Equivalent Immunogenicity of the Highly Attenuated Poxvirus-Based ALVAC-SIV and NYVAC-SIV Vaccine Candidates in SIVmac251-Infected Macaques

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Therapeutic immunization of HIV-1-infected individuals may induce and/or enhance HIV-1-specific immune responses and decrease the dependency on antiretroviral drug treatment. However, repeated immunizations with live-recombinant vectors may induce vector-specific immune responses that interfere with the elicitation of vigorous immune responses to the desired antigen. Therefore, the use of mixed-modality vaccinations may be necessary to induce sustained virus-specific immune responses in HIV-1-infected individuals treated with antiretroviral therapy (ART). Thus, the relative immunogenicity of various vaccine modalities needs to be assessed. Here we compared the immunogenicity of two vaccine candidates, the canarypox-based ALVAC-SIV-gag-pol-env (ALVAC-SIV-gpe) and the vaccinia-based NYVAC-SIV-gag-pol-env (NYVAC-SIV-gpe), in rhesus macaques infected with SIVmac251 and treated with ART by 2 weeks postinfection. Both ALVAC-SIV-gpe and NYVAC-SIV-gpe vaccine candidates induced and/or enhanced a virus-specific CD8+ T cell response to a similar extent, as demonstrated by tetramer staining of Gag-specific CD8+ T cells. Similarly, both vaccines elicited comparable lymphoproliferative responses (LPRs) to the SIV p27 Gag and gp120 Env proteins. Thus, both these vaccine modalities alone or in combination may be suitable candidate vaccines for immune therapy of HIV-1-infected individuals.

INTRODUCTION

Despite the ability of highly active antiretroviral therapy (HAART) to suppress the replication of HIV-1 and to reduce the morbidity and mortality of HIV-1-infected individuals, the eradication of HIV-1 from its latent reservoirs seems unlikely (Chun et al., 1997; Wong et al., 1997). The complexity of HAART regimens (Deeks et al., 1999) and their side effects render long-term adherence difficult. Despite a partial reconstitution of immune competence during HAART in most HIV-1-infected individuals (Autran et al., 1997; Landay et al., 2002; Lederman and Valdez, 2000), virus-specific immune responses decline during prolonged treatment (Casazza et al., 2001; Gray et al., 1999; Kalams et al., 1999; Ogg et al., 1999; Pitcher et al., 1999; Spiegel et al., 2000), presumably due to the decreased exposure to viral antigens. Although suspension of antiretroviral therapy (ART) results in an increase in virus-specific immune responses in most patients, viremia is usually not contained in the absence of HAART (Davey et al., 1999; Ortiz et al., 1999). Since both CD4+ and CD8+ virus-specific T cell responses have been associated with the containment of HIV replication (Koup et al., 1994; Rosenberg et al., 1997), augmenting HIV-specific immune responses by active vaccination before HAART cessation may enhance HIV-specific immune responses and, hopefully, decrease the dependency on drug treatment.

Repeated immunizations will most likely be needed to induce and sustain strong virus-specific responses in infected individuals. In fact, viremia containment in vaccinated ART-treated macaques with long-standing SIVmac251 infection after drug suspension is transient (E. Tryniszewska et al., in press). However, repeated immunizations even with replication-impaired recombinant live vectors may induce sufficient vector-specific immune responses to interfere with their ability to efficiently present the desired antigens (Harrington et al., 2002; Perkus et al., 1995; Yang et al., 1995). In addition, prior vaccination with a live vector that failed to protect from infection may preclude the use of the same live vector for therapeutic vaccination in the same individual. Thus, likely, several live-vector vaccine candidates will be needed for immune therapy of HIV-1-infected individuals. Therefore, it is important to assess the relative immunogenicity of several vaccine candidates in a nonhuman primate model of HIV-1 infection of humans.

An effective therapeutic vaccine against HIV should minimally replenish virus-specific CD4+ T cell responses and possibly elicit CD8+ T cell responses of sufficient breadth to prevent immune escape. The magnitude of virus-specific CD8+ T cell responses and T helper responses has been inversely correlated with viremia con-
tainment (Koup et al., 1994; Ogg et al., 1998; Rosenberg et al., 1997). Similarly, in HIV-1-infected long-term nonprogressors (LTNP) (Kalams et al., 1999; Kent et al., 1997; Ogg et al., 1998; Rosenberg et al., 1997), these responses are usually vigorous. Cell-mediated responses may also protect from HIV infection in individuals at high risk of infection (Mazzoli et al., 1997; Rowland-Jones and Michael, 1995). Further evidence on the importance of virus-specific CD8+ T cell responses in the containment of HIV/SIV infection is provided by the selection of immune escape variants (Allen et al., 2000) or SIV infection of macaques (Hel et al., 2000, 2001) was shown to correlate with containment of viral rebound following the suspension of ART in primary HIV-1 infection of humans (Rosenberg et al., 2000) or SIV infection of macaques (Hel et al., 2000; Lifson et al., 2000, 2001) was shown to correlate with both virus-specific CD4+ (Hel et al., 2000) and CD8+ T cell responses (Lifson et al., 2001).

The highly attenuated vaccinia-based vector NYVAC-SIV-gpe (Benson et al., 1998) is able to induce and expand SIV-specific CD4+ and CD8+ T cell responses in SIVmac251-infected rhesus macaques treated with ART during primary infection (Hel et al., 2000). In addition, the same vaccine used to immunize macaques treated with ART during long-standing SIVmac251 infection significantly ameliorated the virological outcome following ART withdrawal (E. Tryniszewska et al., in press). Herein we aimed to assess the immunogenicity of a canarypox-based ALVAC-SIV-gpe vaccine candidate (Pal et al., 2002) in SIVmac251-infected rhesus macaques treated during primary infection with ART and compare it to the immunogenicity of the NYVAC-SIV-gpe vaccine candidate.

RESULTS AND DISCUSSION

A total of 19 naive rhesus macaques were infected intravenously with ten 50% tissue culture infectious doses (TCID50) of the highly pathogenic SIVmac251 (561) (Pal et al., 2002). All macaques became infected, as evidenced by high plasma viral load at week 2 postchallenge (Fig. 1). Starting at day 15, all macaques were treated with ART as described (Hel et al., 2000). Following the initiation of ART, a rapid decrease of plasma virus levels was observed and in most macaques viral RNA in plasma was undetectable (<2000 copies) by week 6 from treatment and thereafter (Fig. 1). Macaques in group A were immunized three times (weeks 10, 19, and 23) intramuscularly with 10^6 PFU of the mock ALVAC vaccine. The macaques in group B were immunized with the ALVAC-SIV-gpe vaccine candidate at the same time as macaques in group A. In contrast, macaques in group C were immunized only twice (weeks 10 and 19) with 10^6 PFU of NYVAC-SIV-gpe. Therefore, comparison of the immunogenicity of these vaccine modalities will span only the first 21-week interval.

Most of the animals in group A spontaneously developed lymphoproliferative responses (LPRs) specific to the p27 Gag antigen following ART treatment (Fig. 2a), as expected (Hel et al., 2000). A similar finding has been reported in HIV-1-infected individuals treated with ART during primary infection (Rosenberg et al., 2000). In contrast, low to undetectable levels of LPRs specific to the gp120 Env antigen were observed in the macaques from group A (Fig. 2d). The immunizations of group B animals with ALVAC-SIV-gpe resulted in the induction of both p27 Gag- and gp120 Env-specific LPRs (Figs. 2b and 2e) in several macaques. While the gp120 Env LPRs were significantly higher in the ALVAC-SIV-gpe-vaccinated group B animals compared to the control group A throughout the vaccination period \( P = 0.0005 \), weeks 11 to 26, analyzed by repeated measures ANOVA (RM ANOVA); in the case of the p27 Gag-specific LPRs, no significant differences were observed between group A and group B macaques. Similarly, two immunizations with the NYVAC-SIV-gpe vaccine of group C animals induced significantly higher LPRs to gp120 Env \( P < 10^{-6} \), weeks 11–21) but, in the case of p27 Gag, LPRs only approached statistical significance \( P = 0.07 \) when compared to control group A (Figs. 2c and 2f). At the individual level, variability in the ability to respond to the vaccines was observed in both group B and group C macaques as in the case of macaque 769 that failed to mount responses to both p27 Gag and gp120.

To quantitate the vaccine-induced T cell responses, we measured the frequency of CD3+CD8+ T cells specific to the Mamu-A*01-restricted immunodominant epitope Gag181–189 CM9 (Kuroda et al., 1998) in the Mamu-A*01-positive macaques included in the groups (Fig. 3). Vaccination of group B animals with ALVAC-SIV-gpe induced high frequencies of Gag181–189 CM9-specific T cells in a range from 2.5 to 11.2% of the total CD3+CD8+ T cell population (Fig. 3b). In contrast, the frequencies of Gag181–189 CM9-specific cells in the blood of the mock-vaccinated macaques in group A were significantly lower \( (P = 0.007, \) weeks 11–26, RM ANOVA) and remained below 2% throughout the vaccination period (Fig. 3a). Similarly, immunization of group C animals with NYVAC-SIV-gpe induced high numbers of Gag181–189 CM9-specific T cells in both the Mamu-A*01-positive macaques 689 and 690 (up to 8% of the total CD3+CD8+ T cell population).

Last, we compared the extent of virus-specific immune responses elicited by the NYVAC-SIV-gpe and ALVAC-
The p27 Gag LPRs and gp120 LPRs induced by the ALVAC-SIV-gpe vaccine candidate were not significantly different from those induced by NYVAC-SIV-gpe (P = 0.89, P = 0.1, respectively; RM ANOVA). The overall frequency of the CD3⁺CD8⁺ T cells staining the Gag₁₈₁₋₁₈₉ CM9 tetramer did not differ (Fig. 4c) in the Mamu-A*01-positive macaques from groups B and C.
FIG. 2. ALVAC-SIV-gpe and NYVAC-SIV-gpe vaccine candidates induced SIV-specific LPRs in SIVmac251-infected macaques. Mean LPRs calculated as S.I.s to the p27 Gag (a, b, and c) and gp120 Env (d, e, and f) antigens were determined as previously described (Hel et al., 2000).
FIG. 2—Continued

**Group A, mock ALVAC**

- **S.I. gp120**
- **ART**
- **Weeks post-challenge**

**Group B, ALVAC-SIV-gag, pol, env**

- **S.I. gp120**
- **ART**
- **Weeks post-challenge**

**Group C, NYVAC-SIV-gag, pol, env**

- **S.I. gp120**
- **ART**
- **Weeks post-challenge**

**Legend**

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C, although the kinetics of induction of the response appeared to be faster in macaques immunized with NYVAC-SIV-gpe.

Thus, the immunogenicity of the canarypox-based ALVAC-SIV-gpe and of the highly attenuated poxvirus-based NYVAC-SIV-gpe vaccine candidates in SIVmac251-infected ART-treated macaques does not appear to differ significantly.

In this study, the ALVAC-SIV-gpe vaccine candidate elicited substantial CD4$^+$ and CD8$^+$ T cell responses. A study with an equivalent canarypox-based HIV-1 vaccine in HIV-1-infected individuals also elicited both CD4$^+$ and
CD8⁺ HIV-specific immune responses (Jin et al., 2002). However, in that study lower doses of the recombinant ALVAC HIV-1 vaccine were used and only a transient virological benefit was observed in some patients (Markowitz et al., 2002). Here we demonstrate that both the canarypox-based ALVAC and the poxvirus-based NYVAC at 10⁹ PFU per inoculation were highly immunogenic. The relative efficacy of immunization could not be assessed in the studies reported here since ART treatment alone during primary infection results in viremia control following ART suspension in 80% of macaques (G. Franchini, in press; J. Nacsa et al., in press). In addition to being safe (Hel et al., 2000; Jin et al., 2002), these two vectors display limited, if any, immune cross-reactivity with each other (Clements-Mann et al., 1998). In fact, vaccination with recombinant ALVAC-SIV-gpe of monkeys previously immunized with NYVAC-SIV-gpe did not preclude a further enhancement of SIV-specific immune responses and, importantly, appeared to ameliorate the virological outcome following ART cessation (Y.

**FIG. 4.** ALVAC-SIV-gpe and NYVAC-SIV-gpe vaccine candidates induced similar levels of virus-specific immune responses. Mean values of SIs for the p27 Gag (a) and gp120 Env (b) and the frequency of ex vivo Gag₁₈₁₋₁₈₉ CM9 tetramer-staining CD₃⁺CD₈⁺ T cells in the blood of the immunized macaques are shown over time.
Edghill-Smith et al., unpublished data). Thus, it could be envisioned that both vaccine modalities at the dose tested here or at higher doses could be used in sequential immunization regimens of HIV-1-infected individuals.

MATERIALS AND METHODS

Animals, immunizations, and ART therapy

All animals were colony-bred Indian rhesus macaques (Macaca mulatta) obtained from Covance Research Products (Alice, TX). The animals were housed and handled in accordance with the standards of the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). All rhesus macaques were seronegative for simian retrovirus (SRV), STLV-1, and herpesvirus B prior to the study. The macaques were screened by PCR and sequencing for the presence of the Mamu-A*01 allele as described (Hel et al., 2001).

All rhesus macaques were infected intravenously with 10 TCID50 of the highly pathogenic SIVmac251 (561) (Pal et al., 2002). The challenge virus stock was prepared from phytohemoaglutinin (PHA)-activated PBMC obtained from Mamu-A*01-positive macaque 561L previously inoculated vaginally with SIVmac251. SIVmac251 RNA in plasma was quantitated by nucleic acid sequence-based amplification (Romano et al., 1997). The detection limit of this assay is 2×10^3 RNA copies/100 μl of plasma.

Starting at day 15, all macaques were treated with ART consisting of 20 mg/kg/day of (R)-9-(2-phosphonylmethoxypropyl)adenine (PMPA) administered subcutaneously (Tsai et al., 1995), 2.4 mg/kg/day of Stavudine (d4T) divided into two daily doses administered orally, and 10 mg/kg/day of didanosine (DDI) administered intravenously, as described (Hel et al., 2000). The animals were immunized im with 10^9 PFU of mock ALVAC (group A), ALVAC-SIV-gag, pol, env (group B), or NYVAC-SIV-gag, pol, env (group C) at times indicated.

Lymphocyte proliferation assay

The Ficoll-purified PBMC were resuspended in RPMI-1640 medium (Gibco-BRL, Gaithersburg, MD) containing 5% inactivated human A/B serum and antibiotics (Sigma, St. Louis, MO) and cultured at 10^5 cells per well in triplicate for 3 days in an absence or presence of native HPLC-purified SIVmac Gag p27 or Env gp120 proteins (Advanced BioScience Laboratories, Rockville, MD) or Concanavalin A as a positive control. The cells were then pulsed overnight with 1 μCi of [3H]thymidine prior to harvest. The relative rate of lymphoproliferation was calculated as fold of thymidine incorporation into cellular DNA over medium control (SI).

Detection of epitope-specific CD3+CD8+ T lymphocytes by tetramer staining

Fresh PBMC were stained with anti-human CD3 Ab (PerCP labeled, clone SP34, Pharmingen, San Diego, CA), anti-human CD8ε Ab (FITC-labeled, Becton-Dickinson, San Jose, CA), and Mamu-A*01 tetramer complexes refolded in the presence of a specific peptide and conjugated to PE-labeled streptavidin (Molecular Probes, Eugene, OR). Gag181-189 CM9 (CTPYDINQM) (Gag_CM9)- and Tat28-35 SL8 (TTPESANL) (Tat_SL8)-specific tetramers were used. Samples were analyzed on FACS Calibur (Becton–Dickinson) and the data are presented as percentage of tetramer-positive cells out of all CD3+CD8+ lymphocytes.

Statistical analysis

All reported P values are two-sided. RM ANOVA analysis was performed on transformed data as described (Hel et al., 2000). The NCSS (Kaysville, UT) and SigmaStat (version 2.0, SPSS, Chicago, IL) statistical software packages were used for the analyses.

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