ACQUIRED IMMUNE DEFICIENCY SYNDROME

Michael S. Gottlieb and Jerome E. Groopman, Organizers

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Plenary Sessions

Poster Sessions

Epidemiology of Acquired Immune Deficiency Syndrome

0001 EPIDEMIOLOGY OF AIDS IN EUROPE, P. Ebbesen, M. Melbye, Institute of Cancer Research, Aarhus, Denmark and R.J. Biggar, Division of Cancer Cause and Prevention, N.I.H., Bethesda, MD.

As of October 19, 1983 we are aware of 268 cases of AIDS in Europe[1] . Most occur in the largest Western European cities and only 2 cases are Eastern Europeans. Africans from the upper Congo Basin comprise a large group of those reported from France and Belgium. More than a third of African cases are females, only one female is of European origin.

Data on life style of homosexuals[2] resemble the US data and contact with US seems important for the European outbreak[3]. A relationship of the European epidemic to Central Africa is also possible. Early clinical symptoms in European cases resemble those seen in the US. It is unresolved if the clinical picture of Africans in Europe is distinct.

1) "AIDS in Europe" status quo 1983. Report of meeting cosponsored by The Danish Cancer Society, WHO and ECP, Aarhus, Denmark, October 1983. European Journal of Cancer and Clinical Oncology, in press.

2) Ebbesen, P, Melbye, M & Biggar RJ: Sex habits, drug use and recent disease in two groups of Danish male homosexuals. Arch Sex beh. 1983 in press.

3) Biggar, RJ, Melbye, M, Ebbesen, P, Mann, DL, Goedert, JJ, Weinstock, R, Strong DM & Blattner, WA: Epidemiologic evidence for a transmissible agent causing low T-lymphocyte ratios in homosexual men, JAMA 1983 in press

0002 SIMIAN ACQUIRED IMMUNE DEFICIENCY: NATURAL HISTORY AND EXPERIMENTAL TRANSMISSION, Murray B. Gardner, Roy V. Henrickson, Preston Marx, Kent Osborn, Don Maul, Nick Lerche, Martin Bryant, Primate Center and Department of Medical Pathology, University of California, Davis, Davis, California, 95616

Rhesus macaques at two different Primate Centers (UC Davis and New England) suffer from an acquired immune deficiency syndrome (SAIDS). The clinical, immunological and pathological features of SAIDS are remarkably similar to human AIDS. Several outbreaks of SAIDS have occurred over the past decade at the California Center. At both centers and at NIH (Dr. Sever) the simian disease has been experimentally transmitted to healthy rhesus monkeys, either by cage contact or tissue innoculation. The experimentally induced disease closely resembles the natural SAIDS but often exhibits a shorter latent period and more severe course with profound lymphoid depletion and death after only several months. Included among the infective inocula are lymph node extract, whole blood, serum and filtered plasma. Serial transmission with cell-free inocula suggests that the cause of SAIDS is a virus and that serum is a rich source of this agent. However, the virus has not yet been identified. Although highly prevalent, simian CMV is apparently not the primary etiologic agent. On-going efforts to isolate and characterize the causative agent(s) of this simian disease and to determine its natural history and possible relationship to human AIDS will be summarized.

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Lancet, 1:388-390, 1983.
2. Letvin, N.L., et al.
PNAS, 80:2718-2722, 1983.
3. Letvin, N.L., et al.
Lancet, II, 599-602, 1983.
4. London, W., et al.
Lancet, 80:869-872, 1983.
5. Gravell, M., et al.
Science, In Press

Immunology and Immunogenetics

0003 HUMAN T LYMPHOCYTES AND THEIR FUNCTIONAL SUBSETS, Edgar G. Engleman, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

Recognition of heterogeneity in lymphocyte populations has been achieved both through the discovery of selective expression of specific cell surface antigens on lymphoid cells and by findings of different functional properties of cells bearing these antigens. In particular, our understanding of immune regulation has benefited from investigations of two mutually exclusive populations of human T lymphocytes. Mature T cells which express either the Leu 3 (OKT 4) antigen or the Leu 2 (OKT8) antigen comprise the helper/inducer (T_{h+i}) subset or the suppressor/cytotoxic (T_{s+c}) subset, respectively. Upon activation with antigen or mitogen, T_{h+i} cells provide signals necessary for the differentiation of B lymphocytes into antibody secreting cells, and for the differentiation of Leu 2+ cells into mature T_s and T_c. Although T_{h+i} and T_{s+c} populations were thought initially to have nonoverlapping functions, recent studies suggest that the human Leu/OKT phenotype is primarily associated with the ability of the cell to recognize particular products of major histocompatibility complex (MHC) genes. Thus, the Leu 3 population includes cells that can either proliferate in response to MHC class II antigens (eg., HLA DR) and soluble antigens presented in association with class II determinants, or can lyse cells which express specific determinants on class II molecules. In contrast, Leu 2 T cells cannot proliferate in responsible to soluble antigens or class II determinants, but can differentiate under the influence of T_{h+i} cells into cytotoxic effector cells, usually with specificity for determinants on MHC class I (HLA A,B) molecules. Although cells within each major population may share a common MHC receptor, their functional heterogeneity suggests that either a given T cell has the potential of mediating several functions or, alternatively, that there are multiple subsets of T cells, each with a limited functional program. Recent investigations from our laboratory support the latter hypothesis and indicate the existence of multiple functionally distinct subsets within the two major T cell populations. For example, the T_{h+i} population can be divided with monoclonal anti-Leu 8 antibody into one subpopulation (Leu 3+,8-) that upon activation provides potent help to B cells but fails to activate suppressor T cells, and another subpopulation (Leu 3+, 8+) that activates suppressor cells but not B cells. More recently, the T_{s+c} population has been fractionated with another antibody, 9.3, into one subset (Leu 2+,9.3+) that contains all or nearly all precursors of class I MHC restricted cytotoxic cells and another subset (Leu 2+, 9.3-) that fails to kill. On the other hand, the Leu 2+,9.3- subset alone contains cells capable of antigen-specific suppression as well as cells inducible with concanavalin A or histamine into nonspecific suppressor cells. Thus, although the T cell population as a whole performs diverse regulatory and effector functions, individual T cells have limited functional repertoires that correlate with their surface phenotype.

0004 IMMUNOLOGIC ABNORMALITIES IN THE ACQUIRED IMMUNODEFICIENCY SYNDROME, Anthony S. Fauci and H. Clifford Lane, Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD 20205.

The acquired immunodeficiency syndrome (AIDS) is characterized by a profound defect in cell-mediated immune function. This is manifested in vivo by decreased delayed-type hypersensitivity responses, repeated opportunistic infections, and the appearance of neoplasms associated with the immunocompromised state (1). We have studied the scope of the immune defects in patients with AIDS and have delineated the precise nature of the defects of several limbs of the immune response. AIDS patients have a lymphocytopenia with a selective quantitative deficiency in the T4 inducer or helper subset of T cells. The absolute numbers of T8 suppressor/cytotoxic T cells are variable in AIDS patients and may be normal, slightly decreased, or increased. Healthy homosexual men generally have increased numbers of T8 cells in the face of normal T4 cells resulting in a reversal of the T4:T8 ratio. Of note is the fact that AIDS patients have a marked diminution of the T4:T8 ratio as a result of a selective decrease in T4 cells. T8 cells from AIDS patients function normally to suppress T cell-dependent B cell responses. In contrast, T4 cells from AIDS patients which have been highly purified to remove suppressor influences of T8 cells are markedly impaired in their ability to induce B cell function suggesting either a selective depletion of a T4 subset or a global functional impairment of T4 cells in general (2). B cell function in AIDS patients was also markedly abnormal in that they were polyclonally activated with very few, if any, cells in the resting (G_0) phase of the B cell cycle (2). AIDS patients were found to have elevated numbers of cells spontaneously secreting immunoglobulin. In addition, AIDS B cells were deficient in their proliferative responses to T cell-independent B cell mitogens. However, being in a pre-activated state, they responded directly to proliferative signals delivered by T cell-derived B cell growth factors, a characteristic of activated cells and not normal resting cells. The precise nature of this B cell hyperactivity is unclear at present but may represent an in vivo stimulation and/or transformation. Finally, natural killer cell activity and cell-mediated cytotoxicity against viral-infected targets were markedly deficient in AIDS patients, and these deficiencies could be reversed by in vitro incubation with interleukin 2 (3).

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2. Lane HC, Masur H, Edgar LC, Whalen G, Rook, A, Fauci AS: N Engl J Med 309: 453, 1983.
3. Rook AH, Masur H, Lane HC et al: J Clin Invest 72: 398, 1983.

0005 THE THYMUS:AIDS CONNECTION

Allan L. Goldstein, Ph.D. and Paul H. Naylor, Ph.D., Department of Biochemistry, The George Washington University School of Medicine, Washington, D.C. 20037

Acquired immune deficiency syndrome (AIDS) is associated with a progressive crippling of the body's thymic dependent immune system. The profound immunologic abnormalities seen in AIDS are strikingly similar to those found in children with primary immunodeficiency diseases and a number of other diseases associated with thymic malfunction. Significant abnormalities in the thymus of AIDS patients at autopsy have also been observed. In view of the role of the thymus as the master gland of immunity and in light of the evidence to date, a connection between the thymus and AIDS must be considered.

We have demonstrated that thymosin α_1, a hormone of the thymus that is active in mediating T-cell function, is abnormally high in individuals with AIDS. Elevated levels of α_1 (>2 std deviations from the mean) have now also been demonstrated in various at risk groups, including homosexuals, Haitians, hemophiliacs, and children with AIDS, who have received blood transfusions or whose parents are IV drug users and/or bisexuals. In keeping with the viral hypothesis for AIDS and in view of the propensity of the thymus and it's epithelial stroma to house and nurture a variety of viruses, at least three hypotheses could account for the abnormalities of the thymus and the elevated thymosin α_1: 1) end organ (lymphocyte) failure causing abnormal feedback control of α_1 levels, 2) viral invasion of the thymus and the release of viral modified α_1, or 3) production of α_1, and/or an α_1 releasing factor at a non-thymic site due to the infectious agent.

The recent observations that HTLV may be linked to AIDS is especially relevant since:
1) the p19 viral protein of HTLV shares a common determinant with thymic epithelia and;
2) thymosin α_1 is elevated in many HTLV positive individuals.

Since AIDS and pre-AIDS are accompanied by thymus dependent immune deficiencies, a direct link between changes in α_1 and future onset of disease could have significant diagnostic and clinical implications. The α_1 assay may provide a means of early identification of: 1) asymptomatic carriers of AIDS, and; 2) individuals who may benefit from early immune modulation by thymosin or other thymomimetic agents that have the ability to increase immunity.

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Biggar, R.J., et al. N. England J. of Medicine, 309:1, p. 49, 1983.
Naylor, P.H. and Goldstein, A.L., Clinical Immunology Newsletter, 4:9, 126-128, 1983.

0006 IMMUNOLOGICAL STUDIES IN SYMPTOM FREE HOMOSEXUALS, PATIENTS WITH THE AIDS RELATED COMPLEX (ARC) AND PATIENTS WITH ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

by E.M. Hersh, J.M. Reuben, G.R. Newell, A. Rios, J.U. Gutterman, S. Spector and P.W.A. Mansell, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

We have studied 135 homosexual patients immunologically, 20% of whom were symptom free (SF), 60% of whom had ARC and 20% of whom had Kaposi's sarcoma (KS), opportunistic infection (OI), or both and were thus classified as having AIDS. A patient with ARC is defined as one with at least two of the AIDS related symptoms and at least two of the AIDS related immunological abnormalities. The overall results of these studies indicated that as patients progress from SF to ARC to AIDS, the majority of immunological parameters become progressively more impaired. Thus, delayed type hypersensitivity was diminished in ARC and AIDS patients, but not in SF patients. T-11 and T-3 surface markers were reduced mainly in AIDS patients, T-4 surface markers were impaired in all three groups but declined progressively as one moved from SF to AIDS. T-8 levels were neither decreased nor increased significantly in any of the groups. This resulted in a helper:suppressor ratio decline from 1.85 in normals to 1.1 in SF to 0.90 in ARC to 0.54 in AIDS. The TAC receptor expression on peripheral blood lymphocytes stimulated with PHA or PWM was reduced about 50% in AIDS patients. Lymphocyte blastogenic responses to PHA, PWM and CON-A were reduced approximately the same in all three groups. NK cell activity and ADCC were not diminished. The thymosin α_1 level was approximately three fold increased in all three groups. Serum lysozyme was increased in ARC and AIDS but not in SF patients. Serum levels of alpha interferon were detected in AIDS patients only. Monocyte adherence declined progressively from SF through AIDS. IL-2 production and response were decreased in some but not all patients at all stages. In vitro, alpha interferon production in response to HSV was diminished in all groups. In vitro culture with HSV also failed to activate NK cells as it did in cultures of cells from normals. In contrast, HSV induced a proliferative response in vitro in both normals and AIDS. In vitro studies of immunorestoration with isoprinosine, azimexon, and interleukin-2 indicated that these may be useful immunorestorative agents in vivo. The overall results indicate that early parameters of immunodeficiency are detectable in SF and ARC patients which may be useful markers of subjects who would be candidates for immunorestorative immunotherapy in vivo. These parameters may also have major prognostic value.

0007 REDUCED PRODUCTION OF ALPHA INTERFERON BY MONONUCLEAR CELLS FROM PATIENTS WITH AIDS IS CLOSELY ASSOCIATED WITH THE DEVELOPMENT OF OPPORTUNISTIC INFECTIONS (OI), Frederick P. Siegal, Carlos Lopez, Patricia Fitzgerald and Sheldon Landesman, Mount Sinai School of Medicine/CUNY, New York, NY 10129, Sloan-Kettering Institute, New York, NY 10021 and SUNY/Downstate Medical Center, Brooklyn, NY 11203.

Analysis of delayed hypersensitivity, lymphocyte number, T cell subset proportion and number (by monoclonal antibodies), mitogen response and NK cell activity fail to show strong associations with risk of developing OI in AIDS and related syndromes. We found consistent depression of alpha interferon (IFN) production when mononuclear cells of AIDS patients with OI were cultured for 14 hours in the presence of Herpes simplex-infected human fibroblasts. In contrast, cells of patients with Kaposi's sarcoma (KS) without OI usually made amounts of IFN that were comparable to that produced by normal laboratory controls and healthy homosexuals (1). Serial studies in homosexual males and accession of patient material from additional high risk groups have now been done. Laboratory controls ($n = 159$) produced IFN regularly. Cells from 45 homosexual men with OI, 18 with KS + OI and 12 with AIDS-related complex (ARC), 8 children with OI, 5 others with OI (hemophilia, transfusion-associated, no-known-risk factor) and 13/15 Haitians with OI produced diminished amounts of IFN (>2SD below the normal geometric mean), and as a group differed significantly ($p<0.001$) from controls. Two Haitians whose OI (toxoplasmosis) was limited to the central nervous system were normal. Six of 12 with ARC subsequently developed OI (up to 15 months later). In contrast, 21/23 homosexuals with KS without OI, 27/28 with generalized lymphadenopathy and 17/18 without apparent disease; 9 Haitians with localized tuberculosis, 5 with lymphadenopathy and 3 without disease; 11 AIDS contacts and 7 infection controls made IFN within the normal range. These studies indicate close association between failure to produce IFN and propensity to develop OI. Our finding that IFN production is normal in patients with KS who do not develop OI suggests that the processes leading to OI may be intrinsically different from or more advanced than those associated with KS, and separates these observations from those of elevated serum levels of acid-labile IFN seen in both clinical states (2).

(1) Lopez, Fitzgerald, Siegal, J. Inf. Dis., 1983, in press.
(2) Buimovici-Klein, Lange, Klein, Lancet 2:344, 1983.

T Lymphotropic Viruses In Acquired Immune Deficiency Syndrome

0008 CHARACTERIZATION OF A NEW TYPE OF RETROVIRUS ISOLATED FROM PATIENTS WITH AIDS AND FROM PATIENTS AT RISK OF AIDS, J.C. Chermann[1], F. Barré-Sinoussi[1], C. Dauguet[1], F. Brun[2], C. Rouzioux[2], W. Rozenbaum[3], D. Klatzmann[3], J.C. Gluckman[3], E. Vilmer[4], C. Griscelli[4] & L. Montagnier[1]. [1]Institut Pasteur, Département de Virologie, 75015 Paris - [2]Hôpital Claude Bernard, Laboratoire Central-Virologie, 75019 Paris - [3]Hôpital La Pitié-Salpêtrière, Département de Santé Publique et Médecine Tropicale, 75013 Paris - [4]Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75730 Paris Cédex 15.

We have previously reported the isolation of lymphotropic retroviruses from cultured T lymphocytes of 1/ a patient presenting with lymhadenopathy (1) and 2/ of two patients with AIDS, an homosexual man with Kaposi Sarcoma and a B haemophiliac boy (2). These viruses have been characterized as retroviruses on the following criteria : a) Mg^{++} dependent reverse transcriptase activity ; b) density of 1.16 in sucrose gradient ; c) morphology by budding at the cell surface ; d) high molecular weight RNA component.
The major core proteins of the three isolates (P25) are antigenically related, but are not related to the P24 protein of HTLV1. Moreover, the morphology of mature particles ressembles that of D types particles and more closely to that of Equine Infectious Anemia retrovirus. Indeed, some immunological cross-reactivity has been found between the P25 protein of the human viruses and the equivalent protein of the horse virus.
The new virus grows specifically on the $OKT4^+$ subset of human T lymphocytes and their medullary precursors without giving rise to established (immortalized) cell lines. As for other retroviruses, virus production is tightly coupled to cell activation and multiplication.
A sero-epidemiological survey for antibodies against the specific group P25 protein has been started, using radio-immune binding and a specific ELISA test. We have compared the incidence of antibodies in patients with AIDS, patients with lymphadenopathy, healthy homosexuals, haemophiliacs, and a control group of the french population.
Preliminary results indicated a high prevalence of antibodies in patients with lymphadenopathy (63%), a very low incidence in the control group (laboratory workers and blood donors : 2%) and intermediate values in the other groups. The decrease of antibodies positive cases in patients with advanced AIDS may be explained by the alteration of humoral immunity at the end of the disease.
A model for pathogeny of AIDS based on primary infection of T lymphocytes with such lymphotropic retroviruses and involving the need of non specific antigenic stimuli for virus spreading will be presented.

(1) Science 220, 1983, 868-871.
(2) The Cancer Cell 3, Cold Spring Harbor (1983) in press.

DNA Viruses In Acquired Immune Deficiency Syndrome

0009 ONCOGENIC TRANSFORMATION BY HERPESVIRUSES, Denise A. Galloway, Jay A. Nelson, and James K. McDougall, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104.

There are many suggestions that herpesvirus are in some way associated with human malignancies. Most frequently cited is the association of Epstein Barr virus (EBV) with Burkitt's lymphoma and nasopharyngeal carcinoma; herpes simplex virus type 2 (HSV-2) with cervical carcinoma; and cytomegalovirus (CMV) with Kaposi's sarcoma. In general the association has been based on seroepidemiological data and finding viral antigens and nucleic acids in tumors. An important question is to define the oncogenic potential of these viruses. We have chosen to study HSV-2 and CMV in detail, to precisely define the viral sequences that are capable of transforming rodent cells to a malignant phenotype in vitro. These experiments have identified a single fragment of HSV-2 DNA (1,2) and a single fragment of CMV DNA (3,4) with transforming activity. In both cases the viral fragments do not appear to encode a protein suggesting a novel mechanism of transformation not previously described for DNA tumor viruses must occur. A complete molecular description of the transforming fragments will be presented as well as possible models to explain their action.

(1) Galloway, D.A. and J.K. McDougall J. Virol. 42:530-537 (1982)
(2) Galloway, D.A. and J.K. McDougall Nature 302:21-24 (1983)
(3) Nelson, J.A., B. Fleckenstein, D.A. Galloway, J.K. McDougall. J. Virol. 43:550-557 (1982)
(4) Nelson, J.A., B. Fleckenstein, G. Jahn, D.A. Galloway, and J.K. McDougall. J. Virol. (in press) (1984)

0010 CYTOMEGALOVIRUS IN ACQUIRED IMMUNE DEFICIENCY SYNDROME.

W. Lawrence Drew, M.D., Ph.D., Lawrence Mintz, M.D., Richard Miner, Research Associate, Department of Pathology and Laboratory Medicine and Medicine, Mount Zion Hospital and Medical Center, San Francisco, CA 94120. John Ziegler, M.D., Veterans Administration Medical Center, San Francisco, CA 94121. Eng-Shang Huang, M.D., Department of Medicine and Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

The evidence that CMV may contribute to AIDS is:

1. CMV infection is highly prevalent among homosexual men(1).
2. CMV infection is immunosuppressive(2) and causes abnormal T-cell subset ratios(3).
3. CMV is transmitted by blood(4) and semen(5,6).
4. Homosexual men appear to experience multiple episodes of CMV infection as manifested by a high prevalence of IgM antibody(6).
5. Of 164 AIDS patients (including homosexual men, IV drug users and hemophiliacs) tested for CMV antibody, 162 were positive(7).
6. CMV antigen has been detected in 15/27 Kaposi's sarcoma biopsies while cultures have been negative in all but one instance. In addition, normal skin adjoining the tumor or on the opposite extremity has been negative for antigen in 21/22 patients studied. Three of four tumor biopsies have been positive for CMV RNA while virus culture was negative(8).

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7. CDC, preliminary results.
8. Drew WL, Miner RC, Ziegler JL, Gullett JH, Abrams DI, Conant MA, Huang ES, Groundwater JR, Volberding P, Mintz L. Cytomegalovirus and Kaposi's Sarcoma in Young Homosexual Men. Lancet 2:125-127, 1982.

0011 CHARACTERIZATION OF ADENOVIRUS ISOLATES FROM AIDS PATIENTS. Marshall S. Horwitz, Graciela Valderrama, Virgil Hatcher, Ronald Korn and Ilya Spigland, Albert Einstein College of Medicine, Bronx, New York

Adenoviruses (Ad) have been isolated from the urines of 23 patients with AIDS at various stages of illness, including the prodrome. Restriction endonuclease analysis of the viral DNA by the enzyme SmaI has demonstrated that 21 of these isolates were either identical or closely related to Ad type 35. However, serologic characterization by hemagglutination inhibition has shown that these viruses could contain other group B Ad hemagglutinins (HA). Types 7, 11 or 34 HA have been found in addition or in place of the Ad35 HA. Small differences in restriction endonuclease patterns between the Ad35 prototype viral DNA and the AIDS isolates have been demonstrated by the use of another restriction endonuclease, HpaI. Since HpaI cleaves Ad7 but not Ad35 DNA in the gene coding for the HA polypeptide, it has been possible to use HpaI to determine those AIDS isolates that contain domains of the Ad7 HA. These restriction endonuclease and serologic findings can be best explained by recombinational events between a major segment of the Ad35 genome and a short region (approximately 10%) from other types of Group B Ads. We have isolated Ad35 from AIDS patients in New York, Los Angeles, San Diego and from a Zambian woman who died in London. Except for one patient with acute leukemia who underwent bone marrow transplantation, we have not found Ad35 in any control patient without AIDS. Restriction endonuclease fragments of Ad35 have been cloned in prokaryotic vectors so that selected DNA segments can be used to probe AIDS lymphocytes and tissue for residual viral DNA sequences.

0012 IMMUNOREGULATORY DEFECTS IN HEMOPHILIA, JL Sullivan, SH Cheeseman*, DB Brettler*, PH Levine**, Departments of Pediatrics and Medicine, UMass. Med. Schl., Worcester, MA.

Acquired immune deficiency syndrome (AIDS) has been reported in the hemophilia population. We have studied 100 hemophiliacs for serological evidence of Epstein-Barr Virus (EBV), cytomegalovirus (CMV), and hepatitis B virus (HBV) infection.

Age	N	% Seropositive EBV	CMV	HBV
10	15	47	20	100
10-29	54	61	24	96
30-60	31	94	84	100
Total	100	68	42	98

Four patients had chronic hepatitis B antigenemia. Age related acquisition of EBV and CMV antibody was not different from normal controls, however, 70% of hemophiliacs had serological patterns consistent with persistent or reactivation EBV infection (viral capsid antibody 1:320 or early antigen antibody 1:40). Only 25% of young adult controls showed similar antibody patterns. Cell mediated immunity (CMI) was investigated in a subset of hemophiliacs. OKT.4/OKT.8 ratios were significantly decreased in hemophiliacs ($1.36\pm.13$) compared to controls ($1.81\pm.11$). Significant depression of Con A, MLR, and natural killer cell function was observed in hemophiliacs. The few patients studied without evidence of persistent viral infection have normal T cell subset ratios and CMI. Those hemophiliacs with evidence of EBV and CMV infection or persistent hepatitis B antigenemia have marked depression of OKT.4/OKT.8 ratios and depressed CMI. These results suggest that defects in immunoregulation observed in patients with hemophilia may be due to chronic virus infection and might be a predisposing factor in the development of AIDS.

Kaposi's Sarcoma: Biology and Therapy

0013 KAPOSI'S SARCOMA AND AIDS: J. Groopman, Department of Medicine, New England Deaconess Hospital, Harvard Medical School, Boston, MA 02215 and R. Mitsuyasu, Dept. Medicine, UCLA Med Ctr, Los Angeles, CA90024. Kaposi's sarcoma (KS) is occurring in an epidemic fashion in association with the acquired immunodeficiency syndrome (AIDS). KS has been previously described in elderly Caucasian men of Mediterranean or Ashkenazic Jewish origin and in young Central African males. There is seroepidemiologic data supporting an association of Western KS with cytomegalovirus (CMV) infection. KS also occurs in renal transplant patients who are immunosuppressed on an iatrogenic basis. The neoplasm has been reported to spontaneously regress in such transplant patients upon discontinuation of the immunosuppressive therapy. This close relationship between cellular immunity, CMV infection, and KS is seen in AIDS as well. The occurrence of KS in the subset of AIDS patients made up of homosexual men predominantly may relate to the high prevalence of CMV in this population. A cohort of 80 AIDS patients with KS has been extensively studied. The median age of 35 years, predominance of Caucasians (75%) and sexual preference (95% homosexual or bisexual) are similar to that reported nationwide to the Centers for Disease Control. The clinical features of KS in this cohort are variable, with 18% having limited cutaneous involvement and 8% visceral KS without skin lesions. Over half the patients had gastrointestinal KS. Ten percent of this cohort had a normal ratio of phenotypic helper to suppressor T-lymphocytes. Ninety percent of patients had elevated circulating immune complexes. Hypergammaglobulinemia was seen in 85% of patients. Ten percent of KS patients with AIDS were seronegative (less than 1:8) by complement fixation for CMV. 93% of this cohort was Epstein-Barr virus seropositive. All patients had evidence of prior or current infection with hepatitis B virus. Semen was positive for CMV in 76% of patients. Only one patient of 40 tested had antibody to the core protein of human T-cell leukemia virus. Proviral sequences of this latter virus were not detected in peripheral blood mononuclear cells or sperm from this seropositive patient. Several KS lesions appeared to contain DNA that hybridized to specific CMV probes (D. Spector et al: unpublished data). The occurrence of KS in AIDS demonstrates the complex relationships among the immune system, ubiquitous DNA and RNA viruses, and the genesis of certain neoplasms.

0014 ALPHA-INTERFERON THERAPY OF KAPOSI'S SARCOMA IN AIDS, Paul Volberding M.D., Department of Medicine, University of California, San Francisco, San Francisco General Hospital, San Francisco, California 94110

The chemotherapy of Kaposi's sarcoma (KS) in the Acquired Immune Deficiency Syndrome (AIDS) has been limited by incomplete response, early relapse, and especially by a high rate of intercurrent opportunistic infection.[1,2] Because chemotherapy itself may further impair the host immune response, immunomodulatory drugs are of interest. We have treated KS in AIDS with recombinant alpha-interferon (IFN) (Schering Corporation) because of its antiviral, cytotoxic and immune modulating activity.[3] Dose and routes of administration have included $1x10^6$ units SQ (10 patients); $30x10^6$ units SQ (30 patients); $50x10^6$ units IV (30 patients). The toxicity of IFN is minimal at the lowest dose. In the higher doses, subjective side effects (flu-like syndrome) were common and occasionally dose-limiting. Objective toxicity included transaminase elevations and neutropenia. Although these side effects were usually mild and transient (mean nadir WBC=$2.8x10^3$, peak SGOT=98), three patients experienced SGOT greater than 1,000. Objective responses were more common at the higher doses; 4/10 low dose SQ, 13/30 high dose IV, 9/23 high dose SQ. The overall duration of response is greater than 6 months and duration of survival is greater than 8 months. Opportunistic infections have been seen in 20 patients. No evidence of immunorestoration has been seen. IFN can be effective in KS in AIDS and is well tolerated by most patients. Further study of optimal dose and schedule are indicated.

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0015 ASSOCIATION OF HUMAN CYTOMEGALOVIRUS WITH KAPOSI'S SARCOMA. Deborah H. Spector[1], Sydney B. Shaw[1], Lisa J. Hock[1], Donald Abrams[2], and Michael S. Gottlieb[3]. [1]University of California, San Diego, La Jolla CA 92093; [2]University of California, San Francisco, San Francisco, CA 94143; [3]University of California, Los Angeles, Los Angeles, CA 90024.

Until recently Kaposi's sarcoma was a relatively rare tumor found mainly in equatorial African Blacks, in older men of eastern European descent, and in renal allograft patients receiving immunosuppressive drugs. In the last two years there has been a major increase in the incidence of Kaposi's sarcoma primarily in homosexual men with Acquired Immunodeficiency Syndrome (AIDS). Early epidemiologic studies suggested an infectious origin for this tumor and human cytomegalovirus (HCMV) in particular has come under suspicion as being associated with the tumor. Although some hybridization experiments performed in the past have suggested that HCMV genetic information might be associated with Kaposi's sarcoma, there was a question of whether the whole genome was present or only a fraction. In addition, the presence of cell-related DNA sequences in the HCMV genome complicates the interpretation of experiments in which whole virion DNA or cRNA was used as the hybridization probe to detect HCMV-specific sequences. We have examined Kaposi's sarcoma specimens from patients with AIDS for the presence of HCMV DNA using our set of cloned and well characterized subgenomic fragments of HCMV strain AD169 as the hybridization probes. All of the cloned HCMV fragments except EcoRI fragment h hybridize to the DNA of clinical isolates of HCMV and can thus be used to screen patients' specimens for HCMV-related nucleic acid. For these experiments the cloned fragments of HCMV DNA were hybridized individually to Southern blots containing EcoRI digests of DNA extracted from Kaposi sarcoma tissues. Southern blot analysis distinguishes between hybridization to viral sequences and hybridization to normal cellular sequences. In cases where the patient's HCMV isolate is available, these analyses also provide information on which viral sequences are present and whether these sequences are rearranged or integrated into the host genome. The results of our experiments indicated that HCMV sequences were present in Kaposi sarcoma samples at a level which varied from 1 copy/5 cells to less than 1 copy/50 cells. For one patient with Kaposi's sarcoma, HCMV DNA was also detected in uninvolved skin and lung specimens but not in samples of liver, spleen, heart, testis, lymph node, or bone marrow. In situ cytohybridization is necessary to determine precisely which cells the HCMV resides in, and such studies are in progress. We have not detected any evidence indicating selective retention, amplification or integration of specific HCMV fragments in the specimens. Whether HCMV plays a role in the etiology of this tumor or is merely a fortuitous passenger virus remains a critical question yet to be answered.

0016 DNA VIRUS STUDIES IN AIDS AND KAPOSI'S SARCOMA, James K. McDougall, Jay A. Nelson, Patricia P. Smith, David Myerson, and Denise A. Galloway. Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104.

We are studying the distribution of CMV, HSV and HPV in a number of humor tumors, using cytological hybridization methods as well as blotting procedures. In the AIDS and Kaposi's sarcoma tissues particular emphasis is placed upon CMV. The oncogenicity of CMV has been established in experimental systems (1): the virus has been associated with human tumor cells (2) and there are links between CMV infection, virus reactivation and immunosuppression (3). Cytological hybridization using ^{3}H- or biotin-labeled cloned CMV DNA probes has detected CMV DNA and RNA in biopsies of Kaposi's sarcoma and we have found CMV DNA in high molecular weight DNA isolated from tumors in AIDS patients. Studies are in progress to determine the extent and state of the virus genome present. We are also examining tissue samples by electron microscopy to determine the prevalence of unusual cellular structures, e.g., tubuloreticular structures (4) and rosettes (5), which may be related to virus infection.

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0017 AUTOPSY FINDINGS IN THE ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS). Edward A. Smuckler, MD, Kevin L. Welch, MD, Walter E. Finkbeiner, MD, Walter Blumenfeld, MD, Charles E. Alpers, MD, Richard L. Davis, MD, and Jay H. Beckstead, MD, Department of Pathology, University of California, San Francisco, CA 94143.
The medical records, pre-mortem biopsies, and autopsy materials from 34 patients with AIDS were reviewed. All cases met the CDC criteria of biopsy-confirmed Kaposi's sarcoma or life-threatening opportunistic infections in previously healthy (no underlying immunosuppressive condition) persons who are under 60 years. This study includes 40% of all reported AIDS deaths in the Bay Area. The majority of the patients were adult males, 31 of whom had homosexual experience. Also included were 2 adult males (one Haitian and one Caucasian) who denied homosexual experience, a 23-month-old male who developed AIDS after transfusion of blood products, and a 7-month old female. Widespread Kaposi's sarcoma was seen in 8 patients, while tumor was confined to the skin in an additional 11. Four patients had high grade lymphomas. Thirty-three patients had opportunistic infections at some point in the course of their illness. These included: Cytomegalovirus (65%), Pneumocystis carinii (62%), acid fast bacilli (26%), Cryptosporidiosis (12%), Candida albicans (38%), Cryptococcus neoformans (9%), Giardia (6%), Herpes simplex (29%), Entamoeba histolytica (3%), and Histoplasmosis (3%). Many patients had multiple infectious agents. The most consistent and striking autopsy finding was a severe depletion of the lymphoid tissues. Death in the majority (91%) of the patients was attributable to opportunistic infections, most commonly respiratory illness (71%) and meningitis (12%). Three patients (9%) died with widely metastatic Kaposi's sarcoma and secondary hemorrhage.

Opportunistic Infections

0018 **INFECTIOUS COMPLICATIONS IN ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)**
R.G. Douglas, Jr., M.D., R.B. Roberts, M.D., P. Romano, M.D., C. Metroka, M.D., J. Amberson, M.D., R. Soave, M.D., B. Hartman, M.D.Department of Medicine, Cornell University Medical College and The New York Hospital, New York, NY 10021

To put the infectious complications of AIDS in perspective, we reviewed the experience at one medical center. As of May 1983, 63 patients with AIDS and one or more opportunistic infections had been admitted to The New York Hospital -Cornell Medical Center. Patients with Kaposi's sarcoma without opportunistic infection are omitted from this analysis. The diagnosis of AIDS was made by standard CDC criteria. The most frequent opportunistic infection detected was Pneumocystis carinii pneumonia which occurred in 44 patients. Twenty-nine had mucosal candidiasis, 23 disseminated cytomegalovirus infection, 12 disseminated mycobacterium avium-intracellulare infection and 11 herpes simplex perianal ulcerations. Smaller numbers of patients had the following complications: Salmonella bacteremia (3), herpes zoster (3), progressive multifocal leukoencephalopathy (1), disseminated candidiasis (3), disseminated cryptococcosis (5), cryptosporidium enteritis (5) and CNS toxoplasmosis (6). We continue to see 1-2 new patients per week and the distribution of infectious complications remain similar. The data will be updated to January 1984. Clinical presentations, clinical course, treatment and complications will be discussed.

0019 PNEUMOCYSTIS CARINII: BIOLOGY AND MODE OF TRANSMISSION, Walter T. Hughes, Division of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38101.

A taxonomic position for P. carinii has not been established but some features resemble protozoa of the Sporozoa class. While morphologic, tinctorial and ultrastructure characteristics are known, the molecular biology of the organism has not been investigated. Developmental forms include a thick-walled cyst, an intracystic sporozoite and a thin-walled extracystic trophozoite. The small trophozoite (trophic stage) has a nucleus, mitochondria, endoplasmic reticulum and vacuolar spaces. The two-layered cell wall measures 20-30 nm. The inner layer is a plasmalemma composed of trilaminar structure of unit membrane. This stage of P. carinii is characterized by cytoplasmic projections referred to as filopodia or microtubular extensions. The large trophozoite (precyst stage) is oval with few filopodia. The cytoplasm contains ribosomes and glycogen granules as well as mitochondria. The nucleus is seldom seen in this phase. The cell wall is thick and measures 100 nm. The cyst form contains up to eight intracystic structures (sporozoites) which are nucleated. It has been suggested that endocytosis is the mode of macromolecular uptake of P. carinii (1). The organism attaches tightly to host alveolar type 1 cells without discernible changes in the cell membrane of the host or parasite.

P. carinii can be propagated in chick embryonic epithelial lung cells. Isotope labelling studies show that active DNA, RNA and protein synthesis occurs and suggest an interaction between host and parasite. In the reproductive cycle a vegetative cell (trophozoite) attaches by microtubular extension to the host cell, probably for the transport of essential nutrients, and then detaches without entering the cell. The intracystic sporozoites develop within the detached cyst. Excystment occurs through single or multiple breaks in the cyst wall, after which the released trophozoite (trophic stage) develops and attaches to another host cell (2). Vero, Chang liver, MRC 5 and WI 38 cell lines have also been reported to support in vitro propagation of P. carinii.

The only mode of acquisition that has been proven is animal-to-animal transmission by the airborne route. Infection by the oral or parenteral routes has not occurred when attempted in animal models (2,3).

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0020 CRYPTOSPORIDIUM AND CRYPTOSPORIDIOSIS, William L. Current, Department of Zoology-Entomology, Auburn University, AL 36849

Protozoans of the genus Cryptosporidium (Phylum Apicomplexa; Suborder Eimeriorina) are small (2 to 6 μm) coccidian parasites that inhabit the microvillous region of epithelial cells of a variety of animals, including man (1). Until recently, Cryptosporidium infection was considered rare in most animals, and in man it was thought to be the result of a little-known opportunistic pathogen outside its normal host range (2). Our concept of cryptosporidiosis has changed within the past several years to that of an important cause of gastroenteritis and diarrhea in several animal species, especially calves, lambs, goats and humans (1). In immunocompetent humans, Cryptosporidium may produce a short-term, flu-like, gastrointestinal illness (3,4). This contrasts sharply with the prolonged, severe diarrhea in immune deficient individuals who contract cryptosporidiosis (3,5), especially those with AIDS. In immune deficient patients with cryptosporidiosis, fluid loss may be extensive and uncontrollable; 3 to 6 liters per day is common, and as much as 17 liters per day of watery stool has been reported (5). The finding of cryptosporidiosis in immune deficient patients usually carries an ominous prognosis since a variety of therapies have proven uniformly unsuccessful in arresting this disease (5). Recent reports of respiratory (6) and biliary (7) infections demonstrate that Cryptosporidium is not confined to the gastrointestinal tract of immune deficient persons. Experimental infections in farm and laboratory animals have clearly established that Cryptosporidium has little or no host specificity, and that it is transmitted by ingestion of oocysts which are fully sporulated and infective at the time they are passed in the feces (1,3,4). Our recent studies have shown that calves and perhaps companion animals such as kittens, puppies,and rodents serve as potential sources of human infection (3,4), and that human-to-human transmission may occur (8). Thus, cryptosporidiosis has joined the list of more than 150 zoonoses; those diseases, the agents of which are naturally transmitted between other vertebrate animals and man (9). The role of cryptosporidiosis as an etiology of diarrheal illness will be discussed and recent developments concerning the diagnosis, life cycle, and in vitro cultivation of Cryptosporidium will be presented (10,11).

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0021 TOXOPLASMOSIS IN PATIENTS WITH AIDS, Jack S. Remington, Benjamin J. Luft, Robert E. McCabe, Research Institute, Palo Alto Medical Foundation and Stanford Medical Center, Palo Alto, CA 94305.

Until recently, acquired toxoplasmic encephalitis (TE) was restricted to immunocompetent patients. In the past 2 years we have studied serum or tissue specimens from 75 patients with AIDS and TE. Protean manifestations, atypical antibody response and lack of clear histopathologic findings on routine stains hampered diagnosis (1). Signs and symptoms included fever, chills, lethargy, headache, seizures, and depressed mental status. Fifty cases had various serologic tests performed to detect Toxoplasma antibody. 71% (32/45) had Dye test (DT) titers $\leq$1:512; 60% (15/25) had IgG ELISA titers $\leq$1:512; 60% (15/25) had IHA titers $\leq$1:512. Whereas only 27% (7/26) had agglutination (TA) (2) titers $\leq$1:512, 62% (16/26) had TA titers $\geq$1:4096. Elevated TA titers were useful in detecting TE in this population, especially in patients with relatively low DT titers ($\leq$1:512). 13 (76%) of 17 AIDS patients and DT titer $\leq$1:1024 had TA/DT ratios of $\geq$20. None (0%) of 11 immunocompetent patients with comparable DT had TA/DT ratios of $\geq$20; 10(91%) had TA/DT ratios of <10. 13 (72%) of 18 AIDS patients with TE and any DT titer and 4 (20%) of 20 immunocompetent patients with DT titer >1:1024 had TA/DT ratios of $\geq$20. Only 2 (4%) of 50 AIDS patients with TE had IgM antibody and only 4 (24%)of 17 patients had significant rises in serial antibody titers. TA is unique in its ability to detect high levels of Toxoplasma antibodies and may prove valuable as an indicator of TE in AIDS patients. CSF from 8 patients often showed high protein, hypoglycorrha chia and lymphocytic or neutrophilic pleocytosis. The DT was positive in CSF in 10 (67%) of 15 TE AIDS patients. Histologic specimens from 35 TE patients revealed a profoundly destructive, usually well demarcated, necrotizing process. The necrotic center was surrounded by neutrophils, histiocytes and plasma cells. Tissue cysts were rare. Tachyzoites were difficult to identify by routine stains but were demonstrable by the peroxidase anti-peroxidase (PAP) method (3). The PAP method revealed large numbers of tachyzoites and amorphous material which stained positively for Toxoplasma antigens. T. gondii was also identified in the myocardium, lung, stomach, intestine, adrenal glands and pancreas. TE and disseminated toxoplasmosis has recently been occurring in epidemic proportions. This diagnosis must be considered in all AIDS patients with neurologic abnormalities. Particular serologic tests (TA) and immunohistologic (PAP) stains may be invaluable in establishing the diagnosis of TE in these patients.

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Immunologic and Antiviral Therapy of Acquired Immune Deficiency Syndrome

0022 TOXICITY, HALF LIFE AND IMMUNE EFFECTS OF PURIFIED JURKAT DERIVED INTERLEUKIN 2 (IL-2) IN PATIENTS WITH CANCER AND AIDS, Michael T. Lotze, Richard J. Robb°, Lesley Frana, Claudia A. Seipp, Susan O. Sharrow+, Alfred E. Chang and Steven A. Rosenberg, Surgery Branch, Immunology Branch+, National Cancer Institute, NIH, Bethesda, MD 20205 and Glenolden Laboratory°, E.I. duPont, Glenolden, PA 19036.

A total of ten patients, five with the acquired immunodeficiency syndrome, have been treated with Jurkat derived IL-2, purified to homogeneity by affinity chromatography. Patients received 0.25 µg/kg or 2.5 µg/kg by bolus or 24-hour infusion once weekly for four weeks. Two patients received four single doses of 1 mg by bolus injection. The half life of Jurkat derived IL-2 in vivo in patients with AIDS and non-AIDS cancer patients was approximately 7 minutes. This compares with the 3.7 + 0.8 min. half life of murine IL-2 in mice (Donohue and Rosenberg, 1983)[1] and that of human PBL derived IL-2 of 22.5 minutes in humans (Bindon et al., 1983)[2]. IL-2 activity could be detected for up to 4 hours following bolus infusion of the 1 mg dose. Toxicity appeared to be dose related and consisted of transient fever and chills beginning approximately two hours following infusion. No renal, hepatic or hematologic toxicity was seen. Serial assays of T cell phenotype, immunologic response to mitogens and cytotoxicity were performed. No consistent changes in response to phytohemagglutinin, phorbol myristic acetate, IL-2, staph A cowan or keyhole limpet hemacyanin could be demonstrated. Cytotoxicity for fresh human tumor targets could not be demonstrated. No beneficial clinical effects were observed in any of the cancer or AIDS patients.

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Epidemiology and Immunology

0023 IMMUNODEFICIENCY SYNDROME IN CELEBES APES (MACACA NIGRA), Stanley M. Shiigi, Billie J. Wilson, Denis R. Burger, Arthur Malley, Charles F. Howard, Wilbur P. McNulty, Leonard Olson, and David Regan, Oregon Regional Primate Research Center, Beaverton, OR; Veterans Administration Medical Center, Portland, OR; Providence Hospital, Portland, OR.

Celebes apes with a history of recurrent opportunistic infections, autoimmune diabetes, anemia, diarrhea, loss of weight, anorexia, and a high mortality rate were studied to determine whether their immunodeficiency syndrome was similar to human or simian AIDS. Animals were divided into sick and healthy groups on the basis of clinical history. The mitogen and polyclonal IgG plaque-forming cell (PFC) responses of peripheral blood lymphocytes from healthy animals showed significant levels of ^{3}H-thymidine incorporation upon culture with con A and IgG PFCs after activation with pokeweed mitogen. Animals defined as clinically sick showed depressed mitogen and IgG PFC responses. Results of evaluating T4/T8 ratios of Celebes lymphocytes using monoclonal antibodies against human lymphocytes and flow cytometry were unexpected, since the T4/T8 ratios of the sick animals were equal to or higher than the ratios of the healthy animals. These results suggest that the anti-T4 and anti-T8 reagents may exhibit only partial cross reactivity with Celebes lymphocytes. We conclude that the development and characterization of reagents specific for Celebes lymphocyte subpopulations may be necessary to fully exploit this animal model for the study of the mechanisms of immunodeficiency.

0024 *KAPOSI'S SARCOMA IN SUBSAHARAN AFRICA, +John E. Craighead, *Henning Grossman, and +Urs Hess, +University of Vermont College of Medicine, Burlington, VT 05405, *Kilimanjaro Christian Medical Center, Moshi, Tanzania

Kaposi's sarcoma is unusually prevalent among male blacks in Central and Eastern subSaharan Africa. The severity of the disease is variable; in children and some adults it disseminates widely, whereas it is slowly progressive and indolent in most young adults. We have undertaken a study to correlate the clinical-pathological features of the disease with measures of immunological responsiveness (lymphocyte phytohemagglutinin mitogenesis, helper/suppressor T cell ratios, HLA typing) and serological evidence of possible etiological agents. Thus far, almost 150 new patients have been identified and the laboratory studies are underway. This study is designed to demonstrate commonalities and differences between naturally occurring Kaposi's sarcoma in an endemic area and the spontaneous disease in persons with AIDS. Drs. Robert Friedman, Allan Goldstein and Bruce MacPherson are collaborating in these studies.

0025 DEFECTIVE MYELOPOIESIS IN AIDS, Ira Z. Leiderman, Michael L. Greenberg, Bernard R. Adelsberg and Frederick P. Siegal, Mount Sinai School of Medicine, New York, NY 10029

Leukopenia is a frequent finding in patients with the Acquired Immune Deficiency Syndrome (AIDS). To elucidate the mechanism of the leukopenia, the proliferative capacity of the granulocyte-macrophage progenitor cell (CFU_{GM}) was studied in eighteen patients diagnosed with AIDS. Nucleated cells, isolated from bone marrow (BM) aspirates obtained from these patients, were cultured in a semi-solid system in the presence of fetal-bovine serum and giant cell tumor conditioned medium (GCT-CM; Gibco) as a source of CSF, at 37°C in a humidified atmosphere of 5% CO_2/95% air. The cultures, scored on day 10, had 14.3 ±2.3 (mean ±SEM) colonies, significantly less (Student's t-test; $p < 0.01$) than the controls (29 ±1.3 colonies). Co-culture of the patients' BM cells with control peripheral blood mononuclear cells (PB-MNC) in agar revealed significant ($p < 0.001$), sometimes total, colony growth inhibition. Feeder layers containing varying concentrations of patients' BM nucleated cells (0.5, 1.0, 2.0 and 4.0 x 10^5/ feeder layer) were overlayered with a fixed concentration of control PB-MNC. GCT-CM was added to the overlayer but not to the feeder layer. On day 10, decreased colony formation by the normal cells was seen in a dose-response fashion (13.3, 26.7, 66.7 and 79.0% inhibition) with increasing concentration of patient BM cells. These data sugest that BM cells from patients with AIDS release a factor inhibitory to the in-vitro proliferation of normal myelopoietic progenitor cells. Identification and characterization of this factor may permit definition of the proliferative defects seen in this Syndrome and provide clues to its underlying etiology.

0026 SPONTANEOUS AND INTERFERON RESISTANT NATURAL KILLER CELL ANERGY IN AIDS, William M. Mitchell, Robert L. Forti, Alexander R. Lawton, Larry B. Vogler, Charles D. Stratton, and Clark R. Gregg. Vanderbilt University, Nashville, TN 37232

The acquired immunodeficiency syndrome (AIDS) is characterized by severe unrelenting opportunistic infections and/or an aggressive form of Kaposi's sarcoma associated with a dysfunction of cellular mediated immune responses. In addition to anergy of delayed hypersensitivity skin tests and inversion of T-helper/inducer to T-suppressor cell ratios usually observed in AIDS, we have observed in three AIDS cases that spontaneous and interferon (IFN) stimulated natural killer (NK) cell activity against K-562 cells is absent or severely depressed as compared to three male homosexual controls with similar lifestyles. The control group had normal spontaneous and IFN induced NK cell activities and T-helper: T-suppressor ratios approaching unity in contrast to the AIDS patients with inverted T-cell subset ratios and diminished NK cell functional activities. Fluorescence-conjugated HNK-1, a monoclonal antibody that identifies NK and antibody-dependent cytotoxic cells, stained 3-22% of peripheral blood lymphocytes from AIDS patients in comparison to 8-38% of lymphocytes from controls. A larger series will be required in order to ascertain the true incidence of NK cell anergy in AIDS. Although NK cell anergy is frequently quoted as associated with AIDS, we know of only one documented report in the literature in which five AIDS patients with systemic herpes were shown to have anergic NK cell responses against herpes infected human foreskin fibroblasts (New Engl. J. Med. 308:1439, 1981).

0027 HUMAN RECOMBINANT INTERLEUKIN-2 PARTIALLY RECONSTITUTES DEFICIENT IN VITRO IMMUNE RESPONSES IN PATIENTS WITH ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS), Jeffrey D. Lifson, Claudia J. Benike, David Mark, Kirston Koths, and Edgar G. Engleman, Stanford University, Stanford CA, 94305, and Cetus Corporation, Emeryville, CA, 94608

We studied the effects of pure human recombinant Interleukin-2 (rIL-2) produced in E. coli containing the cloned human gene on in vitro immune responses of 16 patients with AIDS and 10 age matched healthy heterosexual men. Following exposure to 1-100 U/ml rIL-2 mitogen and alloantigen induced proliferation, cell mediated lymphocytotoxicity, and natural killer (NK) cell function were increased in most AIDS patients in a dose dependent manner. Patient responsiveness to rIL-2 did not appear to depend upon the primary manifestation of disease (opportunistic infection, Kaposi's sarcoma, or both) or several additional clinical variables. The responses of healthy subjects' lymphocytes were also augmented by rIL-2, but to a lesser degree. NK activity was the function most consistently improved, with deficient patient responses uniformly restored to normal levels following incubation of effector cells with rIL-2.

0028 ANTILYMPHOCYTE SERUM FACTORS IN PATIENTS WITH THE ACQUIRED AMMUNO-DEFICIENCY SYNDROME (AIDS), Denyse M. Simpson and Dobri D. Kiprov, Children's Hospital, San Francisco, CA. 94118

Sera from 40 patients with AIDS and 40 healthy homosexual men were analyzed for the presence of antilymphocyte antibodies (ALA) using an indirect-immuno-flourescence assay and FACS IV and SPECTRUM III Flow-cytometers. ALA were detected in sera from 70% of the AIDS patients. The antibodies were shown to be of IgG class. The serum antilymphocyte activity could be completely removed by absorption with Staphylococcus protein A. Antilymphocyte antibodies were shown to be lymphocytotoxic in a complement-dependent assay. The ALA reacted with 20-25% of helper/inducer T-cells (T4) and did not react with suppressor/cytotoxic T-cells (T8) or B cells. Only 2 of the 40 healthy homosexual men used as controls had ALA in their sera. Both patients had T4/T8 ratio below 0.4. One of these patients subsequently developed AIDS. In addition to anti-lymphocyte activity, sera from patients with AIDS inhibited the mitogenic responses of normal peripheral blood lymphocytes to concanavalin A and phyto-hemoglutenin. It is not clear at the present time whether inhibition of the mitogenic responses are due to the presence of ALA. More than 70% of the patients had a high level of circulating immune complexes. The presence of ALA in sera from AIDS patients may be an important factor contributing to the helper/suppressor imbalance rendering these patients immuno-deficient.

0029 HEMOPHILIC LYMPHADENOPATHY: CLINICAL, HISTOLOGIC, CYTOLOGIC AND CHROMOSOMAL CHANGES IN PATIENTS WITH IMMUNE DEFICIENCY. W.A. Andes, R.D. deShazo,* R.J. Reed,* J.C. Harkin,* N. Wang*. Departments of Medicine and Pathology, Tulane University School of Medicine, New Orleans, Louisiana.

The spectrum of abnormalities in patients at risk for acquired immune deficiency syndrome (AIDS) includes lymphadenopathy, malignancies, and infections. We have seen the development of generalized lymphadenopathy (GL) in 30 of 144 hemophilic patients (21%) evaluated during an 18 month period. Two had factor IX deficiency while 28 had factor VIII deficiency ($p<0.05$) No patient had complete resolution of his GL. We studied mononuclear cell populations in blood and lymph nodes in 4 hemophilic patients with reversed ratios of helper-suppressor circulating T-lymphocytes ($p<0.05$) and GL. Hemophilic nodes from 1-4 cm in diameter revealed nonspecific follicular hyperplasia similar to that in homosexual patients with GL. Electron microscopy revealed no viral particles or 'vesticular rosettes.' Chromosomes from 2 patients' nodes had abnormalities: a tiny acrocentric marker chromosome in one and monosomy of chromosome #21 in another patient. Cells from patients with hemophilia showed distinct differences from cells in normal nodes with a significantly lower OKT4/OKT8 ratio ($p<0.05$). Lymphadenopathy in patients with hemophilia deserves cautious observation to determine the clinical meaning and prognosis. Biopsies in patients with hemophilia may be best reserved for those who have signs or symptoms of other, complicating, illnesses.

0030 THE PROGNOSIS OF ASYMTOMATIC HOMOSEXUAL MEN WITH DECREASED T-HELPER/T-SUPPRESSOR RATIC E. Dickmeiss, J. Gerstoft, K. Bentsen, C.S. Petersen, S. Kroon, S. Ullman, J.O.Nielsen and I. Lorenzen. State Serum Institute and University of Copenhagen, Hvidovre Hospital, Copenhagen.

Screening of 70 asymptomatic homosexual men in Copenhagen revealed that 13 (19%) had T-helper/T-suppressor (H/S) ratios ≤ 1.0. Clinical and immunological follow-up examinations for 2-7 month (mean 5.2 months) disclosed that none of the 13 men developed the acquired immunodeficiency syndrome (AIDS) or AIDS-like symptoms. An increase in H/S ratios to ≥ 1.0 was observed in 11 out of the 13 men during the time of observation.

The decreased H/S ratios were due to an increase in the T-suppressor population. The T-helper population did not at any time differ from that found in 31 male controls. The biological relevance of the observed decrease in H/S ratios was supported by the demonstration of a positive correlation to a decrease in the proliferative response of the lymphocytes. Serological studies did not reveal any specific infectious background for the low H/S ratio found in the 13 men and the reason for the "spontaneous" increase during the time of observation remains unknown. The present results indicate that most asymptomatic homosexual men with a decreased H/S ratio will experience a normalisation of the immunological parameters.

0031 IMMUNOLOGIC STATUS OF ASYMPTOMATIC HAITIANS IN MONTREAL, CANADA, Alix Adrien and Jean-Marie Dupuy, Kellogg Centre for advanced studies in primary care, Montreal General Hospital; "Institut Armand-Frappier", "Université du Québec", Laval-des-Rapides, Laval (Quebec) Canada H7N 4Z3

Most of the patients with acquired immune deficiency syndrome (AIDS) can be placed in groups that suggest a possible means of disease acquisition. The presence of Haitians among the population at risk has not been explained yet. The majority of Haitian patients with AIDS denies homosexual or bisexual orientation or use of intravenous drugs. The demonstration of immunologic abnormalities in apparently healthy homosexuals and symptomatic homophiliacs suggests a spectrum of the disease or different stages in the develpment of classical AIDS. The Haitian community in Montreal is estimated to be 40,000. We tried to determine if laboratory evidence of defective immunity can be found in healthy Haitians in Montreal, Canada.

This was a cross-sectional study. A random sample of healthy Haitians was compared to a Caucasian group matched for age and sex and living in the same neighbourhoud as the Haitians. The same inclusion criteria were used for both groups: health status was determined with a validated questionnaire and individuals with known causes of diminished immune resistance were eliminated. Laboratory investigations included enumeration of T-cell subpopulations, lymphocyte response to phytohemagglutinin, complement components and serum immunoglobulin levels. The study is now in progress.

0032 PURIFICATION OF MOUSE IL-2 AND AN ASSESSMENT OF ITS EFFECTS ON VARIOUS THYMOCYTE SUBPOPULATIONS, Rochelle D. Sailor, James P. Lugo, Santosh N. Krishnan, Barry Caplan and Ellen Rothenberg, California Institute of Technology, Pasadena, CA 91125

T cell development takes place largely within the thymus and is associated with high levels of lymphoid proliferation and death. How the thymus regulates this developmental process, and how it differs in immune-deficient individuals, remains an unanswered question. As one approach to answering this question, we are assessing the effect of the lymphokine Interleukin-2 (IL-2) on various subpopulations of murine thymocytes. Specifically, we wish to determine whether IL-2, purified to homogeneity using conventional as well as reverse phase high performance liquid chromatography, will maintain the proliferation of thymic lymphoblasts in vitro. Preliminary evidence, using partially purified IL-2 has shown that a population enriched in subcapsular thymic lymphoblasts, obtained by centrifugal elutriation, proliferate in response to IL-2 only if the tumor promoting phorbol ester (TPA) is present in the cultures. These results suggest that thymic proliferative signals may include IL-2, the proliferative signal which regulates antigen-specific immune responses, in addition to other factors yet to be identified. Any of these factors could be relevant to the etiology of immune deficiency.

0033 CHARACTERIZATION OF A POSSIBLE SUPPRESSIVE FACTOR IN AIDS, J.C. Nunnink, J. Wiedmeyer J. Hank, S. Kagen, P. Sondel, University of Wisconsin, Madison, WI

A patient with AIDS and Mycobacterium avium sepsis was studied. Her lymphocytes (PBL) were unable to respond to alloantigens, soluble antigens, or mitogens in vitro. Her plasma (PTS) inhibited the response of control lymphocytes to candida (CAN), tetanus (TET), and allogeneic lymphocytes (B_X) (Table 1). Additionally, we assayed the ability of PTS to inhibit a primed lymphocyte (PLT) response. Responder cells were primed with allogeneic irradiated cells or candida and restimulated with these same stimuli. Both of these PLT responses were inhibited by PTS.

We are presently investigating whether this suppressor factor (SF) interferes with class II MHC recognition. In addition we hope to determine whether the SF is an exogenous factor (ie cyclosporin-like compound) produced by an infectious agent in vivo, or produced directly by this patient's lymphocytes.

TABLE 1	6 day ^{3}H-TdR cpm x 10^3				PLT*-3 day ^{3}H-TdR cpm x 10^3		
Responders	Media	TET	CAN	B_X	Media**	CAN**	B_X***
PBL in Normal Plasma	0.4±0.1	30.5±3.2	77.5±9.8	44.5±1.6	1.1±0.1	34.5±5.7	61.1±19.2
PBL in AIDS plasma	1.1±1.0	6.5±2.4	12.8±4.2	12.9±3.8	1.3±0.1	1.6±0.3	4.6±2.9

*Secondary response (PLT) of lymphocytes primed for 12 days with CAN** or B_X***

0034 PARTIAL REVERSAL OF IN VITRO IMMUNOSUPPRESSION IN AIDS, Mark A. Wainberg, Jewish General Hospital, Montreal, Canada

Several laboratories have shown that peripheral blood lymphocytes from patients with AIDS are anergic to both T cell mitogens and to commonly used recall antigens. We have attempted to reverse this immunosuppression in vitro in the case of cells from 8 Haitian AIDS patients, all of whom had histories of genital and/or anal lesions induced by Herpes simplex viruses. Our initial studies involved the simple addition of exogenous T cell growth factor (TCGF) to cultures that had been co-incubated with phytohemagglutinin (PHA), and yielded mixed results. Stimulation indices of 6 or 7, instead of 1 or 2, were reported in about 50% of cases tested, but levels of responsiveness invariably remained much below those of controls. Further to this, we attempted to enrich the population of T helper cells in the cases of AIDS by treatment with anti-OKT8 antibody plus complement for 2 h at 37°C. The surviving cells were then stimulated with PHA in the presence of exogenous TCGF. When this protocol was followed, stimulation indices of 10 or more were obtained in 5 of 8 cases. The PHA-stimulated lymphocytes could then be maintained in growth medium, which had been supplemented with TCGF, for at least 15 days. Finally, in three of five cases studied, these in vitro-maintained lymphocytes were able to be stimulated in a proliferation assay by a Herpes virus antigen preparation routinely used to assess immunity in non-AIDS patients with recurrent Herpes lesions.

0035 ALTERATIONS IN T-LYMPHOCYTE SUBSETS AMONG DANISH HEMOPHILIACS. RELATION TO SOURCE OF FACTOR VIII PREPARATIONS AND HIGH-DOSE FACTOR VIII TREATMENT, J. Gerstoft, K. Bentsen E. Schøibel, J. Dalsgard Nielsen. J. Gormsen and E. Dickmeiss Statens Seruminstitut and University of Copenhagen Rigshospitalet, Denmark.

Screening of 43 healthy Danish hemophiliacs revealed a significantly lower helper/suppressor ratio than in controls. Eight of the hemophiliacs had a H/S ratio $\leq$1.0. A significant negative correlation occurred between the total lifetime factor VIII treatment and the H/S ratio. However, high-dose factor VIII treatment given to patients with antibodies against factor VIII was not associated with immunological abnormalities. Hemophiliacs treated with American preparations did not immunologically differ from those treated with preparations of other origins. 23% had increased serum IgG and this parameter was negatively correlated with the H/S ratio.

The relation of the observed immunological alterations among healthy hemophiliacs to the new disease, the acquired immune deficiency syndrome, warrants further study.

0036 T-LYMPHOCYTE SUBSETS IN HOMOSEXUAL MEN FROM COPENHAGEN. RELATION TO AIDS-PATIENT EXPOSURE AND CMV EXCRETION, Jens O. Nielsen, Jan Gerstoft, Carsten S. Petersen, Susanne Kroon, Susanne Ullman, Kirsten Bentsen, Ebbe Dickmeiss, Hans M. Kerzel Andersen & Ib Lorenzen, Department of Infectious Diseases, Rigshospitalet, University of Copenhagen;

Screening of 66 asymptomatic homosexual men from Copenhagen revealed significantly lower Helper/suppressor (H/S) ratios as compared to controls. Ten (15%) of the homosexuals had a H/S ratio $\leq$ 1.o. The low H/S ratios were the result of an increase in the absolute number of suppressor cells. Homosexuals with many partners and those who reported sexual contact with AIDS patients had significant lower ratios than those without these features. Cytomegalovirus was isolated from urine and/or sputum in 15% and isolation was associated with a H/S ratio $\leq$ 1.o. The observed immunological abnormalities could either represent latent infection with the putative AIDS agent or alternatively be caused by repeated infections and/or exposure to allogenic spermatocytes or lymphocytes.

Virology

0037 AIDS AND ADULT T CELL LEUKEMIA VIRUS, Masao Hanaoka, Institute for Virus Research, Kyoto University, Kyoto 606, Japan

Human T cell leukemia virus (HTLV) has been isolated from several patients with AIDS. The similar retrovirus (ATLV) and/or its genome has been found in neoplastic cells of all patients with adult T cell leukemia (ATL) in the endemic area of Japan. Islands surrounding Carribean basin including Haiti is the second endemic area of ATL, and AIDS has also been reported among healthy heterosexual Haitians who are not drug-abusers. Our interest is the association between AIDS and ATL, because ATL is induced among the population who had infection with ATLV probably at newborn age and resulted the defficiency of cellular immunity. In Japan, however, there is no report on AIDS patients with opportunistic infection, Kaposi's sarcoma and/or B cell lymphoma. According to Dr. Yorio Hinuma's results, anti-ATLV antibody was negative in all sera of AIDS patients in U.S.A. I think more work is needed to confirm the hypothesis that HTLV have close relationship with the induction of AIDS.

0038 Association of Human T-Cell Leukemia Viruses (HTLV) with AIDS. B. Poiesz, R. Tomar, J. Moore, S. Merl, B. Kloster, A. Planas, D. Blair, C. Cabradilla, T. Han, G. Ehrlich, and R. Comis. SUNY UMC, VAMC and S.U., Syracuse, NY 13210; BKRC, Auburn, NY, CDC, Atlanta, Ga., and RPMI, Buffalo, NY. Patients with AIDS or AIDS related complex (ARC) were tested in an ELISA for antibodies to HTLV proteins gp 46, p24, and/or p19 and in a double antibody immunofluorescent assay for antibodies to HTLV transformed cell membrane antigen (HTLV-MA). 9/75 patients had antibody to HTLV protein (+ = >2SD from normal mean) vs 1% of normals. This reactivity could be competed with HTLV proteins, but with kinetics different from those seen with sera from patients associated with prototype HTLV-I. 54% of the patients had antibodies to HTLV-MA (+ = >10% of cells fluorescent at a serum titer of 1/75) vs 0% of normals. This reactivity could be removed by HTLV transformed cells but poorly with non-HTLV infected cells nor purified HTLV viral proteins. Incubation of HTLV transformed cells with HTLV-MA antibody + AIDS sera did not prevent rabbit anti-HTLV sera of monoclonal anti-TAC antibodies from binding to the cells. T-lymphocytes of 13 patients with AIDS or ARC were cultured with lectin-free T-cell growth factor. 5/13 were + in a double antibody immunofluorescent assay, utilizing monoclonal antibody to HTLV p19. 4/5 of these + samples were examined by thin section electron microscopy and found to have extracellular retrovirus-like particles. 3/5 had reverse transcriptase as measured on PEG precipitated cell culture conditioned media using poly (C) oligo dG and 10 mM Mg++ (+ = >4 picomoles GMP incorporated/h/ml precipitant. The data suggest an association between some patients with AIDS or ARC and HTLV. Whether this association is with prototype HTLV-I or a related retrovirus or with cellular protein present in HTLV transformed cells is uncertain at the moment.

0039 Integrated HTLV Proviral DNA in the Splenocytes of a Patient with AIDS, G. Ehrlich, J. Moore, R. Tomar, B. Kloster, S. Merl, J. Turchik, R. Comis, J. Vournakis, and B. Poiesz. SU, SUNY UMC and VAMC, Syracuse, NY, BKRC, Auburn, NY. A 42 year old white homosexual male with AIDS was evaluated for the presence of the human T-cell lymphoma/leukemia virus (HTLV). The patient had severe lymphopenia with a T4/T8 ratio of .15, diarrhea, 100 lb. weight loss, and opportunistic infections with shigellosis, candida, and toxoplasmosis. The patient's sera was negative for antibodies to purified disrupted HTLV proteins (measured in an ELISA) and to HTLV transformed cell membrane antigen (measured by double antibody immunofluorescent assay). His peripheral blood T-lymphocytes cultured in TCGF were negative for HTLV p19 (measured with a monoclonal antibody to HTLV p19) and for reverse transcriptase. At autopsy, the patient's lymph nodes and spleen were removed. DNA was extracted from both tissues and analyzed for integrated HTLV sequencies using [32P]-labelled cDNA from the nick translated cloned probe paTK32, which is specific for the gag-pol region of HTLV. Dot blot hybridization of the patient's splenocyte DNA was significantly positive at both high and low stringency while DNA from his lymph nodes was not. Control DNA's included those from the HTLV-positive cell lines HUT 102 and MT2 and HTLV-negative cell lines HUT 78 and MAC-1 and other fresh human tissues. The data indicate that HTLV proviral DNA was present in the splenocytes of a patient with AIDS but not in his lymph node nor peripheral blood. The data indicate also that such integrated sequences can be present without evidence of anti-HTLV antibodies. The relationship of HTLV to AIDS is under investigation.

0040 INDUCTION OF LYMPHOPROLIFERATION, HYPERGAMMAGLOBULINEMIA, AND IMMUNOSUPPRESSION IN C57BL/6 MICE BY RCN-BM5 MuLV, D. E. Mosier, Research Pathology Section, Fox Chase Cancer Center, Philadelphia, PA 19111

The isolation of a variant of the C57BL/6 radiation leukemia virus termed RCN-BM5 that causes "reticulum cell neoplasms" instead of thymic lymphomas has recently been reported. We have examined this virally-induced disease in mice in more detail and reached the following conclusions. The injection of RCN-BM5 MuLV into normal C57BL/6 mice induces the rapid onset of both T and B cell polyclonal proliferation. The disease is therefore a lymphoproliferative state and not a lymphoma. Many of the proliferating B lymphocytes are induced to high level antibody secretion in virus-injected normal C57BL/6 mice, but virus injection of T cell-deficient C57BL/6 nude mice leads to B cell proliferation without antibody secretion. IgG levels are more elevated than IgM or IgA. The responses of normal mice injected with RCN-BM5 MuLV to T cell mitogens, B cell mitogens, T-dependent and T-independent antigens, and allogeneic cells all are profoundly suppressed within 2-3 weeks of virus inoculation. The ratio of T helper to T suppressor cells is altered, but there is a five-ten fold increase in total T cell number. Mixtures of T cells from virus-infected mice and normal T or B cells did not show a large increase in T cell suppressive activity due to the virus. Infected mice die 3-4 months after inoculation of massive lymphadenopathy and/or intercurrent infections. This murine disease model thus seems to clearly establish that a single virus agent can cause a pleimorphic disease with lymphadenopathy, hyperproduction of IgG antibodies, and profound immunosuppression, a constellation of symptoms seen in AIDS.

0041 VIRAL AND ULTRASTRUCTURAL STUDIES IN LYMPHADENOPATHY, KAPOSIS SARCOMA, AND AIDS, Patricia P. Smith, Leslie E. Caldwell, and James K. McDougall, Fred Hutchinson Cancer Research Center, 1124 Columbia St. Seattle, WA 98104

We have observed by electron microscopy, three unusual structures in lymph node cells of patients with chronic generalized lymphadenopathy, AIDs, and Kaposis sarcoma. Tubuloreticular structures (TS), vesicular rosettes(VR), and replicated basal lamina(RBL), are frequently associated with lymphocytes, plasma cells, and endothelial cells in these specimens. Other reports have described the presence of TS in peripheral blood and lymph nodes of patients with AIDS. TS have previously been described in connective tissue diseases (e.g. lupus erythematosis), cancers, and virus infections (e.g. cytomegalovirus pneumonia). VR have been detected primarily in plasma cells. RBL has been described in patients with diabetes and is suggested to be related to endothelial cell injury.

Although these structures may be indicative of degenerative processes, a relationship to virus infection is possible. TS have previously been suggested to represent incomplete virus particles and are inducable by interferon and interferon-inducers. The tissues are being examined by electron microscopy for evidence of virus particles. DNA extracted from these tissues is being examined for viral nucleic acid sequences by Southern blot analysis, with particular reference to cytogemalovirus.

0042 MOLECULAR BIOLOGY OF HTLV IN A.I.D.S. I. MOLECULAR CLONING OF A NEW HTLV PROVIRUS (HTLV Ib) WITH DIVERGENCE IN THE ENVELOPE AND pX REGIONS. George M. Shaw, Beatrice H. Hahn, Anita LoMonico, Mikulas Popovic, Robert C. Gallo, and Flossie Wong-Staal. Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20205.

Viral isolation and serologic studies have implicated HTLV in the pathogenesis of adult T-cell leukemia (ATL), and more recently, the A.I.D.S. syndrome. We established a cell line (MC) from peripheral blood of a young male homosexual with A.I.D.S. and showed this to be infected with HTLV based on DNA hybridization, viral protein production (p19 and p24), reverse transcriptase activity, and electron microscopy. The integrated provirus was then cloned into Wes/ B, extensively mapped by restriction enzyme digestion, and compared with the restriction maps of HTLV I isolates from patients with ATL, including the predicted restriction pattern of the ATK-1 clone sequenced by Seiki, et al. (PNAS 80:3618, 1983). The cloned A.I.D.S. provirus (MC-1) was found to have a genomic organization characteristic of retroviruses and generally similar to that described in ATL. However, there are 18 differences in the restriction sites mapped for MC-1 compared to ATK-1. Moreover, there is a 1.5 kb segment of genome in the envelope and pX regions where 10 of 12 restriction sites are discordant. We have mapped three of these divergent sites in at least five different isolates from ATL, and in all instances they resemble the ATK-1 clone, not MC-1. Because of its overall similarity with HTLV I, yet differences in the envelope and pX regions, we have identified this new viral isolate as HTLV Ib. The biological significance of these differences and what role, if any, this virus may have in the pathogenesis of A.I.D.S. are under study.

0043 MOLECULAR BIOLOGY OF HTLV IN A.I.D.S.: II. ISOLATION AND CHARACTERIZATION OF HTLV II PROVIRUS IN A PATIENT WITH A.I.D.S. Beatrice H. Hahn, Mikulas Popovic, V.S. Kalyanaraman, George M. Shaw, Anita LoMonico, Flossie Wong-Staal, and Robert C. Gallo. Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20205.

HTLV I is a human retrovirus associated with certain mature T-cell malignancies and may be involved in the pathogenesis of A.I.D.S. syndrome. HTLV II is a related retrovirus which was isolated from a patient with T-cell hairy cell leukemia (Science 218:571, 1982). To date, no other isolates have been reported. We now describe the second isolation of HTLV II from a 45 year old black male prisoner and IV drug user with A.I.D.S. syndrome. A primary cell line derived from peripheral blood mononuclear cells and a cell line derived by co-cultivation with normal human cord blood lymphocytes were reactive with antisera against the HTLV core antigens p19 and p24. The patient's serum contained antibodies which were type-specific for HTLV II (Popovic et al., in preparation). Molecular hybridization of genomic DNA from the patient's cell lines was specific for HTLV II. Preliminary analysis using a probe derived from the 3' end of the HTLV II provirus indicates that the restriction pattern of this second isolate is similar to that of the original HTLV II (Mo). A more detailed characterization of this provirus will be discussed.

0044 SEXUAL TRANSMISSION OF HUMAN T-CELL LEUKAEMIA-LYMPHOMA VIRUS AMONG MALE HOMOSEXUALS WITH AIDS AND THE LYMPHADENOPATHY SYNDROME IN THE NETHER LANDS,Jaap Goudsmit,Frank Miedema,Ria Wijngaarden-du Bois,Marijke Roos, Peter Th. Schellekens,Roel Coutinho,J.van der Noordaa and Cornelis J.M. Melief,Virology department,University of Amsterdam;Central Laboratory of the Netherlands Blood Transfusion Service;Municipal Health Service Amsterdam,the Netherlands

Antibodies to human T-cell leukaemia-lymphoma virus(HTLV)and HTLV-associated antigens(HTLA)were assayed by enzyme-linked immunosorbent assay(ELISA)and indirect immunofluorescence(IF) in sera from 133 immunologically well-characterized promiscuous male homosexuals in the Netherlands,of whom 10 suffered from Acquired Immune Deficiency Syndrome(AIDS),18 from Lymphadenopathy Syndrome(LAS) and 5 from Gay Bowel Syndrome(GBS).Antibodies to HTLV or HTLA were present in 5 out of 10 AIDS patients,in 6 out of 18 LAS patients and in one out of 5 GBS patients.None of the healthy homosexuals with or without T-subset imbalances showed seropositivity for HTLV or HTLA.
A primary HTLV infection in a LAS patient is described after passive anal intercourse with an HTLV seropositive AIDS patient indicating sexual contact as the mode of HTLV transmission in the dutch homosexual community.

0045 STRUCTURE AND FUNCTION OF THE HTLV GENOME, William A. Haseltine, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, Department of Cancer Biology, Harvard School of Public Health, Joseph G. Sodroski, Dana Farber Cancer Institute, Department of Cancer Biology, Harvard School of Public Health

The DNA sequence of selected regions of HTLV proviral genomes isolated from ATLL and AIDS patients is under investigations. Similarities and differences among the viral strains in the LTR, pX and env regions will be discussed.

The nature of proteins encoded by the HTLV genome will also be discussed. The ability of the LTR of several HTLV strains to function as enhancer-promoter elements will be presented. These studies were done in collaboration with Dr. Gallo and Dr. Wong-Staal of the National Cancer Institute.

0046 **IgG ANTIBODIES TO A T-LYMPHOTROPIC RETROVIRUS IN PATIENTS WITH LYMPHADENOPATHY SYNDROME OR AIDS.**

F.Brun-Vézinet, Ch.Rouzioux, F.Barré-Sinoussi, W.Rozenbaum, A.G.Saimot, S.Matheron, L.Montagnier, J.C.Chermann.Hôpital Claude-Bernard, Hôpital Pitié Salpétrière, Institut Pasteur, Paris, France.

A retrovirus has been isolated from the cultured-T-Lymphocytes derived from a lymph node of an homosexual with lymphadenopathy syndrome (LAS). It was named Lymphadenopathy Associated Virus (LAV). Similar isolates have been made from french cases of AIDS. Such retrovirus have been proved distinct from HTLV1 on the bases of their major core protein and morphology. We developped an ELISA to investigate the presence of LAV - Specific - IgG antibodies. The virus prepared from the supernatant of in vitro infected lymphocytes of an healthy donnor was concentrated, purified and disrupted. A crude cytoplasmic extract from the same uninfected lymphocytes was used as a control antigen in order to determine the unspecific binding of sera. Sera at dilution 1/40 were considered as positive when the differential optical density was $\geq$ 0.30. Sera from 27 out of 42 LAS (64 %) and 16 out of 44 AIDS (36 %) were positive for LAV - IgG - antibodies. 8 out of 44 healthy homosexual men (18 %) had LAV antibodies and 1 out of the 70 unmatched control individuals (blood donnors) was positive. Several of these sera were also tested for LAV antibodies by Radio-Immuno-Precipitation-Assay. All the sera were tested for HTLV1, CMV and HBV IgG antibodies by ELISA. Detailed results of these comparative studies will be presented and discussed.

Infection, Neoplasia and Therapeutics

0047 SYSTEMIC INFECTION WITH MYCOBACTERIUM AVIUM-INTRACELLULARE AND THE IMMUNE STATUS OF THE HOST, Frank M. Collins and Ian M. Orme, Trudeau Institute, Saranac Lake, NY 12983. Specific pathogen-free mice infected intravenously with M.avium-intracellulare frequently develop persistent infections lasting for the lifetime of the host. After an early period of limited growth the spleen and liver counts reach a plateau phase while the lung counts continue to slowly increase. Such heavily infected mice fail to respond to the specific M.avium sensitin in vivo and this anergic state is associated with reduced mitogen and alloantigen-induced blastogenic responsiveness in vitro. The latter is partly mediated by a population of adherent cells (macrophages) which appear in a heavily infected spleen as the disease progresses. At this time, substantially increased numbers of activated macrophages were detected in the spleen, with enhanced non-specific antibacterial activity against a Listeria challenge. However, attempts to detect specifically sensitized T-cells in the spleens of the anergic mice by means of an adoptive transfer to T-cells depleted syngeneic recipients were repeatedly unsuccessful. The anergic mice were also sensitized by an i.v. injection of 10^5-10^6 SRBC and the footpad tested with 10^8 SRBC 5 days later. None of these mice developed a delayed (24hr) footpad swelling reaction. Transfer studies indicated that they lacked a population of specifically sensitized T-cells. Increasing the i.v. sensitizing inoculum of SRBC to 10^8 restored this footpad actively suggesting that anergy was due to antigen destruction by the activated macrophages which reduced the normally immunogenic dose of SRBC to non-sensitizing levels. Thus, the observed unresponsiveness may be due to excessive macrophage activation as much as to suppressor cell formation. The possible significance of this finding with respect to the induction of AIDS will be discussed.

0048 THE ROLE OF APHERESIS PROCEDURES AS AN IMMUNOREGULATORY THERAPY IN PATIENTS WITH AIDS RELATED CONDITIONS, Dobri D. Kiprov M.D., David Busch M.D., Randolph Lippert M.D., Ian Lipkin M.D., Denyse M. Simpson Ph.D., Donald Abrams M.D., Richard J. Cohen M.D.

Intensive plasmapheresis was performed in 4 AIDS patients in an attempt to remove immune complexes, anti T-cell antibodies, antiplatelet antibodies and a serum inhibitory factor which suppresses normal lymphoproliferation in vitro. Prompt removal of the abnormal serum immunoreactants was achieved. Increase in the number of white blood cells, lymphocytes and platelets was observed. The percentage of immunoregulatory T-cell subsets did not change. Although some subjective improvement was seen in all 4 patients, this treatment regimen did not change the natural course of the disease. A fifth patient with AIDS was treated with a short course of intensive plasmapheresis followed by maintenance treatments of once a month. Clinical and laboratory parameters improved and sustained in this single patient. A homosexual male patient with lymphadenopathy, low T4/T8 ratio and demyelinating peripheral neuropathy was treated with lymphoplasmapheresis. The patient responded with a fast improvement of his neurological symptoms. A patient with AIDS and advanced disseminated Kaposi's Sarcoma was treated with protein A plasmaperfusion. Gross as well as microscopic changes were observed in some of the tumor lesions.

0049 BONE MARROW TRANSPLANTATION FROM IDENTICAL TWINS IN THE TREATMENT OF AIDS AND KAPOSI'S SARCOMA. R.Mitsuyasu, P.Volberding, J.Groopman, R.Champlin. UCLA Medical Center, Los Angeles, CA, SF General Hospital, San Francisco, CA, and New England Deaconess Hospital Boston, MA. Four male patients, median age 32 (27-39) with the acquired immunodeficiency syndrome (AIDS) and biopsy proven Kaposi's sarcoma were evaluated for bone marrow transplantation (BMT) from their immunologically normal identical twins. Abnormalities of cellular immunity were demonstrated in all patients by cutaneous anergy to recall antigens, abnormal mitogen responsiveness, and reduced T helper to T suppressor (Th/Ts) cell ratio. No patient had received prior treatment for Kaposi's sarcoma. Genetic identity of siblings was confirmed by HLA, ABO, and red cell isoenyzme typing. Two patients received infusion of bone marrow from their twin donor without prior conditioning. Both patients are alive with stable disease 4 and 7½ months post marrow infusion. No improvement in Th/Ts ratio or other parameters of T cell immunity was seen in these patients. A third patient underwent BMT after conditioning with vinblastine, 0.3mg/Kg IV x 2d, and fractionated TBI, 200 rads/d x 5d. This patient had rapid reconstitution of his bone marrow with ANC > 500 by day 10 and had > 50% reduction in the size of his lesions. His Th/Ts ratio remained markedly depressed post transplant. He subsequently developed pneumoncystis carinii and CMV pneumonia and expired on day 41. The fourth patient elected treatment with conventional chemotherapy and has not received BMT. These results indicate that marrow transplantation from identical twins may control the progression of Kaposi's sarcoma initially, but may not immediately restore cellular immunity. Immune dysfunction post transplantation and the risk of concurrent opportunistic infection in patients colonized with many organisms remain major obstacles to the clinical utility of this approach.

0050 E. COLI PRODUCE BIOLOGICALLY ACTIVE HUMAN INTERLEUKIN 2 FROM A SYNTHETIC GENE, T. Boone, L. Souza, Z. Stabinsky, Y. Stabinsky, L. Miller, P. Lai, V. Merluzzi*, K. Welte* and R. Mertelsmann*. Amgen, Thousand Oaks, Ca 91320; *Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

Recently the gene for human Interleukin-2 (IL-2) has been cloned (Taniguchi, et al. 1983. Nature 302: 305]. IL-2 is a lymphokine produced by lectin or antigen activated T-cells which is capable of stimulating the growth of T-cells in vitro (Morgan et al. 1976. Science 193: 1003 and Gillis and Smith 1977. Nature 268:154). We have chemically synthesized the gene for human IL-2. The synthetic gene differs from the natural gene in that the synthetic gene was made with codons optimal for expression in E. coli. The recombinant IL-2 (rIL-2) represents 25% of the total protein of E.coli as determined by densitometric scanning of SDS-polyacrylamide gels (SDS-PAGE) of whole cell extracts. This material has the same apparent molecular weight (15.5Kd) as native IL-2 as measured by SDS PAGE. The rIL-2 differs from the native IL-2 in the addition of one amino acid (met) at the N-terminus of the protein as detected by gas phase microsequencing of the purified synthetic product. Highly purified rIL-2 and human lymphocyte IL-2 are both able to support the growth of human and murine IL-2 dependent T-cells at concentrations >1ng/ml. The defective mitogen responses to PHA and OKT3 antibody in PBL taken from patients with various immunodeficiency states (bone marrow transplants and aquired immunodeficiency syndrome) can be partially or completely restored in vitro with either rIL-2 or native IL-2 as measured by tritiated thymidine uptake. Other in vitro and in vivo biological activity data using IL-2 in various animal systems will be discussed.

0051 YEAST α-FACTOR DIRECTED SECRETION OF HUMAN INTERLEUKIN-2 FROM A CHEMICALLY SYNTHESIZED GENE, Philip J. Barr, R. Chris Bleackley*, Anthony J. Brake and James P. Merryweather. Chiron Corporation, Emeryville, CA 94608 and *University of Alberta, Edmonton, Alberta, Canada.

Interleukin-2 (IL-2) is a lymphokine that promotes the clonal expansion of activated T lymphocytes. Its role as an essential mediator of immune response has been well established and confirmation of the ability of purified IL-2 to function in vivo has suggested its potential therapeutic use in a variety of clinical conditions, including AIDS.

In order to produce large quantities of human IL-2 for further investigation of its biological activity we have utilized a combination of oligonucleotide synthesis and recombinant DNA technology to produce this lymphokine in yeast. Based on the reported cDNA sequence we have chemically synthesized a gene for human IL-2 using codons preferentially utilized in the structural genes for the highly expressed yeast glycolytic enzymes. The synthetic gene was cloned into a vector containing promoter, leader, processing signal and terminator sequences for the yeast α-factor mating pheromone, a naturally secreted polypeptide of 13 amino acids in length. Yeast cells transformed with plasmids containing these sequences gave expression and secretion into the growth medium of biologically active human IL-2.