HIV PATHOGENESIS IN INFANTS AND CHILDREN

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Immunopathogenesis in HIV Infection in Children

QZ 001 EARLY VIREMIA AND IMMUNE RESPONSES IN VERTICAL HIV-1 INFECTION, Katherine Luzuriaga, John L. Sullivan, Department of Pediatrics and Molecular Medicine, University of Massachusetts Medical School, Worcester, MA

Transmission of HIV-1 from infected mothers to the fetus or newborn occurs with a frequency of 13-45%. Two patterns of infection consistent with in utero or intrapartum transmission have been demonstrated. Demonstration of virus in cord blood and subsequent samples suggest in utero infection. Negative viral isolation and PCR studies on cord blood and early neonatal specimens with subsequent detection of viremia by 12 weeks of age is consistent with late in utero or intrapartum infection. Ten children were identified as infected, all within the first 8 weeks of life. Evidence of infection was found in cord blood or subsequent samples from 4 infected children, consistent with in utero infection. Virologic studies on cord blood and early neonatal specimens from the 6 other infected children were negative but became positive by 8 weeks of age, consistent with either late in utero or intrapartum transmission. Evidence of viral replication consistent with primary viremia was observed between 3 and 12 weeks of age. This viremia resolved in the absence of antiretroviral therapy and in the absence of circulating HIV-1-specific CTL or broadly neutralizing antibodies. Following acute infection, there is a progressive immune attrition with loss of CD4 T helper cells. Circulating virus specific cytotoxic T cells are not demonstrable in the majority of infants during the course of disease progression. Deficient immune responses following primary infection may explain the more rapid onset of symptomatic disease following vertical HIV infection.

QZ 002 ROLE OF HIV-1 INFECTION OF THYMOCYTES IN THE PATHOGENESIS OF PEDIATRIC AIDS. Christel H. Uittenbogaart, Deborah J. Anisman, Ingrid Schmid, Esther F. Hays, Grace M. Aldrovandi and Jerome A. Zack. UCLA School of Medicine, Los Angeles, CA 90024.

HIV infection leads to a decline in $CD4^+$ T cell numbers and is characterized by an impaired cellular immune function resulting in the development of AIDS. However, a functional defect in the cellular immune system is detectable early in HIV infection, at a time when numbers of $CD4^+$ T cells are within the normal range. The thymus is essential for normal T cell development and is particularly active during fetal and neonatal life. The great potential of the thymus to generate mature T cells before and after birth may explain the initial lack of low $CD4^+$ T cells in HIV-infected children. Thymic abnormalities have been reported in fetuses aborted from HIV seropositive women. Therefore, we studied the effect of HIV infection on human thymocyte maturation in-vitro and in-vivo in the SCID mouse model. Since thymocytes can be infected with HIV, we investigated which cytokines were involved in augmenting HIV production. Thymocytes were infected with clinical isolates, JR-CSF or JR-FL, and cultured in serum-free medium in the presence of the cytokines IL-2, IL-4 and IL-7 (alone or in combination). Previously, we have shown that IL-2 is a growth factor for mature thymocytes, while IL-4 and IL-7 are differentiation factors for immature thymocytes. We found that after 10-14 days of culture, both IL-4 and IL-7 promote the appearance of large thymocytes with a mature phenotype. Mature cells express high density ("bright") CD3, "bright" CD4 and/or CD8 and "bright" CD45RA. Our results show that IL-2 and IL-4 or IL-4 and IL-7 act synergistically to produce high levels of viral antigen from infected thymocytes. The mechanism of the synergy is under investigation. It is possible that the cytokines cause synergy in HIV production by inducing maturation of different thymocyte subsets or by induction of other cytokines. In order to determine if HIV interferes with thymocyte maturation, we evaluated phenotypic changes of HIV-infected and non-infected thymocytes in parallel cultures. HIV did not impair in-vitro thymocyte maturation. However, there was a decrease in the percentage of $CD4^+CD8^+$ cells which may be related to an increase in apoptosis induced by HIV infection. These changes were observed after infection with live-virus and after culture with a mixture of viral proteins. A decrease in the percentage and number of $CD4^+CD8^+$ cells was also observed when HIV was injected into fetal liver/thymic grafts inplanted under the kidney capsule of SCID mice. Histology of the thymic grafts showed severe depletion of thymocytes in the cortex. Therefore, we conclude that the HIV-infected thymus may export a constant supply of HIV-positive cells to the periphery as long as it is able to generate T cells. However, HIV infection of the thymus might, under some conditions, result in inability of this organ to produce mature T cells to seed the periphery. Both of these situations could have severe ramifications for the developing immune response.

Dysregulation of Growth in HIV-1 Infection

QZ 003 NUTRITION AND HIV INFECTION, Robert M. Suskind, LSU Medical School, Department of Pediatrics, 1542 Tulane Avenue, New Orleans, LA 70112.

The acquired immunodeficiency syndrome (AIDS) has become a global pandemic with an estimated nine to eleven million people being affected with the human immunodeficiency virus (HIV). Of those infected, approximately three million are women and 500,000 are infants and children. Over 80% of children with HIV have acquired the infection vertically from HIV infected mothers. It is estimated that 2.7 million children will die of the HIV pandemic over the next ten years.

Malnutrition is a universal component of AIDS. The primarily malnourished child and the child with HIV have several immunological abnormalities in common that predispose to opportunistic infections such as impaired cellular immunity with a disproportionate decrease in CD-4 T lymphocytes compared to the decrease in CD-8 lymphocytes, a relative sparing of B lymphocytes and a depressed complement system. Even mild protein calorie malnutrition is associated with a consistent impairment of immunity. The genesis and development of malnutrition in children with primary protein calorie malnutrition and malnutrition associated with AIDS is multifactorial and include a decreased caloric intake, malabsorption as a result of opportunistic infections and/or AIDS enteropathy as a result of the impact of HIV virus on the metabolic state of the patient. It is clear from studies in children who have primary protein calorie malnutrition, without the AIDS virus, that the impact of the malnourished state on the immune system is significant. It is felt that the impact of infectious agents such as the HIV virus on the nutritional status of the patient, secondarily impacts on the immune system thereby significantly impacting on the immune system and the host's susceptibility to infection. Parallels between the patient who has the HIV virus and the child who has a primary infection such as chronic diarrhea occur in terms of their impact on the nutritional and immune status of patients. This dicussion will focus on the similarities between the HIV virus and other infections on the nutritional and immune status of the child with a discussion of the potential interventions that may be undertaken to avoid the impact of secondary malnutrition on the child with HIV infection.

QZ 004 GASTROINTESTINAL ASPECTS OF PERINATAL HIV, Harland S. Winter, Tracie L. Miller, Boston City Hospital and Children's Hospital, Boston, MA 02118

Children who acquire HIV-1 infection perinatally experience changes in growth within the first year of life prior to the decline in immune function and development of overt symptoms of malabsorption. Muscle mass as determined by anthropometric measurements appears to decrease despite protein and energy intake that is appropriate for age. The mechanism for these alterations in body composition is unknown, but may relate to altered energy utilization. The aim of these studies is to characterize the mucosal lymphoid response in HIV infection and to determine the expression of brush border enzyme genes. Immunohistochemistry and *in situ* hybridization were used on biopsy material obtained endoscopically from children with diarrhea or growth failure in whom no enteric pathogen could be identified.

Although diarrhea and malabsorption are common in HIV-infected children, unlike HIV-infected adults, enteric pathogens are not identified in many children. Decreased D-xylose absorption should raise the possibility of an enteric infection. Many children have lactose malabsorption that occurs earlier than would be predicted based upon genetic predisposition. The decline in brush border lactase activity is associated with a generalized increase in lamina propria lymphoid tissue with a predominance of CD8 positive lymphocytes. Investigation of the intestinal mucosa for HIV by in situ hybridization reveals scattered lamina propria cells that contain HIV RNA. Although the enterocyte appears histologically normal, there is a marked increase in HLA-DR expression. In addition, the gene for lactase is down-regulated.

One can speculate that alterations in the intestinal mucosal immune system result in production of cytokines, such as γ-interferon and tumor necrosis factor. These immunoregulatory agents interact with enterocytes to modify brush border enzyme expression and ultimately cause malabsorption. The molecular correlate of lactose intolerance may be down regulation of the gene for lactase. Thus, the enteropathy of HIV may be related to cytokine production and not primary HIV infection or replication.

Mechanism of Maternal-Fetal Transfer of HIV Infection

QZ 005 THE ROLE OF MATERNAL AUTOLOGOUS NEUTRALIZING ANTIBODY IN PREVENTION OF MATERNAL FETAL HIV-1 TRANSMISSION, Yvonne J. Bryson, Deborah Lehman, Eileen Garratty, Ruth Dickover, Susan Plaeger-Marshall, Sheryl O'Rourke, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA 90024.

Both increased maternal virus burden and the absence of protective neutralizing antibody (NAB) to HIV-1 during pregnancy have been postulated to increase the risk of vertical HIV-1 transmission; however results have been inconsistent. Because of the heterogeneity of HIV, the use of lab virus strains (HIV IIIB and MN) to assay NAB may not be clinically relevant. We therefore tested sera from HIV positive mothers during pregnancy and at the time of delivery to neutralize autologous virus isolates and their infant's first isolate if transmitted, and correlated this with viral load and vertical transmission. HIV isolates from 24 pregnant HIV infected women were biologically cloned by limiting dils of co-cultured PBMC. We tested 2-fold dils of maternal sera (1:10-1:320) against autologous and infant's isolates and HIV MN at various input MOIs, and measured endpoints in PBMCs by p24Ag inhibition (≥ 50%) vs. controls. Overall 10/24 women transmitted (6 infants, *in utero* and 4 intrapartum). Transmitting mothers were significantly less likely to have auto NAB ≥10 (2/10, 20%) compared to non-transmitters (10/14, 86%) (**p= 0.01**). NAB titers (range <10-320) were higher in non-transmitters vs. transmitters (median 40 vs. 10 respectively) (p=0.05). The 2 mothers with auto NAB transmitted intrapartum and 1 had no NAB against her infant's virus. 6/8 mothers sera from the time of birth had low to undetectable NAB against their infant's isolates. Acid dissociated ICD p24Ag was detected more frequently in transmitters (8/10 vs. 5/14) (p=0.08). In follow-up (2 yrs) some mothers developed NAB against previously transmitted virus. However in sequential pregnancies, 1 mother had no NAB to autologous virus at either birth and transmitted HIV (*in utero*) to both infants. The presence of maternal NAB against autologous HIV seems to be protective against vertical transmission. This suggests that virus which "ecapes" maternal immune mechanisms may play a role in selective HIV-1 transmission to the infant.

QZ 006 IMMUNOPATHOPHYSIOLOGY OF MUCOSAL HIV DISEASE, Phillip D. Smith, Division of Gastroenterology, University of Alabama School of Medicine, Birmingham, AL

Early, intermediate and late phase events within the mucosa underlie the gastrointestinal manifestation of HIV disease. The early phase is initiated with inoculation of HIV into the lower colon. Virus then enters the mucosa through trauma- or infection-associated mucosal breaks or possibly through M cell uptake to infect mucosal CD4+ cells. A transient "mononucleosis-like" syndrome with diarrhea and nausea is experienced shortly thereafter. The subsequent intermediate phase is characterized by a progressive reduction in the number of mucosal CD4+ cells as the mucosa becomes both a reservoir for HIV-infected cells and a site for upregulated viral transcription. Restricted IgA responses, impaired gastric acid secretion, and possible dysmotility predispose the intestine to low-grade bacterial overgrowth as diarrhea and malabsorption become prominent symptoms. The late phase is characterized immunologically by impaired function of mucosal lymphoid cells and clinically by gastrointestinal opportunistic infections and neoplasms. Elucidating these events is critical to understanding the immunobiology of HIV infection and developing an effective vaccine.

Pathogenesis of HIV Infection in Infants and Children

QZ 100 INFECTION OF GOAT BRAIN CELLS IN VITRO WITH CAPRINE ARTHRITIS ENCEPHALITIS VIRUS-63. Oyewole Adeyemo and Lata Phadtare, Department of Microbiology, School of Veterinary Medicine, Tuskegee University, Tuskegee, AL.36087.

Caprine arthritis encephalitis virus (CAEV) is a lentivirus of goats causing a chronic disease including neuromyelopathy and other lesions similar to the acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV). The virus causes neurological dysfunction in young goats similar to HIV infection in man, and joint lesions in the adult goat. CAEV primarily infects the monocyte-macrophage lineage cells. CAEV infection of goats can be used as an animal model for the study of AIDS. To study the mechanism of neuronal dysfunction due to CAEV infection, we established a procedure for culturing the caprine brain. To determine the particular cells infected by the virus, brain tissues obtained from the temporal lobe of 1 to 2-day old neonatal goats were enzymatically dissociated and cultured *in vitro*, enriched for either glial cells or neurons. The brain cultures were infected with virus directly or by co-culturing with virus-bearing macrophages. The CAEV-63 used was propagated in goat synovial membrane cells prepared from 0-2-day old goat kids. The virus was applied to brain cells after 10 to 14 days of culture. The macrophages were isolated from the synovial fluid of swollen carpal joints of CAEV-infected goats by glass adherence method; the isolated cells were positive for non-specific esterase staining. Using the triple-label immunohistochemical technique as adopted by Watkins et al. Science 249:549-553 (1990) combined with phase contrast microscopy, it was possible to identify the astrocytes, microglial cells, fibroblasts and neurons, and to localize the virus. Viral antigen was expressed in microglial cells. Following viral infection, microglial cells fused. To determine if any morphological changes in neurons occurred as a result of contact with cell products of microglial cells infected with the virus, experiments in which neurons are being co-cultured with virus-infected microglial cells were initiated. The results suggest that blood macrophages may be a source of brain infection by CAEV and that the damage of microglial cells may be one of the basis of encephalitis in CAEV infection of goat kids. This *in vitro* system for studying the interaction of lentivirus with brain cells should be particularly useful in investigations of the pathogenesis of brain lesions in AIDS in human neonates and children. Supported by RCMI grant #G12RR03059-01A1.

QZ 101 OVEREXPRESSION OF HIV-1 nef AS A MARKER FOR RESTRICTED INFECTION OF ASTROCYTES IN HUMAN CNS TISSUES, Benjamin M. Blumberg[1], Leroy R. Sharer[2], Yoshihiro Saito[1], Jennifer Michaels[3], Therese A. Cvetkovich[1], Marleece Louder[1], Kadian Golding[1] and Leon G. Epstein[1], Laboratory for Molecular Neurovirology[1], Depts. of Neurology, Microbiology and Immunology, and Pediatrics, Univ. of Rochester Med. Ctr., Rochester, NY, 14642. Depts. of Pathology[2], and Neurosciences[3], UMDNJ, New Jersey Med. School, Newark, NJ, 07103.

In a previous study we found that the HIV-1 nef regulatory gene reading frame was open in 92% of clones derived directly from pathologic brain and spleen tissues of children who died with AIDS-associated progressive encephalopathy, suggesting that nef was expressed. Using immunocytochemistry (ICC) for nef and GFAP, and in situ hybridization (ISH) with monospecific nef probes in postmortem CNS tissues, we have obtained evidence that nef mRNAs and protein are expressed in the cytoplasm of astrocytes. In selected pathological sections from 2 of 6 children, up to 20% of the reactive astrocytes showed evidence of HIV-1 infection. By contrast, using ICC for p24/25 gag and ISH with the pGEM Benn6 probe, we found that HIV-1 structural proteins and their coding mRNAs are localized to macrophages, microglia and multinucleated giant cells, which are the hallmark of productive HIV-1 infection in brain. These findings demonstrate that astrocytes may undergo restricted HIV-1 infection in vivo, as they do in vitro, and imply the existence of a larger reservoir of latently infected astrocytes. Disruption of astrocyte support functions due to HIV-1 infection may contribute significantly to neuronal dysfunction in AIDS encephalopathy.

QZ 102PERINATAL TRANSMISSION OF AN AZT RESISTANCE MUTATION (AZT-R) FROM A MOTHER TO HER INFANT. LM Frenkel, LM Demeter, L Wagner, NA Tejani, JS Lambert, BM Blumberg, LG Epstein, JG Strussenberg, R Reichman, Departments of Pediatrics, Neurology and Medicine, University of Rochester, Rochester, NY 14642

OBJECTIVE: Maternal-infant transmission of AZT-R or lymphotropic syncytia inducing (SI) phenotype has not been described. Therefore, we examined paired mother/infant HIV-1 isolates for the presence of AZT-R and SI.

METHODS: HIV isolates within 12 w of infants' birth were examined for lympho-, monocytotropism, and SI phenotype by co-culture in peripheral blood mononuclear leukocytes (PBML) and MT2 cells. PBML were separated by plastic adherence into populations of lymphocytes and monocytes. PCR-amplified regions of the pol gene were sequenced to determine the presence of reverse transcriptase (RT) mutations at amino acid positions known to be associated with AZT-R (41, 67, 70, 215 & 219).

RESULTS: The infants of 2 of 8 mothers taking AZT prepartum became HIV-infected. Maternal isolate (MI) #1 had RT mutations at 41 (M→L) & 215 (T→Y) and MI #2 at 70 (K→R). Of MI, only #2 was SI. Infant isolate (II) #1 had the 215 mutation and II #2 had no mutations. Both II were non-SI.

CONCLUSIONS: Maternal-infant transmission of AZT-R HIV-1 can occur. The frequency and clinical significance of such transmission remains to be determined.

QZ 103 CORRELATION OF HIV-1 ANTIBODY REACTIVITY AND P24 ANTIGEN WITH SURVIVAL IN PERINATALLY INFECTED CHILDREN. R Geffin[1], C Hutto[1], S Lai[2], S Erscoiu[3], M Master[1], and G Scott[1]. [1]Department of Pediatrics, [2]Biostatistical Working Group Comprehensive AIDS Program, University of Miami School of Medicine, Miami, FL, 33136, [3]The Clinic of Infectious & Tropical Diseases, Dr. V. Babes, Bucharest, Rumania.

The correlation between the antibody response to HIV-1 proteins and survival was evaluated in 30 children followed prospectively from December 1983 to July 1991, as part of a natural history study of HIV-1 infection. Between 2 and 11 sequential serum specimens (median of 5), obtained in each child at approximately 6 month intervals, starting from a median age of 18 months were tested. All children were symptomatic (class P_2), and 17/30 (57%) expired between the ages of 20 and 111 months (median= 39 months). The remaining 13 children are living and their ages range between 61 and 106 months (median 73 months). The following parameters were examined: levels of antibodies to gp160, gp120 and p24 using a radioimmunoassay, p24 antigen utilizing the immune complex dissociation assay (ICD) (Coulter Immunology), total IgG values and CD4+ lymphocyte numbers. Two major patterns of antibody responses were found. In some children, levels of antibody to the various proteins remained stable over time, while in others the levels of antibody initially increased and then decreased over time. In some cases, the decreases in antibody reactivity were greater than fifty fold. Over time, the pattern of antibody response to gp160 and gp120 correlated with survival: children whose antibody reactivity to the two glycoproteins remained low, or decreased with time, had a higher mortality than children whose antibody reactivity increased or remained elevated (P= 0.024 and 0.025 for antibodies to gp160 and gp120 respectively). Using the antibody binding curve obtained with each serum for each of the viral proteins, the dilution required to bind 50% of the antigen was calculated, and arbitrary cutoffs were defined. The highest level of antibody reactivity prior to 36 months of age, in relation to the cutoff, was used to derive Kaplan-Meier survival curves. Children whose antibody reactivity was above the cutoff, survived significantly longer than children whose antibody reactivity was below the cutoff. Antibody levels to all three proteins correlated with survival, but antibody reactivity to gp160 was a better prognostic indicator. The P values for the difference in survival according to the antibody reactivity to each protein were: p24= 0.026, gp120= 0.016, and gp160= 0.0001. p24 antigen levels by ICD were inversely related to levels of antibody to p24 (P< 0.00001). These results suggest that a decline in levels of antibody to all three viral proteins tested occurs in many children over time, that a decline in antibodies to gp160 and gp120 are significant predictors of survival, and that a low level of antibody reactivity by 36 months of age is associated with a poor prognosis.

QZ 104 MULTIPLE MATERNAL GENOTYPES OF HIV-1 ARE TRANSMITTED TO INFANTS BY PERINATAL INFECTION

Maureen M. Goodenow, John W. Sleasman, and Susanna L. Lamers, Departments of Pathology and Pediatrics, University of Florida College of Medicine, Gainesville, FL 32610

Human immunodeficiency virus type-1 (HIV-1) displays considerable genetic variability within env, where five hypervariable domains (V1-V5) with numerous nucleotide substitutions, deletions, or insertions are interspersed by more conserved regions. We showed previously that the V1 and V2 hypervariable domains have distinct sequences in epidemiologically unlinked strains of HIV-1. We have used genetic variation in V1 and V2 to trace HIV-1 transmission from mother to infant and to follow the evolution of the virus over time in related individuals. Our studies are focused on two HIV-1 infected mothers, each with three children infected by perinatal transmission of the virus. Serial samples have been obtained over a two-year period and include paired samples from mother and infant as early as five days postpartum. The polymerase chain reaction and nested env-specific primers were used to amplify V1, V2 and an adjacent constant region in DNA from peripheral blood mononuclear cells. Amplified products were cloned, sequenced, and analyzed.

Results of neighbor-joining analyses of genetic diversity in V1 and V2 defined multiple HIV-1 variants in the mothers. Virus sequences in infants close to the time of birth could be more variable than sequences in mothers. Transmission of multiple maternal variants was apparent in the infants' samples, although not necessarily in the samples obtained closest to birth. Genetic diversity developed independently in the V1 and V2 domains within different individuals. However, virus sequences in children at three to four years of age were recognizable variants of maternal HIV-1 genotypes, reflecting a remarkable conservation of family-specific amino acid signature sequences. Our results support the hypothesis that variability in epidemiologically linked strains of HIV-1 developed in infants from differential outgrowth and genetic drift of multiple maternal HIV-1 genotypes.

QZ 105 EXPRESSION OF COMPLEMENT REGULATORY PROTEIN MCP (MEMBRANE COFACTOR PROTEIN, CD46) AND DAF (DECAY ACCELERATING FACTOR, CD55) WITH HIV INFECTION IN VITRO.

James N. Jarvis, Heide Taylor, P. Michael Long, Vani Gutta, Wayne State Univ. School of Medicine and Children's Hospital of Michigan, Detroit, MI 48201.

Other investigators have shown decreased fluorescent intensity of the complement regulatory protein DAF on FACS analysis of peripheral blood leukocytes from adults with ARC or AIDS. We have shown similar decreases in DAF and the related protein, MCP, in children congenitally infected with HIV. These changes are associated with unusual sensitivity of HIV lymphocytes to C3b deposition. The current studies examined the role of HIV on MCP and DAF expression in vitro.

In the first group of experiments, MCP and DAF were measured by FACS and compared between the T cell lines H9 and $HTLV_{IIIB}$ (H9 cells chronically-infected with HIV). The intensity of MCP and DAF was identical in H9 and $HTLV_{IIIB}$ cells.

In the second group of experiments, peripheral blood mononuclear cells (PBMC's) from healthy adult volunteers were incubated with media from $HTLV_{IIIB}$ cells for 72 hours. Expression of MCP and DAF were then determined by FACS. Significant decreases were seen in intensity of MCP after 72 hours compared to control cells incubated in uninfected media. No alterations in DAF expression were observed. Whether decreases in MCP expression are dependent upon HIV infection of at least some of the cells is unclear. Although small increases in p24 antigen in cell supernates was observed, alterations in MCP expression could not be blocked by neutralizing titers of anti-gp120.

These data suggest that HIV infection does not alter the expression of complement cell-surface regulatory proteins MCP and DAF in homogenous cell populations in vitro. Altered expression of MCP upon HIV infection of PBMC's (a heterogeneous population) may be dependent upon viral infection of some of the cells.

QZ 106 EFFECTS OF HIVIG AND IVIG IN A MURINE MODEL WITH SUBSEQUENT IMMUNIZATION OF OFFSPRING.

Marie T. Jelonek*, Kathelyn Steimer†, Larry Cummins‡, Jennifer Brust* and Margaret Keller*. *Department of Pediatrics, UCLA School of Medicine, Harbor-UCLA Medical Center, Torrance, CA 90509, †Chiron Corporation, Emeryville, CA 94608, ‡Abbott Laboratories, Abbott Park, Illinois 60064.

Proposed strategies to prevent maternal transmission of HIV include administration of HIV Immune Globulin to the mother during pregnancy and to the infant at birth combined with immunization of the infant with HIV vaccines. In a BALB/c model, HIVIG or Intravenous Immune Globulin (IVIG) was administered to postpartum female mice within 24 hours after delivery at a dose of 200 mg/kg, intraperitoneally. The HIVIG or IVIG was efficiently transferred to the offspring via the colostrum. Control and experimental offspring were immunized intraperitoneally with 100 μg of HIV $rgp120_{SF2}$ in complete Freund's adjuvant at 18-21 days of age. At that time, 125 ± 20 μg/ml of HIVIG was still present in the offspring sera. The serum anti-$rgp120_{SF2}$ antibody response was assessed using ELISA with native, glycosylated $rgp120_{SF2}$ used as antigen. An anti-$rgp120_{SF2}$ IgG1 mAb was used to standardize the assay. Both sets of experimental offspring (#1,HIVIG and #2,IVIG recipient mice) were suppressed in serum antibody response compared to the antibody response of control mice 9 weeks after immunization. The mean nine week anti-rgp120 antibody response expressed in mAb equivalent units was 1) 79.43 (50.12-123.02) μg/ml for HIVIG offspring 2) 75.86 (48.98-117.49) μg/ml for IVIG offspring, and 3) 147.91 (100.00-213.80) μg/ml for control mice. P values for HIVIG vs. control and IVIG vs. control groups were 0.03 and 0.02 respectively. There was no suppression of the response to an unrelated antigen, hen egg-white lysozyme by HIVIG. Further studies are necessary to determine the mechanism of suppression of the anti-rgp120 response by HIVIG, as well as IVIG.

QZ 107 HUMAN IMMUNODEFICIENCY VIRUS -1 INFECTION OF HUMAN PLACENTAL MACROPHAGES *IN VITRO*.

Alison M Kesson, Warwick R. Fear, Farhad Kazazi, Jean-Marie Mathijs, Nicholas J.C King‡, and Anthony L. Cunningham. Department of Virology, Westmead Hospital, Westmead, 2145 and ‡Department of Pathology, University of Sydney, 2006 Australia.

Placental macrophages (Hofbauer cells) were isolated and cultured *in vitro* to investigate their susceptibility to HIV infection and possible role in vertical transmission of HIV-1.

Macrophages were obtained from normal human term placentas by enzymic digestion of minced washed tissue followed by sequential density gradient separation on Ficoll and Percoll, and differential adherence to plastic.

After 10 days of in vitro culture more than 80% of adherent cells expressed CD14 as detected by flow cytometry and more than 99% of the cells were non-specific esterase positive. CD4 antigen was expressed at very low levels. CD4 mRNA could be detected in the cells by reverse transcription followed by polymerase chain reaction. The macrophages were infected productively after inoculation with low passage blood isolates of cell free HIV at an m.o.i. of 0.1 /cell. Peak viral titres were detected in the supernatants of most cultures 3 to 7 days post infection by HIV antigen ELISA and reverse transcriptase assay. HIV RNA could be detected in placental macrophages by in situ hybridization 16 days after infection. Multinucleated giant cells were identified in some cultures indicative of an HIV-induced cytopathic effect.

We conclude that placental macrophages can be infected productively with clinical isolates of HIV-1, and such cells may act as a reservoir of virus for transmission to the fetus in utero.

QZ 108 PERINATAL HIV INFECTION MASQUERADING AS SIDS, Lambert J, Metlay L. University of Rochester School of Medicine, Rochester, New York, 14642, USA.

Objectives: The pathology of 3 cases of sudden infant death syndrome (SIDS) are reviewed where the infants were known either pre or post mortem to be HIV infected.

Methods: Hospital charts were reviewed on 3 mothers and infants. All children were determined to be HIV infected by HIV co-cultivation techniques or by diagnosis of an AIDS defining infection. Pathology specimens were reviewed in an attempt to find a common pathophysiologic mechanism for the "SIDS" diagnosis and HIV infection.

Results: In 2 of 3 cases, the spleen was described as having "mild follicular hyperplasia". In all 3 cases, heart examination revealed subendocardial fibrosis.

Discussion: In 2 cases, the infant diagnosis resulted in subsequent diagnosis of HIV in women who were again pregnant. "SIDS" deaths may represent concomitant HIV infection. Further studies are needed to draw conclusions on the pathological findings described.

Case	BA	RD	HB
Heart	subendocardial fibrosis, petechiae	subendocardial fibrosis	subendocardial fibrosis
Lung	congestion, edema	PCP	focal edema, congestion, mild tracheobronchitis
Brain	diffuse astroglial reaction & microfocal mineral deposits	unremarkable	unremarkable
Spleen	congestion, lymphoid depletion	mild follicular hyperplasia	mild follicular hyperplasia

QZ 109 HIV-1 SPECIFIC CTL IN LONG-TERM SURVIVORS OF VERTICAL INFECTION. Luzuriaga K and Sullivan JL. Department of Pediatrics and Molecular Medicine, Univ. of Massachusetts Medical School, Worcester, MA.

While the majority of vertically-infected children develop symptomatic disease within the first 2 years of life, an increasing number of children are being recognized who remain asymptomatic (P1) or mildly symptomatic (CDC P2A) for several years. Study of these children may yield important information regarding virologic and immunologic factors associated with disease progression. HIV-1 specific cytotoxic T lymphocyte (CTL) responses have been studied in 8 long-term survivors (Group 1; > 5 years old with only mild symptoms and preserved CD4 counts for age in the absence of antiretroviral therapy) and in 5 children who developed more severely symptomatic disease (CDC P2B,P2D1) in the first year of life (Group 2). B lymphoblastoid cell lines infected with vaccinia alone or HIV-1 gene products (gag,env) were combined with autologous peripheral blood mononuclear cells in a ^{51}chromium release assay to detect activated CTL. Activated CTL directed against at least 1 HIV-1 gene product were detected in the circulation of the majority of Group 1 children but in only one child in Group 2.

	gag pos	env pos	gag or env pos
Group 1	5/8(62%)	5/6(83%)	6/8(75%)
Group 2	0/5 (0)	1/4 (25%)	1/5(20%)

Activated CTL response rates in Group 1 patients, therefore, are similar to those that we have previously reported in HIV-1 infected hemophilic children and adults who remain relatively well for prolonged periods of time. Our data suggest that the development of a strong activated CTL response is protective against disease progression; early intervention with vaccine therapies to boost these responses may alter the course of disease progression following vertical infection.

QZ 110 MATERNAL IgG1 AND IgA ANTIBODY TO V3 LOOP CONSENSUS SEQUENCE AND MATERNAL-INFANT HIV TRANSMISSION (MIT), Richard B. Markham, James Gomez, Jacqueline Coberly, Andrea Ruff, Julio Desormeaux, Reginald Boulos, Elizabeth Holt, Donald Hoover, and Neal Halsey. Departments of Immunology and Infectious Diseases and International Health, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD 21287.

Other investigators have reported conflicting results regarding the association between MIT and maternal antibodies to small (7-13 aa) V3 loop peptides based on IIIB or MN strains. We examined the association between MIT and levels of maternal isotype antibody to a 41 amino acid consensus peptide representing the entire HIV V3 loop from a population of seropositive Haitian adults. IgG1 V3-loop specific antibodies were measured by EIA in 18 Haitian women who transmitted HIV to their offspring and 124 non-transmitting mothers using sera obtained prior to or at delivery. Log transformation normalized the distribution of antibody levels. The geometric mean IgG1 antibody levels in transmitting mothers (19.5 ug/ml) was higher than the levels observed in non-transmitting mothers (10.9 ug/ml) and this difference was statistically significant ($p=0.036$). However, the differences were not significant ($p=0.18$) by the non-parametric Wilcoxon rank sum test. No significant was observed between association MIT and serum IgA levels by either analysis. The higher levels of IgG1 antibodies in transmitting mothers could be explained by Type I error or by an association with other factors affecting the risk of transmission, such as duration of infection. However, studies to evaluate the possibility that IgG1 antibodies enhance transmission may be indicated. These data strongly indicate no increased risk of MIT associated with lower maternal IgG1 levels and raise the possibility that maternal anti-V3 loop IgG1 antibodies may be positively associated with MIT.

QZ 111 GENOTYPE AND PHENOTYPE CHARACTERIZATION OF THE HIV-1 TRANSMITTED FROM MOTHER TO INFANT. Hongmei Mo, Tuofu Zhu, Yunzhen Cao, Guiling Gu, Richard A. Koup, William Borkowsky, and David D. Ho. The Aaron Diamond AIDS Research Center and NYU School of Medicine, 455 First Avenue, New York, NY 10016.

HIV-1 sequences from the C2-to-V5 region of gp120 and p17 were determined following PCR amplification of DNA from the peripheral blood mononuclear cells of one infected mother plus her newborn and another infected mother plus her pair of identical twins. The viral population found in each infant was homogeneous in the gp120 and p17 sequences. The same minor variant virus was found in each of the twins. Interestingly, the transmitted virus in each case represented only a minor variant of the mother's population of viruses, suggesting selectivity in transmission. Phenotype characterization of the transmitted viruses is now in progress. A better understanding of the virus that is successfully transmitted from mother to child would facilitate the development of interventional strategies to block vertical transmission of HIV-1.

QZ 112 **CD8+ TRENDS IN HIV-INFECTED CHILDREN IN A TRIAL OF INTRAVENOUS IMMUNOGLOBULIN (IG): POSSIBLE BEARING ON IMMUNOPATHOGENESIS OF MORE RAPID DISEASE PROGRESSION IN CHILDREN THAN ADULTS.** Mofenson LM*, Moye J*, Bethel J+, Flyer P+, Nugent R*, for the NICHD IVIG Clinical Trial Study Group. *PAMA, NICHD, NIH, Bethesda, MD and +Westat, Inc, Rockville, MD

OBJECTIVE: In HIV-infected adults, CD8+ lymphocytes show a slow, steady increase above normal until onset of AIDS, after which they decline (*JAIDS* 1989;2:63-9). It is postulated that CD8+ increase in HIV-infected adults without AIDS may reflect the immune system's attempt to control HIV infection (*JID* 1991;163:959-65). We evaluated CD8+ trends in HIV-infected children enrolled in a clinical trial of IG. **METHODS:** Between 3/88 and 1/91, 313 HIV-infected children with entry CD4+ ≥200/μL and mean age 38 mo were enrolled in a randomized controlled trial of IG, 400 mg/kg q 28 days, in which IG reduced rates of bacterial and viral infections, hospitalizations, and CD4+ decline (*JAMA* 1992;268:483-8, *AIDS* 1992;6[S1]:S13). CD8+ counts were measured at entry and every 3 months. Regression slopes were determined for each of 277 children with ≥3 CD8+ values. Age-adjusted regression slopes were determined by comparing study patient CD8+ values to published age-related normal values. A logarithmic model was used to evaluate effect of IG on CD8+ trends. **RESULTS:** At all ages, HIV-infected children had higher entry CD8+ counts than published age-equivalent norms for uninfected children. However, significantly more rapid CD8+ cell loss over time was observed in HIV-infected compared with normal children (excess CD8+ loss of 28 cells/mo, p=0.0001). While a trend toward slower decline in CD8+ count was observed with IG, it was not statistically significant. **DISCUSSION:** In contrast to asymptomatic or mildly symptomatic HIV-infected adults, HIV-infected children with mild-to-moderate symptoms showed progressive loss rather than a stable gain in CD8+ number over time. The more rapid onset of symptomatic disease in HIV-infected children than adults may reflect inadequate cell-mediated immune response to HIV, evidenced by declines in CD8+ number early in infection; deficient HIV-specific CTL responses also have been observed in HIV-infected children (*J Pediatr* 1991;119:230-6).

QZ 113 PRENATAL INOCULATION OF MACAQUE FETUSES WITH SIV/HIV, Hans D. Ochs, William R. Morton, LaRene D. Kuller, Qili Zhu, Che-Chung Tsai, Michael B. Agy, Raoul E. Benveniste, Department of Pediatrics, Regional Primate Research Center, University of Washington, Seattle, WA 98195; National Cancer Institute, Frederick, MD 21702

To determine the effect of simian (SIV) and human (HIV) immunodeficiency virus on the development of the fetus, we have inoculated pregnant *M. nemestrina* and their fetuses at various gestational ages with SIV_{Mne} or with HIV-1_{LAI}. Intra-amniotic inoculation with SIV_{Mne} was performed in six animals at 91-137 days of gestation. All six dams became viremic (reverse infection) and produced anti-SIV antibody. Three pregnancies resulted in nonviable offspring; two were severely autolized and could not be evaluated, one was found to have SIV-Ag positive macrophages in the thymus. Three offspring were viable and were positive for SIV by culture and PCR. All died of SAIDS and, at autopsy, were antigen positive in lymphoid organs, lung, liver and brain. There was striking involution of spleen, lymph nodes and thymus. Three pregnant macaques were inoculated with cell-free HIV-1_{LAI} intravenously and simultaneously intra-amniotically. All dams became HIV positive and developed anti-HIV-1 antibody. One of the three offspring was virus positive at age three days and died at ten days of age with clinical signs of meningitis/encephalitis, pneumonia and enterocolitis; autopsy revealed involution of lymphoid organs; HIV antigen was present in spleen, lymph nodes and thymus. The second infant remains healthy and HIV negative at age 5 months. The third infant was aborted 4 days after inoculation and found to be HIV-1 antigen positive in spleen, lymph nodes and brain. One dam, virus positive after intravenous inoculation, delivered a healthy, culture negative infant and another, inoculated intra-amniotically, remained culture and antibody negative and delivered a healthy now two weeks old baby.

The results of these experiments demonstrate that macaque fetuses can be "timed" infected with SIV or HIV-1, allowing the systematic study of the effect of these viruses on the development of the fetus and newborn.

QZ 114 SERIAL STUDIES OF IMMUNE ACTIVATION IN HIV+ AND CONTROL PREGNANT WOMEN, Susan Plaeger-Marshall, Valentin Isacescu, Jeanne Bertolli, Sheryl O'Rourke, Najib Aziz, Maryanne Dillon, Deborah Wafer, Janis V. Giorgi, Pamela J. Boyer, and Yvonne J. Bryson, Departments of Pediatrics, Medicine, Biomathematics, and Obstetrics and Gynecology, UCLA School of Medicine, Los Angeles, CA 90024

The identification of risk factors for the transmission of HIV infection from mother to child is essential to the design of appropriate preventive strategies. To characterize immunologic and virologic factors that may relate to transmission, we have begun a prospective serial study of HIV-infected and healthy control women during pregnancy (once each trimester) and post-partum (within 2 weeks, 6 weeks, and 3 months). We present here preliminary data characterizing immune activation as reflected in lymphocyte phenotype and serum neopterin levels. Nineteen HIV-infected pregnant women and 14 controls have been studied to date. Of the 19 HIV+ mothers, 13 have given birth. Three of the 14 babies born are known to be infected. Although the number of subjects is too small for accurate statistical analysis, the 3 transmitting mothers had no obvious differences from non-transmitting mothers in expression of immune activation markers or CD4 cell numbers. Compared to controls, the HIV+ mothers at all time points had significantly decreased percentages of CD4 T cells (eg., 26 vs. 43% at tri 3). The percentages of CD4 cells varied during and after pregnancy and in both infected and control women. However, the HIV+ women showed a drop in CD4 cells at 3 months post-partum, whereas control values increased slightly. HIV+ mothers had significantly elevated percentages of CD8 T cells coexpressing HLA-DR and CD38 (eg., 50 vs. 22% at tri 3), and elevated levels of serum neopterin compared to controls. However, control pregnant women generally had higher percentages of HLA-DR+/CD38+ CD8 cells and serum neopterin than did non-pregnant control women, indicating that pregnancy in itself is associated with some level of immune activation. Since T cell activation enhances replication of HIV, this may have ramifications for disease progression in pregnant HIV+ women.

QZ 115 GLUTATHIONE CONTENT AND SURFACE MARKER EXPRESSION OF PERIPHERAL BLOOD MONONUCLEAR CELLS FROM HIV-EXPOSED AND INFECTED INFANTS. Ronald L. Rabin, Ann Petru*, Charles V. Smith, Mario Roederer, Frank J.T. Staal, L.A. Herzenberg, L.A. Herzenberg, and Y.A. Maldonado†. Departments of Genetics and †Pediatrics, Stanford University School of Medicine, Stanford, CA 94305, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, and *Children's Hospital, Oakland, 747 52nd Street, Oakland, CA 94609

Recent studies with cultured T cells and monocytes demonstrate that oxidative stress (redox status) play a major role in regulating signal transduction and HIV expression. Clinical monitoring studies demonstrate that median glutathione (GSH) levels, a measure of oxidative stress, are systematically decreased in T cells from HIV infected adults. These clinical studies also demonstrate significant and early increases of expression of a B cell marker, CD20 on B cells of HIV infected adults and a surprising correlation between CD20 expression and intracellular GSH levels..

To determine whether these findings with HIV infected adults extend to children, we have initiated a study of infants and children who are being followed (at Children's Hospital, Oakland) either for HIV exposure or infection. In addition to flow cytometric analysis of PBMC for relative GSH content, CD20, and other appropriate leukocyte surface markers, we are measuring GSH content of plasma and whole blood. Data from this study will be presented.

QZ 116 RESISTANCE TO ZIDOVUDINE OF HIV ISOLATES OBTAINED FROM PEDIATRIC PATIENTS, Joseph Roberson, William Don Decker, Marilyn Crain, and Robert W. Buckheit, Jr. Retrovirus Research Section, Southern Research Institute-Frederick Research Center, Frederick, MD 21701 and Department of Pediatrics, Children's Hospital, University of Alabama at Birmingham, Birmingham, AL

We have obtained HIV isolates from the plasma, peripheral blood lymphocytes, and peripheral blood macrophages of pediatric patients by established coculture techniques, allowing estimation of the titer of infectious virus in the blood and definition of the biological phenotype of the isolates. We have been especially interested in the appearance of zidovudine-resistant virus isolates in the infected children. We have examined the drug sensitivity of virus isolates obtained prior to and during the course of zidovudine therapy in the infected children. We have also examined the cross-resistance of the zidovudine-resistant strains to other therapeutic agents. The biological phenotype of the isolates has been further defined in terms of their cell tropism, their syncytium-forming ability, their ability to grow in fresh and cultured cells, and their replicative capacity in acute infection and the cytopathicity of the isolates obtained have been correlated to the disease status of the infected child. The results of these experiments will be presented.

QZ 117 B CELL PHENOTYPE CHANGES IN HIV INFECTED CHILDREN.C.Rodriguez, M.D., E. R. Stiehm, M.D., S. Plaeger-Marshall, Ph.D. Dept. of Pediatrics, UCLA School of Medicine. Los Angeles,CA.

B cell activation and related complications (LIP, lymphomas) can be decteted during the course of pediatric HIV disease. We report a pilot study that looks at differences in immunophenotypic peripheral B cell activation, lineage, and maturation markers in HIV infected children at different stages of disease. Subjects were under 13 years of age, 6 asymptomatic (P1), 16 symptomatic (P2) , 10 sero-positive infants status indeterminable (P0), 13 controls (CT) and 15 normal cord blood samples (CB). Using two color laser flow cytometry , we determined the proportion of CD19+ peripheral lymphocytes that expressed the following markers: CD25, CD71, CD23, L-Selectin, CD38, CD69, CD10, CD21, and CD5. Also, absolute CD19(+) B cell number and cell size were obtained. Phenotypic differences observed between the HIV infected subjects and the control group were a significant decrease in the median value of CD19/L-Selectin+ in P2 and CD19/CD23+ in P1 and P2, but no differences in the other phenotypes. There was a decrease in the absolute B cell number in P1 and P2 with respect to the control , P0, and CB and an increase in cell size in the P2 , P0 and CB. CB B cells presented a cell size and L-Selectin percentage similar to P2 but CD71, CD25, and CD10 were expressed at higher levels than any of the other groups.. These preliminary observations suggest that B cell activation in HIV infected children may not be directly expressed by an increase in peripheral B cell activation markers, but indirectly by a decrease of L-Selectin (+) resting B cells. Additionally, peripheral B cells from P2 may be manifesting signs of immaturity suggested by an increase in cell size and a decrease in L-Selectin similar to CB. A decrease in CD23 and B cell number may have disease related significance since it was low only in P1 and P2. The latter may be a sign of stem cell "fatigue"or elimination of mature B cells. In the P0 group, further follow up is required to determine if the differences in phenotype are age or disease related.

QZ 118 ESTIMATE OF THE PERIOD OF PERINATAL HIV-1 TRANSMISSION

C. ROUZIOUX, M. BURGARD, S. BLANCHE, D. COSTAGLIOLA and the HIV infection in Newborns French Collaborative Study
Hôpital NECKER and INSERM Hôpital St ANTOINE - PARIS - FRANCE.

Aims : To determine the timing of materno-fetal HIV-1 transmission and the influence of maternal factors.

Materials and Methods : Virological tests were carried out at birth and within the first three months of life in 95 infected infants born between May 1988 and August 1991 and included in the Cohorte Pédiatrique Française. The virus was detected by means of culture and/or PCR, and by testing for antigenemia. Western blot patterns were compared between the infant and mother in every case, and the timing of transmission was estimated using the Markov model.

Results : Seroconversion was observed in 21 neonates on the basis of antibodies to gag or pol gene products. Markov analysis indicated that transmission occurred in utero during the last two months of pregnancy (95% < 59 days) when the virus was detectable in the first few days of life, and that the remaining infants were contaminated during delivery. The risk of transmission in utero was proportional to the degree of maternal immunosuppression at the time of delivery ($p<0,05$). Finally, the risk of early encephalopathy in the infant was higher following in utero transmission ($p<0,05$).

Conclusion : The results of this study validate attempts to prevent materno fetal HIV-1 transmission in late pregnancy and/or at the time of delivery.

QZ 119 SERUM P24 ANTIGEN LEVELS AND DISEASE PROGRESSION IN HIV-1 INFECTED CHILDREN

Gareth Tudor-Williams[1], Brigitta Mueller[1], Vicki Stocker[1], David Venzon[2], Dorie Marshall[1], Pim Brouwers[1], Karina Butler[1] and Philip Pizzo[1], Pediatric branch[1] and Biostatistics and Data Management Section[2], National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

OBJECTIVE: To assess the utility of monitoring serum p24 antigen as a marker for disease progression in HIV infected children.

STUDY DESIGN: Single center, retrospective case-control study, comparing children with progressive disease to those remaining clinically stable.

PATIENTS and METHODS: From a cohort of 96 children on ddI monotherapy for six months or more, 35 matched pairs were identified. Index cases were taken off ddI because of disease progression (n=24) or death (n=11), independent of p24 antigen data. A comparison group who did not progress (controls) were matched for route of HIV acquisition, prior antiretroviral therapy and initial CD4%. To ensure stability of this group during the same time interval, controls were required to have remained on ddI at least 6 months longer than index cases. p24 antigen levels were measured by Abbott ELISA every 4-8 weeks. 980 values were available for analysis. Running means were computed and compared, using 2-tailed Wilcoxon signed rank tests.

RESULTS: Significantly higher p24 running means were observed in the index cases compared to controls ($p=0.0036$ at time of stopping ddI). The raw mean p24 antigen levels were higher in the index cases in the 90 day period prior to stopping ddI ($p=0.0024$). However they were not significantly different in the 45 days prior to stopping ddI ($p=0.08$, n=26 pairs) or in final means corrected for p24 antigen levels at entry ($p=0.13$).

CONCLUSIONS: Longitudinal p24 antigen data may have greater utility in predicting disease progression in pediatric studies than using single values or mean values over short time intervals. Practical algorithms for the prospective use of p24 antigen data need to be derived. We are assessing whether the Coulter ICD assay offers any advantages over the Abbott ELISA.