CHAPTER 3

CONCLUSION AND PERSPECTIVES

1. Conclusion

HIV/AIDS continues to be a major health problem throughout the world, with more than 60 million HIV-infected cases and 25 million deaths recorded since the first recognized cases of AIDS were reported in 1981 [183]. Despite the number of people in low- and middle-income countries, access to ARV treatment has increased 10-fold in only six years since December 2003, as a result of many countries being involved in the "3 by 5" initiative to provide the treatment necessary to at least 3 million [184]. However, the AIDS pandemic killed an estimated 2.1 million people, including 290,000 children in 2007 [184]. It is estimated that over 6,800 people were newly infected by HIV everyday, most of them being women of childbearing age who transmit the infection to their offspring at a rate of approximately 1,200 cases per day [184].

MTCT is the leading source of HIV infection in children. The transmission may occur during pregnancy (*in utero*), labor and delivery (intrapartum) or after birth, through breastfeeding. Maternal NAbs can cross the placental barrier into the fetal bloodstream, reaching high levels in the fetus at the end of pregnancy and protecting the infant against infection with numerous pathogens [119; 349]. HIV-specific NAbs can prevent either cell-free virus spread by blocking virus entry into the target cells or by killing the infected cells through complement activation or ADCC [12; 14], but their exact role during natural infection is not precisely known. Most HIV-infected individuals rapidly develop NAbs to their autologous HIV strain, typically within two months of infection in natural HIV-infection, [132; 152; 343]. However, those NAbs usually lack broad reactivity and can be overcome by the emergence of neutralization-escape mutants. Several studies have shown that non-transmitting mothers have more frequently detected and higher levels of NAbs responses than transmitting mothers [358; 227; 47; 156; 32], and it seems that the viruses transmitted intrapartum are escape variants resistant to maternal autologous NAbs [109; 410; 432].

In a previous study, we hypothesized that the presence of broadly cross-neutralizing, heterologous NAbs would be indicative of protection towards intrapartum HIV transmission [32]. In that study, our lab identified an association between higher titers of NAbs against a CRF01_AE primary isolate, MBA, and lower rates of intrapartum transmission. However, only one isolate per HIV-1 clade was used at that time.

In our personal present work, we have confirmed the data observed in that previous study. Indeed, our results showed an association between high titers of maternal NAbs against MBA and a lower rate of MTCT of HIV, specifically for intrapartum transmission. We conducted the analysis in a different Thai population of 45 non-transmitting and 45 transmitting mothers selected on highly stringent criteria, and using six primary isolates. Three of these primary isolates were of the predominant clade in Thailand, CRF01_AE and the other three were of the less prevalent clade B. They were representative of the different co-receptor usage phenotypes. No association between NAbs and MTCT was found for the three B strains and for two of the CRF01_AE strains (LEA and C1712). In contrast, non-transmitting mothers had significantly higher NAbs against the MBA strain than the transmitting mothers. The higher levels of NAbs against the MBA strain were also significantly associated with lower rates of intrapartum transmission, but not *in utero* transmission. Therefore our results still suggest that NAbs able to neutralize the MBA strain might be indicators for protection.

We then tried to identify the specific properties of the MBA strain that might explain this association. We found that MBA showed an exceptionally long V2 domain of 63 amino acids including 6 PNGS that might contribute to render MBA more resistant to neutralization when compared to the other two CRF01_AE isolates, LEA and C1712. We compared the neutralization profiles of pseudotyped viruses expressing either wild-type Envs of MBA or LEA or a chimeric Env containing the V2 domain of MBA in a LEA Env backbone. The results demonstrated that the V2 region of MBA increased the resistance of LEA to neutralization by 6 of the 10 tested sera, indicating that the unusual V2 domain of MBA contributed certainly to its neutralization properties, but probably was not the single envelope region involved.

Therefore our findings confirm that NAbs to some viral isolates might be indicators of protection, at least in the MTCT context. This is one more hint that antibodies to HIV are not completely impotent. They also confirm that the V2 region is an important component for sensitivity to neutralization of the HIV-1 envelope glycoprotein

In the second part of our work, we confirmed through the most extensive study that was done in this context to our knowledge, that a genetic bottleneck occurs in MTCT of HIV. Despite the presence of a complex viral population in the mother, only a restricted subset of viruses is transmitted to the infant, regardless of whether transmission occurs *in utero* or intrapartum. We analyzed 353 sequences encompassing almost the entire gp120 *env* gene from 17 mother-infant pairs infected with HIV-1 CRF01_AE strains. We found no difference both in the length of the V1-to-V5 region and number of PNGS of HIV-1 gp120 *env* gene between the viruses of the mothers and infants. This was slightly different from the findings of a recent study that focused on a breastfeeding population, in which the V1-to-V5 lengths of the envelope did not differ between infants and mothers, but fewer PNGS were found in viruses from infants than in those from their mothers infected with viruses of various subtypes, A, C, or C/D and D/A recombinants [432]. However, we identified two PNGS, at positions N301 in V3 and N384 in C3, that had a high degree of conservation in infant viruses, but were significantly less conserved in the maternal

viruses. This suggests that these PNGS may confer a selective advantage on the virus to be transmitted, at least for CRF01_AE viruses, possibly allowing escape to antibody-mediated neutralization.

Moreover, we described for the first time two cases suggesting that recombination probably contributed to adaptation of HIV-1 to its environment to be successfully transmitted from mothers to their infants. Our data showed that the recombinant viruses transmitted to the infants for both pairs harbored V1-V2 and V3 regions issued from different parental sequences. This suggests that, not only V1-V2 region nor V3 region alone, but both of them act additionally or synergistically to provide a specific advantage in selection of the transmitted variant. In addition, it was previously reported that the C2 region of the HIV-1 *env* gene contains a hot spot for recombination [29; 135]. Our data confirmed, in natural *in vivo* conditions, a hot spot for recombination in the C2 region of *env*, and additionnally suggest another hot spot for recombination in the C3 region.

In our study, we analyzed the role of maternal NAbs in MTCT of HIV-1 and we dissected the molecular characteristics of HIV-1 envelope glycoproteins of the CRF01_AE viruses preferentially transmitted from mothers to their infants. The MTCT of HIV is a unique situation in which exposure of the infants to the virus occurs in the presence of passively transferred pre-existing maternal antibodies. It mimics the situation in which exposure to HIV-1 might occur after passive immunization or vaccination. Therefore studies aiming at dissect either the role of maternal NAbs or the molecular characteristics of HIV-1 envelope glycoproteins from viruses transmitted through MTCT of HIV-1, could allow identification of correlates of protection or surrogate markers of protection, and could provide key elements helping in finding ways to prevent HIV infection through vaccination or passive immunization.

2. Perspectives

Our observations of both the role of maternal NAbs and the molecular characteristics of HIV-1 envelope glycoproteins in MTCT of HIV-1 have brought additional knowledge in the context of the perinatal transmission of HIV-1 CRF01_AE strains. To elucidate the specific molecular characteristics of HIV-1 envelope glycoproteins, and to confirm the selective transmission in MTCT of HIV-1 by neutralization resistant strains, we will further test autologous neutralization activity of several selected clones with particular characteristics, trying first to elucidate the role of the two PNGS at positions N301 and N384. To check whether both V1-V2 and V3 regions provide a specific advantage in selection of the transmitted variant in MTCT of HIV-1, we will focus on the informative clones of the intrapatient recombinant (and parental) envelope glycoproteins that we identified in two mother-infant pairs. Pseudotyped viruses harboring the informative envelopes will be generated and their neutralization sensitivity as well their biological properties (infectivity, replicative capacity, binding to receptor and co-receptors, …) will be compared.

Furthermore, the HIV-1 gp120 *env* genes of variants obtained from sequential samples of the infected infants during several months or years after the first time diagnosis of infection will be analyzed in depth. This additional study should allow to identify both the rate and the mechanisms of viral evolution in infected infants.