CRITICAL RESEARCH DIRECTIONS IN PEDIATRIC HIV INFECTION

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Central Nervous System in Pediatric AIDS

WD 001 HIV-1 AND PRODUCTION OF NEUROTOXINS, Dana Giulian, Department of Neurology, Baylor College of Medicine, Houston, TX 77030

Brain dysfunction is a common problem for children infected with HIV-1. However, the cause of AIDS encephalopathy remains uncertain, since the retrovirus spares neurons and infects only microglia and macrophages. This "indirect" disruption of the central nervous system may involve production of neurotoxic factors. Some investigators have proposed that the envelope glycoprotein of HIV-1, gp120, is itself a poison which acts by binding to neurons. Alternatively, my laboratory has found that monocytoid cells infected with HIV-1 are stimulated to release neuron poisons. Some of these neurotoxic agents are heat-stable, protease-resistant molecules with low molecular mass (< 500 Da). Neuron killing by the HIV-1 elicited factors are blocked by antagonists to the N-methyl-D aspartate receptor. Determining the structure and mode of actions for these neurotoxic factors may lead to new strategies for palliative treatment.

Factors Related to Maternal/Fetal Transmission of HIV-1

WD 002 THE ROLE OF THE THYMUS IN THE PATHOGENESIS OF AIDS, Barton F. Haynes, Stephen M. Schnittman, Stephen M. Denning, Sharilyn K. Stanley, Kay H. Singer, Phong T. Le, and Anthony S. Fauci, Duke University Medical Center, Durham, NC 27710 and the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

During infection with the Human Immunodeficiency Virus (HIV), there is depletion and dysfunction in CD4+ T cells. In patients treated with AZT, regeneration of the peripheral T cell pool does not occur, despite suppression of HIV replication. However, the postnatal thymus is capable of regeneration of the peripheral T cell pool in the setting of bone marrow transplantation for congenital T cell immunodeficiency syndromes. Recent studies in animals and man have suggested that the normal thymus continues to function throughout adult life. The lack of regeneration of T cells in AIDS suggests that HIV is adversely affecting central lymphoid microenvironments (thymus, bone marrow) as well as peripheral lymphoid compartments (peripheral blood, lymph node, Peyer's patches), and that there is a defect in the generative function of the thymus in HIV infection. Numerous studies had shown that in AIDS, HIV can be isolated from the thymus, and severe thymic atrophy is present with thymic fibrosis, thymic epithelial cell death, and abnormal depletion of thymocytes. To study mechanisms of HIV modification of intrathymic T cell maturation in man, we have investigated the ability of HIV to infect thymic cell types, and determined the effect of thymic epithelial (TE) derived cytokines to affect HIV replication. Using thymocyte clones, cell lines and freshly isolated thymocyte populations, we found that HIV could infect all TCRαβ+ thymocyte populations that expressed CD4. Interestingly, HIV also infected TCRγδ+ thymocyte clones, freshly isolated CD3-,CD4-,CD8- thymocytes (putative intrathymic T cell precursors) and the CD3-,CD4-, CD8- stem cell line DU528. In each case, infection could be blocked with anti-CD4 mabs, and analysis using flow cytometry and a panel of anti-CD4 mabs demonstrated low level expression of CD4 on each cell type. In contrast, we were unable to demonstrate any expression of CD4 on cultured human TE cells, nor could these cells be infected with HIV in vitro. TE cells produce IL1α and β, IL6, G-CSF, M-CSF, GM-CSF, leukemia inhibitory factor, and TGF-α. TGF-α exerts a potent regulatory role of IL1 and IL6 production via interaction with TE cell EGF receptors. Moreover, IL6 and GM-CSF have been shown to be inducers of HIV replication in chronically infected cell lines. We found that factors in human serum such as γ-interferon upregulate the ability of TE cells to produce IL6 and GM-CSF, and that TE supernatants induced a 20-fold augmentation in HIV expression (as determined by RT assay) in the U1 HIV-infected cell line. Thus, HIV may adversely affect the normal function of the human thymus by infection of CD4lo+ T cell precursors and other CD4+ thymocytes and antigen-presenting cells. Further, HIV infection of CD4+ cells within the thymus may be modulated by TE cell-derived cytokines.

Opportunistic Infections in Children with HIV: Problems and Challenges

WD 003 WHAT WE KNOW ABOUT THE PATHOPHYSIOLOGY, DIAGNOSIS, TREATMENT, AND PREVENTION OF DISSEMINATED MYCOBACTERIUM AVIUM COMPLEX INFECTION (MAI) IN CHILDREN, C. Robert Horsburgh Jr.(1,2), R.J. Simonds(1), Blake Caldwell(1), (1) Division of HIV/AIDS, Centers for Disease Control, and (2) Grady Memorial Hospital, Atlanta GA.

Prevention. The epidemiology of MAI in pediatric AIDS parallels that of adults with AIDS. Between 1981 and 1991, 188 cases of MAI in children under 13 years of age were reported to the CDC (5.7% of all AIDS cases <13 years old). The frequency of MAI as a percentage of AIDS cases did not differ significantly by sex (5.9% of males and 5.4% of females) or race (6.6% of whites, 5.2% of blacks, and 5.5% of hispanics). Frequency was higher in those with hemophilia or transfusion-associated HIV infection than in those with presumed vertical transmission (12.5% and 12.7% vs 4.7%, p<.001). Frequency increased with increasing age (from 1.9% in those with AIDS diagnosed at 1 year to 14.6% in those 6 or more years of age, p<.001); this persisted when stratified by mode of acquisition of HIV. The infection was seen in those with the lowest CD4 cell counts (median 11 cells/mm3) and was the initial AIDS-indicating condition in 17% of cases. We conclude that preventive strategies, such as prophylactic antimycobacterial therapy, should be directed at children with lower CD4 cell counts and a longer duration of HIV infection.

Pathophysiology and diagnosis. The major route of acquisition of MAI for children, as for adults, is via the gastrointestinal tract. Organisms are presumably ingested, and then invade the gut wall and/or the liver with subsequent hematogenous spread to lymph nodes, spleen, and bone marrow. Pulmonary involvement is rare. Clinical presentation reflects this, with fever, diarrhea, abdominal pain and failure-to-thrive the most common symptoms. As in adults, severe transfusion-dependent anemia is also seen. When MAI is suspected, mycobacterial blood cultures should be obtained to establish the diagnosis. However, the sensitivity of this technique when performed on small volumes of blood remains to be established.

Therapy. No trials of therapy of MAI in children have been reported. However, anecdotal reports suggest that responses are similar to those seen in adults. Early identification of MAI is important, since therapy of patients with a large burden of disease is less likely to be successful. Combination regimens are recommended, due to the marked lack of susceptibility seen with in vitro testing of the organisms. Antimycobacterial agents employed include rifampin, amikacin, and ethambutol; experience with clofazimine in children is limited. Newer macrolides, such as clarithromycin and azithromycin, have been used in a few cases with promising results. To date, these agents have not appeared to present special toxicities for the pediatric population.

Critical Research Directions in Pediatric HIV Infection

WD 100 MYCOBACTERIUM AVIUM INTRACELLULARE: A PEDIATRIC AIDS AUTOPSY STUDY ,+*V. Anderson, +M. Sulh, +E. Zevallos, *M.A. Greco, +State University of New York Health Science Center at Brooklyn, Kings County Hospital Center, Brooklyn, NY 11203. *Pediatric AIDS Study Group.

Autopsies performed at SUNY HSCB and KCH on nine children with AIDS had evidence of Mycobacterium avium intracellulare (MAI). All children had congenital HIV infection with failure to thrive. The mean age at death was 4.5 years, (range 14 months to 10 years). MAI was identified in the lymph nodes (6); lungs (5); spleen (4); bone marrow (3); intestines (3) cases. Two children were diagnosed antemortem. One had a positive blood culture; another a positive bronchoalveolar lavage. Additional opportunistic infections included: Candida (5); Herpes simplex (3); and Pneumocystis carinii pneumonia (PCP) (2) cases. Five children had recurrent bacterial pneumonia and one had lymphocytic interstitial pneumonia (LIP). An additional 11 cases were reported to the Pediatric AIDS Study Group. In that series, the mean age at death was 5.4 years, (range 2-11 years). MAI was present in the lymph nodes (10); spleen (8); liver (6); intestine (3); bone marrow (2); lung (1) case. Five children had LIP and six had PCP. PCP and LIP was not seen in the same patient.

MAI occurs as a co-pathogen in older children with a wide range of opportunistic infections. The extent and severity varies greatly and the contribution to morbidity and mortality is uncertain. MAI may appear as a marker of terminal HIV infection in older children. Young children with developmentally naive and HIV infected immune system may succumb to opportunistic infection or overwhelming bacterial infection prior to immunological deterioration which permits MAI to be established. With anti-retroviral therapy, improved clinical management and prolonged survival, an increase in MAI beyond the 10-12% in current autopsy experience may be anticipated. Autopsy studies are essential to identify MAI in tissue.

WD 101 DIMINISHED ABILITY OF PLACENTAL AND NEONATAL MACROPHAGES TO SUPPORT PROLIFERATIVE HIV INFECTION, Sandra K. Burchett, Robert C. Coombs, Lawrence Corey and Christopher Wilson, Department of Pediatrics and Laboratory Medicine, University of Washington School of Medicine, Seattle, WA 98105.

Greater than 85% of cases of pediatric AIDS in the U.S. result from perinatal/vertical transmission of HIV. The potential mechanism of infection includes transplacental passage of free or cell-associated HIV. Maternal/placental factors associated with vertical transmission are unclear. Although HIV infected placentas do not always result in an infected infant, placental macrophages (Hofbauer cells) are likely infected when transmission does occur.

Placental Hofbauer cells and neonatal umbilical cord and adult monocytes were isolated by collagenase/trypsin digestion; ficoll/hypaque and percoll density gradient centrifugation; monoclonal antibody negative selection lysis and adherence to plastic. Cells were cultured +/- PHA prior to infection with 0.1-50 TCID-50 of the LAV IIIb strain of HIV. Proliferative infection was assessed to 4 weeks by p24 Ag assay in the culture supernatant. Although 6 of 7 adult cell preparations supported proliferative HIV infection without prior PHA stimulation, 12 of 16 cord or placental cell preparations did not. After PHA stimulation, all adult and neonatal/placental cell preparations supported proliferative HIV infection. We reasoned that vertical transmission is enhanced at the placental level when HIV is proliferating, resulting in cell free virus. These conditions might include activation by sexually transmitted or trans-activating pathogens. These data allow speculation that the placental Hofbauer cell generally provides an effective barrier to prevention of vertical transmission of HIV unless activating conditions exist at the placental level.

WD 102 SIMULTANEOUS DETECTION OF CMV AND HIV DNA IN DRIED BLOOD SPOTS COLLECTED ON NEWBORN SCREENING BLOTTERS, Sharon Cassol, James Rudnik, Stanley Read, Michael Montpetit, John Gill, Carlos Izaguirre, Teresa Salas, Carol Major, Michael O'Shaughnessy, Federal Centre for AIDS, Health and Welfare Canada; Department of Pediatrics, Hospital for Sick Children, Toronto and the Department of Medicine, University of Calgary, Canada.

Cytomegalovirus is a common cause of life-threatening opportunistic infection in patients with AIDS. In these patients, CMV viremia is predictive of disease progression and CMV organ involvement. To monitor CMV viremia during the course of HIV infection, we have adapted to the polymerase chain reaction (PCR) to the simultaneous detection CMV and HIV DNA in dried blood spot (DBS) specimens. CMV DNA was identified in 14 of 36 (38.8%) adult patients with AIDS or ARC, 1 of 20 (5%) patients with asymptomatic HIV infection and 0 of 21 healthy blood donors, of whom 13 were CMV seropositive and 8 CMV seronegative. There was no cross-reactivity with human genomic DNA or with DNA from other herpes viruses. The sensitivity of CMV DNA detection ranged from 10 to 20 viral genomes/12.5 ul of whole blood. With respect to HIV, 55 of the 56 (98.2%) patients with ARC, AIDS or asymptomatic HIV infection tested HIV DNA positive. All 21 HIV seronegative blood donors tested HIV DNA negative. These results indicate that DBS PCR will provide a sensitive and specific approach for evaluating CMV as a cofactor (or epiphenomenon) in HIV disease. Additional studies are in progress to determine the potential of DBS PCR for the diagnosis and surveillance of congenital CMV in newborns. HIV studies have shown that DBS PCR is valuable for the early diagnosis (or exclusion) of HIV in infants born to seropositive mothers (Cassol et al., J Acq Imm Def Synd, in press). Advantages of DBS PCR include biological stability, reduction of biohazard risk and elimination of the need to maintain the cold chain. Large numbers of DBS samples can be collected under difficult field conditions and shipped to national reference laboratories for retrospective serologic (antibody, antigen) and molecular (PCR) analysis.

WD 103CYTOMEGALOVIRUS (CMV) INFECTION IN CHILDREN WITH HIV INFECTION. ER Cooper, MD; SI Pelton, MD: AM Regan, RN; T Schwartz, MSN and the Pediatric AIDS Program. Department of Pediatrics, Boston City Hospital and Boston University School of Medicine, Boston, Massachusetts, USA.

Our work reflects our interest in co-factors which may be associated with maternal-infant transmission of HIV, especially maternal CMV infection. We have participated in a twin study, and are currently studying transmission patterns in cohorts of children born to infected women. We are presently completing analysis of the role of CMV in HIV transmission and development of opportunistic infection.

We reviewed the records of all children with symptomatic HIV infection followed at Boston City Hospital to identify those children infected with CMV. CMV infection was defined as a positive culture for CMV from urine, throat or biopsy specimen or a positive serologic assay for IgG antibody. Twenty-four (65%) of 37 HIV infected children (P2) were infected with CMV, 17 (71%) were positive on first culture or serologic assay; 12 (50%) had CMV infection documented prior to their 1st birthday. We compared the seroprevalence of CMV in HIV infected children (P2), seroreverters (P3) and in age matched HIV negative controls. CMV infection in HIV infected children was comparable to HIV negative children. Seroreverters were less frequently infected with CMV than HIV infected children (3/24 vs 24/37, $P < 0.01$).

HIV infected children who were also infected with CMV were more likely to have severe encephalopathy, elevated hepatic enzymes associated with AZT and suffer death. CMV disease was identified in 5 children, all with acquisition of CMV early in life.

We conclude that CMV infection occurs in the majority of HIV infected children in our population and adversely effects survival. Most CMV infected children were infected at initial evaluation suggesting acquisition early in life, and influence on transmission of HIV.

WD 104 DEFICIENT DEVELOPMENT AND ACTIVITY OF NATURAL KILLER, NK, CELLS IN HIV+ INFANTS. Susanna Cunningham-Rundles[1], Vincent R. Bonagura[2], Susan Schuval[2], Deborah Sanders-Laufer[1], Paul J. Edelson[1],and Margaret W. Hilgartner[1]. [1]The New York Hospital-Cornell Univ. Med. Ctr, New York, NY and [2]Schneider Children's Hospital, New Hyde Park, NY.

The development of the normal immune system may be different in the HIV exposed infant compared to the normal infant in response to usual developmental signals affecting cells other than CD4+ T cells. We have studied development of the NK system in infants under 1 year in comparison with HIV infected infants as a possible basis for unexplained progression to AIDS in a subset of infants who showed no indication of viral effect as determined by normal CD4 T cell numbers and absence of clinical changes. In our population we have found that among those HIV exposed infants with normal CD4 numbers and CD4/CD8 ratio, 25% may suddenly progress to Pneumocystis carinii pneumonia, PCP. Studies showed that HIV exposed infants showed marked expansion of cells seen during fetal life expressing CD38, a change which was also noted in HIV+ hemophiliacs to be associated with disease progression and decline of CD4+ T cells but was not associated with this change in infants. However infants who had this phenotype (with normal CD4+ T cell number) and progressed to PCP without warning were found to have reduced NK cell activty compared to their age matched counterparts ($p<0.01$)and reduced reponse to interferon alpha in vitro ($p<0.01$) against the K562 target cell. HIV exposed infants had variable numbers of NK cells by phenotyping but not statistically different compared to controls. These studies suggest compromise of natural host defense system in utero during HIV exposure. Supported in part by NIH RO1 HL43781 and The Children's Blood Foundation.

WD 105 ANALYSIS OF HIV ISOLATES OBTAINED FROM PEDIATRIC PATIENTS: BIOLOGICAL PHENOTYPE AND DRUG SENSITIVITY, William Don Decker, Michael Cooney, Marilyn J. Crain, and Robert W. Buckheit, Jr. Retrovirus Research Section, Southern Research Institute and Department of Pediatrics, Children's Hospital, University of Alabama at Birmingham

We have obtained HIV isolates from the plasma, peripheral blood lymphocytes and peripheral blood monocytes of pediatric patients. These isolates were obtained by established co-culture techniques, allowing estimation of the titer of infectious virus in the blood and blood cells and definition of the biological phenotype of the isolates obtained. The phenotype of the isolates has been defined in terms of their cell tropism, their syncytium-forming ability, their ability to grow in fresh and cultured human cells, and their replicative capacity in acutely infected fresh and cultured human cells. The cytopathicity of these isolates were compared with the disease status of the infected child to determine whether their is a relationship between the virulence of the isolate and disease progression. We have been especially interested in the appearance of drug resistant virus isolates in both infected mothers and children and the ability of the pregnant mother to infect the fetus with drug resistant virus. This capability will be of great concern in the successful antiviral therapy of the infected child after birth. We have examined the drug sensitivity of virus isolates obtained prior to and during the course of AZT therapy in the infected children. These ongoing studies will examine the progression of disease in infants born with drug resistant virus isolates and the ability to adequately treat these children with antiviral agents.

WD 106 THE ROLE OF THE CDR-3-LIKE DOMAIN OF CD4 IN VIRAL ENTRY OF LABORATORY VERSUS CLINICAL HIV-1 ISOLATES, Lee E. Eiden, Jeffrey D. Lifson and Dianne M. Rausch, Laboratory of Cell Biology, NIMH and Genelabs, Incorporated, Redwood City, CA 94063

The relative ability of a CD4(81-92)-derived peptide (#30, Kalyanaraman et al., J. Immunol. 145:4072, 1990) and soluble CD4 (sT4) to block viral replication in human peripheral blood mononuclear cells (PBMCs) co-cultured with diluted whole blood obtained from HIV-1-positive human donors was compared to their relative ability to block entry of the laboratory isolate HIV-1HTLVIIIB into CD4-positive lymphoblastoid cells.

Soluble CD4 (sT4) blocked HIV-1 HTLVIIIB entry into CD4-positive CEM-SS cells in culture with an IC50 of ~0.5 µg/ml, whereas #30 blocked infection with an IC50 of ~ 80 µg/ml. In contrast, infection of human PBMCs co-cultured with untitered peripheral blood cells from different HIV-1-positive individuals was blocked by #30 at 250 µg/ml in 3/5 cases while sT4 at10 µg/ml blocked viral replication in 0/5 cases. Thus, #30 inhibited infection by a prototype laboratory isolate, HIV-1HTLVIIIB, and by several primary/clinical isolates at similar doses, while sT4, as previously reported, inhibited infection by primary/clinical isolates at least 100-fold less potently than infection by HIV-1HTLVIIIB.

These data are consistent with the hypothesis that the CDR2- and CDR3-like domains of the CD4 receptor may be differentially engaged during infection by laboratory compared to clinical HIV-1 isolates.

Dr. Philip Pizzo (Pediatrics Branch, NCI) provided clinical pediatric samples used in these studies.

WD 107 PRIMARY CULTURES OF RHESUS PLACENTAL SYNCYTIOTROPHOBLASTS ARE PERMISSIVE FOR TRANSIENT SIV INFECTION, Thaddeus G. Golos, Carolyn Stone Williams and Kevin T. Schultz, Wisconsin Regional Primate Research Center and Department of Pathobiological Sciences, University of Wisconsin, Madison, WI 53715-1299. The mechanisms of fetal-maternal transmission of HIV are unknown, but are likely to involve at least transient infection of placental syncytiotrophoblasts. To understand the events which give rise to fetal infection, we have investigated whether rhesus placental syncytiotrophoblasts can be infected with and support SIV viral replication *in vitro*. Rhesus placental cytotrophoblasts were obtained by trypsin-DNase dispersion of rhesus placental chorionic villous tissue and purified by Percoll gradient centrifugation. Cytotrophoblasts differentiate and fuse in culture to form multinuclear syncytiotrophoblasts which express syncytiotrophoblast-specific genes. Five to seven days after plating, cultures were incubated with 10 TCID of SIV_{mac251} or SIV $Delta_{B670}$. Spent culture medium was analyzed weekly for 4 weeks for the presence of virus by p24/27 antigen capture assay. Placental cells were equally permissive for both viruses, but failed to release virus by the 4th week of culture: levels of viral antigen were 2.77 ± 0.33, 2.10 ± 0.12, 1.21 ± 0.24, and 0.51 ± 0.12 ng/ml ($n=4$) at 1, 2, 3 and 4 weeks postinfection. Viral antigen was undetectable at each time point with nonpermissive A549 cells. Placental cell antigen levels were approximately three-fold lower than with infected rhesus PBLs, in parallel experiments. At 4 weeks, placental cells were lysed and intracellular virus antigen levels were 1.56 ± 0.19 ng/ml. At 2 and 4 weeks postinfection, CEM X174 cells were cocultured with placental cells for 24 h to examine the ability of placental cells to transfer virus by cell-cell contact. Placental cells coinfected CEM X174 cells at 2 weeks as well as 4 weeks, when cells were not shedding virus into the culture medium. We conclude that cultured rhesus placental syncytiotrophoblasts can be infected with two SIV strains, and that although viral replication decreases with time in culture, syncytiotrophoblast may remain latently infected. This culture system will be useful for understanding maternal-fetal immunodeficiency virus transmission in the most appropriate animal model for HIV.

WD 108 UNSTIMULATED FETAL GLIAL CELLS RELEASE SOLUBLE FACTORS WHICH INDUCE HIV EXPRESSION AND WHICH RESPOND DIFFERENTIALLY TO REDUCING AGENTS, Thea Kalebic and Philip A. Pizzo, Pediatric Branch, National Cancer Institute, Bethesda, Md 20892.

Rapid progression of neurologic impairment in young children with perinatally acquired infection with HIV suggests that encephalopathy may originate during fetal development. We worked on hypothesis that fetal glial cells secrete soluble factors which induce the expression of latent HIV harbored in monocytic cells. It is conceivable that infiltrated moncytic cells releasing budding viral particles play a role in pathogenesis of AIDS-related encephalopathy. We also investigated whether the HIV-inducing activity of soluble factors released by fetal glial cells may be suppressed by reducing agents. As a cellular model of HIV latency, we used chronically infected promonocytic U1 cells, which harbor in their genome two copies of proviral DNA. U1 cells were treated with the conditioned media from human fetal glial cell lines SVG and POJ. We found that the conditioned media from both fetal glial cell lines induced the activation of HIV expression in U1 cells in dose dependent manner. We showed that TNFα was one of the factors secreted by fetal glial cells. Pretreatment of U1 cells with GSH or NAC did not suppress HIV expression in the cells stimulated with conditioned media derived from fetal glial cells SVG and POJ. However, the HIV-inducing capacity of TNFα was inhibited by its preincubation with glutathione (GSH), but not with N-acetyl cysteine (NAC). Our observations that unstimulated fetal glial cells may induce HIV expression in vitro suggests that a similar effect may occur in vivo. Further study is needed to identify other HIV-inducing factors released by fetal glial cells and to elucidate the effect of reducing agents on those inducers.

WD 109 MATERNAL VIRAL LOAD AS A RISK FACTOR FOR PERINATAL HIV-1 TRANSMISSION. Keith Krasinski, Yunzhen Cao*, William Borkowsky, and David Ho*. Departments of Pediatrics and Medicine*, New York University Medical Center-Bellevue Hospital Center, and the Aaron Diamond Center for AIDS Research*, New York, NY 10016.

Determinants of HIV-1 perinatal transmission remain to be elucidated. Using standard PBL culture technique, with 1-2 x 10^6 cells, HIV-1 was recovered from 21/33 (64%) of pregnant HIV-1 infected women near the time of birth. There have been 32 children delivered to this cohort of HIV-1 infected women, including: 5 seroreverting children, 11 known HIV-1 infected children, and 16 children of indeterminate (PO) status with negative HIV-1 cultures. Perpheral blood mononuclear cell cultures of 14 mothers at the time of birth indicate 89% transmission among 9 mothers who were PBMC culture positive, and 20% transmission among 5 mothers who were PBMC culture negative. Using quantitative (limiting dilution) PBMC cultures, the geometric mean number (GMN) of maternal PBMC's needed to detect virus in culture among transmitting mothers was 13,220; whereas, it was 3,981,071 for known non-transmitting mothers (This high value results from scoring negative cultures as 10^7 for calculation of GMN). Plasma viremia occurred in 2/7 transmitting mothers, 0/3 non-transmitting mothers and 1/10 mothers of PO children. Plasma viremia was associated with high level (only 10^4 PBMC needed) HIV-1 infection in the two transmitter mothers and the one mother of an infant with indeterminant outcome, in whom plasma viremia was detected.

These preliminary data suggest that plasma viremia and a high multiplicity of cellular infection may be important determinants of maternal-fatal HIV-1 transmission.

WD 110 LACK OF CD4 ANTIGEN OR mRNA EXPRESSION IN PLACENTAL SYNCYTIOTROPHOBLASTS.

M. Lairmore[1], P. Cuthbert[2], C. Morgan[3], C. Dezzutti[1], C. Anderson[4], and D. Sedmak[2]. Departments of Veterinary Pathobiology[1], Medical Pathology[2], Surgery[3], and Internal Medicine[4], The Ohio State University, Columbus, OH.

Critical to the understanding of maternal to fetal transmission of HIV-1 is knowledge concerning the expression of the CD4 receptor on placental syncytiotrophoblasts (STB), the primary cellular barrier between maternal blood and fetal tissues. STB expression of CD4, the principal HIV-1 receptor, is controversial. We examined both frozen placental sections and highly purified human cytotrophoblasts and STBs by immunohistochemical techniques with 3 different monoclonal antibodies to CD4 (OKT4, Ortho; CCT4, Coulter; Leu 3a, Becton Dickinson). Five normal term placentas and 5 immature placentas (18-27 weeks) showed intense staining of Hofbauer cells; however STBs, cytotrophoblasts, stromal cells, and endothelial cells were uniformly negative. Cultured immunopurified cytotrophoblasts, which mature to STBs and produce human chorionic gonadotropin did not express CD4 antigen at any stage of differentiation (N=3 placentas). Northern blot analysis and reverse transcriptase polymerase chain reaction of purified STBs revealed no CD4 mRNA, in contrast to control lymphocytes or Hofbauer cells contaminating STB cultures. These data indicate that human syncytiotrophoblasts do not express the CD4 antigen or mRNA; the transmission of HIV-1 across the placenta must occur by alternative mechanisms.

WD 111 VERTICAL HIV-1 INFECTION: EVIDENCE FOR INTRAUTERINE VS. INTRAPARTUM TRANSMISSION, Katherine Luzuriaga, Patricia McQuilkin, Ariane Alimenti, John L. Sullivan, Department of Pediatrics, Program in Molecular Medicine, University of Massachusetts Medical Center, Worcester, MA 01605. Thirty-three children born to HIV-1 seropositive women have been evaluated sequentially from birth or within the first week of life for evidence of vertical HIV-1 infection. Infection has been documented in five of these children. HIV-1 was isolated from plasma and PBMC, viral genome was detectible by PCR, and p24 antigen was detectible (acid hydrolysis method; ICD p24) from the cord blood and multiple subsequent blood specimens of one child, suggesting intrauterine transmission. Cord blood or early neonatal specimens from four children were negative by plasma and PBMC culture, PCR, and ICD p24 antigen. High titers of HIV-1 were detected in the plasma (100-39,000 TCID/ml) and PBMC (100-5000 TCID/10^6 PBMC) of these four children by eight weeks of age; PCR and ICD p24 were positive at these time points as well. The evolution of these four patients suggests that they were infected perinatally; the rise and subsequent fall of the plasma and serum p24 levels likely represent primary viremia. Three of the four infants were asymptomatic during this primary viremia while one infant was symptomatic with fever, lymphadenopathy, hepatosplenomegaly, and rash. In summary, the methods of quantitative culture, PCR, and ICD p24 can be used to distinguish intrauterine versus intrapartum transmission. Our data suggest that intrapartum transmission occurs more frequently than intrauterine transmission and that the majority of children infected during birth are asymptomatic during their primary viremia.

WD 112 HUMAN PLACENTAL CELL INFECTION WITH HIV-1, Kathleen A. McGann, Ronald Collman, Jerome F. Strauss, Francisco Gonzalez-Scarano, Neal Nathanson. Children's Hospital of Philadelphia and University of Pennsylvania Medical Center, Philadelphia, PA 19104.

Vertical transmission accounts for more than 80% of the cases of pediatric AIDS. Intrauterine transmission is believed to be responsible for a significant proportion of those cases transmitted vertically, and a number of studies have demonstrated virus in the placentae of HIV-infected women. Our laboratory is studying the role of the placenta in intrauterine transmission to determine if active placental infection is a critical step in transmission of HIV to the fetus. We have utilized primary placental cultures, as well as transformed trophoblastic cell lines, and examined their permissiveness for replication using a panel of HIV-1 isolates with varying phenotype.

Primary trophoblast-enriched cultures derived from term human placentae support the replication of five different strains of HIV-1: lymphocyte-tropic strains III B and SF-2, macrophage-tropic strains 89.6 and SF-162, and a pan-tropic strain DV. Varying degrees of permissiveness for different HIV strains are demonstrable with these cells. Infected cultures produce moderate levels of viral p24 antigen (up to 2.5 ng/ml), which are sustained beyond 4 weeks in culture. Immunofluorescent microscopy demonstrates viral antigen in the cytoplasm of both trophoblasts and placental macrophages, but no cytopathic effect has been observed. In contrast, transformed trophoblastic cell lines do not support HIV-1 replication.

Thus, primary trophoblast-enriched cell cultures support a moderate level of replication by a variety of HIV-1 isolates, which suggests that direct placental infection may play a role in the intrauterine transmission of HIV. Studies are underway to further examine the pattern of permissiveness for HIV isolates and to characterize the kinetics and mechanics of placental cell infection by HIV-1.

WD 113 HIV-1 IgA ANTIBODIES IN INFANTS AT RISK, Marisa Mussi-Pinhata*, Cecelia Hutto, Rebeca Geffin, Walter Scott, Gwendolyn Scott, Division of Pediatric Immunology and Infectious Diseases, University of Miami School of Medicine, Miami, FL, 33101, * Department of Pediatrics, Faculty of Medicine at Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil, 14049.
To examine the utility of HIV-1 IgA antibody detection for diagnosis of infection in infants at risk and the development and persistence of HIV-1 IgA in infected infants, 108 sera obtained before 15 months (mo.) of age from 43 infants (36 HIV-1+, 7 HIV-1-) were studied. IgG was depleted with protein G from all sera before testing with an immunoblot assay. A mixture of 5 HIV-1 recombinant proteins was used in the immunoblot. IgA was detected in 27/36 (75%) of infected infants or 12/23 (52%) <3 mo., 15/23 (67%) 3 to 6 mo., 8/13 (62%) 6 to 9 mo., 13/15 (87%) 9 to 12 mo., and 11/12 (92%) infants >12 mo. of age. IgA was detected more frequently in symptomatic (Class P2) than asymptomatic (P1) children, 77% vs. 52%, respectively. None of the 22 sera from the uninfected infants had IgA antibody. For 24 infants, two or more sera obtained sequentially beginning by 6 months of age (mean 2.4 mo.) and followed to a mean age of 11 months were tested. IgA was detected initially in 17/24 (71%) by 6 mo., and 2/24 (8%) by 10 mo. In all but one infant, IgA was present in all subsequent sera after its first detection. Five of 24 infants with 2 to 4 samples each and followed to a mean age of 9.4 months never had detectable HIV-1 IgA. 3/5 had hypogammaglobulinemia consistently after 3 months of age (IgG≤300 mg/dl, IgA≤15 mg/dl). All 3 were symptomatic. HIV-1 IgA antibodies occur in most infected infants, persist for months, but are variable in age at onset. IgA is probably not associated with stage of disease but rather its increased frequency in symptomatic children correlates with age at development. This assay has good specificity, correctly identifying 52% of infected infants by 3 months. Together with other diagnostic assays, IgA seroassays will significantly enhance early detection of HIV-1 in infants.

WD 114 MATERNAL-FETAL TRANSMISSION OF SIV IN MACAQUES. Hans D. Ochs[1], William R. Morton[2], Che-Chung Tsai[2], Qili Zhu[1], LaRene D. Kuller[2], Margaret E. Thouless[2], Mary Welsh[2], Raoul E. Benveniste[3]; [1]Department of Pediatrics and [2]Regional Primate Research Center, University of Washington, Seattle, WA [3]National Cancer Institute, Frederick, MD
To study maternal-fetal transmission of HIV, we have developed a non-human primate model using the simian lymphotropic immunodeficiency virus, SIV/Mne. To date we have inoculated intravenously twelve pregnant *Macaca (M.) nemestrina* with an uncloned isolate of SIV/Mne. All became virus positive by 2-3 weeks after inoculation and all but two developed antibodies to SIV. Most had neutralizing antibodies demonstrable two to three months after inoculation. Three pregnant macaques were inoculated during the first trimester. Two aborted, at 14 and 69 days post inoculation; one delivered a stillborn baby 101 days post inoculation. One of the abortuses was SIV positive by culture and PCR; the SIV status of the other abortus remains unknown; the stillborn was virus negative by culture, PCR and immunohistochemistry. Four pregnant females were inoculated with SIV/Mne between 90-100 days of pregnancy (mid-second trimester). Two offspring are healthy at four months of age and virus negative, by culture and PCR. One offspring, virus positive at birth and throughout life, died at two months of age with symptoms of SAIDS. The fourth macaque delivered a nonviable premature infant three weeks after inoculation; this infant was virus negative (PCR, culture and in situ hybridization). Of three animals inoculated during mid-third trimester, two delivered healthy noninfected infants. The third offspring became virus positive by culture and PCR at one month and remained virus positive until death due to generalized adenovirus infection at six months of age. Except for the first two months when maternal antibody was present, no anti-SIV antibody of infant origin was detected in the SIV-infected infants. To explore the effect of immunosuppression on maternal-fetal transmission, we treated two pregnant animals with cyclosporin A (5 mg/kg/day) and prednisolone (depo-medrol, 5.6 mg/kg/week) starting at day 100 and continuing throughout the pregnancy. Inoculation with SIV was performed on day 110 of pregnancy. One immunosuppressed animal's offspring was in breech position and died during C-section; culture, PCR were negative for SIV. The other delivered a full term infant, which is still SIV negative at 4 months of age. Anti-SIV antibody did not correlate with protection and seems to play a minor role in the protection of the offspring from vertical transmission of SIV.

WD 115 DEVELOPMENT AND SIGNIFICANCE OF ZIDOVUDINE (ZDV) RESISTANCE IN CHILDREN INFECTED WITH HIV. Mark T. Ogino, Wayne M. Dankner and Stephen A. Spector. University of California, San Diego, La Jolla, CA 92093

Although occurence of ZDV resistance is well-established in HIV-infected adults, less is known about the development of resistance in children. Little is known regarding the impact of the development of resistance on disease progression. Using a PBMC based assay to measure ZDV sensitivity, paired HIV isolates from 10 children (mean age: 5 yrs; range 17 mos-10 yrs) were evaluated for their sensitivity to ZDV. All 7 isolates from children without prior exposure to ZDV were sensitive at $\leq$1.0 μM (mean: 0.3 μM; range: 0.001-1.0 μM). In 7 of 10 paired isolates, sensitivity decreased by 10 to 1000-fold after $\geq$5 mos of ZDV (mean: 10.5 mos; range 5-18 mos). The sensitivity of HIV isolates obtained from children on ZDV could be separated into two groups: 4 with isolates sensitive to ZDV at $\leq$1.0 μM and 6 resistant to ZDV at $\geq$10 μM. All 6 children with resistant isolates had progressive disease which included poor growth, serious bacterial infections and significantly declining $CD4^+$ cell counts; 3 children have been discontinued from ZDV because of progressive disease. Of the 4 children whose HIV isolates remained ZDV sensitive, 3 are doing well and 1 developed a lymphoma. Children with ZDV resistant isolates had significantly lower $CD4^+$ cell counts (mean $\pm$ SD/mm^3; 84 $\pm$ 102) compared to children with sensitive isolates (639 $\pm$ 511). These findings suggest: (1) HIV isolates from children without previous exposure to ZDV are sensitive to ZDV; (2) Treatment of some children with ZDV results in resistance; (3) Resistant HIV isolates are more common in children with low $CD4^+$ cell counts; and (4) ZDV resistant HIV strains lead to diminished drug efficacy and more rapid disease progression.

WD 116 VERTICAL HIV INFECTION SUCCESSFULLY DETECTED IN THE FIRST TWO MONTHS OF LIFE BY POLYMERASE CHAIN REACTION, Tamara Rakusan, Carl Brandt, Antonio Sison*, Ela Saxena, Janie Eddy, John Sever, Children's Hospital, The George Washington University, Washington, DC 20010, and *Georgetown University, Washington, DC 20007

We present data indicating that HIV polymerase chain reaction (PCR) using biotinylated probes performed according to the methods we recently reported (J Clin Microbiol 30, Jan. 1992) is a sensitive, specific technique for the identification of HIV-infected infants in the first 2 months of life. Also, the age when blood samples first were found PCR positive suggests the timing of infection.

We tested 90 HIV-seropositive infants in the first 2 months of life. Outcome is now known for 28: 16 are infected with HIV (CDC class P1, P2), and 12 are uninfected (P3, seroreverters). Of the 16 infected infants, all were PCR positive during the first 2 months of life. Of 5 tested at age 1 day, 4 were positive (including 1 trace positive), 1 was negative. Of the 14 tested at 1 month, all were positive as were 2 tested at 2 months. Of the 12 uninfected infants, 1 was tested at 1 week, 7 at 1 month and 6 at 2 months; all tested negative.

PRESENT HIV STATUS	HIV PCR AT AGE 0-2 MONTHS	
	PCR+	PCR-
P0 (Indeterminate)	3	59**
P1, P2 (Infected)	16	0
P3 (Uninfected)	0	12
Total	19	71

** Included is 1 infant who tested negative at 1 month, but positive at 4 and 7 months; 15 infants were tested at 1 day only and have not been retested.

Of 62 infants whose HIV status remains undetermined at this time, 3 were found PCR positive: all of them tested positive at 1 month, and 1 of 2 tested was also positive at 1 day.

We conclude that PCR is a sensitive tool for detection of HIV infection in the first 2 months of life. Detection of HIV DNA in a majority of infected infants tested at age 1 day suggests that vertical transmission primarily occurs during pregnancy rather than during or after delivery.

WD 117 RECONSTITUTION OF NEONATAL SCID MICE WITH CORD BLOOD LEUKOCYTES (HU-CBL-$_{neo}$-SCID MICE) AS A MODEL FOR PEDIATRIC HIV INFECTION. Barbara Reinhardt, Bruce E. Torbett, Peter P. Reinhardt, Richard J. Gulizia, Donald E. Mosier and Stephen A. Spector. University of California, San Diego and Medical Biology Institute, La Jolla, CA.

SCID mice accept xenografts of human peripheral blood leukocytes to yield hu-PBL-SCID mice permitting infection with laboratory and clinical HIV-1 isolates. To develop a model for pediatric HIV infection, adult SCID mice were initially reconstituted with neonatal PBMCs. Although engraftment of human cells permitted HIV-1 infection of some animals, further improvements were necessary. Therefore, we reconstituted neonatal SCID mice with human cord blood mononuclear cells (hu-CBL-$_{neo}$-SCID mice). These mice showed successful reconstitution for at least 12 wks with human lymphoid cells present in the peritoneal cavity, spleen, liver, lymph nodes and circulating in peripheral blood. Further support for engraftment of human cells was the increasing amounts of hu-IgG and hu-IgM observed from 2-8 wks after reconstitution in hu-CBL-$_{neo}$-SCID mice: (mean μg/ml $\pm$ SE) at 2 wks: IgG 590 $\pm$ 245 and IgM 110 $\pm$ 44 compared to IgG 4000 $\pm$ 1000 and IgM 360 $\pm$ 240 at 8 wks. $CD4^+/CD8^+$ ratios in hu-CBL-$_{neo}$-SCID mice were >1 in blood, spleen and lymph node compared with higher levels of $CD8^+$ T-cells in SCID mice reconstituted with adult cells. FACS analyses of T-cells expressing the CD45RA (naive T-cell subset) marker and CD45RO (memory T-cell subset) marker showed that the percentage of CD45RO T-cells increased during the first 4 wks following reconstitution. This finding suggests either selective activation or survival of the memory T-cell subset resembling the immunologic development which occurs in children. Thus, the characteristics of the hu-CBL-$_{neo}$-SCID mouse make it a promising model to study early pediatric HIV infection and therapeutic regimens.

WD 118 PRENATAL INFECTION OF RHESUS MONKEY FETUSES WITH THE SIMIAN IMMUNODEFICIENCY VIRUS: A MODEL TO STUDY LENTIVIRAL PATHOGENESIS DURING ONTOGENY, Ruth M. Ruprecht, Fatemeh Fazely, Prem L. Sharma, Michael S. Wyand, Mushtaq Memon, Norman Letvin and Michael F. Greene, Dana-Farber Cancer Institute, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115 and TSI Mason Research Institute, Worcester, MA 01608.

Because of an increasing prevalence of infection with the human immunodeficiency virus type 1 (HIV-1) among young women, the number of children with the acquired immunodeficiency syndrome (AIDS) will rise. While the timing of maternal HIV-1 transmission is difficult to determine, intrauterine fetal infections have been documented. An animal model would play an important role for elucidating lentiviral pathogenesis during gestation and for evaluating prophylactic strategies. Because maternal transmission of the simian immunodeficiency virus (SIV) occurs rarely, if ever, in rhesus monkeys, SIV_{mac251} was inoculated directly into the fetal compartment. Initially, 4 fetuses were injected intravenously with SIV during hysterotomy. Fetal organ distribution studies revealed a high SIV copy number in spleen, lymph nodes, lung and skin, with lower amounts of virus present in the brain and thymus. Because of a high rate of fetal loss, a less invasive approach was used, and a second group of 4 pregnant animals underwent SIV inoculation of the amniotic fluid under ultrasound (US) guidance. Three out of 4 fetuses became infected, and in the fourth case, the placenta was SIV positive. All fetuses were viable. Several animals born infected showed signs of pediatric AIDS, such as low birth weight, failure to thrive, lymphadenopathy and low CD4 counts. We conclude that US-guided amniotic fluid inoculation is a safe, reproducible means to establish in utero SIV infection at a high rate. This direct approach may be used to study lentiviral pathogenesis during ontogeny and to test different prophylactic strategies.

WD 119 THE REPLICATIVE CAPACITY OF HIV-1 ISOLATES FROM INFECTED MOTHERS CORRELATES TO RISK OF PERINATAL HIV-1 TRANSMISSION, Gabriella Scarlatti*, Jan Albert#, Paolo Rossi** and Eva Maria Fenyö*, *Deptartments of Virology and **Immunology, Karolinska Institute, and #Department of Virology, National Bacteriological Laboratory, Stockholm, Sweden.

The mechanisms involved in mother-to-child transmission of HIV-1 are unclear. Several maternal factors have been investigated to find a correlation to vertical transmission. We have attempted to study the maternal immunological response to the virus by neutralization assay against heterologus viral strains as well as against autologus isolates. The serum of 16 mothers taken during pregnancy, at delivery or short after delivery was tested against their own viral isolate, taken at the same timepoint as the serum, as well as against HIV-MN and HTLV-IIIB. To investigate the presence of broadly reacting antibodies the maternal sera were also tested against the isolates of the other mothers. The mothers were divided in 2 groups: transmitting (n=7) and non-transmitting (n=9), according to the results obtained by polymerase chain reaction and virus isolation in their children. Clinical stage, HIV-antigenemia and CD4+ lymphocyte count of the mothers were known at the moment of sampling (G.Scarlatti et al.,AIDS 1991,5:1173). Furthermore, the replicative capacity of the isolates of 3 transmitting and 6 non-transmitting mothers were tested in a variety of cell lines of T-lymphoid and monocytoid origin (Jurkat, Jurkat tat, CEM and U937 clone2).

Low neutralizing titers (1/80) against the autologus viral isolate were found in 1 transmitting and 2 non-transmissing mothers. Another 2 transmitting and 1 non-transmitting mothers presented a low neutralizing activity against HIV-IIIB or MN. Two non-transmitting mothers showed low titers of neutralizing activity when tested against the isolate of other mothers. No correlation was found between the presence of neutralizing antibodies and the risk of transmission of the virus from mother to child. In contrast, the replicative capacity of the isolates of all 3 transmitting mothers showed a rapid/high pattern, and those of the 6 non-transmitting mothers showed a slow/low pattern.

Thus neutralizing antibodies of the mother appear not to be involved in the protection of the child from HIV-1 infection. However, the rapid/high replicative phenotype of the maternal virus seems to be correlated with an increased risk of perinatal HIV-1 transmission.

WD 120 IN VITRO T CELL IMMUNITY TO HIV SYNTHETIC PEPTIDES AND OTHER ANTIGENS BY PERIPHERAL BLOOD LEUKOCYTES FROM HIV-INFECTED WOMEN AND BY CORD BLOOD FROM THEIR OFFSPRING,

G. M. Shearer*, A. V. Sison#, T. Rakusan*, C. Brandt*, J. A. Berzofsky^, J. L. Sever+, and M. Clerici*, *Experimental Immunol. and ^Metabolism Branches, Natl. Cancer Inst., Natl. Insts. of Health, Bethesda, MD 20892; #Dept. of Obstetrics/Gynecology, Georgetown Univ. Med. Sch., Washington, D.C. 20007; + Dept. of Pediatrics, George Washington Univ., Washington, D.C. 20010.

Early detection of HIV infection in newborns is an important problem. An assay that detects HIV infection prior to or at birth would be valuable for evaluating the status of newborns, and for understanding the pathogenesis of disease. To test whether newborns and their mothers have T cell immunity to HIV peptides and other antigens, peripheral blood leukocytes (PBL) from HIV-seropositive (HIV+) pregnant women, and cord blood leukocytes (CBL) collected at delivery, were studied for in vitro T helper cell (Th) function by antigen- and mitogen-stimulated interleukin 2 (IL-2) production.The pregnant women (15-to-40 years old) were asymptomatic except for one diagnosed with AIDS. Four women were diagnosed HIV+ during pregnancy, and five had a mean CD4 count of 537/µl at delivery. One woman was diagnosed with AIDS. One birth was premature (36 weeks). The immunologic stimuli included: influenza A virus (FLU); allogeneic PBL (ALLO); PHA; and the following synthetic peptides corresponding to the *env* of HIV-1: T1; T2; Th4; P18 IIIB; and P18 MN. Of the16 pregnant women studied: all responded to ALLO and PHA; 14 responded to FLU; 12 responded to at least one HIV synthetic peptide; and seven responded to more than one peptide, in contrast to only 7/132 seronegative controls responsive to any peptide. Of seven CBL samples tested: all responded to PHA; five responded to ALLO; none responded to FLU; and four responded to at least one peptide. CBL from seronegative control deliveries were unresponsive to the peptides.The peptide specificity patterns of the CBL samples were different from those of their mothers, suggesting that the CBL were not of maternal origin. Peptide-specific cytotoxic activity was detected in two of three CBL samples tested. CBL and newborn PBL were tested for evidence of HIV infection by detection of HIV DNA using the polymerase chain reaction, and by detection of virus by culture. Primer pairs used were: SK 145/150; SK 38/39; SK 29/30: and SK 68/69, and detected using biotinylated probes. A specimen was considered to be PCR+ if HIV DNA was detected with at least one primer pair. The only newborn who was HIV+ by PCR was unresponsive to the peptides, and was the offspring of a mother who was unresponsive to recall antigens, including HIV peptides.Our results indicate that: a] 86% of the mothers responsive to FLU were responsive to at least one peptide; and b] T helper and effector immune responses can be generated against HIV peptides in utero.

WD 121 HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) ENVELOPE GLYCOPROTEIN AND ITS PEPTIDE ANALOGS BIND TO CALMODULIN AND INHIBIT CALMODULIN REGULATED ENZYMES. Ranga V.Srinivas, Shamala K. Srinivas, G.M. Anantharamaiah, Jere P. Segrest, and Richard W. Compans. Departments of Microbiology and Medicine, University of Alabama at Birmingham, Birmingham, AL 35294

Calmodulin, the major intracellular receptor for calcium, is intimately involved in early events of T lymphocyte activation, and requires an increase in intracellular calcium for its activity. Calmodulin antagonists inhibit T lymphocyte proliferation. Positively charged amphipathic helical segments have been identified as an important structural motif in recognition of calmodulin (CaM) by different calmodulin-activated enzymes and peptides. The carboxy-terminal domain of the envelope glycoproteins of human and simian immunodeficiency viruses (HIV-1, HIV-2 and SIV) contain regions that can fold into amphipathic helical segments which closely resemble the amphipathic segments found in calmodulin activated enzymes. Here we show that purified HIV-1 gp160, but not gp120 (which lacks the putative amphipathic helical segments) binds to calmodulin. Also, peptide-mimics of amphipathic helical regions (residues 768-788 and 826-854 of HIV-1/WMJ22) from HIV gp160 bind to calmodulin in a calcium-dependent manner. Furthermore, these peptides inhibit calmodulin-regulated activation of phosphodiesterase (PDE). In HIV-infected cells, the putative calmodulin-binding regions of gp160 are located intracellularly, and may therefore interact with the cytosolic calmodulin, thus disrupting calmodulin-regulated cellular functions. Based on these studies, we suggest that the envelope proteins may modulate calmodulin function in HIV-infected cells and contribute to viral cytopathology, and pathogenesis.

WD 122 QUANTITATIVE ASSAY FOR HIV-1 mRNA AND VIRION RNA FROM PERIPHERAL WHOLE BLOOD SAMPLES. G.Tudor-Williams[1], M.Klotman[2], R.C.Gallo[2], P.Pizzo[1]. Pediatric branch[1] and Laboratory of Tumor Cell Biology[2], National Cancer Institute, National Insitutes of Health, Bethesda, Maryland, 20892.

We have developed an assay for quantitating viral burden directly from 1.0 ml. samples of peripheral whole blood. Total RNA is extracted by an acid guanidinium thiocyanate method, which allows storage of samples at 4° C. for up to 48 hours before processing. We have constructed a plasmid containing the 2nd and 3rd exons of *tat* with a 55 base pair (bp) insert within the 2nd exon, under an SP6 promoter. The construct allows transription of quantitative amounts of RNA which are used to spike clinical samples prior to RNA extraction. Reverse transcription, and amplification by the polymerase chain reaction is performed using primers in conserved regions of the 2nd exon. Native mRNA and virion RNA yield 142 bp products which can be distinguished from the synthetic quantitated RNA product of 197 bp by polyacrylamide gel electrophoresis. Electroblots are analysed using a phosphor imager (Molecular Dynamics). Limiting dilution analysis using the synthetic transcripts have demonstrated a linear relationship between input RNA and signal over the range 0.1 pg. to 0.01 µg. By use of different primers, differential gene expression can also be studied quantitatively. The clinical utility of this assay is under evaluation.

WD 123 **HIV PROTEINS INFLUENCE THE DEVELOPMENT OF THYMOCYTES,**

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Previously, we have shown that HIV proteins impair proliferation of $CD4^+$ and $CD8^+$ peripheral blood T lymphocytes by interfering with early events in T cell activation, in particular by increasing cAMP levels. We have found that HIV proteins decrease proliferation and increase cell death in-vitro of human thymocytes. This is accompanied by an increase in intracellular cAMP levels. Others have shown that cell death in rat thymocytes is related to elevations of cAMP. The thymocyte subsets which survived in the presence of HIV proteins matured normally in response to cytokines and became "bright" $CD3^+$, single $CD4^+$ or $CD8^+$ cells. However, HIV proteins caused activation of these cells as reflected by an increase in expression of DR without an increase in expression of IL-2 receptor (p55). These findings may explain functional defects in the cellular immune system early in HIV infection in infants and children. In addition, the decrease in production of mature T cells in the thymus may contribute to the decrease in $CD4^+$ cells later in HIV infection.

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WD 124 SEQUENCE COMPARISON OF THE HUMAN IMMUNODEFICIENCY VIRUS ENVELOPE V3 LOOP AND CD4 - BINDING REGION IN INFECTED MOTHER/INFANT PAIRS, Nancy A. Wade, Don Rathbun and Lorraine Flaherty, Department of Immunology, Wadsworth Center for Labs and Research, New York State Department of Health, Albany, NY 12202 and Department of Pediatrics, Albany Medical College, Albany, NY 12208 Factors involved in the transmission of HIV from infected mother to her infant are not well defined. Mutations in the virus leading to strain variants which escape maternal antibody neutralization or variants with mutations in the CD4 - binding region which could lead to altered attachment to CD4 could be factors which may influence maternal transmission. To study this further we have amplified by polymerase chain reaction a 1000 base segment of the HIV envelope in three mother/child pairs. The amplified segment was cloned into Bluescript vector. Individual clones were sequenced by the dideoxy chain termination method. We present here sequence comparisons covering the V3 loop, the region of the principal neutralizing domain (a.a. 291-331) and the CD4 - binding region (a.a.413-456) in these mother/child pairs. As expected there were significant similarities between strains isolated from mother and child but also some differences. In addition there were nucleotide differences demonstrated between clones of an individual. All infants studied were between ages 3 and 9 months. It is expected that that these differences between mother and child will increase over time.

WD 125 CLONING AND EXPRESSION OF THE GENE FOR THE AVI-3 ANTIGEN OF *Mycobacterium avium* AND MAPPING OF ITS EPITOPES, Ryuji Yamaguchi, Kazuhiro Matsuo, Akihiro Yamazaki, Mitsuyoshi Takahashi, Yutaka Fukasawa, Masako Wada and Chiyoji Abe, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, 210, and Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Tokyo, 204, Japan

Mycobacterium avium-Mycobacterium intracellulare complex (MAC) consists of opportunistic human pathogens which generally infect immunocompromised patients, and the prevalence of MAC diseases has increased dramatically due to the advent of the acquired immunodeficiency syndrome (AIDS). Recent studies have reported that about 50% of AIDS patients are infected with MAC. Although *M. avium* and *M. intracellulare* are genetically heterogeneous, they are biochemically and morphologically indistinguishable. Thus, the development of a new diagnosis which is both rapid and accurate is desirable. The Avi-3 antigen, which is found only in *M. avium* culture sonicates, is species specific and results in strong skin test activity in guinea pigs sensitized with heat-killed *M. avium* but not in those sensitized with heat-killed *M. intracellulare* or *M. bovis* BCG. As a molecular approach to this antigen, its gene was cloned using a previously developed single probe method and was sequenced. The gene encoded a 194 amino acid polypeptide with a molecular weight of 21,500. A recombinant Avi-3 antigen expressed in *Escherichia coli* reacted with monoclonal and polyclonal antibodies raised against the native Avi-3 antigen. To identify epitopes on this protein for immunodiagnostic purposes to MAC diseases, various parts of the Avi-3 antigen were expressed as β-galactosidase fusion protein using pUR and pURS expression vectors. The clones screened by both antibody reactivity and T cell proliferative activity defined fragments with coexisting B and T cell epitopes. A B cell epitope (Asn-176 to Ala-186) and two T cell epitopes (Glu-75 to Ile-86 and Arg-155 to Leu-164) were thus defined. The synthetic polymerized peptides of the T cell epitopes were proven to elicit the delayed cutaneous hypersensitivity reaction in guinea pigs. The recombinant Avi-3 antigen and the synthetic peptide derived from the sequence of B cell or T cell epitopes may be useful for immunodiagnosis and detailed analysis of immune responses to virulent *M. avium*. In general this B and T cell epitope-mapping method would be useful in the development of a subunit vaccine consisting of an immunodominant B cell epitope linked to a T cell epitope in the vicinity.