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Mechanisms underlying reductions in mother-to-child transmission of human immunodeficiency virus type-1 by short-course antiretrovirals

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2007-02-14T10:55:17Z
http://hdl.handle.net/10539/1975

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ABSTRACT

Knowledge of the timing of mother-to-child transmission (MTCT) of HIV-1 is an important issue in reducing the risk of infant infection. Prior to giving birth therefore an HIV-1 positive mother should be provided with anti-HIV-1 drugs (antiretrovirals) during the shortest time possible to ensure both efficacy and minimal toxicity of the antiretrovirals to the newborn. However, in the absence of timeous administration of nevirapine (NVP) or zidovudine (AZT) to the mother at the onset of labour, infants are given post-exposure prophylaxis (PEP). Despite antiviral prophylaxis some infants still become infected. In an attempt to mimic the *in vivo* scenario we investigated, in Chapter Three, the replication ability of a primary isolate (M502L) in peripheral blood mononuclear cells (PBMC) isolated from healthy donors exposed to different concentrations of NVP or AZT either prior to or post-infection, but that reflected mean neonatal plasma concentrations measured following maternal dosing. In phytohaemagglutinin (PHA) stimulated cultures M502L exhibited some growth. Maintaining NVP and AZT in the culture medium resulted in decreased viral growth over time. In contrast to that expected certain donors demonstrated elevated p24 antigen levels in the presence of HIV-1 and NVP or AZT. This suggested that cells were more conducive to HIV-1 replication either because of cellular activation or due to cellular production of cytokines/chemokines. The *in vitro* study highlighted (i) the differential permissiveness of cells from different donors for HIV-1 infection, (ii) different abilities of antiretrovirals (ART) to circumvent infection in different individuals and (iii) immunomodulatory effects of ART *in vitro*.

Commencing in Chapter Four we elected to investigate, *in vivo*, the immunomodulatory consequences of HIV-1 exposure and infection in two groups of HIV-1-exposed newborns whose mothers either received NVP at the onset of labour or who only received NVP as PEP within 72 hours of birth. Short-course antiretroviral drug regimens are known to reduce the risk of MTCT of HIV-1 but mechanisms affording protection of such interventions remain poorly defined. Since T-cell activation is an important factor in productive HIV-1 infection, we tested the hypothesis that single-dose NVP reduces immune activation, which in turn reduces the likelihood of transmission. We compared concentrations of cord and maternal blood plasma immune activation markers, neopterin, β_2-microglobulin (β_2-m) and soluble L-selectin (sL-selectin) in the two groups of HIV-1-

exposed newborns and among HIV-unexposed controls. *In utero* exposure of the infant to HIV-1, regardless of NVP exposure, led to demonstrable increases in levels of immune activation markers, this being most notable in the presence of pre-existing infection. Contrary to what was hypothesized, immune activation was increased by pre-birth exposure to single-dose NVP, with this effect being enhanced in infants already infected at birth. Our data suggest that reductions in immune activation do not explain transmission prevention effects of single-dose NVP. Our data also suggest a biological explanation for why HIV-1 infected infants exposed perinatally to antiretroviral drugs might experience hastened disease progression, namely that the immunological mileau in some HIV-1 infected individuals treated with NVP favours increased HIV-1 replication.

Cytokines and chemokines function to stimulate, or suppress cellular proliferation and differentiation and have unique immunomodulatory properties. Furthermore, they have the potential to protect against HIV-1 infection or to regulate HIV-1 replication. In Chapter Five we therefore questioned whether exposure to HIV-1 or NVP influences cytokine/chemokine levels of infants born to HIV-1 infected mothers. We compared levels of interleukin (IL)-7, IL-10, stromal cell-derived factor: SDF-1α (CXCL12), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein-1α: MIP-1α (CCL3), macrophage inflammatory protein-1β: MIP-1β (CCL4) and regulated upon activation, normal T-cell expressed and secreted: RANTES (CCL5) of the two groups of HIV-1-exposed newborns and among the HIV-unexposed controls. HIV-1 exposure in the absence of single-dose NVP was not found to impact significantly on the levels of IL-7, IL-10, GM-CSF, CXCL12, CCL3, CCL4 or CCL5 and single-dose NVP had no appreciable effect on these cytokine/chemokine levels. Cord blood plasma levels of IL-7, CXCL12 and GM-CSF were found to be independent of mothers' levels. Single-dose NVP reduced the ability of cord blood mononuclear cell (CBMC) to produce GM-CSF spontaneously. Maternal and infant (HIV-1 exposed NVP unexposed) IL-10 levels were significantly correlated. Significantly elevated levels of IL-10 were associated with pre-existing infection in NVP unexposed newborns. CCL3, CCL4 and CCL5 levels in NVP unexposed uninfected infants were not different from those of control infants but correlated significantly with IL-7 levels.

HIV-1 specific cellular immune responses are elicited in a proportion of infants born to HIV-1 seropositive mothers and have been associated with protection from maternal

HIV-1 transmission. In Chapter Six, levels of the immune activation markers neopterin, β_2-m, sL-selectin, the immunomodulatory and haematopoietic factors IL-7, CXCL12, GM-CSF and the immunoregulatory cytokine IL-10 were examined amongst the group of newborns, that received NVP as PEP within 72 hours of birth, of which a proportion had specific cellular responses to HIV-1 envelope (Env) peptides. It was our aim to determine in infants that elicit HIV-1 specific cellular immune responses (Env^+) and those that lack the specific responses (Env^-), whether these factors could predict transmission and whether the former group of infants exhibit unique immune features that might distinguish them from Env^- non-responders. Our data suggested that none of the factors tested were predictive of HIV-1 transmission but confirmed that infants with cellular responses to HIV-1 envelope peptides were associated with lack of subsequent infection. In particular, our data demonstrated an association between HIV-1 specific cellular immune responses, lower maternal viral load and lack of infection suggesting that sustained exposure to antigen (reduced maternal viral load) may be responsible for the strong priming effect. Furthermore, an association between reduced GM-CSF levels and the presence of HIV-1 specific responses was demonstrated, which suggested therefore that newborn infants that elicited HIV-1 specific cellular immune responses exhibited different immune capabilities from those without responses.

Finally, in Chapter Seven we looked at how immune activation and priming impact on thymic output of T-cells in newborn infants. Unfortunately, sample volumes of the two groups of HIV-1-exposed newborns used in the previous three Chapters became limited with the result that we chose to address these questions using anonymously collected cord blood samples from infants, some of which were used to supplement the placebo group of the the UNAIDS-sponsored clinical trial of short-course zidovudine-lamivudine (AZT-3TC). At the time the AZT-3TC trial was conducted short-course antiviral prophlyaxis was not the standard of care for the prevention of MTCT of HIV-1. The thymus is known to be essential for establishing diversity of the T-cell pool, and morphological thymic changes and effects on naïve T-cells and T-cell receptor excision circle (TREC) concentrations have been reported in studies of HIV-1 infected children and adults. As it is not known to what extent *in utero* exposure to HIV-1 and infection affects T-cell division in newborn infants, we elected to determine TREC levels of infants born to HIV-1 seropositive mothers that were not exposed to antiretrovirals. The impact of increased immune activation on TREC levels and the consequence of HIV-1 exposure or infection

on circulating levels of IL-7 (raised levels indicative of T-cell depletion) was also investigated. HIV-1 exposure or infection did not result in significant losses of TREC. TREC levels were not affected by immune activation associated with HIV-1 exposure and infection and IL-7 levels were not raised. Infants that elicited HIV-1 specific cellular immune responses exhibit TREC levels that were similar to those of infants without HIV-1 specific responses. These data suggested that newborn infants of HIV-1 seropositive mothers demonstrated no altered thymopoietic ability compared to control infants. Furthermore, HIV-1 specific immune responses, (indicative of post-thymic memory T-cell expansion) did not influence thymic output measured in newborn infants.

In conclusion, the *in vitro* study demonstrated that there is a high degree of variability between PBMC isolated from different donors with respect to viral replication and drug effectivity which suggests that these phenomena are likely to exist within patient (infant as well as adult) populations. While immune activation is considered central to productive infection we demonstrated that immune activation is increased by HIV-1 exposure and by single-dose NVP. Exposure to HIV-1 alone or with NVP did not influence birth levels of IL-7, IL-10, CXCL12, GM-CSF, CCL3, CCL4 and CCL5. Furthermore, levels of these factors did not predict infection outcome in the infant. Immune activation and haematopoietic growth factors are modulated independently of the mother but maternal factors such as IL-10 and exposure to single-dose NVP, which reduces responsiveness of CBMC, could impact on the infant. HIV-1 specific cellular immune responses at birth, which are elicited in a proportion of infants born to HIV-1 positive mothers, are of immunological significance and can predict lack of subsequent infection. Disturbances in thymic output are not readily detectable at birth when using TREC to assess *de novo* T-cell synthesis, alternatively there is a homeostatic balance between thymic output and peripheral T-cell proliferation in newborns of HIV-1 infected mothers. Overall our data suggests that (i) there are immune consequences of being born to an HIV-1 positive mother, (ii) short-course antiretroviral prophylaxis does impact on the developing immune system of the infant and (iii) while the direct effects of single-dose NVP are not disputed, there are indirect consequences of NVP exposure on immune cells. Despite the consequences of HIV-1 exposure or the result of being born to a HIV-1 seropositive mother or exposure to single-dose NVP, our data proposes that the immune system of newborn infants is capable of responding as demonstrated by the enhanced immune activation. It remains important to determine the correlates of immune protection for the

development of novel immuno-therapeutic and vaccine strategies and maternal-infant transmission of HIV-1 provides a model which can address questions of protective immune processes. Understanding the influence of antiretrovirals on immune processes remains an important component of the drug mechanisms, (aside from their direct antiretroviral activity), that may underlie reductions in maternal-infant transmission of HIV-1. Furthermore, how antiretrovirals influence immune processes and immune development (together with exposure to HIV-1/consequences of being born to an HIV-1 seropositive mother), may impact on subsequent immune responsiveness to infectious organisms or childhood vaccines.