward from 1.35 map units may contain the sequences essential for immortalization and oncogenicity. In tumor cells, the only transcripts detectable from 7.4 kilobasepairs encompassing this region are four small RNAs 70 to 140 nucleotides in length. Two of these small RNAs map between 1.35 and 1.9 map units. Further studies are needed to directly demonstrate the importance of these small RNAs for oncogenic transformation.

D24 ISOLATION OF A T-LYMPHOTROPIC RETROVIRUS FROM HEALTHY MANGABEY MONKEYS, Patricia N. Fultz, Harold M. McClure, Rita Anand, and A. Srinivasan, Centers for Disease Control AIDS Branch and Yerkes Regional Primate Research Center, Atlanta, GA 30333

A T-lymphotropic retrovirus was isolated from peripheral blood mononuclear cells (PBMC) from 14 of 15 mangabey monkeys (Cercocebus atys). The animals were randomly-selected from 88 healthy animals in a colony that was used only for behavioral and reproductive studies. Antibodies to the virus were detected by immunofluorescence in serum samples from all virus-positive animals. The new retrovirus appeared to be related to the human virus HTLV-III/LAV because it was (i) morphologically identical to HTLV-III/LAV by electron microscopy; (ii) serologically related to HTLV-III/LAV by enzyme immunoassay, Western blot, and radioimmunoprecipitation; (iii) composed of proteins with molecular weights very similar to those of the proteins of HTLV-III/LAV; and (iv) cytopathic for $OKT4^+$ cells. However, no homology between the mangabey virus and HTLV-III/LAV was detected by RNA-DNA and DNA-DNA hybridization. The virus isolated from mangabeys also shares the above properties with the simian virus STLV-III that was isolated from diseased macaques; thus, we will refer to the new virus as STLV-III/SMM (SMM for sooty mangabey monkey). In contrast to both HTLV-III/LAV and STLV-III, STLV-III/SMM apparently does not cause clinical immunodeficiency or disease in the host from which it was isolated. Five of 6 rhesus macaques injected intravenously with STLV-III seroconverted within 6 weeks, and virus was recovered from PBMC of the 5 seropositive macaques at 3, 6, and 13 weeks after infection; however, the STLV-III/SMM-infected macaques show no clinical signs of disease as of 13 weeks after inoculation.

D25 SIMIAN AIDS AND TYPE D RETROVIRUS, Murray Gardner, Preston Marx, Don Maul, Nick Lerche, Kent Osborn, Linda Lowenstine, Martin Bryant, Neils Pedersen, University of California Davis, California Primate Research Center, Davis, CA 95616, Gisela Heidecker, Frederick Cancer Research Center, Frederick, MD 21701, Mike Power and Paul Luciw, Chiron Corporation, Emeryville, CA 95608

We have obtained further evidence that a type D retrovirus (SRV-I), related to but distinct from the Mason Pfizer monkey virus, is the cause of an acquired immune deficiency syndrome (SAIDS) in rhesus macaques at the California Primate Research Center (CPRC). Our evidence is based on: 1) induction of fatal SAIDS and profound lymphoid depletion with molecularly cloned infectious SRV-1, 2) strong serologic association of SAIDS with SRV-1 but not with STLV-I or STLV-III and 3) complete prevention of experimental SAIDS using a formalin inactivated SRV-1 vaccine. We have fulfilled Koch's postulates with SRV-I (including molecularly cloned virus) in respect to SAIDS at the CPRC. SRV-I has been completely sequenced (See M. Power et al. abstract, this meeting); it has a unique genetic structure with a distinct protease gene. Although SRV-I is not closely related to the AIDS viruses and has a broader cell tropism, it does cause a naturally occurring and experimentally reproducible, often fatal, acquired immunodeficiency in its natural primate host, the rhesus macaque, and thus, constitutes an important model for AIDS.

D26 DE NOVO SYNTHESIS OF IgG ANTIBODIES TO LAV/HTLV-III WITHIN THE CENTRAL NERVOUS SYSTEM,J.Goudsmit,E.Ch.Wolters,M.Bakker,L.Smit,E.A.H.Hische,J.A.Tutuarima,H.J.van der Helm,Virology and Neurology department,University of Amsterdam;Neurology department,Vrije Universiteit,Amsterdam,the Netherlands.

Matched sera and Cerebro-spinal Fluid(CSF) of homosexual males with and without AIDS were tested for antibodies to LAV/HTLV-III by ELISA and Immunoblotting.No antibodies were present in CSF of seronegative individuals.Antibodies were present in the CSF of seropositive AIDS and non-AIDS patients.By calculating the index of LAV/HTLV-III specific antibody in serum and CSF relative to extrathecally produced protein evidence was obtained for intrathecal synthesis of these antibodies.Longitudinal studies of a seroconverted male corroborated the evidence.This parallels findings in the CSF of visnavirus in sheep and indicates replication of LAV/HTLV-III in the brain,even when signs of AIDS or AIDS encephalopathy are not apparent.

D27 LAV/HTLV-III GAG GENE PRODUCT p24 SHARES ANTIGENIC DETERMINANTS WITH EIAV,BUT NOT WITH VISNA VIRUS OR CAEV,D.J.Houwers,J.Goudsmit,I.M.Nauta,Central Veterinary Institute,Lelystad;Virology department,University of Amsterdam,the Netherlands.

Serological crossreactivity between the lentiviruses Visna virus,Caprine Arthritis Encephalitis Virus(CAEV),Equine Infectious Anemia Virus(EIAV) and LAV/HTLV-III was investigated by ELISA and Immunoblotting.Purified Visna virus,CAEV,EIAV and HTLV-III were used as antigens. Sera from naturally and experimentally infected hosts were used as well as polyclonal rabbit sera specific for gag gene products of Visna virus,CAEV,EIAV and LAV/HTLV-III.
Serological crossreactivity between the gag gene products of Visna virus and CAEV as well as between the gag gene products of LAV/HTLV-III and EIAV was detected.No crossreactivity could be demonstrated between the gag gene products of Visna virus or CAEV on the one hand and LAV/HTLV-III and EIAV on the other.
These data indicate a closer relationship of LAV/HTLV-III with EIAV than with Visna virus or CAEV.

D28 DETECTION OF CONCOMITANT INFECTION OF HTLV-I AND HTLV-III BY IN SITU HYBRIDIZATION, Mary E. Harper, Lisa M. Marselle, Karen J. Chayt, Mark H. Kaplan, Robert C. Gallo and Flossie Wong-Staal, Laboratory of Tumor Cell Biology, NCI, NIH, Bethesda, MD 20892

A sensitive in situ hybridization method has been used to screen primary or short-term cultured mononuclear cells from patients seropositive for HTLV-I and HTLV-III for the presence of both types of viral RNA. From one patient, a 65 yr old bisexual male with a history of OKT8 suppressor cell lymphocytosis, cells cultured for 3-10 days contained a significant percentage (20-50%) of cells expressing HTLV-I RNA at high abundancy (50-200 copies/cell). Primary cells were negative for HTLV-I expression. Primary as well as cultured cells were primarily of the OKT8 type, indicating a suppressor cell lymphocytic leukemia. Cells expressing HTLV-III RNA were also detected in short-term cultures, but at very low frequency (<0.01% of cells). Southern blot hybridization indicated monoclonal integration of HTLV-I in the leukemic cells, but did not detect HTLV-III. This patient represents a direct demonstration of simultaneous infection with HTLV-I and HTLV-III and also constitutes a rare case of HTLV-I infection in an OKT8 leukemia. Additional patients are currently under study to determine the incidence of concomitant infection with more than one HTLV, and to study the interplay of the two viral infections, such as predisposition to the second infection as caused by the primary HTLV. Efforts are also being placed on determining the biologic activity and molecular characteristics of the HTLV members isolated from these patients.

D29 BIOLOGICAL AND MOLECULAR BIOLOGICAL ANALYSIS OF SIMIAN T-LYMPHOTROPIC VIRUS TYPE-I (STLV-I), Masanori Hayami, Institute of Medical Science, Tokyo University, Tokyo.
Non-human primates of African and Asian origins have been found to be naturally infected with retroviruses closely related to HTLV-I and designated as STLV-I. Quite difference of geographical distribution of STLV-I and HTLV-I in Japan suggests the unlikeliness of recent interspecies transmission(1). Ten STLVs-I were isolated from various species of non-human primates(2). Their major components are antigenetically cross-reactive with HTLV-I. They contain virus sequences, gag, pol, env, pX and LTR homologous to HTLV-I. However, the restriction mapping turned out the difference between STLV-I and HTLV-I, and also among STLV-I(3). STLV-I is genomically the closest to HTLV-I, slightly related to HTLV-II and far from HTLV-III. Molecularly cloned STLV-I from an Asian macaque has 90% homology of the nucleotide sequence with that of HTLV-I in each env, pX and LTR region(4), while STLVs-I from a chimpanzee and a monkey of African origin have 95% homology in LTR region with HTLV-I. Leukemogenic potential of STLV-I is indicated by findings of spontaneous adult T-cell leukemia(ATL) like disease in African green monkeys naturally infected with STLV-I(5).
These findings indicate that STLV-I is a member of HTLV family closest toHTLV-I and can be a good model for clarifying the pathobiology of ATL and the origin of HTLV family.

1) Hayami et al., Int.J.Cancer,33,179,1984. 2) Tsujimoto et al., Int.J.Cancer,35,377,1985.
3) Komuro et al., Virol.,138,373,1984. 4) Watanabe et al., Virol.,144,59,1985.
5) Tsujimoto et al., Gann,76,911,1985.

D30 INACTIVATION OF THE AIDS CAUSING RETROVIRUS HTLV III IN ANTIHEMOPHILIC FACTOR VIII CONCENTRATES AND OTHER HUMAN PLASMA PROTEIN PREPARATIONS BY PASTEURIZATION,
Joachim Hilfenhaus[1)], Rudolf Mauler[1)], Alfred M. Prince[2)] [1)] Research Laboratories of Behringwerke AG, D-3550 Marburg, F.R.G., [2)] The Lindsley F. Kimball Research Institute of the New York Blood Center, 310 East 67 Street, New York, NY 10021

Heat treatment at 60°C for ten hours in solution (pasteurization) had been introduced into the manufacturing process of antihemophilic cryoprecipitate, factor VIII, factor IX and factor XIII concentrates as well as of the prothrombin complex and antithrombin III. This procedure has been shown to considerably reduce the risk of transmission of hepatitis to hemophiliacs. We therefore studied whether pasteurization also efficiently inactivates the AIDS virus, HTLV III, in these human plasma protein preparations stabilized with sucrose and glycine. Although we found that low molecular weight substances as sucrose and glycine to a certain extent stabilize infectious HTLV III, efficient inactivation of this virus was achieved by pasteurization. In addition, a group of hemophiliacs only treated with pasteurized factor VIII concentrates did not develop anti-HTLV III antibodies after a three year therapy while in a group of hemophiliacs substituted with conventional factor VIII concentrates 60 % became seropositive. From these experimental and clinical data we conclude that heat treatment at 60°C in solution confers a high margin of safety to human plasma protein preparations regarding AIDS.

D31 Comparative Biochemical and Serological Studies on Structural Polypeptides of Primate Lymphotropic Retroviruses (PLRV)

Hunsmann[1], G., Jurkiewicz[1], E., Nakamura[2], H., Wendler[1], I., and Schneider[1], J.

[1]Deutsches Primatenzentrum, Kellnerweg 4, D-3400 Göttingen, FRG
[2]Institute of Medical Science, 4-6-1, Shirokanedai Minato-ku, Tokyo 108, Japan

Two dimensional tryptic peptide maps of 125-I-labelled core polypeptides p19 and p24 of HTLV-I and eight related simian isolates were compared. The patterns of these peptide maps fall into three groups, HTLV-I and chimpanzee isolates, four macaque viruses as well as the green monkey virus. These data do not support an exchange of PLRVs between distantly related species. Results of a similar analysis with LAV/HTLV-III and serologically related simian viruses will be reported. In addition we will present data of screening for antibodies to PLRVs in more than 500 sera from non-human primates belonging to 27 species.

D32 Mouse Monoclonal Antibodies to HTLV III Structural Proteins. J.C. Hunt, J.S. Webber, L.K. Wray, D.O. Kieselburg, G.J. Dawson, J.M. Casey, P.R. Andersen, C.A. Wood, and S.G. Devare. Departments of Cancer Research, Hepatitis/AIDS Research, and Molecular Biology, Abbott Laboratories, North Chicago, IL 60064

Human T cell lymphotropic virus (HTLV) type III is typical of the group of closely related retroviral isolates which are strongly implicated as the etiologic agents of acquired immune deficiency syndrome (AIDS). Sera from patients with AIDS or AIDS-related complex (ARC) usually contain antibodies to one or more of the major HTLV III structural proteins and/or polyprotein precursors. These include the major structural core (gag) protein p24 and its probable polyprotein precursor Pr55, and the polyprotein precursor Pr160 and its cleavage products gp120 and gp41. Antibodies to these structural proteins and polyprotein precursors are generally considered diagnostic for infection with HTLV III, and current diagnostic blood screening tests for AIDS are designed to detect serum antibodies to HTLV III structural proteins. We have developed several monoclonal antibodies to selected HTLV III structural proteins as possible future diagnostic tools, and for identifying and confirming the relationships between polyprotein precursors and final cleavage products formed during maturation of HTLV III structural components. Specificities of selected monoclonal antibodies were confirmed by (1) analysis on Western Blots containing disrupted HTLV III, (2) precipitation of purified HTLV III proteins, and (3) precipitation of biosynthetically radiolabeled proteins from HTLV III infected HT9 cell lysate.

D33 EXPRESSION AND REARRANGEMENT OF T-CELL RECEPTOR GENES IN CELLS INFECTED BY HTLV-I
R. Jarrett, ¶G. Reyes, *H. Mitsuya,, ¶M. Feinberg, *S. Broder and M. Reitz, Laboratory of Tumor Cell Biology, *The Clinical Oncology Program NCI, ¶Cancer Biology Research Laboratory, Standford University Medical Center

Infection of T-cells by the human T-cell leukemia virus type I (HTLV-I) results in a number of abnormalities of immune function including loss of antigen-specific responsiveness and the acquisition of alloreactivity. We have shown that these changes do not appear to be associated with alterations in the levels of expression of the genes encoding the α- and β-chains of the T-cell receptor (TCR) or with changes in the pattern of rearrangement of the TCR β-chain gene. The expression and configuration of the TCR γ-chain gene is currently being investigated.

The receptor mediated theory of leukemogenesis proposes that a lymphocyte may be driven to continued replication by binding of a retrovirus to a specific receptor on the cell surface, thus expanding the pool of target cells for further leukemogenic events. In order to test the relevance of this to HTLV-I infected cells and the TCR we examined the rearrangement of the TCR β-chain gene in primary tumor cells from patients with adult T-cell leukemia/lymphoma and in cell lines infected by HTLV-I. All of the primary tumor cells and the majority of the cell lines analysed had distinct TCR rearrangements when compared by Southern blot analysis; however this does not preclude the use of the same variable gene segment by different cells. We have, therfore, cloned a TCR β-chain gene from the HTLV-I-infected cell line C91PL and are studying the expression of this particular variable region in a variety of HTLV-I infected cells.

D34 The AIDS virus LTR contains an Sp1-responsive Promoter.
K.A. Jones, J.T. Kadonaga, P. Luciw, and R. Tjian.
Department of Biochemistry,University of Calif., Berkeley, CA 94709.

The Sp1 transcription factor is a sequence-specific DNA-binding protein, endogenous to mammalian cells, that has been shown to activate RNA synthesis in vitro from a class of cellular and viral genes (including the early genes of SV40 and HSV-1). Sp1 has recently been purified to an estimated 95% homogeneity from cultured human (HeLa) cells, with complete retention of binding and transcriptional activation activities. Inspection of the published sequence of the AIDS virus LTR suggested the presence of at least one Sp1 binding site in the promoter region upstream of the RNA start site. Our analysis of the interaction, using purified Sp1 preparations, demonstrate that transcription from the AIDS virus LTR is dependent on Sp1, and binding studies revealed an extended DNase I footprint protection pattern that includes multiple tandem Sp1 binding sites. Site-specific mutations have been targeted to each binding site, and these constructions are being tested for binding and transcriptional activation in vivo and in vitro. The results of our analysis of the interaction of Sp1 and other cellular factors with the defined transcriptional elements of the AIDS virus will be presented.

D35 Simian T-Lymphotropic Virus Type III ($STLV\text{-}III_{AGM}$) in African Green Monkeys and its Relationship to Human Retroviruses in Africa.
P. Kanki[1], F. Barin[1], J. Allan[1], T.H. Lee[1], S. M'Boup[2], M. Essex[1] [1]Dept. of Cancer Biology, Harvard School of Public Health, Boston, MA U.S.A. [2]Bacteriology-Virology Laboratory, Dakar University, Dakar, SENEGAL

It is generally believed that AIDS emerged as a new disease in Central Africa in recent times. We have recently described the isolation and serologic characterization of STLV-III from captive macaques and African Green monkeys. The major viral antigens of $STLV\text{-}III_{mac}$ and $STLV\text{-}III_{AGM}$ are of similar size and cross-reactive with the env, gag, and 3'orf encoded proteins of HTLV-III/LAV. Therefore, it has been posulated that the etiologic agent of AIDS originated in Africa.

To further test the hypothesis that HTLV-III/LAV related viruses in African people might be more closely related to STLV-III, we analyzed the sera from HTLV-III antibody positive people from the U.S. and different areas of Africa to determine their cross-reactivity to $STLV\text{-}III_{AGM}$ viral proteins. Ninety-seven - 100% of HTLV-III antibody positive AIDS patients from Central Africa cross-react to the simian viral antigens. Most importantly, we have recently discovered that certain apparently healthy African people possess serologic evidence for exposure to a virus presently indistinguishable from $STLV\text{-}III_{AGM}$, thus supporting the hypothesis that a simian HTLV-III/LAV related virus may have served as the progenitor of the human AIDS virus. The study of STLV-III may enhance our understanding of the origin of HTLV-III/LAV and its unique pathogenicity.

D36 I-4 ANTIGEN AND T-CELL ACTIVATION MOLECULES ACT AS RECEPTORS FOR HILV-III. Kai J.E. Krohn, Naorong Ruan, Pekka Nieminen and Annamari Ranki, Institute of Biomedical Sciences, University of Tampere, 33210 Tampere, Finland

Mechanism of binding of HILV-III viral proteins to target cells was studied using I-125 labelled purified HILV-III, disrupted by Triton-X or by NP-40 treatment. Target cells were incubated with the radiolabelled virus preparation for 5 - 30 minutes, washed and the bound radioactivity was quantitated. In optimal conditions, 1 - 3 % of the label bound to peripheral blood lymphocytes. Percoll fractions II and III, containing large granular lymphocytes and other large, activated T-cells, showed 4 - 5 times higher binding than small resting T-cells in Percoll fractions V - VI. Macrophages showed low binding capasity. 50 - 60 % inhibition of the binding was noted when the target cells were treated with monoclonal antibodies (Mab) to the T-4 molecule (UKT-4, Leu 3a),characteristic for T-helper cells. A monoclonal antibody (NP-4), recognizing glycoproteins of the T-200 family and thought to be associated with T-cell activation, also inhibited the binding, while no effect was observed with Mabs reacting with T-8, T-3 molecules or with IL-2 receptor. The results suggest, that activated T-helper cells are more susceptible for HILV-III infection than the resting lymphocytes and may explain the mechanism of cofactors regulating the spread of HILV-III infection in vivo.

D37 VARIATION IN REACTIVITY TO THE MAJOR STRUCTURAL PROTEIN P24 MARKS DIFFERENT STAGES OF LAV/HTLV-III INFECTION. J.M.A.Lange, R.A.Coutinho, S.A.Danner, J vd Noordaa, J.Goudsmit, Virology Department, University of Amsterdam, Department of Internal Medicine, University of Amsterdam.

In longitudinal immunoblot studies of persons who seroconverted for LAV/HTLV-III we found the following pattern of IgG protein recognition:
a) antibodies to p24 appeared first, soon followed by antibodies to other gag-encoded proteins (pr55, p18), and gradually followed by antibodies to env-encoded proteins (gp41, gp65, sometimes pr110) and antibodies to the pol gene product (p33); b) the lag time of seroconversion to p24 appeared to be 2-4 weeks; c) in non-AIDS subjects the intensity of the response against all the different visible proteins stabilized or increased in time (even if follow-up extended 3 years); d) in persons in whom AIDS developed this was preceded by a decrease in the intensity of the response against the different proteins, most striking for p24.

D38 HTLV-I Geographic Distribution and Identification of New High-Risk Populations, Paul H. Levine, W. Carl Saxinger, Jeffrey Clark, Robert W. Biggar, Marjorie Robert-Guroff and William Blattner, National Cancer Institute, Bethesda, Md 20892.

Epidemiologic studies indicate that Human T-cell Lymphotropic virus (HTLV)-I, the causative agent of adult T-cell leukemia in Southeast Japan and the Carribean Islands, occurs in a world-wide distribution but with unusual geographic clustering. The current large scale serosurvey was undertaken to quantitate HTLV prevalence in different parts of the world. We analyzed approximately 40,000 serum samples collected from various geographic locales worldwide, 3/4 of these sera from clinically healthy donors. All samples were initially screened by ELISA test and 2165 with high reactivity were further evaluated using a competition assay. Analysis of the sera from normal individuals confirmed the high prevalence of infection in Japan, Africa and several countries in the Carribean basin. Newly recognized areas of possible endemicity included southern Florida, where evidence for infection was found in a group of native Americans whose sera were collected in 1968. It was noted that in certain parts of the world there were particularly important problems in determining specificity of reactivity because of apparent cross-reacting antibodies. Greatest problems were encountered with sera from certain regions in sub-Saharan Africa but no pattern was detected that could explain the cross-reactivity based solely on geographic areas, specific patterns of non-viral parasitic infection, or methods of handling the specimens.

D39 AIDS VIRUS INDUCED GIANT CELLS ARISE THROUGH CELL FUSION INVOLVING THE CD4 MOLECULE, Jeffrey D. Lifson, Michael S. McGrath, Gregory R. Reyes, and Edgar G. Engleman, Stanford University, Stanford CA 94305, University of California, San Francisco, San Francisco, CA 94143, and Gene Labs, Inc., San Carlos, CA 94070

Cultures of T lymphoid cells infected with the AIDS retrovirus typically undergo cytopathologic changes, including formation of multinucleated giant cells, with progression to cell death. We studied several CD4+ T cell lines, including the leukemic T cell line JM (JURKAT), following infection with the AIDS virus to determine the mechanism of giant cell formation and assess the role of the CD4 cell surface molecule in this process. Acute infection of JM cells consistently induced typical cytopathology along with virus production, with the eventual outgrowth of a CD4- population of cells which did not produce detectable virus, did not contain detectable integrated provirus, and was not susceptible to infection by virus. Molecular studies suggest in vitro selection of CD4- cells after infection and death of CD4+ cells as the mechanism leading to the CD4- outgrowth. Two color fluorescent label cell mixing studies using CD4+ and CD4- cell populations demonstrated that the multinucleated giant cells typically observed in infected cultures arise through a process of cell-to-cell fusion. The CD4 molecule appears to be involved in this process as: (1) in contrast to uninfected CD4+ cells, uninfected CD4- cells did not undergo fusion with infected cells, and (2) fusion of uninfected CD4+ cells with infected cells was specifically blocked by anti-CD4 monoclonal antibody. As the giant cells die soon after fusion, this process may represent one of the mechanisms of T helper/inducer cell depletion in infected patients.

D40 HTLV-I Infection May Indirectly Cause B-Cell Chronic Lymphocytic Leukemia. D.L. Mann, P. DeSantis, M. Newman, G. Mark, A. Pfeifer, J. Clark, R.C. Gallo, W.A. Blattner, NCI, NIH, Bethesda, MD.

HTLV-I infection is causually associated with adult T-cell leukemia. In the West Indies, HTLV-I seropositive individuals with B-cell chronic lymphocytic leukemia (CLL) were identified where the CLL cells were negitive for HTLV-I infection by Southern blotting. The CLL cells from 2 patients were fused with a non-immunoglobulin secreting HGRRT-negative lymphoblastoid cell line, azaserine selected and cloned. Supernatants from clones secreting IgM were tested by flow cytometry or ELISA for antibody activity to HTLV-I, HTLV-II and HTLV-III infected cells, the isolated retroviruses and the p19 and p24 gag protein from the 3 virus strains. Supernatants from fusion with CLL from patient 1 reacted specifically with the p24 gag protein of HTLV-I and cross-reacted with p24 from the HTLV-II and HTLV-III. Supernatants from the CLL fusion from the 2nd patient reacted with HTLV-I infected cells only. The specific IgM and Igk immunoglobulin gene rearrangements in the CLL were demonstrated in the fused clones. These data demonstrate that the CLL is a malignant transformation of antigen committed B cells, the antigen being a component of the HTLV-I retrovirus. The results suggest that HTLV-I infection may be directly involved in the etiology of B-cell malignancies.

D41 Cellular Tropism of SRV-1 in SAIDS - D.H. Maul, C. Zaiss, P.A. Marx, M.B. Gardner. California Primate Research Center, University of California-Davis, Davis, CA 95616

Simian AIDS is a disease of rhesus monkeys caused by a type D retrovirus (SRV-1) and characterized by lymphoid depletion, opportunistic infections and depressed immune function indicative of both a humoral and cell mediated immune defect. To understand how SRV-1 infection relates to the immune defect it induces, we purified by panning, B cells (SI_g+), T helper cells (OKT4+), and T supressor/cytotoxic cells (OKT8+) from SRV-1 infected rhesus monkeys. Monocyte/macrophages were isolated by adherence to plastic. Neutrophils were isolated by sedimentation through dextran and platelets were collected by low speed centrifugation. Virus was detected in purified cell populations by syncytia induction in Raji cells (human B cell line). OKT4+, OKT8+ and SI_g+ lymphocytes and monocyte/macrophages were positive for SRV-1 in animals with SAIDS. Neutrophils and platelets had no detectable virus. SRV-1 also infected rhesus lung fibroblasts, rhesus kidney cells, Raji cells, Hut78 cells (human T cell line), and K562 cells (a human myeloid cell line). These findings indicate that SRV-1 has a broad cell tropism corresponding to the clinical syndrome of depressed cellular and humoral immunity. This contrasts with HTLVIII/LAV which has a more specific T helper cell tropism.

D42 THE ROLE OF IMMUNOSPECIFIC RECEPTORS IN HUMAN LYMPHOID NEOPLASIA. M.S. MC GRATH*, J.D. LIFSON**, & I.L. WEISSMAN**, From the Division of Oncology/AIDS UCSF*; Department of Pathology, Stanford University**

The receptor mediated leukemogenesis hypothesis predicted that continuous stimulation of immunospecific receptors by retroviruses may lead to malignant transformation. Utilizing a cytofluorographic HTLV-I binding assay, we have found that labeled HTLV-1 binds to less that 2% of normal T cells or thymocytes, but binds to a variety of HTLV-1 positive and negative T cell lymphomas, including CEM-CCRF, Jurkat, 8402, HPB-ALL, HUT-102, C91/PL and MT-2. Labeled HTLV-1 does not appear to bind at significant levels to the T cell lines HUT-78 or Molt-4. Antibodies to cell surface determinants including leu-1,2,3,4, TAC, HLA and DR framework failed to block HTLV-1 binding. To determine whether the antigen specific T cell receptor (TCR) might be involved in this process, we (in collaboration with Dr. L. Hood) prepared a series of rat monoclonal antibodies to Jurkat and HPB-ALL N-terminal beta-chain peptides. Several antibodies stained the majority of in vitro T lymphomas and binding of labeled HTLV-1 appeared to be specifically blocked by preincubation of target cells with these monoclonal antibodies. Taken together, these data suggest that immunospecific receptors may play a role in human retrovirus mediated leukemogenesis.

D43 SAIDS Induced by an HTLV-III Related Simian Retrovirus. *M. Murphey-Corb, *L.N. Martin, ‡R.C. Montelaro, *S.R.S. Rangan, *G.B. Baskin, *B.J. Gormus, *R.H. Wolf and °W.A. Andes, *Delta Regional Primate Research Center of Tulane University, Covington, LA, ‡Louisiana State University, Baton Rouge, LA and °Tulane Medical Center, New Orleans, LA.

As a part of an ongoing leprosy study, several macaques (Macaca mulatta) were inoculated with a lepromatous leprosy tissue homogenate obtained from a sooty mangabey (Cercocebus atys). One of the recipient monkeys developed a lymphoma 18 months after inoculation. Beginning with this animal, passage of lymphoid tissue from affected animals to healthy macaques has resulted in the induction of lymphoma and/or SAIDS in 11/14 recipient animals. SAIDS D retrovirus was not detected in the recipients by either in vitro coculture with Raji cells or Southern blot analysis of lymphoid tissues. Culture of either tumor cells or peripheral blood lymphocytes, however, resulted in the isolation in 5/7 attempts of a type C retrovirus, STLV-III/Delta, which is morphologically identical to HTLV-III.

Western blot analysis using virion proteins purified from both HTLV-III and STLV-III infected H9 cells demonstrated antibody cross-reactive to HTLV-III in 5/14 infected macaques. However, in the reciprocal experiment, human reference serum failed to recognize simian viral proteins. Macaque antiserum also recognized 4 major STLV-III proteins approximately 16, 26, 53, and 100 kilodaltons in molecular weight. Seroconversion of 3/4 of the recipients of mangabey tissue was noted after inoculation; analysis of the mangabey colony at the DRPRC has revealed a number of seropositive and virus-positive individuals. Taken together, these observations suggest that STLV-III/Delta may reside asymptomatically in the mangabey, an African species whose habitat encompasses a region endemic for HTLV-III in man.

D44 IN VITRO INFECTION OF MONOCYTES WITH HTLV-III/LAV, Janet K. A. Nicholson, G. David Cross, J. Steven McDougal, Centers for Disease Control, Atlanta, GA 30333

Human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) is tropic for human T-helper cells. The virus binds to the T4 antigen or a receptor very closely associated with the T4 antigen, infects the cell, and eventually kills the cell. We explored the possibility that monocytes, which also express the T4 antigen, can be infected with HTLV-III/LAV. We exposed highly enriched human monocytes to HTLV-III/LAV and monitored viral binding as well as infectivity. Virus binds to monocytes, and binding can be partially inhibited with OKT4A monoclonal antibody. Infectious virus can be recoverd from monocytes three days after infection, but only at low levels compared with the levels that can be recovered from T cells. Ten days after infection, both the monocytes and monocyte supernatants have viral activity, suggesting that HTLV-III/LAV replicates in monocytes. We propose that HTLV-III/LAV infects and replicates in monocytes and may help perpetuate HTLV-III/LAV infection of T cells in vivo.

D45 ASSOCIATION OF HTLV-LIKE ANTIBODIES AND ANTIGENS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS. Richard Olsen, James Blakeslee, Melinda Tarr, Lawrence Mathes and Ronald Whisler. The Ohio State University, Columbus, OH 43210

Serological studies demonstrated a high incidence of seropositivity in systemic lupus erythematosus (SLE) to HTLV-I antigens by indirect immunofluorescence (IFA), ELISA, and Western blot analysis. IFA positive sera from U.S. patients also detected HTLV-III/LAV peptides by Western blot analysis. In vitro cultivation of peripheral blood mononuclear cells (PBMC) from 4 of 8 patients with SLE expressed HTLV-I gag antigens after 3 or more days in culture. In addition, 3 of 5 cell culture supernatants tested contained reverse transcriptase activity.

One patient while in the acute phase of the disease, had a positive HTLV-I IFA titer, expressed HTLV-I antigens in PBMC and the sera recognized HTLV-III polypeptides p41, 56 and 65. Following steroid treatment (40 mg Prednisone/day for 4 weeks), the patient's HTLV-I IFA titer was negative, no HTLV-I viral antigens were detected in PBMC and no HTLV-III serum antibodies were detected by Western blot analysis. Thus, evidence of retrovirus infection may be abrogated by therapeutic intervention or correlated with active clinical disease. It is possible that SLE may be associated with or caused by a retrovirus identical to or closely related to the human T lymphotropic viruses.

D46 GENETIC STRUCTURES OF SIMIAN ACQUIRED IMMUNODEFICIENCY SYNDROME (SAIDS) TYPE-D RETROVIRUSES. M.D. Power[1], R.M. Thayer[1], M.L. Bryant [2], P.A. Marx[2], M.B. Gardner[2], P.J. Barr[1], and P.A. Luciw[1]. [1]Chiron Corporation, Emeryville, California, 94608. [2]University of California, Davis, California 95616.

Simian acquired immunodeficiency (SAIDS) in the macaque genus of monkeys is associated with infection by a type D retrovirus, the SAIDS-associated retrovirus (SRV). The genomes of two closely related virus isolates, SRV-1 from a monkey in California and SRV-2 from a monkey in Oregon, have been molecularly cloned and completely sequenced. The LTRs, gag and pol regions of both viruses are highly homologous. Comparisons of the predicted protein sequence of the env gene products shows that the gp70 domains are 80% homologous and the C-terminal transmembrane domains are 95% homologous. Small amounts of homology in parts of gag and pol were noted in comparisons with many other retroviruses. The protease gene (prt) for each virus is encoded in a unique open reading frame (314 codons) located between the gag and pol translation frames. The prt regions of SRV-1 and SRV-2 are highly homologous (90%) to each other and to the prt region of the hamster intracisternal A-type particle genome (60% homology). Interestingly, these prt regions are about twice as long as prt regions of other retroviruses and transposons and show homology with sequences near the active site of cellular aspartyl proteases. These SRV isolates have no notable similarity in either genetic organization or sequence with the human AIDS retroviruses (LAV, HTLV-III, ARV-2).

D47 EVIDENCE OF A RETROVIRUS INFECTION IN MYCOSIS FUNGOIDES AND ITS PRODROMES. Annamari Ranki, Kai Krohn, Maorong Ruan, Jaakko Antonen, Jukka Suni and Kirsti-Maria Niemi. Department of Dermatology, Univ. of Helsinki,Finland and NCI, NIH, Bethesda.

The occurrence of antibodies to HTLV-I, II and III was repeatedly studied in 16 Finnish Mycosis fungoides (MF) patients and in 26 patients with Parapsoriasis en plaque (PP), which often preceeds MF. Also, long term cultures of blood lymphocytes, lymph nodes and tumor-derived cells were established.The malignant cells were positive for a newly described antibody, MF-Y, associated in the binding of HTLV-III to its target cells. Eight MF patients had either positive ELISA or Western blot reactions to HTLV-I and, to a lesser extent, to HTLV-III. A similar finding was seen in eight PP patients, too. However, in the HTLV-I Western blots either a reaction to p19 or to several antigens in the range of 60-120K was seen. None of the patients had any autoimmune disorders or belonged to AIDS risk groups. Fourty patients with other dermatological disorders were antibody negative. In long term cultures, 2 of six patients showed elevated Mg-dependent reverse transcriptase activity after 4 weeks. The results suggest that a retrovirus, closely related but not identical to HTLV-I,II or III is involved in the pathogenesis of MF.

D48 VARIABILITY OF NUCLEOTIDE SEQUENCE OF DIFFERENT ISOLATES OF HTLV-III, M. Reitz[1], C. Gurgo[2], H.-C. Guo[1], A. Aldovini[1], F. Wong-Staal[1], C. Franchini[1] and R.C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health (1) and C.E.O.S., Naples, Italy

Isolates of HTLV-III were obtained from two individuals from the New York area, both of whom were diagnosed in 1984 as having AIDS. These viruses were cloned into the Eco RI site of lambda gt wes.lambda B and full length clones obtained. These were subcloned into M13 and partial nucleotide sequences were obtained by the Sanger method. One of these clones (λPH-1) differed substantially from all the previously sequenced HTLV-III isolates, in keeping with the degree of difference observed among these previous isolates. The second clone (λSL-1), however, was highly similar to λPH-1. The results suggest that viruses obtained from different individuals within a fixed time period and geographic area can have very similar DNA sequences, and that genetic variability within such a population may be more limited than that between such populations.

D49 TRANSMISSION AND EXPRESSION OF THE TAT (PX) GENE OF HTLV-I USING A RETROVIRUS VECTOR

Gregory R. Reyes, Mark B. Feinberg, Susan Knight, Robert C. Gallo and Flossie Wong-Staal
Stanford School of Medicine, Stanford, CA 94305, Laboratory of Tumor Cell Biology, NIH, Bethesda, MD 20205, Gene Labs, Inc., San Carlos, CA 94070

HTLV-I is the etiological agent associated with human adult T-cell leukemia/lymphoma. This nondefective retrovirus does not transduce activated cellular proto-oncogenes, nor does it display site specific integration leading to oncogene activation. Replication competent as well as defective HTLV-I viruses transform (immortalize) naive lymphocyte populations. Our attempts to elucidate the in vitro transformation and in vivo neoplastic properties of this virus have focused on the pX region of the viral genome. Our approach towards the molecular dissection of pX function involves the construction of Moloney murine leukemia virus (MoMLV) derived vectors containing selected DNA segments from both HTLV-I and HTLV-II molecular clones. These vectors contain the selectable gene neo. Rat-2 transfected with the original constructs and selected with G418, displayed multiple proviral integrations of the transfected molecular clone. Defective viruses from this transfected Rat-2 line were rescued into NIH-3T3 using wild type MoMLV and G418 selection. The structure of transduced defective retroviruses and the pattern of expression as assessed by Southern and Northern blotting will be shown. In addition, MoMLV based vectors containing cDNAs encoding pX have been constructed to ensure faithful expression of a non-aberrantly spliced gene. Biological properties of these pX containing viruses are currently under investigation.

D50 **REPEAT ISOLATION OF HTLV-II IN ATYPICAL HAIRY-CELL LEUKEMIA: ETIOLOGIC IMPLICATIONS** by Joseph D. Rosenblatt, William Wachsman, Jerome Lax, David W. Golde and Irvin S.Y. Chen. Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

HTLV-II has been previously associated with a single T-cell malignancy, a case of T-cell variant hairy-cell leukemia diagnosed eight years ago. Two other isolates of HTLV-II were obtained from patients without malignancies; one an AIDS patient, and the second a hemophiliac with pancytopenia. Thus, an etiologic role for HTLV-II in human malignancy has not been established. Recently we identified a second patient with atypical T-cell variant hairy-cell leukemia and demonstrated that this patient also harboured HTLV. Molecular characterization of the virus by hybridization analysis and restriction enzyme analysis demonstrated that this virus was HTLV-II. The possible etiologic role of HTLV-II in this patient was investigated by determining whether the characteristics of the virus in the patient were consistent with the pattern which would be expected for a disease caused by HTLV. HTLV-II was found oligoclonally integrated into DNA from the patient's leukemic cells, similar to malignancies caused by HTLV-I and the related bovine leukemia virus (BLV). Furthermore, no viral RNA expression was detected in the leukemic cells of the patient, also characteristic of HTLV-I- and BLV-associated malignancy. These findings strongly support an etiologic role for HTLV-II in this patient's T-cell malignancy. Further characterization of the virus by molecular cloning will be presented.

D51 MOLECULAR DIFFERENCES IN THE GENOMES OF HTLV-III/LAV VIRUSES ISOLATED FROM A PATIENT WITH LYMPHADENOPATHY (LAD) ON TWO DIFFERENT OCCASIONS, K. Sakai, D. Casareale, and D.J. Volsky, Dept. of Pathol. and Microbiol., UNMC, Omaha, NE.

It has been suggested that HTLV-III/LAV might be able to escape the host immune surveillance by undergoing progressive changes in its envelope proteins. To evaluate this hypothesis, we have isolated and compared HTLV-III/LAV from the same patient at different time points. A homosexual man with LAD donated his blood in Sept., 1984. An HTLV-III/LAV-producer cell line, termed CEM/LAV-N1G, was established as described before. The second blood sample from the same patient was obtained in Dec., 1984. The virus-producer cell line from Dec., 1984 was termed CEM/LAV-N1T. Southern blot analysis of the retroviral DNA present in the CEM/LAV-N1G and CEM/LAV-N1T cell lines revealed similar size and pattern of bands after digestion with EcoRI, PstI or SacI endonucleases. Digestion with SacI generated bands adding up to more than one 10-kb. Therefore, each cell line carried more than one viral genotype. However, the genotypes of the HTLV-III/LAV viruses isolated in Sept. 1984, differed not only between themselves, but also from those isolated three months later. This was shown by comparing internal restriction fragments obtained after digestion of the CEM/LAV-N1G and CEM/LAV-N1T cell line DNA, respectively, with BgL I and Hind III endonucleases, which also cut within the viral LTR.

This restriction enzyme site polymorphism suggests that viral genotypes might undergo molecular changes during the course of infection in vivo, or that the patient has been exposed to genotypically different viruses. We are now cloning the viruses from the both cell lines to obtain detailed information about these HTLV-III/LAV variants.

D52 HOST CELL RANGE OF HTLV-III/LAV: VIRUS BINDING AND INFECTIVITY IN VARIOUS MAMMALIAN CELLS, F. Sinangil, C. Kuszynski, and D.J. Volsky, UNMC, Omaha, Nebraska

HTLV-III/LAV is thought to be specifically restricted to human T-cells, especially the T-helper subset, for binding and infection. This study demonstrates that the binding and infection by HTLV-III/LAV takes place not only on T-lymphocytes but other mammalian cells as well.

The HTLV-III/LAV used in this study was isolated from a cell line derived from the peripheral blood of an AIDS patient whose diagnosis was confirmed by Western blotting in our laboratory. The virus was highly purified by sucrose gradient centrifugation and labelled with fluorescein isothiocyanate (FITC). More than 15 cell lines and fresh cells from human and murine sources were tested for viral binding, using flow cytometric techniques. Infection of these cells was carried out using purified virus or cell free supernatants from producer cells. Infection was assessed at 3 and 10 days by determining viability, LAVA immunofluorescence, and RNA dot blotting.

Our results indicate that many cell lines and fresh lymphocytes of non T-lineage specifically bind the virus. Blocking studies using patient sera indicated the virus binding was, indeed, specific.

D53 REPEATED ISOLATIONS OF LAV-HTLV-III FROM CELL-FREE BREAST MILK SAMPLES OF THREE HEALTHY VIRUS CARRIER MOTHERS

Suzanne Sprecher, Lise Thiry, T. Jonckleer, J. Levy, P. Van de Perre, P. Henrivaux, J. Cogniaux-Leclerc, N. Clumeck.
Institut Pasteur du Brabant, (dep. Virology); Hôpital Saint-Pierre; Université libre de Bruxelles; Hôpital de Bavière, Université de Liège; BELGIUM

HTLV-III virus was isolated from the breast milk of three symptomless mothers who were HTLV-III virus carriers with or without antibodies.The virus was present extra-cellulary and grew on primary human lymphocyte cultures as well as H 9 Cell line. Supernatants of these cultures inoculated on HUT 78 cells transmitted the virus. The viral particals were probably in great quantities because of the short delay between inoculation and the appearance of viral antigens in the cultures as well as because of the efficiency of isolation. (5 of 5 samples tested were positive.

D54 DEFECT IN CELLULAR RESPONSE TO HTLV-III OF INFECTED INDIVIDUALS, Britta Wahren, Linda Morfeldt-Månsson, Per Ljungman, Gunnel Biberfeld and Reinhard Kurth, Departments of Virology and Immunology, National Bacteriological Laboratory, Stockholm, Sweden, and Paul Erlich Institut, Frankfurt, Germany.

The specific lymphocyte and antibody response to HTLV-III (human T-cell lymphotrophic virus) and cytomegalovirus (CMV) was studied in patients with PGL (persistent generalized lymphadenopathy) which may be pre-stages to AIDS (acquired immunodeficiency syndrome). The HTLV-III infected patients who were clinically well had HTLV-III IgG1. None of those had a cellmediated proliferative response to HTLV-III as judged by ^{3}H-dT incorporation in DNA of their lymphocytes. Nine of those patients were CMV seropositive, all with CMV IgG1, eight with CMV IgG3. Seven of the nine had a good CMV-specific lymphocyte response to CMV antigen.

It appears that HTLV-III infected patients have a selective inability of their T4 lymphocytes to respond adequately to HTLV-III antigenic stimulation even early in disease. This defect should be of primary focus in correcting their immune status, since the response to another latent virus of the same persons - cytomegalovirus - was near normal.

AIDS

D55 EXPRESSION OF THE COMPLETE TRANSACTIVATOR GENE OF HTLV-III (AIDS VIRUS) IN E. COLI, Anna Aldovini+, Christine Debouck*, Martin Rosenberg*, Suresh K. Arya+ and Flossie Wong-Staal+, +Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, *Molecular Genetics Department, Smith, Kline and Beckman, 709 Swedeland Rd., Swedeland, PA 19479.

Human T-lymphotropic virus type-III (HTLV-III), also known as Lymphadenopathy virus (LAV) or AIDS-associated virus (ARV), is the etiological agent of the acquired immunodeficiency syndrome (AIDS) and related disorders. One unusual property that unites HTLV types I, II and III related animal retroviruses (bovine leukemia virus and simian T-lymphotropic viruses, type I and the ungulate lenti-retroviruses, is the presence of a viral encoded protein which mediates activation of transcription from the viral long terminal repeat (LTR). Transcriptional activation functions only play a critical role in the biological activities (replication, transformation or cytopathic effects) of this group of viruses. We have expressed in E. coli the complete tat-III protein as well as a truncated form lacking three amino acids from the amino-terminus. The purified proteins are recognised by sera of some, but not all infected individuals, including patients with the acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) and asymptomatic seropositive individuals. Western blot analysis using sera from a large number of individuals in the population with, or at risk for AIDS or related condition found no correlation between the reactivity of the serum with the tat-III protein and the clinical state of the disease. Studies on expression and cellular localization of the tat-III protein in HTLV-III infected cells will be presented.

D56 HTLV-III INFECTED T-HELPER CELLS HAVE THE CAPACITY FOR MITOGEN BUT NOT FOR ANTIGEN INDUCED IL-2 PRODUCTION, Jaakko Antonen, Annamari Ranki and Kai Krohn, Institute of Biomedical Sciences, University of Tampere, 33101 Tampere, Finland

Immunological responses to mitogens and to soluble antigens, as well as the number of peripheral blood T, T-helper (Th) and T-suppressor (Ts) cells were assessed in 22 HTLV-III antibody positive (HTLV-III +ve) and in 57 antibody negative (HTLV-III -ve) homosexual men and in 40 heterosexual controls. HTLV-III +ve men were characterized by low Th values, slightly decreased mitogen and by strongly reduced antigen (PPD) induced proliferative responses, while HTLV-III -ve men sometimes had elevated Ts cells but no significant defect in lymphocyte proliferation. In HTLV-III -ve men, neither the mitogen (Con-A) nor soluble antigen (PPD) induced IL-2 production differed from controls and there was a linear relationship between the amount of IL-2 produced and the absolute number of Th cells. In contrast, HTLV-III +ve men showed normal or only slightly decreased Con-A induced but absent or very low PPD induced IL-2 production. No correlation of PPD induced IL-2 production, but a good correlation of Con-A induced IL-2 production to Th cell values was observed in HTLV-III +ve men. The data indicate a specific defect in HTLV-III infected individuals to respond to soluble antigens requiring class II MLA restricted cooperation by antigen presenting cells.

D57 HUMAN T-CELL HELPER FACTOR AND RECEPTOR ROLES IN IMMUNOREGULATION AND AUTOIMMUNITY. Zvi Bentwich* and Edna Mozes**, *Department of Genetics, Harvard Medical School, Boston, MA 02115 and **Weizmann Institute, Rehovot, Israel

The immune response potential and its genetic regulation in autoimmune diseases can be studied through antigen specific T-cell factors and the T-cell antigen receptor. Thirty-five patients with SLE, 35 with thyroid autoimmune diseases, 80 first degree relatives of the SLE patients and 14 normal controls were studied for their ability to generate a (T,G)-A--L specific helper factor. All of the patients with SLE, in contrast to only 20/35 (57%) of the patients with TAD, produced the factor. The rate of response among the TAD patients and first degree relatives of the SLE patients was similar to that found among normals. These results indicate that the regulation of the specific immune response by T-cells is profoundly altered in SLE but not in TAD and that this is probably an acquired characteristic that is not genetically determined. In a different set of experiments we are now determining the relation of the (T,G)-A--L specific factor to the T-cell antigen receptor and also the expression of the receptor in patients with autoimmune diseases.

D58 HTLV-III REPLICATION AND TRAPPING IN THE IMMUNE SYSTEM. Biberfeld, P.[1&2], Porwit, A.[2], Chayt, K.[1], Harper, M.[1], Biberfeld, G.[3], Gallo, R. LTCB/NCI/ Bethesda (1), Dept. of Pathology, Karolinska Inst. (2) and Natl. Bact. Lab Stockholm, Sweden (3).

Morphological, immunohistochemical, electron-microscopic and in-situ hybridization studies on lymph nodes from HTLV-III seropositive subjects with PGL or AIDS revealed the following findings: Histopathological lymph nodes changes were related to stage and degree of immunodeficiency. In all P.G.L. cases signs of lymph node involution were seen as characteristic alterations in the follicles. In ARC and AIDS patients their changes appeared to progress to complete involution of the follicle B-cell compartment. Follicular involution was accompanied by cytopathic effects on follicular dendritic cells (FDC), infiltration of germinal centers by T cells, expression of virus RNA, focal accummulation of virus antigens and ultrastructural evidence of virus association with FDC. These observations seem to indicate an important role for HTLV-III virus in the involution of the follicular B-cell compartment, possibly by a cytopathogenic effect on FDC. The immunodeficiency in HTLV-III infected subjects not only reflects the cytophatic effects of the virus on T-cells but may also constitute an example of an accessory cell disease.

D59 CHARACTERIZATION OF A HUMAN ENDOTHELIAL CELL LINE FROM AN AIDS PATIENT WITH KS, Peter Biberfeld[1], Shuji Nakamura[2], Judith Swack[2], Robert C. Gallo[2] and S. Zaki Salahuddin[2]. [1]Dept. of Pathology, Karolinska Inst., Stockholm, Sweden [2]LTCB, Bethesda, Maryland 20892

A cell line (N338) was established from a KS lesion in the lung of an AIDS patient, cloned twice and passaged over 70 times. N338 cells were characterized as follows: The morphology is predominantly spindle-shaped in confluent cultures, the cells show contact inhibition, display desmosonal junctions,[1] react with antibodies to angiotension converting enzyme[2] and bind Ulex Europaeus.[3] The karyotype is diploid with polymorphism of chromosome #9 (9q+). The N338 cells release a growth promoting factor to bovine, capillary endothelial cells and to GM-CSF of human bone marrow. They also produce angiogenic factor(s) as shown by a CAM-assay. The N338 cells do not have EBV, HTLV, or FBV, but can be infected with HTLV-I, II and III. The N338 cells appear to represent a human endothelial cell line with interesting properties.

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D60 SPECIFICITY OF ANTI-SYNTHETIC PEPTIDE SERA FOR HTLV-III ENVELOPE GLYCOPROTEIN SUBUNITS, Gordon R. Dreesman, Southwest Foundation for Biomedical Research, Department of Virology and Immunology, San Antonio, Texas 78284

A number of amino acid sequences have been selected from the gp160 precursor glycoprotein associated with HTLV-III with a modified computer algorithm. Areas containing sequences with relatively high degrees of hydrophilicity and beta turns from both the gp120 (envelope glycoprotein) and gp41 (transmembrane glycoprotein) have been selected. These peptides were synthesized by solid phase methodology, conjugated to KLH and subsequently used to immunize rabbits. The resulting antisera have been characterized by radioimmunoprecipitation, by ELISA using both peptide-BSA conjugates and virus infected cell lysates and commercial ELISA kits. In addition the pattern of anti-peptide reactivities with sera obtained from AIDS patients and from experimentally HTLV-III infected chimpanzees has been established. The immunological specifications of each will be summarized and its applications for vaccine development will be discussed.

D61 DETECTION OF HTLV-III ANTIGENS IN LYSATES OF PERIPHERAL BLOOD/LYMPH NODE MONONUCLEAR CELLS. Larry Falk, Deborah Paul and Mark Knigge, Abbott Laboratories, North Chicago and Alan Landay, Bernard Blauuw, Harold Kessler, Robert Chase and David Chudwin. Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois.

HTLV-III antigens (Ag) have been detected in serum of AIDS/ARC patients and in individuals at risk for AIDS by a recently developed EIA test (Paul et al. this conference and manuscript submitted). Employing this assay we have examined freeze/thaw lysates of peripheral blood (PB) or lymph node (LN) mononuclear cells for HTLV-III antigens. Lysates of PB cells and serum from 21 individuals (AIDS/ARC patients or members of high risk groups) were tested: HTLV-III Ag was detected in 5/21 PB lysates and in 20/21 sera. One AIDS patient was PB lysate+ but serum$^-$ and a follow-up specimen from this patient yielded negative results in both PB cells and serum. From 5 patients, all HTLV-III Ag+ in serum, multiple samples were evaluated: 2 remained PB Ag$^-$, 1 remained PB Ag+, and 2 changed from PB Ag$^-$ to PB Ag+. LN cells from 2 AIDS/ARC patients were Ag+. In contrast: PB lysates from 9 asymptomatic, HTLV-III Ab+ individuals were PB Ag$^-$ and serum Ag$^-$. In addition, PB/LN lysates from 40 non-AIDS/ARC/high risk individuals were Ag$^-$. These preliminary studies extend the initial observations of Paul et al. in which HTLV-III Ag was detected in serum/plasma. Thus far a sufficient number of lysate Ag+ samples have not been observed to begin making correlations with pathogenesis of HTLV-III infection.

D62 CHARACTERISATION OF AN INFECTIOUS MOLECULAR CLONE OF HTLV-III: A.G. Fisher, M. Feinberg, M. Harper, L. Ratner, S. Josephs, F. Wong-Staal and R.C. Gallo

Using a plasmid pHXB2D which contains full length HTLV-III proviral sequences, and a novel transfection technique, we have demonstrated that this molecular clone produces HTLV-III virions when introduced into lymphoid cultures. The virus produced is infectious, shows a propensity to infect cells of the OKT4 phenotype and induces multinucleation and other cytopathic effects on T-cells in vitro. We have constructed a panel of variants from the original plasmid clone which contain deletions/insertions in four of the viral genes independantly; SOR (also called orf-I, Q or P'), envelope, 3'orf (also called orf-2, F or E') and TAT. Stable cell lines which contain these mutated genomes and additional naturally occuring HTLV-III variants have been established and are being analysed in order to determine which regions of the genome are essential for virus production per se and to localise the regions that contribute to the cytopathic properties, packaging, replication splicing and integration of the HTLV-III virus.

D63 TESTING FOR AIDS RETROVIRUS (HTLV-III/LAV) ANTIBODIES AND ANTIGENS BY INDIRECT IMMUNOFLUORESCENCE: SPECIFICITY, SENSITIVITY AND APPLICATIONS, M. Hedenskog, B. Ward , S. Dewhurst, D. Casareale, D.J. Volsky. Dept. of Path. & Microbiol., UNMC, Omaha, NE.

Indirect immunofluorescence has been applied to monitor HTLV-III/LAV infection of human T lymphocytes or other virus-susceptible cells. Cells were fixed in acetone at -20°C for 5 minutes at various times after exposure to the virus. The smears were exposed to a well characterized serum from an AIDS patient (titer 1:10,680 as determined by serial dilution), followed by fluorescein isothiocyanate-conjugated goat anti-human IgG (Fab_2 fragment). The HTLV-III/LAV antigens detected by this method were termed LAV-associated antigens (LAVA). 0.1 to 100% of cells were LAVA-positive 1-10 days after infection, depending on the cell type and virus concentration used. This method of monitoring HTLV-III/LAV infection by measuring LAVA expression was validated by nucleic acid hybridization, Western blotting and reverse transcriptase assays on the same cells.

IF was also applied to detect the presence and titers of HTLV-III/LAV antibodies in persons with AIDS or at risk for the disease. As a source of virus antigen, we used acetone fixed smears of the retrovirus-producer cell line, CEM/LAV-N1G, which we established. 72% of sera from male homosexuals were found to be positive by this method, whereas all 16 heterosexual controls were negative. Also, antibodies were detected in 10 of 18 hemophiliacs studied as well as in a number of aboriginal Venezualan Indians and individuals with malaria. Serological status was confirmed by radioimmunoprecipitation and immunoblotting. Our studies show that fixed cell immunofluoresence is a sensitive and specific detection method for HTLV-III/LAV antibodies and antigens.

D64 PRIMARY HUMAN T-LYMPHOTROPIC VIRUS TYPE III (HTLV-III) INFECTION. David D. Ho, M.G. Sarngadharan, Fulvia diMarzo-Veronese, Teresa R. Rota, and Martin S. Hirsch, Massachusetts General Hospital, Boston, MA 02114 and National Cancer Institute, Bethesda, MD 20205.

We report three cases of primary (acute) HTLV-III infection, which were prospectively documented by virus isolation from blood or cerebrospinal fluid during acute illness and concurrent or subsequent HTLV-III seroconversion. All three patients had fevers, rigors, severe arthralgias and myalgias. Additional symptoms included truncal maculopapular rash (2), urticaria (1), abdominal cramps (1), and diarrhea (1). Acute, self-limited lymphocytic meningitis accompanied the febrile illnesses in two patients. One patient has since developed persistent generalized lymphadenopathy, while the other two have remained well. The estimated incubation period (from presumed exposure to acute febrile illness) was 3-6 weeks, and the symptomatic period lasted 2-3 weeks. Seroconversion occurred 8-12 weeks following presumed exposure and was manifested by a characteristic antibody response pattern. Antibodies to gp120 (major envelope protein), gp160 (major envelope precursor), and p24 (major core protein) appeared first. Antibodies to other viral antigens, including p41 (transmembrane protein), were detected four weeks later. Our results suggest that primary HTLV-III infection should be included in the differential diagnosis of prolonged febrile illnesses in individuals at risk for AIDS.

D65 FUNCTIONAL ANALYSIS OF THE HTLV-III LONG TERMINAL REPEAT REGION

S.F. Josephs[1] M.R. Sadaie[1], V. Heisig[1], L.J. Seigel[2], B.R. Franza[3], M.J. Renan[4], S.K. Arya[1], T. Okamoto[1], R. Willis[1], R.C. Gallo[1] and F. Wong-Staal[1] [1]Laboratory of Tumor Cell Biology, National Institutes of Health, Bethesda MD 20205 [2]Frederick Cancer Research Facility, Frederick MD, [3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY [4]National Accelerator Center, Faure, South Africa

An HTLV-III cDNA fragment containing 3' LTR sequences was linked to the chloramphenicol acetyl transferase (CAT) gene. Clones containing deletions extending from the 5' end of the cDNA insert were constructed by Bal 31 digestion from a unique KpnI site 5' to the LTR sequences. Deletions from the 3' end of the cDNA insert were generated by restriction digests followed by T4 DNA polymerase fill-in and blunt end ligation. Transcriptional activity of the clones in HTLV-III infected and uninfected T-cells (H9 cells) as measured by CAT assays showed the region from -118 to +78 nucleotides from the transcriptional initiation start site at +1 to contain major cis-acting elements controlling virus expression. Deletion of the region -103 to -65 eliminated promoter activity in uninfected cells and reduced the transcriptional activation (tat) response in infected cells 36-fold. This suggests the region -103 to -65 likely contains enhancer sequences which are not necessary for tat response. Deletion of the sequences further downstream to -48 eliminated the tat response.

Deletions in clone -117 introduced downstream of the transcriptional initiation site from the BglII site to the SstI site (-117BS) (14 base pairs) and from the SstI site at +34 to the *HindIII* site at +78 (-117SH) *nullified tat response in Cos-1 cells. This confirms that* the integrity of the region downstream of the transcriptional initiation site is essential for transactivation (Cell 41: 813-823, 1985). The promoter activity of the -117BS was similar in uninfected H9 cells and Cos-1 fibroblasts compared to -117. However, the promoter activity of -117SH is increased 24 fold in H9 cells but only slightly reduced in Cos-1 fibroblasts. (The promoter activity of -117SH in the H9 T-cell is less than the Rous Sarcoma Virus (RSV) promoter but greater than the Simian Virus 40 (SV40) promoter in the ratio SV40: -117SH: RSV::7:17:48.) Evidently, a fairly strong promoter activity within the HTLV III LTR is suppressed in H9 cells by the presence of sequences involved in transactivation response.

D66

Molecular Approaches to the Diagnosis of AIDS Retrovirus Infections

Laurence Lasky, Cirilio Cabradilla, Daniel Capon, Donald Dowbenko, and Jerome Groopman

Diagnosis of exposure to the retrovirus which apparently causes the aquired immune deficiency syndrome (AIDS) is important in the prevention of disease transmission via contaminated blood or blood products. Currently available diagnostic assays rely on the use of semi-purified virus as an antigen source for the detection of antibodies directed against the AIDS retrovirus. While these assays are effective, they suffer from several drawbacks including: danger in production, high cost, and a small, but consistent false positive rate. In order to eliminate these problems, we have expressed two antigens from the retrovirus as fusion proteins in the bacteria E. coli and configured these proteins in a clinically-relevant ELISA. The two proteins, the p24 core antigen and a small region of the envelope antigen, were expressed at very high levels in the bacteria and purified using standard techniques. The resultant proteins have been found to effectively recognize AIDS retrovirus antibodies in all of the samples which were found to be positive using the commercially available diagnostic tests. In addition, the bacterially-derived assay was also able to recognize low levels of antibodies in a few patients who were apparently negative in the virus-derived tets, but who were western blot positive. Thus, the bacterially derived assay would appear to be an improvement over the currently available AIDS diagnostic tests.

D67 A NOVEL HEMATOPOIETIC INHIBITORY PROTEIN FROM CULTURED AIDS BONE MARROW

Ira Z. Leiderman, Michael L. Greenberg, Bernard R. Adelsberg, Frederick P. Siegal, Mount Sinai School of Medicine (CUNY), New York, NY 10029

Inhibitors of granulopoiesis have been well described in numerous diseases and syndromes. Many of these inhibitors are T-lymphocyte mediated while others are factors released by specific cell populations. Bone marrow (BM) cells from patients with the Acquired Immune Deficiency Syndrome (AIDS) suppress normal proliferation of the granulocyte-macrophage progenitor cell (CFU-GM), whether the AIDS BM cells are in direct contact with or in feeder layers beneath the normal cells. A cell free conditioned media (CM) prepared by the liquid culture of BM cells from patients with AIDS or the AIDS related complex (ARC) inhibit CFU-GM growth in a manner similar to the BM cells alone. CM prepared from normal BM cells had no such effect. Polyacrylamide gel electropheresis (PAGE) demonstrated a unique band in lanes containing AIDS and ARC CM but not in the control lanes. Periodic acid-Schiff staining revealed it to be a glycoprotein (gp). A molecular weight for this gp was determined to be ≈84 Kd by SDS-PAGE. Eluates of this band from preparative PAGE inhibited hematopoietic proliferation similar to the complete CM. This inhibitory protein did not inhibit mitogenic stimulation of normal T-lymphocytes. CM from AIDS BM cells depleted of adherent cells did not have the unique band though non-depleted CM from the same patients contained the band. We are presently determining if this gp is related to HTLV-III/LAV infection since serum from all patients studied contained antibodies to this virus.

D68 INVESTIGATION OF THE OKT4+ TROPISM OF THE AIDS VIRUS USING A BIOLOGICALLY ACTIVE MOLECULAR CLONE OF HTLV-III: A. LoMonico, A.G. Fisher, R.C. Gallo and F. Wong-Staal.

Human T-lymphotropic retrovirus type III (HTLV-III) has been shown to preferentially infect lymphoid cells of the helper/inducer phenotype, particularly those of bearing the OKT4 antigen. It is postulated that the OKT4 antigen may act as a receptor mediating the entry of HTLV-III into cells. In this study we transfected a biologically competent molecular clone of HTLV-III into a variety of human and murine cells of hematopoietic and non-hematopoietic origin and documented DNA uptake and virus production using standard techniques. Here we provide evidence that intracellular restrictions, in addition to possession of cell surface antigens, limit the type of cell that can be productively infected by HTLV-III.

D69 ASSESSMENT OF POLYMORPHONUCLEAR LEUCOCYTE AND MONOCYTE OXIDATIVE METABOLISM FOLLOWING INCUBATION WITH HTLV-III/LAV, Linda S. Martin, Centers for Disease Control, Atlanta, GA 30333

The ability of human peripheral blood monocytes and polymorphonuclear leucocytes (PMNL) to generate an oxidative burst, [superoxide (O_2^-) production], following incubation with HTLV-III/LAV, was evaluated. Monocytes and PMNL incubated (37°C, 5% CO_2) for 30 and 90 minutes with HTLV-III/LAV produced normal amounts of O_2^-, as compared to controls, when stimulated with opsonized zymosan (Op Z), phorbol myristate acetate (PMA), and f-met-leu-phe (FMLP). After 90 minutes, the cells were washed, resuspended in RPMI plus 10% FCS and incubated overnight. Oxidative metabolism was normal after 24 hours in both infected and uninfected monocytes and PMNL. PMNL incubated for 24 hours exhibited only a slight decrease in the amount of O_2^- elicited by Op Z and PMA while in many cases the response to FMLP actually increased. Monocytes evaluated 8 days post infection, a time at which these cells are capable of infecting PHA blasts, produced superoxide at the same rate as the uninfected controls. Release of b-glucuronidase was not changed by incubation with virus. The evaluation of chemotaxis of normal cells following incubation with HTLV-III/LAV is in progress. Electron microscopy revealed that some PMNL contained viral particles at both 30 and 90 minutes post infection, while monocytes contained many viral particles. Four attempts to culture virus from PMNL 3 days post-infection were negative. Oxidative metabolism of PMNL from AIDS and AIDS related complex (ARC) patients was also evaluated. Two of 10 patients with AIDS and 4 of 9 with ARC exhibited markedly decreased superoxide production when compared to controls.

D70 NATURAL KILLER CELL INFECTION AND INACTIVATION IN VITRO BY HTLV-III/LAV

William M. Mitchell, W. Edward Robinson, Jr., William H. Chambers, Shirley S. Schuffman, David C. Montefiori, and Thomas N. Oeltman. Vanderbilt University School of Medicine, Nashville, Tennessee 37232

In vitro exposure to human mononuclear cells to the French isolate of the HTLV-III/LAV retrovirus in the presence of interleukin-2 results in a rapid loss of natural killer cell functional activity as measured by ^{51}Cr release from target K562 cells. Decreased functional activity can be demonstrated by day 3 with total loss of functional activity by day 27. Expression of HTLV-III/LAV structural antigens following infection requires approximately 14 days, a time comparable to HTLV-III/LAV antigen expression in CEM cells. After 14 days a majority of cells recognized by the anti-natural killer cell monoclonal antibody, Leu IIb, express HTLV-III/LAV antigens as determined by double immunofluorescent microscopy. No evidence was found for the production of a NK cell functional inhibitor by either HTLV-III/LAV infected CEM cells or T-helper lymphocytes. We conclude that natural killer cells as well as T-helper lymphocytes are susceptible to infection by HTLV-III/LAV and that the relative NK cell functional anergy in AIDS is probably secondary to direct HTLV-III/LAV infection.

D71 INHIBITION OF THE IN VITRO INFECTIVITY AND CYTOPATHIC EFFECT OF HTLV-III/LAV BY 2',3'-DIDEOXYNUCLEOSIDES. Hiroaki Mitsuya, Makoto Matsukura, Shuzo Matsushita, Robert Yarchoan, Ruth F. Jarrett, Marvin S. Reitz, and Samuel Broder, Clinical Oncology Program and Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892

Human T-lymphotropic virus type III (HTLV-III)/lymphadenopathy-associated virus (LAV) is a newly discovered lymphotropic retrovirus which is cytopathic for helper/inducer T-cells in vitro. This virus is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) and related diseases. 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 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. With the ribose moiety of the molecule in a 2',3'-dideoxy-configuration, every purine (adenosine, guanosine, and inosine) and pyrimidine (cytidine and thymidine) nucleoside tested suppressed the virus, although the thymidine derivative seemed to have substantially less activity in our system than the others. In general, we observed essentially complete suppression of the virus at doses that were 10- to 20-fold lower than those needed to inhibit the proliferation of the target T-cells and the immune reactivity of normal T-cells in vitro. These observations may be of value in developing a new class of experimental drugs for the therapy of HTLV-III/LAV infections.

D72 REVERSE TRANSCRIPTASE INHIBITORS PROLONG LIFE OF RETROVIRUS-INFECTED MICE. Ruth M. Ruprecht,* Lucia D. Rossoni,* Samuel Broder# and William A. Haseltine.*

*Dana-Farber Cancer Institute, Boston MA 02115, and #National Cancer Institute, Bethesda MD

Retroviruses cannot establish productive infection without functioning reverse transcriptase (RT), so that this enzyme is key to retroviral pathogenesis. We developed murine models and demonstrated that: a) successful inhibition of RT decreases viral titers, b) RT inhibitors must be administered continuously once proviral integration has occurred in order to protect uninfected target cells, and c) continuous administration of RT inhibitors decreases the incidence of disease and prolongs life in animals infected with pathogenic retroviruses.

Newborn NSF/N mice were infected with the T-cell tropic murine leukemia virus SL3-3 and either mock-treated with saline or given suramin at 40 mg/kg/week intravenously. Suramin-treated animals had a median survival of 150 days as compared to 91 days for the mock-treated, virus-infected mice ($p=0.02$ by log-rank test).

We propose that our murine systems are cost-effective animal models for testing the biological effectiveness of candidate drugs directed against the RT of T-cell tropic retroviruses such as HTLV-III.

D73 CHARACTERISATION OF CONSERVED AND DIVERGENT REGIONS IN THE ENVELOPE GENES OF HTLV-III/LAV : B. Starcich, B. Hahn, G. Shaw, S. Modrow, S. Josephs, H. Wolf, R. Gallo, F. Wong-Staal.

Genomic diversity is a prominent feature of different HTLV-III/LAV isolates and is likely to be fundamentally important in the virus's biology and pathogenicity. We determined the nucleotide sequence of an HTLV-III isolate from a Haitian man and analysed the extent and nature of its genomic variation with respect to the published sequences of prototype HTLV-III/LAV viruses. This analysis demonstrated that nucleotide differences occured predominantly within the envelope gene. Furthermore these differences clustered in regions corresponding to the extracellular portion , in particular in areas where antigenic sites are predicted. In contrast, certain other areas of the envelope gene, including parts of the extracellular domain and most of the transmembrane region, were highly conserved. These findings suggest that the envelope glycoproteins of different HTLV-III/LAV viruses may vary substantially in their antigenic properties but also that certain conserved regions exist which may be useful in vaccine development.

D74 EXPRESSION OF HTLVIII GAG AND ENVELOPE PROTEINS IN E. COLI: REACTION WITH AIDS SERA

R. Tritch[1], K. Baumeister[1], M. Chamberlain[1], L. Ivanoff[1], D. Waselefsky[1], D. Reed[1], W. Kenealy[1], D. Tribe[1], L. Strehl[1], B. Hahn[2], F. Wong-Staal[2], and S.R. Petteway[1]; Central Research & Development Department[1], E.I. Du Pont de Nemours & Co., Inc, Experimental Station, Wilmington, DE 19898 USA, Laboratory of Tumor Cell Biology, National Cancer Institute[2], Bethesda, MD 20205

Human T-cell lymphotrophic Virus (HTLVIII) has been implicated as the causative agent of Acquired Immune Deficiency Syndrome (AIDS). In an effort to better understand the structure of viral proteins and their interaction with the immune system, DNA sequences from the gag and env open reading frames of lambda clone BH10 have been inserted into expression vectors and produced in E.coli. Specific restriction fragments or randomly digested viral DNA were engineered into expression vectors and HTLVIII specific proteins produced in E.coli. These recombinant proteins, representing different segments of the gag or env open reading frames and produced as free protein or fusions to β-galactosidase, react specifically with AIDS sera as well as virus specific monoclonal and polyclonal antibodies. This approach has enabled us to identify regions of the gag and env proteins reactive with AIDS sera or virus specific antibodies and map viral proteins to specific regions of the open reading frames. Results from these studies provide knowledge of the structure of the viral proteins and their interaction with the immune system. HTLVIII recombinant proteins are currently being evaluated as candidates for AIDS diagnostics and vaccines. These recombinant proteins provide an understanding of the structure and position of antigenic determinants which should be useful in sero-epidemiology studies, as well as the identification of protein sequences reactive with neutralizing antibodies. These proteins regions will then become candidates in the development of diagnostics and vaccines.

D75 FREQUENT DETECTION AND ISOLATION OF HTLV-III/LAV RELATED RETROVIRUSES FROM AIDS AND ARC CASES IN ITALIAN HOMOSEXUALS, I.V.DRUG ABUSERS AND HEMOPHILIACS.

P. Verani, B.Macchi, M.Federico, A.Orecchia, L.Nicoletti, F.Titti, S.Buttò, G.B.Rossi, Dept.of Virology, Istituto Superiore di Sanità, Rome, Italy.

Human retroviruses have been isolated from the peripheral blood and/or lymphnode lymphocytes from Italian patients with AIDS or with signs or symptoms correlated to AIDS (ARC). Retroviruses have been detected from a total of 39 subjects including 22 of 31 patients with AIDS (6 homosexuals, 10 intravenous drug abusers, 1 transfused), 13 of 37 patients with ARC (2 homosexuals, 8 intravenous drug abusers, 2 hemophiliacs), 3 apparently healthy children born from HTLV-III/LAV positive mothers and 1 of 6 asymptomatic hemophiliacs. For a few patients multiple time spaced samplings yielding retroviruses have been obtained. For representative isolates, supernatant fluids were used for infecting fresh mononuclear cells from normal donors with subsequent production of virus. HTLV-III/LAV antigens were detected in infected cells by specific immunological reagents. For a few isolates, the relationship to HTLV-III/LAV was also determined by immunoprecipitation of ^{35}S-methionine and -cysteine-labeled virus or infected cell lysate with HTLV-III/LAV positive reference sera. These findings provide support for the widespread presence of HTLV-III/LAV among risk group people and their association with ARC and AIDS.

D76 IDENTIFICATION OF p65 AND p51 AS THE HTLV-III/LAV REVERSE TRANSCRIPTASE,

Fulvia di Marzo Veronese[1], M. G. Sarngadharan[1], Anthony L. DeVico[1], Rukhsana Rahman[1], Bridget Joseph[1], Terry D. Copeland[2], Stephen Oroszlan[2] and Robert C. Gallo[3], [1]Bionetics Research, Inc., Rockville, MD 20850, [2]Frederick Cancer Research Facility, Frederick, MD 21701-1240, [3]Laboratory of Tumor Cell Biology, NCI-NIH, Bethesda, MD 20205

By the use of Western blot techniques several antigens in HTLV-III lysates have been recognized as major targets of antibody reactivity with sera from AIDS patients and HTLV-III-infected individuals. While most of these antigens have been identified as HTLV-III encoded proteins, the nature of p65 and p51 was yet to be determined. We approached the question by raising mouse hybridomas secreting monoclonal antibodies to these proteins. One of these hybridomas, M3364, recognized this pair of proteins. Even after extensive cloning, the two antibody reactivities could not be separated, arguing for the presence of two different proteins having common determinants. We purified the antigen through immunoaffinity chromatography using purified M3364 IgG. These were the same antigens recognized by the positive human sera. We subjected the immunoaffinity purified protein to N-terminal amino acid sequencing. Single amino acid residues were identified in 17 successive degradation cycles, demonstrating that p65 and p51 have common amino terminal sequence. The sequence determined is a perfect match with a region of the deduced amino acid sequence of the pol gene of HTLV-III.

D77 ANTI-HTLV-III/LAV ANTIBODIES AMONG NATIVE POPULATIONS IN VENEZUELA, D.J. Volsky, L. Rodriguez[1], S. Dewhurst, F. Sinangil, G. Godoy[2], F. Merino[1], Dept. of Path. & Microbiol., UNMC, Omaha, NE., [1]Dept. Exp. Med., IVIC, Caracas, Venezuela,[2]Dept. of Parasit. & Microbiol., Univ. de Oriente, Bolivar, Venezuela.

The AIDS-related human retrovirus HTLV-III/LAV is thought to have originated in Central Africa. Here we present data pointing to South America as another endemic area for this virus. Serum samples from 1,108 Venezuelans were assayed for antibodies to HTLV-III/LAV by indirect immunofluorescence (IF), using a virus-producer cell line which we established. Sera were only considered positive when IF-reactivity was confirmed by both Western blotting and radioimmunoprecipitation. In all, 30 individuals (12 of them female) were antibody positive by the above criteria. Seropositivity among subpopulations varied from 2.4% in healthy rural populations, 4% in aboriginal Amazonia indians, 4% in Chagas'disease patients, and up to 29.2% in patients suffering from malaria. 0% (0/211) of randomly chosen healthy blood donors from major Venezuelan cities had such antibodies. Among the positive individuals, antibody titers ranged from 1:40 to 1:320 and three such sera dated back to 1968.

The relatively high frequency of seropositivity for HTLV-III/LAV among Venezuelan populations suggests that this virus (or a close relative) is indiginous in non-negroid Latin American and negroid tropical populations. Studies are now in progress to isolate and characterize this virus in order to ascertain its relationship to HTLV-III/LAV. Supported by NIH NCI grant CA 37465 and AIDS Medical Medical Foundation(DJV).

D78 EXPRESSION OF LAV ANTIGENS IN E. COLI, S. Watanabe, W. Cosand, S. McArdle, P. Ward and B. Travis, Genetic Systems Corporation, Seattle, WA. 98121

Sera from some asymptomatic individuals and patients with acquired immunodeficiency syndrome (AIDS) are reactive to proteins produced by a T-cell lymphotropic retrovirus known as LAV, HTLV-III, or ARV. At the present time, detection of these potentially infectious products relies on reactivity to preparations of purified virus. We have taken an alternative approach and produced viral proteins in a prokaryotic system using the E. coli trp operon. Such an approach offers the potential for cheaper, safer production of antigenically important proteins.

Sera from infected individuals are most consistently reactive to the retroviral gag and/or env proteins. For this reason, we concentrated on evaluating these regions for both their level of expression and antigenicity. The entire env protein, excluding the first 47 amino acids, was express both as separate segments of 20 to 30 kD and as a large contiguous sequence. Likewise, the entire gag polyprotein, except for the first 12 amino acids, was expressed as a series of separate, overlapping segments.

All of the recombinant proteins were reactive by Western Blot when assayed with pooled antisera from two LAV-infected individuals. Further screening of approximately 500 individual human sera by ELISA indicated that seropositive and seronegative individuals were easily distinguished by one of our env proteins. The distinctions were not as clear cut with our gag proteins. We suspect that seropositive individuals are more heterogeneous in their response to gag antigens than to env antigens. These results and an analysis of reactivity by clinical group will be presented.

Leukemia Viruses

D79 DETECTION OF HUMAN ANTIGEN HuLym5 EPITOPES ON MOUSE FIBROBLASTS TRANSFECTED WITH CLONED GALV env DNA.
Nicholas J. Deacon, D.F.J. Purcell and I.F.C. McKenzie.
The University of Melbourne, Parkville, Victoria, Australia.

The antigen HuLym5 is a non disulphide linked heterodimer of 60 and 69kd acidic subunits found on the surface of normal human cells in association with HLA Class I molecules. A high molecular weight form (~72kd) is seen in association with tumours. HuLym5 is serologically cross reactive with the envelope glycoproteins of Mason Pfizer Monkey Virus (MPMV) and Gibbon Ape Leukemia Virus (GALV) using the anti HuLym5 monoclonal antibody E4.3. This cross reactivity is not abrogated by the enzymatic removal of N linked carbohydrate from the HuLym5 antigen.

To further investigate the relationship between HuLym5 and primate retrovirus, mouse L cells were transfected with the GALV SEATO genomic clone pGAS2 (M.S. Reitz, N.C.I.), the GALV SF genomic clone λ ChGV 1 and subcloned GALV SF env gene region clone pGV 3 (M.L. Scott, Stanford) and transfectants were analysed for the expression of HuLym5 epitopes. Both stable and transiently expressing GALV env transfectants were identified and shown to express HuLym5 epitopes detectable by rosetting, flow cytometry, immunoprecipitation and Western blotting. Normal human HuLym5 epitopes were observed only on transfectants expressing GALV env gp70, as confirmed serologically and by transfectant RNA analysis with pGV 3 env region hybridisation probes.

The finding that HuLym5 negative mouse fibroblasts can be made HuLym5 positive following transfection and expression of GALV env gene strongly confirms the serological homology of the human cell surface antigen HuLym5 with GALV gp70. This also represents the expression of GALV proteins in a cell type and species for which it is not normally tropic.

D80 FELINE LYMPHOCYTE MARKERS: EXPRESSION ON SOMATIC CELL HYBRIDS AND FELINE LYMPHOMA CELLS. K.L. Hamilton, J.L. Rojko (Columbus, Ohio) J. Sowder, M.D. Cooper (Birmingham, AL), J. Abkowitz (Seattle, WA), W.D. Hardy (New York, NY), and S.J. O'Brien (Frederick, MD).

Markers characteristic of feline lymphocyte subsets have been identified and mapped to feline syntenic groups using panels of monoclonal anti-feline lymphocyte reagents, polyclonal antisera and mouse X feline lymphocyte and hamster X feline lymphocyte somatic cell hybrids. Reactivity has been correlated with expression of surface receptors for guinea pig erythrocytes, erythrocyte-antibody (7S) complexes, erythrocyte-antibody (19S)-complement complexes, and for feline leukemia virus subgroups. The seven feline lymphomas tested have been highly heterogeneous in their expression of all markers except the guinea pig erythrocyte receptor.

Supported by CA 35747. JLR is a Scholar of the Leukemia Society of America, Inc.

D81 **AZIDO THYMIDINE INHIBITS THE REPLICATION OF THE FELINE LEUKEMIA VIRUS.**
W.D. HARDY, JR. [1], E.E. ZUCKERMAN[1], S. NUSINOFF LEHRMAN[2] and D. BARRY[2].
Memorial Sloan Kettering Cancer Center[1], NY, NY and Burroughs Wellcome Co.[2], Research Triangle Park, NC.

The feline leukemia virus (FeLV) is a naturally occurring contagiously transmitted retrovirus of pet cats that induces neoplastic diseases (leukemias) or, more commonly, a feline acquired immune deficiency syndrome (FAIDS). Seroepidemiological studies in this country found that approximately 1 million healthy pet cats are persistently infected with FeLV and are at high risk of developing FAIDS. Due to the virological and clinical similarities of FeLV-induced FAIDS and HTLV-III-induced human AIDS, we have tested compounds for anti-retroviral activity in order to treat FeLV-infected pet cats. The anti-viral effect of azido thymidine (AZT) on replication of subgroups A- and B-FeLV was evaluated by an immunofluorescent focus induction assay using susceptible feline embryo lung fibroblasts (FLF-3). There was complete inhibition of FeLV replication in cultures infected 1 hour prior to treatment with AZT at concentrations of 400, 200, 100 and 50 uM, 90% inhibition at 10 uM, and 50-75% inhibition at 1 uM. No inhibition of replication was observed at 0.1 uM/ml.

Results of AZT therapy of healthy FeLV-infected pet cats will be presented.

D82 X GENES OF BLV AND HTLV: A PUTATIVE OVERLAPPING GENE, Yoji Ikawa and Noriyuki Sagata, Lab. of Molecular Oncology, The Institute of Physical and Chemical Research, Wako (RIKEN), Saitama 351-01, Japan

Bovine leukemia virus (BLV) and human T-cell leukemia virus (HTLV) have a potential transforming gene, termed X (1). The major long open reading frame of these X genes encodes a protein of 38K (for BLV; ref.2) or 40K (for HTLV), which appears to be a nuclear transcriptional activator of the long terminal repeat. In addition to the major open reading frame, the X genes commonly harbor another short open reading frame that overlaps this major one. Both of these open reading frames are found on a single spliced X mRNA in a potentially functional form (3). Circumstantial evidence strongly suggests that they are both translated from the single X mRNA molecule, showing striking similarity to the translation mechanisms of an adenovirus E1b gene mRNA and a reovirus s1 gene mRNA. We note that the short open reading frame has the capability to encode a putative nuclear protein with structural features similar to those of an AIDS virus trans-acting protein. Thus, we propose that the X genes of HTLV and BLV are both an overlapping gene encoding two distinct polypeptides, both of which may be involved in viral replication, cellular transformation, or both, possibly interacting with each other (3).

(1) N. Sagata et al., EMBO J. (1984), 3, 3231-3237.
(2) N. Sagata et al., Proc Natl. Acad. Sci. USA (1985), in press.
(3) N. Sagata et al., FEBS Lett. (1985), in press.

D83 DETECTION OF TRANS-ACTING FACTORS IN HTLV-III-INFECTED CELL LYSATE BY IN VITRO TRANSCRIPTION

T. Okamoto, Y. Taguchi, M.R. Sadaie, S.F. Josephs, R.C. Gallo and F. Wong-Staal
Laboratory of Tumor Cell Biology, National Institutes of Health, Bethesda, Maryland

The trans-acting transcriptional activator protein of HTLV-III (tatIII) is predicted to be a nuclear protein and to bind to nucleic acids based on the presence of an extensive Lysine-Argine-rich region demonstrated by the nucleotide sequence of a functional cDNA clone (S. Arya et.al. Science 229, 69, 1985). The mechanism by which tatIII enhances transcriptional activity may be a direct interaction with target sequences in the LTR or by an indirect mechanism such as stimulation of cellular transcriptional factors. The transcriptional step in which tatIII is involved is also unknown. We have investigated this phenomenon by using in vitro cell-free transcription system.

Whole cell extracts were prepared from HeLa, HTLV-III-infected H9 (H9/III), virus-uninfected H9, and HTLV-I-infected 81/66 cells. A recombinant plasmid, pCD12, which has the U3 and R regions of HTLV-III LTR, was linearized with EcoRI and used as a template. The accurate initiation of transcription from the HTLV-III LTR can be obtained solely with cellular factors present in HeLa cells. However, the abundance of the specific transcripts was up to 10 fold increased in the reaction mixture supplemented by the H9/III lysate. This transcriptional activation was specific for the lysate from H9/III cells. Preincubation experiments indicate that transcriptional activator(s) present in the virus-infected cell lysate are involved in initiation complex formation rather than elongation. Competition assay with an oligopeptide deduced from the Lysine-Arginine-rich region of the putative tat protein of the HTLV-III (tat-III) suggests that tatIII is directly involved in the initiation complex formation.

D84 MYELOPROLIFERATIVE DISEASE INDUCED BY FeLV-GM1, D. Onions[1], N. Testa[2], B. Lord[2], T. Zavaras[3], J. Neil[3]. (1) University of Glasgow, (2) Paterson Laboratories, Manchester, (3) Beatson Institute, Glasgow.

The establishment of persistent feline leukaemia virus (FeLV) infections is associated with a wide range of haematological malignancies. We have isolated a virus FeLV-GM1 from a case of erythroleukaemia which induces myeloid or erythroleukaemia with a latent period of 3 months, when inoculated into newborn cats. When older cats are infected either no disease is seen or, occasionally aplastic anaemia may result.

The induction of leukaemia is associated with a preleukaemic stage characterised by an increase in the myeloid precursor cell population (GM-CFC) from a normal level of $65/10^5$ cells to $200\text{-}300/10^5$ cells. Cell sorter separation (FACS) of colony stimulating factor (CSF) responding cells from CSF producing cells has enabled us to determine that GM-CFC in the preleukaemic phase are hyper-responsive to CSF but differentiate normally in the presence of this factor.

The induction of leukaemia by FeLV-GM1 is associated with the efficient replication of both subgroup-A and B components of this isolate. We have developed a subgroup-B specific probe from the 5' region of the env gene. This probe detects subgroup-B viruses but not A or C subgroups. However, it does detect endogenous FeLV components suggesting an origin by recombination for this subgroup. In DNA from FeLV-GM1 infected FEA cells or tumour cells a novel subgroup-B band can be detected which is distinct from subgroup-B helper virus. We are molecularly cloning this component to determine its structure and its role in the induction of leukaemia.

D85 BIOSYNTHESIS AND CHEMICAL CHARACTERIZATION OF BOVINE LEUKEMIA VIRUS GENE PRODUCTS, S. Oroszlan, T.D. Copeland, A.M. Schultz and Y. Yoshinaka, National Cancer Institute -Frederick Cancer Research Facility, Frederick, MD 21701.

The biochemistry of BLV protein synthesis and the primary structure of the gene products were studied. Utilizing [^{3}H]-myristate labeling of BLV producing FLK cells and immunoprecipitation of cell extracts with antiserum to p24 we identified three myristylated primary translational products, Pr46, Pr70 and Pr150. They are initiated with the same gag initiator and are synthesized in amounts inversely proportional with their size. Pr46^{gag} is the precursor to the structural proteins p15, p24, and p12. Pr70 consists of the entire p15, and p24, most of p12, plus the viral protease and some additional sequences. Pr150 most likely encompasses the region of the mRNA extending from the gag initiator to the terminator of the pol gene. The major gag proteins, p15, p24 and p12, and two env gene products (gp60 and gp30) as well as the viral protease were purified and their partial or complete amino acid sequences determined. Two minor gag proteins, designated p10 and p5, were also characterized structurally. They were shown to be derived from p15 through cleavage by the viral protease; p10 like p15 is myristylated at its N terminus. From both protein and DNA sequence data (Rice et al., Virology 142:357, 1985) the protease reading frame was shown to be different from both the gag and pol frames. We purified and characterized another protein designated p13, and showed that it adjoins the C terminus of the protease and overlaps the open reading-frame for reverse transcriptase. It appears that frameshift suppression rather than splicing is required for the synthesis of Pr70 and Pr150.

D86 NEUTRALISING ANTIBODY CONTROLS LATENT FeLV INFECTIONS
A.M. Pacitti & O. Jarrett, Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow, G61 1QH, Scotland.

Three possible outcomes of feline leukaemia virus (FeLV) infection are: persistent viraemia with a high risk of developing FeLV-associated disease; elimination of virus associated with the appearance of virus neutralising antibodies; or the establishment of a latent infection, also in the presence of neutralising antibodies. Cats with latent FeLV infection are therefore analagous to individuals with HTLV-1 or cattle with BLV infections.

Latent infection may be detected by the spontaneous reactivation of FeLV from cultured bone marrow cells. Reactivation can be prevented by culturing marrow cells in the presence of cat serum containing FeLV neutralising antibodies. By using monoclonal antibodies the determinant involved in the control of reactivation was identified as the epitope on the envelope glycoprotein (gp70) which is employed in virus neutralisation. Titration of those antibodies which inhibited reactivation showed that there was a direct relationship between the highest dilution at which they were effective and their virus neutralising titre. These results suggest that neutralising antibodies have a primary role in maintenance of latency in vivo.

Following exposure to infection the proportion of recovered cats with latent infections is initially more than 50% but drops over a period of three years to about 10%. Although such cats do not appear to develop FeLV-related diseases they may be important in the epidemiology of FeLV since some transmit infectious virus to their kittens in the milk.

D87 IMPORTANCE OF THE ENVELOPE GLYCOPROTEIN gp51 IN THE DIAGNOSIS OF BLV INFECTION AND IN THE DEVELOPMENT OF AN ANTI-BLV SUBUNIT VACCINE, D. Portetelle[1], C. Dandois[2,3], H. Gras[3], A. Tartar[3], M. Mammerickx[4] and A. Burny[1,2]. (1) Faculty of Agronomy, 5800 Gembloux,Belgium;(2) University of Brussels,Belgium;(3) University of Lille, France;(4) National Institute of Veterinary Research, Uccle,Belgium.

The monoclonal antibody against site E of the BLV gp51 was selected to develop a powerful ELISA test for the diagnosis of BLV infection. This test is so sensitive that it allowed identification of one low titre antibody carrier in a pool of 75 control sera. The performance of the test on pooled sera has been appreciated in a large scale survey of the belgian cattle population and its sensitivity allows the detection of infected herds with infection rates lower than 1 %.
Study of the antigenic structure of BLV gp51 with monoclonal antibodies has allowed to define three crucial epitopes F, G, H in the design of an anti-BLV subunit vaccine. The three epitopes: (1) are sensitive to the presence of a reducing agent; (2) are localized on a weakly glycosylated fragment which is the NH_2- half of the molecule; (3) are the only epitopes recognized on a non-degraded virion; (4) are poorly reactive after gp51 purification suggesting that F G H have a three dimensional structure that probably also depends upon spatial relationships in the BLV membrane.
Synthetic peptides were also chosen as an approach to the construction of a BLV subunit vaccine. Anti-peptides antibodies were raised in rabbits and analyzed for their reaction properties towards gp51.

D88 ASSOCIATION BETWEEN A FELINE c-myc GENOTYPE AND THE INCIDENCE OF LYMPHOSARCOMA. Pradip Roy-Burman,[1] Lisa H. Soe,[1] Robert E. Maxson[1] and Edward E. Hoover,[2] University of Southern California School of Medicine,[1] Los Angeles, CA 90033, and Colorado State University,[2] Fort Collins, CO 80523

DNA sequencing of two cloned c-myc alleles (CM2 and CM3) reveals that the polymorphic SmaI site (Soe and Roy-Burman, Gene 31:123-128, 1984) is located 134 nucleotides 5' of the exon III coding sequences. In the 3' 1.2 kb region of the gene, the two clones differ at eight nucleotide positions. Five of these changes occur in intron II and include a two base deletion in CM3 as compared to CM2, and a C to G transversion in CM-3 which results in the loss of the polymorphic SmaI restriction site. The remaining three base changes occur in exon III, two of which are in the third position of the codon and result in no amino acid changes. The other base alteration is a C to T transition in the second position of the codon in CM2 which results in a change from alanine to valine in position 119 from the termination codon. In each of four species (human, murine, feline CM3 and avian) the alanine residue at the polymorphic position along with adjacent amino acids have been tightly conserved. These results suggest an alteration in CM2 affecting the potential function or stability of this allelic form of the c-myc gene product. An examination of 75 normal unselected cats for genotypic frequencies based on SmaI polymorphism, shows a distribution of 0.48 for CM3/CM3, 0.48 for CM2/CM3, and 0.04 for CM2/CM2. Preliminary results of heterozygous breedings suggest a selection against CM2/CM2 genotype. In contrast to the low incidence of CM2 homozygosity, CM3 homozygosity is common in cats. It appears from a study of 40 lymphoma-bearing cats that the occurrence of this type of cancer is more frequently associated with CM3 homozygosity; the odds ratio being 2.01. These results will be discussed in the context of increased susceptibility of this species to leukemogenesis.

D89 POST-EXPOSURE TREATMENT WITH MONOCLONAL ANTIBODIES IN A RETROVIRUS SYSTEM: FAILURE TO PROTECT CATS AGAINST FeLV INFECTION WITH VIRUS NEUTRALIZING MONOCLONAL ANTIBODIES, Kees Weijer, Fons UytdeHaag, Oswald Jarrett, Hans Lutz and Albert Osterhaus, Netherlands Cancer Institute, Amsterdam, The Netherlands.

We have attempted to protect kittens against infection with FeLV by treatment with two virus neutralizing (VN) monoclonal antibodies (MoAbs) directed against the same epitope on the viral glycoprotein gp70.Ten SPF eight-week old kittens were infected with 10^6 ffu FeLV and subsequently inoculated i.m.with MoAbs every 2 days over a 20 day period at different times after infection.The results show that no protection was achieved.It is unlikely that the amount of VN antibodies,the mode and route of their application or the infectious dose of FeLV used account for the failure to protect cats against infection.Other possibilities to explain the lack of a protective effect are that the restricted epitope specificity of the MoAb preparation used may have led to selection of neutralization resistent virus mutants,and that other mechanisms than VN (complement mediated lysis,antibody dependent cell cytotoxicity) that may be involved in protection function less efficiently with MoAb. However, in the light of our finding that an early anti-idiotypic response is observed in all cats following administration of the MoAb preparation, rapid clearance of anti-FeLV MoAb from circulation is a more likely explanation.The data presented support our hypothesis that by administration of MoAb -as compared with polyclonal antibody- a more vigorous anti-idiotypic responseis elicited due to the presentation of only a limited set of idiotypes. This potential drawback of rapid clearance of MoAbs as a consequence of an anti-idiotypic responsemight be overcome by the use of mixtures of MoAbs resulting in a more heterogeneous set of idiotypic determinants.

D90 GENETIC ALTERATIONS INVOLVING FeLV IN VIRUS-NEGATIVE LYMPHOSARCOMAS OF CATS. John H. Wolfe, William D. Hardy, Jr. and William S. Hayward, Sloan-Kettering Institute, New York, NY, 10021

The feline leukemia virus (FeLV) induces lymphosarcomas (LSAs) in cats. Thirty percent of cats with LSAs, however, have no evidence of FeLV in the tumor cells, normal tissues or serum, and no extra FeLV-related sequences have been found in the DNA of LSA cells. In contrast, epidemiologic studies indicate that the virus-negative LSAs (VN-LSAs) arise subsequent to exposure to FeLV, and latent FeLV can be activated from bone marrow cells, but not from LSA cells, of cats with VN-LSAs. Thus, there is strong evidence that VN-LSAs are induced by FeLV. We have compared VN-LSA DNA to DNA from non-neoplastic tissue of the same cat for extra bands containing FeLV sequences. Possible rearrangements involving FeLV have been found in VN-LSA tissue from three out of three cats examined. The rearranged fragments are being analysed to determine their region(s) of homology with FeLV and to determine if any cellular oncogene sequences are present.

Most feline VN-LSAs are B-cell tumors originating in the intestines. We have established the first cell line derived from an intestinal VN-LSA and are examining it, as well as other primary VN-LSA cells, for chromosomal anomalies. The cell line (FL-3754) appears to have a normal feline diploid chromosome number of 38 and has no double minutes.

Papilloma, Hepatitis and Epstein-Barr Viruses

D91 CONTROL OF LATE TRANSCRIPTION IN BOVINE PAPILLOMAVIRUSES. Carl C. Baker and Peter M. Howley, National Cancer Institute, Bethesda, Maryland 20205

Expression of "late" genes of bovine papillomavirus type 1 (BPV-1) occurs only in the differentiated keratinocytes of the fibropapilloma. We have therefore constructed a cDNA library from bovine fibropapilloma mRNA using the method of Okayama and Berg to study transcription during productive infection. Approximately 1.35% of the cDNA clones contain BPV-1 cDNA inserts, and of these, 80% mapped to the transforming region. Six classes of cDNAs have been analyzed. Five of the six classes of cDNAs have a 5' leader encoded within the noncoding region (NCR). This leader has a 5' terminus near n. 7250 and a splice donor at n. 7386 and does not appear to be used in the BPV-1 transformed cell. The upstream sequence GGTACACATCC (n. 7212 to 7222) differs by only 3 nucleotides from the SV40 late promoter sequence GCTACCTAACC. Thus the corresponding BPV-1 sequence is likely to be an important element of the late or wart specific promoter. The late leader is spliced to transforming region message bodies at n. 3225 and 3605. These mRNAs would produce a 103 aa E4 protein and an 80 aa C-terminal E2 protein, respectively. An L1 encoding mRNA splices the late leader to a second noncoding leader encoded from n. 3605 to 3764 and a message body starting at n. 5609. Translation of L1 presumably begins at the initiation codon at n. 5609. The longest L2 cDNA analyzed has a 5' terminus at n. 4096 and may represent a truncated cDNA .

mRNA labeled in vitro in nuclei isolated from BPV-1 transformed cells was analyzed to determine the block to "late" gene expression in the transformed cell. There is negligible transcription from the "late" promoter in the transformed cell. In addition, transcription proceeds at equimolar levels past n. 6000 (almost 2000 n. past the transforming region poly(A) site) and is undetectable in the 200 nucleotides upstream of the "late" poly(A) site. Thus transcription during productive infection differs from transcription in the transformed cells by the use of a "late" or wart-specific promoter and the absence of transcription termination in the late region.

D92 B LYMPHOCYTES IMMORTALIZED BY EBV PROLIFERATE IN RESPONSE TO AN ENDOGENOUSLY PRODUCED IL-1 LIKE FACTOR. Beverly A. Blazar, Mary Roderick, Marshall Strome, Harvard Medical School, Boston, Ma. 02215

Epstein-Barr Virus (EBV) transformed B lymphocytes grow in perpetual cell culture. We have suggested earlier that such continual growth results from a factor dependent cycle. We have also shown previously that the growth stimulator effects T lymphocyte responses. Size exclusion HPLC of growth enhancing supernatants from EBV-LCL yields both enhancing (molecular weight 10-25,000) and inhibitory (molecular weight 75-90,000) activity for growth of EBV transformed cells. These same HPLC fractions also enhance or inhibit IL-1 dependent T cell responses. To question the possible involvement of IL-1 in the autostimulation of EBV-LCL, purified macrophage IL-1 was added to cultures of EBV-LCL. This IL-1 markedly enhanced the growth of EBV-LCL but not normal resting B cells. The combined action of purified IL-1 and the autostimulatory supernatant factor from EBV-LCL was additive or less than additive but not synergistic in effects on growth of EBV-LCL. These data suggest that the cellular response of EBV-LCL to an IL-1 like factor, which these cells appear to produce, may be central to cellular immortalization.

D93 DNA Homology Relationships of Human Papilloma Viruses. Thomas R. Broker, Steven M. Wolinsky, Daniel J. Sporn, and Louise T. Chow. Biochemistry Department, University of Rochester Medical Center, 601 Elmwood Ave., Rochester, NY 14642.

DNA:DNA heteroduplexes between pairwise combinations of many of the human papilloma virus genomes cloned in pBR322-derived vectors were formed at low stringency (Tm = 40o) and examined by transmission electron microscopy at low, moderate, and high stringencies to evaluate the locations and extents of homology relative to the physical and/or genetic maps of the viruses inferred from the known DNA sequences of a few types. The L1 open reading frame encoding the major capsid protein and most of the E1 ORF encoding a DNA replication function are consistently the most conserved. Based on the heteroduplexes, we have created a set of type-specific DNA probes for the genital papilloma viruses which should greatly facilitate identification of the viruses in clinical samples. From the DNA sequences of the genital HPV types 6, 11, and 16, it has been possible to apply the empirical equations relating Tm to some of the factors affecting DNA base pairing stability - AT/GC composition and formamide and salt concentrations - to reevaluate the effects of different kinds of mutational changes such as mismatches and insertion/deletions.

D94 Human Papilloma Virus RNA Transcription in Transfected and Infected Cell Cultures and in Warts. Louise T. Chow, Anthony Pelletier, Ute Brinckmann, Sheila Reilly*, Lorne B. Taichman* and Thomas R. Broker. Biochemistry, Univ. Rochester Med Center, Rochester, NY 14642 and *Dept. of Oral Biology and Pathology, State University of New York, Stony Brook, NY 11794.

Papilloma viruses do not propagate lytically in any available cell culture system. To study messenger RNA transcription and processing of human papilloma virus type 1 (associated with plantar warts) and HPV-6 (associated with genital condylomata and dysplasias), we have cloned their DNAs into expression vectors and obtained mRNAs after transient transfection of SV40-transformed monkey CV-1 cells (COS A2 cells). The vectors utilize either the SV40 early or late promoter, or the Drosophila heat shock hsp 70 inducible promoter. The mRNAs were mapped by electron microscopy of RNA:DNA heteroduplexes. The alternatively spliced species reproducibly cover various portions of open reading frames (ORFs) E6, E7, E2, E4, and L1 deduced from the DNA sequences. The splice patterns and polyadenylation sites are identical to those in RNAs obtained directly from plantar warts or to those extracted from primary foreskin cultured cells infected with HPV-1 virus particles. The native viral promoters in the latter two RNA preparations are located very close to the cloning sites in the expression vectors. RNAs anticipated to encode products from the E1 and L2 ORFs are neither found in vitro nor in vivo. These results suggest that the sequences of the primary transcripts dictate the splicing patterns and relative abundances of the mRNAs.

D95 HEPATITIS B VIRUS GENE EXPRESSION IN TRANSGENIC MICE, Francis V. Chisari, David R. Milich, Alan McLachlan, Pierre Filippi, Richard Palmiter, Carl Pinkert and Ralph Brinster. Scripps Clinic & Research Foundation, La Jolla, CA; Univ. of Washington, Seattle, WA; Univ. of Pennsylvania, Philadelphia, PA

We have produced transgenic mice bearing a subgenomic fragment of the HBV genome containing the pre-S, S and X coding regions under the control of exogenous promoters derived from the mouse metallothionein (pMT-PSX) and albumin (pAlb-PSX) genes. HBV sequences are integrated as tandem repeats with variable copy number at 1 or 2 sites within the mouse genome. Breeding experiments reveal transmission of HBV DNA sequences in a normal mendelian fashion in most animals. Serum hepatitis B surface antigen (HBsAg) concentrations between 0.5-9.0 ug/ml are detectable in 2/6 pMT-PSX transgenics and in 8/8 pAlb-PSX transgenics. The secreted product is particulate (22 nm) and has a buoyant density of 1.19 g/cm^3. Both preS and HBsAg are detectable in liver and kidney as cytoplasmic granular inclusions. Multiple RNA species are present, but 2.3 and 2.5 kbp transcripts consistent with regulation by both endogenous (HBV) and exogenous (MT or Alb) promoters respectively are common to all tissues. As predicted, zinc administration to pMT-PSX mice induces both transcription and tissue antigen concentration while unexpectedly reducing serum HBsAg concentrations to less than 10% of starting values. As expected, all mice tested are immunologically tolerant to HBsAg. To date, all mice are clinically and histopathologically normal which suggests that tissue injury is probably not a direct consequence of preS or HBs antigen expression. Studies that address the pathogenetic role of the immune response to HBV encoded antigens will be possible in this system when HBV gene expression is achieved in inbred strains of appropriate immune response phenotype.

D96 ONCOGENE EXPRESSION IN NON-HODGKIN'S LYMPHOMA: CORRELATION WITH CYTOGENETIC AND IMMUNOLOGIC FEATURES, J. Davis,F. Sinangil, W. Sanger[1], D.J. Volsky, D.T. Purtilo Dept. of Path. & Microbiol., and Pediatrics[1], UNMC, Omaha, NE

Several non-Hodgkin's lymphomas were studied in an attempt to define molecular and cytogenetic events which may govern the conversion from benign, polyclonal lymphocyte proliferation to monoclonal malignant lymphoma and, in some cases, progression to more aggressive malignancies.

The tissues used in this study were obtained through the Nebraska Leukemia/Lymphoma Registry. RNA and DNA were isolated from frozen tissues using guanidium isothiocyanate method. RNA isolates were probed for various oncogene transcripts. DNA isolates were probed for immunoglobulin gene rearrangements and Epstein-Barr Virus genome. The correlation between these data and the cytogenetic, immunologic, and clinical features of each tumor will be presented.

D97 TRANSFECTION OF EBV DNA FRAGMENTS CODING FOR EBNA-1 AND EBNA-2 INTO HUMAN LYMPHOCYTES: GENE EXPRESSION WITHOUT CELL IMMORTALIZATION. S. Dewhurst, K. Sakai, T. Gross, D.J. Volsky, Dept. of Path. & Microbiol., UNMC, Omaha, Nebraska

EBV is a human herpesvirus capable of transforming B lymphocytes into immortal cell lines. All EBV-immortalized cells and EBV-positive tumors are latently infected by the virus. Four major viral transcripts are associated with viral latency: three nuclear antigens, EBNA-1, EBNA-2, EBNA-3 and one latent membrane-associated protein, LMP. The mechanism of cell transformation and roles these proteins might play in this process are unknown. We approached the problem by transfecting EBV DNA fragments containing genes coding for these proteins into human lymphocytes, and studying their expression and effect on cell transformation.

Transfection of the BamHI K fragment, which contains the EBNA-1 gene, into lymphocytes induced EBNA expression but no DNA synthesis, whereas the BamHI D_1 fragment, a portion of the EBNA-2 gene, did not induce EBNA, but resulted in a transient stimulation of cellular DNA synthesis. To investigate the possibility of incorrect or insufficient expression of EBNA-1 and/or EBNA-2, lymphocytes were transfected with the plasmid pSV3neoEBNA-1 that contains the EBNA-1 coding exon SV40 promoter, or plasmid pM780-28, which contains the entire EBNA-2 gene. Significant levels of specific mRNA were detected in cells up to 2 weeks following transfection, and polypeptides could be detected by Western blotting. However, no immortalization was observed with either gene singly or together. Our results suggest that immortalization of lymphocytes by EBV may be a complex process involving many genes, possibly several steps, and may require additional cofactors.

D98 GENE EXPRESSION IN B LYMPHOCYTES IMMORTALISED BY EPSTEIN-BARR VIRUS, Paul Farrell and David Rowe, Ludwig Institute for Cancer Research, MRC Centre, Hills Road, Cambridge CB2 2QH, England

Human B-lymphocytes latently immortalised by Epstein-Barr virus (EBV) express several EBV gene products. Some of these proteins apparently contribute to the immortalised cell phenotype. These gene products include EBNA-1, 2, 3, 4, a protein encoded in the EcoR1-Dhet region of the genome and the EBER RNAs. Analysis of the mRNAs for these genes by S1 mapping, Northern blotting and cDNA cloning (in collaboration with M. Bodescot and M. Perricaudet) indicates that some of these gene products are encoded by highly spliced mRNAs, with exons distributed over large regions of the EBV genome. The relationship between the immunologically defined EBNAs and specific EBV genes is being examined. The detailed pattern of expression of certain of the genes varies substantially between different EBV immortalised lymphocyte lines.

D99 DETECTION OF A CROSS-REACTIVE EPITOPE PRESENT ON EBNA-1 AND RHEUMATOID ARTHRITIS SYNOVIAL MEMBRANE, Robert I. Fox, John Vaughan, and *Gary Pearson, Scripps Clinic and Research Foundation, La Jolla, CA 92037; *Georgetown University, Washington D.C. 20037

EBNA-1 antigen contains repetitive sequences of (glycine-alanine) that are recognized by a monoclonal antibody (MoAb P135) and by antibodies against synthetic peptides derived from the IR3 region of the EBV genome. We have found that MoAb P135 also reacts with synovial living cells of rheumatoid arthritis patients using immunohistologic techniques. In contrast, this MoAb does not react with normal synovial membranes, lymph nodes, liver, spleen or brain. However, the antigen reactive with MoAb-P135 in RA synovial membrane had molecular weight 62 KD in contrast to the 70-85 KD EBNA-1 antigen. Further, the "cellular" 62Kd antigen differed from EBNA-1 antigens in lacking a particular amino acid sequence of (Gly-ala) peptides defined by a rabbit anti-synthetic peptide antibody. Genomic DNA extracted from RA synovial membranes failed to show reactivity with BAM V or BAM M cDNA probes of EBV in Southern blotting. Taken together, these results suggest that RA synovial membranes express a cellular protein that is antigenically cross-reactive with EBNA-1. Since RA patients have previously been shown to have elevated levels of antibodies against the (gly-ala) region of EBNA-1, immune responses against this cross-reactive epitope may play a role in the initiation or perpetuation of synovitis.

D100 THE ROLE OF EBNA-1 IN EPSTEIN-BARR VIRUS (EBV) INDUCED CELLULAR IMMORTALIZATION

T. Gross, K. Sakai, M. Hedenskog and D.J. Volsky, Dept of Path. & Microbol., University of Nebraska Medical Center, Omaha, Nebraska, 68105

When Epstein-Barr virus immortalizes lymphocytes, cells become latently infected and the viral genomes are maintained as closed circular(CC) episomes. An Epstein-Barr nuclear antigen, EBNA-1, is detectable in all EBV-positive tumor cells as well as cells immortalized by EBV in vitro. We have shown that even though EBNA-1 is expressed in lymphocytes following DNA transfection, no immortalization is achieved. If EBNA-1 does not directly transform lymphocytes, we were interested in the function of this viral protein in latency and cellular immortalization.

A plasmid, pSV3neoEBNA-1, was constructed from pSV3neo with an insertion of the Hind III-I fragment of EBV, which contains the complete coding exon for the EBNA-1 gene. pSV3neoEBNA-1 was so constructed that the EBNA-1 gene, as well as the neo gene, could be expressed by the SV40 promoter. P3HR-1 cells were transfected with pSV3neoEBNA-1 and selected for G418 resistance. The selected cells showed a decreased expression of the lytic cycle associated viral antigens, EA and VCA, as detected by immunofluorescence and showed a decrease in amount of linearized viral DNA. The cells are currently being analyzed for differential expression of EBNA-1 and its possible regulation of the viral replicative cycle. Further, the replicative cycle was induced in EBV carrying cell lines by TPA and n-butyrate and studied for the correlation of EBNA-1 expression and relative amounts of CC and linearized EBV genomes.

D101 LYMPHOCYTE POLY(A^+) ENRICHED RNA MEDIATES HEPATITIS B SURFACE ANTIGEN (HBsAg) SYNTHESIS TO MAINTAIN CIRCULATING EFFECTOR CELLS SPECIFICALLY CYTOTOXIC TO HEPATOCELLULAR CARCINOMA.

Anwar A. Hakim. Loyola University Medical Center. Maywood. Illinois 60153.

Several viral agents are known to infect humans and produce hepatitis. The hepatitis B virus (HBV) constitute a family of DNA containing virus that exhibit a marked tropism for hepatocytes. The causes of hepatocellular damage occurring in patients with HBsAg-positive inflammatory diseas have not yet been clarified. Viral hepatitis B (HBV) is a common infection that can progress from acute to chronic liver disease, or a carrier state, and virus persistence may be accompanied by the development of chronic active hepatitis, cirrhosis and primary hepatocellular carcinoma (HCC). Peripheral blood lymphocytes (PBL) from healthy adults with circulating antibodies to the viral surface antigen(HBsAg) and vaccine recipients were examined before and after in-vitro incubation with the viral antigen (HBsAg). These cells were cytotoxic to the hepatocellular carcinoma PLC/PRF/5. A cell line of particular use, since two candidate target antigens, the hepatitis B surface antigen (HBsAg) and the liver surface LSP) are represented on the plasma membrane of this cell line. These target cells also produce and secrete the viral surface antigen (HBsAg). The cytotoxicity of the effector cells was increased extensively after in-vitro incubation in media supplemented with either Interleukin-2 (IL-2) and/or HBsAg. These circulating effector cells specifically form a major defense mechanism against the neoplastic cells, tumor development and cancer metastasis. A Poly(A^+) enriched RNA fraction was isolated from these effector cells. When added to an in-vitro cell-free protein synthesis system, the RNA mediated the synthesis of HBsAg. It also hybridized with both, the DNA from HBV and from PLC/PRF/5 cells.

D102 EXPRESSION OF EBV EBNA PROTEINS USING AN SV 40 LATE REPLACEMENT VECTOR

M-L. Hammarskjöld, J. Dillner, I. Törnberg, S-C. Wang and G. Klein
Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden, S10401

We have constructed an SV 40 expression vector that replicates to high copy numbers in E.coli and transfected eukaryotic cells. This vector has been used to express regions of the EBV genome believed to encode nuclear antigens (EBNAs). A construct containing the BamH1 K restriction enzyme fragment produces 500 to 1000 times more EBNA1 in transfected cells (CV1, Cos1) compared to levels present in latently infected lymphoid cells (Raji). Another construct containing a 5kb fragment from the BamH1 'WYH' region expresses moderate amounts of the 87kd EBNA2. Surprisingly, this construct appears to be toxic to E.coli and gives rise to spontaneous non-toxic deletion mutants mapping within the EBNA2 gene. Some of these clones express large amounts of reduced size EBNA2 upon transfection into eukaryotic cells. Other regions of the EBV genome are currently being cloned into the vector for the purpose of mapping putative additional EBNAs.

D103 REGULATION OF BPV-1 PLASMID DNA REPLICATION. M.Lusky, L.Berg, A.Stenlund, J.Reynolds and M.Botchan. Dept. of Molec. Biology, University of California, Berkeley, CA94720.

To understand the regulation of BPV-1 replication in transformed cells we have attempted to define the viral cis and trans acting factors required. 1. The viral genome encodes for 2 cis acting elements (PMS1, PMS2) which can support plasmid replication (Lusky andBotchan,1984,Cell 36). Tofurther characterize PMS1 which appears to contain the viral origin of replication (Waldeck et al.1984,EmboJ.3) a series of mutations was created by random linker insertions. The resulting mutants were analyzed by transient replication as well as stable transformation assays. Our results show that the PMS1 region can be dissected into 2 domains. Domain 1,mapped to a 200 bp region which is homologous to PMS2, is required for replication activity. Domain 2, located 250bp upstream of domain 1, can act as an enhancer for replication. This sequence can be replaced by other viral transcriptional enhancers. 2. Viral plasmid replication is dependent upon at least 3 genes. Only the rep gene (E1) is absolutely required. Analysis of viral mRNAs indicates that exons located in the noncoding region may contribute to the structure of rep. The cop genes (E6 and E6/7) seem to modulate the viral copy number. Mutations in either of the cop genes can only be complemented completely when the WT allele is introduced together with the mutant DNA into cells. In contrast introduction of the WT allele into cells maintaining already either of the mutants leads only to partial amplification of the resident mutant DNA (Berg et al. ,inpress). Furthermore, stable and transient assays show that cells harboring either cop mutant fail to amplify WT BPV-1 plasmids. This resistance to amplification of both mutant and WT DNA upon stepwise transfection is consistent with the presence of a transacting repressor. Significance and regulation of this repressor will be discussed.

D104 Possible Human Papillomavirus type 6 transcripts elucidated using a retrovirus vector

Dennis J. McCance and Peter M. Chesters, Guy's Hospital Medical School, London. SE1 9RT

HPV6b DNA was cloned into the Bam H1 site of the retrovirus fector Zip. Neo SV (x). After transfection of 2 cells infectious retrovirus virions were used to infect NIH 3T3 cells. Clones of 3T3 cells selected with G418 were picked and fused with con-7 cells. The resulting plasmids were studied for the presence of specific HPV6b regions. Most HPV6b cDNA contained a 4.0kb unspliced region covering most of the early (E) open reading frames. One spliced cDNA of approximately 1.9kb was studied and found to contain regions from the E8 and E6 open reading frames and a large part of the E2 open reading frame. The E2 open reading frame is transcribed in human condylomata acuminata containing HPV6 genomes, and so further mapping of our 1.9kb transcript is in progress.

1. Lehn, H., Ernst, T-M., Sauer, G. (1984) J. Gen. Virol. 65, 2003-2010.

D105 A HEPATITIS B AMPHOTROPIC RETROVIRAL EXPRESSION SYSTEM, Alan McLachlan, Anneke K. Raney, Michael G. Riggs, David R. Milich, Joe Sorge and Francis V. Chisari. Department of Basic & Clinical Research, Scripps Clinic & Research Foundation, 10666 N. Torrey Pines Rd., La Jolla, CA 92037

An amphotropic retroviral expression system has been developed which expresses HBV antigens. The retroviral expression system used is a derivative of CistorNeo (Sorge et al (1984) Mol. Cell. Biol. 4, 1730-1737) which permits HBV antigen expression from unspliced recombinant retroviral transcripts and confers G418 resistance to cells by expression of a spliced retroviral transcript. The unspliced recombinant retroviral transcript can also be packaged to produce infectious recombinant retrovirus particles.

Characterization of HBV surface antigen (HBsAg) produced by this system has demonstrated its properties are the same as surface antigen produced during HBV infection. Recombinant HBsAg has a density of 1.2 g/cm^3 in CsCl gradients and forms 22nm particles as seen by electron microscopy. Polypeptides of 25, 27, 34, 39 and 43 kD are observed by Western analysis. These recombinant retroviruses are currently being used to analyze the expression of these antigens in human cells, mouse cells and mice.

D106 SEQUENCE SPECIFIC BINDING OF EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN (EBNA-1), Gregory Milman, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD 21205

The carboxyl-third of EBNA-1 encoded in the EBV BamH1 K-restriction fragment was synthesized in bacteria [Milman et al., Proc. Natl. Acad. Sci., USA 82:6300 (1985)]. The bacterially synthesized peptide specifically binds to clustered EBV DNA sequence repeats in the Ori-P region required for plasmid maintenance [Rawlins et al., Cell 42 (October, 1985)]. To elucidate the binding properties, a synthetic DNA binding site

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5' GATCTAGGATAGCAT|ATGCTACCCCGGGG    3'
3'     ATCCTATCGTA|TACGATGGGGCCCCCTAG 5'
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was cloned as a monomer, dimer, or trimer into the BamH1 site of plasmid pUC8. Sequence specific binding of EBNA to all three synthetic sequences were detected by mobility retardation on agarose gels. EBNA binding is strongly cooperative even to the monomer sequence. The time required for complete EBNA binding appears to be inversely related to the number of binding sites. Cleavage with Nde1 produces half a binding site to which EBNA does not appear to bind.

D107 RECOMBINATION BETWEEN PAPOVAVIRUSES SV40 AND RFV AND GENERATION OF INFECTIOUS VARIANTS. F. O'Neill, T. Miller and R. Stevens, V.A. Medical Center and University of Utah, Dept. Cellular Viral and Molecular Biology, Salt Lake City, UT 84148 EL SV40, RFV and JCV(HEK) are variants of wt SV40, BKV, and JCV respectively. They contain the viral DNA in a dual or bipartite form. One complementing molecule contains all the early (E) and the other all the late (L) sequences. Permissive BSC-1 cells were co-transfected with molecular clones of E-SV40 and L-RFV, or E-RFV and L-SV40. SV40/RFV complementation was demonstrated by cell killing, T-antigen formation and the appearance of complementing viral genomes in Hirt supernatants. In one combination, E-SV40/L-RFV, growth was very slow in the early virus passages but then accelerated and by passage 7 growth was rapid. Southern blotting before and after cloning in pBR322 showed that by passage 4, the L-RFV molecules had acquired SV40 origins. The evolved L-RF genomes could now be hybridized with SV40 probes. Prototype L-RFV genomes were not cleaved by Bgl I or Sph I but evolved L-RFV genomes were. Detailed analysis shows that the recombinant L-RFV genome contains three copies of an SV40 sequence (~750bp) running from one of the 72 bp repeats to a locus within the SV40 small t-antigen gene. These SV40 origin regions replace one of the L-RFV origins. The late region of L-RFV is intact. Analysis of complementing E-SV40 genomes shows that they remain unaltered. Similar recombinational events occurred in E-RFV/L-SV40 infected cells. The viruses with recombinant RFV genomes show an altered host range, and a marked change in the ability to transform permissive cells. In addition, a new hybrid virus was formed following transfection of human cells with E-JCV(HEK) and L-RFV genomes.

D108 THE PREDICTIVE VALUE OF PAP SMEARS AND COLPOSCOPY IN DETERMINING HPV 16 POSITIVITY. Joel Palefsky, Barbara Winkler, Carolina Braga, Victor Nizet, and Gary Schoolnik, Stanford University School of Medicine, U.C.S.F. School of Medicine.

The presence of HPV 16 DNA in cervical specimens is currently thought to be associated with increased risk of malignant transformation. However, convenient DNA typing procedures are not yet available to the clinician. In an effort to determine how the results of Pap smears and colposcopy correlate with the presence of HPV 16, the colposcopic findings, pathology, and HPV typing of specimens were analyzed for 33 women referred to a colposcopy clinic at U.C.S.F. In all patients, colposcopic findings were limited to the cervix. Ten of 33 patients were positive for HPV 16 DNA on cervical swabs analyzed by Southern blotting. Of these ten patients, 9 (90%) had abnormal colposcopy, whereas 8 of 23 patients HPV 16 DNA-negative patients (35%) had abnormal colposcopy ($p<.01$). Seven of the ten HPV16 DNA-positive patients (70%) had abnormal Pap smears ranging from CIN I to CIN II, while 2 out of 20 HPV 16 DNA-negative patients for whom Pap smear data were available (10%) had abnormal Pap smears, both with CIN I ($p<.01$). The positive predictive values for the presence of HPV 16 DNA with abnormal colposcopy and Pap smear were 53% and 75% respectively, while the negative predictive values with normal colposcopy and Pap smear were 94% and 86% respectively. These data suggest that HPV 16 DNA typing may not be indicated in routine screening of low risk populations, for whom the negative predictive value would be expected to be even higher. One of the questions which remains, however, is the outcome of patients who have negative Pap smears and colposcopy with positive HPV 16 typing. Further studies addressing these issues are currently in progress.

D109 STATUS OF PAPILLOMAVIRUS DNA IN PRE-NEOPLASTIC AND NEOPLASTIC LESIONS OF THE CERVIX. Alan Pater and Mary M. Pater, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NF A1B 3V6 Canada

Papillomaviruses are a heterogeneous subgroup of papovaviruses with a small double-stranded circular DNA as their genome. To date, forty types of human papillomaviruses (HPVs) have been isolated. Specific types of HPVs are associated with specific lesions. The association of PHVs with cervical neoplasia has been well established. Only HPV types 16, 18, 31, 33 and 35 have been found associated with these tumors. The goal of our studies is to see whether progression from dysplasia to neoplasia can be correlated with the status of viral DNA in each type of lesion. We have previously shown that HPV DNA present in several cervical carcinoma cell lines are covalently associated with the DNA of the host (Pater and Pater, Virology, 145, 313-318, 1985). In our present study we are examining the presence of HPV DNA in colposcopic biopsies from patients with abnormal pap smear. About 70-80% of the biopsies examined so far contain HPV DNA sequences. The majority of the positive lesions contain HPV 16. Southern-blot analysis of the undigested DNA from positive lesions reveal the presence of viral DNA in forms I, II and III. None of the samples examined contain integrated sequences. Further analysis of the viral DNA in these lesions is in progress.

D110 FIDELITY OF EPSTEIN-BARR VIRUS-INDUCED DNA POLYMERASE ON SYNTHETIC TEMPLATES. David Peim, Won-Ja Kim and Wendy Clough. Molecular Biology, University of Southern California, Los Angeles, CA 90089.

The fidelity of Epstein-Barr virus (EBV)-induced intracellular DNA polymerase was measured in the presence of various divalent cations and on a variety of synthetic primed templates. There was a greater percentage of nucleotide misincorporation using Cd^{++} and Ni^{++} as compared to Mn^{++}. Mg^{++} was used as the basis for comparison and was the least error-prone of the four divalent cations. The EBV-induced DNA polymerase shows a preference for a Poly dC•oligo dG template over a dA•T combination. The activity and fidelity was measured using different templates with both purine:purine; purine:pyrimidine and pyrimidine:pyrimidine mismatches. There was no measurable turnover activity associated with the EBV-induced polymerase. E. coli polymerase I was used as a comparison on all assays.

D111 TRANS-ACTING FUNCTION THAT MAINTAINS EPSTEIN BARR VIRUS EPISOMES ACTS INTERSPECIES ON HERPESVIRUS PAPIO PUTATIVE CIS-ACTING ORIGIN OF REPLICATION--Rick L. Pesano and Joseph S. Pagano, Lineberger Cancer Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

Herpesvirus papio (HVP) and EBV are closely related biologically and biochemically; both can produce latent infections. HVP is a lymphotropic virus and is able to immortalize both adult human and adult baboon B lymphocytes in vitro (Rabin, 1977). Southern blot hybridization data shows a 40% homology between HVP and B59 EBV; however, the relatedness is not restricted to certain regions of the viral DNA but is dispersed (Lee, et al., 1980). HVP-permissive lymphoblastoid cells contain both unit-length linear and episomal viral DNA. The circular molecules are approximately the same size as episomes found in EBV growth-transformed Raji cells, 170 X 10^3 base pairs (Falk, et al., 1979). The putative origin of replication in EBV (oriP) has recently been assigned to a 1790 base-pair fragment (cis) in the short unique region of the genome which requires a function supplied in trans from elsewhere in the genome (Yates, et al., 1984). We report here the identification of the putative origin of replication (cis) HVP, and have assigned it to the HVP Eco RI J fragment. The results indicate that the HVP origin of replication requires both a cis and a trans-acting function, analogous to that found in EBV. Further, additional data indicate that there is cooperativity of function between the HVP cis and the EBV trans fragments. Plasmid constructs containing the HVP cis fragment can replicate autonomously in cell lines containing an endogenous EBV genome. The results indicate that similar mechanisms and probable conserved sequences operate to maintain latent episomes in EBV and HVP infection.

D112 REGULATION OF PAPILLOMAVIRUS EARLY GENE EXPRESSION, Herbert Pfister and Eva Kleiner, University Erlangen-Nürnberg, Erlangen, F.R.Germany
Bovine papillomavirus (BPV) 1 transformed cells contain extremely low levels of viral mRNAs (15-30 copies of the "most abundant" RNA species (1)). When treating BPV1 transformed C127 cells or transformed DBA mouse embryo fibroblasts with cycloheximide (20 ug/ml medium) for 1h we observed a 15-30 fold increase of viral transcripts. Northern blots revealed no qualitative changes in the RNA pattern. The result indicates that a rather labile protein either represses viral transcription or degrades viral mRNA. When blocking transcription by the addition of actinomycin D (6 ug/ml) the half-life of BPV1 mRNAs seemed to be slightly prolonged. The product of BPV1 open reading frame E2 was recently shown to stimulate transcription of the CAT gene under the control of a BPV1 enhancer from the non-coding region (2). Analyzing cells, which were transformed by a NcoI deletion mutant within E2, we observed 10 fold less viral transcripts than in wild-type transformed cells. Addition of cycloheximide led to an increase of viral mRNAs roughly restoring wild-type conditions, indicating that cycloheximide stimulation is independent of E2 activity. It was interesting to note that cycloheximide had no effect on the amount of human papillomavirus 18 transcripts in cervical carcinoma derived Hela cells thus showing that there exists no comparable control mechanism in this cell line. --- (1) Virology 119, 22-34 (1982), (2) J Cell Biochem Suppl 9C,89 (1985)

D113 FLOWCYTOMETRIC ANALYSIS OF EPSTEIN-BARR (EBV) BINDING TO HUMAN T LYMPHOCYTE SUBPOPULATIONS, Guy Sauvageau and José Menezes, University of Montreal and Ste-Justine Hospital, Montreal, Quebec, Canada H3T 1C5.

An extensive study of the binding of P3HR-1 and B95-8 strains of EBV to human peripheral blood lymphocytes was carried out using the fluorescence-activated cell sorter. The results obtained indicate that, in addition to B cells, the suppressor/cytotoxic ($Leu2a^+/OKT8^+$) T cell subpopulation bound EBV while the helper/inducer did not. To analyse further the interaction between EBV and suppressor/cytotoxic lymphocytes, a double labelling technique using phycoerythrinated Leu2a and fluoresceinated EBV was performed. The results obtained show that: a) over 50% of the Leu2a positive cells (suppressor/cytotoxic) bound both strains of EBV; b) Leu2a positive lymphocytes of both seropositive and seronegative individuals bound equally both strains of EBV; c) this binding could be abrogated by using a known neutralizing monoclonal antibody or by preincubation of the T cells with unlabelled virus. Taken together, these observations illustrate the ability of the Leu2a positive T lymphocyte subpopulation of both the seropositive and the seronegative donors to specifically bind the P3HR-1 and B95-8 strains of EBV. Further studies are needed in order to assess better the importance of these findings in regard to the physiopahology of EBV-related diseases.

D114 THE E5 OPEN READING FRAME OF BOVINE PAPILLOMAVIRUS ENCODES A TRANSFORMING GENE. John T. Schiller, Elliot Androphy, Karen H. Vousden, and Douglas R. Lowy. Laboratory of Cellular Oncology, National Institutes of Health, Bethesda MD 20892.
Previous studies in our laboratory have shown that the early region of Bovine Papillomavirus (BPV-1) contains two non-overlapping segments that, when activated by a retroviral LTR, can independently transform cultured mouse cells. It was demonstrated that the E6 open reading frame (ORF) encodes the transforming gene of the 5' segment and that E6 is expressed as a 15.5 KD protein in transformed cells. The transforming activity of the 3' transforming segment was previously localized to a 2.3 Kb fragment (2.3T) that includes the E2, E3, E4 and E5 ORFs. We now report the construction of a series of linker insertion and deletion derivatives of clone pHLB1, in which a retroviral LTR is linked to 2.3T in the promoter positive orientation, and the analysis of the mutants' ability to induce focal transformation in NIH3T3 cells. The results indicate that the E5 ORF has intrinsic transforming activity in NIH3T3 cells and that the expression of the E2, E3 and E4 ORFs are not required for this activity. Since any of the sequences of 2.3T upstream of the E5 ORF can be deleted without abolishing the transforming activity, we conclude that the transforming fuction lies entirely within E5, which from the first AUG of the ORF would encode a peptide of only 44 amino acids. This peptide is very hydrophobic which may indicate that the putative E5 protein is membrane associated.

D115 IMMUNOLOGICAL CHARACTERIZATION OF REVERSE TRANSCRIPTASE ASSOCIATED WITH NON-A, NON-B HEPATITIS. Belinda Seto and William G. Coleman, Jr., Food and Drug Administration and National Institutes of Health, Bethesda, MD 20892

The infectious viral particles present in non-A, non-B hepatitis patient serum (Inoculum I) can be pelleted by centrifugation. The particle-associated reverse transcriptase from the resultant pellet was purified, following detergent disruption, by affinity chromatography, and characterized by immunoblot analyses.

Using specific antibodies to simian sarcoma virus reverse transcriptase as a probe, a cross-reactive protein of 80,000 daltons was detected in 2 ml of a 10^{-3} dilution of Inoculum I. This inoculum contains a virus titer of 10^{2} infectious particles/ml of serum based on chimpanzee transmission studies with serial dilutions of the inoculum. In contrast, no cross-reactive protein was detected in a control serum.

By means of affinity chromatography of the viral lysate on oligo(dC)-cellulose, the reverse transcriptase was eluted by a linear gradient of 0 to 0.8 M KCl. The reverse transcriptase thus purified cross-reacts with antibodies to reverse transcriptase from type C mammalian retroviruses, namely, simian sarcoma virus, RD114, baboon endogenous virus, and Rauscher leukemia virus. However, no cross-reactivity was observed with antibodies to avian myeloblastosis virus and likewise type B and type D reverse transcriptase viruses. These results indicate that the reverse transcriptase purified from non-A, non-B hepatitis serum share interspecies determinant with other type C mammalian virus reverse transcriptase.

D116 Antibodies to EBNA Peptide 62 in Sera From Patients with Infectious Mononucleosis, Richard S. Smith, John E. Geltosky, Gary Rhodes, Charles A. Horwitz and Alice S. Whalley, Johnson and Johnson Biotechnology Center, Inc.

We compared the diagnostic accuracy of the heterophil and EBV specific procedures to a new ELISA based on detecting antibodies to a synthetic peptide corresponding to a defined region of EBNA. We observed an early and pronounced IgM anti-EBNA synthetic peptide response with clinical sera from 10 heterophil positive and 3 heterophil negative IM patients. The IgG response to the peptide was delayed. A ratio of IgG to IgM anti-peptide antibodies of less than one was indicative of an acute phase infection. The ratio increased to greater than one in the convalescent phase of the infection indicating the rise of the IgG antibodies. We also observed the rise of peptide specific IgA antibodies in ten of 13 acute IM patients and in 74% patients with nasopharyngeal carcinoma. Specificity of this anti-peptide response was demonstrated by competition with purified EBNA. We immunoblotted selected heterophil positive and negative sera on EBV transformed cell lines. The rise of anti-peptide antibodies correlated well with appearance of IgG and IgM antibody to the EBNA protein.

D117 TRANSFECTION OF NORMAL HUMAN LYMPHOCYTES WITH VIRAL ONCOGENES: PLASMID STABILITY AND EXPRESSION, M. Stevenson and D.J. Volsky Univ. of Nebraska Med. Center, Omaha, NE.

Using an efficient Sendai virus envelope-mediated gene transfer technique, we have been investigating the effects of v-myc and v-ras on normal human tonsil lymphocytes. The oncogenes used in our experiments were a pSVv-myc which contains a permuted copy of the MC29 provirus DNA, and a v-Ha-ras plasmid containing a spleen necrosis virus LTR.

The expression of the v-myc plasmid was analysed using a quantitative S1 nuclease assay. In cells transfected with v-myc alone, there was a very low level of v-myc RNA. A significant increase in v-myc RNA was observed in those lymphocytes co-transfected with v-myc and v-ras. Analysis of Hirt DNA extracts showed that the v-myc plasmid was essentially unstable in normal lymphocytes, and as early as four hours after transfection, there appeared to be a breakdown in the plasmid structure with the subsequent appearance of mini-replicons which rapidly replicated to a high copy number. V-myc plasmid stability was achieved when cotransfected with v-ras which may explain the contribution of ras to the enhanced myc RNA expression. In addition, when transfected into immortal cell lines such as NIH 3T3 or a B lymphoblastoid cell line Loukes, the v-myc plasmid was stable, indicating a relationship between plasmid stability and cell phenotype. These results show that other factors bear consideration when studying expression of transforming genes in normal cells.

D118 QUANTITATIVE ANALYSIS OF EPSTEIN-BARR VIRUS (EBV) BINDING TO HUMAN LYMPHOID CELLS BY FLOW CYTOMETRY, Rino Stocco and José Menezes, University of Montrel and Ste-Justine Hospital, Montreal, Quebec, Canada H3T 1C5.

We report the use of fluoresceinated preparations of two strains of EBV (P3HR-1 and B95-8) and of the fluorescence-activated cell sorter (FACS) to analyse quantitatively the presence of EBV receptors on different human lymphoid cell lines and B lymphocytes from both seropositive and seronegative donors. This EBV-cell interaction was also analysed by using neutralized viral preparations obtained by preincubating the virus with a monoclonal antibody with known ability to inhibit EBV infection. FACS analysis also allowed us to approximate the cell surface of the different lymphoid cells utilized. The results obtained indicate that: a) both EBV strains used bound equally to Raji cells while there was no virus attachment to EBV receptor-negative 1301 cells (from a non-B, non-T cell line); b) there was a significantly higher number of Molt-4 cells (T cells) binding B95-8 EBV than P3HR-1 EBV; c) after the difference in cell surface dimensions was corrected, it was found that EBV-bound Raji cells showed a fluorescence intensity which was two-fold higher than that of EBV-bound fresh B cells from an EBV-seropositive donor; d) lymphocytes from an EBV-seronegative donor bound two times less virus than B cells from a seropositive individual. In short, the present observations indicate that there are significant qualitative and quantitative differences in regard to EBV binding to various human lymphoid cells.

D119 Cloning and Expression of HPV 6b Capsid Peptides: Localization of an Immunoreactive Region. David Strike, Robert Rose, H. Reid Mattison, Richard Reichman, University of Rochester, Rochester, New York 14642

Human papilloma viruses (HPV) cannot be grown in tissue culture, and purified homogenous HPV antigens have been difficult to obtain. The recent availability of procaryotic expression vectors capable of high level expression of open reading frame (ORF) DNA now provides a means for the production of viral peptides in E. coli. Such peptides may show immunoreactivity that is similar to that of native viral capsid proteins.

The capsid proteins of HPV arise through translation of mRNA from the "late" genomic ORF's designated L2 and L1. We have cloned several segments of late region ORF DNA from HPV 6b and have achieved high level expression of corresponding peptides using a vector carrying the P_R promoter of lambda phage. With this system, foreign DNA ORF segments are expressed as fusions with bacterial beta-galactosidase. Western blot analysis of these fusion proteins has shown that a specific subregion of the putative L1 protein shows strong reactivity with "group specific" anti-PV antibodies. The full L1 amino acid sequence, as generated by computer from the HPV 6b L1 ORF DNA sequence, has been aligned with the putative L1 amino acid sequences of 6 other PVS, and predictions of local hydrophilicity and secondary structure have been made. The subregion shown to be reactive on Western blots contains beta turns, a highly hydrophilic region and several well conserved stretches with low polarity. Further studies are underway to localize more precisely the immunoreactive portion of this molecule.

D120 CELLULAR IMMUNE REACTION INDUCED BY AN EBV-DETERMINED MEMBRANE PROTEIN EXPRESSED IN LATENT INFECTION, Robert Szigeti, Dov Sulitzeanu, Ada Hatzubai, Joakim Dillner, Kevin Hennessy, Elliott Kieff and George Klein, Dept of Tumor Biology, Karolinska Inst Stockholm,Sweden, Hadassah Medical School, Jerusalem,Israel, Kovler Viral Oncology Lab.,Univ. of Chicago, U.S.A.

Leukocyte migration inhibition (LMI) response of healthy EBV-seropositive (SP) and -seronegative (SN) donors to an EBV-encoded protein, fused with E.coli beta-galactosidase (LT3 protein), was studied. The protein has previously been identified in the cytoplasmic membrane of latently EBV-infected cells. The protein was purified from E.coli and incubated with buffy coat cells from SP and SN donors in direct LMI assay. Although at higher concentration than membrane fractions prepared from Raji cells, the protein induced virus-specific LMI. A rabbit antiserum to the fusion protein abolished most of the LMI effect both of the protein and of Raji membrane fractions, indicating that the predominant immune response in LMI directed to the EBV protein. The blocking by antiserum was reversed by preincubating it with EBV-converted, but not EBV-negative cells. LMI to LT3 and Raji-membranes appeared simultaneously in patients with infectious mononucleosis and together with EBNA-induced LMI. Cross-blocking reaction with LT3 and EBNA revealed that corresponding antisera were effetive in blocking only the LMI induced by the corresponding antigen. The results suggest that the EBV-encoded latently infected cell membrane protein is similar or identical to the antigenically active Raji cell membrane moieties, but not to EBNA-1.

D121 EXPRESSION OF HPV-16 "EARLY" GENES, D. Smotkin and F.O. Wettstein, Division of Gynecologic Oncology and Department of Microbiology and Immunology, UCLA School of Medicine and Molecular Biology Institute, University of California, Los Angeles, CA 90024

In eight cervical cancers and in two cervical cancer derived cell lines (CaSki and SiHa) the HPV-16 DNA was found to be integrated while one cancer contained almost exclusively HPV-16 DNA as plasmid. However, even in this tumor, when analyzed by two dimensional agarose gel electrophoresis a small amount of integrated DNA was detectable. S1 and Exo VII nuclease mapping and primer extension experiments with RNA from the CaSki cells and the cancer with HPV-16 plasmid DNA defined exons which could code for E6, E7 and a partial or complete E2 protein in the Caski cell line and the cancer respectively. All the different RNAs mapped appeared to be generated from the same primary transcript. The 3' end of the transcripts do not extend to the "early" region polyadenylation site, suggesting that they terminate within cellular sequences. Since our DNA analysis revealed intact HPV-16 sequences around this polyadenylation site in the majority of the HPV-16 copies the results imply that only a minor fraction of the viral genes are transcriptionally active. Antibodies to E6 and E7 were obtained by immunizing animals with trypE fusion proteins. The E7 protein was identified by immune precipitation in the S^{35} labeled extracts of the two HPV-16 containing cell lines (CaSki and SiHa) where the protein is present in the cytoplasm. (Cloned HPV-16 DNA was kindly provided by Drs. Schwarz, Gissman, Duerst and Zur Hausen and the trypE vector by Dr. Korner.)

D122 ORGAN CULTURE OF GENITAL CONDYLOMATA, George D. Wilbanks, Jack L. Hawkins, Todd R. Golub, Mary E. Turyk, Rush University, Chicago, Illinois 60612.

Biopsies of cervical and vulvar condylomata were studied in vitro using a modification of the rocking organ culture system developed by Schurch et al (Cancer Res 38:3723, 1978). The explants were grown in a low-calcium (0.072 mM) preparation of Dulbecco's Modified Eagles Medium supplemented with Epidermal Growth Factor, hydrocortisone, Cholera Toxin, insulin, and 2% fetal bovine serum. The cultures were observed for outgrowth of cells onto the flask. Portions of the tissue were sampled at weekly intervals and examined for histologic changes associated with human papilloma virus (HPV) infection and for HPV-specific antigens by immunoperoxidase staining. Results of preliminary studies will be presented.

D123 MULTIFOCAL LOWER FEMALE GENITAL TRACT CONDYLOMA/NEOPLASIA SYNDROME: A CLINICO-PATHOLOGIC + MOLECULAR ANALYSIS B. Winkler MD, B. Krumholz MD, R. Galli BA, V Nizet BS, J. Palefsky MD, G. Schoolnik MD, B. Steinberg Ph.D., University of California, San Francisco, CA, Long Island Jewish-Hillside Medical Center, New Hyde Park, NY, Stanford University, Palo Alto, CA.

Extensive, multifocal condylomatous +/or neoplastic lesions of the lower female genital tract are becoming increasingly common & present as a diagnostic & therapeutic challenge. In this study, 88 tissue samples from 43 women with multifocal lesions were analyzed for HPV types 6,16 + 18 & the results correlated with the clinical history, colposcopic distribution & pathology. The patients had been referred to a specialized colposcopy practice for evaluation of extensive genital involvement refactory to primary treatment. Tissue samples were screened for HPV DNA's by dot blot hybridization at TM-19° & TM-6°. To date, Southern blot hybridization at TM-25° confirms HPV type in 14/14 lesions. There was a high prevalence of mixed HPV types 6 + 16 in 28/43 (65%) of the patients + mixed HPV types 6,16 + 18 in 1/43 (2%). Single viral types were detected in 13/43 (30%) & one patient was negative. Pathologic & HPV-type heterogeneity could be demonstrated in different lesions from the same patient. Although HPV-16 was consistently associated with neoplasia & carcinoma, it was also present in patients with histologically bland condyloma. The results suggest that mixed-type, heterogeneous HPV infections may be associated with multifocality, resistance to therapy & recurrence & that the presence of HPV-16 is not predictive of lesional pathology or biology in this patient group.

D124 TRANSCRIPTION OF RNA FROM BOTH STRANDS OF HEPATITIS B DNA. A.Zelent, P.M.Price, M.A.Sells, & J.K.Christman. Mount Sinai School of Medicine, N.Y., NY 10029.

Characterization of polyadenylated RNAs of a mouse 3T3 cell line (C4.10) transformed with hepatitis B virus DNA has revealed the presence of transcripts off both DNA strands. Whereas RNAs transcribed from the "long" strand of DNA migrate primarily at 2.4 and greater than 3.2 kb on formaldehyde gels, RNAs transcribed from the "short" strand migrate at 2.0, 2.4, and 2.8 kb. Mouse 3T3 polyadenylated RNAs revealed three weak bands that did not correspond with any of those described. Polyadenylated RNAs isolated from PLC/PRF5 cells also contained a weakly hybridizing band of 2.0 kb detectable with "short" strand-specific probes.

Analysis of the 5' end of the short strand-transcribed RNA of C4.10 cells by S1 and primer extension indicated a major initiation site at 1861±2. Analysis of the 3' end of this RNA by S1 nuclease indicated a termination site at 2381±20.

The presence of these transcripts raises the possibilities that, a) they may code for as yet undescribed viral proteins, b) they may act as "antisense" RNAs to regulate translation of viral long strand transcripts or c) they may act as intermediates in the replication process.

This work was supported by grant number CA34818 from the National Cancer Institute.

Diagnostics and Vaccines

D125 EPSTEIN-BARR VIRUS SPECIFIC DEOXYTHYMIDINE KINASE. Kay Azuma-Boykin, Timothy J. Stinchcombe and Wendy G. Clough, Molecular Biology, University of Southern California, Los Angeles, CA 90089.

Epstein-Barr virus (EBV) induces the expression of several unique enzymes associated with viral DNA replication. Recently, our laboratory reported the existence of an EBV-specific thymidine kinase (tk) activity present in EBV producer or superinfected nonproducer B lymphocyte cell lines that was separable from host cell tk using DEAE-cellulose column chromatography (Biochem. 24, 2027-2033). The EBV-specific tk is biochemically distinct from the host cell tk and exhibits properties similar to the Herpes simplex virus tk.

Baer et al. have reported an EBV reading frame that bears a small region of homology with the HSV2 tk gene (Nature 310, 207-211). Experiments are in progress in our laboratory to map the putative EBV tk gene by

(1) computer and Southern blot analysis for sequence homology between the HSV tk and EBV DNA;
(2) calcium phosphate transfection of selected fragments of EBV DNA.

D126 EXPRESSION OF AIDS ASSOCIATED RETROVIRUS GENES IN RECOMBINANT MICROORGANISMS, P.J. Barr, D. Parkes, E.A. Sabin, M.D. Power, H.L. Gibson, C.T. Lee-Ng, C. George-Nascimento, R. Sanchez-Pescador, R.A. Hallewell, K. Steimer and P. Luciw, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608

Using bacterial, yeast and mammalian cell expression systems, we have directly expressed functional domains of each of the major genes of ARV-2, a retrovirus associated with AIDS. Recombinant proteins corresponding to precursors and individual subunits of the gag-pol precursor together with N and C-terminal regions of the env precursor were isolated for analysis. Immunoblotting procedures using these recombinant proteins showed that antibodies in AIDS sera react with domains from each of the gag, pol and env genes. Recombinant env gene products were isolated from yeast and used to immunize rabbits; antibodies from these animals reacted with viral env glycoproteins both in virus and in infected cells. We are currently studying the use of recombinant viral antigens in the analysis of immune responses in infected individuals. In addition, yeast derived env gene products will be useful for the further study of the specificity of immune responses in animal model systems.

D127 EFFICIENT EXPRESSION OF HTLV-III GENES USING A BOVINE PAPILLOMA VIRUS VECTOR
N. T. Chang, C. W. Shearman, G. F. Chen, and T. W. Chang, Centocor, Inc., 244 Great Valley Parkway, Malvern, PA 19355

Subgenomic segments of HTLV-III have been cloned into a eukaryotic expression shuttle vector containing the bovine papilloma virus genome and the neomycin resistance gene. This DNA was then introduced into mouse C-127 cells by DNA mediated gene transfer. Transfection was fairly efficient with 0.1% of the cells being transformed to G-418 resistance. Southern analysis of genomic DNA and "Hirt" extracts of G-418 resistant transformants showed that the plasmid is maintained extra chromosonally and in low copy number. Expression of HTLV-III specific peptides was demonstrated by Western immunoblot techniques using sera of patients with AIDS or AIDS-related complex. Transformants containing the gag gene of HTLV-III produce immunoreactive proteins with molecular weights of 55 kd, 48 kd, 44 kd, 41 kd and 39 kd. Transformants containing the entire HTLV-III coding region produce immunoreactive proteins with molecular weights the same as the gag transformants as well as those of 12 kd, 17 kd, 24 kd, 30 kd, 65 kd and 110 kd. Competition studies with E. coli extracts expressing the gag gene confirmed the complex pattern of gag related proteins. The amount of HTLV-III peptides expressed in mouse C-127 cells has been estimated to be on the order of 5 mg per 10^8 cells. Using the expression system, we hope to express HTLV-III major envelope protein in high levels and to evaluate the application of the recombinant envelope protein as a subunit vaccine against HTLV-III infection.

D128 ELISA USING BACTERIALLY SYNTHESIZED EBNA AND EA: CLINICAL STUDIES OF PATIENTS WITH INFECTIOUS MONONUCLEOSIS AND NASOPHARYNGEAL CARCINOMA, Jessica Halprin, Alan L. Scott, Paul H. Levine and Gregory Milman. Johns Hopkins University and NIH.

A sensitive enzyme-linked immuosorbent assay (ELSIA) was used to quantitate IgG antibodies against bacterially synthesized Epstein-Barr virus (EBV) nuclear associated antigen (EBNA) and early antigen (EA) in sera from 100 healthy North American individuals, 40 North American patients with infectious mononucleosis (IM) and 48 Asian patients with nasopharyngeal carcinoma (NPC). IM patients could be distinguished by ELISA from the healthy population suggesting a potential utility in IM diagnosis. Antibodies to EBNA were detected in 100% of the sero-positive (to EBV viral capsid antigen) healthy population and in none of the IM patients during the first 6 months post-infection. Antibodies to EA were detected at low levels in 70% of the healthy population, but at high levels in IM patients, of whom 55% had anti-EA titers greater than 2 standard deviations above the geometric mean titer of the healthy population. Similar high anti-EA titers were observed for 75% of the NPC patients. The ELISA measurements provide a potentially useful prognostic indicator of survival for NPC patients. The probability of survival for NPC patients showed a significant co. relation ($p<0.02$) with the change in anti-EA titer during the first 12 months following diagnosis. Patients with increasing anti-EA antibodies had a lower probability of survival (probability near 0) than those whose titers remained constant or decreased (probability = 75%).

D129 IgG AND IgM ANTIBODIES TO AIDS RETROVIRUS (ARV) IN GAY MEN. J. Allen McCutchan, Caroline Kennedy, Heli Collins, Phyllis Spechko, Steve Spector, Gerry Boss, and Fred Jensen. University of California, San Diego 92103 and Cytotech, Inc., La Jolla, CA 92121

The significance of IgG and IgM antibodies to ARV was investigated by stratifying 305 gay men into 5 groups: AIDS, AIDS-related complex (ARC), only one ARC sign (usually lymphadenopathy) or symptom (LAS), well with immune abnormalities (Imm Abn), and well without immune abnormalities (Imm NL). The cross-sectional prevalence of these antibodies measured by ELISA for each clinical class is shown in the table.

DIAGNOSIS	AIDS	ARC	LAS	IMM ABN	IMM NL
Number of Subjects	44	30	113	67	51
% IgG +	84	93	69	34	18
% IgM +	0	7	9	13	10
% G or M +	84	93	72	43	22
% M+ of all+	0	7	12	28	45

Prevalence of IgG to ARV is greatest in patients with AIDS and ARC, but mean titers are relatively low and IgM is uncommon in these symptomatic patients. In contrast, IgM to ARV is more common among asymptomatic, seropositive subjects suggesting they have been recently infected. 9% (11/121) of seropositive, asymptomatic (LAS+IMM ABN+IMM NL) and 20% (8 of 40) of seropositive, clinically normal (IMM ABN+IMM NL) gay men had only IgM to ARV. Helper T lymphocytes were increased in those with IgM, but dramatically decreased in those with both IgM and IgG antibody to ARV, suggesting that IgG to ARV may mediate lymphocyte destruction.

D130 DETECTION OF HTLV-III VIRAL ANTIGENS IN SERUM, Deborah A. Paul and Lawrence A. Falk, Jr., Abbott Laboratories, North Chicago, IL 60064

An EIA has been developed which is capable of detecting HTLV-III viral antigens in serum or plasma of infected individuals. The test reacts primarily with the p24 core antigen of the virus, determined using recombinant DNA p24 and p41 proteins, and has a sensitivity of less than 1 ng/ml. Serum samples have been identified which are HTLV-III antigen (Ag) positive and anti-HTLV-III antibody (Ab) negative. In AIDS patients (n=300), 96% were Ab+, with 50% being Ag+; 2.5% of AIDS patients were Ag+/Ab neg. In high risk populations (n=1200), 50% were Ab+, with 25% of these also being Ag+. Overall in the high risk group, 15% were Ag+, 1.84% of which were Ag+/Ab neg. Additionally, the Ag level was seen to vary with time in a given individual. Ag+ samples were confirmed in an Ag inhibition immunoassay by pre-incubation with Ab from various sources. The specificity of the assay was tested using plasma and serum samples from patients with HBV, EBV, and CMV infections, and SLE, multiple myeloma, and leukemia patients. Also lysates from various virus-infected cell lines, including HTLV-I, were tested. No false positives were seen. Thus far, Ag has been identified in immune complexes only when it has also been detected by the EIA. In preliminary testing of peripheral blood lymphocytes from 6 AIDS patients (Ag+/Ab+), 2 lymphocyte lysates were Ag+. Studies correlating HTLV-III serum Ag positivity with infectivity and disease state are in progress. This test should also prove useful for monitoring anti-viral therapy.

D131 IMMUNOLOGICAL CHARACTERIZATION OF HTLVIII RECOMBINANT PROTEINS POTENTIAL AS DIAGNOSTICS OR VACCINES D. Reed,[1] K. Reagan,[1] W. Kenealy,[1] R. Cybulski,[2] R. Tritch,[1] D. Bolognesi[3] and S. Petteway[1]Central Research & Development Department[1] and Biomed Department[2] E.I. Du Pont de Nemours & Co., Inc, Experimental Station, Wilmington, DE 19898 USA, Duke University Medical Center,[3] Durham, NC 27710

Human T-Cell Lymphotrophic Virus (HTLVIII) has been implicated as the causative agent of Acquired Immune Deficiency Syndrome(AIDS). Antibodies to HTLVIII have been detected in patients with AIDS and AIDS related diseases. The availability of individual HTLVIII antigens for seroepidemiology studies, the analysis of viral structure, and the development of diagnostics and vaccines has been limited. In an effort to provide sufficient quantities of viral proteins for these studies we have engineered open reading frame fragments of viral DNA into E.coli expression vectors. A variety of recombinant proteins representing different regions of the gag and env open reading frames have been produced and shown to react specifically with AIDS sera. The recombinant proteins were further characterized by their reactivity with monoclonal and polyclonal antibodies to purified viral proteins and their ability to induce virus-specific antibodies. This approach has been used to define more specifically the antigenic regions of the virus. In an effort to analyze the potential of recombinant proteins as diagnostic tools, we have investigated the reactivity of different AIDS sera with individual recombinant antigens. These studies have implications for the development of specific diagnostics and vaccines.

D132 LATENT FELINE RETROVIRUS INFECTIONS: DIAGNOSIS AND CLINICAL IMPLICATIONS. J.L. Rojko[1] and A.A. Hayes.[2] [1]The Ohio State University, Columbus, Ohio 43210 and [2]The Animal Medical Center, New York, NY 10021.

Thirty pet cats with serological or historical evidence of exposure to the feline leukemia virus (FeLV) were examined for the presence of latent, reactivatable FeLV in bone marrow cultures. Only one of 10 healthy cats had reactivatable FeLV, whereas 8 of 20 cats with FeLV-negative lymphoma, anemia, and other miscellaneous diseases were positive for reactivatable FeLV. The results of serological assays for exposure (ELISA, antibody to the feline oncornavirus-associated cell membrane antigen) were variable.

Supported by CA-35747 and The Animal Medical Center. JLR is a Scholar of the Leukemia Society of America, Inc.

D133 REACTIVITY OF SERA OBTAINED FROM INDIVIDUALS INFECTED WITH THE AIDS RETROVIRUS WITH DIFFERENT DOMAINS OF THE ENVELOPE POLYPEPTIDE PRODUCED IN GENETICALLY ENGINEERED YEAST. K. Steimer, J. Stephans, K. Higgins, C. George-Nascimento, P. Barr and P. Luciw. Chiron Corporation, Emeryville, California, 94608.

Sera were obtained from individuals that were seropositive to the AIDS retrovirus in an ELISA system using purified virus. We have screened these sera for antibodies reactive to different domains of the viral envelope (env) protein by employing env polypeptides produced in genetically engineered yeast. Yeast expression vectors were used to synthesize polypeptides representing the following portions of the AIDS-associated retrovirus (ARV-2) env gene (863 amino acids): 1) env-1, amino acid residues 26 to 276; 2) env-2, amino acid residues 26 to 490; and 3) env-3, amino acid residues 528 to 863. Env-2 corresponds to the major portion of the N-terminal domain of the mature env glycoprotein, gp120, that is external to viral and infected cell membranes. Env-1 is approximately the amino terminal half of the gp120 polypeptide. Env-3 represents a large part of the C-terminal domain, gp41, that spans membranes and serves as an anchor for the env glycoprotein complex. Greater than 95% of serum samples, from seropositive individuals, reacted with both env-2 and env-3. However, only a minority of these sera (approximately 30%) reacted with env-1. We are currently examining the basis for this lack of seropositivity specific to the amino terminal third of the envelope polypeptide.

D134 SPECIFICITIES OF MONOCLONAL ANTIBODIES GENERATED AGAINST A RECOMBINANT ENVELOPE GENE PRODUCT OF THE AIDS RETROVIRUS. J. Stephans, D. Lee, G. Van Nest, P. Barr, P. Luciw, K. Steimer. Chiron Corporation, Emeryville, California, 94608.

Protective immune responses in retrovirus infections are directed at the viral envelope (env) gene product. Yeast have been genetically engineered to produce an env polypeptide (env-2) that represents the predicted amino acid sequence of gp120, the external envelope glycoprotein of AIDS-associated retrovirus (ARV-2). Spleen cells from BALB/c mice immunized with env-2 were fused with the murine cell line P3X63Ag8.653. Two hundred and seventeen stable clones producing antibodies that reacted with purified env-2 in an enzyme-linked immunosorbent assay (ELISA) were obtained. Sixty-eight percent of these clones reacted with env-2 in western blots. A polypeptide (env-1) corresponding to approximately the amino terminal half of gp120 has also been produced in yeast. The majority (84%) of env-2 western blot-reactive monoclonal antibodies also reacted in western blot assays with env-1. In western blots of ARV-2 virus, approximately 25% of the monoclonals raised against env-2 reacted with viral gp120. The remaining 75% of the antibodies did not react with any viral proteins. Among those monoclonals that reacted with viral gp120 were antibodies that recognized each domain. Further studies are directed at characterizing other activities of these monoclonal antibodies such as virus neutralization and cytotoxicity and in evaluating their utility as immunological reagents for studying strain variation within gp120 of independent AIDS retrovirus isolates.

D135 AVIAN RETROVIRAL RECOMBINANT EXPRESSING SUBGROUP A ENVELOPE REDUCES TUMOR FORMATION OF ASV-A-INDUCED SARCOMA, Stephen E. WRIGHT[1,2] and David D. BENNETT[1], [1]Viral Oncology Laboratory, VA Medical Center, [2]Departments of Medicine and Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City, Utah 84148

Avian RNA tumor viruses are members of the retrovirdae family of viruses. Among this family are exogenous tumor-inducing viruses as well as endogenous non-oncogenic viruses. The antigenic difference between these viruses has been shown to be contained within the envelope (env) regions. Envelope variations of tumorogenic viruses are classified by subgroups A, B, C or D. Endogenous viruses embody subgroup E. A chimeric virus was constructed by recombining the env region of the malignant ASV-A virus, subgroup A, with the endogenous virus RAV-0. The hybrid retrovirus has been shown to express the subgroup A envelope while maintaining the non-oncogenic character of RAV-0. To test the immunogenic potential of the recombinant, white Leghorn chickens were inoculated and subsequently challenged with ASV-A. Immunization significantly delayed sarcoma induction by ASV-A. When tumors were formed, significantly smaller volumes resulted.

D136 HUMAN HERPES SIMPLEX VIRUS (HSV) SPECIFIC T CELL CLONES ACTIVATED BY VACCINIA-gD RECOMBINANT VIRUS AND gB AND gD SUBUNIT VACCINES. J.M. Zarling[1], P.A. Moran[1], P.W. Berman[2], L.A. Lasky[2], C. Pachl[3], R.L. Burke[3], and B. Moss[4]. [1]Oncogen, Seattle, WA 98121; [2]Genentech Inc., So. San Francisco, CA 94080; [3]Chiron, Corp., Emeryville, CA 94608; and [4]Nat'l. Inst. of Health, Bethesda, MD 20205.

T cell mediated immunity (CMI) is important in resistance to herpes symplex virus (HSV) infections and therefore a subunit or recombinant virus vaccine should induce CMI against HSV; however, it was not known which HSV antigen(s) elicit CMI in man. We have found that HSV specific human cytotoxic T cell (CTL) clones, generated by stimulation of PBL with HSV-1, recognize HSV glycoproteins gB-1 or gD-1 that were cloned and then expressed in mammalian cells. HSV-1 specific and HSV-2 common CTL clones were also generated by stimulating PBL with purified gB-1 or gD-1 or with a vaccinia recombinant virus that expresses gD-1. The gD stimulated clones were found to lyse autologous target cells infected with the vaccinia-gD recombinant virus, more conclusively establishing that gD serves as a recognition structure for human CTL. These results, demonstrating that gB-1 and gD-1 play a role in T cell mediated immunity to HSV in humans, support the contention that gB or gD subunit or vaccinia-gD recombinant virus vaccines, which have recently been shown to protect guinea pigs or mice from HSV infections, may be of protective value in man.

Late Additions

D137 Ingemar Ernberg, Bengt Kallin, Joakim Dillner, Kerstin Falk, Annika Hedin, George Klein. Depr of Tumor Biology, Karolinska Institute, Box 60400, 10401 Stockholm, Sweden.

Expression and variation of Epstein-Barr virus (EBV) nuclear antigens in cell lines and after B-cell infection.

In Epstein-Barr virus (EBV) transformed immortalized B-lymphocytes four nuclear antigens have been detected by immunoblotting, with apparent molecular weights of 65-92 kD (EBNA 1), 70-85 kD (EBNA 2), 140-160 kD (EBNA 3) and 160-190 kD (EBNA 4). In tumor derived monoclonal cell lines EBNA 2 is missing in 60 % of the lines. This is either due to a deletion of the coding gene (LT-1) or to a variant sequence of the gene. The latter may encode a different as yet non-identified, non-immunogenic protein. We have investigated 27 Burkitt lymphoma derived cell lines for EBNA-2 expression , and 27 wild type virus isolates. All the latter showed an intact EBNA-2 "prototype" sequence and expressed the antigen. Burkitt derived virus isolates which carry the deletion are non-transforming, while those with the variant sequence may produce transforming virus.

All four antigens are detecltable early (24 h) after primary infection of B-lymphocytes, but EBNA-1 can be detected earliest already 8-10 hrs after infection.

We suggest that EBNA-2 has an important role in initiation of transformation of B-lymphocytes, based on these results.

D138 REMOVAL OF HLA DR ANTIGENS FROM HTLV III PREPARATIONS USING IMMUNOAFFINITY CHROMATOGRAPHY, L.O. Arthur*, J.W. Bess, Jr., C. Heffner Barrett*, W.G. Robey+, L.E. Henderson#, P.J. Fischinger+, and R.V. Gilden*, *Program Resources, Inc., National Cancer Institute-Frederick Cancer Research Facility (NCI-FCRF), #Litton Bionetics, Inc., NCI-FCRF, +Office of the Director, Virus Control Selection, NCI-FCRF, Frederick, MD 21701. Supported in part by Contract Number NO1-CO-23910 with Program Resources, Inc. and Contract Number NO1-CO-23909 with Litton Bionetics, Inc. Amino acid sequence analysis of proteins purified form sucrose gradient concentrated HTLV III revealed that proteins of approximately 32 and 38 kilodaltons (p32 and p38) are the α and β chains of HLA DR (Henderson, manuscript in preparation). We have prepared monoclonal antibodies to p32 and p38 which react with unique epitopes on each of the proteins as demonstrated by electroblot procedures. Immunoaffinity chromatography columns constructed with these HLA DR monoclonal antibodies specifically removed p32 and p38 from detergent disrupted HTLV III without affecting the levels of virus antigens. These virus preparations are being evaluated as immunogens in animal inoculation experiments and as antigens in serological screening assays. Serological assays are presently being used to detect the presence of antibody to HTLV III. HLA DR antigens are present in commercial kits used in screening for antibodies to HTLV III/LAV (Kuhnl et al., The Lancet, p. 1222, 1985). Since HLA antigens associated with HTLV III preparations may result in false positive reactions, removal of these contaminating cellular proteins may increase the specificity of HTLV III/LAV antibody assays.