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PAEDIATRIC HIV INFECTION: THE POTENTIAL FOR CURE

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Abstract

Recent anecdotal reports of HIV-infected children who received early anti-retroviral therapy (ART), and showed sustained control of viral replication even after ART discontinuation, have raised the question of whether there is greater intrinsic potential for HIV remission, or even eradication ('cure'), in paediatric than in adult infection. This Review describes the influence of early initiation of ART, of immune ontogeny and of maternal factors on HIV cure potential in children, and discusses the unique immunotherapeutic opportunities and obstacles that paediatric infection may present.

Graphical Abstract

Anecdotal reports of HIV-infected children who have continued to control viral replication after discontinuing anti-retroviral therapy have led to the hope that unique factors associated with paediatric infection might offer greater potential for HIV cure in children than in adults.

The pattern of immune ontogeny in humans has evolved to meet the changing and distinct challenges to the immune system that arise *in utero* and in early life, through childhood and into adolescence and adulthood. The differences observed in disease outcome for a range of infectious diseases, according to the age at which the particular infections arise, illustrate the impact of the distinct phases in immune development on disease susceptibility. HIV

Subject categories

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infection is a case in point (Fig 1). In the absence of antiretroviral therapy (ART) [G], >50% of HIV-infected children have died by 2 years of age¹, whereas in ART-naïve HIV⁺ adults the median survival time is 11 years². In the small subset of ART-naïve individuals infected with HIV who maintain normal CD4 counts and immune function and therefore do not progress to disease, the mechanisms operating in children and adults are markedly different. Non-progressing adult infection, in so-called ‘elite’ controllers [G], is typically characterised by a strong HIV-specific CD8⁺ T-cell response restricted by ‘protective’ MHC class I molecules such as HLA-B*27 and HLA-B*57 [refs 3–5], such that high CD4⁺ T cell counts [G] are maintained as a consequence of successful suppression of viraemia beneath the levels of detection (normally <50 HIV RNA copies/ml). By contrast, non-progressing HIV-infected children (defined here as healthy, ART-naïve children aged >5yrs who maintain absolute CD4 counts in the normal range for age-matched uninfected children, ie above 750 cells/mm³ [ref,6]), maintain normal CD4⁺ T cell counts despite median viral loads of ~30,000 copies/ml plasma⁷, and here the HLA-B alleles that strongly influence disease outcome in adult infection do not play a significant role⁸. It is important here to clarify the distinction between non-progressing adult and paediatric infection, defined above by the maintenance of normal CD4 counts in the absence of ART, and long-term survivors who typically have low CD4 counts and high immune activation⁷ but may be asymptomatic, presenting late, and in the case of children are usually stunted^{9,10}. Non-progressing adult and paediatric infection is characterised in these cases by low levels of immune activation, but through distinct mechanisms. In non-progressing adult infection this is achieved through HLA-mediated suppression of viral replication. In non-progressing paediatric infection, the mechanism by which immune activation remains low in some patients despite persistent high levels of viraemia⁷ is unknown. The phenotype of normal CD4 counts, and low immune activation despite high viral loads, is reminiscent of the natural hosts of SIV infection such as the sooty mangabey and African green monkey, who live normal life spans with normal CD4⁺ T cell counts and low levels of immune activation, with median viral loads of ~10⁵ SIV copies/ml¹¹.

The differences that exist between adult and paediatric HIV infection, not only in terms of disease outcome and the immune response to HIV, but also in terms of mode of transmission, have provided opportunities to learn much about the mechanisms of immune control of HIV. The possibility that these differences might also provide opportunities to learn about mechanisms of HIV eradication or ‘cure’ was initially prompted by the report of the ‘Mississippi child’^{12,13} (Box 1). Although virus levels ultimately rebounded in the Mississippi child 28 months after ART discontinuation, the case was sufficiently unusual to prompt the hypothesis that the combination of early initiation of ART with paediatric infection might provide the setting in which HIV eradication, or remission, could most readily be achieved.

Box 1

Case studies of the ‘Mississippi child’ and the ‘VISCONTI child’

The Mississippi child

The ‘Mississippi child’^{12,13} was infected with HIV *in utero*, with ART having been initiated at 30hrs of age and continued for the first 18 months of life; viral load remained undetectable for more than 2yrs following ART discontinuation.

Other unusual aspects of this case were:

- The healthy CD4⁺ T cell count (644 cells/mm³) and viral load (2,423 copies/ml) of the ART-naïve mother (the median viral load of *in utero*-transmitting HIV-infected mothers is ~90,000 copies/ml [ref 143];
- The low viral load (19,812 copies/ml at 30hrs post-delivery) of the child (the median viral load of *in utero*-infected infants is ~150,000 copies/ml on the first day of life¹⁴³);
- The high percentage (69%) of lymphocytes that are CD4⁺ T cells (the CD4%) of the child 8d post-delivery (in HIV-uninfected infants, the median CD4% is 52% [refs 6,143]);
- Viral rebound in the absence of HIV-specific immune responses in the child to a steady-state value of only 16,750 copies/ml, which is similar to the pre-ART viral load.

The VISCONTI child

The ‘VISCONTI child’²⁸ received ART for the first 6 years of life, likely following *intra-partum* infection (HIV DNA and HIV RNA were undetectable at 3 days of age), and has remained aviraemic (as low as less than 4 copies/ml) for 12 years so far since ART discontinuation.

Other unusual or notable aspects of this case were:

- ART (3–4 drugs) was initiated at 3 months of age, at which time the viral load was 2.17×10^6 copies/ml;
- Two separate episodes of ART non-adherence in the second year of life resulted in viral rebounds to 50,000–100,000 copies/ml;
- After ART discontinuation, there were no apparent increases in viral load other than to 510 copies/ml at 11 years and 48 copies/ml at 13 years;
- Infection was with a rare clade of HIV, subtype H, the significance of which is unknown.

A further anecdotal example of post-treatment control of viraemia was recently described in a French HIV-infected child (Box 1). Although anecdotal cases can be misleading (Box 2), this paediatric example follows the description of post-treatment control in the VISCONTI cohort [G] of adults who received ART during acute infection (but within months not days of infection) and who discontinued ART after a median of 3 years¹⁴. It is estimated that 5–15% of adults who receive ART for this duration, initiated in early infection, become post-treatment controllers, but the equivalent figure in paediatric infection following early initiation of ART is unknown. Several reports have described viraemic rebound within days of ART discontinuation in children in whom ART was initiated in the first week of life and

continued for 2–4 years^{15–18}. In the much larger CHER study [G], in which 250 children were treated for either 40 weeks or 96 weeks from a median age of 7 weeks¹⁹, and in the PEHSS study [G]²⁰, in which 19 infants received 12 months of ART from a median age of 4 weeks, all patients rebounded soon after ART discontinuation (M. F. Cotton, personal communication; P. Goulder, personal communication). In summary, it is not currently known whether early initiation of ART in paediatric infection is any more or less likely to facilitate post-treatment control than early ART in adult infection.

Box 2

Anecdotal evidence of post-treatment control potentially explained by factors other than early ART

Long-term aviraemia following early ART and ART discontinuation in an HIV-infected adult

The original ‘Berlin patient’ was described in 1999 [ref 144] (not to be confused with the more famous Berlin patient, Timothy Ray Brown, who was cured of HIV infection following a stem cell transplant from a donor homozygous for the CCR5 delta-32 deletion); suppression of viraemia was achieved after ART initiation during acute HIV infection, and for 15 years after ART discontinuation, the viral load remained at low to undetectable levels (median 399 copies/ml)¹⁴⁵. Only recently has it emerged that this subject expressed HLA-B*57. The existence of a subset of ART-naïve, HIV-infected adults termed ‘elite controllers’ (Fig 1) was not well-described in the 1990s; however, it is now known that 40–50% of elite controllers express HLA-B*57 [ref 146]. Therefore, a major factor in immune control in the case of the original Berlin patient may have been expression of HLA-B*57.

Long-term aviraemia following early ART and ART discontinuation in an HIV-infected child

ART (ddI and AZT) was initiated at 18 months of age in a child testing HIV-positive in 1991. For the next 8.5yrs, viral loads were undetectable (less than 400 copies/ml) while the patient was on therapy. ART was discontinued at the age of 10yrs, and, with the exception of intermittent increases in viraemia that never exceeded 1,000 copies/ml, viral load remained undetectable for the next 5 years until the case was reported¹⁴⁷. The HLA type of this child did not include ‘protective’ HLA alleles. However the child was heterozygous for the CCR5 delta-32 mutation, which has been shown to delay disease progression in both adult and paediatric HIV infection.

This Review considers the likely impact of early initiation of ART on the potential for HIV eradication in children versus adults, and discusses the influence of immune ontogeny and of maternal transmission in paediatric infection. Finally, we consider the particular opportunities that these features of paediatric infection present in terms of strategies to promote HIV remission or eradication.

Early ART in paediatric versus adult infection

Feasibility

In adult infection, outside of expensive trial settings^{21,22}, the identification of early HIV infection is problematic. By contrast, *in utero* paediatric infection, which mainly occurs late in the third trimester^{23,24}, can be diagnosed using point-of-care testing²⁵ within hours of birth in the children of chronically HIV-infected mothers, and ART can be initiated within minutes of birth, pending results of diagnostic tests. In most cases, therefore, treatment can be started earlier in paediatric infection than in adult infection. However, especially in instances where the mother develops acute infection during pregnancy¹⁶, *in utero* transmission can occur several weeks before birth (Fig 2). In addition, paediatric infection can also arise from *intra-partum* or *post-partum* mother-to-child transmission, and in neither case are these infections detectable at birth. Furthermore, there are added challenges to detecting *intra-partum* or *post-partum* infection for the practical reason that the mother and child have long departed the hospital. Therefore, initiating ART soon after infection following *intra-partum* or *post-partum* transmission may be substantially more problematic than after *in utero* transmission that mostly arises late in the third trimester and is detectable at birth. However, it is not known whether the timing of early ART in infection is critical to the outcome in terms of post-treatment control. SIV-macaque studies indicate very rapid seeding of the viral reservoir [G], within 3 days of infection²⁶. On the other hand, in the VISCONTI cohort subjects, ART was initiated within the first 2–3 months of infection (Fiebig stages I–VI [G])²⁷, similarly in the VISCONTI teenager²⁸, which indicates that ART initiation outside of the first few days of infection does not preclude subsequent post-treatment control in HIV infection.

Reduction of the viral reservoir

It is evident that early initiation of ART reduces the frequency of latently infected T-cells in both adult and paediatric infection. If ART is initiated in chronic adult infection, the latently infected resting CD4⁺ T-cells persist with a half-life of 44 months^{29–31}. However, treatment of adults during acute infection compared with chronic infection results in approximately a 10–100 fold lower frequency of latently infected resting CD4⁺ T-cells, when measured in terms of either HIV DNA or replication competent virus^{32–34}. Similar results have been demonstrated in SIV-infected macaques when treatment is initiated within 3 days compared with 7 days after infection²⁶. In addition, cell-associated unspliced HIV RNA [G], which reflects basal levels of HIV transcription, is significantly lower in individuals who are treated in acute infection compared with chronic infection³⁵. Much of the decrease in HIV DNA and unspliced HIV RNA levels occurs in the initial years of therapy^{36,37}. Latently infected CD4⁺ T-cell subsets have different decay rates, and following long-term ART (more than 10yrs), long-lived central memory T-cells, memory stem cells and naïve T-cells are the major persistent T-cell subsets enriched with HIV^{36,38,39}.

In infants who received ART at a median age of 8 weeks, the half-life of latently infected resting CD4⁺ T-cells was 11 months during the first two years on ART⁴⁰. Levels of both cell-associated HIV DNA and cell-associated HIV RNA are lower in children treated earlier^{17,41–44}. In the CHER study, for example, in which levels of both HIV DNA and cell-

associated HIV RNA were measured, initiation of ART in children before 2 months of age (at a median age of 53 days) resulted in a lower frequency of HIV-infected CD4⁺ T-cells 7–8 years later, compared with children in whom ART was started at more than 2 months of age (at a median age of 170 days)⁴³. Furthermore, whereas in adult infection levels of cell-associated HIV DNA seem to plateau after ~4 years⁴⁵, in children these continue to decline for more than a decade⁴¹. Treatment of HIV infection in children at a median age of 2 months resulted in a significant decrease in HIV DNA but persistence of 2LTR circles [G] and cell-associated unspliced RNA after 96 weeks, and the frequency of HIV DNA was more than 148 fold higher than the frequency of infectious genomes⁴⁶, as previously described following treatment of HIV in adults⁴⁷. Thus, ART can generally be initiated earlier in paediatric *in utero* infection than in adult infection, the half-life of latently infected resting CD4⁺ T-cells is shorter in children, and initial studies indicate that decline in the viral reservoir seems to continue for longer in children on ART.

Impact of immune ontogeny on cure potential in children

Several aspects of normal development will have an important influence on the establishment and size of the viral reservoir, some of which are likely to increase, and others to decrease, the HIV cure potential in infected children. The overall balance of these effects may depend crucially on the timing of ART initiation following birth.

Tolerogenic environment in early life

The immune system *in utero* and in early life is exquisitely adapted to meet the particular challenges of that phase of life. *In utero*, the need to avoid making inflammatory anti-maternal responses during the frequent exposure to non-inherited maternal antigens is facilitated by the high levels of TGF- β in the fetus that direct peripheral differentiation of naïve T cells, in the presence of the cognate antigen, into ‘adaptive’ regulatory T cells (T_{Reg} cells)⁴⁸. These memory T_{Reg} cells are long-lived and can be found in children aged 7–17 years⁴⁸. T_{Reg} cells make up 15% of peripheral T cells in fetal blood, compared with ~5% in adult blood⁴⁹. Also, innate immune cells at birth, compared with adulthood, respond to a panel of TLR agonists by secreting higher levels of IL-6, IL-1 β and IL-23, which supports T_H17 cell differentiation, and lower levels of pro-inflammatory cytokines such as type I interferons, and hence lower levels of IFN- γ , TNF and IL-12, which support T_H1 cell differentiation^{50–52}. The higher levels in neonates of immunoregulatory and anti-inflammatory cytokines (such as IL-10 and TGF- β) and of T_H2 cell supporting cytokines (IL-4, IL-5, IL-9 and IL-13)^{53,54} further function to dampen down Th1 cell responses. Finally, plasma levels of adenosine are higher in neonates than in adults and may contribute to the T_H17 cell bias and low levels of immune activation in early life via increased IL-6 synthesis and decreased TNF production⁵¹. The immune response *in utero* and in early life therefore establishes a tolerogenic environment that is broadly anti-inflammatory and that promotes T_H17 cell-mediated host defence against extracellular bacterial and fungal pathogens, at the expense of a strong T_H1 cell-driven response which, by contrast, is a feature of antiviral cell-mediated immunity in adults.

In the setting of HIV infection in early life, one effect of these immune adaptations is to effectively eliminate the possibility of HLA class I-restricted CD8⁺ T-cell-mediated suppression of viraemia that is observed in adult 'elite controllers'. However, the tolerogenic environment in early life favours low levels of immune activation, which could interfere with the vicious cycle of immune activation, increased bystander cell death via apoptosis and pyroptosis^{55,56}, increased susceptibility of activated CD4⁺ T-cells to further infection, and increased virus production that normally occurs during adult infection (Fig 3). For similar reasons to those hypothesised in adult post-treatment controllers who express disease-susceptible HLA alleles such as HLA-B*35 [ref 11] (discussed further below), the tolerogenic environment of early life may interfere with the establishment of latent infection in the long-lived naive, stem cell-like memory and central memory CD4⁺ T-cells. There is some suggestion that early treatment of children, in contrast to adults, can result in lower levels of these latently infected long-lived T-cell subsets^{36,41}. Reduced infection of long-lived T-cell subsets has also been observed in post-treatment controllers and elite controller adults¹⁴.

Role of Th17 cells

A factor that may also affect the ability of HIV to establish long-lived latency is the high proportion of naive CD4⁺ T-cells *in utero* and in newborns. Indeed, in cord blood the number of activated CCR5-expressing memory CD4⁺ T-cells is close to zero⁵⁷. CCR5 is the co-receptor by which HIV gains entry into CD4⁺ T-cells and activated memory CD4⁺ T-cells expressing CCR5 are the most susceptible targets for HIV infection^{58–60}. By contrast, in the intestinal epithelium *in utero* and at birth almost one-third of the CD4⁺ T-cells express CCR5, many of which also express CCR6 [ref 54], which is characteristic of Th17 cells that are highly permissive to HIV infection^{61,62}. Even resting CD4⁺ T-cells that express CCR6, in the presence of the CCR6 ligand CCL20, have an increased susceptibility to direct infection and establishment of HIV latency⁶³. Thus, the theoretical paucity of CD4⁺ T-cell targets for HIV infection *in utero* and early life is at least partly compensated for by the high numbers of Th17 cells and other CD4⁺ T-cell targets available in the intestinal epithelium.

It is of note that the immune system at birth has a strong Th17 cell bias, given the importance of Th17 cells both functionally, in host defence against extracellular pathogens, and structurally, in maintaining the integrity of the intestinal mucosal barrier⁶⁴. Microbial translocation plays a central role in the vicious cycle of immune activation that results in CD4⁺ T-cell decline during HIV infection, and the preferential loss of Th17 cells observed in HIV infection⁶⁵ is therefore likely to have a disproportionate impact in paediatric infection. It is noteworthy that, in spite of substantial intestinal CD4⁺ T-cell loss following acute infection in both natural^{66,67} and experimental hosts of SIV^{68–70}, Th17 cell numbers in the gut are maintained at normal levels in non-pathogenic SIV infection⁷¹. The presence of higher levels of adenosine in mucosal tissues in non-pathogenic infection⁷² indicates that the adenosine pathway may contribute to limiting Th17 cell loss by reduced immune activation and the promotion of Th17 cell differentiation. In adult HIV infection, if ART is initiated sufficiently early, Th17 cell numbers are preserved/restored and systemic immune activation levels are normalised²². Thus the preferential loss of Th17 cells in the intestinal mucosa during HIV infection *in utero* and in early life may have a disproportionate impact in that

age group, increasing susceptibility to infections with extracellular pathogens, microbial translocation and immune activation. The timing of early ART in paediatric infection may be particularly important, therefore.

Increased immune activation in early life

Working against the tolerogenic environment are factors that predispose to increased immune activation in early life. This is directly relevant to reservoir size because increased immune activation increases viral replication (Fig 3). For example, microbial translocation across the intestinal barrier is increased in neonates, whether HIV infected or not^{73,74}. This is particularly true for premature infants, and more than one-third of babies born to HIV-infected mothers are premature (born before 37 weeks gestation). The changing composition of the intestinal microbiota also contributes to higher levels of immune activation in early life^{73,75}. It is proposed that mixed feeding, as opposed to exclusive breast-feeding, predisposes to increased risk of gastro-intestinal infections and mucosal inflammation, and therefore an increased risk of HIV transmission through breast-milk^{76,77}. Microbial translocation, leading to increased immune activation, is similarly increased by breaches in the intestinal mucosa, and thus it is reasonable to assume the type of feeding received by the child may in this way strongly influence the establishment and size of the viral reservoir prior to ART initiation in the infant.

Immune activation can also be increased by vaccinations and new infections that feature in infancy. The immunisation schedule provides crucial protection against diseases to which HIV-infected children may be particularly susceptible, but certain vaccines may also have unwanted side-effects. *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) is a particular case in point⁷⁸. Given at birth to all South African infants on the first day of life, BCG protects against miliary TB [G] and TB meningitis⁷⁹, and it may also have non-specific benefits in terms of reducing morbidity resulting from non-mycobacterial infections⁸⁰. However the increased levels of immune activation that result from BCG vaccination increase HIV infection risk in uninfected infants and increase disease progression in infected infants⁷⁸. Among the large number of infections to which newborns are exposed, cytomegalovirus (CMV) is noteworthy both for its effect in increasing immune activation⁸¹ and its prevalence in countries where HIV is abundant. By 3 months of age, almost two-thirds of children in sub-Saharan Africa have become infected with CMV⁸¹. CMV coinfection in HIV-infected children results in more rapid HIV disease progression⁸². All of these factors that function to increase immune activation in early life may oppose the benefits in terms of HIV infection and disease progression that would be expected to result from the tolerogenic immune environment described above. However, initiation of ART in the first 24–48 hours of life, prior to many of these factors taking maximum effect, would substantially reduce their impact.

Differential localisation of viral reservoirs

In adults on ART, HIV-infected T-cells are found at higher frequency in the gastrointestinal tract and lymph nodes than in the blood^{83,84}. A detailed comparative analysis of other tissue sites such as the spleen, liver and genital tract has not been carried out but latently infected cells are found in all these tissues⁸⁵. Recent findings also suggest that latently infected cells

can be detected in adipose tissue^{86,87}. Other long-lived infected cells can also persist on ART, including tissue macrophages — in liver as Kupffer cells⁸⁸, in lung as alveolar macrophages⁸⁹ and in the central nervous system as microglia or perivascular macrophages⁹⁰. HIV latency has also been demonstrated in astrocytes in the CNS⁹¹. It is not clear whether HIV DNA that persists in tissue macrophages is replication competent and contributes to viral rebound following cessation of ART.

In HIV-infected children, the location of latently infected T-cells or tissue macrophages has not been explored but may be quite different, given the low frequency of memory T-cells and different levels of expression of key chemokine receptors. Although detecting HIV persistence in tissues is challenging, recent novel methods developed in the macaque model, such as total-body imaging⁹² and RNA/DNA scope *in situ* hybridisation, should aid future studies of HIV persistence in children on ART. This is an important feature to understand, as the mechanisms that lead to HIV persistence in resting T-cells and macrophages differ⁹³ and therefore strategies to activate or eliminate infected cells will need to be different, as we have recently shown in terms of the *ex vivo* response to HDAC inhibitors of virus derived from brain tissue compared with spleen⁹⁴.

Finally, many recent papers have demonstrated that the majority of HIV DNA measured in blood CD4⁺ T-cells from HIV-infected individuals on ART is replication defective^{47,95}. Furthermore, the conventional viral outgrowth assay may underestimate the true number of cells that carry replication competent virus due to the presence of intact proviruses that are not induced through T-cell stimulation⁹⁶. In a single recent study in children treated with ART, it seems that there is a similar relationship between intact and defective viruses in children as in adults⁴⁶ but more studies are required in children using newer molecular strategies to truly define the relationship between defective and replication competent virus in this setting.

T-cell vaccine potential

Children who have received ART in the first few days of life often subsequently lack detectable HIV-specific immune responses. Western blot non-reactivity has been proposed as a marker of early and profound suppression of viral replication⁴¹, and lack of HIV-specific cellular immunity may be similarly indicative. However, it is well established that HIV-specific CD8⁺ T-cell responses are evident at birth in the majority of children infected *in utero*^{97,98}. This suggests that rapid removal of the HIV antigen in infancy or early childhood may result in loss of the memory response even though an immune response was induced. Thus, in early ART-treated HIV-infected children, there may be the potential for a T-cell vaccine to skew the CD8⁺ T-cell immunodominance pattern in a more favourable direction — such as towards a broad Gag-specific response — than would be the case if the pre-existing, sub-optimal responses that are generated in natural infection were predominant.

Successful skewing of the natural immunodominance pattern of the CD8⁺ T-cell response by an HIV T-cell vaccine has been demonstrated in recent analyses of participants in the South African Phambili trial [G]⁹⁹ who subsequently became infected. In individuals expressing the disease-susceptible HLA-B*58:02 allele, recipients of the MRKAd5 HIV-1 Gag/Pol/Nef vaccine made broader and higher magnitude Gag-specific responses than HLA-B*58:02-

positive placebo-recipients¹⁰⁰, who showed the Env-dominated response pattern that is typical of natural infection¹⁰¹. In association with skewing of the CD8⁺ T-cell response towards targeting Gag, viral setpoints in the HLA-B*58:02-positive vaccine-recipients were significantly lower than in the HLA-B*58:02-positive placebo-recipients¹⁰⁰.

In summary, therefore, a range of factors associated with the developmental stage of the child at which HIV infection occurs, prior to ART being initiated, are likely to affect markedly the ultimate size of the HIV reservoir. The overall effect of these mixed influences might depend crucially on the exact timing of *in utero* HIV transmission and of ART initiation. It is likely that if ART can be initiated within the first 24–48hrs of life then the factors predisposing to increased microbial translocation and increased immune activation would be largely mitigated, thereby providing children with an inherent advantage compared with adults in terms of cure potential.

Impact of maternal factors on cure potential in children

In utero and *intra-partum* mother-to-child transmission (MTCT) are effectively prevented entirely if viraemia is suppressed by ART during pregnancy. In countries such as South Africa, prevention strategies have reduced MTCT rates arising *in utero* or *intra-partum* from 25–30% without any intervention to 1–2% currently¹⁰². Where transmissions do occur, these tend to be associated with challenging circumstances. For example, more than 60% of transmitting mothers in South Africa have a daily income of less than \$1, the source of water in more than a third of cases is either a shared standpipe or river water, and toilet facilities for almost a half of mothers is a pit latrine²⁰. In these settings especially, the outcome of the child may have less to do with immunology than with the physical and mental well-being of the mother and the presence or absence of a social support network. There are therefore practical aspects of successful HIV treatment in which adults and children differ, and the dependence of an infected child on the mother to administer ART, in addition to the other needs of childhood, is an added challenge that should not be underestimated.

Maternal HLA type

The risk of MTCT is strongly related to maternal viral load^{103,104}, and therefore transmitting mothers are more likely to express HLA class I molecules that are known to be associated with high viral setpoints and rapid progression to disease^{105,106}. In African populations, these are HLA-B*18:01, HLA-B*45:01 and HLA-B*58:02. In Caucasian populations, the best-studied example is HLA-B*35. As children inherit one HLA haplotype from their mother, *in utero* HIV-infected children as a group are enriched for the HLA class I molecules that are associated with rapid progression in adult infection¹⁰⁶. However, as described above, the HLA-B alleles that have a substantial impact observed in adult infection do not significantly influence paediatric disease outcome⁸. Indeed, it has been proposed in adult studies that HLA class I molecules such as HLA-B*35 that are associated with rapid disease progression in adults may actively facilitate post-treatment control¹⁴. A possible mechanism is suggested by the fact that HLA-B*35 and HLA-B*45:01 bind the leukocyte immunoglobulin-like receptor LILRB2 with high avidity, whereas protective HLA alleles such as HLA-B*27/57/58:01 bind LILRB2 with low avidity¹⁰⁷. LILRB2 is expressed on

cells of the myeloid lineage, such as conventional dendritic cells, and high avidity HLA class I binding by LILRB2 inhibits dendritic cell function. The failure of disease-susceptible HLA class I molecules such as HLA-B*35 to induce broad HIV-specific CD8⁺ T-cell responses against HIV in adult infection, leading to high viral setpoints, may therefore in part be due to the inhibitory action of LILRB2 on dendritic cell activity. However, in the setting of early initiation of ART, factors such as these that reduce antiviral T-cell immunity may tend to reduce immune activation and thereby influence the establishment and cellular localisation of the latent HIV reservoir. If this proves to be the case, the apparent maternally inherited HLA 'handicap' in infected children may in fact predispose towards post-treatment control following early ART.

Virus type transmitted via MTCT

One of the benefits of early initiation of ART in paediatric and adult infection is to suppress viral replication prior to the selection of autologous escape mutants [G]. This early ART might be especially important in adults, in whom cytotoxic T lymphocyte (CTL) escape mutants are selected from the first few weeks of infection¹⁰⁸ and to persist in latently infected T-cells¹⁰⁹. By contrast, CD8⁺ T-cell responses in *in utero*-infected infants, even when restricted by protective HLA alleles such as HLA-B*57/58:01/81:01, seem to be insufficiently functional in the first few months of infection to select escape mutants⁸. However, in MTCT the virus that is transmitted is in many cases pre-adapted to T-cell responses available in the child, as well as to maternal antibodies crossing the placenta. Inheritance by the child of half of the mother's HLA alleles means that, in theory, the virus transmitted from mother-to-child may be pre-adapted to all of the CD8⁺ T-cell epitopes that are restricted by the HLA alleles shared with the mother¹¹⁰. However, for two reasons, this may be less of a problem in reality. First, the mothers of HIV-infected children tend to express disease-susceptible HLA alleles¹⁰⁶ (as described above), and these typically do not select any escape mutants in the crucial Gag epitopes¹¹¹. Second, as prevention of MTCT becomes increasingly effective, the proportion of *in utero* transmissions that result from acute infection of the mother during pregnancy becomes greater (Fig 2). In this scenario, *in utero* transmission may arise before escape mutants have been selected in maternal virus.

In summary, the mother has a crucial role in determining morbidity and survival in the child. If an HIV-infected mother is healthy and sufficient healthcare support is available for mother and child, however, there are reasons to believe that the inheritance by the child of maternal disease-susceptible HLA alleles and the transmission of HIV adapted to maternal immune responses may not be a significant disadvantage in relation to the potential for HIV cure in children versus adults.

Opportunities and challenges for effective cure strategies in paediatric infection

The feasibility of initiating ART within 24–48hrs of birth, the ability of ART to make faster reductions in the size of the viral reservoir that continue for longer in infants compared with adults, and the ability of ART to minimise the effect of factors in early life that would otherwise counteract the reduced immune activation resulting from a tolerogenic

environment, all provide a rationale for the proposition that unique possibilities exist to facilitate eradication or remission of HIV in children. However, there are also specific obstacles that narrow both the window of opportunity and the range of immunotherapeutic interventions that can be used in children. In particular, the very poor levels of ART adherence in adolescence¹¹², coupled with the normal onset of adolescence as early as 8 years and 9 years of age in females and males, respectively^{113,114}, effectively places a time limit on the age by which immunotherapeutic interventions need to be in place (Fig 4).

Early ART alone

The universal failure, so far, of ART initiated in the first weeks of life to result in post-treatment HIV control in paediatric infection, let alone cure, may indicate that ART should be initiated even earlier, and/or for a longer duration; and that there may be additional interventions, other than those related to the timing of onset and duration of ART, that are likely to be required to achieve post-treatment control. Host genetic factors may contribute to the likelihood of post-treatment control, as has been proposed in the VISCONTI adult cohort in relation to HLA class I type. Defining these factors will require large studies to identify and initiate early ART in sufficient numbers of *in utero* infected infants to eliminate some of the misleading confusions that can arise from anecdotal cases (Box 2).

Broadly neutralising antibodies at birth

Broadly neutralising antibodies [G] do not typically neutralise contemporaneous autologous virus¹¹⁵ and tend to be isolated in HIV-infected individuals with high viral setpoints who are progressing to AIDS¹¹⁶. Maternal antibodies crossing the placenta do not neutralise transmitted virus or affect paediatric disease progression¹¹⁷. However, heterologous broadly neutralising antibodies can both prevent infection^{118,119} and enhance subsequent B cell responses in infected infants¹²⁰ and they also substantially reduce viral replication in SHIV-infected macaques (PGT121 antibody)¹²¹ and in HIV-infected humans (3BNC117 antibody)¹²². Expression of broadly neutralising antibodies by adeno-associated virus vectors¹²³ has the potential to prevent infection for life, although long-term protection against infection using this approach has only been demonstrated in animal models so far^{124,125}.

In paediatric infection, broadly neutralising antibodies could be used at birth at the same time as ART is initiated (Fig 3). Human monoclonal antibodies protected against SHIV infection following oral challenge in neonatal macaques¹²⁶. This approach, in combination with ART, may potentially reduce viral loads by 1–2 log₁₀ over 7–10 days after infusion¹²⁷ in addition to the effect of ART. ART alone can reduce a typical starting viral load of 10⁵–10⁶ copies/ml to less than 50 copies/ml in 3–6 months²⁰, whereas ART in combination with a broadly neutralising antibody might achieve aviraemia in days or weeks. The timing of ART initiation and the time to viral suppression are both important, independent factors influencing the ultimate size of the latent viral reservoir⁴⁴. Early suppression of viraemia clearly limits the size of the reservoir but importantly may also limit viral diversity, which thereby might increase the efficacy of any immunotherapeutic approaches adopted downstream, as described below.

Timing of further immunotherapeutic interventions

Having initiated ART early, and achieved suppression of viraemia, sufficient time should then elapse before the introduction of further immunointerventions: first, to enable the latent viral reservoir to be optimally reduced to levels beneath the level of detection using the most sensitive assays; and, second, to allow Th1 cell-polarising innate immune responses to develop more fully, for the reason that cell-mediated immunity is likely to play a key role in eradication of the viral reservoir¹⁰⁹. Although IL-12, a Th1 cell-polarising cytokine, remains at lower levels than in adults even in 12-year-old children⁵², by 2 years of age type I interferons are at adult levels, levels of IL-6 and IL-23 (Th17 cell-polarising cytokines) and of IL-10 (an immunoregulatory cytokine) are lower than in neonates, and T_{Reg} cell numbers are similar to those in adults¹²⁸. Indeed there is evidence that the middle years of childhood represent a ‘honeymoon period’¹²⁹, during which the immune response is optimally adapted against a range of infectious agents, including TB, influenza, malaria, EBV, VZV, mumps, measles, and perhaps also HIV, such that it is sufficient to control the infection but not so much as to cause immunopathology. This period — from approximately 3 years to 9 years of age — may therefore represent the optimal window of opportunity for immunotherapeutic interventions designed to facilitate HIV eradication, prior to the onset of adolescence (Fig 3).

T-cell vaccines

Early generation T-cell vaccines did not reduce viral loads overall in vaccine recipients, and the Ad5 vector used in the Step trial [G] and Phambili trial was associated with increased risk of infection¹³⁰. However, current approaches such as those using prime-boost regimens of chimpanzee adenovirus and modified vaccinia virus Ankara result in T-cell responses that are 1 log₁₀ higher in magnitude and 5–10-fold greater in breadth compared with the T cell responses achieved by MRKAd5 HIV-1 Gag/Pol/Nef^{131,132} (B. Mothe, personal communication). The safety and immunogenicity of these approaches have also been established in Phase I and Phase II malaria trials in children ranging in age from infants (10 weeks old and 5–12 months old) to mid-childhood (2–6 years old) (K. Ewer, personal communication). The induction of protective broad, Gag-specific CD8⁺ T-cell responses in early-treated children by vaccination and/or the use of broadly neutralising antibodies could provide the long-term antiviral immunity that is necessary to contain or even eliminate viral replication from previously latently infected cells, once ART is discontinued.

Latency reversal

Multiple compounds have now been shown to activate latent HIV infection *in vitro*¹³³. These include epigenetic modifiers, PKC agonists, TLR agonists and others. Histone deacetylase inhibitors (HDACi) were the first latency activators to move into clinical trials in HIV-infected individuals on ART; these studies all demonstrated an increase in cell-associated HIV RNA and in some studies an increase in plasma HIV RNA, but no study reported a change in the frequency of latently infected cells^{134–138}. Although HDACi have been widely used for the management of malignancy in adults, there is very little experience in paediatrics and the sustained changes in host gene expression that result from their use would be a serious safety concern in children¹³⁶. More recently, disulfiram, which has been

in clinical practice for decades and with an excellent safety profile was shown to also activate HIV latent infection¹³⁹. This agent could potentially be evaluated in children for latency reversal. It remains unknown whether there are factors that would alter the susceptibility of latently infected cells from HIV-infected children on ART to activation. This needs to be evaluated in *ex vivo* studies to ensure there are no inherent differences in the stability of maintenance of HIV latency in children compared with adults, before the initiation of clinical trials of latency reversal in children.

Other immunotherapeutic strategies

Several additional approaches are currently being developed in adults; some of these show great promise but their efficacy and safety profile in children have yet to be determined. These are summarised in Box 3.

Box 3

Other immunotherapeutic strategies in children

In addition to the early initiation of antiretroviral therapy, and the use of broadly neutralising antibodies, T-cell vaccines and latency reversal agents, other approaches are currently being developed in adults and may be considered in the future for paediatric use.

Immune checkpoint blockade

Blockade of the immune checkpoint molecules PD-1 and CTLA-4 boosts HIV-specific T cell responses *ex vivo*^{148,149}. In a recent case study of CTLA-4-specific antibody (ipilimumab) in an HIV-infected adult on ART with metastatic melanoma, there was a significant increase in cell associated HIV RNA and a cyclical decline in plasma HIV RNA, which is consistent with enhanced virus clearance¹⁵⁰.

Treatment intensification

Studies in adults with HIV infection show that treatment intensification [G] has minimal effects on HIV persistence in patients on ART¹⁵¹. Two studies that intensified standard ART with raltegravir demonstrated residual virus replication in approximately 30% of individuals^{152,153}.

Anti-inflammatory drugs

Anti-inflammatory agents are being evaluated in HIV-infected adults to determine whether reducing inflammation will reduce HIV persistence — by decreasing either residual viral replication or the number of latently infected cells. Current clinical trials in adults include the anti-inflammatory drugs rapamycin, lisinopril, methotrexate, and antibodies targeting IL-1 and IFN (ClinicalTrials.gov NCT02440789, NCT01535235, NCT00000834, NCT02272946).

Gene therapy

Gene therapy using zinc finger nucleases to eliminate *CCR5* expression in peripheral circulating T-cells was recently shown to be safe and approximately 6% of the gene-modified cells persisted in blood and tissue¹⁵⁴. Given safety concerns, it seems unlikely

that *CCR5* gene therapy will be evaluated in children in the near future. Opportunistic stem cell transplantation from a donor with the *CCR5* delta-32 mutation could potentially be considered in a child with the appropriate clinical indication, given the success following transplantation of Timothy Brown (the Berlin patient)¹⁵⁵. However, so far this procedure has not been successful in 6 further adults with HIV infection¹⁵⁶.

Conclusions

The immune system *in utero* and in early life favours low levels of immune activation, at least initially, following HIV infection. However, in most cases it seems that ART would need to be initiated as early as possible to minimise the size of the latent viral reservoir and provide a realistic base from which additional immunotherapeutic interventions can be introduced. The timing of their introduction and a subsequent treatment interruption remains very much open to discussion. In children, as distinct from adults, there is a relatively narrow window of opportunity between the development of an immune response that is sufficient to eliminate activated HIV-infected cells that previously comprised the latent reservoir and the onset of puberty and its accompanying high levels of ART non-adherence. Recent data suggest that even brief ART interruptions in the absence of HIV-specific immunity can lead to a rapid and irreversible rise in the HIV reservoir⁴³, underlying the observation that reservoir size alone does not necessarily predict the time to viral rebound^{33,140}. Defining the factors that determine post-treatment control, and identifying biomarkers that can predict which individuals will have prolonged antiretroviral-free remission¹⁴¹ will require additional work, including clinical trials in children. So far, HIV vaccine trials have been rarely undertaken in children, despite evidence of promising immune responses in children who have been vaccinated¹⁴². Evaluation of early-treated children who receive immunotherapeutic interventions should be a priority.

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GLOSSARY

Antiretroviral therapy (ART)

The combination of usually a minimum of three anti-HIV drugs aimed to suppress viral replication, slow disease progression and minimise the risk of transmission. Pre-exposure prophylactic ART has also been shown to protect against HIV infection.

‘Elite’ controllers

A rare subset (less than 1%) of ART-naïve HIV-infected individuals who have undetectable plasma viral load (by standard assays) and remain clinically healthy long-term. ‘HIV controllers’ is sometimes used synonymously with ‘elite controllers’.

CD4⁺ T cell counts

The number of T-cells expressing CD4 receptor on their surface in a microlitre of blood. These white blood cells ‘help’ the immune system mount a response against pathogens and are the main target cells of HIV.

VISCONTI cohort

A French cohort of 14 adults in whom ART was initiated during primary HIV infection and interrupted after a median of 36.5 months. For a median of 7.4 years after treatment interruption, the viral load remained at less than 400 copies/ml; these 14 individuals are therefore referred to as ‘post-treatment controllers’.

CHER study

The Children with HIV Early Antiretroviral Therapy trial was a randomised phase 3 study that enrolled 6–12-week-old HIV-positive infants in South Africa with a CD4⁺ lymphocyte % of more than 25%, who were assigned either to receive immediate ART for 40 or 96 weeks or to defer treatment until clinical criteria were met.

PEHSS study

Paediatric Early HAART and Strategic Treatment Interruption Study. A feasibility study of 63 perinatally infected infants in KwaZulu-Natal, South Africa, who were randomised to receive either immediate 12-month uninterrupted ART; immediate 18-month treatment with structured interruptions; or deferred treatment.

Viral reservoir

Virus that persists in patients on ART in the form of latent or productively infected cells. Latency is established in long-lived resting memory T-cells as integrated virus that is replication-competent but transcriptionally silent. Latently infected cells persist for decades and are more frequent in tissue compared with blood. Reactivation of virus in these cells (for example, upon ART interruption) is the major obstacle to HIV eradication.

Fiebig stages I–VI

Categorisation of primary HIV infection into six stages based on the detection of different HIV markers. Staging begins with viral detection by PCR (I), then ELISA detection of p24 (II) or HIV-specific antibody (stage III), and then sequential stages of HIV-specific antibody detection via Western blot (IV–VI).

Cell-associated unspliced HIV RNA

Unspliced HIV RNA is detected in cells in individuals on ART from the initial first step of LTR-mediated HIV transcription prior to splicing, host promoter-initiated HIV transcription (or read through transcription) or virus production. By contrast, plasma HIV RNA measures RNA in mature virus particles.

2LTR circles

Circularised forms of unintegrated viral DNA that are by-products of HIV DNA integration into the host genome and exist only in the nucleus. The stability of 2LTR circles is controversial but they are generally thought to be short lived.

Miliary TB

Dissemination of *Mycobacterium tuberculosis* most often throughout the body, including the brain.

Phambili trial

A trial that tested the efficacy of the MRKAd5 subtype B HIV-1 Gag/Pol/Nef vaccine in South Africa, where subtype C virus is dominant. The vaccine increased the risk of HIV acquisition and did not reduce early viraemia.

Escape mutants

Viral variants that reduce recognition by the immune system, typically arising in epitopes targeted by HIV-specific CD8⁺ T cells or neutralising antibodies.

Broadly neutralising antibodies

HIV-specific antibodies that arise during natural infection in 10–30% of individuals and can effectively neutralise diverse viral isolates.

Step trial

A trial that tested the efficacy of the MRKAd5 subtype B HIV-1 Gag/Pol/Nef vaccine in North and South Americas, Australia and the Caribbean, where subtype B virus predominates. The vaccine increased the risk of HIV acquisition.

Treatment intensification

Addition of additional antiretroviral drugs to a standard three-drug suppressive regimen.

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KEY POINTS

Recent anecdotal reports of HIV ‘remission’ in children following a discrete period of ART have prompted the suggestion that HIV cure might be more readily achieved in children than in adults. This Review examines the evidence for such a claim, and discusses the unique opportunities for immunotherapeutic interventions in children to maximise HIV cure potential.

ART can be initiated within minutes of birth following *in utero* infection. Early initiation of ART results in a substantially smaller viral reservoir. The decay half-life of the viral reservoir is shorter following ART initiation in children compared with adults, and the decrease in size of the reservoir seems to continue for longer in children.

The tolerogenic immune environment *in utero* and in early life increases HIV cure potential in infants. Other aspects of immune ontogeny, including the strong T_H17 cell bias at birth, decrease cure potential in children. The overall balance between these opposing influences may depend crucially on the timing of initiation of ART.

Maternal factors, including maternal health (which influences child feeding, general care and ART provision); maternally transmitted infections, such as CMV and TB; and genetic factors, such as the effect of HLA class I alleles on dendritic cell function via LILRB2 binding avidity, could all markedly affect HIV cure potential in children.

Paediatric infection presents particular opportunities for HIV cure via early interventions in addition to ART. Certain challenges are also posed by paediatric infection and the effects of immune ontogeny. The onset of puberty may limit the optimal window of opportunity for immunotherapeutic interventions in children to the ages of ~3–9 years.

Fig. 1. Changes in viral load following HIV infection in paediatric and adult cases

Typical, progressing adult and paediatric infection (upper panels), and non-progressing adult and paediatric infection (lower panels)^{2,7,8,157}. Typically, in HIV infection of ART-naïve adults, a viral setpoint is reached after 6 weeks and the median time to AIDS is 10 years. In typical, ART-naïve paediatric HIV infection, a viral setpoint is reached after 5 years and the median time to AIDS is 1 year. Non-progressing adult infection is characterised by low/undetectable viral loads and low levels of immune activation. The prevalence of non-progressing paediatric infection is 5–10% [ref 7,158,159], and of adult elite control ~0.3% [ref 146]. Non-progressing paediatric infection also features low levels of immune activation, but in the setting of high viral loads (~30,000 copies/ml).

Fig. 2. Risk of Mother-to-child HIV transmission *in utero* and in early life

Mother-to-child transmission (MTCT) risk of HIV in the absence of prophylactic measures is approximately 7% from *in utero* infection, 18% from *intra-partum* infection, and 15% from *post-partum* transmission (through breast-milk; breast-feeding for 18–24 months)^{160–163}. *In utero*-infected infants are viraemic and HIV DNA positive at birth, whereas transmission arising at the time of delivery (*intra-partum*) results in viraemia only some 4 weeks later. Treatment with ART of all mothers testing HIV-positive in pregnancy has reduced combined *in utero* and *intra-partum* MTCT from 25% to 1–2% [ref 102], and prevention of breast-milk transmission via ART also has reduced *post-partum* MTCT rates to 1–2% [ref 164]. The remaining 1–2% MTCT rate results either from ART non-adherence or from acute infection during pregnancy or after birth (*in utero* MTCT risk of ~20% [refs 163,165,166] or *post-partum* MTCT risk of 20–25% [ref 167]), since tests to detect HIV antibody are negative during acute maternal infection when peak viraemia is $\sim 10^7$ copies/ml and transmission risk is high.

Fig. 3. Paediatric factors affecting immune activation and therefore size of the HIV reservoir

The vicious cycle of HIV infection — resulting in intestinal mucosal damage, microbial translocation across the intestinal barrier and increased immune activation, leading to increased CD4⁺ T-cell decline, which predisposes to further co-infections, increased immune activation, HIV replication and CD4⁺ T cell loss — can be prevented by the initiation of antiretroviral therapy sufficiently early in adult and paediatric infection. There are factors specific to paediatric infection that tend to interfere with this vicious cycle, and others that contribute to it, the balance of which may vary between individuals and also may depend crucially on the timing of ART initiation. Aspects of immune ontogeny that favour decreased immune activation include the tolerogenic immune environment *in utero* and in early life and the high proportion of naïve CD4⁺ T-cells. Aspects of immune ontogeny that tend to decrease paediatric cure potential include the large number of Th17 cells in the intestinal mucosa. Maternal factors favouring paediatric cure potential include maternal health, exclusive breast-feeding, lack of co-infections, and expression of HLA class I molecules that bind LILRB2 with high avidity.

Fig. 4. Principal opportunities for immunotherapeutic interventions to maximise the potential for HIV cure or remission in paediatric HIV infection

In utero paediatric infection provides particular opportunities for ART to be initiated with or without additional interventions (such as broadly neutralising antibodies) very early in the course of infection. As cell-mediated immunity is likely to have a key role in eradication of the viral reservoir¹⁰⁹, and T_H1 cell responses take some years to develop fully, a period of time on ART alone may be useful to minimise the size of the viral reservoir prior to further interventions designed to eradicate infection. In late childhood, approximately between the ages of 3 and 9 yrs, immune ontogeny favours the generation of effective CD8⁺ T-cell immunity, which provides a window of opportunity for therapeutic intervention before the onset of puberty with associated behavioural changes including loss of ART adherence and onset of sexual activity.