Maternal chronic viral infections transmitted to infants: from mechanisms to prevention and care

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LECTURE PRESENTATIONS

LI Great challenges to PMTCT in the South: the role of the developed nations in supporting strategies that work

Dorothy Onyango
Women fighting AIDS in Kenya (WOFAK), 00200 Nairobi, Kenya

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Introduction: Prevention of mother to child transmission of HIV (PMTCT) has continued to gain immense significance in the fields of HIV prevention and care, mostly in the last 10 years. This significance has come about due to the growing recognition that HIV is not only in blood and sexual fluids but also in breast milk. The fact that over 58% of all infections in sub-Saharan Africa are found among women in reproductive ages (19–49 years) makes PMTCT a life and death subject.

In Africa, women face the ravages of HIV/AIDS in uneven proportions: they often bear the heaviest brunt of the epidemic, as caregivers, as persons living with HIV/AIDS and as the pivotal points of every society. When they are infected, their roles in society do not change much, as often, the society still expects them to play their parts as ever before. It is estimated that about 30% of all babies born to HIV positive mothers will acquire the virus. Our experiences show that this percentage only talks of the ones that will get the virus either in the womb or during delivery. We suspect that a bigger fraction get the virus through breastmilk.

In developed countries, almost all HIV infected women will receive good PMTCT care. With the best treatment and formula feeding, the chance of HIV being transmitted to the baby is less than two percent. In developing countries it is very different. While a few developing countries have launched effective responses, on average less than ten percent of women receive even the most basic PMTCT services. (Source: The Stop AIDS in Children Campaign).

As a result, around half a million children are infected with HIV every year. Why are so many pregnant women not receiving the help they need? Most often it is because PMTCT services are unavailable or inefficient, or because women are unable to access them. Stigma and fear may also play a role by making a woman unwilling to take an HIV test, or unwilling to take PMTCT drugs. These problems, though challenging, can be overcome. Some developing countries, such as Botswana and Brazil, are already providing PMTCT services to most of their pregnant women.

PMTCT: The grim picture in the South: Africa South of the Sahara is a grim picture of the struggle of mothers living with HIV/AIDS to deliver safe uninfected babies. The African woman who is living with HIV/AIDS and does not have her rights to making informed choices on sexuality and child-bearing, the woman who does not have her own money to go for treatment, to access competent antenatal and postnatal care services, who does not have the ability to travel long distances to access HIV testing and related care, will most certainly beget a baby who is infected with HIV.

In many African Countries, uneven distribution and infrastructural inadequacies of existing PMTCT services make them ineffective and unattractive to many would-be –beneficiaries. Access to PMTCT services and community knowledge around mother to child transmission (MCT) remain low. The existing PMTCT sites are battling with what has been christened as “minimum package”, that is to say, that at least “something” that would help reduce chances of mothers transmitting the infection to their babies. It is the height of all inadequacies. For how long will women contend themselves with “minimum package” of PMTCT?

The role of the developed countries: The reality of PMTCT in Sub-Saharan Africa is that many women in the reproductive ages still continue getting infected and many are still unable to access competent treatment and PMTCT and care services. It is still a fact that there are structural inadequacies in the current setups of PMTCT sites as they have aimed too low at getting “a minimum package”, thus losing great opportunities to reduce mother to child infections to around 2% as in developed countries. The developed world has a moral duty to support scaling up strategies that have been proved to work. These strategies include:

• Improving distribution of PMTCT centres, especially with facilitation of the community based organizations that have the capacity to undertake this.
• Improving quality of PMTCT services to move away from “minimum package” to a near-comprehensive package, thus drastically reduce chances of mother to child transmission of HIV.
The near-comprehensive position of PMTCT that we call for includes but not limited to the following:
• Supporting mothers to access competent and timely treatment services, including ART and other drugs for opportunistic infections.
• Supporting mothers to access adequate nutrition that also includes supportive supplements such as multivitamins.
• Supporting mothers to access safe delivery services including caesarean section as per the advice of qualified personnel.
• Supporting mothers to access alternative feeding opportunities for their babies if they wish, even though this option still remains quite controversial.

**Conclusion:** African women living with HIV/AIDS have a burning desire to beget children who are free from HIV/AIDS. Their resolve is unparalleled, their readiness is unquestionable. What they lack are the resources to achieve these aims. It is our appeal that PMTCT should get more elaborate support from the developed world, to ensure that we shall no longer talk of “minimum package of PMTCT” but a comprehensive package that ensures that babies are born without HIV infection.

**L2**

**Virologic and host determinants of breastfeeding transmission of human retroviruses**

Philippe Van De Perre

University Montpellier 1 and CHU Montpellier, France

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Breastfeeding (BF) transmission is responsible for approximately 300,000 new paediatric HIV infections each year. Mechanisms of transmission of HIV by BF are difficult to decipher due to the progressive maturation of the neonate/infant defences and to the evolving content of human milk over different stages of lactation. HIV is distributed and diversifies according to the constraints of anatomic sites. This is particularly true for the mammary gland and breast milk. The portal of entry of HIV on infant’s mucosal surface remains largely mysterious. A rabbit model suggests that M cells may transport HIV particles by transcytosis to the lamina propria of Peyer’s patches. In an ex vivo human model, HIV enters human enterocyte from HIV-infected cells through an agrin-dependent viral synapse, is transported by transcytosis across the enterocyte’s cytoplasm and delivered in the vicinity of lamina propria. A macaque model suggests that tonsils crypts may behave as portal of entry for HIV. Cell-associated HIV in milk is the source of infection in most of the early transmission events and that free virus in milk is more frequently involved later on. Latently HIV infected T cells in breast milk are considerably more prone to enter viral cycle after ex vivo activation and to produce viral particles than their blood counterparts. This strongly suggests that local microenvironment in milk may favour transcription of integrated viral DNA from latently infected cells and its translation into proteins and new virions. Human milk contains also DCSIGN-expressing monocytes and dendritic cells that are able to transport and propagate RS viruses in breast milk. It contains also HIV-specific MHC class I-restricted CD8+ Cytotoxic T lymphocytes that may play a role in the clearance of HIV-infected cells in breast milk. Human milk is extraordinary rich in soluble factors, some of them with immunomodulating or antiinfectious properties. Lactoferrin, Lewis X factor, SLPI, Interleukin-7 and α-defensins have all been suggested either in vitro or in vivo to modulate transmission. Breast milk of HIV infected women contains high concentration of HIV antibodies. HIV-specific secretory IGA and IgM have been associated with an absence of breast milk transmission in some but not all studies. Transmission of HIV by BF is a clearly multifactorial. The exact picture remains unclear but certainly involves a complex intercompartment cell trafficking, fuelled with complex viral populations, and modulated by a richly diversified microenvironment. Knowledge of the mechanisms of mucosal transmission of retroviruses should help designing new preventive interventions.

**L3**

**Determinants of maternal infection associated with virus infection of the fetus and newborn infant**

William J Britt

Depts of Pediatrics, Microbiology, and Neurobiology, University of Alabama School of Medicine, Birmingham, AL, USA


Congenital infection with human cytomegalovirus (HCMV) is most common intrauterine acquired virus infection in infants in the developed world. Rates of congenital HCMV infection range from 0.2%–2.0% in live births. Although maternal infection with this virus rarely results in clinical symptoms, approximately 10% of infants born with congenital HCMV infection can exhibit symptomatology ranging from mild hepatitis to severe multiorgan dysfunction, including damaging central nervous system infection. CNS infection can result in microcephaly, retinitis, and abnormalities in psychomotor function. More commonly, infants are clinically asymptomatic at birth but between 10–20% of infected infants will exhibit long term deficits in neurological function, with hearing loss being the most common long-term sequelae. Congenital HCMV infection is thought to be the most common non-familial cause of hearing loss. Although these characteristics of congenital HCMV infection are well described in studies from the developed world, little is known about the natural history of this infection in resource constrained countries. However, several studies have demonstrated that the incidence of congenital HCMV infection increases with increasing maternal infection. Recent findings from Brazil and India are consistent with these earlier studies and indicate that congenital HCMV infection occurs in 1–2% of newborn infants in most of the world’s populations. Because HCMV infection is universal in these maternal populations and infection is acquired early in life, these findings indicate that maternal immunity to this virus is insufficient to prevent infection and perhaps can only modulate the incidence of disease in infected infants. Studies from the US have demonstrated that infected infants born to women with preconceptional immunity exhibit rates of developmental abnormalities similar to those infants born to women with primary infection acquired during pregnancy. The mechanisms that account for the inability of maternal immunity to prevent infection and limit disease in the developing fetus infected with HCMV have not been defined, but reinfec tion with new strains of virus has been shown to occur in normal host following community exposure. Reinfection of previously infected hosts with new strains of virus is common in animal models and recent studies in normal women suggest that rates of reinfection could...
significant variations of > 25% have been reported in other well described and appear to arise from minor sequence variation (1–3%) in some envelop glycoproteins such as gB whereas significant variations of > 25% have been reported in other envelop glycoproteins (gN). Whether these differences account for reinfection with different strains of HCMV and contribute to disease in congenitally infected infants remains to be determined. However, the apparent contribution of reinfection to the natural history of this congenital infection in the developing world suggests that HCMV could be a significant cause of morbidity in infants in resource-constrained countries as well as in the developed world.

L4
Mechanisms by which co-infections modify HIV-1 transmission
Grace C John-Stewart
University of Washington School of Medicine, Seattle, WA 98195, USA
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Women with HIV-1 are frequently co-infected with other pathogens that may influence transmission of HIV-1. Bacterial, helminth, and viral infections are prevalent in settings with high HIV-1 prevalence and may be associated with immune activation, increased HIV-1 replication and genital shedding. Discerning the contribution of co-infections to HIV-1 transmission is difficult because co-infections are more prevalent with advanced HIV-1, a scenario in which transmission is concurrently elevated due to increased systemic HIV-1 burden.

Maternal plasma HIV-1 RNA level is a key determinant of vertical HIV-1 transmission. In addition, mucosal HIV-1 RNA levels in maternal secretions to which the infant is exposed (genital secretions and breastmilk) correlate with transmission risk, independent of plasma HIV-1 levels. Co-infections that cause local inflammation (STDs, mastitis) may increase local mucosal HIV-1 RNA and increase transmissibility of HIV-1. It is plausible that co-infections may contribute to some loss of efficacy of HAART regimens if local HIV-1 shedding occurs despite systemic suppression of virus. In a study with frequent serial sampling of women on HAART, episodic detection of breastmilk HIV-1 RNA occurred despite adherence suggesting local inflammation.

To date, published studies have noted associations between HSV-2, helminth, and TB infections and mother-to-child transmission of HIV-1. Of these, the evidence base is strongest for HSV-2, which has been associated with increased systemic and genital HIV-1 shedding and mother-to-child transmission of HIV-1. Decreased systemic and genital HIV-1 RNA has also been demonstrated following anti-HSV-2 treatment (valacyclovir). There is more limited evidence for helminth and TB infections, in which single studies for each have noted increased risk of vertical transmission among mothers with the co-infection. CMV co-infection is almost universally present in HIV-1 infected women and CMV co-infection is associated with disease progression in infant HIV-1 infection.

The mechanisms by which co-infections exert their effects on infant HIV-1 acquisition and progression are likely to differ. Sexually transmitted infections, in particular HSV-2, are likely to increase transmission via increases in genital HIV-1. Bacterial, helminth, CMV, or TB infections may increase immune activation and systemic HIV-1 replication, which in turn may increase infectivity. A comprehensive approach to maternal care, including management of co-infections will be useful to minimize HIV-1 transmission, morbidity, and progression in infants born to HIV-1 infected women.

L5
Mother-to-child transmission (MTCT) of persistent viral infections: pathogenesis and prevention
Katherine Luzuriaga
Pediatrics and Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA
Several viral infections, including human immunodeficiency virus, hepatitis B and C viruses, and cytomegalovirus can be transferred from mothers to their infants; these viruses may establish persistent infections and are a significant source of child morbidity and mortality globally. The development of successful preventive antiviral or vaccine strategies is dependent upon better understanding of the infection process and transmitted viral strains, along with defining immune correlates of protection against infection or natural disease. Unique features of MTCT may be particularly helpful in understanding correlates of immune protection. Neonatal vaccination may not only prevent mother-to-child transmission but may also provide lifetime immunity. It is thus also important to define how best to elicit durable protective immune responses in infants.

L6
Neonatal adaptation of the immune system
Per Brandtzæg
Laboratory for Immunohistochemistry and Immunopathology, Department and Institute of Pathology, University of Oslo, Rikshospitalet-Radiumhospitalet Medical Center, Norway
Introduction: The vast majority of immunological challenges confronting the body make contact with mucosal surfaces, including infectious agents and foreign proteins. To maintain homeostasis in the extensive and vulnerable mucosae, they are protected by specialized mechanisms of immune protection. Numerous genes are involved in the regulation of both innate (natural) and adaptive (acquired) immunity, with a variety of modifications introduced over million of years.

Two-layered adaptive mucosal immunity: In the process of evolution, the mucosal immune system has generated two layers of non-inflammatory defence: (i) immune exclusion performed by secretory IgA antibodies to modulate or inhibit surface colonization of microorganisms and dampen penetration of potentially dangerous soluble factors; and (ii) suppressive mechanisms to avoid local and peripheral hypersensitivity to innocuous antigens, particularly food proteins and components of commensal bacteria. When induced via the gut, the latter
phenomenon is called ‘oral tolerance’, which appears to be a rather robust adaptive immune function in view of the fact that large amounts of food proteins pass through the gut, while overt and persistent food allergy is not so common.

**Neonatal immune adaptation:** The neonatal period is particularly critical, both with regard to infections and priming for allergic disease. This is so because the mucosal barrier function and the immunoregulatory network are poorly developed for a variable period after birth, and successful neonatal immune adaptation to exogenous stimuli is crucial to health. Notably, immunological homeostasis depends on appropriate microbial colonization as well as adequate timing and dose of foreign food proteins when first introduced in the diet. Dendritic cells are decision makers in the immune system when they perform their antigen-presenting function, thus linking innate and adaptive immunity by sensing the exogenous impact (e.g. conserved microbial molecular patterns) on the mucosa. A balanced indigenous microbiota is required to drive the normal development of both mucosa-associated lymphoid tissue, the epithelial barrier with its secretory IgA system, and mucosally induced tolerance mechanisms such as the generation of regulatory T cells. Moreover, the properties of dendritic cells also appear to be influenced by dietary factors including vitamin A. Other dietary factors such as lipids, particularly the polyunsaturated n-3 fatty acids in fish oil, can likewise in several ways have a beneficial effect on the developing immunophenotype of the infant. The same is true for breast milk, which provides both immunoregulatory factors and secretory antibodies reinforcing the infant’s mucosal barrier.

**L7**

The natural history of vertically acquired HCV infection
Pier-Angelo Tovo
Department of Paediatrics, University of Turin, Turin, Italy


The World Health Organization estimates that about 3% of the world population is infected with HCV and 3 million individuals are infected each year. Most of those infected develop chronic liver disease leading, in some cases, to liver failure or hepatocellular carcinoma. Little information is available on the epidemiology, natural history and responsiveness to antiviral therapy of HCV infection in children. The epidemiology has changed substantially in recent years. In Italy, for example, in the 90’s most children were affected by post-transfusional hepatitis. In contrast, in the new millennium most have been due to mother-to-child transmission. The total number of vertically infected children has also been decreasing, because the screening of blood donors has reduced the spread of the virus to women of child-bearing age. However, in the USA approximately 7000 new cases of vertical infection are estimated to occur over the next decade. The screening of blood donors has also changed the relative prevalence of different genotypes in infected children, with a decrease in transfusion-associated type 1b genotype and an increased percentage of genotypes 3 and 4; this has relevant therapeutic implications since the latter are more sensitive to specific treatment. Newer population-based studies on the epidemiology of paediatric infection in different parts of the world are needed.

During primary infection no vertically infected infant becomes icteric or developed signs. Interestingly, only one third of infected infants are viraemic at birth. The PCR assay proved highly specific with a good sensitivity (about 80%) from the first month of life. Based on the presence of viraemia over time, three groups of vertically infected children can be identified: a) with persistent viraemia, b) with intermittent viraemia, and c) seropositive children in whom serum HCV RNA was never detected. At times, children with intermittent viraemia may be PCR-negative but with increased ALT levels. Initially, ALT levels mirror a primary infection that is normal or mildly enhanced values during the first months of life, with a subsequent increase. ALT concentrations decline after the first two years of life, presumably reflecting better viral control by a more effective adaptive response after infancy. Overall, the enhancement of ALT levels was less frequent and pronounced than in adults.

HCV-associated clinical manifestations were observed in a minority of vertically infected children, with only a quarter developing hepatomegaly in the first decade. Furthermore, all children grew regularly, with no variations from the normal height and weight ranges. A high frequency of autoantibodies has been reported also in childhood, although with the bias of a selected population recruited by tertiary care centres, while the incidence of autoimmune reactions in vertical chronic infection remains to be established.

Wide ranges of histopathologic abnormalities have been found in children with vertical infection. Although to different extents, most patients had signs of chronic hepatitis. Based on signs of structural alterations, inflammatory activity, and necrosis the grade of disease usually varies from minimal to moderate, though some children have a certain degree of fibrosis. No direct correlation was found between underlying liver disease and increased ALT levels, suggesting that these are not accurate prognostic markers. Some cases of advanced liver disease in infected children have however been described, including the need for liver transplantation. In general, patient and allograft survival are suboptimal in transplanted children with chronic HCV infection, with a high risk of recurrence requiring re-transplantation with a poor prognosis.

Taken together the data suggest that about 20% of vertically infected children, being repeatedly PCR negative the last times they were tested, with no symptoms or ALT abnormalities, apparently recover from infection. Half of the children remain asymptomatic with chronic infection, fluctuations of viraemia and ALT activity. The remaining 30% have chronic active infection with persistent viraemia, abnormal ALT activity and, sometimes, hepatomegaly. Among these a fraction may develop severe liver damage.

This heterogeneity in disease progression implies the existence of virus-related and/or host-related factors conditioning the liver injury, which require specific research. Most studies agree that genotypes and viral load do not have a significant impact on the evolution of infection, although some authors suggest a worse outcome with genotype 1. Several investigations focused on the diversification of HCV quasispecies in vertically infected children. In general, only one or a low number of variants are present in the first months of life, then seroconversion leads to the development of many quasispecies. The pressure due to the humoral response is consistent with the low or no viral diversification observed in hypogammaglobulinemic subjects, who have severe disease progression. In one study, biochemical
Main infections in the children under 5 years old in developing countries: reports and perspectives

Christian Courpotin

SOLTHIS, Paris, France

SOLTHIS, Paris, France

各国の子供における主要な感染症状: 報告と視点

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Spectacular progress in reducing under 5 mortality achieved in the last few decades is projected to continue. There were about 11 million such deaths in 1997 compared to 21 million in 1955. The under-5 mortality rates per 1000 live births was 210 in 1955 and is projected to be 37 in 2025. Today about 29,000 children under the age of five – 21 minute – die every day of the 10.5 million deaths in children under five a year. Two-thirds of deaths occur in just 10 countries and almost half of them occur in sub-Saharan Africa, where progress has slowed due to lack of preventive care and treatment, fragile health systems, and socio-economic stagnation due to conflicts, instability and AIDS. According to the 2005 World Health's report near by 90% of the less than 5 years old children’s deaths are related to six major diseases: acute newborn’s problem (mainly prematurity, apparent neonatal death, infections (37%)) lower respiratory tract infections mainly pneumonia (19%), diarrhoea (18%), paludism (8%), measles (4%) and HIV/AIDS (3%). About 50% of deaths among children under 5 are associated with malnutrition. But disease isn’t inevitable, nor do children with these diseases need to die. Research and experience show that six million of the almost 10.5 million children who die each year could be saved by low-tech, evidence-based, cost-effective measures such as vaccines, antibiotics, micronutrient supplementation, insecticide-treated bed nets and improved family care and breastfeeding practices. In 2000, the millennium goals where defined by WHO. The goal number 4 is to reduce child mortality by two-thirds, from 93 children of every 1,000 dying before age five in 1990 to 31 of every 1,000 in 2015. Are we on the way to succeed?

ORAL PRESENTATIONS

O1
Primary HIV-1 infection during pregnancy: high rate of HIV-1 MTCT in a cohort of patients in southern Brazil

Karín Nielsen-Saines1, Marineide Melo2, Ivana Varella2, Rosana Fonseca2, Rita Lira2, Maria Lourdes Turella2, Ivete Canti2, Claudio Campello2, Ana Maria Moreira2 and Breno Riegel Santos2

1Department of Pediatrics/Infectious Diseases, David Geffen UCLA School of Medicine, Los Angeles, CA 90095, USA
2Department of Infectious Diseases, Grupo Hospitalar Conceição, Porto Alegre, Brazil

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Background: Mother to Child HIV-1 Transmission (MTCT) is frequent among women who receive highly active antiretroviral therapy (HAART) throughout pregnancy [1]. Women who undergo primary HIV-1 infection during pregnancy, however are at high risk of perinatal transmission [2].

Materials and methods: MTCT rates among patients receiving antenatal HAART at our institution were evaluated and compared to MTCT rates among women identified as HIV-1 infected late in pregnancy including women who became HIV-1 seropositive during gestation. Rapid HIV testing of pregnant women was performed on all women admitted in labor to our institution who had negative HIV results > 3 months prior or with unknown HIV-1 serostatus. Neonatal infection was ascertained using RNA and DNA PCR at several time points.

Results: Over two years, deliveries at our institution totalled 11,241 with 318 (2.9%) occurring in HIV-1 infected women. The incidence of HIV-1 seroconversion was 0.8/1,000 (CI 95% 0.4-1.5/1,000). The study population consisted of 256 HIV+ women who delivered at our institution having received prenatal care at our hospital and HAART during pregnancy or were identified as HIV-infected at delivery. Of these, 212 women had infants with known HIV outcomes. To 168 women on HAART, 142 infants (84%) had diagnosis ascertained: 1 child was HIV-infected (0.6%). Of the remaining, 6 (3.6%) deaths occurred in the neonatal period; 5 (3.0%) women miscarried and 15 (8.9%) infants were lost to follow-up. Eighty-eight women received no antiretroviral treatment being identified as HIV-1 infected at delivery. Infant diagnosis was ascertained in 70 cases. For the remainder, 4 (4.4%) neonatal deaths occurred before diagnosis, 7 (8.0%) women miscarried and 7 (8.0%) infants were lost to follow-up. Of these, 61 had unknown HIV-1 seroconversion time and 9 had proven seroconversion during pregnancy. In women with no treatment and unknown seroconversion time, there were 5/61 transmissions (8.2%) and in those with proven seroconversion 3/9 (33%). No women breastfed.

Conclusions: In southern Brazil, an area of high HIV-1 prevalence, seroconversion during pregnancy is not an unusual phenomenon and is associated with extremely high HIV-1 MTCT rates. Strategies should be implemented for repeat testing of patients later in pregnancy in addition to testing of partners early in pregnancy in order to identify patients at risk of seroconversion.
O2

Role of R5 phenotypic variation in mother-to-child transmission of HIV-1

Mariangela Cavarelli1, Ingrid Karlsson2, Marisa Zanchetta3, Liselotte Antonsson4, Anna Plebani5, Carlo Giaquinto6, Eva Maria Fenyo2, Anita De Rossi1 and Gabriella Scarlatti1

1Viral Evolution and Transmission Unit, DIBIT, Fondazione Centro San Raffaele, Milan, 20132, Italy
2Division of Medical Microbiology/Virology, Department of Laboratory Medicine, Lund University, Lund, 223 62, Sweden
3Department of Oncology and Surgical Sciences, Unit of Viral Oncology, AIDS Reference Center, University of Padova, 10V-IRCCS, Padova, 35022, Italy
4Division of Cellular and Molecular Pharmacology, Department of Experimental Medical Science, Lund University, Lund, 223 62, Sweden
5Department of Pediatrics, University of Milan, Clinica De Marchi, Milan, 20122, Italy
6Department of Pediatrics, University of Padova, 35022, Italy


Background: R5 viruses were shown to have an intrinsic phenotypic variation, as demonstrated by their capacity to differentially infect in vitro target cells expressing CCR5/CXCR4 chimeric receptors [1, 2]. In this study we have explored the hypothesis that the phenotypic variation of R5 viruses of pregnant women could play a role in mother-to-child transmission (MTCT) of HIV-1.

Materials and methods: Virus isolates obtained from 59 mothers (24 transmitting and 35 non transmitting) and from 24 infected children were tested for their ability to infect U87. CD4 cells expressing the wild type chemokine receptors CCR5 or CXCR4, or the six CCR5/CXCR4 chimeric receptors.

Results: Transmitting mothers (7 out of 24) carried more often viruses able to use both CCR5 and CXCR4 coreceptors than non transmitting mothers (3 out of 35) (p = ns). The analysis of the chimeric receptor usage showed that 57.1% of maternal R5 isolates displayed an R5narrow phenotype (28 out of 49 R5 viruses), as they exclusively used wild type CCR5 and none of the chimeric receptors and were similarly distributed in transmitting and non transmitting mothers. Multiple chimeric receptor using viruses (R5broad) were more frequent in non-transmitting mothers than in transmitting mothers (in 15 and 6, respectively; p = ns), and utilized more frequently one specific chimeric receptor (FC4b) (13/15 vs. 2/6, respectively; p = 0.056), but were independent from transmission event.

To understand if selective processes occur during transmission, we compared the phenotype of the virus isolates of 21 mother-child pairs. All ten mothers harbouring an R5narrow virus had children who displayed the same viral phenotype. Interestingly, the six mothers carrying R5broad viruses transmitted in all but one case a virus with an identical or similar broad chimeric receptor usage. On the contrary, the five mothers with an R5X4 virus transmitted the whole spectrum of virus phenotypes: two R5narrow, two R5broad and one R5X4.

Conclusions: Our results show that the presence of an R5broad virus appears not to be prognostic of MTCT of HIV-1. The majority of viruses replicating at a time point close to infection are restrictive to the use of wild type CCR5, however, transmission of R5broad viruses is not limited.

References


O3

Bile-salt stimulated lipase in human milk binds DC-SIGN and inhibits HIV-1 transmission

Martijn J Stax1, Marloes A Naarding1, Dave Speijer2, Olle Hernal3, Georgios Pollakis1 and William A Paxton1

1Laboratory of Experimental Virology, Academic Medical Center, University of Amsterdam, 1105AZ Amsterdam, the Netherlands
2Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, 1105AZ Amsterdam, the Netherlands
3Department of Clinical Sciences, Umea University, SE-901 87 Umea, Sweden


Background: Approximately 20% of HIV-1 infected breastfeeding mothers transmit virus to their infants. It has been hypothesized that dendritic cells expressing C-type lectins, such as DC-SIGN, play an important role in the establishment of infection with HIV-1 and several other pathogens. Within our laboratory we have identified that Bile Salt Stimulated Lipase (BSSL) is able to bind to DC-SIGN and block HIV-1 transmission via dendritic cells. The C-terminal part of BSSL contains a highly polymorphic repeat section coded by exon 11 of the gene and is composed of an array of 11 amino acid repeats.

Materials and methods: We have studied a large number of human milk samples from HIV-1 negative mothers. The BSSL protein was analyzed by size fractionation and iso-electric focusing. We studied the genomic structure of the gene through PCR amplification and sequencing of the BSSL repeats for a group of selected mothers.

Results: Milk samples from a large number of different mothers (n = 25) were identified that demonstrated variant levels of inhibition to viral transfer. We have studied specific BSSL genotypic as well as phenotypic properties in order to identify what provides for the large variation of milk binding DC-SIGN. The tested milk samples were divided into weak binding and strong binding groups based on their DC-SIGN...
binding capacity. When comparing the PCR results for the BSSL repeat number we identified a link between the number of repeat domains and inhibition, with the more repeats binding less efficiently. A selection of weak and strong binders with identical repeat numbers was also made. Sequencing of this selected group revealed a mutation in the repeat section of an extreme weak binder on an interesting position with regards to BSSL glycosylation. Further analysis at the proteomic level was performed with a higher molecular mass for BSSL in the weak binding group being identified. Analysis of the DIGE results showed a shift in pI of BSSL in the weak binding group for 3 out of 5 cases.

**Conclusions:** Our results demonstrate that multiple factors contribute to the differential binding of human milk to DC-SIGN. Variation in the DC-SIGN binding capacities of BSSL may provide for alterations in transmission patterns of pathogens or in altering the immune mediated responses mounted in children against milkborne pathogens. Furthermore, understanding these differences in BSSL could aide in the development of new agents aimed at preventing pathogen transmission across a mucosal barrier.

**O4**

**Human herpesvirus 8 (HHV-8) “in vitro” infection of human placental histocultures**

Mariantonietta Di Stefano¹, Maria Luisa Calabro², Iole Maria Di Gangi³, Santina Cantatore³, Massimo Barbierato⁴, Luigi Chieco-Bianchi⁴, Pantaleo Greco⁵, Loreto Gesualdo¹, Elisabeth Menu⁶ and José Ramon Fiore⁷

¹Laboratory of Molecular Medicine, University of Foggia, Foggia, Italy
²Istituto Oncologico Veneto, IRCCS, Immunology and Diagnostic Molecular Oncology, Padova, Italy
³Laboratory of Histology, School of Medicine, University of Foggia, Italy
⁴Department of Oncology and Surgical Sciences, Oncology Section, University of Padova, Padova, Italy
⁵Department of Surgical Sciences, University of Foggia, Foggia, Italy
⁶Unité de Régulation des Infections Rétrovirales, Institut Pasteur, Paris, France
⁷Department of Clinical and Occupational Health, University of Foggia, Foggia, Italy

**Background:** Most human Herpesvirus infect placental cells and may be harmful in pregnancy, leading to obstetrical and/or neonatal complications. Although a correlation between human herpesvirus 8 (HHV-8) infection and abortion or low birth weight in children has been reported [1, 2] presently no information has been published regarding HHV-8 tropism for placenta.

**Materials and methods:** In this study, a placenta histoculture system was used to evaluate the susceptibility of placental cells to “in vitro” HHV-8 infection. Quantitative detection of HHV-8 was performed by real-time PCR, and virus expression was evaluated by immunohistochemistry for latent and lytic HHV-8 antigens.

**Results:** Increasing amounts of HHV-8 DNA were detected in placental tissues and culture supernatants and immunohistochemistry analyses demonstrated that both cyto- and syncitio-

**Figure 1 (abstract O4)**

Immunohistochemical detection of the HHV-8 LANA protein in placental histocultures. Specific reactivity was visualized with immunoperoxidase staining using anti-LANA-1 monoclonal antibodies with a DAB developer (brown colour) and haematoxylin counterstaining. (A) HHV-8-infected CRO-AP/3 cells showed a strongly positive nuclear immunostaining. (B) HHV-8-infected placental histocultures showed positive immunostaining in cytotrophoblasts (yellow arrow), syncytiotrophoblasts (blue arrow) and endothelial cells (white arrow). (C) Mock-infected placental histocultures. Original magnifications, X100.
trophoblasts, as well as placental endothelial cells, expressed latent (see Figure 1) and lytic antigens. In addition, relevant apoptotic phenomena were observed in infected histocultures.

Conclusions: We here demonstrated for the first time that HHV-8, like other human herpesviruses, may productively infect placental cells in vitro, thus providing evidence that this phenomenon might influence vertical transmission and pregnancy outcome in HHV-8-infected women.

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References


O5 HIV-1 internalization in polarized human trophoblasts occurs through a peculiar endocytic pathway
Michel J Tremblay and Gaël Vidricaire
Centre de Recherche en Infectiologie, Centre Hospitalier de l'Université Laval, and Département de Biologie médicale, Université Laval, Quebec (QC), Canada


Background: In human trophoblastic cells, a correlation between early endosomal trafficking of HIV-1 and virus infection was previously documented. However, if HIV-1 is massively internalized in these cells, the endocytic pathway(s) responsible for viral uptake is still undefined.

Materials and methods: The process through which HIV-1 is endocytosed was studied using different reagents (e.g. chlorpromazine, cholera toxin B, water-soluble cholesterol, colchicine, cytochalasin B, filipin, jasplakinolide, methyl-beta-cyclodextrin, paclitaxel, and vinblastine) and experimental strategies (e.g. transfection of JAR cells with various expression vectors, virus internalization test, infection assay, confocal laser scanning, colocalization analysis and digital image preparation).

Results: Amongst all the putative endocytic pathways present in polarized trophoblastic cells, we demonstrate that HIV-1 infection of these cells is independent of clathrin-mediated endocytosis and macropinocytosis. Importantly, treatment with the cholesterol-sequestering drug filipin severely impairs virus internalization, whereas the cholesterol-depleting compound methyl-beta-cyclodextrin has no impact on this pathway. Moreover, viral internalization is unaffected by overexpression of a mutant dynamin 2 or treatment with a kinase or tyrosine phosphatase inhibitor. Thus, HIV-1 infection in polarized trophoblastic cells occurs primarily via a clathrin-, caveolae-, and dynamin-independent pathway requiring free cholesterol. Notably, even though HIV-1 did not initially co-localize with transferrin, some virions migrate at later time points to transferrin-enriched endosomes, suggesting an unusual transit from the non-classical pathway to early endosomes. Finally, virus internalization in these cells does not involve the participation of microtubules but relies partly on actin filaments.

Conclusions: We demonstrate that HIV-1 internalization in polarized human trophoblastic cells occurs primarily via a clathrin-, caveolae-, and dynamin-independent pathway which is sensitive to a cholesterol-sequestring drug.

O6 A major susceptibility locus for HTLV-1 infection in childhood maps to chromosome 6q27
Sabine Plancoulaine1,2, Antoine Gessain1, Patricia Tortevoye2, Anne Boland-Auge3, Alexandre Vasilescu2, Fumihito Matsuda3 and Laurent Abel3
1U550, INSERM, Paris, France; Human Genetics of Infectious Diseases, Université Paris Descartes, Paris, France
2Unité d’Épidémiologie et Physiopathologie des Viruses Oncogènes, Institut Pasteur, France
3Centre National de Génotypage, Evry, France


Background: Human T-cell leukemia/lymphoma virus type 1 (HTLV-1) is a human oncoretrovirus causing adult T-cell leukemia/lymphoma (ATL) and chronic neumyelopathy. We showed previously, by segregation analysis, that a dominant gene controls HTLV-1 infection through breast-feeding in children of African origin.

Materials and methods: To map this locus, we performed a genome-wide linkage analysis, based on the genetic model provided by segregation analysis, in five pedigrees (46 subjects with available DNA) of African origin with HTLV-1-seropositive children. A total of 382 microsatellites markers spanning the whole genome were typed. Two attractive positional genes located within the linked regions were further studied through an association analysis in an independent sample of 59 cases (24 HTLV-1 infected children and 25 ATL) and 48 controls (27 HTLV-1 seronegative but exposed children and 21 HTLV-1 seronegative young individuals) of African origin.

Results: Significant evidence for linkage (lod-score of 3.36, p = 0.00004) was obtained for chromosomal region 6q27. Another maximum lod-score of 2.79 (p = 0.0002) was obtained for chromosome 2p25. This result was entirely due to the largest pedigree of our sample, which alone gave a lod-score of 2.90 (p = 0.00013). The role of exonic variants of CCR6 on 6q27 and ID2 on 2p25 was excluded.

Conclusions: Our results, mapping a major susceptibility locus to chromosome 6q27 and suggesting genetic heterogeneity with another locus at 2p25, pave the way to determination of the molecular basis of predisposition to HTLV-1 infection in children. [1]

Reference
Results: The number of peptide pools showing positive CTL reactivity.

Variations in antigenic specificity and breadth of antigenic recognition, variations that could be explained by sequential waves of expansion and contraction of CTL clones of mixed antigenic specificity and/or continuing viral escape from CTL responses. These results provide a novel understanding of the dynamics of HIV-specific CTL responses during pregnancy and may help to promote maternal immunization as a strategy to prevent MTCT of HIV-1.

O8 HCV-specific T-cell responses during acute Hepatitis C Virus infection in pregnancy
Jonathan Honegger1, Mona Prasad2, David Colombo2, David Bowen1 and Christopher Walker1,3

Materials and methods: A 34 year old pregnant woman was diagnosed with acute genotype 1a HCV infection when she presented with jaundice at 24 weeks gestation. She delivered at 34 weeks gestation. T-cell immunity, plasma viremia, and liver function (ALT) were assessed serially from week 28 gestation through week 26 post-partum. HCV-specific T-cell activity in peripheral blood was quantified by ex vivo interferon-gamma ELISpot using overlapping peptides spanning the entire HCV polyprotein.

Results: During pregnancy, HCV viral load (IU/mL) rose from 1.3 million at 24 weeks gestation to 25.4 million at 34 weeks gestation (delivery). High viremia during pregnancy was associated with low frequency T-cell responses restricted to HCV NS3 and NS5b proteins. At week 8 post-partum the viral load fell sharply to 11,400, but rebounded to 16.6 million at week 26. The transient 1,000-fold drop in viremia in the early post-partum period was accompanied by a transient 5-fold increase in T-cell frequency with broadening of the response to most viral proteins. T-cell activity returned to low levels by week 26 post-partum. Failing T-cell immunity accompanied by a climb in viral load in the months after delivery may predict a persistent course of infection for the mother. Preliminary data also indicate that the baby was infected in the perinatal period. Comparisons of HCV evolution and immune responses in the mother and child are ongoing.

Conclusions: In this case of acute HCV infection during pregnancy, T-cell responses increased prominently in frequency and breadth in the early post-partum period, and were associated with a sharp fall in viral load. This surge occurred several months after the onset of symptoms, suggesting that it was not the initial primary T-cell immune response to HCV, but perhaps related to a post-partum phenomenon. This finding needs to be corroborated in further studies of HCV infection during pregnancy, as it may
afford new insights into T-cell control of HCV and maternal influences on vertical HCV transmission.

O9 Toxoplasmosis reactivation following HAART introduction associated with foetal death in a severely immune suppressed HIV-infected woman: an immune reconstitution inflammatory syndrome (IRIS) consequence?

Fabienne Caby1, Delphine Lemerrier2, Fériel Touafek3, Luc Paris3, Romulus Grigorescu1, Marie Gonzales4, Guislaine Carcelain2, Michèle Pauchard1, Ana Canestri1, Christine Katlama1, Marc Domergues1 and Roland Tubiana1

1Department of Infectious Diseases, Pitié-Salpêtrière hospital, Paris, France
2Department of Obstetrics, Pitié-Salpêtrière hospital, Paris, France
3Department of Parasitology, Pitié-Salpêtrière hospital, Paris, France
4Department of Feto PATHology, Trousseau hospital, Paris, France
5Department of Immunology, Pitié-Salpêtrière hospital, Paris, France


Background: Congenital toxoplasmosis usually results from acquired infection in non-immune pregnant woman. Some cases have been described in immunodeficient women, as a result of Toxoplasma gondii infection reactivation [1, 2, 3]. We report the case of a fetal death probably related to a congenital toxoplasmosis in an HIV infected pregnant woman during immune restoration following HAART initiation.

Case report: An HIV infection was diagnosed in a 26 years old African pregnant woman at 10 weeks of pregnancy. The CD4 cell count was 7/µl (1%), the HIV viral load was 108.000 cp/ml, and the toxoplasmic serology was positive for IgG (18000 UI/ml) and IgM negative, the retrospective toxoplasma detection by PCR was negative at entry.

HAART consisting in a combination of lopinavir/r and zidovudine plus lamivudine was introduced at 12 weeks of pregnancy and resulted in a rapid immune restoration 2 weeks later: At that time the CD4 cell count increased up to 185/µl (10%) and the plasmatic HIV viral load dropped to 1.222 cp/ml. A miscarriage occurred 7 weeks after HAART introduction. At this time, toxoplasma PCR was positive in the mother plasma as well as in the amniotic fluid, concomitantly with an anti toxoplasma IgG increase (81.000 UI/ml) and occurrence of anti toxoplasma IgM, amniotic fluid HIV viral load was undetectable.

The post-mortem analysis dated the foetus death around 16 weeks of pregnancy that was 4 weeks after the HAART introduction and showed evidence of non specific inflammatory histologic lesions.

Conclusions: The chronological parallelism between toxoplasmosis reactivation and rapid CD4 cell count increase makes us wonder if this miscarriage case could not be an immune reconstitution inflammatory syndrome manifestation associated with toxoplasmosis reactivation. According to this hypothesis, we should take into account IRIS risk factors in HIV infected pregnant woman when introducing HAART, not only for the mother but also for a healthy foetal development.

References

O10 High prevalence of cytomegalovirus (CMV) infection in infants born to HIV infected mothers—ANRS French Perinatal Cohort (EPF)

Gaelle Guibert1, Marianne Leruez-Ville2,3, Laurent Mandelbrot1,6,7, Stéphane Blanché3,8, Jean-Paul Teglas1,9, Yassine BenMbarek1,10,11, Jérôme Le Chenadec1,9, Josiane Warszawski1,9,10,11, ANRS French Perinatal Cohort (EPF)

1Inserm, U822, Le Kremlin-Bicêtre, France, F-94276
2AP-HP, Virology Department, Necker Hospital, Paris, F-75015 France
3EA 3620, Univ Paris Descartes 5, Paris, France
4AP-HP, Department of infectious diseases, Hôpital Pitié Salpêtrière, Paris, F-75651 France
5INSERM, U543, Paris, France
6Univ Paris 7, Paris, France
7AP-HP, Gynecology and obstetrics department, Hôpital Louis Mourier, Colombes, France, F-92700
8AP-HP, Unité d’Immunologie Hématologie Pédiatrique, Necker Hospital, Paris, France, F-75015
9INED, Paris, France, F-75020
10AP-HP, Epidemiology department, Hôpital Bicêtre, Le Kremlin-Bicêtre, France, F-94276
11Univ Paris-Sud, Faculté de Médecine Paris-Sud, Le Kremlin-Bicêtre, France, F-94276

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Background: In developed countries, 0.3% to 0.5% of all newborns are congenitally infected by cytomegalovirus (CMV) with the risk of sensorineural hearing loss or mental retardation [1, 2]. Few results about congenital CMV infection in infants born to HIV-infected women have been reported [3]. Rate of disease progression and central nervous system disease was found to be higher in HIV-I-infected infants who acquire CMV infection in the first 18 months of life than those infected with HIV-I alone [4]. We aimed to estimate the prevalence of neonatal CMV infection in children born to HIV-infected mothers between 1993 and 2004 enrolled in the ANRS French Perinatal Cohort (EPF).

Materials and methods: EPF is a national prospective multicenter cohort of mother-to-child HIV transmission. As part of the standardized follow-up of infants born alive between 1993 and 2004 in EPF sites, a urine sample was obtained within
the ten first days of life. These samples were used to screen for congenital CMV infection, using rapid culture from 1993 to 2001 and real-time PCR since 2001.

**Results:** Between 1993 and 2004, 4995 of the 7878 newborns included in EPF were screened for CMV. The prevalence of CMV infection was 2.4% (119 positive tests; 95% confidence interval: 2.0–2.8). Thirteen of the 119 CMV infected newborns were also infected with HIV. The prevalence of CMV infection was higher in HIV-infected newborns (10.2%; 95% CI: 4.9–15.5) than in HIV-uninfected newborns (2.2%, 95% CI: 1.8–2.6, p < 0.01).

**Conclusions:** The prevalence of congenital CMV infection was high in children born to HIV-infected mothers and was significantly higher in HIV-infected children than HIV-uninfected children.

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**References**


**O11**

**Hepatitis C virus quasispecies evolution during pregnancy and between consecutive pregnancies: influence of maternal immune responses**

Myriam Troesch1,2, Isabelle Meunier1,2, Christine Lepage1,2, Johanne Samson3, Normand Lapointe1,2, Marc Boucher1,5 and Hugo Soudeyns1,2,4

1Unité d’immunopathologie virale, CHU Sainte-Justine, Montreal, Quebec, Canada, H3T 1C5
2Department of Microbiology & Immunology, Université de Montréal, Montreal, Quebec, Canada, H3C 3J7
3Centre maternel et infantile sur le SIDA, CHU Sainte-Justine, Montreal, Quebec, Canada, H3T 1C5
4Department of Pediatrics, Université de Montréal, Montreal, Quebec, Canada, H3C 3J7
5Department of Gynecology & Obstetrics, Université de Montréal, Montreal, Quebec, Canada, H3C 3J7

**Background:** Hepatitis C virus (HCV) can be transmitted from mother-to-child during pregnancy and childbirth. Most importantly, the rate of vertical HCV transmission is increased four-fold in the presence of maternal coinfection with human immunodeficiency virus type 1 (HIV-1). To gain insight into the evolution of HCV disease during pregnancy and to better understand the influence of HIV-1 coinfection, HCV quasispecies composition was characterized longitudinally in a group of 17 pregnant women infected with HCV, including 13 subjects coinfeected with HIV-1, 4 of whom were followed during 2 consecutive pregnancies.

**Materials and methods:** HCV RNA was extracted from serum and E2 hypervariable region 1 (HVR1) and flanking regions (positions 1278–1889) were amplified by RT-PCR. Amplicons were cloned and plasmid DNA from transformants was sequenced unidirectionally. Sequences were aligned using Clustal X. A mean of 18.8 cDNA clones of HVR1 were analyzed per time point (1,337 clones representing 1,101 non redundant sequences). Mean genetic distance (p distance) and its standard deviation were calculated using MEGA2.1. dN/dS ratios were computed according to the Nei-Gojobori method. Normalized Shannon entropy (Sn) was used to account for the frequency of each different variant in the quasispecies. Diversification of viral variants was assessed using phylogenetic reconstructions built according to the neighbour-joining method and the Kimura two-parameter model.

**Results:** Median aspartate aminotransferase and HCV RNA levels were higher in coinfected subjects throughout pregnancy, with 3rd trimester HCV RNA levels significantly higher than those observed in subjects infected with HCV alone (p = 0.0283, Mann-Whitney U test). Quasispecies complexity based on numbers of HVR1 variants or Shannon entropy was higher in subjects treated with and responding to antiretroviral therapy (treated-responders; n = 11) than in untreated-non-responders (n = 6) or subjects infected with HCV alone (n = 4) at all time points examined. Analysis of dN/dS ratios revealed that intrahost selective pressure was consistently larger in treated-responders than in HCV-only subjects, and always higher in HCV-only than in untreated-non-responders. Finally, the level of diversification of HVR1 observed between consecutive pregnancies was incompatible with linear genetic drift during the inter pregnancy interval.

**Conclusion:** Overall, these results indicate that: a) coinfection with HIV-1 leads to reduced immune pressure on HVR1; b) this immunosuppressive effect is overturned by antiretroviral treatment; and c) pathological events observed in HCV-infected women in late pregnancy are immune-mediated. This study will lead to a better understanding of therapeutic and immune factors that influence viral evolution and the clinical outcome of hepatitis C during pregnancy.

**O12**

**Risk factors for human cytomegalovirus (HCMV) infection in infants born to HIV-1 infected mothers in Thailand**

Wootitichai Khamdung1,2, Wasna Sirirungs2, Gonzague Jourdain1,3, Baptiste Leurent1, Kenneth McIntosh1, Karin Pagdi2, Rosalim Somsamai5, Surat Sirinontakan6, Tensmir Hinjiranananda1, Wanna Ardong8, Marc Lallemant1,5, Rosalin Somsamai5 and Nicole Ngo-Giang-Huong1,3

1Institut de Recherche pour le Developpement UMI 174/Programs for HIV Prevention and Treatment (PHPT), Chiang Mai, Thailand, 50100
Background: In Thailand where virtually all pregnant women are infected with HCMV, about 2% of infants are congenitally infected with HCMV [1]. However, the transmission rate among infants born to HIV-1 infected mothers is not well known. Our objectives were to evaluate HCMV transmission rates in infants born to HIV-1 infected mothers, and to identify maternal and newborn risk factors associated with infant HCMV infection.

Materials and methods: Ninety-seven HIV-1 transmitting mothers were matched on maternal plasma HIV-1 RNA before zidovudine prophylaxis initiation with 194 non-transmitting mothers enrolled in PHPT-I [2], an HIV prevention trial in Thailand. Infant HCMV infection was assessed by anti-HCMV serology at 18 months. Congenital HCMV infection was defined as the presence of HCMV IgM and/or a positive DNA PCR within 10 days of life. Univariate odds ratios (95% confidence intervals) were calculated for potential risk factors among maternal (age, HIV and immunological stage, pregnancy history, past/present sexual transmitted diseases, CD4/CD8 T-lymphocyte counts) and infant characteristics (HIV status, sex, prematurity and birth weight). Adjusted odds ratios were calculated using logistic regression with stepwise selection of variables with less than 0.20 p value association.

Results: The prevalence of congenital HCMV infection was 16% (10/62) in HIV-1 infected infants and 5% (5/105) in uninfected infants, p = 0.013. The prevalence of HCMV infection by 18 months of age was 83% (62/75) in HIV-1 infected infants and 62% (112/182) in uninfected infants, p = 0.001. Upon univariate analysis, among the maternal factors, only vaginal delivery was associated with HCMV infection in infants (OR: 2.5; 95%CI: 1.3–4.7). Among infants’ factors, HIV infection (OR: 3.3; 95%CI: 1.7–7.0) and prematurity (OR: 3.5; 95%CI: 1.0–18.8) were associated with HCMV. Upon multivariate analysis only vaginal delivery (OR: 2.5; 95%CI: 1.3–4.5) and infant HIV infection (OR: 3.3; 95%CI: 1.7–6.4) remained independently associated with HCMV infection in infants.

Conclusions: Infant HIV infection and vaginal delivery are the main risk factors for HCMV infection in children born to HIV-1 infected mothers. The clinical consequences of congenital and postnatal HCMV infection on HIV disease progression need to be assessed.

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References

Co-infection with Trypanosoma cruzi (Chagas’ disease agent) decreases HIV-1 transcription in human placenta

Guillermina Dolcini1, María Elisa Solana2, Guadalupe Andreani1, Ana María Celentano2, Ana María Donato3, Alberto Del Río3 and Liliana Martínez Peralta1

1 National Reference Centre for AIDS, Microbiology Department, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina
2 Laboratory of Parasitology, Microbiology Department, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina
3 Endocrinology Service, Clinic Biochemistry Department, José de San Martín Hospital, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

Background: Several factors determine the risk of HIV mother-to-child transmission (MTCT), such as co-infections in placentae from HIV-1 positive mothers with other pathogens [1, 2]. One of the most important endemic zoonosis in Latin America is Chagas’ disease, caused by the protozoa Trypanosoma cruzi. MTCT of T. cruzi is today one of the main transmission routes in big cities [3]. The aim of the study was to determine whether T. cruzi modifies HIV infection at tissue or cellular level of the placenta.

Material and methods: Simple and double infections were carried out on a placental histoculture system (chorionic villi isolated from term placentae from HIV and Chagas negative mothers) and on choriocarcinoma BeWo cell line. We used trypomastigotes of T. cruzi (VD lethal strain, isolated from a child with MTCT), either purified from mice blood or from Vero cell cultures, 24h-supernatants of blood and cellular trypomastigotes, and the viral HIV-1 DenLuc+VSV-G pseudotype. Viral replication was evaluated by luciferase activity quantification. Tissue viability was evaluated by hCG hormone secretion in histoculture supernatant. Quantification of soluble factors protein secretion and mRNA expression in histocultures were carried out by ELISAs and real time PCR respectively.

Results: Whole trypomastigotes, either from mice blood (mean ± SD: –92.13 ± 4.85) or from cell cultures (–97.33 ± 0.58), in co-infection with viral pseudotype decreased luciferase activity in placental histocultures. Similar results were obtained on BeWo cells. When supernatants from blood trypomastigotes were used on placental histocultures, luciferase...
activity presented a decrease of $\approx 85.50\% \pm 2.12\%$, while supernatants from culture trypomastigotes presented lower effects ($\approx 65.50\% \pm 2.12\%$). Tissue viability was not modified by viral and/or parasite infection. In co-infected histocultures, both protein secretion and mRNA expression of IL-10 were down regulated. Surprisingly, RANTES was increased.

**Conclusions:** Acute infection with *T. cruzi* and HIV-1 in this placenta culture system as well as in the trophoblast cell line decreased HIV-1 transcription. *T. cruzi* activity on HIV-1 replication seems to be caused not only by active infection but also by soluble factors shed by the parasite. A balance between proinflammatory and inhibitory cytokines/chimiokines might have a role in this phenomenon. Ongoing experiments are being conducted in order to elucidate the mechanisms involved in the impairment of HIV replication by *T. cruzi* and their role in MTCT of both pathogens.

**References**


**O14 Plasmodium falciparum** and placental cytokine profiles among pregnant women in relation to their HIV-1 status: possible implications for mother-to-child transmission (MTCT) of HIV-1 in Cameroon

Anfumbom Kftuwah1, Jean Yves Mary2, Brigitte Lemen1, Robert Leke3, Dominique Rousset1, Françoise Barré-Sinoussi1, Eric Nerrienet4, Elisabeth Menu5 and Ahidjo Ayobua1

1Virology laboratory, Centre Pasteur du Cameroun, BP 1274 Yaoundé, Cameroon
2INSERM U717, Université Paris 7, DBIM, Hôpital St Louis, 75010 Paris, France
3Centre d’Animation Sociale et Sanitaire (CASS) North, Yaounde, Cameroon
4The maternity of the Central Hospital, Yaoundé, Cameroon
5Unité de Régulation des Infections Rétrovirales, Institut Pasteur, 75015 Paris, France


**Background:** Placental cytokines play vital roles in establishing and maintaining pregnancy as well as protecting the fetus from in utero infections. Previous studies have strongly suggested the implication of co-infections such as *P. falciparum* in the in utero MTCT of HIV-1 H[1,2,3]. This study was designed to assess the impact of *P. falciparum* on the influence of HIV-1 infection on placental cytokine profile and the association of these profiles with clinical factors known to be related to HIV-1 MTCT.

**Materials and methods:** *P. falciparum* was tested in the peripheral and/or placental blood from 50 and 80 HIV-1 negative and positive women respectively. Cytokines (proteins) were quantified in the supernatants of 24 hours culture of placental explants by ELISA while cytokine mRNAs were quantified in placental tissue by real time PCR. Antibodies to the DBL3γ domain of GPIEMP1 that binds *P. falciparum* infected red blood cells to placental CSA were titrated by ELISA in sera. The comparisons of the levels of cytokine proteins and mRNAs, as well as of anti-DBL3γ antibodies between HIV-1 negative and positive women who were either *P. falciparum* negative, or positive in the periphery or placenta, were tested through non-parametric tests, as well as the associations between cytokine profiles and clinical factors.

**Results:** Placental and peripheral *P. falciparum* infection was comparable in both HIV-1 negative and positive women (from 18 to 24%). Conversely, *P. falciparum* parasitemia was significantly higher in the HIV-1 positive group. Large individual variations were observed in placental cytokine proteins and mRNA expression in each group. No significant differences were observed between placental cytokine median levels (protein and mRNA) in HIV-1 negative and positive women. However, among *P. falciparum* negative women, we observed significant differences in several cytokine median levels (TNF-α, IL-10, IL-16, IL-7, LIF, and RANTES) between HIV-1 negative and positive women. Median levels of antibodies to DBL3γ were significantly higher in the HIV-1 negative group (p = 0.03) and was dependent of peripheral and placental *P. falciparum* infection. TNF-α among the HIV-1 positive women was the only cytokine associated with clinical parameters linked to HIV-1 MTCT (premature rupture of membranes, number of pregnancies and parity; p = 0.04).

**Conclusions:** Altogether these results highlight the reciprocal influence of both infections at the materno-fetal interface that might have possible implications for in utero HIV-1 MTCT in areas where HIV-1 and *P. falciparum* co-circulate.

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**References**

O15
VSV/MVA vaccine rapidly elicits SIV antibodies and local and systemic SIV T cell responses in macaque neonates but does not prevent SIV dissemination after oral challenge
Kristina Abel1, Koen KA Van Rompay1, Juliet Eastlick1, Joseph Moore2, Mathieu Lemieux3, Kimberly Schmidt1, Patricia Earl2, Linda Buonocore-Buzzelli2, Bernard Moss1, Nina Rose4, John Rose5, Michael B McChesney1, Pamela Kozlowski6 and Marta L Marthas1
1Virology and Immunology Unit, California National Primate Research Center, Davis, CA, 95616, USA
2Laboratory of Viral Diseases, National Institute of Allergy & Infectious Diseases, Bethesda, MD, 20892, USA
3Department of Pathology, School of Medicine, Yale University, New Haven, CT, 06520, USA
4Department of Genetics and Gene Therapy, Health Sciences Center, Louisiana State University, New Orleans, LA, 70122, USA

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Background: Despite availability of antiretroviral therapies, a neonatal vaccine is needed to prevent HIV-1 breast milk transmission in resource-poor settings. Given the relative immaturity of the infant immune system and the frequent, long-term exposure to HIV in breast milk, a neonatal HIV vaccine must induce quick, strong, and long-lasting anti-HIV immunity. Our prior studies demonstrated that intramuscular immunization with attenuated poxvirus-based SIV vaccines gave infant macaques partial protection against oral SIV challenge. We hypothesized that a vaccine vector that can replicate after oral administration may induce better mucosal immunity and be more effective.

Objectives: To test the safety, immunogenicity and efficacy of a recombinant vesicular stomatitis virus (VSV)-SIV gag, pol env (SIVgpe) prime/modified vaccinia Ankara (MVA)-SIVgpe boost vaccine regimen in infant rhesus macaques.

Materials and methods: VSV-SIVgpe was orally administered at birth, followed by intramuscular injection with MVA-SIVgpe at 2 weeks of age to eight rhesus macaques. All vaccinated and eight unvaccinated infant macaques were challenged at 4 weeks of age by a repeated oral low-dose SIVmac251 inoculation regimen to mimic breast milk exposure. Lymphocyte subsets and SIV-specific T cell responses were assessed by multiparameter flow cytometry. Antibody levels were measured by whole SIV-lysate and SIV gp130 env ELISAs. SIV RNA levels were measured by SIV branched chain DNA assay.

Results: The VSV/MVA-SIVgpe vaccine elicited SIV-specific plasma antibodies (IgG and IgA) as well as CD4+ and CD8+ T cell responses in several oral and systemic lymphoid tissues. Despite the persistence of SIV-specific antibodies and T cell responses after SIV challenge, these responses were insufficient to prevent rapid virus dissemination. Plasma viral RNA levels in most vaccinates were indistinguishable from controls. Vaccinates with highest SIV gp130 antibody levels at the time of oral SIV infection had lowest viremia. Although few SIV-specific CD8+ T cells were observed, these cells were activated and had cytotoxic activity (CD107 expression after in-vitro SIV antigen stimulation). T cell activation in tonsils was associated with lower numbers of regulatory T cells in this tissue.

Conclusions: Although this oral + injected SIV vaccine regimen succeeded in rapidly eliciting virus-specific antibodies and T cell responses in neonatal macaques, these immune responses did not limit virus dissemination after oral SIV inoculation. These data underline some of the challenges a vaccine must overcome to protect against HIV breast milk transmission

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O16
Is access to care different for women from sub-Saharan Africa than for French women according to prevention of mother-to-child HIV transmission in France?
Carine Jasseron1,2, Josiane Warszawski1,2,3,4, Stéphane Blanche5,6, Christine Rouzioux6,7, Jérôme Le Chenadec1,4, Catherine Dollfus8, Albert Faye9, Karima Hamrene1,2, Jean-Paul Teglas2,4, Roland Tubiana9,10,11, Laurent Mandelbrot12,13, and ANRS French Perinatal Cohort EFP
1Inserm, U822, Le Kremlin-Bicêtre, France, F-94276
2AP-HP, Epidemiology department, Hôpital Bicêtre, Le Kremlin-Bicêtre, France, F-94276
3Univ Paris-Sud, Faculté de Médecine Paris-Sud, Le Kremlin-Bicêtre, France, F-94276
4INED, Paris, France, F-75020
5AP-HP, Unité d’Immunologie Hématologie Pédiatrique, Necker Hospital, Paris, France, F-75015
6EA 3620, Univ Paris Descartes 5, Paris, France
7AP-HP, Virology Department, Necker Hospital, Paris, F-75015 France
8AP-HP, Service d’Hématologie et d’oncologie pédiatrique, Hôpital Trousseau Paris, F-75571 France
9AP-HP, Service de Pédiatrie Générale, Hôpital Robert Debré, Paris, F-75019 France
10AP-HP, Department of infectious diseases, Hôpital Pitié Salpêtrière, Paris, F-75651 France
11INSERM, U543, Paris, France
12Univ Paris 7, Paris, France
13AP-HP, Gynecology and obstetrics department, Hôpital Louis Mourier, Colombes, France, F-92700

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Background: Among HIV-infected women delivering in France, two thirds are immigrants from sub-Saharan Africa. A high proportion of immigrants live in precarious conditions [1, 2], which may be a potential obstacle to adequate prevention of HIV Mother-to-child transmission (MTCT). We aimed to compare indicators of access to prevention of MTCT between African and French-born women.

Materials and methods: We used data from the French Perinatal HIV Cohort (EFP), a multicenter prospective cohort of HIV-infected pregnant women and their children. Women enrolled in EFP were included in our analysis if they were infected by HIV-1, and delivered in mainland maternities from 1985 to 2004: 9245 pregnancies in 7090 mothers (3292 from Sub-Saharan Africa and 2766 from mainland France). Comparison of indicators of access to prevention of MTCT according to prevention of mother-to-child HIV transmission in France?

Results: Compared with French women, African women had later access to care, whatever the study period. During the HAART era period, a higher proportion of African women discovered their HIV infection during pregnancy (6.8% vs 1.2%
after 28 weeks, respectively), started prenatal care in the third trimester (14.1% vs 9.8%) and started antiretroviral treatment late, after 33 weeks (7.6% vs 4.1%). The association with late treatment initiation disappeared when adjusting for the time of HIV diagnosis and first prenatal visit (adjusted OR: 1.0 : 95%CI : 0.8–1.5). Geographic origin was not associated with management in contradiction with French recommendations, such as monotherapy or vaginal delivery with uncontrolled delivery viral load, lack of intrapartum and postpartum treatment or breastfeeding. Highly active antiretroviral therapy during pregnancy was as frequently used in African and in French women. Conclusion: African women had a higher proportion of late HIV screening in pregnancy than French women, but access to MTCT prevention, once the infection was diagnosed was similar in those two groups.

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O17 Prevention of mother-to-child transmission of HIV-1 through breastfeeding by treating infants or mothers prophylactically with antiretrovirals in Dar es Salaam, Tanzania: the MITRA and MITRA PLUS studies

Charles Kilewo 1, Katariina Karlsson 2, Matilda Ngarina 3, Augustine Massawe 4, Elgious Lymuaya 5, Rosina Lipyoga 6, Georgina Msomo 7, Muhamad Bakarji 8, Andrew Swai 9, Fred Mhalu 10 and Gunnal Biberfeld 2

1 Department of Obstetrics and Gynaecology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
2 Department of Immunology and Vaccinology, Swedish Institute for Infectious Disease Control (SMI) and Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, SE-171 82, Stockholm, Sweden
3 Department of Obstetrics and Gynaecology, Muhimbili National Hospital, Dar es Salaam, Tanzania
4 Department of Pediatrics, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
5 Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
6 Department of Pediatrics, Muhimbili National Hospital, Dar es Salaam, Tanzania
7 Department of Internal Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
8 Department of Internal Medicine, Muhimbili National Hospital, Dar es Salaam, Tanzania

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O18 Prevalence of HIV-1 drug resistance mutations in antiretroviral naive pregnant women from Cambodia, Cameroon, Central African Republic and Vietnam

Achiedy Ayoubia 1, Lénair Le Fouler 2, Truong TX Lien 3, Nary Ly 1, Valérie Maréchal 5, Aurelia Véssière 4, Anfumbom Kftuwah 1, Jean Marc Reyens 4, Elisabeth Menu 6, Eric Rerrihet 1, Muriel Vry 2, Hervé Fleury 7 and Françoise Barré-Sinoussi 6

1 Virology laboratory, Centre Pasteur du Cameroun, Yaoundé, Cameroon
2 Unité d’épidémiologie des maladies émergentes, Institut Pasteur, Paris, France
3 Virology Laboratory, Institut Pasteur, Ho Chi Minh Ville, Vietnam
4 Laboratory of HIV and Hepatitis, Institut Pasteur, Pnom Penh, Cambodia
5 Virology Laboratory, Institut Pasteur, Bangui, Central African Republic
Background: Since 2003 seven laboratories of the International Network of Pasteur Institutes have implemented HIV-1 genotypic drug resistance tools. These tools were then applied to study primary antiretroviral (ARV) resistance in drug-naïve HIV positive pregnant women living in central Africa (Cameroon, Central African Republic or CAR) and South-East Asia (Cambodia, Vietnam).

Methods: HIV-1 positive ARV naïve pregnant women who gave their written informed consent participated to the study. In addition to clinical examination, EDTA blood samples were collected for T-CD4 count, plasma HIV-RNA viral load determination and ARV genotypic resistance testing following the French ANRS AC11 guidelines for both reverse transcriptase and protease genes. HIV genetic sequences were analysed with the latest version of the ANRS, Stanford HIVdb and IAS algorithms. HIV-1 subtypes were determined by phylogenetic analysis and BLAST search. Statistical comparisons of the results were performed through the Fisher’s exact test or Kruskall-wallis test, whenever appropriated.

Results: Overall, 362 women participated: 95 in Cameroon, 93 in CAR, 122 in Cambodia, and 52 in Vietnam. At inclusion, the median age was 26 years (IQR: 22–30). For most of the women (94%), HIV status was known for less than a year and 31% were in their first pregnancy. The median CD4 count was 358 cells/mm3 (IQR: 220–551). The median HIV-RNA viral load was 4.6 Log RNA copies/ml (IQR: 4-5) in Cameroon and CAR. Phylogenetic analysis revealed that 96% and 98% of viral sequences from Cambodia and Vietnam, respectively, belonged to the CRF01_AE subtype. In Cameroon, 60% of the samples clustered with CRF02_AG subtype, but 10 other subtypes were encountered. In CAR, 40% and 22% of samples belonged to CRF11 and A1 subtypes respectively. Seven strains of 362, 1.9%, (4 from Cambodia, 2 from Vietnam and 1 from Cameroon) harboured mutations associated to major ARV resistance in RT gene: 4 with V75M (resistance to D4T), 1 with the K101E (resistant to NVP and EFV) and the last one with the mutation L210W/T215S (weak resistance to AZT/D4T). In the protease gene, 3 strains (1 from Cameroon and 2 from Cambodia) bore N88 alone or associated to M46I, conferring resistance to IDV and NFV.

Conclusion: This four-sites study confirmed the high genetic diversity of HIV-1 circulating in Central Africa compared to the hegemony of CRF01_AE in South-East Asia. Drug resistance mutation survey revealed prevalence below 5% in this sentinel population, consistent with the recent introduction of ARV in these countries.

Acknowledgements
This work is presented on behalf of the FSP/RAI/ARV Study Team.

O19 Low birth weight is associated with maternal nevirapine based antiretroviral therapy in Abidjan, Côte d’Ivoire: the Ditrame Plus project and MTCT-Plus initiative (2001-2007)

Patrick Coffie1, Renaud Becquet2, Besigim Tonwe-Gold1, Apollinaire Horo1, Petry Touré1, Stéphane Blanche1, François Dabis1, Valeriane Leroy1 and Didier Ekouévi1

1ANRS DITRA ME PLUS Project, PACCI Collaboration, Abidjan, Côte d’Ivoire

2Unité INSERM 593, Institut de Santé Publique d’Épidémiologie et de Développement (ISPED), Université Victor Segalen, Bordeaux, France

3ACONDA, MTCT-Plus programme, Abidjan, Côte d’Ivoire

4Service de Pédiatrie, Centre Hospitalier Universitaire Necker Enfants Malades, Paris, France

O20 Tolerance and viral resistance after single-dose nevirapine (NVP) and short-course of tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) to prevent mother-to-child transmission (PMTCT) of HIV-1: the TEmAA ANRS 12109 phase II trial, step 1

Elise Arrivé1, Stéphane Blanche2, Marie-Laure Chaix3, Eric Herrieu1, Christine Rouzioux1, James McIntyre2, Glenda Gray2, Patrick Coffie1, Kruyl Leang Sim4, Didier Ekouévi1 and François Dabis1

1Equipe VIH Internationale, INSERM U593, ISPED, Université Victor Segalen, Bordeaux, France

2Service d’Immunologie et Hématologie Pédiatrique, Hôpital Necker Enfants Malades, Paris, France

3Laboratoire de virologie, Hôpital Necker Enfants Malades, Paris, France

4Laboratoire HIV/Hépatites, Institut Pasteur du Cambodge, Phnom Penh, Cambodia

Background: Viral resistance occurs with high frequency after single-dose nevirapine (sdNVP) for PMTCT and alternative regimens are urgently needed [1]. The objective of this study was to evaluate the safety and resistance profile of a combination of TDF (300 mg) and FTC (200 mg) in HIV-1 infected pregnant women and their newborns.

Methods: The TEmAA ANRS 12109 trial is an open label phase II trial conducted in Ivory Coast, Cambodia and South Africa. sdNVP (200 mg) and 2 tablets of TDF/FTC were given to HIV-1-infected pregnant women at the beginning of labor. One daily tablet of TDF/FTC was given for 7 days postpartum. All women received zidovudine (ZDV, 300 mg BID) from the day of enrollment, between the 28th and 38th week of gestation, until the beginning of labor. All infants received sdNVP syrup on Day 2 (2 mg/kg) and ZDV syrup (4 mg/kg BID) for 7 days. Mothers and infants were followed for two months. Serious adverse events (SAEs) and HIV-1 infection status of the infants at 3 days and 4 weeks of life were assessed using plasma RNA PCR. Maternal HIV-1 RNA plasma viral load (VL) was determined at enrollment, day 2 postpartum (PP) and at week 4 PP. Genotypic resistance tests were performed at week 4 PP.

Results: Thirty-eight HIV-1 infected pregnant women were enrolled (19 in Abidjan, 12 in Phnom Penh and 7 in Soweto): median age 27 years (interquartile range [IQR]: 23–30), median CD4 count 450 cells/mm³ (IQR: 314-596) and median HIV-1 RNA VL 4.08 log copies/mL (IQR: 3.60-5.03). All women received TDF/FTC at a median of 4.9 hours before delivery (IQR: 3.0-8.2). Nine (24%) transient grade 3/4 biological events occurred in mothers during postpartum follow-up (3 anaemia, 5 neutropenia and 1 elevation of liver enzymes). Among 39 livebirths (one pair of twins), 9 infants had clinical SAEs (23%) and 2, transient grade 3 anaemia (5%). Four children died (1 meningitis, 1 gastroenteritis with malnutrition, 1 intestinal occlusion and 1 unexplained neurological disease) while the other SAEs, with infectious origin (gastroenteritis, bronchopneumonia, meningitis, conjunctivitis and neonatal sepsis), resolved. SAEs and deaths were unlikely to be related to TDF/FTC. Two infants out of 38 tested at 4 weeks of life had detectable RNA plasma viral load, also detectable at D3, suggesting in utero HIV infection (5.3%, 95% Confidence Interval [CI]: 0.6–17.8). Median maternal HIV VL was 3.3 log₁₀ copies/mL at day 2 PP. and 4.2 log₁₀ copies/mL at week 4 PP. No viral resistance mutations to ZDV, NVP, FTC, and TDF were found in 19 mothers and infants at 3 days and 4 weeks of life were assessed using plasma RNA PCR. Maternal HIV-1 RNA plasma viral load (VL) was determined at enrollment, day 2 postpartum (PP) and at week 4 PP. Genotypic resistance tests were performed at week 4 PP.

Conclusion: A TDF/FTC combination for PMTCT was well tolerated in women and exposed newborns with no intrapartum HIV transmission reported. Providing 7 days of additional PP antiretroviral exposure with TDF/FTC immediately after sdNVP + TDF/FTC extended the suppression of viral replication avoiding a PP exposure to sdNVP. The second step of the trial will now look for the optimal neonatal dose of TDF and FTC to introduce in this PMTCT regimen.

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Reference


O21 Microtransfusion and viral exposure in uninfected infants born to HIV-infected women

Julia Warning 1, John Ziegler 1 and Rosemary Ffrench 2

School of Women’s and Children’s Health, University of New South Wales, Randwick, New South Wales, 2052, Australia
2Viral Immunology Group, Burnet Institute, Melbourne, 3001, Australia

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Background: HIV-specific cellular immune responses have been detected in some uninfected infants born to HIV-infected women, indicating transient virus exposure or replication has occurred. Small quantities of maternal blood pass into the infant’s circulation during childbirth, termed “microtransfusion”, potentially facilitating viral exposure. We sought to determine the occurrence of microtransfusion and HIV-specific immune responses as an indicator of viral exposure in a cohort of uninfected infants born to HIV-infected women, in the context of interventions.

Materials and methods: 46 uninfected infants born to HIV-infected women were included in this study. Infants were grouped according to interventions utilised by the mother: none or antiretroviral therapy (ART; group A, n = 16), ART with elective caesarean section (eCS; group B, n = 12), highly active antiretroviral therapy (HAART) only (group C, n = 7), and HAART with eCS (group D, n = 11). HLA-A and -B alleles were typed for all mother-baby pairs to identify the non-inherited maternal alleles (NIMA). To detect microtransfusion, sensitive flow cytometry and qPCR-based assays specific for the NIMA were developed and applied to umbilical cord blood and peripheral blood collected from HIV-uninfected infants born to HIV-infected women. HIV-specific immune responses were detected using the chromium51-release and IFN-γ ELISpot assays.

Results: Microtransfusion was detected in the umbilical cord blood of 10 of 11 infants tested, and remained detectable in the peripheral blood of 4 of the 10 infants up to 1 week of age. 10 of 46 infants had HIV-specific cellular immune responses, 7 were in group A, 2 in group B, and 1 in group C, while no infants in group D had detectable responses (p = 0.02, Fisher's exact test). Blood samples from 3 of these 10 infants were available for the analysis of microtransfusion. Microtransfused maternal cells were present in the umbilical cord blood of all 3 infants, and remained in the peripheral blood of 1 infant up to one week of age. The remaining 7 infants with microtransfusion did not have detectable HIV-specific immune responses.

Conclusions: In this study, the number of infants with detectable HIV-specific immune responses decreases with the use of MTCT interventions, suggesting that these interventions reduce viral exposure in these infants. This is the first study to observe both microtransfusion and HIV-specific immune responses in uninfected infants, indicating that the passage of
maternal blood may be a source of viral exposure and subsequent development of potentially protective HIV-specific immune responses in infants born to HIV-infected women.

O22
The frequency of HIV-specific CD8 T lymphocytes is inversely correlated to HIV DNA levels in untreated infected children
Florence Buseyne1, Stéphane Blanche6, Marianne Burgard3, Catherine Milliancourt3, Daniel Scott-Álara4, Christine Rouzioux3 and Yves Rivière1
1Unité postulante d’Immunopathologie Virale, Institut Pasteur, Paris, France
2Fédération de Pédiatrie, Hôpital Necker, Paris, France
3Laboratoire de Virologie, EA3620 Université René Descartes, Hôpital Necker, Paris, France
4Unité de Régulation des Infections Rétrovirales, Institut Pasteur, Paris, France


Background: HIV infection during the perinatal period differs from infection during adulthood by clinical progression, dynamic of viral replication, level of thymic activity and maturity of the immune system at time of infection.

HIV-DNA level in PBMC has recently been shown to be an independent predictor of disease progression. In PBMC, HIV-DNA is present as provirus and as non-integrated DNA in recently infected cells.

Most studies report the absence of association between the frequency of HIV-specific IFN-γ producing CD8 T lymphocytes and plasma viral load. However, CD8 T lymphocytes recognize cell-associated viral peptides but not free virions. Therefore, we investigated whether any association could be found between HIV-DNA load in PBMC and the HIV-specific CD8 T lymphocytes frequency.

Method: A cross-sectional analysis of immune and viral parameters was performed on 44 HIV-infected children (median 7.8 yrs, 0.08–18.6) that were untreated at time of biological assessment. Frequency of IFN-γ producing CD8 lymphocytes in response to Env-Gag-Pol antigens was determined using the ELISPOT technique. HIV-RNA level was determined by PCR (monitor 1.5, Roche). HIV DNA level was determined in total PBMC, using real time PCR in LTR (ANRS method). Associations between quantitative variables were defined by Pearson’s partial correlations. Partial correlation measures the strength of relationship between two variables, controlling for the effect of one or more other variables.

Results: HIV-DNA level in PBMC was directly correlated with HIV-RNA level in plasma (r = 0.542, p < 0.003), inversely correlated to CD4% (r = 0.328, p < 0.04) and was independent of age. The frequency of HIV-specific CD8 lymphocytes was directly correlated to age (r = 0.402, p < 0.02), indirectly correlated to HIV-DNA (r = 0.338, p < 0.04) independently of plasma HIV-RNA, CD4 and CD8 levels. No correlation was observed between HIV-specific CD8 T lymphocytes and HIV-RNA, CD4% or CD8%.

Conclusion: In untreated HIV-infected children, the intensity of the HIV-specific CD8 response increases with age. The negative association between the frequency of the HIV-specific CD8 T lymphocytes and HIV DNA levels in PBMC supports an antiviral role of these immune effectors.

O23
Primary CMV infection: a co-factor HIV-1 disease progression in African infants?
Jennifer A Slyker1,2, Barbara L Payne2,3, Grace C John-Stewart2, Elizabeth Obimbo3, Sandy Emery4, Barbara Richardson5, Tao Dong1, Dorothy Mbóri-Ngacha4, Julie Overbaugh4, Vincent C Emery6 and Sarah L Rowland-Jones1
1MRC Human Immunology Unit, Oxford University, Oxford, OX3 9DS, UK
2Department of Medicine, University of Washington, Seattle, Washington, 98104, USA
3Department of Paediatrics, University of Nairobi, Nairobi, Kenya
4Fred Hutchinson Cancer Research Center, Seattle, Washington, 98109, USA
5Department of Biostatistics, University of Washington, Seattle, Washington, 98104, USA
6Centre for Virology, Department of Infection, Royal Free and University College Medical School, London, UK


Abstract not submitted for publication

O24
Early diagnosis of HIV-1 infection in newborns, in the context of prevention of mother-to-child transmission with HAART (Perinatal Cohort ANRS Co 01)
Véronique Avettand-Fenoël1, Marie-Laure Chaix1, Stéphane Blanche1, Marianne Burgard1, Josiane Warszawski2 and Christine Rouzioux1
1AP-HP, CHU Necker, EA 3620 Université Paris-Descartes, France
2INSERM U822, Le Kremlin-Bicêtre, France


Background: In 2007, less than 1% of the children born in France to HIV-1 positive mothers are infected, as preventive HAART is used. A high HIV-1 genetic diversity is observed, with nearly 60% of non-B subtypes, especially among women of African origin. The objective of this study was to evaluate new molecular technologies developed for HIV-1 diagnosis in babies, using HIV-DNA and HIV-RNA detection in babies’ blood samples.

Material and methods: All infants born between May 2005 and April 2007, with samples sent to Necker’s Virology Laboratory, were included in this study. Early diagnosis was based on viral detection on samples taken in the first days of life, at 1, 3 and 6 months. A new protocol using Real Time PCR technology (LTR) was developed to facilitate HIV diagnosis on whole blood that used HIV-DNA quantification in babies, using HIV-DNA and HIV-RNA detection in babies’ blood samples.

Results: 1135 infants were included in this sub-study of the National EPF cohort, with 3133 samples received in the Laboratory during the study period. The specificity of HIV-DNA and HIV-RNA real time PCR assays was estimated at 100% and 98% respectively; 1126 children were not infected, nine were infected as they presented with positive results on two
consecutive samples; five of them were in utero infected (1st sample at birth: median HIV-DNA = 2.2 log copies/10^6 leukocytes [min < 1.6 log, max: 3.7 log], median HIV-RNA = 3.5 log copies/mL [min: 1.8 log, max: 4.3 log]), while four infants were intra-partum infected: the first sample negative with both techniques and the second one positive: median HIV-DNA = 2.85 log [min: 2.1 log, max: 3.7 log], median HIV-RNA = 5.1 log [min: 1.6 log, max: 6.4 log].

Six patients had low viral levels (8 samples with HIV-DNA < 2.5 log; and 8 samples with HIV-RNA < 4.0 log). Lastly, two infants had discordant results, one with HIV-DNA negative and HIV-RNA at 1.8 log; inversely, the 2nd infant with HIV-RNA negative, while HIV-DNA was at 2.0 log, underlying the necessity to perform both assays in the context of preventive HAART.

Conclusions: Despite HAART, few cases of infection are still occurring in newborns of northern countries. The HIV-1 primary infection occurs in infants under antiretroviral pressure, reducing viral replication’s level, so making the diagnosis more difficult than previously. Our results show that, in the context of preventive HAART, very sensitive and specific techniques are necessary to detect very low viral levels before 60 days of life, both in plasma and PBMC of HIV-infected infants.

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This work is presented on behalf of the Co 01 ANRS Cohort Study Group.

O25 Early vs deferred highly active antiretroviral therapy in HIV infected infants: a European Collaborative Cohort Study
Tessa Goetghebuer1, Edwige Haelterman1, Jerome Le Chenadec2, Catherine Dolfus3, Diana Gibb4, Katherine Boyd4, Ali Judd4, Luisa Galli5, Clara Gabiano5, Jose Ramos6, Claire Thorne7, Magdalena Marcynska8, Olivia Keiser9, Luminita Ene10, Marc Hainaut1, Henriette Scherpbiere11, Uwe Wintergerst12, Veronique Schmitz13, Gwenda Verweel14, Carlo Giaquinto15, Josiene Warszawski2 and Jack Levy1
1 Paediatric Department, CHU St Pierre, Brussels, Belgium
2 Institut National de la Santé et de la Recherche Médicale, Paris, France
3 AP-HP, Hôpital Trousseau, Service d’Hématologie et d’oncologie pédiatrique, Paris, F-75571 France
4 MRC Clinical Trials Unit, London, UK
5 Italian Register, Department of Paediatrics, University of Florence, Italy
6 Hospital 12 Octubre, Madrid, Spain
7 ECS, institute of Child Health, London, UK
8 Infectious Disease Hospital, Medical University Warsaw, Poland
9 Data Center of the Swiss HIV Cohort Study, Lausanne, and Institute of Social and Preventive Medicine, University of Bern, Switzerland
10 Hospital for Infectious Diseases “Dr. Victor Babes”, Bucharest, Romania
11 Emma Children’s Hospital, Academic Medical Center, Amsterdam, The Netherlands
12 University Children’s Hospital, Munich, Germany
13 Hôpital La Citadelle, Liège, Belgium

Figure 1 (abstract O25)

Time from birth to AIDS/death comparing children treated before 3 months of age and children not treated before 3 months of age.
pooled. The risk of AIDS/death was estimated by Kaplan-Meier survival analysis, and compared between the groups of infant treated or not treated before 3 months of age. Cox regression was used to estimate hazard ratios.

Results: Among the 210 children, 21 developed AIDS and 3 died. The exposure to treatment was heterogeneous among cohorts. Overall ART and Highly active ART were initiated in 59% and 48% of the infants before 3 months of age and in 87% and 76% by one year, respectively.

Treatment was initiated before the age of 3 months in 124 infants. There was no significant difference in demographic, pregnancy and delivery characteristics between the two groups. Moreover the proportion of infants with early treatment did not vary significantly over time. As shown in figure 1, we found that the risk of developing AIDS/death at one year was 1.6% in infants treated before the age of 3 months compared to 11.7% in infants who started treatment later (p < 0.001). At 5 years the risks were 4.6% and 21.5% respectively. Deferred treatment was associated with a five-fold higher risk of AIDS has compared with treatment before 3 months of age (crude hazard ratio = 5.0; 95% CI: 2.0–12.6). Adjustment for ethnicity, birth weight, breast feeding, number and class of neonatal prophylaxis, number and class of drug in first treatment did not substantially affect the hazard ratio.

Conclusion: The preliminary results of this retrospective collaborative study suggest a significant association between ART started before the age of 3 months and a lower subsequent incidence of AIDS/death in infancy.

Acknowledgements

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References


O26

Once-a-day pediatric HAART with DDI+3TC+EFV in Burkina Faso. A phase II trial (ANRS 12103 trial)

Philippe Msellati1, Boubacar Nacro2, Emmanuelle Zoure2, Hervé Hien3, Hassan Tamboura2, François Rouet3, Serge Diagbouga3, Adama Ouiminga3, Ali Drabo3, Souleymane Yaméogo3, Hélène Peyrièrè4, Olivier Mathieu4, Joëlle Nicolas4 and Philippe Van de Perre4

UMR 145 IRD, Bobo-Dioulasso, Burkina Faso

2Department of Pediatrics, CHU de Bobo-Dioulasso, Burkina Faso

3Centre Muraz, Bobo-Dioulasso, Burkina Faso

4Laboratory of Medical Pharmacology and Laboratory of Virology, Montpellier University Hospital, Montpellier, France


Background: Simplification of the administration of HAART in children should improve compliance and efficacy of these treatments. Combination of 3TC + DDI + EFV in a single daily oral administration is one option. However, pharmacokinetics, tolerance and efficacy of such once-a-day HAART have not been assessed in children. The ANRS 12103 trial is a phase II on pharmacokinetics, tolerance and compliance of a once-a-day pediatric DDI+3TC+EFV in Burkina Faso.

Material and methods: ANRS 12103 is an ongoing Open Phase II Trial with a 12 months follow up. 50 HIV-1 infected children eligible for HAART had to be included. Inclusion criteria were: weight = 10 kg and aged 30 months to 15 years, naïve of ART treatment and eligible for HAART. Children received efavirenz + 3TC (8 mg/kg) + DDI (240 mg/m²). Clinical examination was performed weekly until M3, then monthly. Pharmacokinetics (plasma Cmin and Cmax) was done at day 15. Quarterly RNA HIV-1, CD4 counts, haematology and biochemistry are performed.

Results: Enrolment was from February to November 2006 and 98 HIV-infected children have been screened for eligibility criteria: 45 were excluded, one died before inclusion and 52 were included. Among included children there were 21 girls and 31 boys, mean age was 6.8 years, Zscore Weight for Age (W/A) and H/A were respectively 1.37 and 1.99 at inclusion. Mean CD4 was 355/µl (mean CD4 percentage 9%), and median HIV-1 RNA 5.50 Log10 cp/ml. Among the 52 expected samples for minimum concentration of efavirenz, 50 were obtained. Thirty children were in the expected ranges (60.1%), 11 (22%) below therapeutic levels and 9 (18%) above therapeutic levels. Maximum concentrations were in the expected ranges in 23/49 children (47%), below therapeutic levels in 3/49 children (6%) and over in 23/49 children. Dosages of 3TC and DDI are ongoing.

At 9 months of follow-up, two children had died, Zscore W/A and H/A were respectively –1.37 and –1.62, and mean CD4 was 893/µl (mean CD4 percentage 20%). HIV-1 RNA was below detectable level (300 copies/ml) in 39 children (76%) and below 1000 copies/ml in two children (4%). Two HAART adverse effect occurred. One cutaneous eruption with a temporary stop and successful reintroduction and one increase in liver enzymes.

Conclusion: Preliminary data of this phase II trial suggest that once-a-day DDI+3TC+EFV in children provides satisfactory
plasma concentration for EFV, and is associated with virological success and immune restoration. At 9-month follow up, it is effective and well tolerated.

O27 Early virological suppression despite high frequency NNRTI resistance following perinatal prophylaxis in HIV-infected African infants

Andrew Prendergast1, Wendy Mphatswe2, Gareth Tudor-Williams1, Natasha Blanckenberg2, Ayanda Cengimbo3, Prakash Jeena2, Mpho Rakgotho4, Visa Pillay5, Christina Thobakgale2, Sharon Reddy5, Zenele Mncube6, Mary Vanderstoek5, Noel McCarthy1, Krista Dong2, Hoosen Coovadia2, Lynn Morris4, Bruce D Walker1,2,5 and Philip Goulder1,2,5

1Department of Paediatrics, University of Oxford, Oxford, UK
2HIV Pathogenesis Programme, University of KwaZulu-Natal, Durban, South Africa
3Department of Paediatrics, Imperial College London, London, UK
4National Institute for Communicable Diseases, Johannesburg, South Africa
5Partner AIDS Research Center, Boston, MA, USA
6Howard Hughes Medical Institute, Chevy Chase, MD, USA

Results: All 63 HIV-infected infants were exposed to sd-NVP. 20/51 (39%) infants with baseline genotyping results had NNRTI resistance (most frequently Y181C; 20%). Median pre-ART viral load was 952,000 copies/mL. 43 infants were randomised to immediate ART. Of these, 3 were lost to follow-up pre-ART; 40 started ART (median day 28; range 8–164) and 36/40 completed 1 year of ART. 20 infants were randomised to deferred ART. 16 reached the treatment threshold of CD4% (at median day 99) and 13/16 started ART during infancy (on median day 142; range 81–227). Verbal and measured adherence was assessed at every appointment by caregiver verbal recall and by measured medication returns.

Methods: Infants born to HIV-infected mothers in Durban, South Africa, were tested on days 1 and 28 of life to determine intraterine and intrapartum HIV infection, respectively. HIV-infected infants received randomised immediate or deferred (once CD4% 4 drug ART (zidovudine, lamivudine, nevirapine and nevirapine) in a dedicated study clinic, with free outpatient and inpatient treatment of illness. Genotyping for NNRTI resistance mutations was undertaken pre-ART. Monthly follow-up to 1-year post-ART included viral load (VL) and CD4 count measurement. Adherence was assessed at every appointment by caregiver verbal recall and by measured medication returns.

Results: All 63 HIV-infected infants were exposed to sd-NVP. 20/51 (39%) infants with baseline genotyping results had NNRTI resistance (most frequently Y181C; 20%). Median pre-ART viral load was 952,000 copies/mL. 43 infants were randomised to immediate ART. Of these, 3 were lost to follow-up pre-ART; 40 started ART (median day 28; range 8–164) and 36/40 completed 1 year of ART. 20 infants were randomised to deferred ART. 16 reached the treatment threshold of CD4% (at median day 99) and 13/16 started ART during infancy (on median day 142; range 81–227). Verbal and measured adherence was 99% and 95%, respectively. One year post-ART, 49/49 (100%) infants had VL < 400 copies/mL and 46/49 (94%) had VL < 50 copies/mL. 9 infants (18%) required second-line ART due to virological failure (n = 4). TB treatment (n = 4) or both (n = 1). Time to VL < 50 correlated with maternal CD4 (r = −0.42; P = 0.005) and infant pre-ART VL (r = 0.64; P < 0.001). NNRTI mutations had no significant effect on virological suppression. Infants starting immediate compared to deferred ART had fewer illness episodes (median 7 vs 12 illness episodes per infant; P = 0.003), but no significant difference in mortality, virological suppression or CD4% repletion.

Conclusions: Excellent adherence and virological suppression are achievable in infants, despite high-frequency NNRTI mutations, high viral loads and rapid disease progression. Infants are currently relatively neglected in roll-out programmes and ART provision must be expanded. Immediate therapy may be preferable to delayed ART, to reduce morbidity and prevent loss to follow-up.

O28 Virus phenotype variability during disease progression of HIV-1 infected children

Mariangela Cavarelli1, Stefania Dispineri1, Chiara Ripamonti1, Ingrid Karlsson2, Liselotte Antonsson2, Anna Plebani3, Eva-Maria Fenyo2 and Gabriella Scarlatti1

1Viral Evolution and Transmission Unit, DIBIT, HSR, Milan 20132, Italy
2Division of Medical Microbiology/Virology, Department of Laboratory Medicine, Lund University, Lund, Sweden
3Division of Cellular and Molecular Pharmacology, Department of Experimental Medical Science, Lund University, Lund 223 62, Sweden
4Department of Pediatrics, University of Milan, Clinica de Marchi, Milan 20122, Italy

Results: All 63 HIV-infected infants were exposed to sd-NVP. 20/51 (39%) infants with baseline genotyping results had NNRTI resistance (most frequently Y181C; 20%). Median pre-ART viral load was 952,000 copies/mL. 43 infants were randomised to immediate ART. Of these, 3 were lost to follow-up pre-ART; 40 started ART (median day 28; range 8–164) and 36/40 completed 1 year of ART. 20 infants were randomised to deferred ART. 16 reached the treatment threshold of CD4% (at median day 99) and 13/16 started ART during infancy (on median day 142; range 81–227). Verbal and measured adherence was 99% and 95%, respectively. One year post-ART, 49/49 (100%) infants had VL < 400 copies/mL and 46/49 (94%) had VL < 50 copies/mL. 9 infants (18%) required second-line ART due to virological failure (n = 4). TB treatment (n = 4) or both (n = 1). Time to VL < 50 correlated with maternal CD4 (r = −0.42; P = 0.005) and infant pre-ART VL (r = 0.64; P < 0.001). NNRTI mutations had no significant effect on virological suppression. Infants starting immediate compared to deferred ART had fewer illness episodes (median 7 vs 12 illness episodes per infant; P = 0.003), but no significant difference in mortality, virological suppression or CD4% repletion.

Conclusions: Excellent adherence and virological suppression are achievable in infants, despite high-frequency NNRTI mutations, high viral loads and rapid disease progression. Infants are currently relatively neglected in roll-out programmes and ART provision must be expanded. Immediate therapy may be preferable to delayed ART, to reduce morbidity and prevent loss to follow-up.
RSX4. The remaining two children showed R5<sup>broad</sup> phenotype during the whole follow-up.

**Conclusions:** Our results show that HIV-1 with broad chimeric receptor use is not hampered in transmission, and is more frequent close to birth in FP than in SP children. Viruses from LTNP show a similar phenotypic evolution though at later age.

**References**

**POSTER PRESENTATIONS**

**P1**

A guinea pig model for cytomegalovirus congenital infection: dose-effect and vertical transmission rate

Guillaume Benoist<sup>1</sup>, Yves Ville<sup>1</sup>, Christine Rouzioux<sup>2</sup>, Anne-Lise Delezio<sup>3</sup>, Pierre Flori<sup>4</sup> and Mariane Leruez-Ville<sup>2</sup>

<sup>1</sup>Service de Gynécologie Obstétrique, Hôpital de Poissy-St-Germain, 78300 Poissy
<sup>2</sup>Laboratoire de Virologie, EA 36-20 Université Paris-Descartes, Faculté de Médecine de Necker, CHU Necker-Enfants-Malades, AP-HP, Paris, France
<sup>3</sup>Laboratoire de biologie du développement, Hopital Robert Debré, AP-HP, 75935 Paris cedex 19, France
<sup>4</sup>Laboratoire de biologie du développement, Hopital Robert Debré, AP-HP, 75935 Paris cedex 19, France

**Backgrounds and objectives:** Animal models are necessary to test new antiviral drugs to prevent CMV materno foetal transmission [1]. Among the small animal CMV models, the guinea pig CMV (GPCMV) has the ability to cross the placenta [2], causing disease in utero [3, 4]. The objective was to study the relation between inoculum doses of guinea pig cytomegalovirus (GPCMV) and the natural history of congenital disease in the pregnant guinea pig model, by means of updated ultrasound and virological methods.

**Methods:** 1) Development of ultrasound examination for precise assessment of the gestational age based on fetal crown-rump length (CRL) measure. 2) Development of a real time PCR Taqman® GPCMV based on the amplification of a sequence of the UL83 gene and determination of its sensitivity. 3) Subcutaneous administration at mid-gestation of two doses (10<sup>6</sup> DI50 in group 1 and 10<sup>8</sup> DI50 in group 2 of GPCMV strain obtained by cell culture in CMV-seronegative pregnant Hartley strain guinea pigs. Serial sacrifice of the animals were performed in the second part of the gestation up to collect maternal tissues, maternal blood, amniotic fluid, placenta and fetal tissues and to determine the GPCMV load. Conventional histological examination of the fetal infected tissues was performed.

**Results:** 18 pregnant guinea pigs were included in group 1 and nine in group 2.

1) Ultrasound examinations allowed the diagnosis of gestations in the totality of the cases. A growth curve of fetuses based on CRL was built.
2) The real time PCR GPCMV developed had sensitivity at 50% and 95% of 200 copies/mL and 2500 copies/mL respectively, with a wide linear ranges up to 10<sup>6</sup> copies/mL.
3) CMV infections were observed in 14/18 females in group 1 and in 9/9 females in group 2. GPCMV maternal viremia was observed in 28% and 70%, the median viral load was 100 and 700 copies/mL in groups 1 and 2 respectively. The proportion of female with at least one infected fetus in their litter was 0/18 and 2/9 (22.2%) in group 1 and 2 respectively (p = 0.103, Fisher Exact Test).

**Conclusions:** We developed an effective animal model using ultrasound for determination of gestational age and a sensitive real time GPCMV PCR for the diagnosis of GPCMV neonatal infections. The acute infection with GPCMV in the guinea-pig was reproduced in this model with materno-foetal transmission. We show that the kinetics and the intensity of the primary infection in the pregnant females as well as the vertical transmission rate are related to the inoculum’s viral load. This model will be useful for further antiviral CMV drug assays.

**References**

**P2**

Transmission of hepatitis B virus infection is predominantly perinatal in the Indian subcontinent

S Hissar, K Manoj, N K Syed, S Sanjay, GT Kumar, C Ranjeet, S Didar, S Puja and K S. Shiv

G. B. Pant Hospital, New Delhi, India

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Abstract not submitted for publication.
P3
Preventable zidovudine overdose during post-natal prophylaxis in healthy children born to HIV-1-positive mothers
E Chiappini
Department of Paediatrics, Firenze, Italy

Abstract not submitted for publication.

P4
Genetically engineered live-attenuated cytomegalovirus (CMV) vaccines improve pregnancy outcome in the guinea-pig model of congenital CMV infection
Mark R Schleiss1, Alistair McGregor1, Yeon Choi1, Jodi Anderson1, Mike Leviton1, Xiaohong Cui2 and Michael McVoy3
1Center for Infectious Diseases and Microbiology Translational Research, Minneapolis, MN, USA
2Medical College of Virginia and Virginia Commonwealth University, Richmond, VA, USA
3Department of Paediatrics, Firenze, Italy

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Background: Congenital CMV infection is a major cause of disability in newborns. An effective preconception vaccine is a major public health priority. The guinea-pig cytomegalovirus (GPCMV) model was utilized to evaluate the efficacy of live, attenuated CMV vaccines generated using a bacterial artificial chromosome (BAC) approach.
Methods: The GPCMV genome was cloned as a BACmid in E. coli and used to regenerate a wild-type viral vaccine (wt), and a highly attenuated recombinant vaccine deleted of the gene encoding the dominant T-cell target, UL83 (pp.65). Seronegative animals were immunized with a two-dose series of each vaccine (0- and 3-week schedule), or placebo. Following establishment of pregnancy, dams were challenged with salivary gland-passaged (SG) GPCMV (5 x 105 pfu) in the second trimester, and pregnancy outcomes were compared.
Results: Vaccinated dams seroconverted to GPCMV antigen. ELISA titers were significantly higher in the wt (2.8+/−0.3 log10) compared to the 409 group (2.5+/−0.2 log10; p < 0.05). Vaccination resulted in highly significant reductions in the magnitude and duration of DNAemia post-SG challenge, and was associated with improved pregnancy outcomes. Among 13 litters in the control group, there were 29 live and 22 dead pups (43% mortality, mean pup weight of 89 g), compared to 45 live and 14 dead pups born to 15 litters in the vaccine group (26% mortality, mean pup weight 106 g; p < 0.05 vs. control). The two vaccines were comparable in reducing GPCMV transmission at the placental and fetal levels.
Conclusions: Live, attenuated CMV vaccines are effective at preventing congenital infection and disease in the guinea pig model. Of interest, although UL83 is an effective subunit vaccine in guinea-pigs, immune responses to UL83 are not essential for fetal protection in the context of a live-virus vaccine. Recombinant CMV vaccines with targeted mutations of pathogenesis or immune evasion genes warrant further consideration in clinical trials.

P5
Characteristics of HIV-1 gp120 env sequences in mother-child pairs infected with HIV-1 subtype CRF01_AE
Tanawan Samleerat1,2, Martine Braibant2, Gonzague Jourdain3,4, Alain Moreau3, Nicole Ngo-Giang-Huong3,4, Pranee Leechanachai1, Jittapol Hemvuttiphan6, Temsiri Hinjiranandana5, Tikamporn Changchit5, Boonyarat Warachit5, Veera Suraseraniyong4, Marc Lallemant3,4 and Francis Barin2
1Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand
2Université François-Rabelais, INSERM ERI 19 & CHRU de Tours, Tours, France
3Harvard University, IRD 054, Chiang Mai, Thailand
4Institut de Recherche pour le Développement, UMI 174, Chiang Mai, Thailand
5Phayao Hospital, Phayao, Thailand
6Somdej Pranangchao Sinkit Hospital, Chonburi, Thailand
7Phan Hospital, Chiang Rai, Thailand
8Hat Yai Hospital, Songkla, Thailand
9Bhumibol Adulyadej Hospital, Bangkok, Thailand

Background: Previous studies have suggested that mother-to-child transmission of HIV-1 is often characterized by acquisition of a homogeneous viral quasispecies in the infant [1, 2, 3]. Suggestive of a genetic bottleneck. In this study, we have analyzed the molecular characteristics of transmitted HIV-1 viruses in a homogeneous population infected by CRF01_AE variants in Thailand.
Materials and methods: Seventeen mother-child pairs were studied. The infants were not breastfed. Six infants were infected in utero and 11 infants were infected intrapartum. The env sequences covering the entire gp120 were amplified from both the proviral DNA of maternal PBMC collected at delivery and the plasma viral RNA at first positive time point of infants. The amplified products were cloned and sequenced. A total of 353 clones were available for analysis.
Results: Phylogenetic analysis indicated 2 patterns of transmission: 14 mothers transmitted a single variant and 3 mothers transmitted multiple variants to their infants. The mean genetic distance of viruses from the mothers was significantly higher than those from the infants (2.7% vs. 0.6%; P < 0.01), but without any difference according to timing of transmission, either in utero or intrapartum. The length of gp120 and number of potential N-linked glycosylation sites (PNGS) were similar in both the entire gp120 and individual regions of gp120 of all mother-child pairs. However, our data indicate that 4 PNGS positions (N241, N301, N354, and N384) that appeared conserved in all infant variants but were irregularly present in the mothers might be associated to a selective advantage. In addition, we report the first case to our knowledge of transmission to an infant of a recombinant virus issued from variants from his mother.
Conclusions: Our results provide additional evidence that despite a complex viral population in the mother, only viruses of a restricted subset are transmitted to the infant, independently of the timing of transmission, in utero or intrapartum. We did
not find that shorter gp120 or fewer PNGS were characteristics of viruses transmitted from mother to infant, as it was suggested for sexually transmitted viruses at least for a few subtypes [4, 5, 6, 7]. Four PNGS were selected for transmitted viruses, supporting the role of the “glycan shield” of the HIV-1 envelope in conferring a selective advantage.

References

P7
In vitro HIV-1 infection of human uterine mucosa during pregnancy
Romain Marlin1, Marie-Thérèse Nugeyre1, Claire de Truchis2, Nadia Berkane3, Amélie Gervaise2, Daniel Scott-Algara3, Françoise Barré-Sinoussi1 and Elisabeth Menu1
1Unité de Régulation des Infections Rétrovirales, Department of Virology, Institut Pasteur, Paris, France
2Service de gynécologie-obstétrique, A. Beclère Hospital, Clamart, France
3Service Maternité, gynécologie et obstétrique, Tenon Hospital, Paris, France


Background: During the first trimester of pregnancy, Natural Killer cells represent the main leukocyte population (70%) in the human decidua (maternal uterine mucosa during pregnancy). These decidual NK cells (dNK) have a distinct phenotype from their peripheral blood counterpart. Decidual leukocyte populations contain also antigen-presenting cells (dAPC) like dendritic cells and macrophages, as well as regulatory and gamma-delta T cells.

In vitro, peripheral NK cells inhibit HIV-1 replication by the release of chemokines or cytolytic activity. Furthermore, HIV-1 infected cells, through their altered-ligands-expression, are able to trigger NK cell activation. As an upregulation of NK cell activation has been reported in exposed-non-infected individuals, we thus hypothesized that dNK could play a role in the control of HIV-1 in utero transmission by interacting with infected dAPC at the materno-fetal interface.

Materials and methods: Deciduas were obtained from HIV-1 negative women undergoing elective abortions between 6–10 weeks of pregnancy. dNK cells and dAPC were isolated respectively by negative and positive selection using Miltenyi microbeads after collagenase digestion of the tissue. Cell subpopulations were checked for purity and characterized by FACs analysis using specific monoclonal antibodies. Infections were performed in vitro with HIV-1 primary isolates, HIV-1 strains carrying a GFP reporter gene or HIV-1 pseudotypes bearing a luciferase reporter gene. Decidual histocultures were also performed and infected with the same strain viruses.

Results: Phenotype of dNK was CD3+ /CD16+/CD56+ (up to 90%) and they expressed the activation markers CD69 (100%) and NKP30. Most of dNK expressed the inhibitory receptor CD94/NKG2A but they expressed activating receptors CD94/NKG2C. The phenotypes of the main dAPC subpopulations were CD14+/HLA-DR+/CD123–/ and a few percentages were HLADR+/CD83low.

dNK cells were not permissive to HIV-1 infection in vitro, even when they were pre-activated with IL-2. In contrast, dAPC subpopulations as well as decidual histocultures were susceptible to R5 and X4R5 HIV strains but not to X4 strains.

Conclusions: In decidual tissue, NK cells have a phenotype of activated cells that might indicate their interaction with dAPC. The infection of dAPC subpopulations by HIV-1 that we observed in vitro might impact the cross-talk between dAPC and dNK, especially through receptor and soluble factor expression modifications. It is thus important to further study the consequences of HIV-1 infection of human uterine mucosa during pregnancy.
infection on dNK and dAPC interactions to gain new insights into the role of these immune cells in controlling HIV-1 infection at the materno-fetal interface.

P8
Permissivity to HCMV infection in early and term human placentae with a new ex vivo model of histocultures
Charlotte Casper1,2, Hélène Lopez1, Talal Al Saati1, Isabelle Dugas-Neulat1, Alain Berrebi1, Jean-Luc Davignon1 and Christian Davrinche1
1Inserm U563, CPTP, CHU Purpan, 31000 Toulouse, France
2Neonatology unit, Children’s Hospital, 31000 Toulouse, France
3Department of Anatomopathology, CHU Purpan, 31000 Toulouse, France
4Department of Obstetrics, Paule de Viguier Hospital, 31000 Toulouse, France

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Objective: To evaluate a new ex vivo model of long term placental histocultures in order to investigate the permissivity of early and term placenta to HCMV.

Material: First trimester placentae were obtained following elective abortion and term placentae were obtained after elective caesarean section.

Method: Two methods of culture were established. Fresh placental chorionic villi were isolated, washed and distributed on collagen sponge gels as previously described [1]. Endotheliotropic HCMV strain VHL/E was left in contact with the microexplants overnight (Method A). The culture medium was collected and fresh medium added on day 6, 10, 15 and 21. In method B, villi blocks were distributed on sponge gels in plates covered with subconfluent VHL/E infected layers of MRC5. After 5 days, the sponge gels were transferred to plates with fresh uninfected medium only. The culture medium was collected and fresh medium added on day 6, 10, 15 and 21. Viral plaque assay in MRC5 cells was performed on each collection day. Tissue was fixed and embedded in paraffin for histopathological and immunohistochemical study with detection of immediate early antigen (IEA).

Results and conclusion: Viral infection could be seen in tissue sections of infected villi blocks after day 10 for method A in early placentae but remained negative in term placentae. Viral plaque assay showed a productive and increasing replication in supernatants at days 15 and 21 for early placentae (mean 6200 PFU/mL) but remained negative for term placentae. Method B was efficient both for infection of early and term placentae. This is the first long term ex vivo placental model of histocultures with effective HCMV production. Our findings suggest that early placentae are more permissive to HCMV infection than term placentae.

Reference

P9
A Plasmodium falciparum antigen increases HIV-1 replication in a human placenta-derived cell line
Ahidjo Ayoub1, Cyril Badaut2, Anfumbom Kfutwah3, Claude Cannou1, Alexandre Juillerat4, Stéphane Gangnard4, Charlotte Behr4, Odile Mercereau-Puijalon4, Graham A Bentley2, Françoise Barré-Sinoussi1 and Elisabeth Menu1
1Unité Régulation des Infections Rétrovirales, Institut Pasteur, Paris, France
2Unité d’Immunologie Structurale (C.N.R.S. U.R.A 2185), Institut Pasteur, Paris, France
3Virology laboratory, Centre Pasteur du Cameroun, BP 1274 Yaoundé, Cameroon
4Unité d’Immunologie Moléculaire des Parasites (C.N.R.S. U.R.A 2581), Institut Pasteur, Paris, France


Background: Malaria is endemic in countries of sub-Saharan Africa where there is also a high prevalence of HIV-1 infection, with pregnant women being the population most at risk to both infections. Epidemiological data and indirect evidence have established a link between placental malaria and an increased risk for HIV-1 mother-to-child transmission (MTCT), by unknown mechanisms.

Material and methods: The placenta-derived choriocarcinoma cell line BeWo and monocyte-derived macrophages (MDM) were infected with varying doses of luciferase reporter HIV-1 pseudotyped with the vesicular stomatitis virus protein G. A recombinant DBL3γ domain, derived from a placental Plasmodium falciparum Erythrocyte Membrane Protein 1 (PfEMP1) adhesin domain (DBL3γ-732), that binds to chondroitin sulfate A (CSA) and a non-CSA-binding PfEMP1 adhesin domain, DBL1α-varO domain were used to stimulate infected cells. In some experiments, DBL3γ-732 was preincubated with the Fab fragment of a specific or an irrelevant monoclonal antibodies (mAb) that inhibits, or not, binding to CSA. TNF-α was measured in the culture supernatants and luciferase activity was quantified at different time points in cell lysates to evaluate viral replication.

Results: Addition of DBL3γ-732 to BeWo cells, led to a dose-dependent increase of HIV-1 replication of up to 400 times the control level in the absence of DBL3γ-732. This enhancement was specific since it was inhibited by Fab fragment of an anti- DBL3γ-732 monoclonal antibody but not by the Fab of an irrelevant mAb. In contrast, the addition of DBL1α-varO domain does not increase viral replication. In MDM which presents surface CSA, both DBL domains strongly inhibit viral replication. The effect of DBL3γ-732 on HIV-1 replication is most likely mediated by TNF-α as this cytokine is significantly increased by DBL3γ-732 binding to BeWo cells and MDM.

Conclusions: This study shows, for the first time, a direct link between a P. falciparum antigen and an increase of HIV-1 replication in placental cells in vitro. If this is occurring in vivo, the presence of both infections could lead to a higher risk of HIV-1 in utero transmission. These data underline the importance of efficient malaria prophylaxis and antiretroviral
interventions for pregnant women in areas where HIV-1 and malaria co-circulate.

**P10**

**Mother-to-child transmission of HIV-1 drug resistance in a French cohort**

Frédéric Benizri, Véronique Schneider, Ali Kara, Sabine Guessant, Anne-Geneviève Marcelin, Roland Tubiana, Marie-Dominique Tabone, François Hervé, Geneviève Vaudre, Axelle Dehee, Guy Leverger and Catherine Dollfus

APHP, Tenon hospital, Paris, France

APHP, Pitié Salpêtrière hospital, Paris, France

APHP, Armand Trousseau hospital, Paris, France

Background: The use of antiretroviral drug therapies in HIV-1 infected pregnant women and their infants has resulted in significant reductions in the rates of mother-to-child transmission (MTCT), although emerging resistances become a growing concern. The aim of this study was to characterize resistance patterns of HIV-1 strains for mother-infant pairs among the residual cases of vertical transmission.

Materials and methods: HIV-1 infected infants by vertical transmission diagnosed in their first year of life in a single teaching hospital and born between January 1997 and May 2006 were enrolled. Viral genotypes were performed on available samples of plasma RNA-HIV, at the time of diagnosis for infants and the closest possible to delivery in their respective mothers. HIV-1 genotype drug resistance interpretation was based on the ANRS algorithm of July 2006. Whenever possible, genotypic drug resistance profile of HIV-1 was also obtained for the father.

Results: 18 mother-infant pairs were included, 11 identified through MTCT prevention follow-up (MTCT rate: 11/947 = 1.2%), 7 cases in various contexts with mothers diagnosed for HIV-1 beyond delivery. Samples were available for genotypes in 14 of them. In 4/14 (29%) infected HIV-1 newborns, resistant virus to at least one antiretroviral drug were observed. For these four cases, two genotypic analyses of the father supplemented the analysis of mutation transmission.

In the first mother-infant pair, mutation M184V/M was identified in the sample of the newborn (unexposed postnatally to 3TC) at 14 days of life although not detected in its mother’s sample at 40 days before delivery. In the second mother-infant pair, poor compliance of multiregimen treated virus explained vertical transmission of multidrug-resistant HIV-1 (AZT, 3TC and IP). In the third case, identical mutations conferring resistance to AZT (T215Y) and NNRTI (K103N+Y181V) were observed in the infant and both parents. The last case resulted from a documented primary HIV-1 infection in late pregnancy with vertical transmission of a K101E resistant mutation isolated also in father and mother, both being naive to ARVs.

Conclusions: Over almost 10 years, 18 cases of vertical transmission of HIV-1 were registered in this cohort with an important proportion of infected infants who acquired drug-resistant virus (4/14 – 29%). The results of this study raise the importance of HIV screening of pregnant women and partners. Viral genotyping can guide prophylaxis regimen and/or treatment of infected infants.

**P11**

**Successful reduction of mother-to-child transmission of HIV-1 by nevirapine and non-breastfeeding in Hanoi and Haiphong, Vietnam**

Tran Thi Thanh Ha, Pham Le Tuan, Nguyen Huy Bao, Nguyen Mai Anh, Phung Duc Cam, Francesca Chiodi and Annika Ehrnst

National Institute of Hygiene and Epidemiology, Hanoi, Vietnam

Hanoi Health Service, Vietnam

Haiphong Obstetric and Gynaecology Hospital, Vietnam

Hanoi Obstetric and Gynaecology Hospital, Vietnam

Department of Microbiology, Tumour and Cell Biology (MTC), Karolinska Institute, Stockholm, Sweden

Background: The rate of transmission of HIV-1 from mother-to-child increases over time during pregnancy, delivery, and breastfeeding [1, 2]. The latter is about as common as the other two combined. However, breastfeeding is also crucial to decrease infant mortality from infections. Vietnam is a developing country. In the cities the access to clean water and understanding of sanitation is good, providing an opportunity to introduce formula feeding of children, born to HIV-infected mothers [3].

Subjects and methods: A prospective study was performed in 137 HIV-1 infected pregnant women from Hanoi and Haiphong. Nevirapine prophylaxis was given to the women at delivery and to the newborn child. Counselling was provided, focusing on the importance of follow up of the child for early diagnosis and the value of formula feeding to avoid HIV-1 transmission from breast milk. Peripheral EDTA blood samples were collected at birth (1-2 days), at 1, 3, 6, 12, 18 months. Polymerase chain reaction (PCR), specific for the detection of the pol gene, was used as a diagnostic tool during the first year of life. Serology was added at 12 and 18 months.

Results: All 137 women accepted to provide formula feeding. Nevirapine prophylaxis was given to 107/137 (78%). In total eleven children (8.03%) were infected. Treatment reduced transmission by 50 % from 4/30 (13.3%) among untreated to 7/107 (6.5%) in the treated group. Six of 135 (4.4%) children had a positive PCR test at birth, as evidence of intrapartum transmission [2, 4]. Three children of 135 (2.2%) were negative at birth but positive at one month, as evidence of intrapartum transmission [5]. Two of these children had been subject to nevirapine prophylaxis. Another two children were not tested at birth and at one month, but came for diagnosis at 12 and 18 months, respectively [5]. In theory they could have been transmitted by breast milk, but the mothers denied having fed them breast milk. Three of 11 (27.3%) of the HIV-1 infected children died in AIDS. There was no death among the uninfected children during the first 18 months of life (p=0.0004).

Conclusions: Using a focused counselling on the role of nevirapine prophylaxis for prevention and breastfeeding for transmission, it was possible to reduce transmission to a similar low level as in developed countries, while using a similar intervention strategy. There was no sign that the uninfected children had been exposed to a medical danger by the lack of breastfeeding, rather lives were saved.
References

P12 Assessing the emergence of drug resistance in a cohort of HIV infected pregnant women under HAART for prophylaxis of mother-to-child transmission (MTCT) followed in a referral center in Rio de Janeiro, Brazil
Jose-Henrique Piletto1,2, Beatriz Grinsztejn1, Valdilea Veloso1, Jose-Carlos Couto-Fernandez1, Adriana Rodrigues-Pedro1, Geane Flores1, Jorge-Eurico Ribeiro2, Elizangela Lima2, Ruth Khalili1, Sandra Muri2, Ronald Ismerio1 and Mariza G Morgado1
1Laboratory of AIDS & Molecular Immunology (IQC) and Evandro Chagas Clinical Research Institute (IPEC), Oswaldo Cruz Foundation, RJ, Brazil
2Department of Infectious Diseases, Hospital Geral de Nova Iguaçu, RJ, Brazil

Clinical background: Brazil stands out among developing countries due to its program of universal access to antiretroviral drugs and clinical care exams. According to the national guidelines, women initiating ART during pregnancy for the prevention of MTCT of HIV who do not otherwise meet the requirements for continued receipt of therapy discontinue ART after delivery [1]. There are significant concerns that this strategy could jeopardize future treatment options for these women [2]. The objective of the present study was to evaluate the impact of treatment discontinuation following delivery for the emergence of genotypic resistance.

Patients and methods: Since January 2005, a prospective cohort of HIV infected pregnant women, identified during prenatal care visits have been established at Hospital Geral de Nova Iguaçu, RJ. Antiretroviral prophylaxis with HAART was adopted for the prevention of HIV vertical transmission. Clinical and laboratory parameters, such as flow cytometry for CD4 counts, HIV-RNA levels (Nuclisens, Biomerieux) and genotyping (ViroSeq, Celera-Abbott), were determined at baseline and follow-up visits (6–8 weeks after HAART, at delivery and postpartum [15 days, 1, 6 and 12 months]).

Results: Until August 2007, 195 women ARV naïve and their babies have been enrolled and followed. Data from the first 120 ARV naïve pregnant women have already been analyzed. The median age was 26 years (SD = 6.6); 72% of the women were non white and the median gestational age at prenatal care initiation was 26 weeks (IQR, 20–31). The median CD4 cell count at baseline was 439.5 cells/mm^3 (IQR, 300–586) and VL was 3.91 log_{10} (IQR, 3.22–4.46). 25% of the women received a NVP-based ARV regimen (ZDV, 3TC and NVP) and 75% have received a Nelfinavir based ARV regimen (ZDV, 3TC, and NFV). The median time on ART was 75 days (IQR, 42–107). The median VL reduction between 6–8 weeks following initiation of ART was 2.01 log_{10} copies/ml. 64.2% (43/67) of women achieved undetectable VL (< 80 copies/ml) and 89.5% (60/67) had less than 1,000 copies/ml at the time of delivery. 76% (56/74) of women had a cesarean section delivery and 24% (18/74) had a vaginal delivery.

Conclusions: No cases of MTCT of HIV have been reported so far. Low levels (<5%) of drug resistance have been detected until now at baseline and after ARV. HIV-1 infections were due to subtypes B (78%), F (8%) and BF (10%), BD (3%) and BC (1%) recombinant viruses. From this naïve cohort, 8.6% (17/194) became pregnant within the first year of follow up after their delivery.

References

P13 Difficulties in assessing HIV-prevention interventions in resource-limited settings by randomized controlled trials (RCT) – The example of prevention of mother-to-child transmission (PMTCT)
Isabelle De Vincenzi, Philippe Gaillard and Tim Farley
Department of Reproductive Health and Research, World Health Organization, Geneva, Switzerland

Testing new interventions to reduce the MTCT risk in resource-limited settings with an RCT faces various challenges. Pregnant and lactating women with an advanced HIV disease require long-term antiretroviral treatment (ART) for their own health, which reduces substantially MTCT risk. For those not eligible for ART, PMTCT interventions from the 3rd trimester with combination ARVs and modified infant feeding practices greatly reduces risk. At present such MTCT-prevention interventions remain too complex to be considered as standard of care in resource-limited settings. However, in an RCT those interventions must be provided to all participants in the control arm. The residual
MTCT risk is therefore low (considerably less than 10%), and the sample size needed to demonstrate the benefit of any new intervention is now much larger (at least 1000 mother-infant pairs) than when pioneer MTCT-prevention interventions were developed.

HIV prevention research, and MTCT in particular, is a rapidly evolving field. However, conducting an RCT requires time: protocols often have to be adapted to reflect interim findings from other studies, thus delaying the conduct of an RCT and making interpretation of results more complex. When finally available RCT results may be less relevant, as practice evolves, frequently based on results from observational studies. The cost of such RCTs is high due to: the large sample size needed; the need to ensure HIV care during and after the trial; the large multidisciplinary team needed to ensure care of the participants, analyse specimens and data, and ensure adequate documentation for Good Clinical Practice standards; the need for a multi-site study to achieve the sample size as rapidly as possible, thus increasing staff and infrastructure. The expected benefit/cost ratio of conducting an RCT for testing MTCT-prevention interventions may therefore become ethically questionable. Securing funding for such trials becomes more difficult as most funds are directed at implementing and expanding PMTCT interventions already proven effective and expanding access to HIV care. Therefore, future RCTs on MTCT prevention in resource-limited settings may be hard to implement. The same may apply to other HIV-prevention research.

P14
Impact of highly active antiretroviral therapy (HAART) on clinical outcomes of vertically HIV-1 infected children
Claudia Palladino, Jose Maria Bellon, Laura Diaz Munoz, Dolores Garcia Alonso and M Angeles Munoz-Fernandez
Laboratory of Molecular Immuno-Biology, Hospital General Universitario “Gregorio Maranon”, Madrid, Spain


Introduction: The use of antiretroviral therapy produced a decrease in morbidity and mortality rates in human immunodeficiency virus type 1 (HIV-1)-infected children.

Objectives: To update a previous epidemiological survey that demonstrated the benefits of HAART on the clinical outcome of HIV-1 vertically infected children.

Materials and methods: We carried out a retrospective observational survey involving 346 HIV-1 vertically infected children (17 years old) living in the Autonomic Community of Madrid. The analysis was stratified in 5 calendar periods (CP) on the basis of the changing antiretroviral treatment protocols: CP1 (80-89): not treatment was used in this period; CP2 (90-93): the standard of care was monotherapy with nucleoside analogue reverse-transcriptase inhibitor (NRTI); CP3 (94-96): when dual-NRTI therapy was administrated; CP4 (97-98): the antiretroviral regimens was HAART with a combinations of three or more drugs; CP5 (99-06): when > 60% of children were on HAART and less than 10% were untreated. We assumed that vertical transmission occurred on the birth day. We calculated the mean CD4+ T cells count and log_{10} viral load per year, and we studied their trend over time. Then, we estimated the Kaplan-Meier curve to analyze the occurrence of AIDS and death. Finally, we performed a Cox regression analysis to calculate the relative risk (RR) for absence of AIDS and survival.

Results: We observed an increase of the mean CD4+ T-cell percentage and a concomitant decrease of the plasma log_{10} viral load since 1997. A total of 205 kids had a diagnosis of AIDS (59.2%) and 122 died (35.3%). The last two periods (CP4 and CP5) had significantly fewer AIDS cases compared to the previous (P < 0.001); in particular, the relative risk of the CP5 compared to the CP1 was of 5.5 (95%CI: 3.60–8.51). In the CP5 there were less death cases compared with the other periods (P < 0.01) and the RR was of 9.7 (95%CI: 3.49–27.2) compared to the CP1, when the event of death occurred in more than the 20% of the children.

Conclusions: We confirmed the benefits of HAART in reducing adverse clinical outcomes as AIDS and death in HIV-1 vertically infected children in our cohort of kids living in the Autonomic Community of Madrid.

Acknowledgements
The abstract is being presented on the behalf of the Spanish Group of Pediatric HIV Infection.

P15
Age-related standards for CD4+ T-lymphocytes in healthy non-infected infants born to HIV-1 infected mothers from AMATA study in Rwanda
Gilles Francois Ndayisaba Patrick, Cyaga Ndimumbanzi, Claude Rutanga, Emmanuel Havuga, Christine Omes Karasi and Cecile Alexandra Peltier
Esther project, Lux-Development, Kigali, Rwanda


Background: CD4 cell count reference values have not yet been established in Rwanda.

Objectives: Describe the evolution of CD4+ subsets in HIV-exposed-uninfected children from birth to 7 months.

Methods: Pregnant HIV-infected women received information on PMTCT and AMATA study. Those giving informed consent were enrolled from 28 weeks of pregnancy and were given HAART systematically. The child’s HIV status was assessed by DNA polymerase chain reaction. Infants aged 15 days to 7 months came for regular visits at day 1, 15, 45, month 3 and 7. Within 72 hours after birth and at every visit, peripheral blood was collected for CD4+ cell counts and PCR. CD4+ T-lymphocytes subsets expressed in absolute numbers and percentages were recorded.

Results: 521 infants were studied prospectively at four health centers in Rwanda. By day 1, the median CD4+ percentage is 54% (5th to 95th percentile, 37% to 67%) and the average absolute number of CD4+ cells is 1712 [164–5736]. Forty (7.7%) non-infected newborns have CD4+ cell count less than 1000. By day 15, the median CD4+ percentage is 50% (5th to 95th percentile, 31% to 62%) and the average absolute number of CD4+ cells is 2430 [407–7065].

By day 45, the median CD4+ percentage is 41% (5th to 95th percentile, 24% to 56%) and the average absolute number of CD4+ cells is 2151 [862–5776]. By 3 months, the median CD4+ percentage is 37% (5th to 95th percentile, 25% to 49%) and the average absolute number of CD4+ cells is 2071 [326–8202].
By 7 months, the median CD4+ percentage was 38% (5th to 95th percentile, 22% to 52%) and the average absolute number of CD4+ cells is 2241 [614–6236].

Conclusions: Normal lymphocyte subset values among Rwandan children do not differ from those in other populations. CD4 counts are very variable for the same child.

**P16**

The switch from the standard proteasome to the immunoproteasome in the mononuclear cells of children with vertically acquired HCV infection

Alida Alfarano, Antonella Versace, Gabriellla Bertolotto, Simona Vatrano and Pier-Angelo Tovo

*Departement of Paediatrics, University of Turin, Turin, Italy*


**Background:** The mechanisms responsible for the HCV escape to the immune system remain largely unknown. A strict interaction between NS3, a non-structural protein of HCV, and the LMP-7, a subunit of the immunoproteasome, has been described suggesting that this may result in an impaired antigen presentation. Proteasomes are intracellular multicatalytic complexes whose primary function is the degradation of abnormal or foreign proteins. In particular, they regulate the presentation of viral antigens onto MHC I molecules. The 26S proteasome has a 20S catalytic core and two 19S regulatory complexes. Three subunits (β1, β2 and β5) are responsible for the main proteasomal peptidase activities (PGPH, trypsin-like, and chymotrypsin-like activity, respectively). With certain cytokines, such as interferon-γ (IFN-γ), the three β subunits of the standard proteasome (PS) are replaced by inducible subunits (LMP-2, MECL-1 and LMP-7) to form the immunoproteasome (IP), which improves the antigen processing and presentation.

**Materials and methods:** Total RNA was extracted from PBMCs of 13 HCV infected children and 50 healthy controls. 1µg RNA was reverse-transcribed and a real-time quantitative PCR was used to evaluate the expression of standard proteasome genes (β1, β2 and β5) and of immunoproteasome genes (LMP-2, MECL-1 and LMP-7). The values obtained were normalised using Abelson as the control gene and the results were expressed using the DDCT method. The results were compared by Wilcoxon’s test.

**Results:** In the control group the median expressions of PS genes were: β1 = 1.75 (range 0.70–2.80), β2 = 1.29 (range 0.67–1.91) and β5 = 1.08 (range 0.63–1.54); the median expressions of IP genes were: LMP-2 = 1.79 (range 0.59–3.0), MECL-1 = 1.58 (range 0.58–2.58) and LMP-7 = 1.32 (range 0.48–2.17); the median value of a subunits was 1.04 (range 0.37–1.72). In HCV infected children the median expressions of PS genes were: β1 = 0.52 (range 0.17–1.19), β2 = 1.0 (range 0.44–3.52) and β5 = 0.48 (range 0.49–1.53) and those of IP genes: LMP-2 = 1.56 (range 0.27–2.43), MECL-1 = 1.33 (range 0.14–2.14) and LMP-7 = 1.53 (range 0.25–1.40). The difference was significantly higher for LMP-7 expression in HCV infected children.

**Conclusions:** There is a significant higher expression of IP genes in HCV infected children. This switch from PS to IP presumably reflects an adaptive response aimed at increasing antigen presentation and T cell reactivity. However, the interaction of NS3 protein with LMP-7 might frustrate this effort allowing the virus to escape. Conversely, the switch from PS to IP may lead to an increased presentation of self-antigens onto MHC I molecules, ultimately resulting in enhanced autoimmune responses.

**P17**

Alterations in immunoglobulin levels in uninfected children born to HIV infected women

Madeleine Bunders1, Lucy Pembrey2, Taco Kuipers1 and Marie-Louise Newell3

1 Division of Pediatric Hematology, Immunology and Infectious diseases, Emma Children’s Hospital (AMC), Amsterdam, The Netherlands
2 Centre of Epidemiology for Child Health, UCL Institute of Child Health, London, UK
3 UK & Africa Centre for Health and Population Studies, University of Kwazulu-Natal, South Africa


**Background:** Immunoglobulin levels are known to be elevated in HIV infected children. However, little is known about the effect of maternal HIV infection and the maternal altered immune system on immunoglobulin levels in uninfected children. As few data are available on immunoglobulins from young healthy children, we used data from uninfected children born to hepatitis C virus (HCV) infected women as a comparison.

**Methods:** Prospective data on immunoglobulin levels were available from birth to 5 years for children enrolled in the European Collaborative Study (ECS) of children born to HIV-1 infected women and from birth to 24 months for children enrolled in the European Paediatric HCV Network (EPHN). Children born to HIV/HCV co-infected women were excluded. Smoothers (running means) illustrated patterns of immunoglobulins over age by infection status. Associations between infant and maternal factors and child log10 total IgG, IgM and IgA levels were quantified in linear regression analyses allowing for repeated measures within child. Further analyses were performed using only data of HIV exposed uninfected children to investigate associations between child immunoglobulins and maternal immunological and virological factors and anti-retroviral therapy exposure.

**Results:** 1751 HIV uninfected, 190 HIV infected children (ECS), 173 HCV uninfected and 30 HCV infected children (EPHN) were included. HIV infected children had higher levels of all immunoglobulins compared to uninfected children over all ages. HIV uninfected children had significantly higher IgG, IgM and IgA levels than HCV uninfected children up to at least 24 months, adjusting for gender, prematurity and race. Prematurity was associated with significantly lower levels of immunoglobulins up to 24 months. Children born to African women had higher IgG and IgA levels up to 24 months than those born to white women but lower IgM in the first 6 months. Among HIV uninfected children higher IgG levels were associated with elevated maternal IgG levels, as well for measurements from 18 months to 5 years of age. No significant effect of maternal CD4 count was observed. ART exposure was associated with significantly lower IgG levels at 6–24 months. Race was not associated with immunoglobulin levels in multivariable analyses in this sub-group.
Conclusions: These findings indicate significant alterations in immunoglobulin levels in uninfected children born to HIV-infected women. This suggests that exposure to an activated maternal immune system is associated with an altered humoral response in children without antigen stimulation, and warrants further research.

P18
The evolution of vertically acquired HCV infection
Antonella Versace, Federica Mignone, Luisella Lazier, Clara Gabiano, Carlo Scalfaro, Alda Alfarano and Pier-Angelo Tovo
Department of Paediatrics, University of Turin, Turin, Italy
Retrovirology 2008, 5(Suppl 1):P18

Background: The natural history of vertically acquired HCV infection is unclear, with few studies regarding children prospectively followed from birth at a single centre and mostly with a short follow-up. This limited information casts doubts on the use, in childhood, of antiviral drugs commonly employed in adults with chronic HCV infection.

Materials and methods: All children with vertically acquired HCV infection who came to our attention and were regularly followed from the first months of life were included in this analysis. One child with double HCV and HIV infection was excluded. HCV associated clinical manifestations, viral genotype, persistence of viremia and HCV antibodies, liver biochemistry (ALT levels, bilirubin, alpha fetoprotein, coagulation parameters), liver ultrasound and other investigations to identify extrahepatic manifestations (e.g. the appearance of auto-antibodies or cryoglobulins, C3 and C4 concentrations) were evaluated.

Results: Twenty-five children were studied; their median age at the last check was 8.7 years (range 2.0—17.4 years). One child developed hepatomegaly, all the others were asymptomatic. Genotypes 1 and 3 were those most commonly found. Serum HCV-RNA was persistently detected in 68% (17/25) of patients, while viremia was no longer detected in 8 (32%) children in the last several determinations. Anti-HCV antibodies persisted over time in all but one child, who had two positive PCR in the first months of life and then seroreverted. Most children had increased ALT levels in the first three years of life (median of 64.0 mU/mL, range 11—678 mU/mL). Subsequently ALT levels normalised in 12 children (median 22.75 mU/mL, range 15.6—39.5) and remained enhanced in 10 (median 64.2 mU/mL, range 47.8—92.7). The liver ultrasound was constantly normal in all but two cases, who showed signs of steatosis. Seventeen patients (68%) developed auto-antibodies: 14 anti-smooth muscle (SMA), I anti-nuclear antibody (ANA), I anti-liver-kidney microsomal Type I (LKM1) antibody, and I ANA + LKM1. Cryoglobulins were transiently detected in 7 children (28%), including one who had mixed type II cryoglobulinemia, though with no concomitant clinical manifestations. C3 and C4 levels were always normal.

Conclusions: Our findings confirm that vertically acquired HCV infection is usually asymptomatic in childhood and ALT levels remain within the normal range in a large proportion of cases after the first period of life. However, viremia persists in at least two thirds of children and, of these, a significant percentage develops autoantibodies and/or cryoglobulins.

P19
Hepatitis B vaccine efficacy in HCV vertical infected infants
Małgorzata Aniszewska1,2, Barbara Kowalik-Mikola- jewska1,2 and Maria Pokorska-Lis1,2
1Department of Children's Infectious Diseases, Medical University of Warsaw, Poland
2Regional Hospital of Infectious Diseases, Warsaw, Poland

Aim: The aim of the study was to evaluate the immunogenicity of hepatitis B vaccine after primary immunization in children vertically infected with HCV.

Patients and methods: 105 infants born to HCV infected mothers were vaccinated against hepatitis B virus (recombinant vaccine, 10 g, 0-1-6 month schedule). The diagnosis of HCV infection in infants was based on positive viral RNA in serum by PCR (Amplicor v 2.0 Roche) on at least two separate specimens in the first year of life. HCV infection was confirmed in 18 children. 87 children who were HCV uninfected formed the control group. Anti-HBs titers were measured 3—6 months after the third dose of immunization in HCV infected children and in control group. Seroconversion was considered if anti-HBs level was ≥ 10 mIU/mL. Non-responders were tested for HBV infection by PCR.

Results: 13/18 (73%) HCV infected children achieved seroconversion (anti-HBs =10 mIU/mL) compared to 80/87 (92%) in the control group. 5 (27%) HCV infected children and 7 (8%) healthy subjects were non-responders (p = 0.04). Antibody titers ≥ 100 IU/1 following vaccination were observed in 10/18 (55%) HCV infected and in 56/87 (64%) uninfected children. The mean level of anti-HBs in hepatitis C infected children was lower than that found in control group (180 mIU/ml and 306 mU/ml respectively). None of non-responders were infected with HBV.

Conclusions: The immunogenicity of HBV vaccine seems to be lower in infants infected with HCV. Further studies are necessary to establish the role of cellular response and immune memory in protection of HCV-infected individuals without anti-HBs antibodies after vaccination.

P20
Immunological response in congenital cytomegalovirus infection
Elio Freda1, Maria Luisa Romiti1, Giusy LiPira2, Fabio Casciano1, Alessandra Simonetti3, Fabrizio Manca4, Andrzej Krzyzsztioflak5, Paolo Rossi1,3, Patrizia D' Argenio6 and Caterina Cancrini1,3
1Cattedra di Pediatrica, Università di Roma Tor Vergata, Roma, Italia
2Cellular Immunology Unit, Advanced Biotechnology Center, Genova, Italia
3U.O.C. di Immuno-Infettivologia Ospedale Pediatrico Bambino Gesù, Roma, Italia
4Clinical and Experimental Immunology Laboratory, G. Gaslini Institute, Genova, Italia

Background: Human cytomegalovirus (CMV) is the main cause of congenital viral infection. There are not early and certain prognostic markers to define infection /disease course
and no standard treatment of children with symptomatic congenital infection is available as yet. Indeed, a small number of infants present severe neurological complications and isolated visual and hearing impairments. The aim of our study is to verify possible correlations between immunologic alterations and clinical/therapeutic aspects. Eighteen eligible infants were enrolled in our study. Eight of them were symptomatic, showing neurological alterations.

**Methods:** Lymphocyte proliferation was detected by co-culture with mitogens (Phytohemagglutinin and Pokeweed), anti-CD3 monoclonal antibody, recall (Candida) and CMV-specific antigens. T-cell receptor (TCR) repertoire of CD8+ and CD4+ T-cell subsets was analysed by Spectratyping after RNA extraction and cDNA synthesis and amplification with a SuperScript One-Step RT-PCR kit (Invitrogen) by 24 different Vβ primers combination with a 3 Cβ labelled primer. IFN-γ production after CMV lystate and peptides pool stimulation was evaluated by cellELISA in 384 wells microplates.

**Results:** Standard immunological investigations as immunoglobulins levels and cellular immunity did not show any alteration in both groups. All symptomatic patients (8/8) did not show any specific CMV response in lymphoproliferative assay. Six out of ten asymptomatic patients showed a good CMV specific response (Stimulation Index > 3). TCR spectratyping analysis on CD8 T-cell subset showed a various degree of alteration in all symptomatic patients and in six out of nine analysed asymptomatic patients. CellELISA assay on CD4 and CD8 T-cell subset was performed and the evaluation of results is ongoing.

**Conclusions:** Our preliminary data suggest a possible correlation between a lack of CMV specific response and higher degree alteration of TCR spectratyping analysis in symptomatic versus asymptomatic patients.

**P21 Surveillance of perinatal AIDS and HIV diagnoses in France**
Florence Lot, Françoise Cazein, Josiane Pillonel, Roselyne Pinget and Caroline Semaille
Département des maladies infectieuses, Institut de veille sanitaire, Saint-Maurice, France

Retrovirology 2008, 5(Suppl 1):P21

**Background:** Since the use of zidovudine to reduce the mother-to-child HIV transmission, the number of children perinatally infected has dramatically declined. HIV/AIDS surveillance provides data to assess the effect of prevention on the perinatal HIV transmission.

**Materials and methods:** In France, mandatory surveillance of first AIDS diagnoses and new HIV diagnoses was implemented in 1986 and 2003 respectively. Paediatric notifications are reported by clinicians with an anonymous code to the Institut de veille sanitaire. We analysed trends in perinatal AIDS and HIV cases reported by 12/31/2006, adjusted for reporting delays. Characteristics of perinatal AIDS diagnoses were analysed for 2 periods (before and since 1998) and new HIV diagnoses since 2003.

**Results:** By December 2006, a total of 703 children were reported with perinatally acquired AIDS. The annual number of cases declined from 69 in 1994 to 8 in 1998, since when it has remained stable. Before 1998, mean age of children at AIDS diagnosis was 2.4 years, one third of the HIV-infected mothers were infected through injection drug use and one quarter were of French origin.

Comparatively, between 1998 and 2006, mean age of children was 7.3 years, no mother was injecting drug user and 6% were of French origin. The most common AIDS indicative diseases were oesophageal candidiasis (16%), *Pneumocystis carinii* pneumonia (15%), HIV encephalopathy (15%), cytomegalovirus disease (10%), lymphoid interstitial pneumonitis (9%) and tuberculosis (9%).

Between 2003 and 2006, 106 new HIV diagnoses were notified in children perinatally infected, with a decrease from 35 in 2004 to 19 in 2006. Half of the children were born in sub-Saharan Africa and 40% in France. Mean age at diagnosis was 5.3 years. For the 42 children born in France (23 since 2003), the mother’s geographic origin was as follows: sub-Saharan Africa (19), Caribbean (13), metropolitan France (6) and other (4).

22 mothers were not known as HIV-infected at pregnancy and the infection was known for the 20 others: a complete antiretroviral treatment (prenatal, intrapartum and neonatal) was given in 13 cases, the treatment was incomplete in 5 cases and 2 children didn’t receive any treatment.

**Conclusions:** Paediatric cases are probably under-reported, but exhaustivity could be improved by the modifications of the system introduced in July 2007. Although number of AIDS/HIV diagnoses is low in the last years, a few cases could still have been avoided by screening and treatment of all HIV+ pregnant women. Furthermore, early testing should be systematically proposed for children from endemic countries.

**P22 Early diagnosis of HIV-1 infection in Cambodian infants**
Sophie Ngin1, Sim Leang Kruy2, Olivier Segera3,7, Viseth Srey Horm1, Ly Meng Ek2, Im Sethiak2, Vara Ouk2,3, Vibol Ung3, Olivier Marcy3,5, Christine Rouzioux6, Jean François Delfraissy3,7 and Eric Nerrienet1
1HIV/Hepatitis Laboratory, Institut Pasteur du Cambodge, Phnom Penh, Cambodia
2Calmette Hospital, Phnom Penh, Cambodia
3ESTHER/Calmette Hospital, Phnom Penh, Cambodia
4National Paediatric Hospital, Phnom Penh, Cambodia
5French Red Cross, France
6Laboratoire de virologie, EA 3620, Université Paris-Descarte, CHU Necker-Enfants Malades, Paris, France
7Clinical Immunology Department, CHU Bicètre, Kremlin Bicêtre, Paris, France


**Objective:** In Cambodia, national programs to prevent mother-to-child transmission (MTCT) of HIV, scaled up since 2001, were hampered by lack of access to HIV early diagnosis in infancy. A low cost strategy to assess HIV-RNA viral load, was implemented in 2005 and applied to the early diagnosis in infants. Our objectives were i) to generate information on the early diagnosis of HIV infection in Cambodia, and ii) to estimate the in utero and perinatal MTCT rates of HIV-1 among mothers delivering at Calmette Hospital, Phnom Penh.

**Methods:** Detection of HIV-RNA in infants was determined using the ANRS second-generation (G2) real-time RT-PCR test.
Results: i) Between May 2005 and February 2007, 755 plasma samples from children (mean age: 5.5 months [1–18]), born from HIV-infected mothers were screened for HIV infection. Samples originated from Phnom Penh (57.8%) and from 5 provinces (42.2%). Sex Ratio F/M was 0.97. Data showed that 134/755 (17.7%) samples were HIV-RNA positive (mean viral load: 6.4 Log10 [3.9–8.6]). ii) During the study period, 157 HIV-infected pregnant women (mean age: 27, IQR [25–31]) attended antenatal care and delivered at Calmette Hospital. Among those, 77 (49%) on HAART delivered 77 babies of whom 2 were HIV-RNA positive (MTCT: 2.6%). Twenty-eight (18.1%), not eligible for HAART, received AZT then sdNVP during labour: 3/28 (10.7%) babies were HIV-RNA positive. Forty-one (26%) received only sdNVP during labour because of HIV late testing: 6 infants (14.6%) were diagnosed HIV-RNA positive. Finally, 11 mothers did not receive any prophylaxis: 2 infants (18.1%) were HIV-RNA positive.

Conclusions: This study confirms the usefulness of the HIV RNA ANRS (G2) real-time RT-PCR test to diagnose early HIV-1 infection in infancy and to monitor PMTCT programs. This study also highlights the urgent need to expend the early diagnosis at the national level to improve medical care of HIV infected Cambodian children and to expand HAART access for women during pregnancy to reduce MTCT.

P23

Spatial pattern of HIV-1 cases of vertical transmission in Madrid (Spain): impact of demographic and socioeconomic factors

Claudia Palladino, Jose Maria Bellón, Rosa Resino, Laura Díaz Muñoz, Dolores García Alonso and Mª Ángeles Muñoz-Fernández

Laboratory of Molecular Immuno-Biology, Hospital General Universitario “Gregorio Marañon”, Madrid, Spain


Introduction: The Autonomic Community of Madrid is the area most affected by the human immunodeficiency virus type 1 (HIV-1) in Spain, with a total of 17,667 AIDS cases up to December 2006, the 23.9% of the cases registered at national level.

Objectives: To depict the spatial evolution of the HIV-1 cases of mother to child transmission (MTCT) and to assess the possible impact of demographic and socioeconomic characteristics of the population of the Municipality of Madrid on the HIV epidemic in the paediatric population.

Materials and methods: We performed a study of a retrospective observational cohort of 224 HIV-1 vertically infected children (17 years old) living in Madrid, born between 1980 and 2006. The analysis was stratified in 5 calendar periods (CP) on the basis of the changing antiretroviral treatment protocols: CP1 (80-89); children were untreated; CP2 (90–93): the standard of care was monotherapy with nucleoside analogue reverse-transcriptase inhibitor (NRTI); CP3 (94–96): children were receiving dual-NRTI therapy; CP4 (97–98): the antiretroviral regimen was HAART with a combination of three or more drugs; CP5 (99–06): more than 60% were on HAART and less than 10% were still untreated. We assumed that vertical transmission occurred on the birth day. We georeferenciated the HIV-1 MTCT cases and we elaborated maps representing the prevalence of HIV-1 MTCT cases by the 21 districts of Madrid, with the support of ArcView Geographic Information Systems Version 3.1. Afterwards, we carried out an ecological analysis to assess the association between demographic and socio-economic characteristics of the population of the different districts of Madrid and the spatial distribution of HIV-1 MTCT cases.

Results: The districts with the higher prevalence of HIV-1 MTCT were: Usera, Puente de Vallecas, San Blas in the Southern area of the city, and Hortaleza in the Northern area. We observed a significant correlation between the prevalence of HIV-1 MTCT cases and i) the percentage of migrants in 1996 ($\rho = -0.54$; $P = 0.011$) and in 2001 ($\rho = -0.54$; $P = 0.011$); ii) the percentage of illiterates in 1996 ($\rho = 0.49$; $P = 0.025$) and in 2001 ($\rho = 0.59$; $P = 0.005$); and iii) the percentage of unemployed women in 1996 ($\rho = 0.48$; $P = 0.029$) and in 2001 ($\rho = 0.58$; $P = 0.005$); 4) the mean annual incoming in 1996 ($\rho = -0.51$; $P = 0.019$) and in 2000 ($\rho = -0.51$; $P = 0.018$).

Conclusions: We observed the highest prevalence of HIV-1 MTCT in the geographical areas with a lower socio-economical status.

Acknowledgements

The abstract is being presented on the behalf of the Spanish Group of Pediatric HIV Infection.

P24

Effectiveness of a early initiation of protease inhibitor-sparing antiretroviral regimen in human immunodeficiency virus-1 vertically infected infants

Dimitri Van der Linden1, Marc Hainaut1, Tessa Goetghueber1, Edwige Haelterman1, Veronique Schmitz2, Philip Maes3, Alexandra Peltier1 and Jack Levy1

1CHU Saint Pierre, ULB, Brussels, Belgium
2CHR de La Citadelle, ULg, Liège, Belgium
3AZM Koningin Paola Kinderziekenhuis, Antwerp, Belgium


Background: Vertically HIV-1-infected infants are at high risk of severe manifestations of the disease in the first year of life [1]. For this reason, we have elected, since 1996, to treat all infants born to HIV-1-infected mothers with a combination of 3 reverse transcriptase inhibitors as soon as the diagnosis of vertical transmission is established.

Material and methods: This is a cohort study of the effectiveness and tolerance of therapy in the 17 HIV-1-infected infants followed from birth in 3 belgian paediatric reference centres since 1996. All of them had been treated according to these guidelines.

Results: Treatment was initiated in all patients before 66 days of life. All but one were asymptomatic at initiation of therapy. Median follow-up was 56 months (range: 26–103). Twelve out of the 17 patients (70.6%), including 11/13 (85%) infants treated with the combination of zidovudine, lamivudine and nevirapine, experienced a complete viral suppression (<50 copies/mL) with their first drug regimen. Lack of compliance was acknowledged by the parents of 3 of the 5 infants whose initial regimen failed. At last follow-up, 12 patients were asymptomatic, two were CDC stage A and three were stage B; 15 had HIV-1 RNA levels of < 50 copies/mL and 14 had =25% CD4 lymphocytes. Among them 11 were still treated with their first line regimen (Table 1).
Table 1. (abstract P24) Initial and Current Antiretroviral Regimen and Long-Term Outcome of the 17 Children Included in the Study

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<th>Age at Initiation of Therapy (d)</th>
<th>First Antiretroviral Regimen</th>
<th>Lowest VL with Initial ART</th>
<th>Age (y) at Last FU</th>
<th>VL at Last FU</th>
<th>CDC Classification</th>
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ID, patient identification number; VL, viral load; ART, antiretroviral therapy; FU, follow-up.

One child experienced a transient severe side effect, another child had clinical lipodystrophy and 4 developed hypercholesterolemia.

Conclusions: Early initiation of treatment with 3 reverse transcriptase inhibitors appeared to be highly effective in this cohort of vertically HIV-1 infected infants. Parental adherence is crucial to the effectiveness of therapy.

Acknowledgements

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Reference


P25
Clinical management of children diagnosed with HIV and hepatitis C virus infection
Kirsty England1, Claire Thorne1, Lucy Pembrey1 and Marie-Louise Newell1,2
1MRC Centre of Epidemiology for Child Health, Institute of Child Health, University College London, London, WC1N 1EH, UK
2Africa Centre for Health and Population Studies, University of KwaZulu Natal, Mtubatuba 3933, South Africa


Background: In the absence of evidence-based guidelines, we investigate current European practices for the clinical management and treatment of children coinfected with HIV and Hepatitis C virus (HCV).

Material and methods: A semi-structured questionnaire survey in clinical centres from 7 European countries who were either enrolling in the European Collaborative Study of HIV-infected pregnant women and their children or the European Paediatric HCV Network of children born to HCV-infected mothers.

Results: To date we have received responses from clinicians from 16 centres, caring for a total of 35 HIV/HCV coinfected children. Four centres were not currently following any coinfected children. Only one centre had a written policy for the management and treatment of HIV/HCV coinfected children and in most other centres (8/11, 72%) decisions regarding the care of coinfected children were taken at the hospital or departmental level with only 3 out of 11 centres relying on regional or national decision-making. In addition to standard laboratory tests for monitoring HIV disease (HIV RNA quantification, CD4 counts etc), 6/12 centres also performed HCV RNA PCR tests at least annually and 6/12 centres carried out ALT and AST tests at least every 6 months in coinfected children. Liver biopsy was rarely performed but liver ultrasound was performed in 5 out of 12 centres at least annually. 91% of 11 centres performed HCV genotyping on all HIV/HCV coinfected children and 88% of 8 centres monitored for evidence of hepatotoxicity in coinfected children receiving antiretroviral treatment for HIV disease. Only one centre had ever treated coinfected children for HIV and HCV infections concurrently but 4/7 other centres stated they would consider treatment at the same time if necessary. In the situation of an HIV/HCV coinfected child, 3/7 respondents stated that they would treat HCV infection before starting HIV therapy.

Conclusions: In the absence of guidelines for the clinical management of children coinfected with HIV and HCV, practices throughout Europe vary widely. Individual centres see relatively few coinfected children and therefore a lack of experience in the management of this group and the lack of evidence-based policy may be a barrier to achieving optimal care and treatment. This survey highlights the importance of research focused on this group of children to inform guidelines for their best possible care.

Acknowledgements

We thank all the clinicians who participated in this survey.