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Containment of Infection in Tat Vaccinated Monkeys After Rechallenge with a Higher Dose of SHIV89.6P$_{cy243}$

Alessandra Borsetti,[1] Silvia Baroncelli,[1,3] Maria Teresa Maggiorella,[1] Sonia Moretti,[1] Emanuele Fanales-Belasio,[1] Leonardo Sernicola,[1] Antonella Tripiciano,[1] Iole Macchia,[1] Zuleika Michelini,[1,3] Roberto Belli,[1] Stefania Farcomeni,[1] Maria Rosaria Pavone-Cossut,[1] Donatella Negri,[1,4] Antonella Caputo,[2] Stefania Bellino,[1] Stefano Butto,[1] Fausto Titti,[1] Aurelio Cafaro,[1] and Barbara Ensoli[1]

Abstract

We previously reported that cynomolgus monkeys vaccinated with the human immunodeficiency virus (HIV)-1 Tat protein controlled infection after challenge with the simian human immunodeficiency virus (SHIV) 89.6P$_{cy243}$ for up to 2 y of follow-up. To evaluate the breadth of the protective immunity elicited by the Tat protein, the vaccines along with the naïve monkeys were intravenously rechallenged with a fivefold higher dose (50 MID$_{50}$) of the same SHIV-89.6P$_{cy243}$. The vaccinated monkeys exhibited a statistically significant and long-lasting reduction of viral replication compared to control monkeys. This effect was associated with a strong anamnestic response to Tat, while responses to Gag and Env were nearly undetectable. Taken together, these data provide further evidence for the usefulness of Tat-based vaccines.

Introduction

The development of a human immunodeficiency virus (HIV) vaccine capable of inducing an efficient antiviral immunity that is able to control viral replication and keep under control the ongoing disease in infected individuals is of extreme importance. Early vaccine strategies based on inactivated or attenuated virus have been abandoned as ineffective or unsafe, respectively (8,22). To date, Env-based vaccines have failed because of the complex structure of Env, its high variability, and the difficulty of generating broadly reactive high-titer neutralizing antibodies (9). Moreover, vaccines based on various combinations of the structural proteins Env, Gag, and Pol were partially protective in preclinical studies, yet have never provided sterilizing immunity (6). HIV vaccine research has turned towards the more conserved regulatory proteins Tat, Rev, and Nef as possible components of a new combined vaccine. In particular, several studies have suggested that an immune response to Tat has a protective role and may control the progression of disease *in vivo* (3,13,15,18,24). In addition, in natural infection the presence of humoral responses against Tat correlates with the control of disease progression (15,16,25). In particular, longitudinal studies in a cohort of HIV seroconverters followed up to 14 y have indicated that none of the persistently anti-Tat antibody-positive individuals progressed to AIDS, suggesting that the immune response to Tat represents a correlate of protection (15). Similarly, cytotoxic T lymphocytes to Tat have been frequently detected in asymptomatic HIV-1-infected individuals, and also have been shown to inversely correlate with progression to AIDS (21). Moreover, Tat has the major advantage of being highly conserved in its functional and immunogenic domains throughout the viral clades (12). We have previously shown that vaccination of monkeys with the HIV-1 Tat protein elicited immune responses that controlled replication of the cynomolgus monkey-derived SHIV89.6P-$_{cy243}$ (2) to undetectable levels, preventing CD4 T-cell decline and disease onset (4,5). Protection was prolonged up to 2 y after challenge, as shown by the absence of provirus in peripheral blood mononuclear cells (PBMCs) or in lymph nodes, and of virus plasma RNA. Moreover, by assessing the animals for unintegrated forms of the SHIV genome, which are thought to be a sign of active but occult virus replication, no virus reservoir was detected in PBMCs or lymph nodes (10). This protection was associated with long-term memory immune responses to Tat in the absence of consistent humoral responses to Env or Gag, and cellular immune responses against Gag, which were low and transient or undetectable (4,10). We concluded that vaccination with the Tat protein contained primary infection at its early stages, preventing or

[1]National AIDS Center, Istituto Superiore di Sanità, Rome, Italy.
[2]Department of Histology, Microbiology and Medical Biotechnologies, University of Padova, Padova, Italy.
[3]Department of Drugs and Evaluation, Istituto Superiore di Sanità, Rome, Italy.
[4]Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy.

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reducing virus spread in blood and tissues. In the present study we assessed the extent of the resistance of Tat-vaccinated monkeys to a new exposure to a higher dose (50 MID_{50}) of the same virus SHIV89.6P_{cy243} to determine whether specific Tat immune responses, still present 2 y after vaccination in the absence of any vaccine boost, could influence reinfection with much higher virus doses.

Materials and Methods

Animals, immunization, and challenge

Cynomolgus monkeys (*Macaca fascicularis*) were singly housed according to European Guidelines for Non-Human Primate Care (EEC Directive No. 86-609, Nov. 24, 1986). Euthanasia was performed by IV injection of Tanax (Intervet Italia, Milan, Italy) after sedation. Necropsy in all animals was performed and tissues of different organs were fixed in 10% formalin for histological analysis. PBMCs as well as cells from spleen and lymph nodes were recovered for further study. In this study four cynomolgus macaques (54844, 54879, 54899, and 54240) vaccinated with Tat protein in alum or RIBI adjuvant (4,5,10), and four naïve monkeys (9406, 9610, 9503, and 9104) were used. All animals were inoculated IV with 50 monkey infectious dose (50 MID_{50}) of SHIV89.6P_{cy243} that was generated as previously described (2). Briefly, SHIV-89.6P_{cy243} was obtained by infecting a cynomolgus macaque (monkey 55111) with 50 MID_{50} of the SHIV-89.6P obtained from Letvin's laboratory (5,14). Blood, lymph node, and spleen mononuclear cells were collected and $CD8^{+}$-depleted cells were stimulated with PHA and interleukin-2 to induce massive virus replication. At the peak of RT activity (95000 cpm/mL), determined as previously described (20), the cell-free supernatant was stocked and frozen. The new viral stock was termed SHIV-89.6P_{cy243}. This viral stock was titered *in vitro* on human cell lines (CEMX174 and C8166) given a titer of 5.6×10^{3} and 1.6×10^{4} $TCID_{50}$/mL, respectively, and on PBMCs from four naïve monkeys given a mean titer value of 3.23×10^{3} $TCID_{50}$/mL. To titer the virus *in vivo*, eight cynomolgus monkeys were intravenously exposed to serial dilutions of SHIV-89.6P_{cy243}.

Detection of anti-SIV, anti-HIV Env, and anti-Tat antibody titers

Anti-SIV antibodies (Ab) titers were determined by endpoint dilution of plasma samples using an HIV-2 enzyme-linked immunosorbent assay (ELISA) (Elavia AC-Ab-Ak II Kit). Ab titers against the HIV-1 Env were detected by an HIV-1 ELISA assay (HIV-1/HIV-2 Third Generation Plus; Abbott, Chicago, IL). The mean of the negative control plus 3 SD represented the cut-off value. Anti-Tat Ab titers were determined by ELISA as previously described (3,4).

Plasma viral RNA measurements and proviral copy detection

Plasma levels of SHIV-89.6P_{cy243} were quantified using a highly sensitive quantitative competitive RNA-polymerase chain reaction assay (19) with a threshold limit of detection of 50 RNA Eq/mL. To determine the cell-associated viral load, DNA was extracted from 400 μL of whole citrated blood using the QIAamp DNA blood Mini kit (Qiagen, Milan, Italy) according to the manufacturer's instructions. SHIV proviral copy number was determined by a semi-quantitative DNA PCR utilizing 1 μg of DNA and amplifying a 496-bp region of the *gag* gene of SIVmac239, as previously described (5).

Lymphocyte subset determination

Citrated peripheral blood cells were stained with phycoerythrin-conjugated anti-CD4 (Biosource International, Camarillo, CA) and peridin chlorophyll protein-conjugated anti-CD8 monoclonal antibodies (Becton-Dickinson, Mountain View, CA), and analyzed with a FACScan cytometer and software (Becton-Dickinson). Absolute cell numbers were calculated from the blood cell counts.

T-cell proliferation assays

Ficoll-purified PBMCs (2×10^{5}/well) were seeded in flat-bottomed 96-well microtiter culture plates in triplicate in a final volume of 200 μL of RPMI containing 10% FCS, and cultured either alone or in the presence of PHA (2 μg/mL), TT (5 μg/mL; Connaught, Ontario, Canada), Tat protein (5 μg/mL), SIVmac251 p55 *Gag* protein (5 μg/mL), SIVmac239 p27 *Gag* 15mers peptides (aa 226–240, 231–245, 236–250, 283–297, 289–303, and 295–309 at 5 μg/mL each), or buffer control (PBS containing 0.1% BSA). After 5 d of incubation at 37°C in 5% CO_2, the cell cultures were pulsed with 1 μCi/well of $[^{3}H]$thymidine (Amersham Life Sciences, Buckingamshire, U.K.), and the incorporated radioactivity was measured 18 h later. A stimulation index >3 was considered positive.

Interferon-γ (IFN-γ) ELISPOT assay

IFN-γ production was measured using a commercial kit (Human IFN-γ ELISPOT; Euroclone Ltd., Paignton, U.K.) following the manufacturer's instructions. Briefly, PBMCs (2×10^{5}/well, in duplicate) were cultured with medium alone or in the presence of PHA (2 μg/mL), TT (5 μg/mL), Gag or Tat protein (5 μg/mL), or reconstitution buffer in flat-bottomed 96-well plates previously coated with anti-IFN-γ monoclonal antibodies (Euroclone Ltd.). After 18 h the cells were removed and locally produced IFN-γ was revealed by an immunoenzymatic reaction in a gel matrix as colored spots. The spots, corresponding to IFN-γ-producing cells (SFC), were counted under light microscopy and expressed as the number of SFC per million cells upon subtraction of background SFC. Based on data obtained with PBMC from 24 naive monkeys, a response was considered positive when the number of SFC was >20 SFC per million PBMC.

Statistical methods

Viral load (expressed as $\log_{10}$ copies/mL) and CD4 T-cell counts were evaluated for different phases of infection. The acute phase includes all values within the fourth week, and the post-acute and chronic phases include all determinations made between the ninth and 20th weeks and between the 24th and 52nd weeks, respectively. Peak viral load was obtained in the second week, and in the acute phase the maximum CD4 T-cell decline until the fourth week was obtained using the last CD4 T-cell count prior to challenge as baseline. To compare Tat-vaccinated versus control monkeys, a regression model for correlated data was applied for

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TABLE 1. SUMMARY OF THE VIROLOGICAL STATUS OF VACCINATED ANIMALS AT THE TIME OF RECHALLENGE

Monkey	*Group*	*Plasma viral RNA*[a]	*DNA-PCR*[b]	*Anti-HIV*[c] *IgG (titer)*	*Anti-SIV IgG (titer)*
54844	Tat + RIBI	<50	<1	Neg	Neg
54879	Tat + RIBI	<50	<1	Neg	Neg
54899	Tat + alum	<50	<1	Neg	Neg
54240	Tat + alum	<50	<1	Neg	Neg

[a]Cut-off of plasma viral RNA (as determined by quantitative-competitive RNA-polymerase chain reaction) was 50 RNA copies/mL.
[b]Proviral load was determined by semi-quantitative DNA PCR utilizing 1 μg of DNA and amplifying a 496-bp region of the *Gag* gene of SIVmac239.
[c]Anti-HIV and anti-SIV Abs were determined by the use of commercial kits (see materials and methods for details).
This table shows the results of analysis at the day of rechallenge.

each phase of infection separately: The smoothed curves of viral load and CD4 T cells were estimated using a smooth local regression (LOESS), with a quadratic fit and a span of 0.3. Statistical analyses and data processing were performed using SAS® software (SAS Institute, Cary, NC). All statistical tests were performed at a two-sided 5% significance level.

Results

Tat-vaccinated monkeys control reinfection with 50 MID_{50} of the $SHIV89.6P_{cy243}$

Four vaccinated macaques that had contained virus replication at the first challenge and four naive animals were injected IV with 50 MID_{50} of the same SHIV $89.6P_{cy243}$. At the time of rechallenge, plasma viremia levels, proviral DNA, and antibodies to SIV and to HIV-1 were undetectable in all vaccinees (Table 1). Upon rechallenge, all animals became infected; however, significant differences in viral RNA levels among the groups were observed (Fig. 1a). Viral RNA levels in vaccinees and controls were analyzed and compared at four different time points, corresponding to: peak viremia (week 2), the acute phase of infection including the peak of plasma viremia 2–4 wk post-infection (p.i.), the post-acute period (weeks 9–20), and the chronic phase of infection (weeks 24–52). As shown in Fig. 1a, at 2 wk p.i. the peak of viral RNA was significantly higher in the controls than in the vaccinees ($p < 0.0001$), although during the acute phase of infection, differences in plasma viremia between the two groups of animals was near the significance level ($p = 0.0539$). Then, in the post-acute phase (weeks 9–20), a statistically significant reduction in plasma RNA levels was observed in the vaccinees compared to the controls ($p = 0.0454$). Similarly, during the chronic phase of infection (weeks 24–52), the mean levels of plasma viremia showed significant differences between the vaccinees and the controls ($p = 0.0346$). According to these results, the smoothed local regression line of plasma virus level clearly confirmed that the peak of plasma viremia was at week 2 p.i., and that the viral long-term set-point level was reduced in the vaccinees compared to the controls (Fig. 1b). Analysis of the $CD4^+$ T-cell levels in the two groups of monkeys was also performed over the acute, post-acute, and long-term set-point periods. As shown by the trend line of the smoothed local regression of the absolute $CD4^+$ T-cell number, all animals suffered a progressive and profound $CD4^+$ T-cell decline by 4 wk p.i. (Fig. 1c). After that, a partial recovery of $CD4^+$ T cells was observed in the vaccinees, but not in the controls,

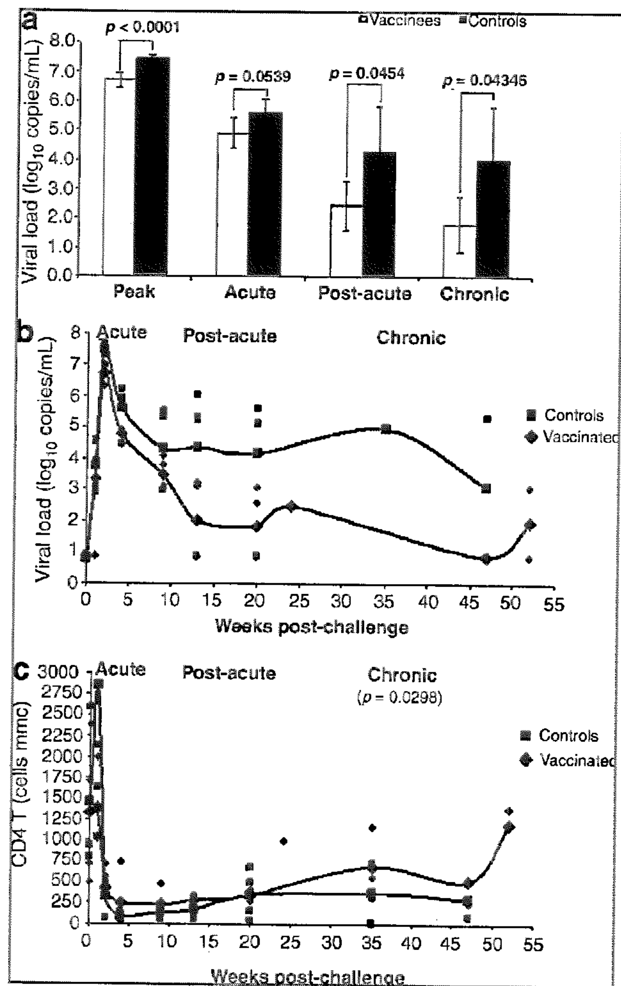

FIG. 1. Plasma viral RNA levels and $CD4^+$ T-cell numbers after rechallenge with $SHIV89.6P_{cy243}$. Change in viral RNA levels during the acute, post-acute, and chronic phase post-infection of vaccinees and controls. (a) The mean log viral load with a 95% confidence interval is indicated; the p values were calculated by applying a regression model for correlated data for each infection phase separately: peak (week 2), acute (weeks 1–4), post-acute (weeks 9–20), and chronic (weeks 24–52). (b) The trend line is a LOESS smoothed average analysis of plasma viremia, and of absolute $CD4^+$ T-cell counts calculated for the acute, post-acute, and chronic phase set-points. (c) A significant difference in CD4 T-cell numbers between vaccinees and controls is indicated ($p = 0.00298$).

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even though no significant differences were observed during the post-acute phase of infection between the groups. Conversely, the CD4$^+$ T-cell counts in the vaccinees was significantly higher than in the controls ($p = 0.0298$) during the chronic phase (Fig. 1c).

Consistent with the containment of infection and CD4 T-cell recovery, no signs or symptoms of AIDS were ever detected in the vaccinated animals (Table 2). Necropsy confirmed the healthy clinical status of three of the four vaccinees, which also showed low (54879) or undetectable (54844 and 54899) levels of plasma viral RNA, and a low number of proviral copies in the peripheral blood. In contrast, evidence of liver and pulmonary pathology was found in animal 54240, which also had detectable plasma viremia (1×10^3 copies/mL), and high levels of proviral DNA copies in the spleen. These virological data were confirmed by the CD4 T-cell counts in the different compartments (Table 2). Specifically, all vaccinated animals displayed partial to complete restoration of peripheral blood CD4$^+$ T-cell numbers, from 40–100% with respect to values at the day of rechallenge (Table 2). Similarly, a limited depletion of CD4 T cells was observed in the spleens (mean CD4$^+$ T-cell percentage: 9.9 ± 1.0 versus 24.04 ± 5.93) of naive cynomolgus macaques (unpublished data), and in the lymph nodes (mean CD4$^+$ T-cell percentage: 27.3 ± 1.98 versus 51.57 ± 6.71) of uninfected macaques (unpublished data) of the three and the two vaccinees that could be analyzed, respectively (Table 2). In contrast, all controls showed progression and clinical signs of AIDS, including frequent diarrhea and hematological alterations, such as anemia (animals 9503 and 9610), and thrombocytopenia (animal 9104) (Table 2). The CD4$^+$ T-cell recovery in the peripheral blood was either absent (9610) or impaired (animals 9104 and 9503) in three of the four naive animals, ranging from 1–30% compared to the prechallenge values (Table 2). Similarly, a severe depletion of CD4$^+$ T cells was detected in the lymph nodes and spleens of animals 9104 and 9610, and to a lesser extent in animal 9503 (Table 2).

At necropsy, signs of enterocolitis were found in all naive animals, indicating compromised intestinal mucosa, a finding consistent with the frequent diarrhea observed in these animals. Active viral replication was detected in the blood (viremia levels ranging between 1×10^4 and 1×10^5 copies/mL) from 3 out of 4 naive macaques. In the fourth naive macaque, animal 9406, viral RNA levels were undetectable and the percentages of CD4$^+$ T cells in the peripheral blood and in lymphoid tissues were comparable to those observed in the vaccinated monkeys (Table 2). However, necropsy revealed a generalized atrophy of all lymphoid tissues (Table 2). Finally, high levels of proviral DNA were detected in the lymphoid tissues from 2 out of the 4 naive macaques (Table 2).

Control of reinfection correlates with memory Tat-specific immune responses

After infection all animals developed anti-SIV or anti-HIV Env antibodies, with no significant differences between vaccinated and naive animals (Fig. 2a–d). Anti-Tat immunoglobulin G (IgG) titers remained stable in two vaccinees (animals 54844 and 54240), and increased in the other two animals (animals 54879 and 54899), and remained unde-

TABLE 2. CLINICAL STATUS, NECROPSY FINDINGS, AND VIROLOGICAL AND IMMUNOLOGICAL STATUS OF VACCINATED AND NAIVE MONKEYS AT THE STUDY'S END

Monkey	Group	Clinical signs seen during follow-up after rechallenge	Week of sacrifice	Necropsy findings	Viral RNA (copies/mL)	Proviral DNA (copies/μg DNA)			CD4$^+$ T cells% (cells/μL)			CD4$^+$ T-cell recovery (%) from pre- to rechallenge
						Peripheral blood	Spleen	Lymph nodes	Peripheral blood	Spleen	Lymph nodes	
54844	RIBI + Tat	None	52	None	<50	57	102	853	23.3 (1387)	9.9	25.9	84
54879[a]	RIBI + Tat	None	24	None	3×10^2	<1	2	<1	20.5 (1001)	10.9	ND	41
54899	Alum + Tat	None	50	None	<50	8	ND	ND	7.9 (244)	ND	ND	49
54240	Alum + Tat	Liver dysfunction[c]	52	Pneumonia, hepatopathy	1.2×10^3	9	476	ND	30.2 (1209)	8.8	28.7	100
9104	Naive	Diarrhea (12/30),[d] thrombocytopenia	47	Pneumonia, enterocolitis	2.1×10^5	10	>1000	>1000	2.6 (87)	1.6	3.9	16
9503	Naive	Diarrhea (21/30), anemia	32	Sclerotic spleen, enterocolitis	9.1×10^4	10	>1000	10	24.2 (728)	5.9	18.4	30
9610	Naive	Diarrhea (22/30), anemia	34	Sclerotic spleen, enterocolitis	9.2×10^4	70	100	8.0	0.5 (20)	0.1	0.3	1
9406	Naive	Diarrhea (20/30)	48	Generalizedatrophy of lymphoid tissues, enterocolitis	<50	6	68	33	20.7 (497)	8.6	28.6	66

[a]This animal was sacrificed because of abnormal behavior. Euthanasia was decided by the veterinary staff according to Italian laws on animal welfare.
[b]This animal died of gastric dilatation, and data are from the last bleeding.
[c]High levels of GOT and GPT.
[d]Number of diarrhea episodes/number of observations in the last month before euthanasia.

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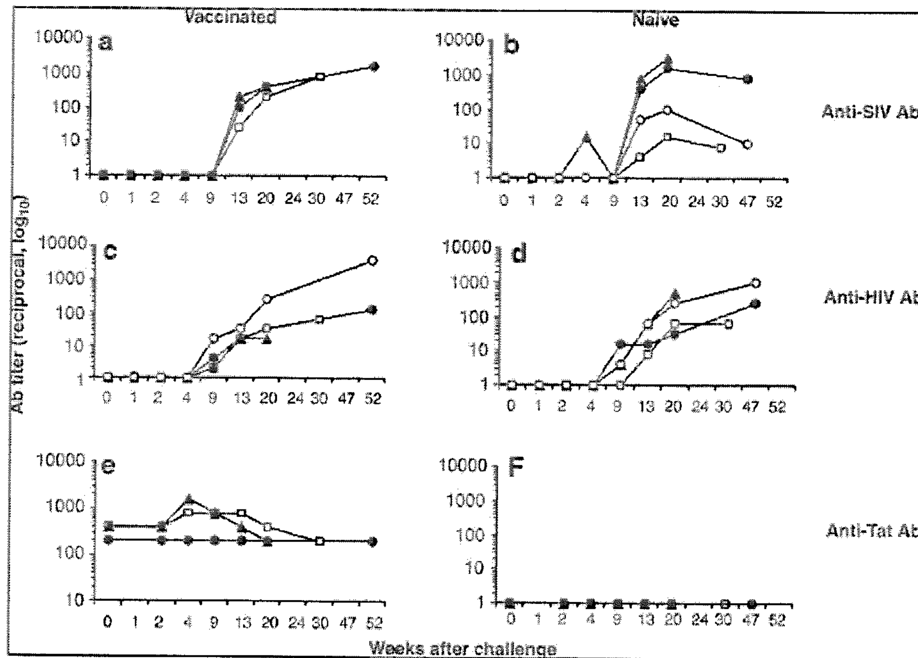

FIG. 2. Antibody responses to viral antigens in monkeys after rechallenge. (**a** and **b**) Anti-SIV IgG, (**c** and **d**) anti-HIV IgG, and (**e** and **f**) anti-Tat IgG after the rechallenge as determined by ELISA in vaccinated (54844 [solid circles]; 54879 [open squares]; 54899 [solid triangles]; 54240 [open circles]) and naive (9406 [solid circles]; 9610 [open squares]; 9503 [solid triangles]; 9104 [open circles]) monkeys, respectively. No significant difference between the two groups was observed for anti-SIV ($p = 0.880$) or anti-HIV ($p = 0.461$) antibody levels.

tectable in the controls (Fig. 2e–f). Similarly, Tat-specific T-cell responses (lymphoproliferation and IFN-γ ELISPOT) were boosted by the rechallenge in the vaccinated animals and remained high, whereas they were sporadic or undetectable in the naive group (Table 3 and Fig. 3a). Of note, the vaccinated animal 54240, which had undetectable or low Tat responses prior to rechallenge, showed no proliferation and only sporadic IFN-γ production to Tat after rechallenge (Table 3 and Fig. 3a).

Responses to SIV Gag were poor both in vaccinated and naive monkeys, as indicated by the absence of specific proliferative responses (Table 3), and IFN-γ SFC in response to

TABLE 3. PROLIFERATIVE RESPONSE TO TAT OR GAG OF VACCINATED AND NAIVE MONKEYS PRIOR TO AND AFTER RECHALLENGE

Animal	*Group*	*Stimulus*	*Pre- to rechallenge (week −8)*	*Post- to rechallenge (week +9)*
54844	RIBI + Tat	Tat	16.0	59.0
		Gag	>3	>3
54879	RIBI + Tat	Tat	21.0	104.0
		Gag	>3	>3
54899	Alum + Tat	Tat	34.0	26.0
		Gag	>3	>3
54240	Alum + Tat	Tat	4.0	3.0
		Gag	>3	>3
9406	Naive	Tat	>3	ND
		Gag	>3	ND
9610	Naive	Tat	3.0	ND
		Gag	>3	ND
9503	Naive	Tat	>3	>3
		Gag	ND	>3
9104	Naive	Tat	>3	8.3
		Gag	>3	<3

PBMC were stimulated with either Tat (5 μg/mL) or Gag (5 μg/mL). Results are expressed as stimulation index (SI). The SI was calculated as the ratio between mean cpm value of the Tat-stimulated sample and mean cpm value of the unstimulated controls. Values above the cut-off (SI >3) are indicated as positive.

ND, not done.

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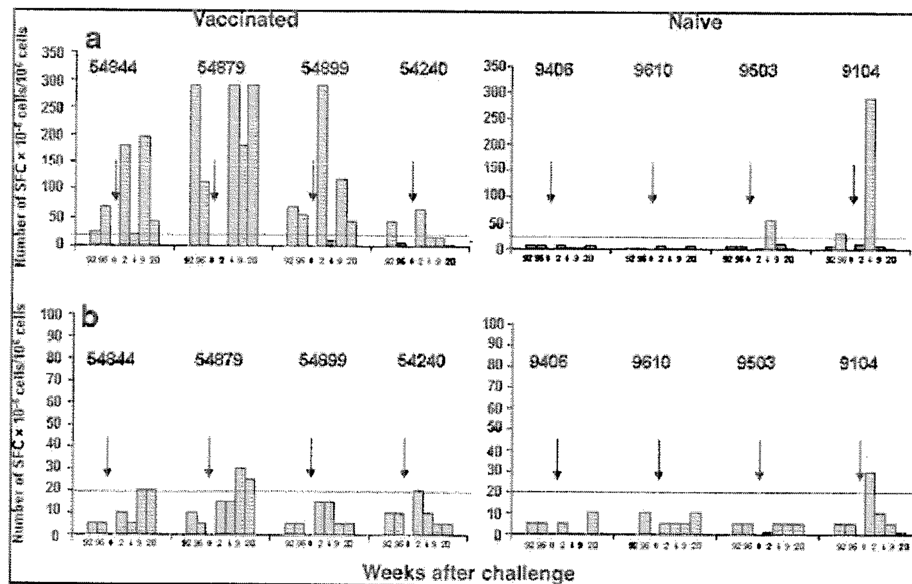

FIG. 3. Tat- and Gag-specific responses as evaluated by IFN-γ ELISPOT assay. Pre- (weeks 92, 96, and 0) and post rechallenge (weeks 2, 4, 9, and 20) frequencies of IFN-γ SFC/10^6 cells. PBMC from vaccinated or naive monkeys were stimulated with either Tat (**a**) or Gag (**b**) antigens. The cut-off was calculated as the mean number of SFC per million PBMC in unstimulated samples. The arrows indicate the day of challenge with 50 MID_{50} of the SHIV89.6P_{cy243}.

Gag was observed only in one vaccinated (54879), and in one naive monkey (9104) (Fig. 3b).

Discussion

The results presented herein indicate that vaccination with Tat protein elicited an immune response that correlated with a significant containment of viral replication upon two challenges with escalating SHIV89.6P_{cy243} virus doses and with prevention of AIDS development. This scenario is accompanied by the absence of detectable humoral immune responses against Env or Gag, and cellular responses to Gag. However, for an exhaustive analysis, the effects of the first challenge should also be considered. Different mechanisms might have played an important role in the establishment of the protection from re-challenge virus, such as viral interference with superinfection (7). A way to address the effectiveness of immunity induced by viral infection is to examine whether infection with one strain can provide protection against superinfection with a second strain after partial containment of the first virus. Experimental models of AIDS in animals have provided convincing evidence that heterologous superinfection can occur long after an initial infection (6,23). In this study it has been reported that immunity induced by vaccination with an attenuated virus, a condition that may mimic the non-sterilizing immunity obtained after the first challenge, was not always sufficient to protect from a subsequent virus re-challenge (23). On the other end it has been suggested that the persistence of virus replication, such as the exposure to a low dose of virus, might be responsible for the protection against subsequent heterologous infection (6,11). In our study, during the 54 wk after the first challenge and later on (weeks 71–104), vaccinees were aviremic, showing undetectable levels of p27 antigenemia and plasma viral RNA, and numbers of proviral copies that were sporadic or below the threshold limit of detection (<10 viral copies/μg DNA) (4,10). Monkeys mounted a very weak and transient or undetectable antibody response to SIV antigens and no antibodies to HIV-1 Env. Moreover, immune responses to Gag became undetectable in all animals beginning at week 18 p.i. On the other hand the humoral and cellular response to Tat antigen were more consistently detected in the immunized animals compared to the controls (7). All together, these data accounted for an undetectable level of viral replication in the vaccinees, at least at the systemic level. Indeed, we could not exclude the occurrence of occult replication at viral niches such as intestinal mucosal sites. At the time of rechallenge, levels of plasma viremia and proviral DNA in the vaccinees were below the detection limits. Monkeys were re-infected with a high dose of virus (50 MID_{50}), which likely overcame the potential benefit, if any, of the previous exposure to the lower dose of virus (10 MID_{50}). Indeed, all animals became plasma viremic after the second challenge, thus ruling out both the potential effects of the previous exposure to the virus and the cellular resistance to superinfection. In agreement with these findings, the pattern of seroconversion after rechallenge was similar to that detected in the controls, suggesting that in the vaccinees B-cell memory against HIV-1 Env and SIV antigens either did not develop, or waned soon after the first challenge. Similarly, in these animals SIV Gag-specific cellular immunity was low and transient or undetectable after the first challenge. Moreover, at the time of rechallenge no Gag-specific cellular immune responses were observed in the vaccinees, and no evidence of T-cell memory to Gag was seen after rechallenge. In contrast, Tat-specific proliferation and IFN-γ production

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as measured by ELISPOT revealed a strong anamnestic response after rechallenge, which correlated with the outcome of the infection and with the healthy clinical status of the animals, with the exception of animal 54240, in which the partial signs of progression observed were associated with low or undetectable levels of anti-Tat responses prior to and after rechallenge. With regard to the control monkeys, a Tat-specific T-cell response was observed only in animal 9104 at 4 and 9 wk after re-challenge for Tat-specific IFN-γ ELISPOT and Tat-specific proliferation, respectively. The significance of the Tat-specific T-cell responses seen in naive monkeys is presently under investigation. One possibility is that during natural infection monkeys develop a cellular response against Tat, as do humans. In fact, cytotoxic T lymphocytes to Tat are frequently detected in asymptomatic HIV-1-infected individuals (1), and also have been shown to inversely correlate with progression to AIDS (21). The relevance of the anti-Tat immune response was also shown previously in vaccinated unprotected animals, in which vaccination with the Tat protein induced a broad antiviral response, as demonstrated by the reduced ability to develop escape mutants, which is known to help in the control of viral replication (17). Thus, it is possible that the immune response to Tat accounts for achieving sustained control of infection, even if the establishment of persistent infection in these animals was not prevented. Although we could not exclude the role of other factors such as the release of soluble antiviral factors (6), we concluded that the significant control of viral replication in vaccinees after the second challenge was due to the pre-existing activity and to the re-challenge–driven expansion of anti-Tat humoral and cellular immune activity. It is important to note, however, that the Tat-based vaccine is not directly aimed at the elimination of latently infected cells, but is rather intended for containment of infection. Our results imply that it may be possible to achieve sustained viral control, provided that adequate immune responses to Tat protein are still present.

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Disclosure Statement

The authors declare that no financial conflict of interests exists.

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Address reprint requests to:
Dr. Barbara Ensoli
National AIDS Center
Istituto Superiore di Sanità
V.le Regina Elena 299
00161 Rome, Italy

E-mail: barbara.ensoli@iss.it

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