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Nonhuman primate models and the failure of the Merck HIV-1 vaccine in humans

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Abstract

The recent announcement that a replication defective adenovirus-type 5 Gag-Pol-Nef HIV-1 vaccine developed by Merck failed in the STEP human Phase IIb efficacy trial to either prevent HIV-1 infection or to suppress viral load in subjects who subsequently became infected, was predicted by studies that had evaluated analogous vaccines in the simian immunodeficiency virus (SIV) challenge/rhesus macaque model. In contrast, vaccine protection studies in macaques that used a chimeric simian-human immunodeficiency virus (SHIV89.6P) challenge failed to predict the human trial results. Adenovirus-vector based vaccines did not protect animals from infection after SHIV89.6P challenge but did cause a substantial reduction in viral load and a preservation of $CD4^+$ T-cell counts post-infection, findings that were not reproduced in the human trials. While disappointing for the clinical development of Merck's vaccine candidate, these studies now enable vaccine designers to utilize the SIV-challenged macaque model with more confidence, thus facilitating the future prioritization of candidate vaccines. Vaccine designers must now develop T-cell vaccine strategies that reduce viral load after heterologous challenge.

Introduction

Passive transfer studies with broadly neutralizing antibodies in non-human primates (NHPs) provide a proof of principle that immunological protection against HIV-1 is possible [1–3]. Moreover, natural history studies in cohorts of HIV-1-infected humans, and analogous studies with simian immunodeficiency virus (SIV) in NHPs, show that cellular immune responses can control primate immunodeficiency virus replication [4]. It is generally agreed that an effective HIV-1 vaccine will probably need to elicit broadly neutralizing antibodies (NAbs) and robust cellular immune responses to provide protection from infection and/or disease and to reduce transmission.

The failure of VaxGen's AIDSVAX HIV-1 vaccine was announced in 2003 [5]: this envelope-specific, gp120-based vaccine induced antibodies that did not neutralize primary HIV-1 isolates *in vitro,* did not prevent HIV-1 infection of humans, and had no effect on viral load in trial participants who became HIV-1 infected. To date, no HIV-1 vaccine in clinical trials has induced broadly active NAbs; this "neutralizing antibody problem" remains the primary obstacle to a safe and effective vaccine, and is being addressed by a number of groups and

consortia including IAVI's Neutralizing Antibody Consortium [6]. The HIV-1 vaccine field has also been developing cytotoxic T lymphocyte (CTL)-based immunogens, encouraged by data from natural history studies and NHP models demonstrating control of virus replication by CTL (see below). To that end, an efficacy trial of a prime-boost regimen consisting of a canarypox vector prime and an AIDSVAX protein boost started in late 2003, amidst considerable controversy as to its likely outcome [7,8]; we still await the results of this trial, now expected in 2009. Unfortunately, the most promising approach for inducing CTL responses tested clinically to date, an adenovirus-based vaccine regimen, has recently failed in human efficacy trials [9,10]. This candidate, developed by Merck, Inc., elicited cell mediated immune (CMI) responses against the HIV-1 Gag, Pol, and Nef proteins, in safety and immunogenicity trials [11]. However, on average, individual volunteers mounted relatively weak responses (10%–20% of that seen in HIV-1 infected individuals controlling viral replication). Furthermore, vaccinees recognized a total of only three epitope-specific responses in the Gag, Pol and Nef immunogens, which may not be adequate for protection. It is also possible that some or even all of these responses were rendered ineffective by HIV-1 sequence diversity, since the viruses to which human vaccinees were exposed differ by ~10% even when the clade of the vaccine strain matched the one most prevalent within the trial site. Sequence mismatches are a particularly relevant concern because analyses of variability in regions of the virus outside Env have shown that the majority of amino acid replacements are selected for by CTL [12,13]. Hence, we can anticipate that many circulating viruses incorporate mutations that allow them to escape from immunodominant responses induced by vaccines of limited breadth.

Advancement of candidate AIDS vaccines from Phase I/IIa safety and immunogenicity trials to Phase IIb/III efficacy trials has been empirical. We discuss below the hopes for a T cell-based vaccine and how the SIV-rhesus macaque challenge model predicted the failure of the Merck vaccine. We also propose mechanisms for the future prioritization of candidate HIV-1 vaccines.

The Role of CTL in Control of Immunodeficiency Virus Replication

The first indications that CTL could suppress HIV-1 replication *in vivo* were observations that the reduction in viremia in acute infection was temporally associated with the appearance of HIV-1-specific CTL [14,15]. A NAb response usually occurs subsequent to this initial CTL response, after viremia has been controlled. The important role of CTL was further suggested by work in the SIV-macaque model of HIV-1 infection. When anti-CD8 monoclonal antibodies were used to transiently deplete circulating $CD8^+$ T lymphocytes. The resulting loss of $CD8^+$ T cells significantly impaired immunological control of SIV replication in both the acute and chronic phases, leading to substantial increases in plasma viremia [16–19].

A Vaccine Should Reduce Disease and Transmission

The best long-term solution to the HIV-1 pandemic is a vaccine that prevents infection completely ("sterilizing immunity"). A less desirable, but still valuable, alternative is a vaccine that substantially reduces HIV-1-induced disease and the risk of transmitting infection to a new host. The latter was the most realistic goal of the Merck vaccine and of other CTL-inducing inducing vectors that do not induce NAbs. The risk of HIV-1 transmission is greatest when viremia is highest, i.e., during acute infection and chronic infection with elevated viral load [20–22]. Any HIV-1 vaccine that cannot provide sterilizing immunity should, therefore, aim to limit peak viremia in acute infection and to reduce chronic-phase viral loads from the median value of ~30,000 copies/ml in untreated subjects, to levels at which transmission is unlikely. In an observational study, infected individuals

with viral loads below 1,500 copies/ml had a substantially reduced risk of infecting their seronegative partners (Fig. 1) [23–25].

Conventional Vaccines have had Limited Success against SIV Challenge

Unfortunately, few CTL-based vaccine regimens have significantly lowered viral load or affected disease course in macaques challenged with the most stringent SIV strains, SIVmac239 and SIVmac251 [26–29]. SIVmac251 is a swarm whereas SIVmac239 is a clone derived from an SIVmac251-infected animal [30]; for the purposes of this discussion, these viruses can be considered equivalent. Both are equally pathogenic, with comparable peak and chronic phase replication in Indian rhesus macaques, the animal model of choice for SIV researchers. Encouragingly, several vaccine regimens including modified vaccinia Ankara (MVA), canarypox virus (ALVAC) or New York Vaccinia Virus (NYVAC) encoding SIV proteins have exerted modest levels of control over SIVmac251 replication [31–36].

MVA, NYVAC, ALVAC and fowlpox vectors have also been used alone or after a DNA prime to vaccinate macaques that were subsequently challenged with SHIV89.6P. In 2001, Amara *et al.* [37] used a DNA prime, MVA boost strategy to control SHIV89.6P replication, as manifested by viral load reduction and preservation of peripheral $CD4^+$ T cells. Studies conducted by Merck and others had similar outcomes [38–40]. Although several and serious doubts have long been raised about the suitability of SHIV89.6P challenge for testing CTL-based vaccines [41,42], some researchers considered control of SHIV89.6P replication in macaques to be sufficient to warrant further evaluation of analogous vaccines in humans. The outcome of the Merck/HVTN trial clearly showed that such views were flawed. The failure of the Merck vaccine to control HIV-1 replication was, however, mimicked by macaque studies that used SIVmac challenge (see below). But although failure against SIVmac challenge predicted failure in humans, a more complete validation of this model will require that amelioration of disease course in vaccinated macaques successfully predicted the efficacy of an analogous HIV-1 vaccine in humans. Since only live attenuated SIV has thus far suppressed viral load at set point in macaques challenged with heterologous SIV, improved vaccine candidates will be required to further validate the SIV-rhesus macaque model, and of course to bring us closer to an effective HIV-1 vaccine. Although the SIV challenge model is incompletely validated, we propose below that its expanded use can help facilitate the prioritization of candidate HIV-1 vaccines, ensuring that resources are focused on the most promising candidates.

DNA/Ad5 is Superior to Ad5/Ad5 at Controlling SIV Replication In Macaques of a certain MHC type

Previous studies have demonstrated that macaques vaccinated with a DNA prime / rAd boost were more effective at controlling SIV infection than macaques immunized with a rAd prime / rAd boost. However, even with the DNA prime / rAd5 boost, only macaques expressing *Mamu-A*01* showed any control of SIVmac239 replication (Fig. 2A). Vaccination with rAd/rAd was ineffective in both *Mamu-A*01* positive and negative macaques (Fig. 2B), and *Mamu-A*01* negative animals vaccinated with DNA prime/rAd boost failed to control virus replication [43,44].

DNA Priming followed by Ad5 Controls Replication of SIVmac251 and SIVmac239

A DNA prime Ad5 boost regimen expressing SIVmac239 Envelope, Gag and Pol has been tested by the Vaccine Research Center (VRC) in Indian macaques [45,46]. This vaccine

resulted in transient control (until day 112) of the homologous SIVmac251 challenge virus, amelioration of memory $CD4^+$ T cell loss during the acute phase and increased survival of vaccinees. However, given the inclusion of a matched Envelope construct in the vaccine regimen and marginal reduction of replication of the homologous challenge virus, the prospects for the success of this type of vaccination in humans are not compelling.

Whether vaccine-induced cellular immunity in the absence of any Env-specific antibodies can control viral replication was studied by using multiple low-dose challenges with the highly pathogenic SIVmac239 isolate [47]. In this experiment, eight *Mamu-A*01* positive Indian rhesus macaques were vaccinated with SIV Gag, Tat, Rev and Nef using a DNA prime, adenovirus boost strategy. Peak viremia ($p = 0.007$) and the chronic phase, set point viral load ($p = 0.0192$) were significantly decreased in the vaccinated cohort, out to one-year post infection (Fig. 3). Of note is that only one of the eight vaccinees had developed Env-specific NAbs by one year after infection. Thus, vaccine-induced CMI responses can clearly exert significant control over replication of a primate immunodeficiency virus in the complete absence of NAbs. This finding supports the idea that a vaccine that induces only CMI responses might be able to control viral replication.

Even our Best NHP Vaccine is only Partially Effective against a Heterologous SIV Challenge

Immunization with live-attenuated SIV has consistently protected rhesus macaques against challenge with a homologous, pathogenic SIV [48–50]. However, only a few small studies have addressed whether this type of vaccine can control replication of a heterologous SIV, with mixed results [51–53]. Recently, we investigated whether macaques vaccinated with SIVmac239ΔNef could control an intravenous challenge with the highly pathogenic, heterologous swarm virus SIVsmE660, in a large-scale study designed to achieve sufficient statistical power (Reynolds *et al.*, Keystone presentation 2007 and manuscript submitted). Tests under equal group variances revealed that plasma viral loads were significantly reduced in the ten vaccinees compared to the ten MHC-I-matched controls at 2–16 weeks post-challenge. Hence, it is possible to achieve a reduction in virus replication post-infection, even after heterologous challenge.

Why did the Merck Vaccine Fail?

The vaccine's failure to control HIV-1 replication may have been due to the Ad5 vector, the choice of the HIV-1 transgenes or a combination of these two factors. It is possible that a replication-defective Ad5 vector is simply unable to stimulate cellular immune responses of sufficient breadth to control HIV-1 infection. While pre-existing Ad5-specific antibodies will restrict the number of Ad5 particles that can infect target cells and produce transgene-derived proteins, pre-existing Ad5-specific $CD8^+$ T-cell responses could potentially reduce the potency and breadth of vaccine-induced HIV-1-specific $CD8^+$ T-cell responses. In individuals previously exposed to an adenovirus, anamnestic adenovirus-specific CD8+ T cells will dominate the initial response to the Ad5 vaccine. Many different factors might affect the preferential expansion of these Ad5-specific $CD8^+$ T cells thereby diminishing the expansion of the HIV-1-specific precursors [54–58]. Moreover, the selection of the HIV-1 transgenes (Gag-Pol-Nef) may be insufficient for control. In contrast to the limited number of HIV-1 gene products expressed by the Ad5 vector, the live attenuated SIV that protects against heterologous SIV challenges is a persistently replication-competent (albeit weakened) virus that expresses every SIV antigen with the exception of parts of Nef. Developing the next generation of improved vaccine candidates will require that we address the following important issues.

Vaccines Should Broaden Immune Responses Rather than Relying on Natural Immunodominance Patterns

Volunteers in the Merck/HVTN Ad5 vaccine trial mounted only a limited, and possibly inadequate, number of epitope-specific CTL responses against the HIV-1 Gag, Pol and Nef transgene products, and that number may not be adequate for protection. While several factors can contribute to the anti-viral efficacy of $CD8^+$ T cells, including functional avidity [59], killing efficiency [60], polyfunctionality [61], evolutionary constraints on the epitope sequences [62,63], and the kinetics of antigen presentation [64,65], it is becoming increasingly apparent that not all CTL are functionally equivalent. The CTL responses present in natural infection may not be the most efficacious, and we still do not know which ones might be. Hence, one possible approach to a vaccine is to induce as many CTL responses as possible. It may be necessary to alter the natural immunodominance patterns of HIV-1- or SIV-specific $CD8^+$ T-cell responses to reveal sub-dominant responses of potentially greater efficacy [66,67].

We Need New T cell Assays

We must identify which of the many different CTL responses that arise during HIV-1 infection actually contribute to reducing viral replication [68]. To do this, new and sensitive methods of assessing CTL function should be developed. We have previously relied on autologous EBV-transformed B cell lines pulsed with high concentrations of peptides to assess $CD8^+$ T-cell function. Similarly, conventional ELISPOT or ICS assays assess the ability of $CD8^+$ T cells to secrete interferon gamma (IFNγ) in response to rather high peptide concentrations. None of these assays necessarily measures the ability of CTL to suppress the replication of the HIV-1 in autologous $CD4^+$ T cells or cell lines. However, such assays have been developed very recently [65,69–73] and should help us determine, for the first time, which of the many CTL responses can actually control HIV-1 replication, *in vitro* and *in vivo*.

HIV-1 Sequence Variability

The enormous variability of HIV-1 is among the major hurdles that must be overcome if an effective vaccine is to be successfully developed. Accumulated nucleotide changes within the highly mutable *env* gene are important in classifying HIV-1 into different groups (M, N, and O) and then into subtypes or clades. Sequence analysis shows that *env* nucleotide sequences may vary by up to 35% between clades, and by up to 20% even within a clade [74]. Hence, many CMI-based HIV-1 vaccine designs have abandoned Env as an immunogen, to focus on more conserved proteins (e.g. Pol and Gag). However, even relatively minor variations in these proteins may have grave implications for vaccine efficacy; single amino acid differences can impair or even eliminate antigen recognition by vaccine-induced antibodies or $CD8^+$ T-cells [75,76].

Summary and Conclusions

The results of the Merck/HVTN trial, while disappointing, were consistent with, and arguably predicted by, studies of analogous SIV vaccines in rhesus macaques. However, because no macaque study has predicted a positive result in humans (there has been none), we must still exercise caution in interpreting NHP challenge studies. Nonetheless, the concordance of outcomes from vaccine trials in NHP that use SIV challenge viruses and human efficacy trials does increase the potential for using SIV challenge of monkeys as a valuable filter for advancing vaccine candidates to clinical trials. But, how stringent should the conditions for product advancement be?

First, it should be noted that SIV antigens are different from HIV-1 antigens and that MHC types differ between humans and macaques. Thus, epitope-specific CTL responses against SIV epitopes may be irrelevant to CTL responses against HIV-1 epitopes. There are also several other variables that might affect the outcomes of vaccine trials in NHPs, including the species of macaque, the choice of the challenge virus, the distribution of MHC class I alleles in the study animals, and the route of challenge. The factors outlined below should therefore be considered when judging whether a vaccine should enter large-scale Phase III efficacy/licensing trials.

1. Safety/immunogenity trials in humans: The vaccine candidate should be shown to be safe and immunogenic in Phase I/IIa trials. Immunogenicity should be based on validated assays that should demonstrate that a vast majority of volunteers immunized with the vaccine show positive responses in such validated assays. The quality and/or quantity of the immune responses to a CMI vaccine should be a significant improvement over those elicited by the Merck Ad5 product.

2. Protection conferred by an analogous vaccine in the SIV-rhesus macaque challenge model: Whenever feasible, the analogous SIV vaccine should be designed and tested in rhesus macaques, prior to advancing the candidate to a Phase II Screening Test of Concept trial (see #3 below). This may not always be possible: Some candidate vaccines, e.g. epitope-based concepts, some bacterial-vectored delivery systems and some viral vectors that are species-specific for humans cannot be appropriately modeled in SIV-NHP challenge studies. For those candidates that can be evaluated in the SIV rhesus macaque model, the vaccine should suppress viral load by a minimum of 1.5 logs (peak and setpoint) compared to control animals, when tested for its efficacy against a heterologous repeated low-dose mucosal SIV challenge.

3. Protection of humans in a Screening Test of Concept (STOC) trial: STOC trials could rapidly screen a limited number of leading HIV-1 vaccine candidates, enabling the most promising to be prioritized to large-scale, Phase III efficacy licensing trials. The primary endpoint of a STOC trial is plasma HIV-1 RNA viral load at set-point (about 3–6 months post-infection) in participants who become infected with HIV-1. In a STOC trial, ~30 incident HIV-1 infections are enough to detect a minimum of a 1-log suppression of viral load with sufficient statistical power. Any candidate vaccines that demonstrate a >1.5 log suppression of viral load for greater than one year duration should be considered for advancement to Phase III licensing trials.

4. Feasibility for large-scale manufacture and distribution: Candidate vaccines which have fulfilled criteria 1 and 2, or 1 and 3, should be advanced to Phase III licensing/efficacy trials, provided that they can be manufactured on a large-enough scale to enable their widespread distribution if they turn out to be effective.

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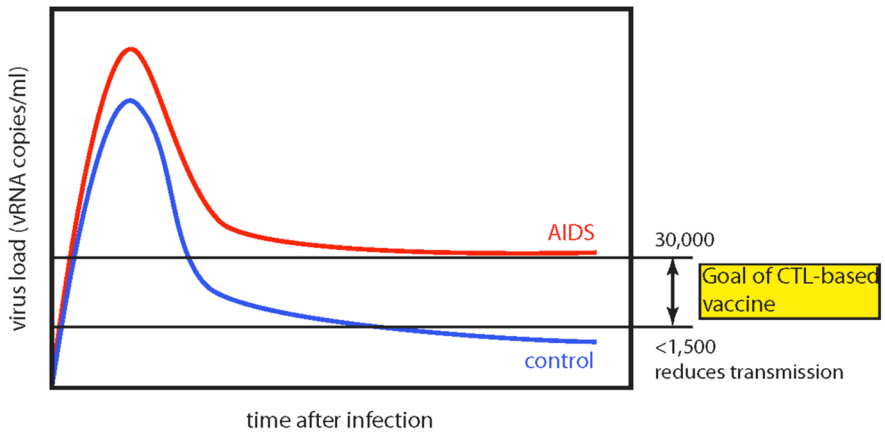

Figure 1.

The goal of a successful CTL-based vaccine is to reduce HIV-1 replication to a level that reduces or eliminates transmission. In practice, this is about a 1.5 log reduction, from a set point of 30,000 RNA copies/ml of plasma to less than about 1,500. A similar numerical reduction in SIVmac239 infection of rhesus macaques would be from about 10^6 RNA copies/ml to <30,000, although whether this would be sufficient to reduce any hypothetical transmission of SIV from macaque to macaque is unknown.

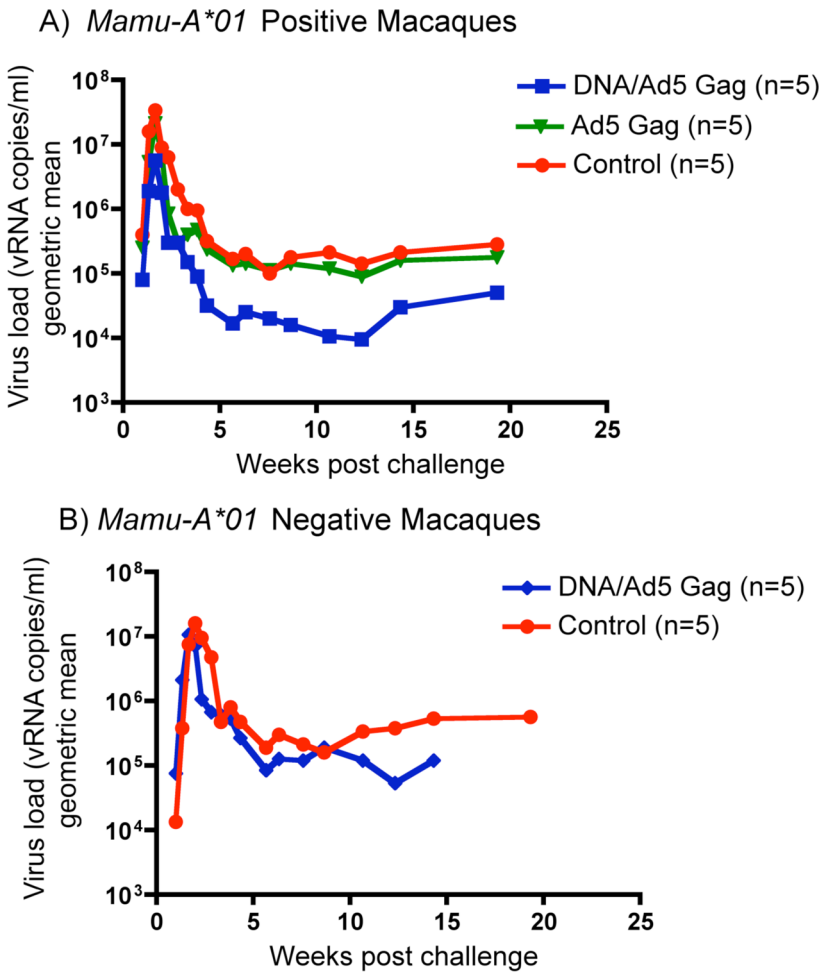

Figure 2.

DNA/Ad5 Gag vaccination only shows protective effect in *Mamu-A*01* positive rhesus macaques; Ad5 Gag vaccination has no effect. A) *Mamu-A*01* positive macaques were vaccinated with Gag, and then challenged with a high dose of SIVmac239 i.r. The animals were either primed three times with DNA, then boosted with Ad5 (DNA/Ad5), or were primed three times with Ad5 and boosted with Ad5 (Ad5). In animals primed with DNA Gag, the peak of viremia was 6 times lower than in control animals and the early chronic set point was 15 times lower. There were no differences in either peak viremia or the early chronic set point in animals primed with Ad5 Gag, compared to control animals. B) *Mamu-A*01* negative macaques were primed with three doses of DNA Gag, and then boosted with Ad5 Gag. The vaccinated animals had a peak of viremia that was 3.2-fold lower than in control animals, but no difference was observed in viral loads at any subsequent time points, indicating that *Mamu-A*01* has only a moderate protective effect in Gag-vaccinated animals [43].

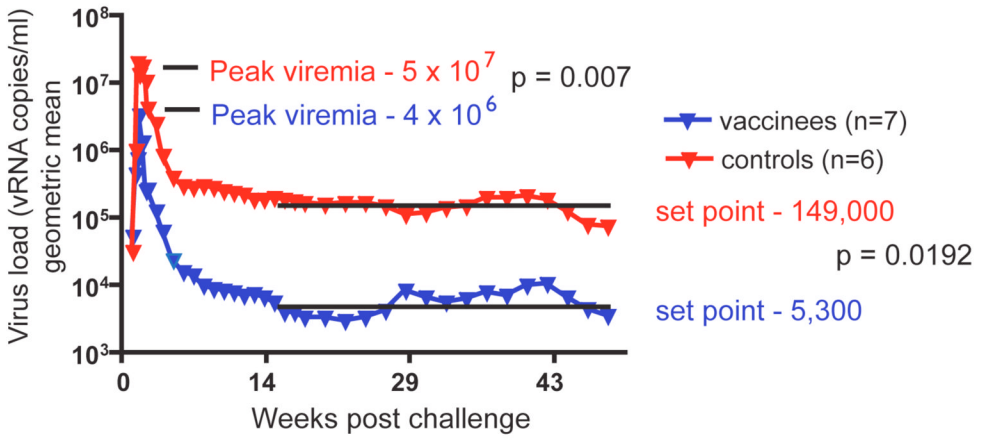

Figure 3.

Durable suppression of SIVmac239 replication in *Mamu-A*01* positive macaques vaccinated with DNA/Ad5 encoding Gag, Tat, Ref and Nef. *Mamu-A*01* positive rhesus macaques were primed with DNA encoding Gag, Tat, Rev and Nef three times, then boosted with an Ad5 vector encoding the same four proteins before a repeated low dose i.r. challenge. Both the peak and the set point viral loads were significantly lower in the vaccinees than in control animals [47].