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Early antiretroviral therapy in children perinatally infected with HIV: a unique opportunity to implement immunotherapeutic approaches to prolong viral remission

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From the use of antiretroviral therapy to prevent mother-to-child transmission to the possibility of HIV cure hinted at by the Mississippi baby experience, paediatric HIV infection has been pivotal to our understanding of HIV pathogenesis and management. Daily medication and indefinite antiretroviral therapy is recommended for children infected with HIV. Maintenance of life-long adherence is difficult and the incidence of triple-class virological failure after initiation of antiretroviral therapy increases with time. This challenge shows the urgent need to define novel strategies to provide long-term viral suppression that will allow safe interruption of antiretroviral therapy without viral rebound and any associated complications. HIV-infected babies treated within a few days of birth have a unique combination of a very small pool of integrated viruses, a very high proportion of relatively HIV resistant naive T cells, and an unparalleled capacity to regenerate an immune repertoire. These features make this group the optimum model population to investigate the potential efficacy of immune-based therapies. If successful, these investigations could change the way we manage HIV infection.

Introduction

Study of the pathogenesis and management of HIV in paediatric populations has contributed pivotally to the collective understanding of the pathogen, from use of antiretroviral therapy to prevent mother-to-child transmission[1] to the possibility of cure suggested by the circumstances surrounding the Mississippi baby.[2] Babies infected vertically with HIV and treated within a few days after birth represent a unique opportunity to study novel approaches to HIV management and particularly therapeutic vaccines. These babies have a very small viral reservoir, rarely exhibit HIV-specific immunity, but still seem to maintain normal immune development.[3,4] The unique combination of a very small pool of integrated viruses,[5] a very high proportion of relatively HIV resistant naive T cells,[6] and an unparalleled capacity to regenerate an immune repertoire[7,8] makes this group the optimum model population to investigate the potential efficacy of immune-based therapies.

Infants born with HIV infection have access to potent combinations of antiretroviral therapy, so that increasing numbers of children are surviving to adolescence and older. Despite this optimistic outlook, several questions still need to be addressed (panel 1). An estimated 3–4 million children are living with HIV, more than 90% of whom are in sub-Saharan Africa, and almost all of these infections were acquired through mother-to-child transmission. As a result of widespread use of preventive interventions such as the administration of antiretroviral drugs to mothers and their babies, elective caesarean section, and bottle feeding, vertical HIV transmission has diminished to less than 2% from mother to baby in resource-rich countries. Similar results have been achieved in resource-poor settings, in which these strategies have also been implemented. Although new HIV infections in children declined by 53% from 2001 to 2012 because of the effective implementation of techniques to prevent mother-to-child transmission, about 250 000 HIV-infected infants are still newly infected every year.[9] Antiretroviral therapy has very effectively prevented mortality when initiated in infancy[10] and international guidelines now recommend initiation of antiretroviral therapy in all infants younger than 12 months infected with HIV, irrespective of clinical and immunological variables.[11] Thus, research can now focus on the effect of viral reservoirs in different antiretroviral therapy regimens started in early life. In terms of long-term viral control, evidence is growing to suggest that regimens containing lopinavir, if tolerated, started within the first year of life might be better than nevirapine regimens.[12] Moreover, a potential role for the use of integrase inhibitors during infancy has been suggested.[13] How the use of different or novel combinations of antiretroviral drugs will affect viral reservoirs is still unclear.

What did we learn from the Mississippi baby?

The Mississippi baby led scientists to think that very early and aggressive antiretroviral therapy in vertically infected infants could be sufficient to ensure HIV remission, defined as a prolonged period of undetectable plasma viraemia without antiretroviral therapy. The attempt to replicate this case with very early antiretroviral therapy (started within 48 h after birth) represents the objective of the IMPAACT1115 trial[14] announced by the National Institute of Health. The Mississippi baby initiated antiretroviral therapy at 30 h after birth during the acute phase of the infection. The child had a spontaneous antiretroviral therapy interruption at age 18 months followed by a period of 27 months with undetectable

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Panel 1: **Essential research questions to be solved to develop an effective immunotherapeutic strategy for use in children infected with HIV**

- What are the mechanisms that drive the long-term viral remission reported in the so-called Mississippi baby?
- How frequently can prolonged HIV viral remission be established in neonates infected with HIV who have antiretroviral therapy initiated within 48 h of birth?
- What sampling should be done to adequately assess the HIV reservoir in children?
- What are the best methods to evaluate the HIV latent reservoir?
- What biomarkers could be used to guide drug interruption in seronegative children infected vertically with HIV?
- Which immunological responses should be elicited in a therapeutic vaccine study to achieve HIV viraemic control?
- Is it ethical to interrupt antiretroviral therapy supported by an immunotherapeutic approach?
- What affects the decisions of parents to allow their infant to participate in a therapeutic vaccine trial?
- What enrolment criteria and endpoints should be considered in a therapeutic vaccine trial that targets paediatric patients with HIV?

plasma HIV RNA and no replication competent virus in CD4 T cells with only traces of HIV DNA in peripheral blood. These data led scientists to believe that a functional cure, or at least sustained control of HIV in the absence of antiretroviral therapy, could be achieved with very early treatment—namely, within a few hours after birth. However, at age 4 years, the child had a rebound of HIV RNA to nearly 20 000 copies per mL and antiretroviral therapy was resumed.[1,15]

This case shows that acute HIV infection targets could lead to a significant reduction in HIV reservoirs.[3,16] However, the reasons for the long duration of viral suppression without antiretroviral therapy for 27 months in this child still need to be elucidated (panel 1) and thus far, these factors are difficult to reproduce with antiretroviral therapy alone. In most early treated cases that interrupt antiretroviral therapy, plasma viral rebound is recorded in less than 2–4 weeks after interruption.[17,18] This outcome suggests that early antiretroviral therapy alone might not be sufficient for HIV remission. Furthermore, the instruments used to detect low numbers of HIV-infected cells[19] and undetectable HIV DNA, such as those used with the Mississippi baby, might not show whether all infected cells are cleared. Moreover, HIV RNA and DNA in the peripheral blood are insufficient biomarkers for viral clearance in these children as the virus could persist in different anatomical compartments and cell types. As shown in the adult population, the gut-associated lymphoid tissue[20,21] and the CNS[22] play a crucial part in contributing to the viral reservoir[23] and serving as a possible source of viral rebound after treatment interruption.[24] The ethics of tissue biopsy collection and of lumbar punctures for research in children who are not able to provide consent are challenging, and thus far, have restricted the ability of researchers to examine these reservoir compartments (panel 1).[25] However, use of these medical procedures in a subset of patients, such as in early treated, long-term virally suppressed adolescents,[26] could guide therapeutic strategies for cure.

Virological and immunological benefits of early antiretroviral therapy in children

Emerging evidence suggests that the use of early antiretroviral therapy not only reduces HIV-1 related mortality but also preserves immune function and long-term control of viral production. Early antiretroviral therapy restricts the number of long-lived CD4 T cells that harbour HIV-1 DNA and viruses that are competent of replication.[4,26] Importantly, early treatment also preserves the predominant naive CD4 cell populations and restricts the generation of memory cells. Data in early treated children suggest that within the small population of memory cells that do exist, the contribution of the proviral reservoir is greater in the short lived transitional memory than the long lived central memory CD4 T cells or naive CD4 cells pool,[26] a profile reported in the post-treatment controllers from the VISCONTI cohort.[27] Furthermore, early antiretroviral therapy is advantageous to restrict viral diversity and reduce escape mutations, both secondary to the absence of viral evolution over time. Immunologically, early control of viral replication through antiretroviral therapy preserves the normal development of the memory B-cell and T-cell compartments as shown in several cohort studies.[28–30] Additionally, Schuetz and colleagues[20] reported that early antiretroviral therapy initiation prevents the functional and quantitative loss of mucosal Th17 cells in addition to the induction of a normalisation of local and systemic T-cell activation.

Well established evidence suggests that most children who achieve sustained viral suppression since the first days of life show undetectable HIV antibodies and little, if any, cellular HIV-specific responses attributed to the absence of antigen stimulation.[3,4,16,24,31–33] The few studies that investigated the relationship between serostatus and viral reservoir show a direct association between the concentration of HIV-specific antibodies and the size of the viral reservoir.[3,34]

One implication of the use of early antiretroviral therapy worldwide is the increased number of HIV-infected children in therapy for many years who remain seronegative, leading to a growing demand by parents and patients to interrupt therapy. Whether a safe way can be achieved to interrupt treatment has become a pertinent and urgent question in the paediatric community. Paradoxically, although children who are seronegative are most likely to achieve a period of drug-free viral

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remission, as seen with the Mississippi baby, they might also be more susceptible to uncontrolled viral replication in the absence of HIV-specific immune responses.[18,23,32,34]

Therapeutic HIV vaccine research in early treated children

Reasons to implement therapeutic HIV vaccine research in children are summarised in panel 2. Life-long drug treatment for antiretroviral therapy is recommended for the paediatric population.[36] Maintenance of life-long adherence to the therapy is difficult, the risk of viral resistance, and as yet unknown long-term toxic effects, represent major concerns. Children infected vertically with HIV have a progressively increased risk of developing triple-class virological failure after 5 years of antiretroviral therapy.[37] Because of the absence of reliable biomarkers that can predict safe antiretroviral therapy interruption in these children, such a strategy will need to be closely monitored and should ideally be investigated in clinical trials. The PENTA 11 trial[40] reported that although short periods of treatment interruption might be well tolerated and seem safe long term, they are unlikely to provide any long-term immunological advantages.[40] Thus, the need to define strategies to provide long-term viral suppression and durable host immune control of HIV is urgent for safe interruption of antiretroviral therapy without viral rebound and the associated complications.

Panel 2: Reasons to implement therapeutic HIV vaccine research in children

Reasons to do therapeutic HIV vaccine studies in children infected with HIV perinatally

- Knowledge of timing of HIV exposure in infants allows for prompt diagnosis and treatment[25]
- All international guidelines recommend early antiretroviral therapy in all newly infected infants[35]
- The antiretroviral therapy recommended for the paediatric population needs lifelong medication with high risk of long-term toxic effects[36]
- A large number of children living with HIV will continue to need care and treatment after 2020[12]
- Vertically HIV-infected children have a progressively increased risk of developing triple-class virological failure after 5 years of antiretroviral therapy[37]

Reasons why early antiretroviral therapy treated children represent a unique model population to investigate immunotherapeutic strategies

- Children have a much more active thymus than adults and a greater capacity for immune regeneration which permits evaluation of vaccines specific immune responses[38–40]
- Children treated early with antiretroviral therapy can become seronegative and not have HIV-specific cellular immune responses leading to uncontrolled viral replication[4,31,33]
- Young infants have high immune tolerance, low immune activation, high naive CD4+ T cells, and low central memory cells, all features that are, conducive for a reduced and less diverse HIV reservoir and a great potential for effective response to vaccinations[23,41]

Immunotherapeutic strategies tested in HIV-infected adults

Several immunological strategies have been proposed to achieve viral remission without antiretroviral therapy. These include passive immunisation with broadly neutralising antibodies, which have been tested in primates,[42] in infected human beings,[43] and with a selected potent human monoclonal anti-CD4 binding site.[44] Another passive immunisation strategy targets the infected cell with a combination of antiretroviral drugs and monoclonal antibodies.[45] These approaches could reduce viral loads in human beings, but only for a short time (4–12 weeks). Strategies aimed at patients with prolonged expression of neutralising antibodies from use of an adeno-associated viral vector are being investigated with promising results.[46]

Immunotherapeutic approaches to enhance host immunity to control viral replication have so far shown small clinical effects or reduction in viral load in the context of treatment interruption. One pioneering study[47] in adults with HIV on single drug therapy (zidovudine) showed an improved 2-year survival with frequently repeated immunisations of the gp160 glycoprotein produced in a baculovirus expression system. HIV-1-specific immune responses elicited by dendritic cell vaccines significantly changed HIV RNA concentrations after antiretroviral therapy interruption in patients treated in the early stages of the disease.[48] Patients treated with antiretroviral therapy were given dendritic cells loaded ex vivo with HIV lipopeptides with good control of viral replication related to the efficacy of the vaccine.[49]

The efficacy of peptide-based HIV-1 vaccines have also been disappointing. The Vacc-4x vaccine was shown to significantly lower the viral setpoint after treatment interruption in adults, but without clinical benefit.[50] Li and colleagues[51] reported one therapeutic trial with a recombinant adenovirus-5-based HIV-1 gag vaccine was associated with only a slight transient effect on residual viraemia.[51]

These studies with single vaccines have not shown convincing long-term efficacy in controlling HIV in the absence of antiretroviral therapy. Therefore, our view is that a prime DNA-boost (vector-based) schedule could be more promising for early treated children. Such approaches have been shown to induce broad and long lasting specific cellular immune responses and functional antibodies in healthy individuals.[52–55] DNA vaccines are safe, immunogenic in prime-boost strategies, stable, easily stored, and can be manufactured on a large scale. The HIVIS vaccine, consisting of a multigene, multi-subtype A, B, C HIV-DNA vaccine, has been tested in

	Infant (0–6 months)				Child (6–16 years)
	PACG 230[72]	PACTG 326[73]	PedVacc 001[74]	PedVac 002[75]	PedVacc[76]
Vaccine type	Prophylactic	Prophylactic	Prophylactic	Prophylactic	Therapeutic
Vaccine compounds	rgp120 (MN) + adjuvant (alum/MF-59)	vCP1452 ± rgp120	MVA.HIVA (HIV-1 clade A gag p24/p17 + CD8 T-cell epitope)	MVA.HIVA (HIV-1 clade A gag p24/p17 + CD8 T-cell epitope)	HIVIS DNA (HIV-1 subtypes A, B, and C, encoded env, rev, gag, and RT)
Number of patients	188	30	48	73	20
Population under study	Exposed infants	Exposed infants	Healthy infants	Exposed infants	Vertically HIV infected children
Study design	Multicentre, phase 1/2, randomised, placebo-controlled study	Multicentre, phase 1/2, randomised, double blinded, placebo-controlled study	Single centre, phase 1, open-label, randomised, no treatment controlled study	Single-site phase 1/2, open-label, randomised-controlled trial	Phase 1/2 open-label controlled, randomised trial
Schedules	Birth and ages 2 or 4, 8, and 20 weeks	Birth and ages 4, 8, and 12 weeks	Aged 20 weeks	Aged 20 weeks	Week 0, 4, 12 with a boosting dose at week 36
Immunogenicity	Induce robust, durable env-specific IgG responses, including anti-V1V2 IgG; higher anti-V1V2 IgG responses in infant recipients of rgp120 (MN) + MF59 than in adult recipients of RV144[77]	Induce robust, durable env-specific IgG responses, including anti-V1V2 IgG	Did not alone induce sufficient HIV-1-specific responses	Not sufficiently immunogenic to induce HIV-1-specific, interferon-γ-producing T cells	Higher HIV-specific cellular immune responses were noted transiently to gag compared with age-matched control group; lymphoproliferative response to the gag virion antigen (HIV-1 MN) were higher in children than in adults[78]

rgp120=recombinant gp120. vCP1452=HIV-1 canarypox vaccine 1452. MVA=modified vaccinia virus Ankara. HIVIS DNA=multiclade multigene HIV DNA vaccine. RT=reverse transcriptase.

Table: **HIV vaccine studies in paediatric settings**

HIV-infected adults and elicits novel HIV-specific cellular immune responses, especially to gag antigens.[56] Mosaic genes, including strong immunogens of several HIV strains, are of particular interest.[57] A mixture of two mosaic HIV env genes, which included the single strains HXB2 and MN, the M group consensus sequence, and the two-valent and three-valent mosaic sequences, increased both cellular and antibody responses in macaques[58] whereas mixtures of plasmids encoding three selected HIV-C gp140 envs elicited high concentrations of neutralising antibodies in guinea pigs.[59]

Another genetic vaccine approach links together the optimum 18-mer T-cell epitopes of gag, pol, vif, and nef. This construct has shown good induction of CD8 cells in animals but has yet to be tested in human beings.[60] In healthy adults, broad and strong cellular immunity was shown with a heterogeneous vaccination strategy that primes with DNA and then boosts with recombinant vaccinia virus or adenovirus-based HIV genes, with or without adjuvant.[54,61,62]

Among several vectors that could carry HIV gene inserts, vaccinia virus, BCG, and measles virus have been included in non-HIV vaccination programmes for children and could therefore be advantageous as boosting strategies in future paediatric HIV vaccine studies.[63–65] Modified vaccinia virus Ankara might be a good vector for genetic vaccination because the safety record in human trials is good[66] and can boost long-term memory responses (more than 3 years) in healthy adults without limiting the opportunity to reboost because of vector immunity.[67] Rhesus cytomegalovirus as a vector induces unusual T cells that control simian immunodeficiency virus infection in macaques.[68,69] Clinical trials are continuing to investigate the potential use of this vector in HIV therapy in human beings (such as NCT01931358 and NCT02315703). Cytomegalovirus and adenoviral vectors generate extraordinarily good cell mediated immunogenicity, but with potential toxic effects that could restrict their use in children.

Among these different strategies, the selection of the first prime-boost approach to be used in early treated HIV infected children will have to be on the basis of safety and ability to induce effective cell mediated and humoral immune responses. To date, even the most promising strategies for HIV treatment have only been tried in adults. Although such results cannot necessarily be extrapolated to children, one or more of these approaches have the potential to achieve a durable viral suppression in children infected with HIV.

Early treated children with perinatal HIV-infections as unique models to evaluate therapeutic HIV vaccine

A major limitation of the adult cohorts studied so far is the absence of a uniform population to analyse in terms of timing of antiretroviral therapy, in addition to the immunological and virological status of the participants. Immunological impairment that occurs during the early phases of the infection and pre-existing immunity to HIV can severely bias the interpretation of immunological results, as described by Robb and Kim.[70] Children vertically infected with HIV and treated with antiretroviral therapy during the acute infection in the first 2 months after birth, correspond precisely to this profile since they have a very small pool of integrated viruses and an unparalleled capacity to regenerate a functional immune repertoire (panel 2).[5,38–40] Furthermore, many of these children are seronegative and do not have HIV-specific cellular

immune responses that facilitate a comprehensive investigation into vaccine specific responses. For these reasons, we think that studies on new immunotherapeutic strategies in paediatric populations might be more informative than those in adults, even in the context of prophylactic vaccination as advocated by Fouda and colleagues.[71]

Present scenario and future perspectives of therapeutic HIV vaccines in childhood

Only five randomised clinical trials of HIV vaccine have been done in paediatric settings in the past 20 years and only one in the specialty of therapeutic vaccination (table).[72–76] Two prophylactic HIV-1 vaccines in exposed infants were safe and immunogenic in phase 1 trials.[72,73] These vaccines can induce robust and durable env-specific IgG responses, including antibodies (anti-V1V2 IgG) associated with a reduced risk of HIV-1 acquisition in the RV144 adult vaccine trial.[77] Of note, the few data available that compare immunogenicity of similar HIV vaccines between adults and children show significantly better immune responses in the paediatric population.[77,78]

An initial attempt to increase HIV-specific responses during the infection, with the aim to control viral replication, has been tested in children by planned repeated controlled exposure to the autologous virus. This approach resulted in increased CD8 T-lymphocyte responses to HIV antigens and a reduction of viraemia; however in these individuals large amounts of viral diversification was noted. This heterogeneity was attributed to either increased viral replication or immunological pressure.[79]

To date, the only therapeutic HIV vaccine trial in children was a phase 2a open-label study with the DNA-based HIVIS vaccine (table).[76] In this population, a better cellular response was transiently recorded in HIV-specific antigens compared with an age-matched group of children infected with HIV and treated with antiretroviral therapy. The vaccine was also shown to be safe.[80] Although, to obtain broader and durable responses in every child, the priming DNA should be followed by a boosting vector-based vaccine, as previously described in healthy adults.[56]

The model of early treated children with perinatal HIV infections will contribute to the understanding of the mechanisms behind HIV remission, which will be translated into novel and more effective immunotherapeutic strategies than at present. An important first step will be to extensively characterise existing cohorts of early treated children as a potential trial population, including timing of treatment, size of viral reservoir, and presence of HIV-specific immune responses. These data might drive the choice of a personalised or a generalised immune intervention approach. In these future trials, the immunological and virological endpoints must be defined and closely monitored; these should include several relevant immune responses related to viral control, immune activation markers, and estimates of the viral reservoir. The latter should assess intracellular HIV DNA and RNA and detect HIV RNA in plasma with ultrasensitive methods.[81] Moreover, inclusion of the assessment of replication-competent viral reservoirs and analysis of viral evolution is crucial. A systems biology approach in combination with a mechanistic mathematical model could be used to help to predict vaccine induced immunity in the context of early treatment to improve trial design.[82,83]

Together these approaches could provide unique insights into the effect of immunotherapy on the viral reservoir and define the basis for achieving an effective immunotherapeutic strategy to prolong viral remission in perinatally infected individuals.

Contributors

All authors helped with the conception of this Personal View. NK and PP wrote the first draft, and managed all subsequent revisions. KL, SP, EN, DMG, PRoj, WB, SB, PZ, VC, AC, BW, CF, MAM-F, ADR, JA, DP, CG, and PRos provided comments on the draft. All authors reviewed the article and approved the final submission.

Declaration of interests

We declare no competing interests. The views expressed by JA are those of the author and should not be construed to represent the positions of the US Army or the Department of Defense.

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