Pediatric HIV-1-infection: perspectives on vaccination strategies and immune reconstitution during long-term antiretroviral therapy
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Chapter 9

Discussion
Before 1997 an estimated 23% of HIV-1-infected infants developed AIDS before the age of 1 year, and nearly 40% by 4 years of age. Ten percent died before reaching 1 year of age and 28% before the age of 5 [1]. In 1996 HAART was introduced in the treatment of HIV-1 infected adults [2], a year later combination antiretroviral therapy was also introduced in the treatment of pediatric HIV-1 [3]. Treatment with HAART has substantially improved survival and quality of life for HIV-1-infected children [4,5]. The aim of this thesis is to define the long-term efficacy of HAART to suppress HIV RNA production and improve immunity and vaccination responsiveness in pediatric HIV-1-infection.

In this thesis, it is shown that HAART can be as effective in children as in adults. HIV replication can be successfully suppressed in children and immune reconstitution, defined as an increase in CD4+ T cell count and T-cell proliferation, occurred normally. However, despite HAART, the in vivo function of the immune system may not completely restore, as reflected by primary and secondary vaccine failure, continued replication of CMV and the prolonged presence of a high EBV DNA load in both B and non-B lymphocytes in many HIV-1-infected children.

**Virologic response to HAART**

In Chapter 2, a long-term follow-up study of 2 NRTI’s and nelfinavir is described. Virologic success was achieved in 74%, 66%, 58%, and 54% after 48, 96, 144, and 240 weeks after start of HAART, respectively. During a follow-up of almost 7 years a growing number of children failed to suppress HIV-1 replication while medication was prescribed. From these data it seems likely that all children will eventually fail on the regimen started and they will need to switch to other combinations of antiretroviral drugs. To date, studies on the efficacy of HAART in the treatment of HIV-1-infected children have reported highly variable response rates. Virologic success, defined as plasma HIV RNA below the lower limit of detection, was reached in 11% to 87% of the children [6-8]. Moreover, time of follow-up in most of these studies was limited to less than 1 to 2 years after initiation of HAART.

Treatment with HAART does not eradicate the virus and cure the HIV-1 infection [9]. Chronic use of antiretroviral medication is indispensable when started for well-defined clinical or immunologic reasons. The consequences of the long-term use of antiretroviral therapy in adults and children are becoming apparent [10]. Lipodystrophy [11], atherosclerosis, cardiovascular events [12], low bone mineral density and bone metabolism impairments [13] are among the most commonly reported side-effects. Chapter 2 reports on eleven children who developed clinically evident lipodystrophy during treatment with a nelfinavir-containing regimen. Regimens should be tested for these side effects and the role of these drugs in child development should be considered when choosing a regimen.

In suboptimal suppression of the virus, HIV-1 strains are selected that carry mutations which make the virus less sensitive to the antiretroviral drugs. This could lead to virologic failure and the CD4+ T-cell count may again decline. Suboptimal suppression in HAART-treated patients is correlated with non-adherence to the very strict regimens
Sustained suppression of HIV-1 can be improved by improving the patient’s compliance. Lower pill burden and a lower frequency of intake could help to improve the adherence to the antiretroviral medication. In 1997 patients had to take their medication three times a day. Once daily regimens are now available. Further efforts should be made to increase the compliance and to prolong the virologic suppression thereby saving antiretroviral-drugs regimens for future use.

As has been described in Chapter 3, treatment with an alternative, newer regimen containing an NNRTI instead of a PI, resulted in potent suppression. The combination of drugs in this regimen is assumed to cause fewer side effects and can be administered once daily. The study showed virologic failure free survival rates of 76% and 67% after 48 and 96 weeks. An increasing number of the children developed resistance during the previous PI-containing regimen. Virologic suppression in HAART pre-treated children was equal to that in children who started naive for antiretroviral drugs. Recently it was shown that in children who switched their regimen from PI-containing to PI-sparing HAART, NNRTI treatment can still be effective in continued HIV-1 suppression [17]. Our study extends these data and shows that children failing on their primary HAART regimen can be successfully treated with an alternative NNRTI-containing 2nd-line regimen.

HAART in children is assumed to be less effective than in adults. The higher pVL could partially explain the reduced virologic effectiveness of HAART at young age. Viral decay after starting HAART is used as a measure for the viral turnover during HAART [18,19]. In Chapter 4, we described a correlation between pVL and age. In contrast, no correlation between viral decay and pVL or age was found upon start of HAART.

From these analyses we conclude that the pVL in young children was not higher as a result of a higher viral turnover. Other possible explanations include the immature immune system at young age, having more target cells available for HIV-1 infection [20]. Alternatively, compartmentalization could explain the differences between young children and older HIV-1-infected individuals. Gastrointestinal mucosa (gut-associated lymphoid tissue [GALT]) is an initial and principal site for viral replication during acute HIV-1 infection [21]. If the pVL only reflects the activity of the virus in the lymphoid system, it could be that the higher pVL reflects a higher concentration of RNA. This is produced at the same rate as in adults, but in relatively more lymphoid tissue per kg body weight compared to the plasma volume. However, this hypothesis is difficult to explore since these compartments are hard to sample in children, mainly for technical and ethical reasons. Finally, other cells than the usual target cells, not susceptible for infection and entry of HIV-1 (any longer) in adults, may be involved in the increased pVL and rapid dissemination of HIV-1 in children.

**Growth and CD4+ T cell count during HAART**

When untreated or failing on HAART, HIV-1-infected patients show continuous viral replication and subsequent loss of CD4+ T cells, which may cause a severe immunodeficiency with opportunistic infections as a consequence. Most children that are diagnosed with an HIV-1 infection suffer from opportunistic co-infections and
show growth retardation. In a minority of these children severe failure-to-thrive can be observed [1,22]. CD4+ T cell count and growth retardation are predictors for the progression of the disease in children [23,24]. Treatment with HAART has a major impact on these parameters. During a follow-up of 5 years, described in Chapter 2, both numerical immunologic recovery and clinical improvement in growth and development occurred in all, mainly during the first years after initiation of HAART, even in children that failed to sustain viral suppression. This is in line with recent findings that CD4+ T cell numbers reached a plateau after 2 years of HAART in HIV-1-infected children [25].

In Chapter 3, no difference was found in growth parameters between children that started naive to antiretroviral drugs and children that were not pretreated with other regimens, other than the preexisting differences at baseline. This was due to the effect of viral suppression achieved during the prior use of HAART [26]. This study also showed that children starting HAART, clearly showed an increase in weight-for-height in the first 24 weeks. The increase in height-for-age became more obvious after the first year of treatment.

From these chapters it can be concluded that HIV-1-infected children treated with HAART are able to suppress the virus effectively. The concomitant catch-up growth and increase in CD4+ T cell numbers is present in all children on HAART, irrespective of eventual virologic failure during follow-up. However, it is unknown whether the increase in CD4+ T cells or the absolute number of certain lymphocyte subsets correlates with or reflects a full recovery of the immune system in HIV-1-infected children.

Immune response in children
Vertical HIV-1-infection occurs at a time when the functional capacity of the infant’s immune system is still reduced because of immaturity. At birth infants have a relatively high number of T cells, but the lymphocytes are mainly naive in function and phenotype. An immune response is mounted upon encountering antigens from the environment or antigenic triggers during infection. With increasing age the absolute numbers of CD4+ T cells decline towards adult values and undergo phenotypic changes that reflect previous activation and acquisition of a memory effector function in vivo [27,28]. In children the thymus is still functional, which may account for the ability of rapid immune reconstitution at young age. During adolescence the thymus starts to shrink. The production of new T cells in adults is reduced compared to children, although it does continue throughout life at a very low level [29]. Adults become HIV-1-infected at an age that the immune system is fully developed. In contrast, most children become HIV-1-infected before childhood infections have triggered and shaped the immune system. The impact of early infections on the immunologic development has hardly been studied.

Vaccination in HIV-1-infected children
Varicella-zoster virus (VZV) infection in HIV-1-infected patients can cause severe chickenpox with major morbidity and mortality. In the era before HAART became available, a high proportion of the patients had to be hospitalized after contracting VZV [30,31]. HIV-1-infected individuals are recommended to be immunized with the live-
attenuated VZV vaccine if seronegative or without a clinical history of prior chickenpox [32]. However, when the patient is severely immunocompromised by HIV-1-infection vaccination against VZV is contra-indicated, because of the potential for dissemination of the attenuated vaccine strain [32]. However, the *in-vitro* T-cell proliferation restores during the first weeks after start with HAART, as shown in Chapter 2. This indicates a functional reconstitution of the immune system. Previously immunocompromised HIV-1-infected children were therefore vaccinated against VZV during treatment with HAART. The children were closely monitored for clinical symptoms as well as the presence of VZV-vaccine DNA load in their blood. In an attempt to improve protection, a family-based vaccination strategy was initiated. All VZV-seronegative household members were offered active immunization to reduce the chance of household contact to wild-type VZV for the HIV-1-infected children.

VZV vaccination is safe in HIV-1-infected children during HAART, as was shown in chapter 5. The only report on VZV-vaccination in HIV-1-infected children restricted inclusion of eligible VZV-seronegative children to the mildly affected HIV-1-infected children (i.e. CDC-N0 and -A1) [33]. Based on this report, it was stated that HIV-1-infected children who still have a well-maintained immune system, can be safely immunized [32]. We extend these data to HIV-1-infected children previously diagnosed with CDC-C. As shown in our VZV vaccination study, immunization with the live-attenuated VZV-Oka strain seems a safe procedure and should be considered in children on HAART.

However, the immune response upon vaccination is not comparable to healthy children. Only 60% of the HIV-1-infected children seroconverted after 2 vaccinations, compared to 100% of the healthy siblings. In contrast to VZV-antigen-specific humoral responses, we could not find a difference in the cellular response between HIV-1-infected children and their healthy siblings. The function of CD4<sup>+</sup> T cells and CD19<sup>+</sup> B cells after immune recovery during HAART seemed not comparable to the function of these cells in healthy children. The suboptimal response upon vaccination despite immune reconstitution raises the question about the durability of the serologic protection of these children after natural infection and vaccination.

**Immunologic memory in HIV-1-infected children**

In Chapter 6 it was shown that specific antibodies to the viral components of the MMR vaccine were gradually lost in HIV-1-infected children, even during treatment with HAART. Moreover, also the long-term serologic protection after natural VZV infection in HIV-1-infected children declined over time. In a median of 161 weeks 21% of the children lost their protective VZV antibodies. MMR specific IgG titers waned over time in 40% of the children, as did the serology against CMV in 7% of the children.

Upon recovery of the immune system, antigen specific immune reactivity remains strongly reduced compared to HIV-negative controls. Moreover, the CD4<sup>+</sup> T cells are apparently also not able to maintain stable and protective serologic levels as has been reported in healthy children. Comparison of the MMR serology with specific antibodies against herpes viruses after natural infection shows that many HIV-1-infected children
may lose antibodies against certain antigens. This was not a general observation, because the EBV antibodies were not lost in any of the children during HAART.

From our data it remains unclear whether CD19⁺ B cells are affected in their specific responsiveness by HIV-1 in a direct way or indirectly by the loss of CD4⁺ T cell helper activity. It had been shown before, that the perturbations in B cell responsiveness were not only due to impaired CD4⁺ T cell help, but also intrinsic to the detrimental changes in the B cell compartment itself [34]. The impaired response of B cells upon stimulation correlated to pVL. Reduction in pVL was shown to improve B-cell responses upon various stimuli in vitro [35]. Direct T-cell responses seem to be comparable to those in healthy children. This would explain the lack of severe clinical reactions after administration of the live-attenuated VZV-Oka strain and the decline in opportunistic infections in the era of HAART [4,5]. However, even during treatment with HAART, both primary and memory humoral responses [36] remain disturbed as shown by our studies in Chapter 5 and 6. Also under continuous use of HAART, regular testing and revaccination, seems mandatory though.

The role of CD4⁺ T cells during primary infection in helping to establish immunologic memory of both CD8⁺ T cells and CD19⁺ B cells seems crucial. In HIV-1 related research much attention has been focused on the role of cytotoxic T cells. This can be explained by the finding that after primary infection the decline in pVL [37-39] and CMV in whole blood [40] is paralleled by an increase in HIV-1-specific CD8⁺ T cells. Additionally, patients progressing to AIDS show a decline in HIV-1 specific CD8⁺ T cells. Apart from the disturbances in CD8⁺ T cell function it is again becoming clear that also the B cell function is affected by the loss of CD4⁺ T cell help or by a direct effect of the virus to these cells [41,42].

While the short-term restoration of the immune function seems to largely protect HIV-1-infected persons from major opportunistic complications of advanced disease, the functional immune restoration and immunologic fine-tuning is incomplete and the long-term significance of this sub-clinical immune deficiency remains as yet unclear. At the same time, there is uncertainty regarding the optimal timing for the initiation of HAART, in children in particular. The age and concomitant exposure to various primary viral infections and bacterial colonization make the decision on the start of lifelong use of antiretroviral drugs difficult. The doubts about long-term side effects during development and growth will not make such decisions any easier.

**Clinical implications**

Measles, mumps and rubella are among the viruses that could be a threat to adults as well. Especially in pregnant women these infections may have devastating consequences. Moreover, mothers pass their IgGs to their newborn. These IgGs protect in the period that the infants are not yet able to vigorously fight these infections by themselves due to their immature immune system. The extent and time in which the loss of serologic memory to these viruses may occur in adult HIV-1-infected patients, is not exactly known. Memory to other vaccine-preventable diseases such as influenza virus and
bacterial strains such as *Haemophilus influenzae* type b and pneumococcal infections in HIV-1-infected patients treated with HAART is also unclear [43,44].

Our VZV vaccination study in HIV-1-infected children indicates that more extensive studies are warranted. Vaccination studies ought to be primarily focused on the induction of sufficient immune protection, but the level of protection and the need for repeated boosting should be checked regularly, both in larger cohorts of children as well as in adults. Testing for childhood virus infections and VZV vaccination of adult HIV-1-infected patients should be considered as routine procedure as brought forward by our pediatric studies in the current thesis. Additional caution is at place for migrating people from tropical areas to areas with more temperate climate, since these less exposed individuals[45] are more at risk of severe chickenpox [46] and should therefore be tested for VZV antibodies. Pregnant HIV-1-infected mothers should also be monitored for protective levels of MMR and revaccinated if needed.

**Immune reconstitution and latent herpes virus infections**

The impact of HIV-1-infection on the immune reactivity in children was explored for two latent herpes viruses, CMV and EBV. CMV was a marker for HIV disease progression in children infected at birth [47]. Even in the era of HAART, detection of CMV identified patients with a poor prognosis [48]. Immune activation was shown to be associated with HIV disease progression in the era before HAART [49]. In Chapter 7 it was shown that CMV has a great impact on the numbers of differentiated T cells in HIV-1-infected children. During immune reconstitution the number of terminally differentiated CD8$^+$ T cells was increased. Some of the children showed continuous replication of CMV during HAART, as reflected by CMV shedding in the urine. These children had more terminally differentiated CD8$^+$ T cells and an increase in CMV-specific IgG. Regarding CMV-specific functional reactivity of the T lymphocytes, we demonstrated that T cells showed less IFN-$\gamma$ production upon stimulation in vitro. It seems that the immune system was not able to contain the virus, resulting in replication, and increased antigen exposure, elevated numbers of CD8$^+$ T cells and high titers of CMV-specific IgG.

Before the start with HAART, the EBV DNA load was elevated in HIV-1-infected children, as shown in Chapter 8. An elevated EBV load is associated with the development of lymphoproliferative disease after bone marrow or solid organ transplantation [50]. Similarly, HIV-1-infected individuals are at increased risk of lymphoproliferative disorders [51-54].

EBV loads in children treated with HAART remained elevated for years in most children. Only some children suppressed the EBV DNA load below the lower limit of detection for as yet unknown reasons. The elevated levels of EBV DNA in whole blood of HIV-1-infected children could be an explanation for the sustained serology by continued exposure to EBV antigen. Importantly, it seems that HAART did not restore the immunity against EBV, because of the observed failure to clear the viral DNA, as seen in otherwise healthy children. Also in adults after treatment with HAART, EBV
DNA remained detectable [55]. Loss of EBV-specific T cells and immune activation were found to correlate with the elevated EBV loads in HIV-1-infected individuals.

In healthy individuals EBV infects the oropharyngeal mucosa and subsequently CD19+ B cells in which it establishes life-long latency [56,57]. In our cohort we found that EBV can be detected both in CD19+ B cells as well as in CD4+ and CD8+ T cells. These findings in HIV-1-infected children raise questions about the interplay between HIV-1 and EBV. EBV also infects other blood cell types besides the B lymphocytes during chronic active EBV (CAEBV) and acute EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH) [58]. However, the presence of serious clinical symptoms and a high mortality rate characterize these EBV-related diseases [59], in contrast to chronic EBV infection in HIV-1-infected patients during HAART. Whether the balance of EBV tropism between B and T cell compartment defines the clinical symptoms remains unclear.

CMV and EBV remained present in most children during treatment with HAART. The long-term implications of this failure of the immune system to recover fully upon start of HAART are as yet unknown.

**HIV-1 vaccine**

A vaccine against HIV is now being developed to fight HIV-1-infection. Improvement or restoration of this CD4+ T-cell helper function could improve the effectiveness of these vaccines on the long term. Whether the incomplete recovery of CD4+ T-cell help as demonstrated in our studies in pediatric HIV-1-infected patients will limit the efficacy of HIV-1 vaccines has yet to be shown. The recovery may have little impact unless strongly boosted by adjuvants or enhanced immunogenicity of the vaccine to be supplied.

**Conclusions**

HAART has changed pediatric HIV-1-infection from an inexorably progressive and fatal disease into a chronic largely controllable disease. The number of opportunistic infections decline with major reductions in morbidity and mortality [4,5,60]. CD4+ T cell numbers increase to levels nearly comparable to otherwise healthy children. Although immunization with live-attenuated vaccines are safe, primary and booster vaccine responses are not fully restored during HAART. Also immunological memory functions are not restored upon start of HAART as demonstrated by the loss of specific antibodies as well as the fact that containment of latent viral infections are still affected in HAART-treated children. More research is needed to value the clinical significance of these observations.

**References**


