

Immune intervention strategies for HIV-1 infection of humans in the SIV macaque model

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Abstract

Studies in the SIVmac macaque model have demonstrated that the extent of virus-specific CD4+ and CD8+ T-cell responses induced by vaccination prior to virus-challenge exposure correlate with viremia containment following establishment of infection. These findings led to the hypothesis that active immunization with vaccines able to induce virus-specific T-cell responses following the establishment of infection could also ameliorate the virological outcome. Here, we will review the relative effect of ART and vaccination during primary SIVmac infection of macaques.

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Keywords: Macaque model; HIV immune therapy; Cytokines; Vaccines

1. Introduction

The introduction of ART has resulted in effective suppression of viral replication and decreased morbidity and mortality of HIV-1-infected individuals [1]. The decreased morbidity appears to be associated with the reconstitution of immune responses to pathogens, such as cytomegalovirus and Epstein-Barr virus [2–4]. However, HIV-1-specific immune responses decline during ART treatment of adults and children [5–9], perhaps because of a decrease of HIV-1 replication and antigen exposure under ART. The wide use of ART has also resulted in a better appreciation of the limitations of this daily multidrug combination treatment, such as compliance [10,11], due to the complexity of treatment and life-threatening side effects after long-term treatment [12–22].

The limitations of ART coupled with anecdotal findings that in a few cases ART interruption resulted in the maintenance of low plasma virus levels [23] particularly when ART treatment was initiated early during acute infection [24] have led to organized efforts to evaluate the clinical benefits of

ART interruption (STI) in HIV-1-infected individuals. The “therapeutic” use of STI was proposed with the dual purpose of decreasing drug toxicity and increasing virus-specific responses through auto-vaccination (viral rebound after STI) [25]. Recent data, however, indicate that, even though STI is associated with an increase in virus-specific immune response [26], it does not provide long-term benefits, except possibly when ART is initiated very early in acute infection [24,27]. The quality and/or quantity of the HIV-1-specific immune response increased by viral rebound may not be sufficient to maintain immune control of viral replication in the absence of ART and the risk of development of drug resistance and viral immune escape during STI is not negligible, as demonstrated in the macaque model [28].

In parallel, it has become appreciated that both HIV-1-specific CD4+ and CD8+ T-cells are important in the immune control of HIV-1 replication during primary and long-standing HIV-1 infection [29,30]. In fact, in HIV-1-infected individuals who do not require ART because they are able to contain viremia naturally, vigorous CD4+ and CD8+ T-cell responses have been demonstrated [8,30]. Therefore, a logical consequence of this collective knowledge has moved forward the concept that increasing the host immune response to the virus before ART is suspended may ameliorate the clinical outcome in the absence of ART [31–35]. However, progress in this area has been relatively slow due in part to the small number of vaccine modalities available for human use.

Abbreviations: ART, antiretroviral therapy; STI, structured treatment interruption; MHC I, major histocompatibility complex class I; CTL, cytotoxic T-lymphocytes; GALT, gastrointestinal lymphoid tissues; *gpe*, *gag-pol-env*

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2. SIV macaque model

SIVmac infection of macaques closely parallels HIV-1 infection of humans [36]. SIVmac-infected macaques experience an acute phase of infection within the first months following exposure, which at times is accentuated by rash and lymphadenopathy, as reported in primary infection of humans by HIV-1 [37]. Plasma virus levels during acute and chronic SIVmac251 infection of macaques [33,38,39] parallel the variability observed in humans and a portion of macaques are able to contain viremia spontaneously and progress to disease slowly, as in HIV-1-infected long-term non-progressors [40]. Both in humans [41] and macaques [42], fast progressors to disease have been described. In macaques that progress to disease, high plasma virus levels (usually above 10^5 to 10^6 copies/ml) go hand in hand with failure to gain weight, and impairment in response to recall antigens is observed in macaques that progress to disease [43]. Importantly, as in untreated HIV-1-infected individuals, in SIV-infected macaques a progressive decrease in the absolute number of CD4+ T-cells occurs. Usually, as CD4+ T-cells reach or fall below 100 CD4+ T-cells/mm³, opportunistic infection, loss of appetite, and loss of more than 10% of the body weight occur. Lymphoma can be observed occasionally.

The usefulness of the SIVmac model has been greatly increased by the characterization of MHC I molecules in Indian rhesus macaques [44], the identification of SIVmac peptides presented by these molecules [45], and the development of tetramers for CD8+ T-cells [44,46] as well as other quantitative and qualitative functional assays [47] to accurately measure virus-specific CD4+ and CD8+ T-cell responses induced by vaccination or viral infection. Similarly, accurate assays to measure neutralizing antibodies to primary and laboratory-adapted viruses have also been developed in the SIV model [48]. Because of these technical advances, it has been possible to assess the relevance of cell-mediated and humoral immunity in the control of SIVmac infection.

As in humans, the immune response to SIVmac during primary and chronic infection differs [49–52] and evidence of immune escape has been provided in both systems [28,53,54]. The importance of CD8+ T-cells in viremia containment has been demonstrated by depletion studies [55–57] and the role of virus-specific CTLs has been inferred by inverse association with their frequency and viremia containment [58,59]. Although less is known about CD4+ T-cells, their importance in immune control of SIV replication has been observed in studies whereby the infusion of autologous naive CD4+ T-cells ameliorated virological outcome in SIVmac251-infected macaques [60]. In addition, an inverse correlation between virus-specific CD4+ T-cell responses and viremia containment has also been observed in prophylactic [58] and therapeutic studies in macaques [71].

Similarities between the SIVmac model and HIV-1 infection of humans include the demonstration of a lower susceptibility of macaques with expression of a specific MHC I molecule (Mamu-A*01) to disease induction by the SIVmac251 (561) stock [38], as observed in humans carrying the HLA-B*5701 or HLA-B*2701 molecule [61–63]. In both [complexes] macaques and humans [62] carrying these MHC I, a broad and robust immune response to the virus has been observed [50,64].

Treatment of SIVmac infection with antiretroviral drugs within minutes or hours from infection results in virus clearance [65–67]. Delayed treatment after exposure up to 6 weeks results in most cases in long-term viremia containment [33,68,69]. The observation that HIV-1-infected individuals treated with ART during the acute symptomatic phase of infection results in containment of viremia in some individuals [24,70] validates once again the relevance of the SIVmac macaques model for human AIDS.

ART treatment alone of macaques several months from exposure does not result in virological benefit after ART cessation [25,71], as observed in an increasing number of HIV-1-infected individuals undergoing ART interruption [26,72]. All together, these data validate the SIVmac model as a good predictor for humans of the effects of vaccines in both the prophylactic and therapeutic settings.

3. Prospects for immune therapy of HIV-1/SIV infection

In principle, a therapeutic vaccine for individuals infected with HIV-1 and treated with ART should be able to restore functional virus-specific CD4+ T-cells in order to maintain CD8+ T-cell function, broaden the immune response to subdominant epitopes in order to minimize viral immune escape after ART cessation, and revert the vicious cycle that maintains chronic infection. The loss of CD4+ T-lymphocytes in long-standing HIV-1/SIV infection coupled with defects in the number and, perhaps even more importantly, in the function of virus-specific CD4+ and CD8+ T-cells [64,73–78] may represent, however, serious limitations for immune intervention. Therefore, immune-based strategies may need the help of cytokines to correct deficiencies in the number of immune cells in the host and/or in the quality of the immune response. The quality of the immune response may be a predictor of disease progression [52,79].

An important limitation of therapeutic immunization is that vaccination likely will expand mainly preexisting memory responses and, particularly in the case of CTLs, these responses may be irrelevant because of viral immune escape. In addition, because of continuous genetic variation in HIV-1 proteins encoded/presented in the vaccine, a “fixed” immunogen may not elicit immune responses relevant to the virus present in the host at the time of immunization. Another possible limitation resides in the decreased number and

possibly the function of virus-specific CD4+ T-cells [77] since these cells are a preferential target for HIV-1 [80,81] and key in the generation and maintenance of functional CD8+ T-cell response.

Several investigators have proposed that, among the underlying defects in HIV-1 infection, the production of and response to IL-2 may be limiting [77,82–88]. Therefore, IL-2 has been used extensively in human trials of ART-treated individuals in HIV-1 infection [89–102]. However, IL-2 alone in the absence of exogenous antigens does not appear to increase virus-specific response. In IL-2-treated HIV-1-infected individuals, cessation of ART does not confer immunological control of viral replication in the long term. In contrast, the effect of IL-2 as an adjuvant to a vaccine has not been extensively studied in humans.

Here, recent information on ART treatment, vaccination, and IL-2 treatment in macaques with SIVmac251 infection will be summarized. Because immunological events during primary and long-standing SIVmac251 infection may differ substantially, the intervention during these stages of infection likely provides different challenges. Since most of the data available at present are derived mainly from studies on

intervention in primary infection, this will be the major focus of this review.

4. Long-term containment of viral replication following early ART treatment of primary SIVmac251 infection

Since the 1990s, it has been clear that intervention with a single drug (PMPA) within a few minutes and up to 48 h from infection can prevent the establishment of SIVmac chronic infection [65–67], indicating that inhibition of viral replication and spreading of infection (within the first 48 h) may allow the host to clear the virus. ART can also prevent viral SIVmac infection in newborn macaques [103].

Since the development of adaptive immune responses requires at least 4–5 days [104,105], the data obtained in adult macaques suggest that, if any host response is involved, the innate immune response in combination with drug-reduced viral replication and seeding may play a role.

Later intervention, within a few weeks following infection, with antiretroviral drugs occurs in a setting whereby adaptive humoral and cellular immune responses have occurred. In untreated macaques, these responses are

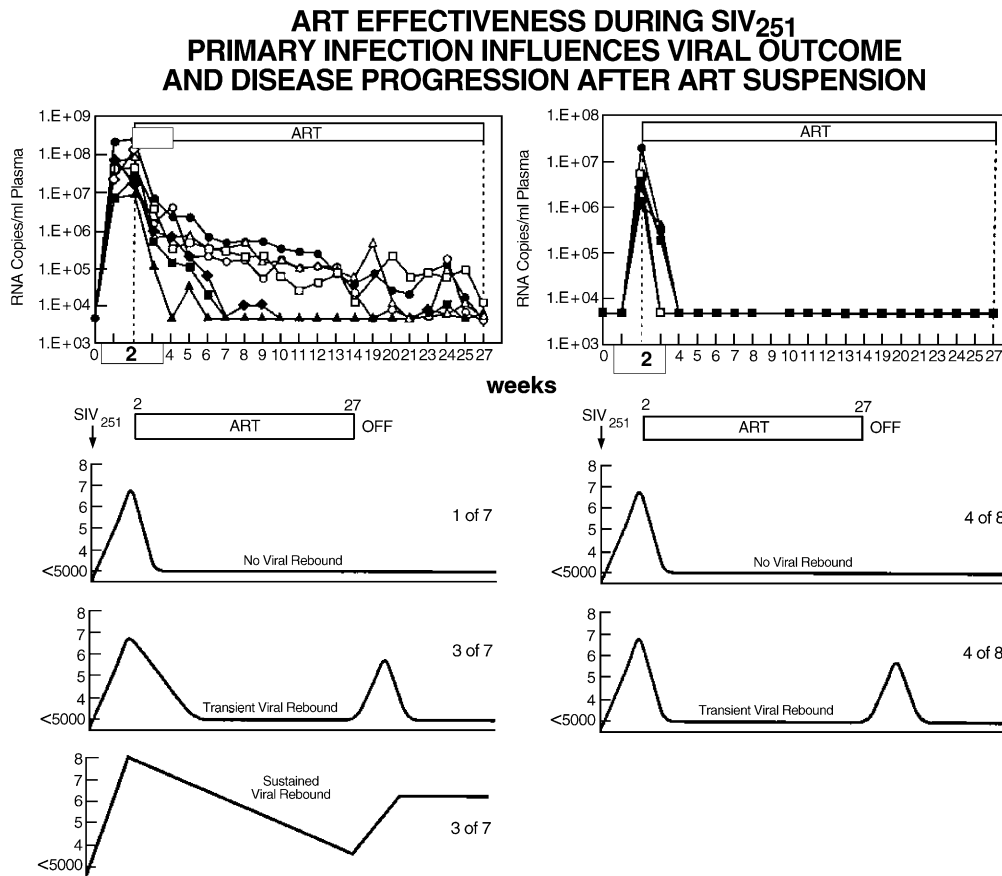


Fig. 1. Virological outcome following ART cessation in macaques treated with different ART regimens: On the top two panels virus plasma levels during ART in macaques treated with a less effective (left top panel) or more effective (right panel) ART treatment are shown for each macaque. The panels in the bottom of each treatment graphically represent the virus load in macaques following ART treatment. The logs of the copies of viral RNA/ml of plasma are depicted on the y-axis. The x-axis represents time in weeks. ART was initiated at week 2 and suspended in all macaques at week 27.

nevertheless unable to clear viral infection and chronic infection is established. In macaques treated between 2 and 6 weeks from infection with ART, however, the disease course is substantially delayed [33,68,69]. Depletion of CD8+ T-cells after early treatment demonstrates the role of CD8+ T-cells in the control of viremia [106]. Of interest, the virological outcome appears to depend in part on the extent of ART suppression of viral replication. In our experience, the virological outcome of early intervention with ART is related to the effectiveness of treatment. Macaques exposed intravenously to SIVmac251 and a less effective ART regimen at week 2 post-infection, which resulted in failure to suppress viremia below the threshold of detection within the first 2–3 weeks of treatment, tended to fare less well after cessation of antiretroviral drugs (i.e. sustained viral relapse occurred; Fig. 1).

Although the overall frequency of CD8+ T-cell responses to the SIVmac-dominant Gag_{181–189} CM9 (p11C, C → M) Gag epitope did not differ in macaques treated with a more or less effective ART regimen, a clear difference was observed in the restoration of virus-specific CD4+ T-cell responses during ART, which was much lower in macaques

treated with a less efficient ART (Fig. 2). These data appear to suggest that an effective early intervention may preserve the number and/or the function of virus-specific CD4+ T-cells. Early intervention may limit the profound depletion of CD4+ T-cells, particularly in the GALT [107], which is observed in this macaque model. The maintenance of a pool of virus-specific memory helper cells in concert with the CD8+ T-cells may contribute to viremia containment. An alternative and not mutually exclusive interpretation could be that containment of the initial viral burst may restrict the generation of viral quasi-genes and lessen the chance of viral immune-escape selection. In humans, effective suppression of HIV-1 replication in early infection is also associated with restoration of HIV-1-specific responses [26,108].

Our cumulative experience in the macaque model demonstrated that early (2 weeks following infection) treatment of 15 macaques with ART alone for a minimum of 22 weeks resulted in sustained viral rebound in only three of them (20%). Interestingly, all macaques that experienced a sustained viral rebound were among those treated with a less effective ART regimen [33,109,110].

RESURGENCE OF PROLIFERATIVE RESPONSES CORRELATES WITH ART EFFECTIVENESS (Viremia Suppression)

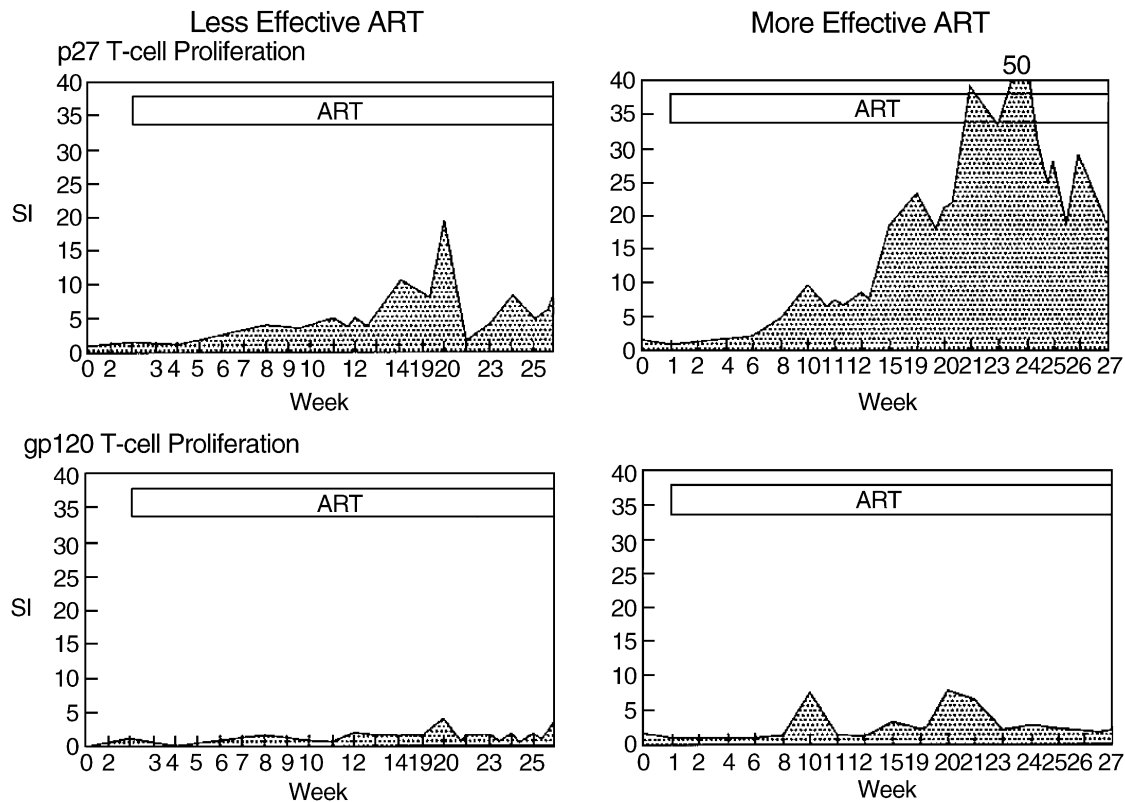


Fig. 2. Restoration of p27 Gag and gp120 Env proliferative responses during ART. The stimulation indices are presented on the y-axis and the weeks of treatment with the different ART regimens on the x-axis. The gray area exemplifies the maximum response among seven macaques (left panel) and eight macaques (right panel) for p27 Gag (top panels) and gp120 Env (bottom panels).

5. Active vaccination of macaques during primary SIVmac251 infection

Experiments were designed to allow the establishment of infection at first, and then the macaques were vaccinated in the presence or absence of ART (initiated at week 2). The vaccine modalities used in those studies were NYVAC-SIV-*gpe* [111] and ALVAC-SIV-*gpe* [38,112]. Both vaccines were able to expand CD8+ Gag-specific tetramer-staining T-cells and proliferative responses to p27 Gag and gp120 to the same extent in the infected macaques [33,109]. However, the ability of NYVAC-SIV-*gpe* (and presumably also ALVAC-SIV-*gpe*) to expand virus-specific CD4+ and CD8+ T-cells appeared to depend on the level of suppression of plasma virus by ART. In fact, in the absence of ART, no expansion of virus-specific CD8+ T-cells was observed following vaccination [33]. Interestingly, the extent of proliferative responses induced by vaccination was also dependent on the effectiveness of viral suppression by therapy, as demonstrated by different levels of reconstitution of CD4+ T-helper responses in the macaques

treated with the two regimens of ART demonstrated in Fig. 3.

The virological outcome following vaccination of macaques during primary infection did not differ significantly from macaques treated with ART alone, regardless of the vaccine used. Out of a total of 19 macaques (11 vaccinated with NYVAC-SIV-*gpe* and eight with ALVAC-SIV-*gpe*), only four (21%) experienced sustained viremia following ART suspension, indicating that vaccination did not appear to provide better protection from sustained viremia than ART alone.

6. IL-2 as a vaccine adjuvant

The effect of IL-2 as adjuvant of the NYVAC-SIV-*gpe* vaccine as well as alone was also assessed. IL-2 at a 120,000 IU daily subcutaneous dose was chosen following a pharmacokinetic study [28] and started at the time of immunization with mock or NYVAC-SIV-*gpe* vaccine. IL-2 treatment was continuous and maintained up to week 28,

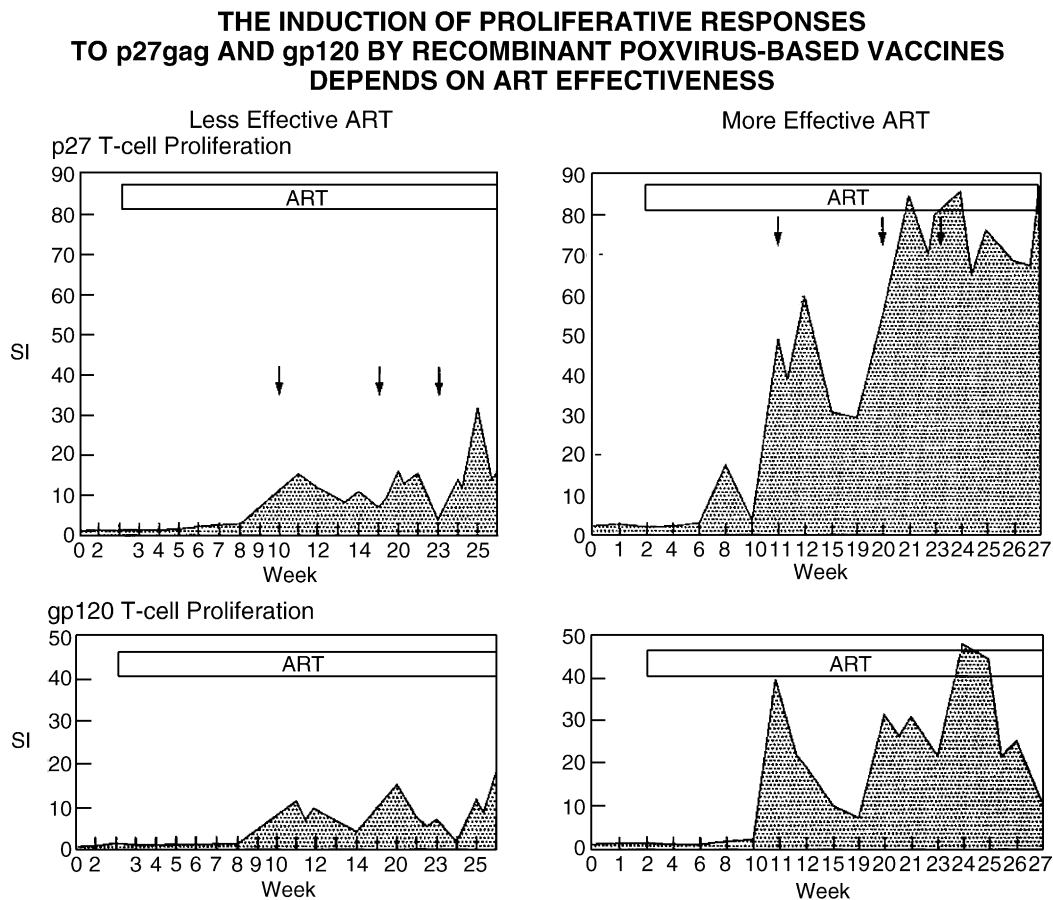


Fig. 3. The vaccine-induced proliferative responses are depicted on the level of viremia suppression. Macaques treated with both ART regimens were vaccinated with either NYVAC-SIV-*gpe* (left panel) or ALVAC-SIV-*gpe* (right panel) at the weeks indicated by the arrows (10, 19, 23). As in Fig. 2, the gray area represents the maximum response among eight macaques in each group. The difference observed does not relate to differences in the immunogenicity of these vaccine modalities since a comparison of the ability of NYVAC-SIV-*gpe* and ALVAC-SIV-*gpe* to induce these responses demonstrated no significant differences between the two vectors [109].

6 weeks after cessation of ART. IL-2 increased the functionality of vaccine-induced CD8+ T-cell-dominant Gag response [28] and, for unclear reasons at present, decreased the vaccine-induced as well as the proliferative responses that are reconstituted during ART. Out of the 12 macaques treated with IL-2, only two (20%) experienced sustained viral rebound following ART cessation. Vaccination, whether alone or in combination with IL-2, did not appear to influence the virological outcome (data not shown). Of interest, a number of macaques experienced a detectable and transient plasma level increase only after suspension of the cytokine, suggesting that the maintenance of cytokine treatment may have contributed to viremia containment (data not shown). Recent data demonstrate indeed that IL-2 is limiting not only in humans [77,82–102] but also in the macaque model. The demonstration of a decreased production of IL-2 in CD4+ memory and effector cells in SIVmac-infected macaques is particularly relevant [113].

7. Discussion

All together, these data indicate that early initiation of ART significantly modifies disease progression. The effect of vaccination, however, was not obvious in these studies. Possibly the effect of ART treatment and the relatively small number of macaques in the studies may account for the findings. Alternatively, in the presence of the large adaptive immune response that occurs during primary infection, further exposure to antigens (by vaccination) may not contribute significantly to the immune control of viral replication. Importantly, however, all these data also indicate that the activation of the immune system induced by these vaccine modalities does not appear to be deleterious and result in a worse virological outcome.

In conclusion, ART intervention during primary infection in the macaque model consistently allows for long-term control of viral replication and in most cases maintenance of normal CD4+ T-cell count. Thus, early intervention in HIV-1 infection may as well change the clinical course of HIV disease in humans and data on a few patients treated very early in infection appear to support this concept [24].

In contrast, in long-standing SIV/HIV-1 infection, it is quite clear that prolonged ART treatment neither eradicates infection [114,115] nor results in the restoration of immune responses able to control viral replication [26,27], indicating the need for alternative approaches. Immunization during ART of macaques with long-standing SIVmac251 infection is providing encouraging results [71], but immunization alone likely may not be sufficient to revert the virus-induced damage in individuals with low CD4+ T-cells. The appropriate cytokines in combination with the appropriate dose of vaccine or mixed vaccine modalities [116,117] need to be modeled to obtain an immune response of sufficient breadth and potency to control HIV-1 infection in the long term. The fact that a percentage of humans infected with HIV as well

as macaques infected with SIV (long-term non-progressors) is able to do so indicates that it may be possible to develop the appropriate combination of treatments.

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