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Rational Basis for an Immune Intervention in the Treatment of Primary HIV-1 Infection

Alain Lafeuillade, Gilles Hittinger, and Stéphane Chadapaud

Unité d'Infectiologie, Hôpital Chalucet, Toulon, France

Abstract

During acute HIV-1 infection, a strong cellular antiviral response develops, allowing a decrease in viral replication and the establishment of a set-point in HIV-1 RNA levels within a few months. However, HIV-1 persists due to the formation of a pool of latently infected cells and immune escape mechanisms. Combination antiretroviral therapy initiated at the time of acute infection has demonstrated the capacity to block HIV-1 replication < 20 copies/mL in most cases and protect from immune depletion. Some immune responses can improve, as can responses against recall antigens and CD4 anti-HIV specific immunity, but cytotoxic T cell activity generally wanes and plasma HIV-1 RNA rebounds each time therapy is stopped. The adding of an immune intervention to antiretroviral drugs is the next step to be studied. Interleukin-2 has demonstrated its potential benefits in chronic disease and its capacity to decrease the pool of latently infected cells. Specific vaccination against HIV-1 proteins using canarypox vectors is currently being tested in combination with HAART and IL-2 during acute infection. Cytotoxic drugs, like hydroxyurea, which act by antiviral and immunologic mechanisms, could also be of interest. The next few years will tell us whether these strategies initiated early in the course of HIV-1 infection are capable of inducing viral remission.

Key words

Primary HIV infection. Immune intervention. Treatment strategies.

Introduction

Primary HIV-1 infection (PHI) is an acute illness which is often under-recognized as clinical symptoms are not specific and risk factors not always obvious at the time of the first consultation. However, physiologically it represents the first contact with a previously normal immune system and the virus. Studies of the first virological and immunological events seen during PHI are consequently extremely important for understanding the mechanisms of the disease, and therapeutic interventions at this stage are expected to produce long-term benefits.

For the past 4 years, highly active antiretroviral therapy (HAART) initiated during chronic disease has led to significant decreases in morbidity and mortality related to HIV1 but it has also been shown unable to induce viral eradication or remission[2]. Recently, immune-based interventions have been proposed to reach this goal[3]. In this paper, we shall review currently available data demonstrating that PHI is the ideal stage of the disease to test these new approaches. As more than 40,000 new cases of HIV infection occur each year in the United States[4], 5,000 in France, and PHI is symptomatic in 40 to 90% of these cases, extensive efforts must be developed in parallel to diagnose this syndrome as early as possible.

Correspondence to:
Alain Lafeuillade
Unité d'Infectiologie
Hôpital Chalucet
83056 Toulon, France

The acute retroviral syndrome

Within days to weeks following exposure, non-specific symptoms can appear, including fever, headaches, rashes, pharyngitis, and oral or genital ulcers[4]. The major pitfall for diagnosis is that these signs will resolve spontaneously within a few days or weeks and usually in less than 14 days. Consequently, HIV-1 infection is often discovered several years after, when immune depletion has appeared.

This acute retroviral syndrome reflects early dissemination of the virus and intense viremia. In fact, animal models have shown that following sexual exposure to HIV the first cellular targets for the virus are Langerhans' cells; tissue dendritic cells found in the lamina propria subjacent to the cervicovaginal epithelium. Within 2 days, these cells infect CD4+ lymphocytes and HIV can be detected in regional lymph nodes. Shortly thereafter, systemic dissemination occurs and HIV can be recovered from blood within 5 days following infection. This early rise in viremia, often to levels of more than 1 million RNA copies/mL, explains why HIV dissemination is always massive, not only in lymphoid organs[5] but also in CNS and other organs. HAART initiated during PHI is consequently unable to prevent this spread, as at least a few days will always be necessary to confirm diagnosis. Viremia then spontaneously declines, reaching a "set-point" within 4-6 months following the acute retroviral syndrome. Resolution of clinical signs is followed by transition to chronic disease which can remain asymptomatic for many years.

This spontaneous decline in viremia has been attributed to a decrease in the number of target cells[6] and specific immune responses. There is a temporal relationship between the appearance of the cellular immune response against HIV and decreasing viral load. On the contrary, the humoral neutralizing immune response is more delayed and is detectable only weeks or months after the acute syndrome. Pantaleo *et al.* have demonstrated that the qualitative nature of the primary cellular immune response against HIV influences subsequent clinical outcome[7]. The authors analysed the Vβ repertoire of 21 patients diagnosed with PHI longitudinally and found different patterns of disturbance. In some cases, only a single family was expanded, in others 2 Vβ families were expanded, and in others the activation was polyclonal. In the following weeks, the rate of disease progression, measured as the rate of CD4+ T cell depletion, increased when Vβ expansion during PHI was limited.

In other viral infections, such as lymphocytic choriomeningitis virus (LCMV) infection in mice, as many as 70% of total CD8+ T cells are cytotoxic lymphocytes (CTL). During PHI, it has been estimated that 1 in 17 CD8+ T cells in blood are CTL targeting the virus. This reflects an attempt by the host to control viral replication, but leads only to decreased levels of viremia. However, another important mechanism is the T helper cell specific response against HIV[8]. Murine models with LCMV show that viremia is brought under control by CTL only when this helper cell response is present. Specific CD4+ T cells are hence critical for maintaining an effective CTL response.

However, initially expanded CTL clones have been shown to disappear with the establishment of chronic infection[9]. Furthermore, CTL activity, when detectable, decreases exponentially when HAART is initiated during chronic disease[10]. Specific CD4+ activity also usually disappears during established infection and can only be detected in patients with transient episodes of viremia[11,12]. Prolonged suppression of plasma viremia with HAART is unable to restore this specific response[13].

Viral persistence mechanisms

Despite the strong antiviral response observed during PHI, the persistence of HIV-1 infection rules the transition to chronic disease. This can be explained by virological and immunological mechanisms.

Virological mechanisms

Within a few days following contamination by HIV-1, antigen presenting cells migrate to regional lymph nodes where immune responses are generated. There, active recruitment of susceptible target cells leads to rapid spreading and amplification of HIV infection[14].

It has also been demonstrated that a pool of latently infected CD4+ T cells is established early during PHI. Chun *et al.* have studied 10 patients with PHI, some of them treated with HAART as early as 10 days following the onset of symptoms[15]. Remaining CD4+ T cells could be isolated in each case and infectious HIV-1 titers were not influenced by the time HAART was initiated or for the duration of therapy. This latently infected cell pool is therefore probably established before the appearance of an HIV-specific immune response. Thereafter, this pool remains stable and sheltered from the effects of host immune responses.

During the transition from acute to chronic disease, the formation of immune complexes leads to the concentration of HIV virions at the surface of the follicular dendritic cells in lymph nodes, which infect cells transiting through the lymphoid organs[16]. This constitutes a stable viral reservoir and a continuous source of infection for new cells.

It is now clearly demonstrated that HIV-1 replication also persists despite HAART, albeit at low levels. Firstly, using ultra-sensitive techniques, HIV-1 RNA has been measured in the plasma of 22 patients with sustained levels < 50 copies/mL[17]. Secondly, episomal intermediates[18], HIV-1 RNA and messenger RNA[19, 20] have been found in peripheral blood mononuclear cells of patients under effective therapy. Finally, viral evolution has also been demonstrated in such patients[21, 22]. During acute infection, it has been suggested that viral dynamics are mainly the result of Long-Range Transmission, although Proximal Activation and Transmission re-

mains the predominant mechanism during the chronic phase[23, 24]. Local bursts of infection persist in lymphoid tissues and antiviral drugs are less effective in blocking Proximal Activation and Transmission than Long-Range Transmission[23].

Immunological mechanisms

The CTL response shows early qualitative and quantitative changes leading to diminished efficiency.

Although high levels of viral replication take place in lymphoid organs, higher frequencies of *in vivo*-activated HIV-specific CTL are found in peripheral blood[14]. In fact, CTL cross rapidly from the lymph nodes into circulation; a physiological mechanism which serves HIV infection.

CTL also undergo clonal exhaustion after the massive expansion seen during acute disease[9]. This phenomenon is different from escape mutations and could be induced by the persistence of high antigenic levels.

The frequent occurrence of CTL escape mutants during HIV infection has been extensively documented. CTL kill infected cells via recognition of viral peptides (epitopes) derived from proteolytically processed viral proteins present at the cell surface within the HLA class I binding cleft[25]. This then triggers CTL to kill the infected cell via direct recognition mediated by the T-cell receptor on the CTL. CTL are able to recognize infected cells before progeny virus have been produced. The precise viral proteins targeted by CTL differ among individuals, depending on the particular class I molecules expressed on the surface of cells in a given person. Each HLA class I allele is slightly different; the viral peptides that can bind in the peptide binding groove are therefore different. Over 50 class I alleles have now been defined, and there are at least as many different viral peptides that bind to them. The measurement of specific CTL has been facilitated in the past few years by new sensitive technologies like Tetramer staining. Tetramers consist of 4 molecules of synthetic peptide-class I bound to a fluorescently labelled streptavidin, which allows direct binding to CTL through the T-cell receptor and direct visualization by flow cytometry. This technique has permitted the correlation of CTL and viral load in several cohorts. It has also been demonstrated that CTL responses exert selective pressure leading to epitope sequence changes, such as that exerted by antiretroviral therapy for the selection of resistant mutants. In a recently published study performed on monkeys, Evans *et al.* demonstrated that this is the result of a positive selection rather than a random mutation[26]. Furthermore, there is also evidence that some mutated epitopes can act as peptide antagonists[14]. Other viral mechanisms can also down-regulate the CTL response. For example, the viral *Nef* protein can diminish HLA class I expression, which can affect both the generation of antigen-specific immune responses and the recognition of virus-infected target cells by CTL[14].

In addition to CTL, cellular immune response is also associated with the generation of specific T-helper cells. These cells recognize viral peptides associated with class II molecules via antigen-associated cells; they are then activated and interact with other immune cells directly and through the release of cytokines. It has been clearly demonstrated that this T-helper cell response is necessary for maintaining an effective CTL response[8]. However, these cells are soon qualitatively and quantitatively impaired during the acute retroviral syndrome. This deficit exists not only in blood, but also in lymphoid tissue and persists with transition to chronic infection[27]. This defect can be explained by direct virus-induced pathology and indirect mechanisms such as increased apoptosis. The measurement of specific T-helper cells' activity is generally performed using lymphoproliferative assays against HIV-1 antigens. However, other recent techniques used to measure the intracellular induction of cytokines expression by specific antigens have found HIV-1 specific CD4+ T cells in most individuals with active infection[28]. This discrepancy underlines the fact that functional defects in these cells are probably as important as their previously-found reduced frequency.

In addition to these cellular immune responses, the humoral immune response may also contribute to viremia control. Neutralizing antibodies directly neutralize free virus and are targeted at epitopes in the envelope glycoprotein in highly-variable regions. However, the appearance of neutralizing antibodies is delayed during HIV infection and the maturing of the humoral response is slow[14].

Special situations

Further evidence supporting the effects of the immune system in controlling HIV-1 viremia comes from studies on long-term non-progressors and exposed but uninfected individuals.

Long-term non-progressors are characterized by HIV-1 RNA levels 4-10 fold lower in lymph nodes, and 20 fold lower in plasma than patients with progressive infection[29]. HIV-1 proviral load is also lower and lymph node architecture is preserved[29]. These patients demonstrate strong CTL activity and T-helper cell activity[11, 28, 29].

Strong CTL and non-cytotoxic CD8+ specific responses have also been detected in heavily exposed but uninfected persons like commercial sex workers[30, 31]. Regular exposure to HIV-1 antigens is probably required to maintain these responses.

Acute HIV-1 infection therapy

Several trials have been published recently on the effects of HAART administered since PHI. Hoen *et al.* have treated 64 patients with a combination of Zidovudine, Lamivudine and Ritonavir[32]. The proportion of patients with plasma HIV-1 RNA < 50 copies/mL by month 21 was 72%, but half of them still had quantifiable PBMC HIV-1 RNA and 2 out of 3 patients who underwent lymphoid tissue analysis had detectable viral RNA at this level. A subset of 17 patients was analysed in terms of CTL activity with this HAART regime[33]. Anti-HIV CTL were de-

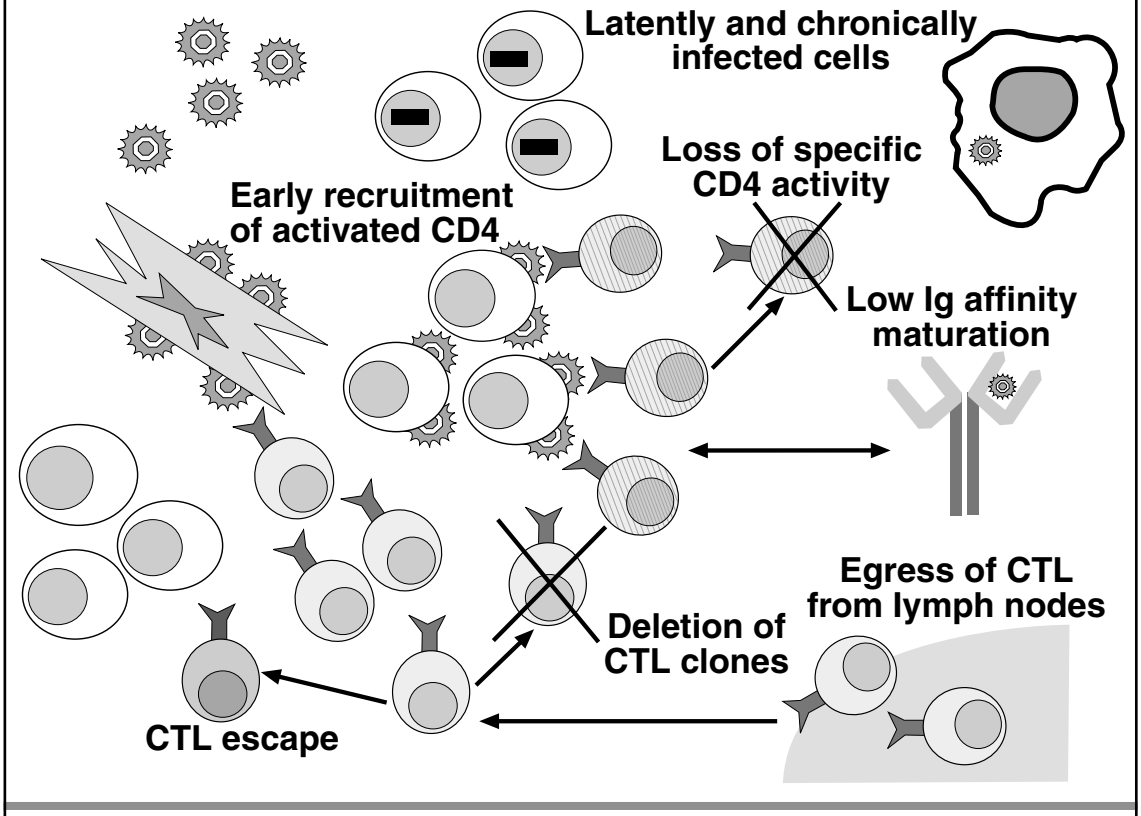

Fig. 1. *Early virological and immunological events involved in HIV-1 persistence during acute infection.*

tected initially in 15 patients. In 6 cases, CTL disappeared rapidly and persistently after initiation of HAART. In 3 cases, CTL disappeared transiently and in the last 6 cases CTL activity remained detectable and a clear correlation was demonstrated, with less efficient control of HIV-1 replication. In another study, the same antiretroviral combination was given to 12 patients diagnosed with PHI[34]. Patients adherence and tolerance was poor, mainly due to Ritonavir side effects. Plasma viremia persistently remained below 50 copies/mL in compliant subjects. HIV-1 RNA was detected in PBMC of some subjects and HIV-1 DNA remained consistently detectable. Expression of HIV-1 messenger RNA, either multiple-spliced, thereby indicating ongoing viral replication, or unspliced, indicating the presence of virions regardless of infectivity, was studied in gastro-intestinal associated lymphoid tissue in some cases. Proviral DNA was consistently detected at this level, as was unspliced RNA, although multiple-spliced RNA was undetectable. Fresh, unstimulated CTL anti-HIV activity was very low, and did not change during the trial. CTL precursors were undetectable in some patients, or detectable in others where viral replication was demonstrated in some compartments. The humoral immune response remained lower than anti-HIV antibody titers generally found in chronically infected persons. Of note, similar results have been obtained in PHI using triple therapy without a protease inhibitor, e.g. Zidovudine + Didanosine + Lamivudine, with better tolerance and acceptability[35]. However, Miro *et al.* have recently demonstrated that even using triple therapy with protease inhibitors, plasma HIV-1 RNA levels drop < 5 copies/mL in only 22% of cases after a mean period of 18 months[36].

More recently, published trials using HAART in PHI have better analysed the evolution of functional immune responses with therapy. The extra CD4 cells appearing with a quadruple HAART regime have been demonstrated to bear similar phenotypic properties to cells existing at baseline, but a higher proportion are capable of synthesizing IL-2[37]. In 13 patients receiving triple therapy for 1 year following PHI, average CD4+ cell counts were comparable to HIV-uninfected controls, with naive CD4+ T cells, memory CD4+ T cells and T cell receptor Vβ subsets all within normal ranges[38]. On the contrary, CD8+ T cell counts remained high and activated (HLA-DR+ CD38+) and CD8+ cells remained higher than in uninfected subjects, although rapid decreases were found with therapy. This was correlated with the persistence of HIV-1 in all cases, as demonstrated by a similar PBMC proviral burden in PHI treated and untreated patients at week 52. Naive CD8+ T cells were maintained with therapy, and memory CD8+ T cells decreased; a different situation from that observed when HAART is initiated during established infection. The evolution of T cell proliferative responses in patients receiving HAART since PHI has recently been extensively studied by Malhotra *et al.*[39]. Forty-one patients with PHI were analysed: 30 untreated and 11 receiving Zidovudine, Lamivudine and Indinavir. Initial lym-

Table 1. *Evolution of phenotype and function of immune cells in HIV-1 infection.*

	PHI without therapy	PHI on HAART	Chronic infection without therapy	Chronic infection on HAART
CD4 count	Decrease	increase	decrease	increase
CD4+CD45RO+	Decrease	increase	decrease	increase
CD4+CD45RA+	Decrease	increase	decrease	delayed increase
CD8 count	Increase	decrease	increase	decrease
Lympho-proliferation to recall antigens	Decrease	+	+/-	++/-
Lympho-proliferation to HIV antigens	Decrease	+	-	-
Anti-HIV CD4 activity	+	++	-	-
Anti-HIV CTL activity	+	++	+/- according to viral load	-

phoproliferative responses to recall antigens, Candida and tetanus, were absent in most of them. These responses increased 14-23 fold with therapy, although they increased less in untreated patients. Lymphoproliferative responses at baseline were observed against p24 in 7% of cases and envelope in 10%. The induction of lymphoproliferative responses was demonstrated in 60% of patients after 100 days of HAART against p24 and was less marked against gp-160. Untreated PHI patients rarely developed p24 specific lymphoproliferative responses. HIV-1-specific pCTL frequencies were variable in time but sustained in the majority of treated patients.

The fact that HAART initiated during PHI was capable of preserving anti-HIV CD4 specific activity was first pinpointed in 1997 by Rosenberg *et al.* in 3 cases[11]. This observation has recently been reinforced by analysing 8 additional patients[40]. Although patients who initiated HAART more than 6 months after seroconversion had no detectable HIV-1 specific CD4+ T cell responses, these responses were consistently found in early treated patients. HIV-specific CD8+ T cells were also preserved physically and functionally, contrary to the decline in tetramer-stainable HIV-specific CD8+ T cell populations found in patients with delayed initiation of HAART. Of note, even a short course of HAART during PHI of 1-4 weeks was capable of maintaining these CD4+ and CD8+ specific responses.

Rapid rebound of plasma HIV-1 RNA is observed following discontinuation of HAART initiated since PHI, even after more than 3 years of therapy[41]. However, in some anecdotal cases, this rebound has been delayed. The most extensively studied case is known as the "Berlin patient"[42], who received a combination of Hydroxyurea, Didanosine and Indinavir since PHI but stopped therapy at day 15 due to epididymitis. Plasma HIV-1 increased and HAART was resumed at day 22. A second therapeutic interruption occurred between days 128 and 137 due to acute hepatitis A, without plasma HIV-1 RNA rebound. Therapy was finally discontinued permanently at day 175 and plasma HIV-1 RNA remained undetectable during the following 551 days of the observation period. This patient was found to have a normal CD4/CD8 ratio, detectable CD4+ T cell specific activity, detectable CTL activity, but potentially infectious HIV-1 remained recoverable from cultured CD4+ T cells, although at very low levels. In another study, 6 patients receiving HAART for more than 3 years, including 5 treated since PHI, were followed up after therapy was discontinued[43]. In 3 cases, all treated since PHI, the rebound in plasma viremia was delayed for 4-24 months after discontinuation of HAART. These patients presented broad and strong HIV-1 specific CTL responses.

Overall, therapeutic interventions performed currently in PHI have demonstrated that long-term suppression of HIV-1 replication can be obtained, lymphocyte CD4+ subsets normalized, abnormal lymphocyte activation greatly reduced and specific anti-HIV cellular immune responses preserved, but that proviral DNA persists systematically and HIV-1 replication resumes almost every time HAART is stopped. Consequently, immune intervention could be a necessary adjuvant to HAART to obtain similar results to the "Berlin patient".

Potential candidates for immune modulation in PHI

No data are available at present in the context of PHI and lessons on immunotherapy in HIV-1 infec-

tion have been learned from established disease. The main potential candidates are soluble cytokines and therapeutic vaccination.

Cytokines

IL-2 has been used for many years in HIV-1 infection but the most interesting results come from studies using IL-2 in conjunction with HAART. This cytokine undoubtedly increases CD4+ T cell counts, including CD4+ cells with a memory and also a naive phenotype[44]. Of note, IL-2 also has an impact on CD8+ subsets by increasing CD8 +CD28+ cells; this is not observed with HAART alone[45]. However, little is known of its effects on lymphocyte functions. In some studies[45,46], no increase in CTL activity was found, although an increase in perforine and granzyme expression in CD8+ T cells was demonstrated in others[47]. Concerning lymphoproliferative responses to recall antigens, some authors have found an increase with HAART + IL-2 compared to HAART alone[45] although others have not[46]. More recently, IL-2 therapy has been used in association with HAART to attempt to decrease the pool of latently-infected CD4+ T cells. It is well known that administration of IL-2 can induce transient increases in viral replication, and *in vitro* studies have shown that combining IL-2 with TNF-α and IL-6 is able to purge this cellular pool[48]. An average number of 10 cycles of IL-2 was administered by Chun *et al.* to 14 patients on HAART and compared with 12 receiving HAART alone[49]. In the HAART + IL-2 group, a significant reduction in the pool size was obtained, with 6 patients reaching undetectable levels of potentially infectious virus in the remaining blood CD4+ T cells and 2 also having undetectable infectious virus in lymph node cells. However, when HAART was discontinued in these 2 patients, plasma HIV-1 RNA rebounded within a few weeks[50]. This therapeutic strategy was hence able to target the remaining pool but unable to decrease it below a theoretical threshold controllable by the immune system alone[2]. More aggressive approaches have been attempted for immuno-activation in 3 patients using IL-2 and anti-OKT3 antibodies, but tolerance was poor and HIV-1 DNA persistence was demonstrated in each case[51].

Other potentially interesting cytokines are Interferon-α, Interferon-γ and IL-12. Interferon-γ can increase CD8 functions and stimulate macrophages. IL-12 is of high theoretical interest for potentiating the generation of new specific immune reponses[3] but its use has been limited by toxicity.

Therapeutic vaccination

The use of specific HIV antigens is another way of attempting to obtain immune control of viral replication. Several vaccine candidates are already available and others will reach clinical trials in the near future. Remune* is an inactivated whole virus vaccine that has been depleted of the envelope protein. It is derived from a virus originally obtained in Zaire and contains a clade A envelope and clade D *gag*. It has been shown to be capable of inducing *gag*-specific T-helper cell responses in HIV-infected patients[52]. Furthermore, the production of β-chemokines (RANTES, MIP-1α, MIP-1β) is also increased after administration of this immunogen[53].

Recombinant viral proteins have been studied in several trials but the results were disappointing. Some envelope protein vaccines have induced HIV-specific T-helper cells and neutralizing antibodies, but these responses are not broadly cross-reactive. Randomized clinical trials using recombinant gp-160 in several hundred HIV-infected patients over 2-5 years have been unable to demonstrate any benefit in terms of viral RNA, CD4+ cell counts or clinical outcome[54, 55]. However, the possibility that these vaccines could be improved using a lipopeptide version is under investigation. Another approach involves the use of CTL peptide epitopes identified within the viral proteins; a strategy capable of providing protection against bovine leukaemia virus in animal models[56]. Canarypox vectors, similar to vaccina virus but with limited ability to replicate in human cells, are also used to express HIV-1 proteins and have demonstrated their ability to induce CTL responses and neutralizing antibodies[57-59]. New recombinant canarypox viruses expressing HIV proteins and regulatory genes of cytokine expression will be available soon. Preliminary data have shown that delayed rebounds in plasma HIV-1 RNA can be observed after stopping HAART in patients having received antiretrovirals plus this vaccine after PHI[60]. Finally, the use of DNA vaccines is currently being studied in phase I trials and has so far been shown to stimulate multiple immune responses, including Th1 type cellular responses[61-63].

Structured therapeutic interruptions

This method for stimulating anti-HIV immune responses involves using the patient's own viral strain. However, it can currently only be envisaged in the framework of clinical trials as no definite answers are available on its potency and lack of adverse effects[64]. Although stimulation of cellular immune responses against HIV has been demonstrated in anecdotal cases, it is possible that they wane with repeated therapeutic interruption and the question of the possible replenishment of the latently-infected cell pool with small increases in viremia still remains open.

Other approaches

Gene therapy could constitute another approach for converting cells into anti-HIV CTL[65]. Lymphocytes from patients are transducted with a vector resulting in the expression of the external portion of the CD4 molecule linked to the intracellular portion (zeta chain) of the T-cell receptor. These cells are capable of inhibiting HIV replication *in vitro* as efficiently as HIV-specific CTL derived from infected persons[66].

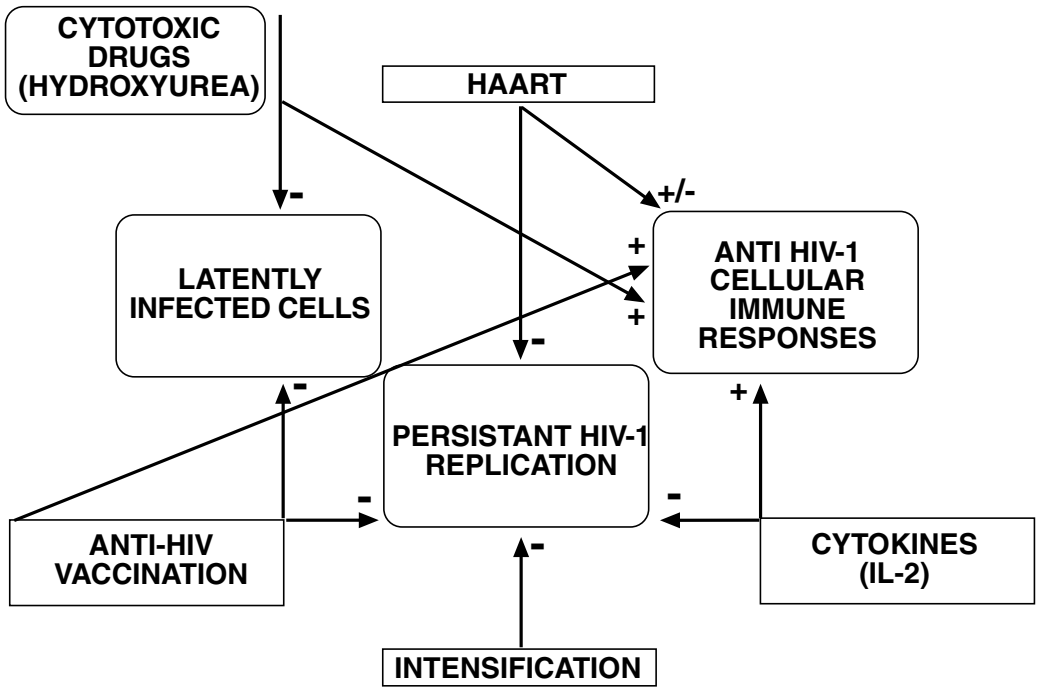

Fig. 2. *Potential strategies for therapeutic interventions in acute HIV-1 infection.*

Although hydroxyurea is mainly a cytotoxic agent, its immune modulatory effects have recently been underlined[67]. Associated with a possible action on the pool of latently infected CD4+ T cells, these immune modulatory effects could be of importance in the context of PHI.

Conclusions

Combined therapeutic interventions during acute HIV-1 infection (Fig. 2) may achieve the goal of long-term viral control. However, these strategies have to deal with ongoing viral replication and viral persistence enabled by immune exhaustion and escape. Intensification of therapy is probably necessary to obtain an optimal control of HIV-1 replication in all body compartments, but adjuvant therapies aimed at increasing anti-HIV immunity have to be combined in order to allow HAART interruption in some cases.

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