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Host factors and early treatments to restrict paediatric HIV infection and early disease progression

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Abstract

A body of evidence indicates that a threshold level of the virus is required to establish systemic and persistent HIV infection in the host and that this level depends on virus–host interactions. Mother-to-child transmission (MTCT) of HIV is the main source of paediatric HIV infection and occurs when the host's immune system is still developing. Thus, innate resistance and immunity, rather than adaptive immune response, may be the main drivers in restricting the establishment of HIV reservoirs and the long-lived persistence of HIV infection in infants. Genetic variations in HIV co-receptors and their ligands, as well as in Toll-like receptors and defensins, key elements of innate immunity, have been demonstrated to influence the risk of perinatal HIV infection and disease progression in HIV-infected infants. Early treatments with combined antiretroviral therapy (cART) restrict paediatric infection by reducing the level of the transmitted/infecting virus to below the threshold required for the onset of immune response to the virus and also significantly reduce HIV reservoirs. However, despite long periods with no signs and symptoms of HIV infection, all early cART-treated children who later discontinued cART had a rebound of HIV, except for one case in whom a period of viral remission occurred. Which parameters predict viral remission or viral rebound after cART discontinuation? Could early cART prevent rather than just reduce the establishment of viral reservoirs? And, if so, how? Answers to these questions are also important in order to optimise the use of early cART in infants at high risk of HIV infection.

Keywords: perinatal HIV infection, HIV reservoir, prevention of chronic HIV infection, innate immunity, early cART

Introduction

Currently, there are approximately 3.2 million children living with HIV worldwide [1]. Although the number of new infections in children has decreased in the last few years, 240,000 new paediatric HIV infections still occurred in 2013, mainly in resource-limited countries [1]. Mother-to-child transmission (MTCT) is the main source of paediatric HIV infection. Most cases of MTCT occur around the time of delivery, but may also occur *in utero* or postnatally through breastfeeding; without prevention, the overall risk of transmission via breastfeeding is up to 40% [2]. Disease progression in HIV-infected infants is more rapid than in HIV-infected adults and, in the absence of antiretroviral treatment, about one-third of perinatally HIV–infected infants progress to early AIDS within 2 years of life [3,4].

The introduction of combined antiretroviral therapy (cART) has dramatically reduced MTCT of HIV to less than 2% in high–income countries and has also given rise to substantial improvements in terms of survival and quality of life in HIV–infected children [5]. Despite these advances, about 200,000 children still died from HIV-related causes worldwide in 2013 [1] and many efforts are still required to implement prevention of MTCT (PMTCT) and reduce disease progression in HIV-infected children.

In recent years, several virological and immunological findings have suggested that a threshold level of the virus is required to establish systemic and persistent HIV infection in the host. This level depends on virus–host interactions. The paediatric model affords several advantages for studying virus–host interactions, including knowledge of viral source and time of exposure. While the use of cART at birth may impede or reduce the level of the transmitted/infecting virus, the host's genetic variants of viral co-receptors and innate immunity, the earliest response to microbial entry and injury, may affect perinatal HIV infection and early disease progression. The effects of innate immunity/genetic resistance to HIV may be of particular importance in infants because they are exposed to HIV and acquire infection when their adaptive immune system is still developing.

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Mother-to-child transmission of HIV and establishment of perinatal HIV infection: critical questions

A large number of factors are involved in MTCT of HIV [6–11]. The type and length of exposure and maternal level of plasma viraemia are key determinants for MTCT. In the European Collaborative Study, the risk of transmission increased from 2- to 3.5-fold for each log increase of maternal plasma viraemia and, adjusted for maternal viral load, vaginally delivered immature neonates (born before 37 weeks of gestation) have a 10-fold higher risk of acquiring infection than infants born at term by Caesarean section [6]. HIV infection acquired during pregnancy or postpartum and co-infections, which target the placenta, fetal membranes, genital tract and breast tissue, also increase the risk of transmission [10,11].

It is noteworthy that a large percentage of infants born to HIV–infected mothers escape HIV infection, even in the absence of ART-based prophylaxis, thus providing evidence of an innate resistance/immunity to HIV. The failure to establish a persistent chronic infection may be due to 'irreconcilable incompatibilities' between virus and host. A few studies in newborns have indicated that, although MTCT of HIV does occur, the virus cannot establish a persistent infection in the host, thus raising the possibility of transient infection followed by natural clearance of HIV. In most of these cases, when the virus was detected early in life, either by virus isolation or proviral sequence detection by the polymerase chain reaction, HIV could no longer be detected in peripheral blood cells a few weeks later. All children remained

seronegative and without signs of infection [12–16]. It should be stressed that the clearance of the virus was based on the lack of HIV detection in peripheral blood cells combined with the lack of immunity against HIV. However, it was not clear whether these cases represented true transient infections or whether the virus was persistently below a threshold level in some cellular sanctuaries. Notably, one infant, who apparently cleared the virus within the first few months of life, showed a marked fall in CD4+ T cell count over 9 years of follow-up, in the absence of other laboratory and clinical signs of HIV infection [17]. Unfortunately, no further data are available on this patient. Persistence of HIV DNA integrated in the genome of peripheral blood cells has been described in a few children at 2 years of age belonging to a paediatric cohort of HIV-seronegative children born to HIV-seropositive mothers [16]. However, the possibility of a transient infection is debatable, as the particular HIV life–cycle (i.e. reverse transcription of viral RNA genome into proviral DNA once the virus has entered the host cell, and integration of proviral DNA into the host genome) and the natural clearance of HIV is still an open question.

ART-based prophylaxis, including treatment of mothers during pregnancy, delivery, and post-delivery in neonates, is well known to reduce the rate of MTCT by 98%. When short regimens of prophylaxis were initiated in neonates within 3 days of birth, the rate of infection fell by 20–30% [18]. A single dose of nevirapine administered within 24 hours after birth to infants born to cART–untreated HIV-infected mothers has been shown to reduce rate of MTCT by 40% [19]. This reinforces the concept of efficacy of post-exposure prophylaxis and the possibility that, in certain settings, the transmitted virus is unable to establish a systemic chronic infection.

Once HIV infection in infants is established, it is characterised by a rapid increase in HIV RNA in plasma and HIV DNA in peripheral blood cells within the first 2–3 months of life. Unlike in adults, where the primary infection is followed by a viral decline associated with serological and cellular immune response to HIV, in children plasma viraemia declines more slowly and remains at high steady-state levels in infants who rapidly progress to early AIDS by 2 years of age [20]. The immune system of neonates/infants is still developing, and thus the adaptive immune response against HIV is delayed compared to adults. This concept is supported by the low adaptive immune response observed in HIV-infected infants during the first year of life [21], and by the lack of autochthonous antibody production in HIV–infected infants who initiated cART within 3 months of life [22,23]. Thus, other factors may contribute to controlling viral replication soon after primary infection in non-rapid progressor infants.

The key aspect of life-long persistence of HIV infection is the presence of HIV reservoirs. HIV preferentially infects memory CD4+ T cells. Limited HIV infection of central memory and stem cell memory CD4+ cells is associated with lack of progression in viraemic individuals [24]. Factors that prevent/limit the establishment of HIV reservoirs are critical to prevent/restrict paediatric HIV infection and disease progression (Figure 1).

Role of HIV co-receptors and their ligands

HIV is characterised by a high degree of genetic and phenotypic variability, including its exploitation of receptors to enter target cells. Among the panoply of molecules known to work in conjunction with the CD4 molecule, the main receptor for HIV, two chemokine receptors, CCR5 and CXCR4, have been identified as the major HIV co-receptors. According to co-receptor use, the

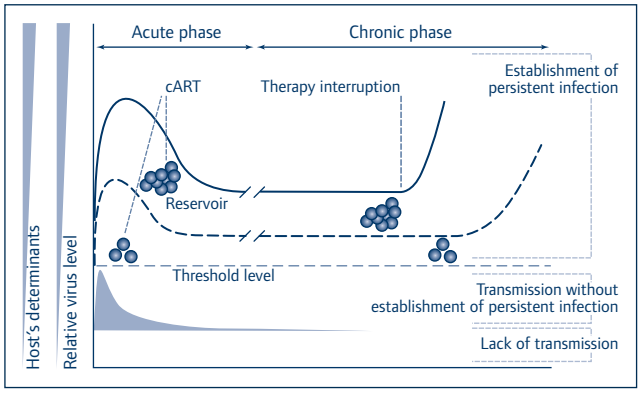

Figure 1. Model of host factors and early treatment to prevent/restrict HIV infection in infants. Post-exposure cART-based prophylaxis and host factors, e.g. specific variants of HIV co-receptors and key components of innate immunity, may prevent establishment of HIV infection (- - -). Early cART in HIV-infected children reduces HIV reservoirs and HIV replication to under the level required for onset of specific HIV-immune responses. According to the size of the viral reservoir, which may also be dependent on the time of cART initiation, interruption of cART is followed by variable HIV response, i.e. from prompt rebound of plasma viraemia (——) to a long period of viral remission (- - -), after cART suspension.

viruses isolated from patients, i.e. the primary isolates, have been classified as R5 and X4, respectively. The origin of infection is monophyletic: only one or a few variants are transmitted from mother to child, and these variants are mainly of R5 type, even when the mother harbours a mixture of R5 and X4 viral variants [25]. Although the few infants born with X4 type isolate rapidly develop AIDS, most cases of early AIDS are due to the R5 type variants [26–28]. Genetic polymorphisms of HIV co–receptors and their ligands may restrict virus infection (Table 1). A 32-nucleotide deletion (Δ32) in the *CCR5* gene–coding region prevents protein expression on the cell membrane and confers resistance to infection by R5-type isolates [29]. Haplotye P1/P1 and specific single nucleotide polymorphisms (SNP), which increase or decrease CCR5 expression, influence the risk of perinatal HIV infection and/or the onset of early AIDS [26,28,30–33]. A SNP in the *CCR5* regulatory region is in linkage disequilibrium with an SNP in the coding region of the *CCR2* gene: rs1799864A/A (*CCR264I*) is associated with a low risk of early AIDS [30,32].

Chemokines, natural ligands of viral co-receptors, can inhibit HIV infection [34]. The β-chemokines CCL3, CCL4 and CCL5 are natural ligands of CCR5; their over-expression in exposed uninfected infants suggests their possible role in mediating inhibition of perinatal infection [35]. While multi-copies of the *CCL3L1* gene confer a slight increase in the CCL3 level [36], a reduced ability to produce this chemokine is associated with increased susceptibility to perinatal infection [37,38].

HIV variability increases over time in both rapid and non-rapid progressors; viruses using CXCR4 co-receptor emerge during the course of infection and are detected in nearly half the cases of late AIDS. Stromal cell-derived factor-1 (SDF1) is an α–chemokine, a natural ligand of the CXCR4 receptor, and it may interfere with HIV infection by X4 type isolates. The *SDF1* gene exhibits genetic polymorphisms, including the SNP 3′G/A, located in the 3′ untranslated region of the gene, which may serve as a target for *cis*-acting factors, thus influencing the expression of the chemokine. This SNP does not influence the risk of MTCT and early AIDS, mainly due to R5 type isolates, but it accelerates disease progression and increases the risk of late AIDS [28,30,39].

Dendritic cell-specific ICAM-3 grabbing-nonintegrin (DC-SIGN) is an HIV receptor that enhances virus transmission to T cells and is expressed on placental macrophages. Specific haplotypes of *DC-*

Table 1. Genetic variants associated with risk of perinatal HIV infection and/or early disease progresson

Gene	Genotype	Study population (children)	Main findings	Refs
HIV co–receptors and their ligands				
CCR5	rs333wt/Δ32	European and African Hispanic, non-Hispanic	Low risk of early AIDS Low risk of early AIDS	[26,32] [30]
	Haplotype P1/P1	Italian	High risk of early AIDS	[26,28]
	rs1799987A/A	Hispanic, non-Hispanic sub-Saharan African	Rapid disease progression High risk of perinatal HIV infection	[30] [31]
	rs1800023A/A	Brazilian	Low risk of perinatal HIV infection	[33]
	rs41469351C/C	sub-Saharan African	High risk of perinatal HIV infection	[31]
CCR2	rs1799864A/A	Hispanic, non-Hispanic European and African	Low risk of early AIDS Low risk of early AIDS	[30] [32]
CCL3L1	Low gene copy number	South African	High risk of perinatal HIV infection	[37,38]
SDF-1	rs1801157A/A	Italian	Accelerated disease progression	[39]
		Hispanic, non-Hispanic	Accelerated disease progression	[30]
	rs1801157G/A	Caucasian	No impact on early AIDS, high risk of late AIDS	[28]
DC-SIGN	Haplotype H2	Zimbabwean	Low risk of perinatal HIV infection	[40]
	Haplotype H4; H6		High risk of perinatal HIV infection	
DC-SIGNR	Haplotypes H1/H1; H1/H3; H3/H3	Zimbabwean	High risk of perinatal HIV infection	[41]
Key elements of innate immunity				
TLR9	rs352139A/A	Caucasian	Slow disease progression	[50]
	rs352140A/A	African	High risk of perinatal HIV infection	[51]
	rs352140A/G	Caucasian	Rapid disease progression	[50]
	Haplotype rs352139G/rs352140A	Caucasian	Low risk of perinatal HIV infection	[49]
	Haplotype rs352139G/rs352140G	Caucasian	Rapid disease progression	[50]
β-defensin 1	rs11362G/G	Caucasian	No impact on perinatal HIV infection	[52]
		Brazilian	Low risk of perinatal HIV infection	[53]
	rs1800972C/C	Caucasian	High risk of perinatal HIV infection	[52]
		Caucasian	Rapid disease progression	[50]
	rs1799946A/A	Brazilian	High risk of perinatal HIV infection	[53]
	rs1799946G/G	Caucasian	Low risk of perinatal HIV infection	[54]
	Haplotype rs1800972G/rs352140G	Caucasian	Low risk of perinatal HIV infection	[54]
		Caucasian	Slow disease progression	[50]
MBL2	Haplotype XA/XA	Argentinian	High risk of perinatal HIV infection Rapid disease progression	[58]
TNF-α	rs1800629G/G Haplotype rs1800629G/rs361525G	Indian	Low risk of perinatal HIV infection	[59]
IL-1 gene cluster	Haplotype rs2234650T/rs1800587C/rs16944C/rs1143634C/rs315952T	Indian	High risk of perinatal HIV infection	[60]
HLA	B*4901, B*5301	White, African American, Hispanic	No impact on perinatal HIV infection	[64]
	B*18	Kenyan	Low risk of perinatal HIV infection	[63]

SIGN and of DC-SIGN-related gene (*DC-SIGNR*) influence the risk of intrauterine and intrapartum MTCT of HIV [40,41].

An intriguing role is exerted by the genetic variants of the *MDR1* gene, which codes for the drug transporter P-glycoprotein (P–gp). Besides their impact in modulating P-gp expression and drug response, *in vitro* assays have indicated a decreased HIV infectivity in cells over-expressing P-gp by affecting viral fusion and possibly viral release. SNPs and specific haplotypes have been associated with slow disease progression, but have no effect on HIV perinatal infection [42].

Role of innate immunity

Innate immunity provides the first line of defence against a wide range of micro-organisms before the development of adaptive immune responses: its role in disease outcome is particularly important in children who acquire infections when their adaptive immune response is still developing. Toll-like receptors (TLRs) and defensins, initiators and effectors of innate immunity, have been described to influence infection and disease progression in paediatric HIV/AIDS.

TLRs are type 1 transmembrane proteins differentially expressed in immune cells, and can recognise and bind pathogen-associated

molecular patterns (PAMPs), shared by large groups of micro–organisms. Following interactions with their ligands, TLRs trigger activation of signalling pathways that ultimately induce cytokine production [43]. TLR7 and TLR8 are expressed on dendritic cells and monocytes, and recognise the guanosine- and uridine-rich single-stranded (ss) RNA of HIV-1. After triggering by viral ssRNA, TLR7 and TLR8 interact with several adaptor proteins to activate transcription factors, leading to production of inflammatory cytokines and antiviral compounds such as interferon-α [44]. TLR9 recognises the cytidine–phosphate–guanosine (CpG) DNA motifs that are present in many bacteria and viruses. Activated TLR9 alerts the immune system, triggering the activation of pro-inflammatory reactions and inducing dendritic cell maturation and production of cytokines [45]. Defensins are small cationic peptides mainly produced by leukocytes and epithelial cells; according to their size and binding patterns, they are subgrouped into α, β, and θ [46]. β–defensin 1 is constitutively expressed by epithelial cells, whereas expression of β-defensins 2 and 3 can be induced by pro–inflammatory cytokines. The antiviral activity of β–defensins involves several mechanisms, including direct interaction with viral envelopes and target cells. β-defensins 2 and 3 inhibit HIV spread and replication through viral inactivation and downregulation of CXCR4, co-receptor of X4 HIV strains [47].

SNPs in TLR and defensin genes have been associated with increased susceptibility or protection against several infectious diseases [47,48]. Genetic polymorphisms and specific haplotypes of *TLR9* [49–51] and β-defensin 1 [50,52–54] influence the risk of perinatal HIV infection and disease outcome in HIV-infected children (Table 1). An important mechanism by means of which TLRs and defensins may modulate disease progression is their role in chronic immune activation. During the course of HIV infection, the CD4+ cell count (or CD4+ cell percentage in children under 5 years of age) is the most important indicator of disease progression. The gut is one of the major sites of CD4 cell depletion; its damage impairs the mucosal barrier and allows microbial translocation [55]. Specific variants of β-defensins may protect against disease progression by increasing defensin expression at mucosal level. Microbial translocation allows bacterial components to enter the bloodstream and trigger TLRs with consequent production of pro-inflammatory cytokines and induction of a chronic state of immune activation. This immune activation persists in children who do not respond well to cART, and is a hallmark of HIV pathogenesis [56] (Figure 2).

Mannose-binding lectin (MBL) is synthesised by the liver and secreted into the bloodstream, where it plays an important role in the innate immune defence against invading micro-organisms. MBL is a plasma pattern-recognition receptor that can mediate the elimination of pathogens [57]. Specific genetic variants of *MBL2* have been described to affect the risk of perinatal HIV infection and progression to AIDS [58].

Genetic variants of cytokines have recently been described to influence perinatal HIV infection and disease progression. The rs1800629GG genotype, responsible for the low expression of TNF-α, is associated with a lower risk of perinatal transmission [59]. A specific haplotype of interleukin *(IL)-1* cluster gene has been found to be associated with increased risk of perinatal infection [60].

Human leukocyte antigen (HLA) molecules regulate the cellular immune system by detecting and presenting peptides derived from infectious agents to T cells. The HLA region includes 128 genes, and a great number of allele variations occur among individuals. Multiple *HLA* class 1 alleles have been linked to the rate of HIV disease progression: HLA alleles B*27, B*58, B2701 are potentially protective [61,62]. HLA B*18 has been associated with low risk of perinatal infection [63]. HLA B*4901 and B*5301 in mothers have been found to be associated with a lower risk of MTCT, but in children these alleles are not associated with infection risk [64]. The HLA-G molecule is of particular interest, as it is a non-classical MHC class I molecule highly expressed in the trophoblasts at the maternal–fetal interface. Several SNPs of *HLA-G* have been associated with a decreased risk of perinatal HIV infection [65].

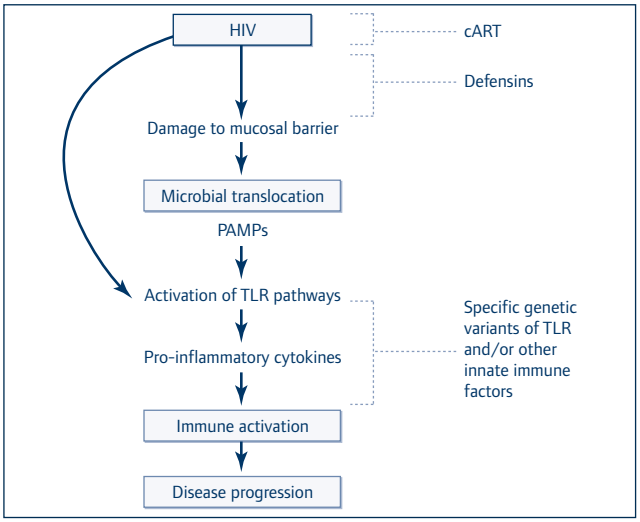

Figure 2. Model of host factors and early treatments to prevent early disease progression. Defensins may counteract damage to mucosal barriers due to HIV. Microbial translocation generated by damage of mucosal barriers, and HIV itself, activate Toll-like receptors (TLRs) that recognise and bind pathogen-associated molecular patterns (PAMPs), shared by large groups of micro-organisms. Following interactions with their ligands, TLRs trigger activation of signalling pathways that promote pro-inflammatory cytokines and thus immune activation, a hallmark of disease progression. Specific genetic variants of TLRs that modify their activity and other components of innate immunity may restrict immune activation and disease progression

Notably, there are important geographic and ethnic variations in the frequency of genetic variants, encoding products that may interfere with HIV infection and the outcome of HIV disease.

Role of early treatments in paediatric HIV/AIDS

HIV preferentially infects activated memory CD4+ cells that express the chemokine co-receptor CCR5. The majority of infected cells die quickly, but a small number revert to a resting state and persist life-long as a latent reservoir [66]. Because cART blocks nearly all new infection events, the reservoirs that exist at the time of cART initiation are those which persist throughout life and impede the eradication of chronic HIV infection.

As recommended by World Health Organization (WHO) [67], Department of Health and Human Services [68], and Paediatric European Network for Treatment of AIDS (PENTA) [69] guidelines for treatment of paediatric HIV infection, cART should be started as soon as possible in all HIV-infected children, irrespective of clinical or immunological status. The risks of drug resistance and early toxicity are markedly outweighed by the reduction of both short–term mortality and disease progression, especially prevention of irreversible HIV encephalopathy [70–74] (Table 2).

Evidence from four children, who initiated early cART before the age of 2.6 months, showed no HIV plasma viraemia for more than 10 years, had no immune response to HIV, and had replication–competent virus in only one case, all supports the concept that early cART significantly reduces HIV reservoirs [23]. Even cART initiation before 6 months of age seems to reduce viral reservoirs; in fact, 15 children who initiated cART before the age

Table 2. Viro–immunology parameters in early–treated infants

Study population	Viro-immunological parameters	Main findings	Refs
Multicentre nationwide case–control study 30 infants with early cART (<6 months) *vs* 103 infants with deferred cART (>6 months)	HIV plasma viraemia CD4 cells CD8 cells	• 73% early cART *vs* 30% deferred cART reached undetectable viral load • Higher % CD4 cells in early cART than in deferred cART • Lower % CD8 cells in early cART than in deferred cART • No disease progression in early cART *vs* 43% disease progression in deferred cART (median follow-up 4.1 years)	[70]
European Collaborative Study 124 infants with early cART (<3 months) *vs* 86 infants with deferred cART (>3 months)		• Lower risk of AIDS/death in early ART than in deferred ART (1.6% *vs* 11.6% at 1 year follow-up)	[71
96 infants with early cART (<3 months) *vs* infants with deferred cART (22: 3–6 months and 21: 6–12 months)	HIV plasma viraemia CD4 cells	• Lower decline of % CD4 cells over first year of life in early cART than in deferred cART groups • Time from cART initiation to first virological suppression shorter in early cART than in deferred cART groups • Trend to lower HIV plasma viraemia between 12 and 48 months in early cART than in deferred cART groups	[72]
4 infants with early cART (<2.6 months) *vs* 4 children with late cART	HIV plasma viraemia HIV DNA HIV serology Virus culture CMIR to HIV CD4, CD8 cell subsets	• Plasma viraemia undetectable in all early-treated • HIV DNA in peripheral blood cells lower in early-treated than in late–treated HIV seropositivity in 1/4 early-treated *vs* 4/4 late-treated CMIR to HIV epitopes in 0/4 early-treated *vs* 4/4 late-treated • Normal level of CD8 activated cells in early-treated	[23]
15 infants with early cART (<6 months)	HIV plasma viraemia HIV DNA 2-LTR HIV serology CMIR to HIV	• Median time of suppression plasma viraemia: 6 years • 60% with undetectable HIV DNA in CD4+ cells • No detectable 2-LTR circles • 47% HIV-seronegative, • Only 1 with detectable CMIR to HIV epitopes	[75]
6 infants with early cART (<3 months) discontinuation of cART in 2 children at 3–4 years of age	HIV plasma viraemia Cell-associated HIV RNA HIV DNA Virus culture HIV-serology CMIR to HIV CD4, CD8 cell subsets	• Decline of HIV plasma viraemia to undetectable levels in all cases • Persistence of HIV DNA in 4, and cell-associated HIV RNA in 2 • Lack of HIV autochthonous antibodies in 4 children • Lack of CMIR to HIV • All viro-immunological parameters persistently negative in 2 children for up to 4 years follow-up • cART interruption in 2 children, 1 positive and 1 negative for all viro-immunological parameters: rebound of HIV plasma viraemia in both	[22]
CHER Trial 252 infants with early cART (<3 months: 126 cART for 40 weeks; 126 cART for 96 weeks) *vs* 125 infants with deferred ART		• Lower risk of death (4% *vs* 16%) and disease progression (6% *vs* 26%) in early cART than in deferred cART group at median 40–week follow–up	[73]
4.8 years follow-up Substudy		• 25% early ART-40W, 21% early ART-96 W and 38% deferred ART reached the primary endpoint (failure of first-line cART) or death	[74]
12 infants with cART initiation <2 months *vs* 8 with cART initiation >2 months	HIV plasma viraemia HIV DNA Cell-associated HIV RNA	• After 7–8 years of continuous cART, children starting cART <2 months of age had lower HIV DNA and cell-associated HIV RNA than children who started cART >2 months of age	[77]
4 infants with early cART (within 72 hours of life)	HIV plasma viraemia Cell-associated HIV RNA HIV DNA, CD4 cells Virus culture HIV serology CMIR to HIV HLA genotype	• At 2.5–7.5 years of follow-up: negative for all parameters (HIV serology, CMIR to HIV, ultrasensitive HIV plasma viraemia, HIV DNA in CD4 cells) • Low level of cell-associated HIV RNA • Normal CD4 cell count • Viral rebound in 2, due to poor adherence to cART	[78]
1 infant with early cART (initiated by 30 hours of life and discontinued at 18 months)	HIV plasma viraemia HIV DNA Virus culture HIV serology CMIR to HIV HLA genotype CD4 cells, CD8 cells	• HIV seronegative at 24, 26 and 28 months of age • No HIV-specific cellular immune response • No rebound of HIV plasma viraemia & HIV DNA after therapy interruption • Normal CD4 and CD8 cell count after therapy interruption • After 46 months' follow-up (27 months after cART discontinuation) rebound of HIV plasma viraemia and onset of autochthonous HIV–specific antibodies	[79] [80]
1 infant with early cART (initiated at 12 hours and discontinued at 3 years)	HIV plasma viraemia HIV DNA Virus culture HIV serology, CD4, CD8 cell subsets	• Persistently undetectable plasma viraemia after the initial decline • Negative for HIV DNA, virus culture and HIV-serology at 3 years • Increased number of activated CD4 and CD8 cells at 3 years • Rebound of HIV plasma viraemia within 2 weeks after cART interruption	[81]

CMIR: cell–mediated immune response

of 6 months showed a median duration of 6 years of undetectable plasma viraemia; 60% of them had undetectable integrated HIV DNA, and none had detectable 2-LTR circles, a marker of residual viral replication during cART. Of these children, 47% were seronegative and all except one had undetectable cell–mediated immune response against HIV epitopes [75]. While early cART is efficient in reducing mortality and disease progression, the need for life-long therapy due to the early establishment of latent HIV reservoirs is a problem.

Studies on the natural history of paediatric HIV infection suggested that cART in neonates may prevent the establishment of HIV reservoirs and systemic chronic HIV infection. The first study that specifically addressed this aspect involved six infants who started cART within 3 months of life [22]. All of them showed a decline in plasma viraemia to undetectable levels. HIV DNA persisted in the peripheral blood cells in four of these children, but only two had detectable intracellular HIV mRNA. All viral parameters remained persistently negative in two children. Only two children produced HIV antibodies; the others, after having lost maternal antibodies, remained seronegative. Therapy was interrupted in two children: one a 3-year-old, positive for all viro-immunological parameters, and one a 4-year-old, negative for all viro–immunological parameters. In particular, the seronegative child had received zidovudine at birth and started cART at 11 weeks of age. At 13 months, the child lost maternal antibodies and remained seronegative. Persistent undetectable HIV plasma viraemia, proviral HIV DNA and intracellular HIV mRNA, plus no autochthonous HIV antibody production by IVAP assay (at 18 months) and no HIV-specific immune response by EliSpot assay (at 38 months) all strongly suggested viral clearance [22]. Further analyses with CD25 and CD38 markers on CD4+ and CD8+ lymphocytes (at 38 and 46 months) disclosed no signs of immune activation [76]. Rebound of HIV plasma viraemia occurred within 2 weeks after ART interruption, was more rapid than that observed in the seropositive child, and preceded detection of HIV DNA and HIV mRNA in peripheral blood cells, thus indicating that the HIV reservoir was outside the peripheral blood compartment [22].

Of particular interest is the Children with HIV Early Antiretroviral Therapy (CHER) trial [73,74]. In this trial, 377 HIV-infected infants with a median age of 7.4 weeks were randomly allocated to one of three groups: deferred cART (ART-Def), immediate ART for 40 weeks (ART-40W) or immediate ART for 96 weeks (ART-96W) with subsequent interruption of treatment. Criteria for ART initiation in the ART-Def group and re-initiation after interruption were CD4% less than 25% in the first year; otherwise the criteria were CD4% less than 20% or stage B or stage C. Median time to cART initiation in the ART-Def group was 20 weeks; the time to restarting cART after interruption was 33 weeks in ART-40W and 70 weeks in ART-96W group. At the median follow-up of 40 weeks, the early-treated children had lower risk of death and disease progression than infants in the ART-Def group [73]. At the median follow-up of 4.8 years, 38% in ART-Def, 25% in ART-40W and 21% in the ART-96W group reached the primary end-point (immunological, clinical or virological failure of first-line cART) or death. Thus, early cART had better clinical and immunological outcomes than deferred ART. Additionally, children randomised to 96W of ART had a longer period off therapy compared with those allocated to 40W [74]. Some of the children initially randomly allocated to early-limited ART in the CHER trial did not interrupt cART; a substudy conducted in 20 of these infants who initiated cART <8 weeks of age (n=12) or >8 weeks of age (n=8) and remained for 7–8 years with undetectable HIV plasma viraemia (below 400 HIV RNA copies/mL) showed that the children who initiated cART earlier had lower levels of HIV DNA and intracellular HIV RNA than children who started later [77]. This finding may indicate that starting cART a few days/weeks after birth may change the size of the HIV reservoir. Studies on HIV serology and kinetics of HIV rebound after therapy interruption in the CHER trial will be very important to understand this mechanism.

In another study, initiation of cART within 72 hours after birth was carried out as perinatal exposure prophylaxis in high-risk situations (i.e. mothers with detectable viral load and/or poor adherence to therapy) [78]. In this study, four of 12 infants recognised to be HIV-infected achieved sustained virological suppression. These children, after having lost maternal antibodies, remained HIV-seronegative and the EliSpot assay revealed no detectable cell–mediated immune response against several HIV epitopes. At 2.5–7.5 years, all four children were negative for HIV DNA and had a low level of cell-associated HIV mRNA in their peripheral blood CD4+ cells. Three of these children had the B*58 allele and HLA–B sequence variations associated with better HIV control. Two of them underwent viral rebound, due to poor adherence after several years of effective therapy [78].

An interesting case was the infant termed the 'Mississippi Baby' in whom cART had been started at 30 hours of life. After cART discontinuation at 18 months, the infant remained negative for HIV plasma viraemia, HIV DNA in peripheral blood cells, HIV antibodies and cell-mediated immune responses against HIV gag and nef epitopes [79]. However, after 27 months of cART discontinuation, the child had a rebound of HIV plasma viraemia together with the onset of HIV-specific antibodies [80]. This case suggested that very early cART may alter the establishment of long-term persistence of HIV infection; the rebound of plasma viraemia after a substantial period of viral remission may be consistent with the model of HIV latency in long-lived resting memory CD4+ cells. This case indicates that early cART restricts, but does not eradicate HIV reservoirs, once they have been established.

A case has recently been reported in which zidovudine and nevirapine prophylaxis initiated within 12 hours of birth was followed by cART a few days later, when the infant was recognised to be HIV-infected [81]. At 3 years of age, the child was seronegative, had undetectable HIV plasma viraemia, undetectable HIV DNA in peripheral blood cells, but had an increased number of activated cells together with HIV-specific cytotoxic T lymphocytes. When cART was discontinued, HIV plasma viraemia rebounded in 2 weeks [81]. The authors suggested that presence of immune activation, impairment of naïve/memory cells and cytotoxic T lymphocytes may predict rebound rather than remission of HIV after cART discontinuation. The solution is not so simple. Indeed, as observed previously, rebound of plasma viraemia after cART discontinuation also occurred in an early-treated child without any virological and immunological signs of HIV infection [22]. Thus, the absence of virological and immunological signs of HIV infection in early–treated infants is important, but not sufficient to exclude viral rebound after therapy interruption.

Conclusions

A few cases have suggested that, although MTCT does occur, the virus cannot establish persistent infection in the infant, thus raising the possibility of transient infection followed by natural clearance of HIV [12–16]. Virus–host interactions contribute towards defining the threshold level required for the establishment of life-long persistence of HIV infection. HIV is

characterised by a high degree of genotypic and phenotypic variability, which involves a multitude of interactions with the host. In neonates who acquire infection when their immune system is still developing, innate resistance and/or immunity may be the principal drivers in restricting perinatal infection and early disease progression.

Several genetic variants of viral co-receptors and their ligands influence perinatal HIV infection and the onset of early AIDS, according to the co-receptor usage of transmitted/infecting viral strains. Regardless of the biological and genetic variability of HIV, data indicate that the genetic variants of TLRs and defensins, key elements of innate immunity, influence the risk of MTCT, the establishment of perinatal infection, and the outcome of paediatric HIV disease. Although the mechanism by means of which these variants influence HIV interaction with the host are still largely unknown, TLR and defensins may play a critical role in the onset and persistence of immune activation, a hallmark of HIV disease.

The main effect of early cART is to decrease the HIV load to below the level required for the onset of the immune response to HIV. The majority of children who initiated cART within 3 months of age, after having lost passively transferred maternal antibodies, did remain seronegative and did not develop immune responses against HIV epitopes [22,23,75,78]. Early cART also significantly reduced HIV reservoirs [23,75,77]. During continuous cART, early-treated children remained persistently HIV seronegative, with undetectable HIV plasma viraemia and undetectable or very low levels of cell-associated HIV DNA and HIV RNA. Despite long periods with no signs or symptoms of HIV infection, all early cART-treated children who underwent cART discontinuation had rebound of HIV in within 2–3 weeks [22,81], except for one child who had a 27-month period of viral remission [80]. In addition, early cART may restrict but not eliminate HIV reservoirs. Thus, early cART is quite efficient in decreasing mortality and disease progression, but is unable to eradicate infection. Could cART prevent the establishment of HIV reservoirs and life-long establishment of HIV infection? cART interruption with a long follow-up may be the only practical way of determining the effect of early treatment, but which parameters should be analysed in children with no signs of HIV infection before stopping cART? Answers to these questions are also important in order to optimise the use of early cART in infants at high risk of HIV infection.

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