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

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Chapter 1

Introduction
Background

25 years ago, the first cases of the disease that came to be known as AIDS were reported in the Weekly Morbidity and Mortality Report of the US Centers for Disease Control [1,2]. Since then, 20 million people have died of AIDS and a further 40 million people have been infected with HIV-1, the virus that causes AIDS [3]. Human immunodeficiency virus (HIV) primarily infects T-lymphocytes and uses the cell machinery for its replication. After the production of new virions the cell releases the virions and new cells get infected. During the infection a high number of new virions is produced daily [4,5]. At the same time the CD4\(^+\) T cell numbers decline progressively. With declining numbers of CD4\(^+\) T cells the risk of opportunistic infections increases [6]. These infections are called AIDS-defining events. At that moment an infected individual is clinically diagnosed as having AIDS.

Replication of the virus can be blocked by antiretroviral therapy. By the end of 2005, 21 antiretroviral drugs were registered for adult treatment; 13 of these have been approved for the use in treatment of children. All the antiretroviral drugs interfere with the replication pathway of the virus. The drugs can be divided into 4 different classes based on their working mechanism. Given the high rates of viral replication and the frequency of mutations occurring during each replication cycle, drug-resistant viral strains may appear under the selective pressure of antiretroviral therapy [4]. This process can only be prevented by completely blocking viral replication. This is achieved by combining at least 3 drugs from 2 different classes. This combination is called Highly Active Antiretroviral Therapy (HAART). HAART is now the standard of care in the developed world and its use is being scaled up in developing countries. HAART is also used in children since 1997. A combination of at least two nucleoside reverse transcriptase inhibitors (NRTIs) with either a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) is recommended [7,8].

The number of CD4\(^+\) T cells increases after the start with HAART and subsequent inhibition of viral replication, both in adults and in children. This immune reconstitution decreases the chance of infection by opportunistic agents. As the cure of HIV-1 infection by total elimination of the virus is not achieved using this strategy, long-term treatment with HAART is indispensable.

Adherence to the regimen is an important prerequisite for successful treatment. Suboptimal levels of medication, after irregular intake, can result in resistance and treatment failure after which the regimen can no longer be used for successful suppression of HIV replication. Studies in both adults and children have shown that adherence is a major factor determining the degree of viral suppression achieved in response to antiretroviral therapy [9,10]. Poor palatability, dependency on others for medication and interference with normal social life are some of the factors that can negatively influence adherence in children. However, treatment of children poses additional difficulties. The majority of the children becomes infected during labor, delivery or lactation; they are infected before their immune system has fully matured. The plasma viral load of young children tends to be higher [11] and progression to AIDS is faster [6,12,13], in part because of the
immature immune system. This underlines the necessity of a robust, trustworthy and palatable regimen of antiretroviral drugs. Treatment in infants is difficult, because drug absorption, interactions and metabolism differ from older children, and higher doses may be required to achieve adequate drug levels. In the growing individuals the dose of the medication must be adjusted regularly to their increasing height and weight in order to maintain effective drug levels.

In adult studies viral efficacy of HAART ranged from 40-70% (below 50 copies/mL) to 55-80% successful suppression of the virus (below 400 copies/mL) after 1 year of HAART, depending on treatment, adherence and the definition of successful suppression used [14]. The viral efficacy of HAART is assumed to be less in children than in adults. Several studies were done to describe the effectiveness of HAART in pediatric HIV-1. Most studies describe a follow-up of 48 weeks or 96 weeks after the start with HAART. Long-term efficacy data are needed.

The pediatric Amsterdam cohort on HIV-infection (PEACH) was established in 1997 when HAART became available for children. PEACH is an ongoing prospective cohort of all children and adolescents under the age of 18, infected with HIV-1 who are treated and followed at the AMC hospital. This thesis describes analyses of data from this observational cohort. In Chapter 2 a twice-daily PI-containing regimen used for a median of almost 5 years is described. The virologic effectiveness and the immunologic response and growth of the children are studied.

Since new drugs have become available, new regimens can be proposed and applied. Recently, simplification of the regimen by reducing the number of pills and frequency of intake has become possible [15]. Drugs associated with long-term side effects such as lipodystrophy, osteopenia and blood lipid disturbances [16] can now be substituted with other drugs. These combinations are to be tested in children for their side effects as well as for their efficacy. Once-daily regimens have the potential of better compliance than twice-daily regimens. NNRTIs may have less long-term side effects than PI's. In Chapter 3 a once-daily NNRTI-containing regimen used for up to 2 years is described. Children previously treated with other antiretroviral medication and children that started this regimen naive to antiretroviral drugs were compared.

During untreated chronic HIV-1-infection virion production and degradation are in equilibrium, resulting in a relatively stable viral set-point. After starting HAART viral replication comes to a nearly complete stop. The decline of HIV RNA is used as a measure of the viral turnover before starting HAART. The cause of the high viral loads in young children has not yet been elucidated. To test the hypothesis that higher viral loads in children are caused by a higher viral turnover the decline of HIV RNA after the start with HAART was studied. The results are described in Chapter 4.

The second part of this thesis focuses on the role of the immunologic memory function during the treatment with HAART. How efficacious is HAART in pediatric HIV-1-
infection? Does it result in functional immune reconstitution after CD4+ T cells have increased?

**Immunology**

Host defense against infectious agents is secured through a combination of physical barriers, including the skin, mucous membranes, mucous blanket, and ciliated epithelial cells, and the various components of the immune system. The immune system consists of T lymphocytes, B lymphocytes, natural killer (NK) cells, dendritic and phagocytic cells, and various humoral components such as immunoglobulins, cytokines and complement proteins. The immune system also serves to protect against autoinflammation or autoimmune diseases and may help to prevent the occurrence of malignant diseases [17].

Microorganisms that penetrate the epithelial surfaces of the body for the first time are met immediately by cells and molecules that can mount an innate immune response. Common constituents of many bacterial surfaces bind to surface receptors of phagocytic macrophages and complement factors. A whole cascade is activated. Cytokines, chemokines and small peptides are released by the activated macrophages. This gives local inflammation and attracts circulating leukocytes. This cascade of reactions is a first line of defense against many common microorganisms and is called the innate immune system [18].

The adaptive immune system’s response is more specific to the microorganism, but it needs more time to react upon the first entry of a microorganism. The induction of the adaptive immune system begins when an immature dendritic cell in the infected tissue ingests a pathogen. The dendritic cell becomes activated, and travels to a nearby lymph node. By then the activated dendritic cell has matured to an antigen-presenting cell. T lymphocytes that have not yet been triggered by their specific antigen are called naive T cells. Naive T cells are continually migrating through the peripheral lymphoid tissues. In the MHC molecule on its surface the antigen-presenting cell presents degraded parts of the microorganism, called antigens. A naive T cell that recognizes antigens bound to the MHC molecule can be activated by co-stimulatory signals on the surface of the antigen-presenting cell. The T cell matures into an effector cell. The cell can now act on any target cell that displays the specific antigen on its surface. T cells can be divided into CD4+ and CD8+ T cells, depending on their surface molecule and their function. CD4+ T cells have cytotoxic effects, killing cells that have the specific antigen expressed on their surface, secrete inflammatory molecules and can help in activation of CD8+ cytotoxic T cells and CD19+ B cells, another lymphocyte subset. CD8+ T cells can act as cytotoxic cells. CD19+ B cells mature into immunoglobulin secreting plasma cells upon activation with a specific antigen. These plasma cells home to the bone marrow and secrete IgM, IgA, IgG or IgE into the extra cellular space. These immunoglobulins bind to the pathogenic antigens and facilitate uptake by macrophages and neutralize the pathogen by opsonisation.

With these systems acting together the host can fight the infection and try to eliminate the microorganism. After primary infection and the production of armed lymphoid cells,
memory T and B cells remain. These cells are able to mount an enhanced secondary response after re-infection, both by their increased number and the lower threshold required for their activation in contrast to naive B-cells. This provides immunity to that particular microorganism.

**Immunology in children**

Immediately after birth, neonates have high numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, all of which are naive. During the first years of life, as any child encounters several infections, the numbers of activated and memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells increase progressively toward adult values [17]. Via the placenta and through lactation, if given, the mother passes specific IgG and IgA to her child. Only IgG is actively transported over the placenta to the fetus [17]. These antibodies offer a degree of protection to infections up to about 12 months of life. After encountering infections the child builds up a cellular and serologic adaptive immune system.

**Vaccination**

Vaccination is an artificial way to protect an organism against an invading organism. With passive immunization a person gets immunoglobulins to prevent an infectious disease upon exposure. This strategy mimics the production of antibodies by plasma cells. However, no immunologic memory is formed. In contrast, the development of immunologic memory and subsequent immunity to diseases is used in active vaccination. A live attenuated or inactivated microorganism is administered to a person. An adaptive immune response, as described above, is mounted and subsequent immunologic memory develops. This concept was first used a century ago with cowpox and is now used to vaccinate against various diseases [19].

**Herpes viruses**

Herpes viruses form a family of viruses that share common features. After the initial infection, that often takes place during childhood, the viruses of this family establish a latent infection in their natural host and persist for life [20]. Equilibrium between the virus and the immune system exists. The virus can hide, thus preventing it from being eradicated. In order to persist the viruses reactivate from time to time, with or without symptoms (e.g. VZV causes herpes zoster upon reactivation). The viruses can then spread to other susceptible persons and a high degree of infection in humans is established. On the other hand the immune system prevents further spread of the virus over the body. The herpes family can be subdivided into 3 subfamilies, alpha-, beta- and gamma-herpes viruses.

Due to their ability to evade the immune system and maintain latency for life and to reactivate after initial infection herpes viruses can cause serious problems in immunocompromised patients, like in HIV-1 infected patients. The equilibrium between herpes viruses and the host is out of balance, as was observed before the availability of HAART. Treatment with HAART has substantially improved the survival and quality of life of HIV-1-infected children [21,22]. However, does HAART improve the immune
system and hence completely restores the balance between the host and the many pathogen microorganisms?

Varicella zoster virus (VZV) and Herpes simplex virus (HSV) are alpha-herpes viruses. VZV causes chickenpox. The virus is very common in temperate climates, where about 95% of the young adult population has serologic evidence of previous infection [23]. Whereas primary VZV infection in children is usually benign and self-limiting, primary infection encountered during adulthood is associated with higher morbidity and mortality. The highly contagious virus is less common in tropical climates with an infection rate of 70% or less [24]. After migration to colder climates the patient’s risk of getting varicella infection at older age increases, which poses an additional threat to the host. Complications of varicella are bacterial super-infection, cerebellitis and encephalitis. After the initial infection it establishes latency in the dorsal route ganglia. Reactivation is thought to occur when alterations in the balance between the virus and host factors allow local replication of the virus in the ganglion and axonal transport to the skin, resulting in zoster also called shingles [25]. This is mostly restricted to one or several sensory dermatomes [20].

**VZV in HIV-1-infected individuals**

VZV infection in HIV-1-infected individuals can cause severe chickenpox with major morbidity and mortality. Before HAART became available a high proportion of the patients had to be hospitalized after contracting VZV [26,27]. Recurrence and persistence was found to be the most occurring problem [26]. Even during treatment with HAART HIV-1-infected adult patients are found to have an increased risk of reactivation of VZV, which causes shingles [28]. HIV-1-infected patients who are susceptible to VZV should avoid exposure to persons with chickenpox or shingles. After close contact to a person with chickenpox or shingles passive immunization with varicella-zoster hyper immune immunoglobulin (VZIG) should take place within 48-72 hours [29].

In 1972 a live-attenuated VZV vaccine was developed [30]. In the US and Japan this vaccine is part of the routine vaccination schedule in children [31]. Susceptible adults are also encouraged to be vaccinated. In contrast, in Europe the VZV vaccine is not part of the routine vaccination; the vaccine has only recently been licensed in the Netherlands. Vaccination of immunocompromised individuals is not recommended by the Centers for Disease Control and Prevention (CDC) because of fears of vaccination-induced chickenpox [32]. However, after exposure to VZV these patients can develop chickenpox and have a higher risk of morbidity and mortality. Immunocompromised children would benefit the most from protection through vaccination. Vaccination of this group could be considered, if vaccination is safe. The safety and efficacy of VZV vaccination in HIV-1-infected children that had severe immune deficiency before the start with HAART are described in Chapter 5.

**Measles, mumps, rubella**

Since 1957 all children in the Netherlands are offered vaccination against a spectrum of common and severe diseases in the state vaccination program (Rijksvaccinatie
programma, RVP) [19]. The schedule has undergone several changes since then and more diseases were added. In 1973 rubella vaccination for all girls was introduced, all children born after March 1976 are vaccinated against measles and in 1987 a trivalent vaccination against measles, mumps and rubella (MMR) was introduced for all children. Children get this MMR vaccination at the age of 14 months. Because after the first vaccination a small proportion of the children does not show complete seroconversion to all 3 components, the vaccination is repeated at the age of 9 years. Almost all immunocompetent children have antibodies against all 3 components after the second vaccination [23]. Life-long immunity is assumed. Children that are not vaccinated or otherwise not immune are generally protected by herd-immunity. This means that the virus cannot spread in a population where the vaccination rate is high. However, the vaccination rate has declined in recent years due to a decline in the acceptance of the vaccination by the parents [33,34]. Sporadic outbreaks of the diseases still occur in Europe. This underlines the need for vaccination in high-risk patients.

In the era before the introduction of HAART it was shown that HIV-1-infected children showed a decline in titers of antibodies and subsequent loss of protection against several diseases after vaccination [35,36]. Does HAART stop this loss of antibodies? What happens to the antibodies against viruses that maintain latency and are kept for life-long, such as herpes viruses? Serologic memory is one of the functions of immunologic memory. Memory against MMR, CMV, EBV and VZV is studied in Chapter 6.

**Cytomegalovirus**

Cytomegalovirus (CMV) is a beta-herpes virus [20]. Primary infection, lifelong latency and intermittent shedding resulting from reactivation commonly occur without any marked disease consequences in otherwise healthy individuals. Young children are mostly asymptomatic during primary infection, whereas adolescents and adults can suffer from mononucleosis-like symptoms, such as fever, headache and malaise. In immunocompromised individuals this virus can cause a very serious disease with symptoms like retinitis, pneumonitis and encephalitis. Despite successful treatment with HAART some children tend to continue to spread CMV in the urine. In Chapter 7 we explore the effects of continuous CMV replication on the outgrowth of CD8+ memory T cells.

**Epstein Barr virus**

Epstein Barr virus (EBV) is a gamma-herpes virus that infects over 90% of humans worldwide. As with CMV infection, young children are mostly asymptomatic during primary infection, whereas adolescents and adults can suffer from mononucleosis. During latency EBV transforms latently infected B cells into proliferating blasts and convert these cells into long-lived memory cells [37]. The virus is intermittently shed in saliva of healthy individuals. The virus is associated with several forms of lymphoproliferative disorders, including Burkitt’s lymphoma and non-Hodgkin lymphomas [38]. The virus has the ability to immortalize B cells that it infects. Possibly these immortalized cells start to proliferate under circumstances of immunosuppression by medication or disease, thus making lymphoproliferation possible to occur overtly. Before starting HAART,
EBV is associated with lymphoproliferative disorders due to immunodeficiency. The biology of EBV after the start with HAART in children is unknown. In Chapter 8 we follow the biology of EBV during the treatment with HAART.

References


