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LECTURE PRESENTATIONS

L1

Human genome variability and host-pathogen interactions

Lluís Quintana-Murci

Institut Pasteur/CNRS, Paris, France

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Natural selection is a major force behind the shaping of patterns of human genome variability. Inferences concerning the action of selection in the human genome provide a powerful tool for predicting regions of the genome of major functional importance. Genetic variants influencing human susceptibility to disease are likely to affect the fitness of the organism, unless the disease concerned begins late in the life. There is therefore an intimate relationship between disease and selection that can be exploited for the identification of candidate disease loci. To date, some of the strongest evidence for selection in the human genome has been obtained for genes involved in the immune response or host-pathogen interactions. Indeed, before the advent of antibiotics and vaccines, infectious diseases have been paramount among the threats to health and survival for most of human evolutionary history. I will review our most recent studies searching for the footprints of natural selection in the human genome. These studies, which go from global genomewide scans to more fine-tuned analyses in specific genes, highlight how the identification of selected loci or variants may provide insight into host genes or pathways playing an important role in pathogen resistance. For example, we have shown that natural selection has significantly driven the processes of population differentiation in modern human populations. Specifically, we have identified a number of genes under strong geographically-restricted positive selection, some of them involved in host-pathogen interactions. In addition, I will present our most recent data on the Toll-like receptor (TLR) signalling pathway. Our evolutionary data indicate that the different members of TLR family differ in their ecological relevance and increase our understanding of how variation in these genes results in different contributions to the outcome of infectious diseases. More generally, I will show how the identification of the extent and type of selection acting upon human genes involved in host-pathogen interactions make it possible to define the redundant and non-redundant functions of individual immunity-related genes in the natural setting.

L2

Perspectives on pathogenesis and prevention of congenital herpes virus infection

Mark R Schleiss

Pediatric Infectious Diseases, University of Minnesota Medical School and Center for Infectious Diseases and Microbiology Translational Research, Minneapolis, MN, USA

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Each of the 8 members of the human herpesvirus family identified to date has been associated, to varying degrees, with congenital and perinatal transmission to the fetus and newborn infant. The problem of prevention of maternal-to-child transmission of herpesvirus infections is complicated by a lack of understanding of correlates of protective immunity for both the mother and the fetus. Herpesviruses encode multiple immune evasion genes, and establish lifelong, persistent infection in the host. These issues render the design of protective vaccines highly problematic.

In spite of these challenges, progress has been made in recent years toward the development of new vaccines for herpesviruses, in particular the viruses that pose the greatest risk to the newborn – herpes simplex virus (HSV) and cytomegalovirus (CMV). Recent strategies undergoing evaluation in clinical trials include both the approach of attenuated, live-virus vaccines as well as recombinant, subunit vaccines that target immunodominant virally-encoded proteins. A recombinant subunit vaccine against genital HSV infection, based on the viral glycoprotein gD, has demonstrated efficacy in placebo-controlled studies, and merits further investigation. A benefit of immunization against genital HSV for prevention of neonatal HSV infection is inferred, although this endpoint may ultimately be difficult to demonstrate in clinical trials. Progress has also been made for immunization against congenital infection with CMV. Given the neurodevelopmental injury associated with congenital CMV infection, such a vaccine is a major public health priority. Recently, subunit vaccination with the CMV glycoprotein, gB, has demonstrated efficacy against acquisition of infection in a phase II trial in young women. In a placebo-controlled study, vaccination had a significant impact on the probability of a study participant remaining CMV seronegative through the 42-month follow-up period. These data are the first that demonstrate significant efficacy of a CMV vaccine for prevention of infection, and will drive interest in future gB vaccine studies using other expression technologies. Although these results are encouraging, ultimately, several major issues must be resolved before CMV vaccines can

be optimized. First, the phenomena of re-infection of CMV-immune hosts with new strains must be understood, since non-primary CMV infections account for the major disease burden of congenital CMV. Presumably, vaccine design will need to account for the heterogeneity in clinical isolates that may account for most re-infections. Secondly, the role of viral immune evasion genes in abrogating host immunity must be clarified: a better understanding of this process may, in turn, allow design of optimized live, attenuated vaccines. Finally, the role of immune responses that may potentially control infection at mucosal surfaces needs to be elucidated, and this knowledge may in turn facilitate development of novel vaccines that block primary infection at mucosal sites.

L3 Mother and fetal immunity to cytomegalovirus

Arnaud Marchant

Institute for Medical Immunology, Université Libre de Bruxelles and ImmuneHealth, Charleroi, Belgium

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The pathogenesis of cytomegalovirus (CMV) transmission in utero remains poorly understood. Identification of the mechanisms involved and of biomarkers of transmission would help in the design and in the “proof of concept” evaluation of vaccine candidates. Primary CMV infection during pregnancy induces the differentiation of CD4 and CD8 T lymphocytes producing antiviral cytokines. In contrast, proliferative responses of CD4 T cells are acquired slowly and are associated with a reduced risk of transmission. Defective CD4 T lymphocyte responses could allow the replication of CMV in organs involved in transmission and may limit the development of neutralising antibody responses. The development of severe symptoms and the prolonged viral excretion observed following CMV infection during fetal life may be related to defective immune responses. Mature CD8 T lymphocyte responses to CMV can be detected in infected newborns. In contrast, CMV induces the expansion and the differentiation of fetal CD4 T lymphocytes that are unable to produce anti-viral cytokines or proliferate in response to viral antigens. Our current understanding of mother and fetal immunity to CMV suggests that the virus interferes with the acquisition of specific functions of CD4 T lymphocytes, particularly during the early phase of the infection. The different expression of this interference in mothers and fetuses may involve the immaturity of antigen-presenting cells.

L4 The art and science of preventing and treating HIV disease in children: a life cycle approach

Elaine Abrams

Department of Paediatrics, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA

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Dr Abrams will review current scientific knowledge in the area of pediatric HIV infection from infancy through adolescence and will identify critical gaps that significantly impact successful care of children with HIV disease. She will explore three interconnected themes: evolution of physical and psychological development throughout childhood; the relationship between the child and her family; and importance of psychological, behavioral, and social

issues as they affect successful treatment of the HIV infected child as well as vulnerability of the uninfected adolescent to acquisition of HIV infection. Combining experiences from HIV programs in resource rich and resource constrained settings, Dr. Abrams will highlight a number of pressing questions warranting urgent scientific inquiry.

L5 Pharmacological issues in antiretroviral therapy during pregnancy: a clinician's perspective

Laurent Mandelbrot

Université Paris 7 – Diderot, APHP-Department of Obstetrics-Gynecology, Hôpital Louis Mourier, Colombes, France

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All antiretroviral drugs are eventually used in pregnant women, either as prevention of mother-to-child transmission (PMTCT), or as long-term therapy for the woman's own health. Yet, pregnancy data is lacking for recent drugs. Four types of pharmacologic issues should be addressed:

1) Does PK/PD change during pregnancy due to physiological factors (absorption, plasma volume, plasma proteins such as albumin and alpha acid glycoprotein, cytochrome induction), and can this lead to reduced efficacy? Plasma concentrations are decreased during the 3d trimester for several protease inhibitors (PIs). This has led to 2 strategies, increasing the dose or performing therapeutic drug monitoring, however there is debate as to whether such measures are useful when maternal viral replication is under control.

2) Does the drug penetrate into the genital tract, and does this play a role in prevention of transmission? Preliminary data shows that the penetration of drugs into the genital tract differs widely between drugs, and is very poor for some PIs. Since one of the mechanisms of MTCT is vertical transmission, and since viral replication in the vaginal compartment can persist despite undetectable plasma HIV RNA, there is concern as to whether low intravaginal drug levels may lead to an increased transmission risk, especially during vaginal delivery.

3) Does it cross the placenta, and what are the potential benefits and risks of fetal exposure? For the fetus, placental transfer may either be harmful because of toxicity or protective (PrEP). Most NRTIs diffuse across the placenta, the fusion inhibitor enfuvirtide does not, and there are wide differences between the various PIs. A number of factors are known to limit drug transfer (some of which are unique to the placenta), including molecular size (impermeable >1000 kDa), low lipid solubility of drug, pKa, low protein binding, low placental blood flow, placental fixation, placental metabolism, fetal acidosis. Efflux pumps such as P-glycoprotein (MDR1) present in the trophoblast are involved in protecting the fetus from xenobiotics in the maternal compartment. They play an important role in decreasing transfer of PIs to the fetus. Genetic polymorphisms may account for inter-individual differences. Thus, placental transfer cannot be entirely predicted from the physico-chemical properties of the drug, nor from its transfer across other barriers. Several methods can be used to determine placental transfer: clinical data (sampling at delivery for cord blood/maternal blood ratio), animal models, in vitro models, ie. cell cultures, placental tissue slices, and the ex vivo human perfused cotyledon. The perfused cotyledon model, which determines a clearance index, is

generally predictive. Clinical data must be interpreted cautiously because only a single paired cord/maternal sample is available, and the ratio can change according to timing of delivery after the last administration of the drug. Population pharmacokinetics allows for more accurate modeling with this type of data.

4) Is it metabolized by fetus, and how does this influence its toxicities? Metabolism differs from adults due to immaturity of fetal CYP3A (CYP3A7), as well as excretion in the urine and gut. Little is known about the clearance of drugs and metabolites from the fetus back through the placenta. Thus, consequences on the fetus cannot be predicted simply from placental transfer. In conclusion, placental transfer differs between ARVs and is influenced by many factors. Poor placental transfer may be an advantage, in order to avoid toxicity to the fetus, or a disadvantage for PrEP. In order to help clinicians and patients to choose the optimal regimen, placental transfer should be evaluated as part of the licensing procedure for medications.

L6 Prevention of breastfeeding transmission of HIV-I by infant peri-exposure prophylaxis

Philippe Van de Perre^{1,2}

¹University Montpellier 1, EA 4205 and CHU Montpellier laboratory of bacteriology-virology, Montpellier, France

²On behalf of the ANRS 12174/Promise-PEP trial consortium

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Transmission of HIV-I by breastfeeding is responsible for at least 250,000 yearly acquired HIV-I paediatric infections worldwide. However, it remains largely out of reach of short perinatal prophylactic regimens. All HIV-I infected women eligible for highly active antiretroviral therapy (HAART), lactating or not, should be offered treatment for their own health, which will also reduce transmission to their babies. Recent studies suggest that residual transmission can occur through HIV-I cellular reservoirs in breast milk of these women despite successful HAART. Prevention of breastfeeding transmission of HIV-I from women non eligible for HAART remains a dilemma. Prophylactic maternal HAART restricted to the lactation period is an option. Peri-exposure prophylaxis (PEP) consisting in the administration of an antiretroviral (ARV) drug to the infant during exposure to HIV-I through breastfeeding as a prophylaxis, is another option. Proof of concept studies (two randomised trials, PEPI in Malawi and SWEN in Uganda, Ethiopia, India as well as the observational cohort MITRA in Tanzania) have confirmed the efficacy of PEP in preventing transmission. Infant PEP regimen included nevirapine (NVP) for 6 weeks in SWEN, NVP or NVP plus zidovudine for 14 weeks in PEPI, and lamivudine for 6 months in MITRA. Part of the preventive benefit of infant PEP was lost if the babies were still breastfed and exposed to HIV-I after prophylaxis was stopped. PEP regimens were associated with minimal side effects, but in the SWEN study, PEP failure was associated with a very high rate of NVP resistance in babies, which may challenge the first-line HAART regimen recommended as soon as infant HIV infection is diagnosed. PEP offers the potential advantages of sparing ARV exposure in women non eligible for HAART (avoidance of side effects and of selection of resistance), of being independent of HIV-I reservoirs in breast milk, of being potentially cheap and easy to administer, and of allowing the promotion of optimal infant feeding practices to all women, regardless of their HIV status. These advantages have to be

balanced with the risk of developing side effects in uninfected babies during extended exposure to the PEP drug and of selecting ARV drug resistant viruses in babies who acquire HIV-I despite prophylaxis. PEP is a promising intervention to prevent breastfeeding transmission of HIV-I. More research is needed to identify the PEP drug regimen having the optimal benefit/risk ratio (including long acting drugs) and to synergise PEP with promotion of best practices of infant feeding.

L7 Universal antiretroviral therapy for pregnant and breastfeeding HIV-infected women: towards the elimination of mother-to-child transmission of HIV-I in resource-limited settings?

Renaud Becquet¹⁻³

¹INSERM, Unité 897, Centre de Recherche "Épidémiologie et Biostatistique" Bordeaux, France

²Institut de Santé Publique Epidémiologie Développement (ISPED), Université Victor Segalen Bordeaux 2, Bordeaux, France

³On behalf of the ANRS 12200 Study Group

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Several unmet scientific needs that account for the partial failure of MTCT prevention efforts in resource-constrained settings, particularly in Africa. Single-dose and short-course antiretroviral (ARV) regimens are only partially effective and have failed to achieve wide coverage despite their apparent simplicity. More potent ARV combinations are restricted to pregnant women who need treatment for themselves but are also infrequently used. Furthermore, postnatal transmission via breastfeeding is a serious additional threat. Modifications of infant feeding practices aim to reduce breast-milk HIV transmission: replacement feeding is neither affordable nor safe for the majority of African women, and early breastfeeding cessation (e.g. prior to 6 months of life) requires substantial care and nutritional counselling to be practised safely. We argue that the recent roll out of highly active ARV treatment (HAART) has changed the paradigm of prevention of MTCT. To date, postnatal ARV interventions that have been evaluated target either maternal ARV treatment to selected breastfeeding women, with good efficacy, or single-drug post-exposure prophylaxis for short periods of time to their neonates, with a partial efficacy and at the expense of acquisition of drug-related viral resistance. We hypothesize that a viable solution to eliminate paediatric AIDS lies in the universal provision of HAART to all HIV-infected women through pregnancy, delivery, and covering the entire breastfeeding period.

We suggest the active promotion of the universal maternal HAART approach as a way towards elimination of MTCT in resource-limited settings. We argue that HAART should be made available to all HIV-infected pregnant women in resource-limited settings, irrespective of their CD4 count or clinical stage, and even to those who present late in pregnancy. This universal ARV-based strategy will be accompanied by proper pharmacovigilance systems. It should consider the breastfeeding cessation around six months of age, which implies the need for a proper and systematic nutritional support. Continuing investigations will compare the safety, acceptability, feasibility and efficiency of various maternal HAART regimens for preventing MTCT throughout the entire exposure period in order to rank them according to the best risk-benefit balance.

L8**Abstract withdrawn**

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L9**Tuberculosis in infancy**

Ben J Marais

Tygerberg Children's Hospital and Stellenbosch University Cape Town, South Africa

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Mother to child transmission of tuberculosis (TB) is not restricted to the first year of life, but it is a high risk period that illustrates all the important principles involved. The pregnant state increases a woman's vulnerability to develop TB and this is greatly amplified by co-existent HIV-related immune compromise. Mothers can transmit *M. tuberculosis* to their infants in three ways: 1) haematogenously via the placenta, 2) with aspiration and/or swallowing of infectious secretions during delivery and 3) post-partum via inhalation of small infectious particles. Haematogenous spread is most likely to occur if the mother develops primary infection with occult dissemination or frank disseminated disease during pregnancy. Infants represent the group at highest risk to develop severe forms of tuberculosis following primary infection, which justifies the provision of preventive chemotherapy following documented TB exposure and/or infection. The high risk experienced by HIV-exposed infants and the value/importance of infection control and various other strategies to reduce mortality and morbidity will be discussed.

L10**Memorial lecture: viruses and the developing nervous system**

Marc Tardieu

Université Paris Sud 11, Neurologie pédiatrique et centre de référence maladies inflammatoires du cerveau, Hôpital Bicêtre Assistance publique hôpitaux de Paris, Inserm U802, France

Retrovirology 2009, 6(Suppl 1):L10

The next Dominique Dormont International Conference will cover the consequences of viral infections on the *developing* nervous system, a subject usually not studied per se. The term of "developing nervous system" should be understood as the development of brain and spinal cord from the end of 1st month post conception to the end of the 2nd year of life. Such a complex subject will require a clinico-biological approach, evaluating the parallel development of immune and nervous systems and considering the variety of clinical situations resulting from virus-related insults to the developing nervous system. Several major events occur during the long interval of time we consider: first trimester of gestation: neural precursors proliferation and initial mesenchymatous/microglia invasion; second trimester: neural cell migration to constitute the cortical layers; last trimester: glial cell multiplication, dendritic network disposition and angiogenesis; first two years of life: oligodendrocytes/myelin maturation and blood-brain barrier constitution.

Clinical consequences of any insult to the developing nervous system will be highly dependent on time of the injury during development. For example, CMV infection occurring during the second trimester will induce abnormal cortical malformations

(polymicrogyria or pachygyria), whereas HIV-1 transmission during last trimester does not result in brain malformation but in reduced brain size at birth.

Virus-induced insults are of multiple mechanisms. The most frequent ones are indirect insults either through modification of vessels or induced inflammation/immune responses. Early occurring HIV-1 related encephalopathy is most likely the consequence of both virus-related and immune-related toxic effect occurring during late pregnancy and initial weeks of life. Less studied, but an important field to be explored, are virus-induced disturbances of angiogenesis and of blood-brain barrier maturation. CMV, VZV, HIV infections can induce alterations of brain vessels, as does autoimmune disorders such as antiphospholipid syndromes. Finally, viruses may infect directly neuroectodermal cells and induce cytopathic effect and tissue necrosis. Herpes simplex infection of the brain, although observed at any age, is more frequent during the first two years of life. This is, at least in part, the consequence of genetic variations in the inflammatory and immune response to herpes simplex virus with a specific dependence of neural cells. Immunogenetic is another partner in this highly complicated interaction between developing brain, developing immune system and viruses.

ORAL PRESENTATIONS**O1****Effects of HPV-16 early proteins on trophoblastic cells**Selma Boulouar¹, Christine Weyn¹, Melma Van Noppen¹, Moussa Ali Mohamed¹, Françoise Bex², Agnès Noël³, Yvon Englert¹ and Véronique Fontaine¹¹Université Libre de Bruxelles, Bruxelles, Belgium²CERIA-ULB, Bruxelles, Belgium³Ulg, Liège, Belgium

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The trophoblastic cell represents the main functional unit of the placenta. It proliferates, migrates, and invades the maternal tissue in a way that is similar to malignant tumors. Nevertheless, these processes are tightly controlled by stringent spatial and temporal confines. Therefore, the trophoblastic cell, as 'a well-behaved tumor', represents an ideal model system to investigate several oncogenic processes. Several studies reported that HPV viruses could infect trophoblasts during pregnancies. Surprisingly, HPV can replicate *in vitro* in trophoblasts. Higher HPV infection frequency has been reported to be associated with some spontaneous abortion and gestational trophoblastic diseases.

In this study, we have studied the impacts of HPV-16 early proteins, mainly E5, E6 and E7, on the viability, adhesiveness, migration and invasion of trophoblastic cells.

Our results showed that the hydrophobic E5 protein is localized in many interne membranes compartments of the transfected trophoblast. E5 affects the viability of transiently and stably transfected trophoblastic cells. The viability seemed to be restored or even increased in the presence of E6 and E7. These observations were also confirmed by transfection in C33a cells, the HPV-negative human cervical carcinoma cell line. In addition, E5 decreased the adhesiveness of the trophoblastic cells to the support and to the endometrial cells. Cells expressing metastasis E6, E7 and in less extend E5 favour chemotactic migration and matrigel invasion

compared to the cells expressing the LacZ control. These effects are also observed when early proteins are expressed under the control of their own viral promoter (LCR). Our findings show that HPV-16 early proteins can affect the adhesiveness, the migration and the invasion of trophoblastic cells, key properties involved in placentation and metastasis.

O2

Activation of PPAR γ by human CMV for de novo replication impairs invasiveness of cytotrophoblast from early placenta

Benjamin Rauwel¹, Bernard Mariamé¹, H  l  ne Martin¹, Dani  le Evain-Brion², Thierry Fournier² and Christian Davrinche¹

¹INSERM 563, Toulouse, France

²INSERM 767, Paris, France

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Human cytomegalovirus (HCMV) contributes to pathogenic processes in immuno-suppressed individuals, in fetuses and in neonates. Infection during pregnancy is known to cause miscarriages and low-birthweight newborns and we know that in this case infection of the placenta precedes transmission to the fetus. HCMV was shown to benefit from inflammatory conditions by using the cyclooxygenase-2 (Cox-2)-dependent prostaglandin pathway for transcription of the essential immediate-early gene IE2. The fact that Cox-2 activation could serve as a source of ligand for the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR γ), which is known to play a pivotal role in controlling human trophoblast invasion, led us to hypothesize that HCMV could impair placentation through activation of PPAR γ .

By using reporter gene activation assays and confocal microscopy in the presence of specific antagonist, we provide the first evidence that PPAR γ was activated in infected cells. We demonstrated that PPAR γ antagonist dramatically impaired IE2 mRNA expression and virus production and that the major immediate-early promoter (MIEP) contained PPAR response elements (PPRE) able to bind PPAR γ , as assessed by electrophoretic mobility shift and chromatin immunoprecipitation assays. By using an *in vitro* model of primary culture of extravillous cytotrophoblasts isolated from early placentas we demonstrated that HCMV could dramatically impair cytotrophoblasts invasiveness and migration processes through activation of PPAR γ . Our data provide new clues to explain how infection during the first trimester of pregnancy could impair implantation, placentation and therefore embryonic development.

O3

Mother-to-child transmission of HTLV-I: *in vitro* study of HTLV-I passage across a tight human epithelial barrier

Sandra Martin-Latil¹, Nina Gn  dig¹, Adeline Mallet², Marie-Christine Prevost², Marion Desdouits¹, Antoine Gessain¹, Simona Ozden¹ and Pierre-Emmanuel Ceccaldi¹

¹EPVO Unit, Institut Pasteur, Paris, France

²PFME, Institut Pasteur, Paris, France

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Human T-Cell Leukemia Virus type I (HTLV-I), that infects around 15 million people world wide, is the causative agent of

adult T-cell leukemia/lymphoma and HTLV-I Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). Besides horizontal transmission, HTLV-I is transmitted vertically mainly through breastfeeding. This maternal transmission via breast milk appears to be the dominant mode of HTLV-I spread in the high endemic areas, and is correlated with the presence of HTLV-I infected cells (lymphocytes, epithelial cells...) in the milk of infected mothers.

We developed an *in vitro* model of epithelial barrier (Caco-2 human enterocytic cell line) to assess the mode of passage of HTLV-I through the digestive tract. Integrity of the epithelial barrier was checked by ultrastructural approach (immunofluorescence for tight junction proteins, electron microscopy), measurement of the trans-epithelial resistance (TER), and diffusion of fluorescently labeled molecules (fluorescent dextran) through Transwell devices.

When the enterocytic cell line was co-cultured with HTLV-I-infected lymphocytes, no structural modifications could be detected in the tight junctions between enterocytes. Moreover, the functional integrity of the epithelial barrier was maintained since no change in TER was detected in the presence of infected lymphocytes. Similarly, the passage of small molecules (4 kDa fluorescent dextran) was unaffected. No increase in the passage of HTLV-I infected lymphocytes (vs uninfected) across the epithelial barrier was observed.

Although enterocytes were not found to be susceptible to HTLV-I infection, free infectious HTLV-I virions were detected in the basolateral compartment, and such a passage was shown to be temperature-dependent. These results suggest a transcytotic passage of virions across the enterocytes.

Our present data indicate that HTLV-I may cross the tight epithelial barrier without disruption or alteration of its integrity, in the absence of enterocyte infection. The role of dendritic cells in HTLV-I passage through the epithelial barrier is currently under investigation in our *in vitro* model, to further delineate the mechanisms of HTLV-I transmission during breastfeeding.

O4

Dendritic cells sample HIV-I through an intestinal epithelial cell monolayer

Mariangela Cavarelli¹, Chiara Foglieni¹, Maria Rescigno² and Gabriella Scarlati¹

¹San Raffaele Scientific Institute, Milan, Italy

²European Institute of Oncology, Milan, Italy

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The intestinal mucosa is a preferential portal of entry for HIV-I during mother-to-child transmission. Oral infection is also a well documented route for transmission of HIV-I in neonates. Neonates can acquire the disease by breast-feeding, moreover presence of blood in gastric aspirates of neonates born to HIV-I infected mothers has also been incriminated as a risk factor in the transmission of HIV-I. Multiple mechanisms for mucosal HIV-I transmission have been proposed, however the exact role played by dendritic cells in facilitating viral passage across intestinal epithelium have not been fully defined. We had hypothesized that sub-mucosal dendritic cells (DCs) can mediate mucosal transmission of HIV-I through a process similar to bacterial sampling through gastrointestinal epithelium (Rescigno M., Nat.Immun.2001).

An *in vitro* transwell co-culture system of colonic cell line Caco-2 and DCs was developed. DCs collected on transwell after incubation on the apical side of Caco-2 monolayer with cell-free HIV-1 (R5 and X4 phenotype), were able to transfer infection efficiently to susceptible target cells. Abundant HIV-1 replication (as measured by p24 antigen ELISA until day 25 of DCs-T cells co-culture) was reproducibly observed, suggesting that DCs sampled the virus and transferred it to target cells. DCs retained infection capability for at least 4 days. Confocal microscopy showed intense migration of DCs through the tight junctions of Caco-2, following incubation with HIV-1, at a level comparable to LPS treated cultures (positive control), thus indicating that HIV-1 promotes DCs migration through an epithelial monolayer. This process, already evident after 30 min, reached a peak at 4 h 30 min. GFP-labeled HIV-1 or p24 antigen was detected on the apical side of the Caco-2 monolayer and inside the migrated DCs. Transmission electron microscopy confirmed the localization of HIV-1. Thus, our results show that in the *in vitro* model DCs migrate through intestinal epithelial cells (IECs) to explore the luminal surface in the presence of HIV-1, are able to uptake the virus and to infect susceptible target cells. The molecular mechanisms underlying HIV-1 induced DCs migration across IECs, and consequent implication for mother-to-child transmission, will be discussed.

Our results clarify the earliest events in the establishment HIV-1 infection at mucosal level and so are of outmost relevance for the development of an effective preventative vaccine.

O5

Unusual natural killer cell responses to HIV-1 peptides are associated with protection against maternal-infant transmission of HIV-1

Caroline Tiemessen¹, Sharon Shalekoff¹, Stephen Meddows-Taylor¹, Diana Schramm¹, Maria Papathanasopoulos², Glenda Gray³, Gayle Sherman^{4,2}, Ashraf Coovadia^{5,2} and Louise Kuhn⁶

¹National Institute for Communicable Diseases, Johannesburg, South Africa

²University of the Witwatersrand, Johannesburg, South Africa

³Perinatal HIV Research Unit, Soweto, South Africa

⁴National Health Laboratory Services, Johannesburg, South Africa

⁵Coronation Women and Children Hospital, Johannesburg, South Africa

⁶Columbia University, New York, USA

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Natural killer cells play a pivotal role in innate and adaptive immunity. Innate immune defense pathways are classically distinguished by rapidity of response and "pattern" recognition of pathogens. Epitope specificity and memory are normally attributed to the adaptive compartment. In a study of HIV-specific T cell responses and maternal-infant HIV-1 transmission we have encountered robust responses among CD3-negative cells to HIV-1 peptides which we determined to be NK cells. HIV-specific T cell and non-T cell responses among 79 HIV-1 infected women and their 76 infants were evaluated for responses to HIV-1 subtype C peptide pools (Gag, Pol, Nef, Env, and Reg [Tat, Rev, Vif, Vpu, Vpr combined] proteins) using an whole blood intracellular cytokine assay that measures IFN-gamma. Overall, 43% and 22% had CD3 neg responses to

Env and Reg, respectively, and these same regions were targeted in slightly smaller proportions of their infants (15.8% and 5.3%). Minority targeting by CD3 neg cells occurred against Pol (2 HIV-1 infected mothers and one infant) and Nef (one infant). Importantly, no peptide-specific CD3 neg responses could be detected in either the 20 HIV-1 uninfected control mothers or their infants. Amino acid regions targeted by NK cells were identified on gp160, Vpu and Tat by whole genome peptide mapping of 5 HIV-1 infected women with CD3 neg responses using a whole blood ICS assay that utilizes a peptide pool and matrix design.

To establish if HIV peptide-specific CD3 neg responses may be protective in maternal-infant HIV-1 transmission, we compared 49 non-transmitting (non-TM) and 15 transmitting (TM) mothers and 44 exposed uninfected (EU) and 18 HIV-infected infants. Twenty-eight of 49 (57.1%) non-TM mothers and 13/44 (29.5%) EU infants had detectable HIV-specific CD3 neg responses. In comparison, 1/15 (6.7%) and 1/18 (5.6%) of TM mothers and infected infants, respectively had these responses (P = 0.001 and P = 0.049 for mothers and infants respectively). Adjusting for maternal viral load, the association between the presence of HIV-specific CD3 neg responses in the mothers and transmission remained statistically significant (P = 0.01). No other factors including maternal CD4 count, mode of delivery, infant birth weight or antiretroviral prophylactic regimens explained the association.

Therefore the presence of NK cells that respond with remarkable specificity and high magnitude to HIV-1 peptides was significantly associated with lack of maternal-infant HIV-1 transmission, suggesting a protective role of HIV-specific NK responses within this mode of HIV-1 transmission. These findings identify an important new measure of protective immunity to HIV-1 that highlights the importance of innate immunity in preventing the establishment of HIV-1 infection.

O6

CD14+ cells are the main targets for HIV-1 infection in first trimester pregnancy human uterine mucosa

Romain Marlin¹, Marie-Thérèse Nugeyre¹, Claire de Truchis², Nadia Berkane³, Amélie Gervaise², Françoise Barré-Sinoussi¹ and Elisabeth Menu¹

¹Institut Pasteur, Paris, France

²A. Beclere Hospital, Clamart, France

³Tenon Hospital, Paris, France

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During the first trimester of pregnancy, HIV-1 mother-to-child transmission (MTCT) is relatively rare despite the permissivity of placental cells to cell-to-cell HIV-1 infection. The invasive placental cells interact directly with the decidual cells (cells of the maternal uterine mucosa during pregnancy) in the first months of pregnancy; however the role of the decidua in the control of HIV-1 transmission remains unknown. The decidua has a characteristic leukocyte population containing natural killer cells (dNK), antigen-presenting cells (dAPC) and T lymphocytes. The aim of this study was to determine whether decidual cells could be potential targets to HIV-1 and could thus represent a risk for HIV-1 MTCT in utero.

To determine the permissivity of decidual tissue to HIV-1 infection, we adapted and validated a histoculture model for

the decidua basalis and decidua parietalis (which are located at the implantation site and surrounded the uterus respectively). Deciduas were obtained from HIV-1 negative women undergoing elective abortions between 6–10 weeks of pregnancy. Infections were performed *in vitro* with HIV-1 primary isolates. Tissue sections over the course of the culture and a functional test based on TNF- α secretion upon LPS stimulation indicate that decidual histocultures remain viable for approximately 15 days. Decidual tissue is more susceptible to infection by an R5 tropic HIV-1 (BaL) than an X4 tropic HIV-1 (LAI). Moreover, we show that the level of infection is lower in the decidua basalis compared to the decidua parietalis. Infected cells were identified by flow cytometry analysis of isolated decidual mononuclear cells using CD3, CD14 and CD56 specific monoclonal antibodies. Double immunohistochemistry staining was also performed on infected decidual tissue sections to confirm *in situ* the flow cytometry analysis. The results show that subpopulations of dAPC expressing CD14 are the main target of HIV-1 infection in the decidua.

The permissivity of decidual tissue to HIV-1 infection *in vitro* suggest that *in vivo* a first level of control of HIV-1 *in utero* mother-to-child transmission occurs, preceding and in addition to the control previously demonstrated by the placenta. The role of dNK cells in this control is currently under investigation.

O7

Phenotypic characterization of HIV-specific CD8 T cells during acute infant HIV infection

Jennifer Slyker^{1,2}, Tao Dong², Grace John-Stewart¹, Barbara Lohman-Payne^{1,3}, Marie Reilly⁴, Ann Atzberger², Stephen Taylor², Elizabeth Maleche-Obimbo³, Dorothy Mbori-Ngacha³ and Sarah Rowland-Jones²

¹University of Washington, Seattle, WA, USA

²Oxford University, Oxford, UK

³University of Nairobi, Nairobi, Kenya

⁴Karolinska Institutet, Stockholm, Sweden

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Objective: Infants infected with HIV-1 fail to contain viral replication as efficiently as adults. In the absence of antiretroviral therapy (ART), opportunistic infections are common and mortality rates range between 10–45% in HIV-infected infants. To understand better the factors underlying rapid infant HIV-1 progression, we examined HIV-specific CD8 T cells during the acute and chronic phases of infection.

Methods: HIV-infected pregnant Kenyan women were recruited from 1999–2003. Other than antenatal prophylaxis, neither women nor infants received ART. Serial blood specimens were obtained at delivery and months 1, 3, and quarterly thereafter until death or two years. IFN- γ -producing CD8 T cells were quantified with ELISpot assays using HLA-matched HIV-1 peptides as antigens. In a subset of 7 infants, HIV-specific CD8 T cells were quantified using class I HLA tetramers. Cellular phenotype was described using multicolour flow cytometry; PBMC were stained with tetramers and antibodies to cellular proteins.

Results: ELISpot assays were performed in 67 infants who acquired HIV-1 before 1 month of age. HIV-specific IFN- γ release was detected 39% of infants at 1 month of age, and 58% at 3 months. The magnitude of responses to individual peptides was low, but within the range observed in adults (median 230

HIVSFC/million PBMC, range 50–2040 HIVSFC/million PBMC). High frequencies of HIV-specific CD8 T cells were detected during acute infection using tetramers (median 0.67%, range 0.045–3.8%). Over time, the frequency of cells identified by tetramer staining declined and the frequency of cells producing IFN- γ increased. Neither IFN- γ production nor frequencies of tetramer-stained cells correlated with HIV-1 viral load. During acute HIV-1 infection, the phenotype of infant HIV-specific CD8 T cells was similar to that observed in adults; HIV-specific CD8 were activated, CD27+CD28-, CD45RA-, CD95+ and contained low levels of perforin. Similar to adults, during chronic infection infant HIV-specific cells transitioned to a resting phenotype and increased expression of CD57, suggesting the accumulation of senescent cells. In contrast to adults, the majority of infant HIV-specific CD8 cells expressed CD95 during chronic infection, suggesting ongoing susceptibility to apoptosis. Also unlike adults, perforin declined to very low or undetectable levels HIV-specific CD8 cells, suggesting low cytotoxic potential.

Conclusion: The relatively poor control over HIV-1 viral replication during infancy may be explained by differences in T cell functionality between infants and adults, which may include higher susceptibility to Fas-mediated apoptosis and low cytotoxic potential.

O8

Human Immunodeficiency Virus-specific B cells in human breast milk

Edouard Tuaillon¹, Diane Valea², Yassine Al Tabaa¹, Pierre Becquart¹, Nicolas Meda², Karine Bollore¹, Jean-Pierre Vendrell¹ and Philippe Van de Perre¹

¹Université Montpellier 1 and CHU Montpellier, Montpellier, France

²Centre Muraz, Bobo-Dioulasso, Burkina Faso

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Background: Breast milk is a component of the mucosal immune system, and contains specific antibodies and lymphocytes that may modulate the infectivity of milk, and therefore the risk of HIV-1 transmission via breastfeeding. While secretory antibodies (Ab) have been extensively explored in human breast milk, the existence, features, and function of B lymphocytes remain to be described in this compartment.

Methods: We analysed breast milk and blood lymphocytes from 12 HIV-1-infected lactating women. All women were treated by anti-retroviral therapy or have been recently exposed to anti-retroviral drugs for prophylaxis of mother-to-child transmission. Milk samples were collected 5 to 42 days post partum. Phenotype of breast milk cells were analyzed by flow cytometry and cells function by ELISpot assays.

Results: In contrast to their blood counterpart, naive B cells remained largely underrepresented in breast milk. Breast milk B cells mostly consisted of IgD⁻ memory B cells. They displayed a phenotype of class-switched memory B cell, with few IgD⁺ memory and naive B cells. As compared with blood, higher percentages of activated B cells (CD38⁺), large size B cells, plasmablasts and plasma cells (CD19⁺, CD20^{low/-}, CD27^{high}, CD138⁺) were found. This indicates that a significant proportion of breast milk B cells underwent terminal plasma cell differentiation. We also observed a higher frequency of cells secreting spontaneously Ig in breast milk. Among these cells, IgG-secreting cells (SCs) predominated over IgA-SCs as measured by

Ig-ELISpot assays. Specific Ab-SCs were investigated following polyclonal activation using the CD40L ligation. The detection of anti-HIV-1-SCs demonstrated the existence of B cells specific to HIV-1 Ag in breast milk from HIV-1-infected women. Finally, we observed that breast milk B lymphocytes bore a unique profile of adhesion molecules (CD44⁺, CD62L⁻, $\alpha4/\beta7^{+/-}$, $\alpha4/\beta1^{+}$) suggesting that these cells may originate from the gut-associated lymphoid tissue (GALT).

Conclusion: Breast milk from lactating women infected by HIV-1 contains activated B cells including cells specific to HIV-1 antigens. These cells display a phenotype strikingly different from blood, with a mucosal homing profile related to B cells located in the GALT.

O9

Transmission of Hepatitis B virus (HBV) minor variants in children born to HBV/HIV co-infected mothers

Wootichai Khamduang^{1,2}, Catherine Gaudy-Graffin², Alain Moreau², Nicole Ngo-Giang-Huong³, Gonzague Jourdain³, Marc Lallemand³, Surachai Pipatnakulchai⁴, Chaiwat Putiyanun⁴, Sura Kunkongkapan⁴, Pornpun Wannarit⁴, Surat Sirinontakan⁴, Wanna Ardong⁴, Wasna Sirirungsi¹ and Alain Goudeau²

¹Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand

²INSERM U966, CHU Tours, Faculté de Médecine, Université François-Rabelais, Tours, France

³Institut de Recherche pour le Développement UMI 174/Programs for HIV Prevention and Treatment (PHPT), Chiang Mai, Thailand

⁴Ministry of Public Health, Nonthaburi, Thailand

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Background: Since 1992, the Ministry of Public Health has integrated newborns HBV vaccination into the national expanded program on immunization. However, some children acquire HBV infection despite immunization.

Objective: To characterize HBV vaccine escape mutants in infants born to HBV/HIV-1 co-infected mothers.

Methods: Of 1433 HIV-infected women participating in the perinatal HIV prevention trial (PHPT-1), 107 were HBsAg positive. Five transmitted HBV to their children despite HBV vaccination in their children were documented. Blood samples collected from mothers during pregnancy and children at 4 and 6 months of age were analyzed by direct PCR sequencing of the S gene ("a" determinant region and flanking regions). HBV variants were sequenced after cloning of PCR products into pGEM-T[®] easy vector (20–25 clones by samples). Sequencing was performed using the BigDye Terminator V3.1 sequencing kit, Applied Biosystem. Sequence alignments were performed using Bioedit software. HBV serotype was inferred from results at codons 122, 127, 160, 177 and 178 of the S gene.

Results: Complete samples series were available for 3 mother-child pairs, all infected by HBV genotype C. Infant virus direct sequencing showed no known vaccine escape mutation. However, direct sequencing identified the sK122R mutation in 2 infants but not in their mother. The predicted dominant HBV serotype in the 2 mothers was *adrq+*, while it was *ayr* in the 2 children at 4 months of age. Although sK122R was not

detected by direct sequencing, further analysis of maternal clones showed that the 2 mothers harbored this minor variant at very low frequency (1 of 65 clones and 2 of 67 clones, respectively). Analysis of children HBV clones showed an increase of *ayr* variants from 4 months to 6 months.

Conclusion: Although the impact of the sK122R mutation on HBV vaccine escape is unknown, this study suggests that HBV minor maternal variants defining serotype can be transmitted to children who received HBV vaccine. This observation justifies the systematic virological evaluation of children infected despite active immunization and their mother.

O10

Very early diagnosis of HIV infection in newborn at day 0–day 3 on DBS in Cambodia

Sopheak Ngim¹, Sim Kruey Leang^{1,2}, Chhunly Kong^{1,3}, Sethikar Im^{1,2}, Vannith Lim^{1,2}, Meng Ly Ek^{1,2}, Denisa Augustinova^{1,4}, Kanal Koum^{1,5}, Christine Rouzioux^{1,6} and Eric Nerrienet¹

¹Institut Pasteur, Phnom Penh, Cambodia

²Calmette Hospital, Phnom Penh, Cambodia

³Chey Chumneas Hospital, Takamao, Kandal province, Cambodia

⁴Magna Children at Risk, Phnom Penh, Cambodia

⁵NMCHC Hospital, Phnom Penh, Cambodia

⁶EA 3620, Université Paris-Descartes, CHU Necker-Enfants Malades, Paris, France

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Background: In resource constrained settings where prevention of mother to child transmission of HIV-1 (PMTCT) programs are in place, the proportion of residual *in utero* transmission (rIUT) versus *peri partum* transmission (PPT) is unknown and the morbidity/mortality related, or not, to MTC transmission of HIV-1, within the first 6 weeks of life, poorly documented.

Main objective: To assess the feasibility and the contribution of the very early diagnosis on Dried Blood Spot (DBS), conducted at day 0–day 3 of age in improving the medical care of HIV-1 exposed newborn within the first 6 weeks of life.

Methods: Very early diagnosis was explained and proposed, before and/or after delivery, to HIV positive mothers delivering at Calmette and hospitals and health centers supported by Magna Children at Risk (NMCHC, Chey Chumneas Hospital and 3 Municipality Health Centers). A first negative HIV-DNA negative DBS at day 0–day 3 was followed up by a second DBS at W6. HIV-DNA positive DBS at d0–d3, or at week 6 were followed up by a venipuncture as soon as possible for HIV-RNA quantification (Kit G2 ANRS) and CD4 count.

Preliminary results: Heel prick blood specimens were spotted on DBS for 272 newborns (ratio M/F = 1.3) at d0–d3. HIV DNA was detected in 3 of 272 babies (rIUT rate: 1.1%). One of them died before week 6. The two others presenting detectable HIV-1 RNA viral loads at week 6 (6.4 and 6.9 log₁₀ copies/ml with CD4 at 19% and 21%, respectively) started first line ARV regimen and became HIV-1 RNA undetectable after 10 and 4 months of treatment. Among the 269 HIV-DNA negative newborn at d0–d3, 228 (84.5%) have been already seen at week 6 for virological confirmation, 23 are still waiting for the visit of the week 6, 14 were lost of follow up and 4 died without any AIDS clinical symptoms. 226 of 228 DBS were confirmed HIV-DNA negative at week 6 whereas 2 infants became

HIV-DNA positive (PPT rate: 0.8%). Both were confirmed HIV-RNA positive two weeks later (5.8 and 6.2 log₁₀ copies/ml with CD4 at 33% and 26%, respectively) and will soon begin their ARV treatment.

Discussion: The rIU and PP transmission rates were low in this study (1.1% and 0.8% respectively). 5 new born were diagnosed HIV infected. One died before W6, and 2 already started ARV treatment. Further investigations are undergoing to understand why 14 newborn were lost of follow up. These preliminary results demonstrate the feasibility of a minimally invasive very early diagnosis, done shortly after birth. The small amount of blood required, the ease of collection, storage, and transport of samples, and the low cost of the test make it ideal for HIV-I testing of infants in remote maternities in Cambodia.

O11

High frequency of belated HIV diagnoses in pediatric population in Buenos Aires, Argentina

Moira Vignoles¹, Graciela Barboni², María Rosa Agosti³, Mariel García³, Silvia González Ayala³ and Horacio Salomón¹

¹National Reference Center for AIDS, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina

²Clinical Immunology Division, "Dr. Pedro de Elizalde" General Children's Hospital, Buenos Aires, Argentina

³Sup. Sor María Ludovica Children's Hospital, La Plata, Argentina

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Background: The ready availability of HIV prevention, testing and treatment services has lowered HIV mother-to-child transmission (MTCT) rates to less than 2% in high-income countries. Argentina has laws that guarantee universal and free antiretroviral therapy (ART) to every HIV-infected person and makes the offering of an HIV test to every pregnant woman mandatory. In spite of this, HIV MTCT is still present. The aim of this report is to call the attention of general paediatricians of belated HIV diagnoses in children.

Materials and methods: From December 2004 to May 2008, HIV diagnoses in children were evaluated in 2 pediatric hospitals (referral for infectious diseases) of Buenos Aires. Those children who initiated HAART were followed-up during treatment, analyzing immunological, virological and clinical parameters to evaluate disease progression.

Results: During this period 101 children were HIV-diagnosed. Of them, 31 were diagnosed at age less than 12 months (median: 4 months, range: 0.5–11 months, IQR: 3–7 months), while 70 aged more than 1 year old (median: 6.4 years, range: 1–14 years, IQR: 3–10 years). Most of them had been previously assisted at primary health care centers. Around 60% of them presented moderate or severe immunological suppression and stage B or C clinical signs at the time of diagnosis. Our follow-up results of 40 of these children (median follow-up time: 24.3 months, IQR: 17.7–34.7 months) indicate that delayed diagnosis of HIV-I infection was associated with virological failure, lower CD4 percentages which did not increase during the study period despite receipt of HAART, and more advanced clinical disease which did not improve in 50% of the subjects here studied.

Conclusion: It is imperative to highlight that a large proportion of these children (70%) had a belated diagnosis and became the "index case" for their families. The possibility of HIV-I infection

was frequently overlooked at primary health care centers, and the diagnosis of HIV-I infection was made at referral centers only later (once the child was referred with advanced HIV-I clinical symptomatology and severe immunosuppression). This has been happening since the beginning of the epidemic in Argentina and apparently has not improved as much as necessary. Thus, training to increase awareness of the possibility of HIV-I infection in children should be emphasized among general pediatricians at all primary health care sites. Even though it should be considered that the follow-up time might have not been enough to see immunological improvements, these results highlight the importance of early diagnosis and early initiation of ART.

Therefore, in a country such as Argentina, where adequate prevention resources and clinical care have been available for more than a decade, it is urgent to achieve early HIV diagnosis by generating the awareness of the possibility of HIV-I infection in children among the pediatricians community.

O12

New approach for congenital CMV infection diagnosis in neonates: sensibility and specificity of CMV detection in dried blood spots

Marianne Leruez-Ville^{1,6}, Christelle Vauloup-Fellous², Sophie Couderc⁴, Sophie Parat³, Salima Oucherif³ and Jean-François Magny⁵

¹Hôpital Necker-Enfants-malades, AP-HP, National Reference Center for Cytomegalovirus, Paris, France

²Hôpital Antoine Bèclère, AP-HP, Virologie, Clamart, France

³Hôpital Necker-Enfants malades, Maternité, Paris, France

⁴Hôpital de Poissy, Maternité, Poissy, France

⁵Institut de Puériculture, Néonatalogie, Paris, France

⁶Université Paris-Descartes EA 36-20, Paris, France

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Background: Detection of CMV DNA in DBS (Guthrie cards) has been proposed for neonatal diagnosis of CMV congenital infection.

Objectives: To evaluate the in vitro sensitivity of 2 methods of CMV DNA detection in DBS. To evaluate the specificity and the sensitivity of these 2 methods for congenital CMV diagnosis in comparison to the gold standard method (CMV detection in urine).

Methods: To study in vitro sensitivity, "test cards" were prepared with dilutions of pre-quantified whole blood samples. To study in vivo specificity and sensitivity, 215 neonates who had CMV congenital infection diagnosis done by PCR or culture in a urine sample collected in the first week of life were included prospectively. Forty-five of these neonates had positive CMV detection in their urine (by PCR (Necker, Poissy, Bèclère) or by rapid culture (IPP)). CMV DNA was detected in the Guthrie cards by 2 methods. Method 1 consisted of DNA extraction in a whole DBS with NaOH 0.32% lysis followed by QIAamp DNA Blood Mini Kit and amplification by an in house real time PCR in duplicate. Method 2 was a phenol/chloroform extraction of a whole DBS followed by amplification with the CMV PCR kit (Abbott, France).

Results: The 95% sensitivity of the 2 methods was 4000 and 2000 copies/ml respectively. In neonates, sensitivity and specificity of method 1 were 100% (45/45) and 96.9% (160/165) when at least one duplicate was positive and 88.8% (40/45) and 100% (165/165) when the two duplicates were positive. Sensitivity and

specificity of method 2 were 95.1% (39/41) and 97.5% (158/162) respectively. Results were discordant (negative detection in urine and positive PCR in DBS) in 8 cards from 8 different neonates (4 with method 1 and 4 with method 2), these false positive were not repeatable when retested. Mean viral load of the 8 false positive were 376 [280–500] and 31 [9–53] copies/ml with method 1 and method 2 respectively. In one case, the CMV PCR in DBS was repeatedly positive with the 2 methods, whereas it was negative in the urine at birth by rapid culture. This case was considered as a false negative of the rapid culture and was therefore excluded from the analysis.

Conclusion: Sensitivity of CMV DNA detection in DBS was very high when PCR was done in duplicate. However, when only one duplicate was positive it could be a false positive result. Low positive results needed to be confirmed by a second testing. In these best conditions, we think that these 2 methods are sensitive and specific enough for neonatal diagnosis of CMV congenital infection and for retrospective diagnosis in children presenting with hearing loss.

O13

Use of chloroquine in reducing mother to child transmission of HIV-1 during breastfeeding

Marloes Naarding¹, Stanley Luchters², Joseph Vyankandondera³, Ferdinand Wit², Nienke Veldhuijzen², Brigitte Kankindi³, Rolf Sparidans⁴, Jos Beijnen⁴, Georgios Pollakis¹, Johan Boelaert⁵, Joep Lange² and William Paxton¹

¹Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands

²International Antiviral Therapy Evaluation Center, Amsterdam, Netherlands

³Centre Hospitalier Universitaire de Kigali, Kigali, Rwanda

⁴University of Utrecht, Utrecht, Netherlands

⁵Algemeen Ziekenhuis St-Jan, Brugge, Belgium

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Chloroquine (CQ) has been shown to inhibit HIV-1 replication *in vitro* as well as *in vivo*, thereby indicating its use as an effective drug in limiting mother to child transmission of HIV-1 during breastfeeding. We show that HIV-1 replication on CD4⁺ T-lymphocytes can be reduced in the presence of CQ and show that the reduced replication is producer cell specific and that viruses generated in the presence of CQ are not inhibited for their subsequent infectivity and replication. By analysing the gp120 envelope protein sequences from virus passaged over a long period of time in the presence or absence of CQ we demonstrate variant evolution patterns of the envelope. We also demonstrate that HIV-1 produced in the presence of CQ have a reduced capacity for the transfer by Raji-DC-SIGN cells to CD4⁺ T-lymphocytes indicating another means whereby virus replication may be reduced *in vivo*. We conducted a Phase I/II, randomized, placebo-controlled study to evaluate chloroquine (CQ) administration to reduce HIV-1 RNA levels in human milk. Thirty HIV-1 positive pregnant Rwandese women (CQ n = 20; placebo n = 10) were enrolled in a 16 week study, with the treatment group receiving a 200 mg oral dose of CQ daily. Baseline plasma viral load (pVL) measurements and CD4 counts were determined prior to delivery with pVL, breast milk VL (bmVL) and CQ levels measured at wk0, wk8 and wk16. For selected mothers the envelope C1C4 gp120 region was DNA sequenced

and analyzed. A higher concentration of CQ in breast milk compared to plasma (over 2.5 fold) was observed after 8 and 16 weeks of treatment. CQ levels in plasma correlated to those in the left and right breast milk (P = 0.002 and P = 0.003, respectively). Additionally, a link between high CQ concentrations in plasma and high CD4 counts (P < 0.001) was observed. No alterations in bmVL were observed with CQ treatment whilst pVL increased significantly (P = 0.001). In over half of the CQ treated individuals there was a greater than 5 fold increase in pVL which was observed by week 8 of treatment. No specific alterations in the gp120 envelope sequences could be associated with CQ treatment. Our results indicate that CQ administration is associated with increased pVL in early breastfeeding mothers from Rwanda which cautions against the use of CQ in such individuals.

O14

High emergence of drug resistance after HAART interruption at delivery in a cohort of HIV+ pregnant women submitted to antiretroviral treatment to prevent mother-to-child transmission in Rio de Janeiro, Brazil

Jose Henrique Pilotto^{1,2}, Beatriz Grinsztejn¹, Valdilea Veloso¹, Jose Carlos Couto-Fernandez¹, Adriana Rodrigues-Pedro¹, Carlos Augusto Velasco-de-Castro¹, Jorge Eurico Ribeiro¹, Ruth Khalili¹, Sandra Muri¹, Ronaldo Ismerio¹, Judith Courrier³ and Mariza G Morgado¹

¹Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

²Hospital Geral de Nova Iguaçu, Nova Iguaçu, RJ, Brazil

³Univ. of California, Los Angeles, CA, USA

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Background: Brazilian guidelines stipulate that HIV+ pregnant women initiating HAART for PMTCT, who do not otherwise need ARV for their own health, discontinue antiretrovirals after delivery. However, it is unknown how frequent drug resistance is with this strategy.

Objectives: to evaluate HIV-1 primary resistance at baseline visit, the impact of antiretroviral discontinuation, following delivery for the emergence of genotypic resistance, HIV-1 subtype, HIV-1 recent Infection using the Calypte BED Incidence EIA, and the rates of HIV-1 vertical transmission in this population of HIV-1 pregnant women.

Methods: Since January 2005, an HIV+ pregnant women cohort has been established at Hospital Geral de Nova Iguaçu. HAART for PMTCT was used according to the Brazilian Guidelines. Clinical/lab evaluations (CD4, HIV-RNA and genotyping were performed at baseline, 6–8 weeks after HAART, delivery and postpartum [15 days, 1 month and 6 months]).

Results: 139 women/babies have been enrolled and followed. Median age is 25.3 years; 69.8% women are non-white, median gestational age at prenatal care initiation is 24 weeks. Median CD4 cell count at baseline is 518 cells/mm³ and HIV-1 VL 7.800 copies/mL. A NNRTI and PI based regimen was prescribed for 22.3% and 77.7% of the women, respectively. The median time on ART was 84 days; 76% had HIV-1 RNA <400 copies/ml at delivery. The prevalence of HIV primary drug resistant was 11.3% at baseline visit, and 13.8% of the women developed new mutations after ART interruption at delivery. HIV-1 recent infection was detected in 13.9% pregnant women at baseline visit. In the

multivariate analysis, HIV-1 primary resistance was independently associated with the development of new resistance mutations at delivery/post-partum. Pregnant women with detectable HIV-1 RNA at delivery had a RR of detection of mutations at delivery/post-partum 3.5 times the one for women with undetectable HIV-1 RNA at delivery. The length of time of HAART use was independently associated with the incidence of resistance after ART initiation for PMTCT.

Conclusion: High Prevalence of HIV-1 primary resistance at baseline visit; 13.8% of the women developed new mutations after ART interruption after delivery. HIV-1 recent infection was detected in 13.9% pregnant women at baseline visit. HIV subtype B was the most prevalent. There was no HIV-1 vertical transmission.

O15

Risk factors for HCV infection in HIV positive pregnant women and rate of HCV perinatal transmission in Thailand

Nicole Ngo-Giang-Huong¹, Luc Decker¹, Wasna Sirirungsit², Sophie Le Coeur³, Gonzague Jourdain¹, Woottichai Khamduang^{2,1}, Suparat Kanjanavanit⁴, Wanmanee Matanasaravoot⁵, Chaiwat Putiyanun⁶, Francis Barin⁷ and Marc Lallemand¹

¹IRD 174/PHPT, Chiang Mai, Thailand

²Chiang Mai University, Faculty of Associated Medical Sciences, Chiang Mai, Thailand

³UMR 196 CEPED (INED-IRD-Université Paris Descartes), Paris, France

⁴Nakornping Hospital, Chiang Mai, Thailand

⁵Lamphun Hospital, Lamphun, Thailand

⁶Chiang Kham Hospital, Chiang Kham, Thailand

⁷Laboratoire de Virologie, INSERM ERI 19, and Centre National de Référence pour le VIH, Université F Rabelais, Tours, France

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Objectives: To assess the prevalence of HCV in HIV-1 infected and uninfected pregnant women; to determine factors associated with maternal HCV infection in HIV-infected pregnant women; and to evaluate the rate of HCV transmission in infants born to HIV-HCV co-infected mothers in Thailand.

Population: 1435 HIV-1 infected pregnant women were enrolled from 1997 to 1999 in the Perinatal HIV Prevention Trial (PHPT-1), multicenter study performed in 27 hospitals throughout Thailand. The control group was composed of 448 HIV uninfected pregnant women randomly selected with a 1:3 ratio in the same hospital sites.

Methods: Maternal HCV serology was assessed at 26 weeks of gestational age or later, using an EIA (Murex anti-HCV kit v. 4.0) and confirmed with a recombinant immunoblot (Bioblot HCV). HCV RNA load was quantitated using the Cobas Amplicor HCV Monitor Test, v. 2.0 (Roche Molecular Diagnostics). Infants were considered HCV infected if positive for HCV serology at 18 months of age or positive for HCV RNA before the age of 6 months. We tested the association of socio-demographic and medical characteristics with maternal HCV infection in HIV-infected mothers.

Results: Of the 1435 HIV infected pregnant women, 42 (2.9%, 95% CI 2.1–3.8%) were HCV infected versus 2 (0.5%, 95% CI 0–1.1%) of the 448 HIV uninfected pregnant women (P = 0.001).

Among HIV infected women, 3 (33%) of those with a history of IV drug use were HCV infected, versus 38 (2.8%) among the 1338 others (p = 0.002). HCV co-infected and not co-infected women did not differ for their CD4 count or HIV viral load. Of the 42 HIV-HCV co-infected women, 30 (71%) had circulating HCV RNA (range 3.61–6.12 log₁₀ IU/mL). The rate of HCV perinatal transmission was 10% (4/40) but there was no HIV-HCV co-transmission. All HCV transmitting mothers had an HCV viral load greater than 5.5 log₁₀ IU/ml.

Conclusion: The prevalence of HCV infection was six times higher in HIV-1 infected women compared to HIV-1 uninfected women. IV drug use was the main risk factor for HCV infection. The low number of HCV infected children did not allow for the investigation of the risk factors for HCV perinatal transmission.

O16

Barriers to HIV testing among mothers at a maternity ward in Phnom Penh, Cambodia

Kanal Koum¹, Sasaki Yuri^{1,2}, Kakimoto Kazuhiro^{1,3}, Sathiarany Vong¹, Shirayama Yoshihisa^{1,2}, Ali Moazzam^{1,2}, Kuroiwa Chushi^{1,2} and Shibuya Kenji^{1,2}

¹National Maternal and Child Health Center, Phnom Penh, Cambodia

²Dpt of Health Policy and Planning, University of Tokyo, Tokyo, Japan

³International Medical Center of Japan, Tokyo, Japan

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Introduction: As a response to the shift in epidemic transmission, prevention of mother to child transmission (PMTCT) services have been expanded in Cambodia. However, the improvement of the acceptance for HIV testing and counselling is still challenging even after the ministry of health in Cambodia adopted provider-initiated HIV testing and counselling (PITC) policy. The objective of this study was to investigate the accessibility of HIV testing and its barriers among mothers at maternity ward in Phnom Penh where the PITC approach had been widely offered.

Methods: This study was a quantitative and cross-sectional assessment of mothers after delivery at National Maternal and Child Health Centre (NMCHC) in Phnom Penh. Randomly selected 599 mothers from October through December 2007 were asked to participate in a half hour's face-to-face interview by trained interviewers.

Results: Of the 599 mothers, 455 (76%) had an experience of HIV testing. Most of the mothers got information about HIV testing from health care provider (360: 79.1%) and mass media (86: 18.9%). After adjusting by the multivariate logistic regression, need of partner's permission for HIV testing (OR = 0.27, 95% CI = 0.14–0.51, p < 0.01), low knowledge on HIV prevention and treatment (OR = 0.38, CI = 0.22–0.66, p < 0.01), ANC place out of Phnom Penh (OR = 0.35, 95% CI = 0.21–0.58, p < 0.01) were found to be barriers to HIV testing.

Discussion: Three factors were found to be barriers to HIV testing: partner's permission, low knowledge on HIV prevention and treatment, and place of ANC. It is necessary to provide education and quality counselling not only for mothers but also for their partners even in PITC strategy. In addition, access of pregnant women to HIV testing and counselling services in rural areas should be improved through strategic approaches such as efficient utilization of human resources.

O17**PMTCT activities implementation: case of Côte d'Ivoire, from ACONDA's experience**

Hervé Prao Aka Kouamé¹, Nicole Dakoury Dogbo¹, Siaka Touré¹ and Valentin Noba²

¹NGO ACONDA VS, Abidjan, Cote D'Ivoire

²Center for Disease Control and Prevention, Abidjan, Cote D'Ivoire

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Background: With a HIV prevalence of 8.6% among pregnant women and 661,000 births per year, Cote d'Ivoire has an estimated 55,000 HIV-infected women delivering per year who need PMTCT services. The national HIV program's strategic plan calls for integration of PMTCT services into antenatal clinics, maternity and Family Planning units. ACONDA's extension of decentralized prevention and care for pregnant women and PLWHA is based on a district approach that integrates basic health care, PMTCT, and ART services in maternal and child health centers. In 2008, ACONDA implemented this approach in 65 ANC clinics in the 26 health districts of Côte d'Ivoire.

Methods: Health workers were trained. After, the program strategy consisted in coaching the care providers at the sites in VCT techniques with rapid HIV testing for women with unknown HIV status in the labor-and-delivery rooms.

Drawing up and spreading simple technical procedures helped the care providers in the implementation of PMTCT.

ARV drugs are packed up at the sites to get PMTCT kits ready to be distributed.

The combined prophylaxis was offered to HIV-infected mothers and their newborns, as recommended by national program, and then pregnant women who were tested HIV positive got initial biological exams. Those who were eligible received a readjusted treatment. Those who were ineligible continued the current disease prevention. A psychosocial supports for treatment adherence, was provided by counselors. Nutritional advice were provided to the mother and the follow-up of the exposed child was systematic. A support group helps to identify and resolve problems of disclosure to partner, lost to follow up, etc. A child's early HIV diagnosis by PCR is made after 6 weeks of postnatal follow up.

A reference and counter-reference system links all HIV-infected women to the medical doctors in the reference health centers.

Results: From January through Aout 2008, PMTCT services were integrated into 60 ANC clinics covering seven districts, with 80 trained health workers. Of 26,488 pregnant women using antenatal services, 21,588 (81.5%) received HIV counseling and testing; 1,977 (9.1%) were HIV-positive; and 1,779 infected pregnant women (90%) received their test results. 80% of HIV-infected women received the mother and child combined prophylaxis against 68 % in 2007. Among the HIV-infected women, 282 were eligible for ART according to the WHO criteria.

Conclusion: HIV counseling and testing is possible in labor and delivery rooms, with a high acceptance rate in Côte d'Ivoire and providing the combined prophylaxis from the disclosure of test results is essential if we noticeably want to reduce the Mother to child HIV Transmission for the scaling up.

O18**Abstract withdrawn**

Retrovirology 2009, 6(Suppl 1):O18

O19**Effectiveness of a district-wide programme for the prevention of mother-to-child transmission of HIV in Cambodia. Experience from six maternity units in Phnom Penh and Kandal**

Denisa Augustinova¹, Andrea Stranska¹, Kanal Koum², Sokha Ean¹, Satiarany Vong², Chhunly Kong³,

Sopheak Ngin⁴, Eric Nerrienet⁴ and Martin Bandzak¹

¹Magna Children at Risk, Bratislava, Slovakia

²The National Maternal and Child Health Center,

Phnom Penh, Cambodia

³Chey Chumneas Referral Hospital, Takmao, Cambodia

⁴HIV/Hepatitis Laboratory, Institute Pasteur,

Phnom Penh, Cambodia

Retrovirology 2009, 6(Suppl 1):O19

Background: Despite the great advances in developing and implementing effective interventions to prevent HIV transmission from infected mothers to their infants, almost 2,000 infants are infected every day through MTCT in resource-poor countries. In these settings, where prevention of mother to child transmission of HIV-1 (PMTCT) programs are in place, the uptake and effectiveness of the PMTCT comprehensive program need to be regularly monitored.

Main objective: To estimate the field efficacy of a district-wide PMTCT programme, in Phnom Penh and Kandal province.

Methods: Six maternity units were included: five in Phnom Penh (NMCHC, Daun Penh HC, Tuol Kork HC, Pochentong HC) and one in CCH Takhmao, Kandal Province. Individual and group counseling were conducted. Prenatal HIV testing were performed using rapid tests. HAART, or preventive ARV drugs, were administrated to the mothers and children according to the national recommendations. Diagnosis of HIV infection in babies (heel prick blood spotted on DBS and/or EDTA blood specimen) was done at the Pasteur Institute of Cambodia by mean of HIV-1 DNA or RNA real time PCR (ANRS kits).

Results: From March 2006 to December 2008, 38,398 pregnant women visited the ANC (total number of ANC attendees = 117,739). 33,811 (88%) accepted pre-test counseling and 33,436 (98%) the HIV testing. 28,236 received post-test counseling (84%). Finally 320 women joint the PMTCT programme and 215 delivered. 201 (93,48%) received an effective PMTCT intervention. Eighty-eight received HAART (3TC+d4T+NVP) during pregnancy. Zidovudine (AZT) was provided antenatally from week 28 of gestation and during labour with single dose of Nevirapine (sdNVP) to 104 women, including 7 enrolled in the TEmAA ANRS clinical trial who received AZT and both sdNVP and Truvada[®] at the onset of labour. Six women received AZT only, and 2 a single dose of NVP only. Finally, 14 did not receive any antiretroviral interventions. 177 infants received a sdNVP and AZT twice a day for 7 days, 33 received a sdNVP and AZT for 1 month, 1 received sdNVP and AZT on delivery only, 2 only sdNVP and 1 no treatment. Infant formula milk was provided to 214 mothers choosing the formula feeding option and 1 was exclusively breastfed. Nine out of

215 infants (4.2%) were early diagnosed and confirmed HIV-1 infected before the age of 2 months. Two of them were born from mothers receiving no therapy, 2 from mothers receiving sdNVP only. One was on HAART and 4 received AZT.

Conclusion: The 4.2 % rate of MTCT of HIV-1 we report here confirms the feasibility and effectiveness of a large-scale PMTCT programme in an urban and sub urban settings.

O20

Is marital status and information of the father associated with access to prevention of mother-to-child HIV transmission?

Carine Jasseron^{1,3}, Laurent Mandelbrot^{13,12}, Stéphane Blanche^{7,8}, Roland Tubiana^{5,6}, Jérôme Le chenadec^{1,4}, Jean-Paul Teglas^{1,4}, Catherine Dolfus⁹, Albert Faye¹⁰, Christine Rouzioux^{7,11} and Josiane Warszawski^{1,2}

¹Inserm U822, Le kremlin-Bicêtre, France

²Univ Paris sud, Faculté de médecine Paris Sud, Le kremlin-Bicêtre, France

³AP-HP, Hôpital Bicêtre, Epidemiologie and public health service, Le kremlin-Bicêtre, France

⁴INED, Paris, France

⁵AP-HP, Hôpital Salpêtrière, Department of infectious diseases, Paris, France

⁶INSERM, U543, Paris, France

⁷EA 3620, Univ Paris Descartes 5, Paris, France

⁸AP-HP, Hôpital Necker, Unité d'immunologie Hématologie pédiatrique, Paris, France

⁹AP-HP, Hôpital Trousseau, Service d'Hématologie et d'oncologie pédiatrique, Paris, France

¹⁰AP-HP, Hôpital Robert Debré, Service de pédiatrique générale, Paris, France

¹¹AP-HP, Hôpital Necker, Virology Department, Paris, France

¹²Univ Paris 7, Paris, France

¹³AP-HP, Hôpital Louis Mourrier, Gynecology and obstetrics department, Colombes, France

Retrovirology 2009, 6(Suppl 1):O20

Background: A previous survey conducted between 1997 and 2004 in the COI-ANRS French Perinatal Cohort (EPF) showed that geographical origin of the mothers was associated with late diagnosis of HIV during pregnancy. We did not take into account marital status and socio-economical characteristics of both mothers and fathers in that survey. Such data are available since 2005 in EPF.

Objective: We aimed to describe socio-economical and marital status of HIV-infected pregnant women, and information of the father about his and her virological status. We also studied whether such factors were associated with late diagnosis (at third gestational trimester), late treatment (initiation of antiretroviral therapy after 31 gestational weeks) and/or detectable viral load (≥ 50 cp/mL) at delivery.

Methods: All HIV-infected pregnant women, enrolled in 2005 and 2006 in EPF were eligible for this analysis.

Results: Among the 1423 mothers included, 75% originated from sub-Saharan African countries, 36% were unemployed, 37% lived alone. In 5% of cases, the father was unknown. Among the others, 64% came from sub-Saharan African countries and 15% were unemployed. In 18% of cases, physicians did not have information about the virological status of the fathers. For the

others, they reported that 15% were not tested, 28% were infected by HIV and 57% were non infected. For one fifth of the pregnant women, the father was not informed about the HIV-infection of the mother. Geographical origin remained associated with diagnosis during pregnancy however the difference was smaller than previously. Compared with women living in couple, women living alone had more often late diagnosis during pregnancy (3.7% vs 1.6%; $p = 0.03$), late treatment (8.9 vs 5.8; $p = 0.04$) and detectable viral load at delivery (37.2% vs 32.4%; $p = 0.07$). Late diagnosis, late treatment and detectable viral load at delivery were also more frequent when the father was not tested for HIV (compared with both infected and non infected fathers), and when the father was not informed of the HIV-infection of the woman.

Conclusion: These preliminary results suggest that marital situation of the pregnant women and knowledge of the fathers concerning his and her HIV status are associated with optimal strategies for prevention of mother-to-child transmission of HIV.

O21

Why do HIV negative mothers refuse to participate in a clinical research involving HIV positive mothers in Cameroon?

Mathurin Cyrille Tejiokem¹, Georgette Guemkam², Chantal Same Ekobo³, Ida Penda⁴, Annie Nga³, Patricia Mbida⁵, Marie-Louise Belinga³, Cassandre Tocko⁴, Francis Ateba Ndongo², Jean Audrey Ndongo², Melanie Mekoudjou⁴, Anfumbom Kfutwah¹, Dominique Roussset¹, Josiane Warszawski⁶ and Albert Faye⁷

¹Centre Pasteur du Cameroun, Yaoundé, Centre, Cameroon

²Centre Mère et Enfant de la Fondation Chantal Biya, Yaoundé, Centre, Cameroon

³Centre Hospitalier d'Essos, Yaoundé, Centre, Cameroon

⁴Hôpital Laquintinie de Douala, Douala, Littoral, Cameroon

⁵Hôpital Central de Yaoundé, Yaoundé, Centre, Cameroon

⁶Unité INSERM U822, CHU de Bicêtre, Kremlin Bicêtre, Paris, France

⁷Hôpital Robert Debré, Service Pédiatrie Générale, Paris, France

Retrovirology 2009, 6(Suppl 1):O21

Background: Adhesion of participants is critical for clinical study recruitment and success. The PEDIACAM-ANRS I2140 survey, including babies born both to HIV positive mothers and HIV negative mothers started in Cameroon at the end of 2007 and is currently ongoing. The objectives are to evaluate the feasibility of early antiretroviral multitherapy on HIV infected infants and the humoral response to vaccines of the expanded program of immunization (EPI). HIV negative mothers are more numerous than expected to refuse to participate to this survey. It should be important to investigate reasons for refusal in order to improve recruitment and better understand attitudes of general population towards clinical research on HIV/AIDS. We present here the preliminary results on reasons for refusal and socio-demographic characteristic associated with early lost-to-follow up in HIV-negative mothers

Methods: Inclusion of children in the PEDIACAM survey is proposed to all HIV-infected mothers in participating maternities before the 7th day of life. Each time a child born to HIV infected mother is included (index child), we propose, the survey to the

following HIV-negative mothers who delivered in the same maternity with a child of same gender than index, until acceptance of one control mother.

Results: During the first year of the study, 9723 deliveries occurred in the three study sites, with a maternal HIV prevalence estimated at 6.6% (636). The preliminary data showed that two kind of initial refusal were reported: 1) the mother did not explicitly refuse but told that she has to obtain opinion of her family (husband, mother in law) prior to accept and never came back; 2) the mother refused for various reasons: fear of stigmatization due to the participation in a study involving HIV infected persons or practitioners known to take care of HIV patients; refusal to act as a guinea pig; lack of time for follow-up. Among HIV negative mothers who accepted initially to participate, 36% (211/582) of HIV negative mothers had not returned more than two weeks after the visit planned at 6 weeks of age, in spite of telephone recall. Early lost-to-follow up was not significantly associated with age, marital status, number of child alive and sex of the child. However, it was lower among high- than low-educated HIV negative mothers (21% vs 33%, $p = 0.003$).

Conclusion/perspectives: This study raises some of the obstacles to the participation of HIV negative mothers to clinical research, for the greater part due to their perception of HIV infection. Even after initial acceptance to participate, a high rate of parents did not return for first follow up. The anthropological study associated to PEDIACAM which will start in March 2009 may contribute to better understand representations of parents towards clinical care and research in HIV infection.

O22

High incidence of invasive group B streptococcal infections in uninfected infants born to HIV-1-infected mothers

Cristina Epalza¹, Tessa Goetghebuer¹, Marc Hainaut¹, Patricia Barlow¹, Anne Dediste¹, Pierrette Melin² and Jack Levy¹

¹St Pierre Hospital, Université Libre de Bruxelles, Brussels, Belgium

²Centre de Référence des Streptocoques du Groupe B, Centre Hospitalier Universitaire de Liège, Liège, Belgium

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Background: Practice guidelines recommend the follow up of all children born to HIV-infected women to ascertain the absence of vertical transmission and to detect possible adverse effects of exposure to ART. The occurrence of an unusual number of invasive group B streptococcal (GBS) infections in uninfected HIV-exposed infants in our center prompted this study.

Objective: To describe the incidence and clinical presentation of GBS infections in a cohort of uninfected HIV-exposed infants born between 2001 and 2008 in comparison to the population of infants not exposed to HIV born in the same hospital during this period.

Methods: The medical charts of all uninfected HIV-exposed infants prospectively followed since birth in our center and the microbiology laboratory records were reviewed to identify invasive GBS infections.

Results: 8 episodes of invasive GBS infection occurred in 7 out of 397 uninfected HIV-exposed infants. Ninety seven % of the mothers were treated with antiretroviral agents during preg-

nancy, including 6 of the 7 mothers of children with GBS infection. The median gestational age of these infants was 36 weeks. Five episodes occurred later than 7 days of life (days 9, 26, 33, 64 and 72). One of the infants had a recurrent episode 28 days after completion of the antibiotic treatment of a first GBS infection. When the analysis was restricted to the infants born in our centre, GBS invasive infection occurred in 5/322 (15.5/1000 live births) HIV-exposed infants compared to 16/20158 (0.79/1000 live births) infants in the control population (OR = 19.6 $p < 0.0001$). In the latter, median age of onset of GBS infection was 1 day and the median gestational age 40 weeks.

Conclusion: Between 2001 and 2008 the incidence of invasive GBS infection was significantly higher in the uninfected infants born to HIV-infected mothers than in the control population born in our centre. The majority of GBS infections in HIV-exposed infants were late or very late onset and 1 child had a recurrence; 2 features that were strikingly different than in the general population.

O23

Perinatal transmission of Cytomegalovirus (CMV) in children born to HIV positive and negative women in Cameroon

Anfumbom Kfutwah¹, Mathurin Tejjokem¹, Albert Faye², Martial Yonga¹, Josiane Warszawski³, Stephane Blanche⁴, Claire Rekacewicz⁵, Francis Ateba⁶, Jean Ndongo⁶, Georgette Guemkam⁶, Chantal Same-Ekobo⁷, Christine Rouzioux⁴, Marianne Leruez-Ville⁴, Pascal Boisier¹ and Dominique Rousset¹

¹Centre Pasteur, Yaounde, Cameroon

²Hôpital Robert Debré, Service de Pédiatrie Générale, Paris, France

³Unité Inserm U822, CHU de Bicêtre, Bicetre, France

⁴Hôpital Necker Enfants Malades, Paris, France

⁵ANRS, Paris, France

⁶Centre Mère et Enfant de la Fondation Chantal Biya – Maternité Principale Hôpital Central de Yaoundé, Yaounde, France

⁷Centre Hospitalier d'Essos à Yaoundé, Yaounde, Cameroon

Retrovirology 2009, 6(Suppl 1):O23

Background: Cytomegalovirus (CMV) infection is considered as a risk factor in disease progression in HIV co-infected infants. The objective of this study was to evaluate the perinatal transmission of CMV in children born to HIV positive women compare to HIV negative women and to evaluate the prevalence of CMV in HIV infected infants.

Methods: Children born to HIV positive and negative women who participate in ANRS 12140 survey (Pediacam) in Yaoundé (Cameroon) were included in this study. Whole blood samples were collected from the children at the moment of inclusion into Pediacam. DNA was extracted using the Qiagen blood extraction kit and CMV DNA was quantified using a real time in house CMV PCR (ABI Prism 7700, Applied Bio systems). Two groups of HIV infected children diagnosed before 6 months were included: children followed since birth with early diagnosis (group I; $n = 17$) and those not followed since birth and diagnosed generally later (group III; $n = 29$). There were two groups of HIV uninfected children followed since birth: those born to HIV-infected mothers (group II; $n = 11$) and those born to seronegative mothers (group IV; $n = 12$).

Results: Among the 69 children who participated in this study (median age; 15 weeks [range 3–34 weeks]) 31 (45%) had a positive CMV detection. CMV prevalence was 31 and 65% in groups I and III respectively and 0 and 64% in groups II and IV. All infants of group IV were breastfed while the other groups consisted of children who were either breast-fed or bottle-fed. As expected, CMV prevalence was higher in the breastfed children 21/33 (66%) than in the bottle-fed children 7/34 (21%) ($P < 10^{-4}$) (Information not available for 2 children.)

There was a 30% increase in CMV prevalence among HIV infected children compared to non infected children in both the breastfed group (81.2 % versus 50%) and the bottle fed group (29% versus 0%). The median CMV viral load was 3.6 log copies/ml [range 1.2 to 6.5]. Viral load was higher in group III (4.6 log copies/ml) compared to groups I, II, and IV (3.1, 0, and 2.8 log copies/ml respectively) ($P = 0.01$). Among the infants of group III who presented with non specific symptoms, CMV was detected in 12/16 (75%). Group III presented subtle differences in CD4 cell percentages between CMV infected and non infected children 23% vs. 31%.

Conclusion and perspective: This preliminary study showed a high incidence of CMV infection in HIV infected infants in Cameroon. As previously described, breastfeeding and HIV transmission irrespective of mode of feeding were associated with CMV infection. Most of the HIV symptomatic children were infected with CMV. This co-infection may be a major factor of morbidity/mortality in children with severe HIV disease in the African setting.

O24

Frequency of resistant virus and options for a second-line treatment for HIV-1 infected children under HAART in Mozambique

Marie-Laure Chaix¹, Ilesh Jani², Eugenia Macassa², Dulce Bila², Adolfo Vubil², Soren Andersson³, Christine Rouzioux¹, Paula Vaz² and Stéphane Blanche¹

¹EA 3620 Université Paris Descartes, CHU Necker, Paris, France

²Hospital Central de Maputo, Instituto Nacional de Saude, Mozambique

³Karolinska Institute Stockholm, Sweden

Retrovirology 2009, 6(Suppl 1):O24

Background: Resistance outcome for treated children in low resource countries is scarce. We aimed to describe the frequency and the profile of resistant virus in children treated with at least 12 months of WHO advised highly active antiretroviral therapy (HAART) in a large access program in Mozambique.

Methods: Between December 2003 and December 2006, 515 children (median age: 36.8 months) were included, 97% received a combination of d4T plus 3TC and nevirapine. HIV-1 RNA was transversally performed once using the Roche Amplicor v1.5 test. HIV-1 genotypic resistance tests were performed on available plasma samples when HIV-1 RNA was $> 3 \log_{10}$ copies/ml. Drug resistance was interpreted according to the 2007 French ANRS resistance algorithm.

Results: Viral load was available for 498 out of the 515 children. Among them, 134 (27%) had a viral load $> 3 \log_{10}$ copies/ml and genotypic resistance test could be performed for 87 children. The overall frequency of viruses showing genotypic

resistance to at least 1 antiretroviral drug was 90%. The prevalence of children infected with virus with ≥ 1 major mutation conferring drug resistance to NRTIs and NNRTIs were 85% and 90%, respectively. M184V conferring resistance to lamivudine was the most common NRTI mutation. Thymidine analogs mutations (TAMs) conferring resistance to ZDV or d4T were observed in 15%. Resistance to Tenofovir, Abacavir and ddI were described in 6%, 5% and 3.5% respectively. The NRTIs Multi Drug Resistance complex (Q151M) was found in 3 cases. Unexpectedly, five children (6%) had developed extensive resistance to NNRTIs inducing resistance to the new NNRTI etravirine (TMC 125). The only factor identified by multivariate analysis as being associated with this broad spectrum resistance was the duration of treatment: aOR: 10.15 [95% CI 1.59–64.94], $p < 0.05$ for treatment for longer than 24 months. The level of viral replication at the time of genotyping was not predictive.

Conclusion: After experiencing failure with HAART containing two drugs with low genetic barrier, almost all children have at least lamivudine and NNRTI resistance. Broad spectrum resistances to second NRTIs line (ddI, ABC, TDF) concerned 6% of children. Multi drug resistance to all NRTIs was found in 3.5%. Resistance to etravirine was described in five children (6%).

O25

Lopinavir/ritonavir-based second line antiretroviral treatment in children at National Pediatric Hospital, Phnom Penh, Cambodia

Sophan Sam¹, Vibol Ung¹, Chantheany Huot¹, Bunthny Chan¹, Kdan Yuvatha¹, Christian Courpotin², Guillaume Adam², Sopheak Ngim³, Eric Nerrienet³ and Y Meng Chhour¹

¹National Pediatric Hospital, Phnom Penh, Cambodia

²French Red Cross, Paris, France

³HIV/Hepatitis Laboratory, Institute Pasteur, Phnom Penh, Cambodia

Retrovirology 2009, 6(Suppl 1):O25

Background: Cambodia has scaling up a large national ART program using 1st line therapy (d4T or AZT+3TC+NVP or EFV). According to NCHADS, as December 31st 2008, 3,067 children were on HAART in Cambodia, 746 of them were followed-up in Child Health Improvement Clinic (CHIC) at the National Pediatric Hospital, (NPH), with French Red Cross technical support. Fifty-three out of 746 already switched on LPVr-based 2nd line regimen.

Objective: The aim of this study was to evaluate virological and immunological outcomes of these children on second line.

Methods: Retrospective analysis based on data and medical records from a cohort followed at CHIC to 31st December 2008 was conducted. Patients meeting the Cambodian National Guidelines for the Use of Pediatric ART for treatment failure were evaluated. First line treatment failure was confirmed based on clinical and immunological failure and/or virological failure. Plasma viral load has been assessed by HIV RNA real time PCR using 2nd generation ANRS Kit. Genotypic resistance analysis was done at Institute Pasteur according to ANRS algorithm (v.sep.07).

Results: 53/746 patient (7.1%) switched to 2nd line were enrolled in this study (33.9% were females). Median age was 10.9 years (2.1–17.9). Median duration on the 1st line was 2.2 years (0.6–6.3). Median of CD4 percentage at switch was 8.0%

and VL was 5.1 log₁₀ (4.0–6.3) with ± clinical failure. At switch, 38/53 patients were tested for HIV drug resistance. HIV Drug resistance analysis revealed that 97.3% (37/38) children were resistant to NVP/EFV, 78.9% to AZT/d4T/3TC/FTC, 47.3% to ABC/ddI, and 10.5% to TDF. Thirty-six of 53 patients (67.9%) received standard 2nd line regimen (ABC/ddI/LPV/r), 9 (16.9%) received 3TC/TDF/LPV/r, 4 (7.5%) were on 3TC/AZT/LPV/r, and 2 (3.7%) on 3TC/ddI/LPV/r. At evaluation, median duration on 2nd line was 1.0 years (0.1–3.3). Median CD4% gain on 2nd line regimen were 13.0% (1–31%) at M6 (n = 34); 17.0% (1–33) at M12 (n = 27); 19.5% (12–29) at M18 (n = 12); 20.0% (16–32) at M24 (n = 7); and 18.0% (17–28) at M30 (n = 3). Children who achieved undetectable VL (VL < 2.4 log₁₀) at M2 were 71.0% (n = 38); 85.2% at M6 (n = 34); 88% at M12 (n = 25), 77.7% at M18 (n = 9); 85.5% at M24 (n = 7) and 100% at M30 (n = 3).

Conclusion: These preliminary data on Cambodian HIV infected children on LPV_r-based second line HAART regimen indicated good virological/immunological responses.

POSTER PRESENTATIONS

PI

The trophoblast: a model to study HPV transcription, replication and host cell interactions

Véronique Fontaine, Selma Boulenouar, Christine Weyn and Yvon Englert
Université Libre de Bruxelles, Bruxelles, Belgium

Retrovirology 2009, 6(Suppl 1):PI

Background: Hallmarks of HPV infection include a restricted tropism for human epithelial cells and a viral life cycle tightly linked to the differentiation program of the host cells. This has hampered the study of the HPV vegetative life cycle. Previous studies reported that the tissue and differentiation dependence seemed to be dictated by viral transcription rather than viral DNA replication.

Objectives: 1. To compare HPV transcription in cervical and trophoblastic cells and to study in those models the regulation of the LCR activity by various hormones and the viral early proteins. 2. To study the impact of the early viral proteins, especially E5, E6 and E7, on cell adhesiveness, migration and invasiveness.

Methods: To study transcription, we analyzed the activation of a reporter gene under the control of the HPV-16 LCR. To study replication, we measured, in RT-PCR after DpnI/MboI digestions, the amount of replicated DNA. Cellular properties were studied using various biological assays.

Results: The LCR activity was similar in both cell types and could be regulated by various hormones. To analyze the effect of all early proteins expression on the LCR activity and on viral replication in both cell types, the reporter plasmid was cotransfected with a plasmid allowing the expression of the entire early coding region under the control of its own HPV-16 LCR. Viral early proteins activated viral transcription and replication. Using various plasmids harboring point mutation in E1 or E2 ORF, we were able to observe that neither E1 nor E2 did play a role in the increased viral transcription. Early proteins could also modify the adhesion, the migration and the invasion of trophoblastic cells.

Conclusion: We will discuss about the interest of this model to identify new cell host (trophoblast)/pathogen (HPV) interactions.

P2

Evolution of HIV-1 envelope sequences and coreceptor tropism during pregnancy

Doris Ransy^{1,2}, Josiane Couto^{1,2}, Bertine Akouamba^{1,2}, Johanne Samson¹, Normand Lapointe^{1,3}, Marc Boucher^{1,4} and Hugo Soudeyns^{1,2}

¹CHU Sainte-Justine, Montreal, Quebec, Canada

²Department of Microbiology and Immunology, Université de Montréal, Montreal, Quebec, Canada

³Department of Pediatrics, Université de Montréal, Montreal, Quebec, Canada

⁴Department of Gynaecology and Obstetrics, Université de Montréal, Montreal, Quebec, Canada

Retrovirology 2009, 6(Suppl 1):P2

Background: HIV-1 Env mediates viral entry into target cells and defines R5 or X4 phenotype. HIV tropism is important in pathogenesis and is associated with mother-to-child transmission. Env is under strong selective pressure within the host. The aim of this study was to determine whether changes in maternal immunity associated with initiation and progression of pregnancy influence Env sequence variation and HIV-1 coreceptor usage.

Methods: A longitudinal study of Env sequence variation was performed in 25 pregnant women infected with HIV-1 of clade B (n = 16) or non-B (n = 9). Viral RNA was extracted from plasma, the env gene (nucleotide positions 6430–7374) was PCR-amplified and subcloned, and a mean of 20 clones were sequenced per trimester of pregnancy. HIV tropism was predicted *in silico* using PSSM_{X4R5}, PSSM_{SINSI}, SVM, geno2pheno and charge rule algorithms. Phylogenetic reconstructions were built, sequence diversity was estimated using *p* distances and selective pressure (dN/dS) was computed.

Results: In study subjects, HIV-1 viral load decreased progressively during the course of pregnancy. Envelope sequences were amplified at all trimesters in 63% of the cases. Phylogenetic analysis revealed at least partial clustering of sequences per trimester in 4 out of 9 patients. Nucleic acid *p* distances were negatively correlated with CD4⁺ T cell counts at study entry (*r*² = 0.66, *p* = 0.02). In all clade B variants examined, R5 phenotype was predicted by all algorithms. Interestingly, while predictions were not as consistent with non-B subtypes, R5 and X4 variants were shown to coexist during pregnancy in 4 of 5 cases. Of note, evolution of tropism from R5 to X4 was observed in one subject, and evolution from dual tropism to exclusive X4 was observed in another subject between two consecutive pregnancies. Higher levels of genetic diversity in the V2 region were observed in subjects infected with non-B subtypes as compared with clade B variants. Finally, selective pressure on V2 tended to decrease with time in subjects infected with the B subtype but remained relatively high in sequences derived from carriers of non-B subtypes.

Conclusion: This study provides insights into the possible interplay between viral population dynamics and selective pressures exerted by maternal HIV-1 specific immune response during pregnancy, insights that could prove invaluable for the development of active and/or passive immunization strategies to prevent mother-to-child transmission of HIV-1.

P3**Abstract withdrawn**

Retrovirology 2009, 6(Suppl 1):P3

P4**Abstract withdrawn**

Retrovirology 2009, 6(Suppl 1):P4

P5**Maternal neutralizing antibodies against a CRF01_AE primary isolate are associated with a low rate of intrapartum HIV-1 transmission in Thailand**

Suzie Thenin¹, Tanawan Samleerat^{1,2},
Gonzague Jourdain^{3,4}, Nicole Ngo-Giang-Huong^{3,4},
Alain Moreau¹, Pranee Leechanachai², Marc Lallemand^{3,4},
François Barin¹ and Martine Braibant¹

¹Université François Rabelais, Tours, France²Chiang Mai University, Chiang Mai, Thailand³Harvard School of Public Health, Boston, USA⁴Institut de Recherche pour le Développement, Chiang Mai, Thailand

Retrovirology 2009, 6(Suppl 1):P5

Background: Mother-to-child transmission (MTCT) of HIV-1 provides a model for studying the role of passively acquired antibodies in preventing infection. We previously hypothesized that broadly neutralizing heterologous antibodies (NAbs) would protect babies against intrapartum (IP) transmission. We measured NAb titers against primary isolates of various clades in sera from pregnant Thai women, and identified an association between higher titers of Nabs against a CRF01_AE primary isolate, MBA, and lower rates of IP transmission. Here, we extended our previous study using three CRF01_AE strains in a different Thai population, to confirm the association previously observed. We also investigated the molecular characteristics of the MBA envelope glycoprotein (Env) that might explain this association.

Methods: We measured and compared the titers of NAbs against six primary isolates (3 CRF01_AE and 3 clade B strains) in sera from 45 transmitting (T) and 45 nontransmitting (NT) Thai mothers matched for baseline viral load and duration of zidovudine prophylaxis, the two main independent factors associated with MTCT. We cloned and sequenced the env gene of the three CRF01_AE strains and compared the neutralization profiles by mothers' sera of pseudotyped viruses expressing wild type or chimeric Env proteins.

Results: Among CRF01_AE strains, MBA was more resistant to neutralization than the two other strains, LEA and C1712. The three clade B strains displayed similar neutralization profiles. We did not find an association between NAbs and MTCT for the three B strains or for LEA and C1712. In contrast, higher levels of NAbs against MBA were significantly associated with lower rates of IP transmission. The Env of this strain showed an unusually long V2 domain of 63 amino acids including six potential N-linked glycosylation sites. Using pseudotyped viruses expressing either MBA or LEA wild-type Env or a chimeric Env containing the V2 domain of MBA in an LEA Env backbone, we

showed that the extended V2 domain contributed to the higher level of resistance to neutralization by mothers' sera in this strain.

Conclusion: This study confirms that higher titers of maternal NAbs against a CRF01_AE primary isolate, MBA, are associated with a lower IP risk of HIV-1 transmission in Thailand, and that the V2 domain of gp120 seems to have a major role in the neutralization process. We suggest that some primary isolates may be useful indicators for identifying protective antibodies.

P6**HCV infection in a sample of pregnant women in central Poland: seroprevalence and risk factors**

Malgorzata Aniszewska^{1,3}, Barbara Kowalik-Mikolajewska^{1,3}, Maria Pokorska-Lis^{1,3} and Monika Kalinowska²

¹Department of Children's Infectious Diseases, Warsaw Medical University, Warsaw, Poland²Department of Obstetrics and Gynecology, Warsaw Medical University, Warsaw, Poland³Hospital of Infectious Diseases, Warsaw, Poland

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Background: Vertical transmission from HCV infected mothers seems to be one of the main modes of infection in children. Approximately 6% of hepatitis C virus-positive women transmit HCV to their offsprings. There has been no information about anti-HCV prevalence in pregnant women in Poland to date. Routine universal antenatal screening for hepatitis C infection has not been shown to be cost effective. Selective antenatal screening is used on the basis of risk factors for exposure to HCV. There is no effective intervention to prevent vertical transmission of HCV during pregnancy, but HCV infection could be reduced by a course of therapy prior to conception. Aim: 1. To determine the frequency of HCV infection in pregnant women in central Poland 2. To estimate knowledge about HCV infection in childbearing women. 3. To identify risk factors for HCV infection among pregnant women.

Methods: Study in two separate parts: Part I/: Blood samples were collected from 544 pregnant women in central Poland and tested with anti-HCV ELISA third-generation tests. Part II/: Data of risk factors of HCV infection, reason of diagnostics were assessed through structured interview and review of available medical records in 281 women infected with HCV who came to Department of Children's Infectious Diseases to examine their infants for HCV infection.

Results: Part I/ Eleven pregnant women (2.02%) were infected with HCV. One of them (1/11) knew about her infection before examination. The seroprevalence varied by city/country living (2.2% vs 1.0%). The mean age of tested women was 29.9 years, infected women 29.8 years. Part II/ 247(88%) infected women lived in cities, 34(12%) in country. 24% indicate a history of blood products transfusion (all before 1992), 23% surgical, gynaecological procedures, transplantations, 15% intravenous drug use, 8% hospitalization without surgical procedures, 7% exposures of health care personnel, 3% infected mother, 6% other risk factors like: tattoos, sexual partner or other member of family infected with HCV. Histories taken from 14% women did not include any risk factors. HCV infection in women were diagnosed: 186(66%) before pregnancy, 61(22%) during preg-

nancy, 34(12%) after delivery. 95/281(34%) did not know about their HCV infection before pregnancy. All women were Caucasian, Polish nationality.

Conclusion: The prevalence of anti-HCV in pregnant women in central Poland is 2.02%. There is a number of childbearing HCV infected women who are not identified as HCV positive. Selective HCV testing to woman at high risk of HCV infection should be encouraged prior to conception. Following the introduction of blood product screening (in Poland: anti-HCV in 1992, HCV-RNA RT-PCR 2001) – transfusion has not been a possible route of HCV infection.

Detailed epidemiological anamnesis plays an important role in the diagnostics, but not always allows identifying the risk group.

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P7
CD4⁺ T lymphocyte response to primary CMV infection

Pierre Antoine¹, Véronique Ollislagers¹, Sandra Lecomte¹, Corinne Liesnard², Catherine Donner² and Arnaud Marchant¹

¹Institute for Medical Immunology, Charleroi, Belgium

²Hôpital Erasme, Brussels, Belgium

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Primary cytomegalovirus (CMV) infection induces rapid expansion of CMV-specific cytokine-producing CD4⁺ T cells but slow acquisition of proliferative responses. In utero transmission of CMV following maternal infection is associated with low proliferative responses. We have observed that pregnant women with primary CMV infection have high frequencies of CD4⁺ T cells expressing low levels of Bcl-2. The frequency of these cells returns to that of chronically infected subjects after the first months of infection. As Bcl-2 controls cell survival and proliferation, its regulation could play an important role in the control of CMV-specific CD4⁺ T cell responses. The aim of the project is to characterise the phenotype, the antigen specificity and the functions of Bcl-2_{low} CD4⁺ T cells in pregnant women with primary CMV infection. We have observed that Bcl-2_{low} cells are more activated and differentiated than Bcl-2_{high} cells. In particular, the low expression of Bcl-2 is tightly associated with loss of CD28 expression, decreased CD127 (IL-7 receptor α chain) expression and increased PD-1 expression. The low expression of CD127 is associated with low STAT-5 activation in response to IL-7 stimulation. These results indicate that primary CMV infection regulates the expression of Bcl-2 by CD4⁺ T lymphocytes and that this phenomenon is associated with the modulation of other surface receptors controlling cell proliferation. Further studies will define the functional consequences of these phenotypic alterations.

P8
Abstract withdrawn

Retrovirology 2009, 6(Suppl 1):P8

P9
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Retrovirology 2009, 6(Suppl 1):P9

P10
Early diagnosis of HIV-1 infection in infants using RNA Quantitative PCR in Burkina Faso

Hermann Somlare^{1,2}, Lassana Sangare^{1,3}, Saydou Yamegogo^{1,2}, Carine Ouedraogo², Yolande Dembel¹, Monique Soro², Parfait Some⁴ and Caroline Yonaba⁴

¹Laboratory of Bacteriology and Virology of Hospital University Center Yalgado Ouédraogo, West Africa, Burkina Faso

²UFR Sciences de la Vie et de la Terre of University of Ouagadougou, West Africa, Burkina Faso

³UFR Sciences de la Santé, West Africa, Burkina Faso

⁴Paediatric clinic of Hospital University Center Yalgado Ouédraogo, West Africa, Burkina Faso

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Aim of study: To diagnose HIV infection in African infants born to HIV-1 infected mothers using Abbott RealTime PCR (Abbott Molecular).

Materials and methods: From January to December 2008, 114 infants born to HIV-1 infected mothers were referred to the University Hospital Yalgado Ouédraogo (Ouagadougou, Burkina Faso), for early diagnosis of HIV infection. Two to 3 mL of blood sample were collected on EDTA/K3 microtubes from each infant. After centrifugation, the plasma samples were stored at -80°C, until their use. HIV-1 RNA was detected in each sample using Abbott RealTime HIV-1 Assay (Abbott Molecular) and m2000rt protocol: the RNA detection threshold was 40 copies/mL (1.6 Log). From each infant with RNA positive result, a second plasma sample was collected 4 weeks later to confirm the previous RNA result Sociodemographic data were collected from the infants and analysed.

Results: The mean age of the newborns was 4,5 months, and the sex ratio was 0.92. The HIV RNA PCR assay was positive in 14/144 (9.7%) newborns in both samples tested: the mean viral load was 4,135,853 copies/mL (6.6 Log copies/mL), and the mean CD4 percentage was 20.25%. Nineteen children and their mothers did not receive dual antiretroviral prophylaxis (AZT +NVP) for the prevention of mother to child transmission (PMTCT): 13 (68.4%) of them were HIV-positive against only 1 (0.8%) among the 125 who received the prophylaxis. The HIV-1 transmission rate was significantly higher in children without PMTCT ($p = 0.00$). Ten (71.4%) HIV-positive infants were breast-fed exclusively, 2 (14.3%) received mixed breast-feeding and 2 (14.3%) received formula: exclusively breast-feeding could be a higher risk of HIV transmission than the 2 other routes.

Conclusion: These results showed that Abbott RealTime HIV-1 assay for the quantitation of HIV-1 can be used for the early diagnosis in HIV-exposed infants, even in newborns who received antiretroviral prophylaxis. The exclusive breast-feeding appears as high risk of HIV transmission from infected mothers to their children.

P11**Couple issues in the last pregnancy experience of HIV+ women knowing their status before pregnancy**

Nadine Trocmé, Marie-Dominique Tabone, Mary-France Courcoux, Laurence Stengel and Catherine Dollfus
Hôpital Trousseau Service d'Hématologie, Paris 75012, France

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Background: Even in mothers knowing their HIV status before pregnancy, many women who recently delivered demonstrate a high level of stress regarding medical, social, and psychological questions. We decided to research more deeply into the specificities of vulnerabilities of these women.

Patients and methods: Women attending a MTCT clinic at a teaching hospital within 3 months after delivery were systematically offered the possibility of meeting a psychologist. A number of relevant issues were systematically discussed. Immediately after each guided interview, maternal words were transcribed on the computer. In this study, we selected the subgroup of women who were aware of their HIV status before pregnancy.

Results: 37 women have been interviewed; Their median age was 33.5 years (18;44).

32/37 women (86.5%) were of African origin. They have been knowing their HIV status for a median time of 3.5 years (2;20). Among 26 who had previous pregnancies, 15 had a total of 20 children who did not live with them. Only 3 pregnancies/37 were unintended.

22/37 mothers live with the child's father; 20/22 fathers (90%) are aware of maternal HIV status; they have been immediately informed by the mothers either when they met or when she discovered her HIV status. 15/20 of these fathers are HIV negative.

Among the 15 mothers who do not live with the fathers, only 8/15 fathers are informed of the status. All the mothers (4) who disclosed their status during pregnancy were abandoned by the child's father.

25 women got pregnant through unprotected sex (7 fathers being HIV positive, 10 being HIV negative and refusing condom use, 8 unaware of maternal status), 9 used self-insemination after protected intercourse, 1 used AMP, 2 reported condom rupture. 27/37 express frustration of not having been allowed to breast-feed their infant.

Despite information on HIV transmission, only 11/37 do not fear the risk of transmitting HIV to their infant through casual contacts (touching and kissing...).

Conclusion: In women aware of their HIV status for over 3 years before last pregnancy, secret remain strong in a noticeable number of couples. 9/37 had children without their partner being aware of their status, and 18/37 got pregnant through unprotected sex.

27/37 regret not to be able to breast-feed and 26/37 fear to transmit HIV to their infant through casual contact.

Both HIV and secret regarding HIV are detrimental for family life of HIV positive women.

P12**Evolution of the biological follow-up of efficiency and tolerance of a once daily antiretroviral treatment with 3TC+DDI+EFV in children infected with HIV-1 (CLINICAL TRIAL ANRS 12 103)**

Boubacar Nacro¹, Yaya Diasso², Emmanuelle Zoure¹, Potiendi Serge Diaboug³, Philippe Van de Perre⁴, Aly Drabo³, Adama Ouiming³, François Rouet³, Dramane Kania³, Souleymane Yameogo⁵, Alain Hien⁵, Hervé Hien⁶ and Philippe Msellati⁷

¹Pediatric Department CHUSS, Bobo-Dioulasso, Burkina Faso

²Laboratories Department CHUSS, Bobo-Dioulasso, Burkina Faso

³Immunology laboratories Centre Muraz, Bobo-Dioulasso, Burkina Faso

⁴Bacteriology and Virology laboratories CHU Arnaud de Villeneuve, Montpellier, France

⁵Pharmacy Department CHUSS, Bobo-Dioulasso, Burkina Faso

⁶Epidemiology Department Centre Muraz, Bobo-Dioulasso, Burkina Faso

⁷URM 145, IRD, CReSS, Aix en Provence, France

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Objective: To study the evolution of biological parameters of efficiency and tolerance of the anti retroviral treatment (3TC, DDI, EFV) in child.

Methods: A total of 52 HIV1 infected children have been included in the study and have receiving a once daily dose of Lamivudine, Didanosine and Efavirenz. The follow up biological parameters have been collected quarterly in a period of one year.

Results: The median age was 6.83 years, with an age range of 2.5 to 14.5 years. The clinical stages at the enrolment were the following: 1 at stage N, 18 at stage A, 27 at stage B, 6 at stage C. At the biological level, the median gain of CD4 in the children, after a period of one year of follow-up was of 14% (8.6% in inclusion). At the 12th month of follow-up, 51% of the patients had an undetectable viral load (<50 copies/mL) and 78% (<300 copies/mL). The mean values of the biologic parameters in blood such as platelets, ALAT, bilirubine, the blood sugar, cholesterol, the triglycerid and the creatinine were always in the norms. Despite a meaningful increase to M12, the mean rate of haemoglobin was always inferior to the norms (10.60 ± 1.01 to M12). Regarding the biological perturbations, grade III or IV, it has been observed an increase of the transaminases in 1% of the patients and mainly an elevation of the amylasemia in 3.9%, 2%, 13.7% and 10% of the children respectively on M3, M6, M9 and M12.

Conclusion: The biologic tolerance and the efficiency of the treatment are satisfactory. The rate of haemoglobin, the transaminases, and the amylasemia appear to be the determining criteria for the follow-up of the moderate toxicity of this short-term treatment.

P13**Transient elastography (Fibroscan) in HIV-1 vertically infected children.****A cross-sectional study**

Fabrice Monpoux¹, Emilie Huguon¹, Régine Truchi²,
Amandine Rubjo¹, Valérie Riolo¹, Anne Deville¹,
Patrick Boutte¹ and Albert Tran²

¹Hôpital de l'Archet II – Pédiatrie, Nice, France

²Hôpital de l'Archet II – Clinique des maladies du foie,
Nice, France

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Progressive liver toxicity is a concern in HIV-infected patients. Although liver biopsy remains the gold standard for liver assessment, its invasiveness, sampling errors, variability in interpretation and expense do not make it an ideal routine follow-up exam. During the last decade, new non-invasive tools have been developed for the assessment of hepatic fibrosis in HCV and HIV/HCV co-infected patients.

The aim of this cross-sectional study was to evaluate the feasibility of transient elastography (TE) measuring liver stiffness in chronically HIV-infected children. Inclusion criteria were: materno-foetal transmission, age 8 to 18 years old, informed parental consent and patient assent. Twenty-one HIV-1 chronically infected children were included. There were 11 girls and 10 boys, with a median age of 13.2 years (8.3–17.3). Five were HIV stage N, 3 were stage A, 6 were stage B and 6 suffered from AIDS definition illness (stage C). For one orphan child HIV CDC status could not be determined. Mean weight was 46.7 kg (18.4–83.5), mean height was 151.2 cm (116–175). Mean CD4 T-cell count at inclusion was 669 cells/mm³ (256–1,252) or 29.9% (9.4–45%), mean viral load was 3.88 log₁₀ copies/mm³ (1.60–4.83). Eight children had undetectable viral load (<40 copies/mm³). At the time of enrolment, 10 patients received conventional HAART with two NRTIs (nucleoside reverse transcriptase inhibitor) and 1 PI (protease inhibitor), 4 received 2 NRTIs + 1 NNRTI (non-nucleoside reverse transcriptase inhibitor), 2 patients were on dual NRTI association, 1 was on triple NRTI combination and 1 were receiving 1 drug of each class (NRTI+NNRTI+PI). Three children were on planned treatment interruption.

All patients underwent a Fibroscan exam. The two youngest children failed in having a TE measure because of technical difficulties due to their small corpulence. The average measurement success rate was 96.7%. HIV-infected patients had significantly higher TE results than matched healthy control children (5.92 ± 1.60 versus 4.34 ± 1.10 kPa) (p < 0.02). Furthermore, loss of elasticity assessed by TE measures tended to increase with age in a linear manner (adjusted R²: 0.208, p < 0.03). This correlation was found only in the HIV-infected group. We therefore hypothesized that HIV infection and/or continuous exposure to antiretroviral treatment were responsible for this relation.

Our results showed that 1 – evaluation of liver stiffness is feasible in most HIV-1 chronically infected children. 2 – Patients had significantly higher TE results than matched healthy control children 3 – the loss of elasticity tended to increase with age in a linear manner. Liver injury should be monitored on a regular basis. The place of TE in the management of these children must be further defined.

P14**Motherhood in HIV+ women infected in early childhood**

Catherine Dollfus, Nadine Trocme,
Marie-Dominique Tabone, Geneviève Vaudre,
Christian Courpotin and Guy Leverger
HOPITAL Armand Trousseau, APHP, Paris, France

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Background: As HIV infected girls enter adolescence and adulthood, the ability to become a mother is a question of utmost importance.

Due to major advances in treatment efficacy and MTCT strategies, a growing number of adolescents and young adults are facing the reality of motherhood. We wish to describe our experience of this relatively recent issue.

Methods and patients: Patients are all identified mother-infant pairs followed in a paediatric care reference center specialized in HIV where the mothers have been cared for as a child with HIV and their babies in the context of PTME follow-up.

Data used are those systematically collected in the patients' medical files, with treatment and evaluation following national guidelines.

Results: 8 mothers had 18 pregnancies, resulting in a total of 9 live – born children.

6 of them experienced one or more elective abortions (1–3); in only one instance the decision was based on medical reason (patient conceiving under Efavirenz treatment).

3 mothers were younger than 18 at their first pregnancy although the median age was 19.5 years (16;28). The median age at child birth was 21.5 years (17;31).

6/8 mothers had been infected through blood transfusion in their early childhood; 2/8 by vertical transmission. 7/8 fathers were informed of maternal status.

All mothers took ARV treatment during pregnancy. In all cases maternal viral load close to delivery was < 500 c/ml.

Six women delivered vaginally, although 3 underwent caesarean section.

Children were born between 1996 and 2008; all of them benefited of post natal antiretroviral prophylaxis (6 AZT, 1 AZT +3TC, 1 Lopinavir/Ritonavir).

All children are HIV-uninfected (minimum 3 DNA PCR) and healthy, with a current median follow-up of 2 years (4 months; 5 years).

Conclusion: These adolescent females who had been diagnosed as HIV-infected during childhood demonstrated a strong willingness to be pregnant. They delivered uninfected and healthy children, defying predictions that were made at the time of their own diagnosis. Long-term medical and psychological support remains needed for these young mothers.

P15**Health care of children diagnosed HIV positive before 18 months in Chea Chumneas Hospital, Takhmao, Cambodia**

Denisa Augustinova¹, Born Ban¹, Chhunly Kong²,
KimSong La¹, Suos Prem Prey³, Mealin Kong²,
Sopheak Ngin⁴, Eric Nerrienet⁴ and Martin Bandzak¹

¹Magna Children at Risk, Bratislava, Slovakia

²Chey Chumneas Referral Hospital, Takmao,

Kandal province, Cambodia

³The National Centre for HIV/AIDS Dermatology and STDs, Phnom Penh, Cambodia

⁴HIV/Hepatitis Laboratory, Institute Pasteur, Phnom Penh, Cambodia

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Background: Effective health care delivery to the majority of HIV exposed infants worldwide, including those enrolled in prevention of mother-to-child transmission programs, is hampered by lack of access to an HIV diagnosis in infancy. Early testing can help HIV infected infants access treatment, provide psychosocial benefits for families of uninfected infants, and help programs for prevention of mother-to-child transmission of HIV monitor their effectiveness.

Main objective: The aim of the study was to report the impact of the early testing of HIV on the improved health care of the HIV-infected children and to describe the treatment outcomes in children (< 18 months of age).

Methods: Analysis of cumulative data gathered between April 2006 and December 2008 at Chey Chumneas Hospital including children born under PMTCT and the others attending pediatric services, which general pediatric out-patient include i) general pediatric out-patients, ii) nutrition rehabilitation centre, iii) in-patients care, iv) OI/HAART access and trained medical staff supported by NCHADS and Magna Children at Risk.

Preliminary results: During the study period, 174 HIV-exposed infants, less than 18 months, attending pediatric services were screened for HIV-1 infection. 137 (78,7%) had an undetectable HIV-1 DNA/RNA viral load confirmed on a second blood samples. 37 (21,2%) were found and confirmed HIV-1 infected, including 9 who received a PMTCT intervention. Four out of 37 HIV positive children were lost of follow up, 4 were transferred to another service, 7 died (two were already on HAART), 13 children were on OI prophylaxis only. Finally 12/37 infected children started already HAART (3TC+d4T+NVP).

Conclusion/perspectives: The virological efficacy of treatments for the 12 children on HAART is currently under evaluation and will be presented. According to the new WHO guidelines for Paediatric HIV/ART Care, all the others infected children (n = 13) will get HAART as soon as possible. The results of this study confirm that early testing is crucial to improve health care delivery to HIV-infected children in low-resource settings right now. Well adapted pediatric services are also needed for the complex follow-up of HIV infected children.

P16

A Ukraine birth cohort of children with vertically acquired HIV infection

Saboura Mahdavi¹, Claire Thorne¹, Ruslan Malyuta², Igor Semenenko² and Tatyana Pilipenko²

¹UCL Institute of Child Health, London, UK

²PPAI, Odessa, Ukraine

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Background: HIV prevalence in Ukraine is estimated at 1.6%, with women comprising around half of the HIV-infected population, the vast majority of childbearing age. MTCT rates declined to 7–10% in 2007 following implementation of a national prevention of MTCT (PMTCT) programme in 2000, which to date has been based on single dose NVP (sdNVP) and/or short-

course zidovudine. Little information is available on the natural and treated history of HIV infection in children living in Eastern Europe.

Methods: Within the European Collaborative Study, HIV-infected pregnant women and their infants were enrolled in 6 cities since 2000. Data on HIV-infected children followed from birth to December 2008 were analyzed from this ongoing study.

Results: There were a total of 162 infected children (47% female, 52% male), with average age at last follow-up of 19.1 months (IQR 9.1–44.0). A quarter had low birth weight, 19% (n = 31) were preterm and 25% delivered by Caesarean Section; 37% (n = 60) of mothers had an injecting drug use history. Most (n = 160) infants were bottlefed; four were breastfed briefly. Most (147, 91%) children were infected despite PMTCT prophylaxis (mostly single dose NVP, 62%). Ninety-two children had ≥ 1 CD4 counts; based on nadir CD4 count to date, half (n = 49) were classified in CDC immunological stage I, with 34% (n = 31) and 13% (n = 12) in CDC stage II and III, respectively. Forty-one percent (n = 66) of children had started highly active antiretroviral treatment (HAART) by most recent follow-up, at a median CD4 count of 1340 cells/mm³; of those with viral load measurements, 15.5% (n = 13/84) on treatment had achieved an undetectable viral load to date. Seventy-seven (48%) received PCP prophylaxis (mainly cotrimoxazole) and 8 children received TB prophylaxis. Overall, 3% of children were ever anaemic, with median haemoglobin of 11.5 gm/dl (range, 3.9–23.2 gm/dl). Twenty-two (14%) children had died by last follow-up, with a median age at death of 14.5 months (range, 1–47 months); nearly half (10, 45%) had developed AIDS before their death. Of the children who died, only one had been started on HAART (and had received cotrimoxazole). The most common causes of death were pneumonia (n = 5) and sepsis (n = 5). Overall, 7% (n = 11) children developed AIDS during follow-up. From survival analysis, estimated AIDS-free survival rates were 94%, 90% and 87% for children at age six, twelve and eighteen months, respectively. Survival analysis indicated a significantly improved survival rate among children born in 2004–2008 compared with those born earlier (P = 0.0002). The mothers of nine infected children were known to have died.

Conclusion: Less than half of our cohort of infected children had received HAART and/or PCP prophylaxis. However, the improvements in AIDS-free survival in more recent years reflect the scale-up of paediatric treatment in Ukraine.

P17

Adherence in HIV-1 infected children taking once daily HAART with Didanosine + Lamivudine + Efavirenz in West Africa. ANRS 12103 clinical trial

Jerôme Somé¹, Boubacar Nacro¹, Hervé Hien², Hassane Tamboura¹, Emmanuelle Zouré¹, Serge Diagbouga², Adama Ouiminga², Aly Drabo², Alain Hien¹, Souleymane Yaméogo¹, Philippe Van de Perre³ and Philippe Msellati⁴

¹Service de Pédiatrie, CHU Sourô Sanou, Bobo-Dioulasso, Burkina Faso

²Centre Muraz, Bobo-Dioulasso, Burkina Faso

³Laboratoire de bactériologie-Virologie, CHU de Montpellier, Hôpital Arnaud de Villeneuve, Montpellier, France

⁴UMR I 45, IRD, Centre de Recherche Cultures Santé Sociétés/IFEHA, Université Paul Cézanne, Aix en Provence, France

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Objective: Assess adherence and determine the reasons of poor adherence in HIV-1 infected children taking once-a-day paediatric HAART with DDI+3TC+EFV in Bobo-Dioulasso, Burkina Faso.

Methods: Fifty-two HIV-1 infected children were followed during a 12-month period of the II phase clinical trial. Adherence was assessed using monthly pill counts. A questionnaire was administered quarterly in order to investigate reasons of poor adherence.

Results: During the 12 months of follow up, two children died. Seven became resistant and 118 questionnaires were administered. Only two caregivers declared missed takings the previous week, which was contrasting with the counting. The difficulties reported by the caregivers were related to the time slot, and the instruction to take the pills on an empty stomach, the length of the treatment, the ARV's form and palatability.

The adherence rate was 98% and 32% of the patients had always had an adherence rate \geq 95%. The adherence was not correlated to the socio-demographic factors and to the immuno-virological response.

Conclusion: Good adherence of this once daily's treatment study. No association between adherence, the socio-demographic factors and the infection's markers.

P18

Vertical transmission of Hepatitis C Virus in children born to HIV/HCV coinfecting mothers in Poland

Sabina Dobosz, Magdalena Marczyńska, Agnieszka Oldakowska, Małgorzata Szczepanska-Putz and Konrad Zawadka

Department of Children's Infectious Diseases, Warsaw Medical University, Warsaw, Poland

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Background: The risk of vertical hepatitis C virus (HCV) transmission is estimated at 3–6.2%. In children born to HIV/HCV coinfecting mothers the vertical HCV transmission is as high as 15–36%.

Aim: To evaluate the incidence of mother-to-infant HCV transmission in children of HIV-infected mothers.

Patients/methods: 103 children born to HIV/HCV coinfecting mothers were studied from June 2001 to October 2008. The follow-up in children was from 6 months to 5 years 6 months. None of them was breast-fed. History of IVDU had 94/103 mothers. Following tests were performed in all children: anti-HCV antibodies (at the age of 4 weeks and above 12 months), GPT level (every 3 months of life), HCV-RNA in serum and peripheral blood mononuclear cells (at least twice in the first year of life). HCV-Ab and RT-PCR HCV were detected with ELISA III tests (Pointe) and Amplicor HCV, v2.0 (Roche), respectively. In case of PCR-HCV(+) or increased GPT level examination was repeated. HCV infection diagnosis was based on at least two positive PCR HCV results, one above 6 months of life.

Results: Maternal HCV-Ab were detected in 99/103 (96%) of children. HCV RNA was detected in 13/103 (13%) children, in 6 children spontaneous clearance of HCV-RNA was diagnosed, 7 were chronically HCV infected. HIV infection was diagnosed in

16 children, HIV/HCV coinfection was not confirmed in any cases.

Conclusion: • Children born to HIV/HCV coinfecting mothers require thorough diagnostics of vertical HCV transmission.

• Observation of children born to HIV/HCV(+) mothers should last at least 18 months after birth.

• The risk of vertical HCV transmission in children born to HIV/HCV(+) mothers is high (13%).

P19

Are recommended doses of efavirenz optimal in young children? (ANRS 12103)

Déborah Hirt^{1,2}, Saïk Urien², Mathieu Olivier⁴, Hélène Peyrière⁴, Boubacar Nacro⁵, Serge Diabougou⁶, Emmanuelle Zouré⁵, François Rouet⁶, Hervé Hien⁶, Philippe Msellati⁷, Philippe Van de Perre^{8,9} and Jean-Marc Tréluyer^{3,1}

¹Université Paris-Descartes, Paris, France

²Hôpital Tarnier, Paris, France

³Hôpital Cochin-Saint Vincent de Paul, Paris, France

⁴Hôpital Lapeyronie, Montpellier, France

⁵CHU Sourô Sanou, Bobo Dioulasso, Burkina Faso

⁶Centre Muraz, Bobo Dioulasso, Burkina Faso

⁷Université Paul Cézanne, Aix en Provence, France

⁸Hôpital Arnaud de Villeneuve, Montpellier, France

⁹Université Montpellier I, Montpellier, France

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Background: Pediatric studies suggested that the actual recommended efavirenz dosage produced insufficient plasma concentrations in children. In the context of a phase II trial on once-a-day pediatric HAART, the aims of this study were to describe efavirenz concentration-time courses in treatment naïve children, to study the effect of age and bodyweight on efavirenz pharmacokinetics and to test relationships between doses, plasma concentrations and efficacy.

Methods: Efavirenz concentrations were measured in 48 children after 2 weeks of didanosine – lamivudine – efavirenz treatment, and samples were available in 9/48 children between month 2 and 5 of treatment. A total of 200 efavirenz plasma concentrations were collected and a population pharmacokinetic model was developed with NONMEM. The influence of individual characteristics was tested using a likelihood ratio test. Estimated minimal (C_{min}), maximal (C_{max}) concentrations, area under the curve (AUC) were correlated to the decrease in HIV-1 RNA levels after 3 months of treatment. The threshold C_{min} (and AUC) improving efficacy was determined. The target minimal concentration of 4 mg/L was considered for toxicity. An optimized dosing schedule was simulated in order that the higher percentage of children is in the effective and not toxic concentrations interval.

Results: Efavirenz pharmacokinetics was best described by a one-compartment model with first order absorption and elimination. Mean efavirenz apparent elimination clearance and volume of distribution were respectively 0.21 l L/h/kg and 4.48 L/kg. The elimination clearance significantly decreased with age. With the recommended doses given to 46 out of the 48 children, 19 % had a minimal concentration below 1 mg/L; they were 44 % under this limit in the less than 15 kg children. A significant higher percentage of children with $C_{min} > 1.1$ mg/L (or AUC > 51 mg/L.h) had a viral load decrease greater than 2 log₁₀ copies/mL

after 3 months of treatment, compared to children below these values.

Conclusion: To optimize the percentage of children with a C_{min} between 1.1 and 4 mg/L, children should receive the following once daily efavirenz dose: 25 mg/kg from 2 to 6 years, 15 mg/kg from 6 to 10 years and 10 mg/kg from 10 to 15 years. These assumptions should be prospectively confirmed.

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Didanosine population pharmacokinetics in West African HIV-infected children administered once daily tablets in relation to efficacy after one year of treatment (ANRS I2103)

Déborah Hirt^{1,2,6}, Christophe Bardin⁷, Serge Diabougoua⁵, Boubacar Nacro⁴, Hervé Hien⁵, Emmanuelle Zouré⁴, François Rouet⁵, Adama Ouiminga⁵, Saïk Urien², Vincent Foulongue⁸, Philippe Van de Perre^{8,10}, Jean-Marc Tréluyer^{2,3} and Philippe Msellati⁹

¹Université Paris Descartes, Paris, France

²Hôpital Tarnier, Paris, France

³Hôpital Cochin-Saint Vincent de Paul, Paris, France

⁴CHU Sourô Sanou, Bobo Dioulasso, Burkina Faso

⁵Centre Muraz, Bobo Dioulasso, Burkina Faso

⁶Hôpital Lapeyronie, Montpellier, France

⁷Hôtel Dieu, Paris, France

⁸Hôpital Arnaud de Villeneuve, Montpellier, France

⁹Université Paul Cézanne, Aix en Provence, France

¹⁰Université Montpellier 1, Montpellier, France

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Background: Didanosine is a potent nucleoside reverse transcriptase inhibitor used for HIV infection treatment. A once daily administration of chewable/dispersible didanosine tablets (Videx[®]) was available for children in Burkina Faso but few pharmacokinetic data were reported with these galenic form and administration scheme. This study is part of a phase II trial on once-a-day pediatric HAART. The objectives were to describe

didanosine pharmacokinetics and to establish relationships between doses, plasma concentrations and treatment efficacy in children.

Methods: Didanosine concentrations were measured in 40 children after 2 weeks and in 9 children after 2 to 5 months of didanosine lamivudine efavirenz combination. A total of 166 didanosine plasma samples were measured using an HPLC assay with detection by UV absorbance. A population pharmacokinetic model was developed with NONMEM. The link between maximal concentration (C_{max}), area under the curve (AUC) and the decrease in HIV-1 RNA levels after 12 months of treatment was evaluated. The threshold AUC and C_{max} improving efficacy were determined and an optimized dosing schedule was simulated.

Results: Didanosine pharmacokinetics was best described by a one-compartment model with first order absorption and elimination. Mean population pharmacokinetic estimates with the corresponding inter-subject variabilities (%) were: apparent elimination clearance $CL/F = 146$ L/h (90%) and apparent volume of distribution $V/F = 356$ L (122%). CL/F and V/F were increased, probably due to a lower bioavailability with tablets than with pediatric powder. Clearance increased with body surface area. The decrease in viral load after 12 months of treatment was significantly correlated with didanosine AUC and C_{max} ($p \leq 0.02$) during the first weeks of treatment. An AUC > 0.85 mg/l.h was significantly linked to better viral load decrease (3 vs. 2.4 \log_{10} copies/ml, $p < 0.03$) and higher percentage of children with undetectable viral load after one year of treatment (95% vs. 68%, $p = 0.056$). Four children developed viral resistances to didanosine before one year of treatment, all of them had an AUC < 0.85 mg/L.h. A $C_{max} > 0.4$ mg/L was also correlated to a better viral load decrease and to the absence of viral resistance to didanosine.

Conclusion: A 360 mg/m² ddl dose administered as tablets should be a more appropriate dose to improve efficacy than 240 mg/m² in these children. However data on adverse events with this dosage are missing.