JOURNAL OF VIROLOGY, Feb. 1997, p. 866–872 Vol. 71, No. 2
0022-538X/97/$04.00+0

Expression of Gamma Interferon by Simian Immunodeficiency Virus Increases Attenuation and Reduces Postchallenge Virus Load in Vaccinated Rhesus Macaques

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Received 12 July 1996/Accepted 17 October 1996

Simian immunodeficiency virus (SIV) infection of macaques is a model for human immunodeficiency virus (HIV) infection. We have previously reported the construction and characterization of an SIV vector with a deletion in the *nef* gene ($SIV_{\Delta nef}$) and expressing gamma interferon (SIV_{HyIFN}) (L. Giavedoni and T. Yilma, J. Virol. 70:2247–2251, 1996). We now show that rhesus macaques vaccinated with SIV_{HyIFN} have a lower viral load than a group similarly immunized with $SIV_{\Delta nef}$. Viral loads remained low in the SIV_{HyIFN}-vaccinated group even though SIV expressing gamma interferon could not be isolated after 6 weeks postimmunization in these animals. All immunized and two naive control macaques became infected when challenged with virulent SIV_{mac251} at 25 weeks postvaccination. In contrast to the two naive controls that died by 12 and 18 weeks postchallenge, all vaccinated animals remained healthy for more than 32 weeks. In addition, postchallenge cell-associated virus load was significantly lower in SIV_{HyIFN}-immunized animals than in the group vaccinated with $SIV_{\Delta nef}$. These findings indicate that cytokine-expressing viruses can provide a novel approach for development of safe and efficacious live attenuated vaccines for AIDS.

Simian immunodeficiency virus (SIV) infection of macaques is the best model currently available for human immunodeficiency virus (HIV) infection (12). Inactivated SIV or recombinant subunit vaccines have provided limited or no protection against infection with SIV (1, 15, 27). To date, only live attenuated vaccines, containing $SIV_{\Delta nef}$ (SIV_{mac239} with a deletion in the *nef* gene) and $SIV_{\Delta 3}$ (lacking *nef*, *vpr*, and upstream sequences in U3), have provided complete protection to rhesus macaques against challenge with a high dose of virulent SIV_{mac251} (8, 32). However, $SIV_{\Delta nef}$ persists indefinitely in macaques, provides limited or no protection until a year or more after immunization (32), and has been reported to be pathogenic to neonatal macaques (3); these characteristics limit its use as a vaccine. In our efforts to develop a safer and more efficacious live attenuated vaccine for AIDS, we have constructed and characterized a replication-competent $SIV_{\Delta nef}$ that expresses high levels of human gamma interferon (HuIFN-γ) (SIV_{HyIFN}) (14).

IFN-γ is a lymphokine with potent immunoregulatory, antineoplastic, and antiviral properties. The in vitro antiviral activity of IFN-γ against a number of viruses, including encephalomyocarditis virus, vaccinia virus, and HIV, has been demonstrated (5, 7, 16, 17, 19). More recently, experiments using mice with disrupted IFN-γ or IFN-γ receptor genes showed an increase in susceptibility to infection by intracellular parasites, including viruses, despite normal cytotoxic and T-helper cell responses (18). We and others have clearly demonstrated the immunity-enhancing and attenuating activities of lymphokines such as IFN-γ and interleukin-2 (2, 11, 19, 28, 33). Vaccinia virus recombinants expressing IFN-γ are attenuated 1 million-fold for nude mice (13, 21), although the mechanism for the most part remains unknown. In this study, we show that the prospects for the development of a safe and efficacious vaccine for AIDS can be improved by the expression of lymphokine genes by attenuated live vectors.

MATERIALS AND METHODS

Cells and viruses. CEM-x-174 cells, rhesus peripheral blood mononuclear cells (PBMCs), and lymph node cells (LNCs) were used for SIV isolation and propagation; these cells were maintained in RPMI 1640 supplemented with 10% fetal

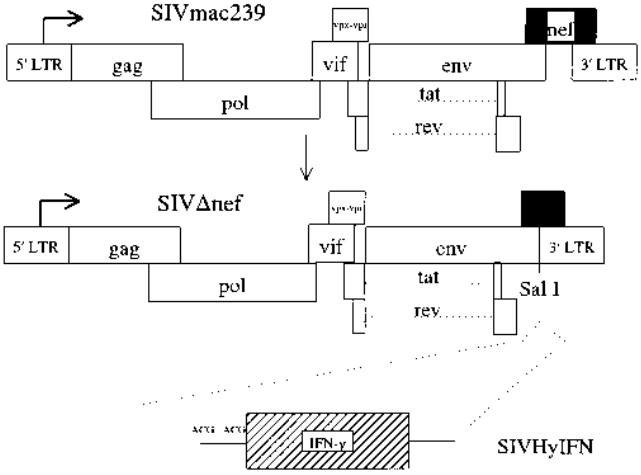

FIG. 1. Schematic representations of the genomic organizations of $SIV_{\Delta nef}$ and SIV_{HyIFN}. A 186-base fragment of the *nef* coding sequence was deleted, and a unique *Sal*I cloning site was incorporated for the insertion of the HuIFN-γ gene. To increase the expression levels of IFN-γ, the two in-frame *nef* start codons were mutated without altering the Env amino acid sequence. Details are provided in reference 14. LTR, long terminal repeat.

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TABLE 1. Cell-associated virus load in PBMCs and LNCs of vaccinated rhesus macaques

Animal	Vaccine	$TCID_{50}/10^6$ PBMCs (LNCs)						
		1 WPV	2 WPV	4 WPV	6 WPV	8 WPV	12 WPV	25 WPV
26595	SIV_{HyIFN}	<1	47 (10)	16 (22)	3	1	3 (<10)	<1 (47)
26704	SIV_{HyIFN}	<1	214 (316)	7 (316)	3	1	1 (32)	316 (<10)
26919	SIV_{HyIFN}	<1	316 (10)	32 (2,138)	32	1	1 (<10)	<1 (<10)
27047	SIV_{HyIFN}	<1	3 (100)	32 (214)	2	10	<1 (<10)	10 (32)
27078	SIV_{HyIFN}	<1	<1 (22)	<1 (100)	<1	<1	<1 (<10)	<1 (<10)
27178	SIV_{HyIFN}	<1	1 (22)	3 (214)	148	2	10 (<10)	18 (<10)
26720	$SIV_{\Delta nef}$	68	676 (4,677)	10 (422)	10,000	25	3 (32)	1 (32)
26740	$SIV_{\Delta nef}$	5	1,795 (1,710)	316 (3,162)	2,371	21	1 (32)	10 (32)
26890	$SIV_{\Delta nef}$	468	316 (10,000)	2,138 (3,162)	100	10,000	4,677 (316)	316 (316)
27149	$SIV_{\Delta nef}$	47	1,000 (10,000)	100 (316)	3,162	31,623	316,230 (2,153)	316 (316)

bovine serum. Human A549 cells were propagated in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum and antibiotics.

We have previously described the construction and characterization of $SIV_{\Delta nef}$ and SIV_{HyIFN} (14). Briefly, the pathogenic molecular clone SIV_{mac239} (20) was used to construct $SIV_{\Delta nef}$ vectors (Fig. 1). To generate SIV_{HyIFN}, a 186-base fragment of the *nef* coding sequence was deleted, and a unique *Sal*I cloning site was incorporated for the insertion of the HuIFN-γ gene. HuIFN-γ is fully active in monkey cells, induces an antiviral state in Vero cells, and enhances major histocompatibility complex class II expression in macaque PBMCs (25, 26). To increase the expression levels of IFN-γ, we mutated the two in-frame *nef* start codons without altering the Env amino acid sequence. $SIV_{\Delta nef}$ and SIV_{HyIFN} were propagated and titered in CEM-x-174 cells. SIV_{mac251}, a pathogenic biological isolate, was kindly provided by R. Desrosiers (Harvard Medical School); the virus was propagated in rhesus PBMCs, and titers were determined in rhesus monkeys (22). Encephalomyocarditis virus, used for the antiviral assay of HuIFN-γ, was propagated in human A549 cells.

Vaccination of rhesus macaques. Colony-bred, juvenile rhesus macaques (*Macaca mulatta*) seronegative for simian type D retroviruses, simian T-cell leukemia virus, and SIV were used in this experiment. They were housed in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines. A group of six macaques were vaccinated intravenously with 1 ml of RPMI 1640 containing 10^4 50% tissue culture infective doses ($TCID_{50}$) of SIV_{HyIFN}; a second group of four animals received a similar dose of $SIV_{\Delta nef}$.

Cell-associated viral load. Cell-associated virus, latent or productive, was measured by limiting-dilution assay (four replicates per dilution) of PBMCs and LNCs with CEM-x-174 cells in 24-well plates (30). Twice weekly, aliquots of the culture medium were assayed for the presence of the SIV major core protein (p27) by enzyme-linked immunosorbent assay (ELISA) (23). When p27 antigen was detected at two consecutive time points, cultures were recorded as virus positive. Endpoint cultures were maintained and tested for 4 weeks before being scored as negative. Virus levels were calculated according to the method of Reed and Muench (29) and expressed as $TCID_{50}$ per 10^6 cells.

Plasma SIV p27 antigen levels. Plasma p27 antigenemia was measured by a commercial SIV core antigen capture ELISA (Coulter Corporation, Hialeah, Fla.) as instructed by the manufacturer.

Detection of proviral SIV DNA. DNA was isolated from 2×10^5 cells (PBMCs, LNCs, or CEM-x-174 cells) by using a DNA isolation kit (Qiagen, Chatsworth, Calif.). The presence of SIV proviral sequences was confirmed by PCR amplification of the *env*-3′ long terminal repeat region, using primers A (5′GTACC ATGGCCAAATGCAAG3′, sense primer, nucleotide 8720) and E (5′AAATC CCTTCCAGTCCCCCC3′, antisense primer, nucleotide 9710). The proviral DNA was hot started with Mg^{2+} beads (Invitrogen Corp., La Jolla, Calif.) at 94°C for 5 min, annealed at 65°C for 1 min, and extended at 72°C for 2 min; then the denaturation time was reduced to 1 min, and the cycle was repeated 35 times.

Lymphocyte phenotyping. PBMCs were stained with anti-human monoclonal antibodies to CD4 (phycoerythrin-conjugated OKT4; Ortho Diagnostic Systems Inc., Raritan, N.J.) or to CD8 (Leu 2a-fluorescein isothiocyanate; Becton Dickinson Immunocytometry Systems, San Jose, Calif.) as instructed by the manufacturers, and immunofluorescence was measured with a dual-laser flow cytometer (FACScan; Becton Dickinson).

Analysis of the humoral immune response of rhesus macaques. Anti-Gag and anti-gp160 antibodies were measured in the plasma of monkeys by ELISA, using 96-well plates coated with baculovirus-produced SIV Gag or gp160 antigens (1). Titers were calculated as the reciprocal of the dilution that produced an absorbance at least twice the value of the negative control plasma. Both prechallenge and postchallenge anti-Gag and anti-gp160 titers are presented for each animal.

Challenge of rhesus macaques. Vaccinated macaques were challenged with SIV_{mac251} 25 weeks postvaccination (WPV); two naive controls were included at the time of challenge. The viral inoculum, 100 50% monkey infective doses (MID_{50}), was given intravenously in a single 1-ml dose. Animals were euthanized when they showed three or more of the following clinical observations: (i) weight loss greater than 10% in 2 weeks or 30% in 2 months; (ii) chronic diarrhea unresponsive to treatment; (iii) infections unresponsive to antibiotic treatment; (iv) inability to maintain body heat or fluids without supplementation; (v) persistent, marked hematologic abnormalities, including lymphopenia, anemia, thrombocytopenia, or neutropenia; and (vi) persistent, marked splenomegaly or hepatomegaly.

Statistical analyses. Virus loads in the three different groups of macaques were statistically analyzed. Cell-associated virus levels for each group were compared by calculating the area under the curve (AUC) of time versus viral load (without logarithmic transformation) for each animal; the AUC was analyzed by using the Wilcoxon rank-sum test (9).

RESULTS

Vaccination of rhesus macaques. We conducted a comparative study in two groups of rhesus macaques vaccinated intra-

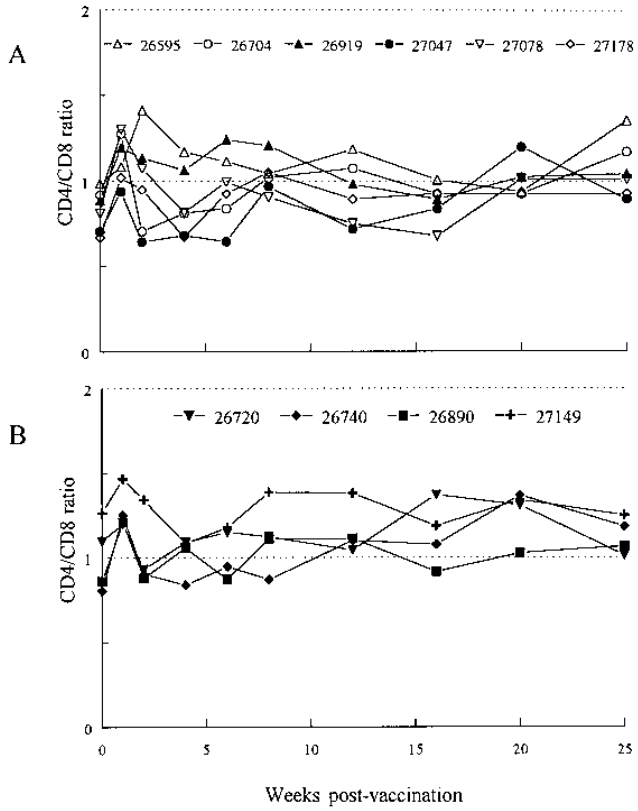

FIG. 2. Prechallenge variations in CD4/CD8 ratios after vaccination with live-attenuated SIV. The data represent the ratio between the percentage of $CD4^+$ versus $CD8^+$ cells for animals inoculated with SIV_{HyIFN} (A) or $SIV_{\Delta nef}$ (B).

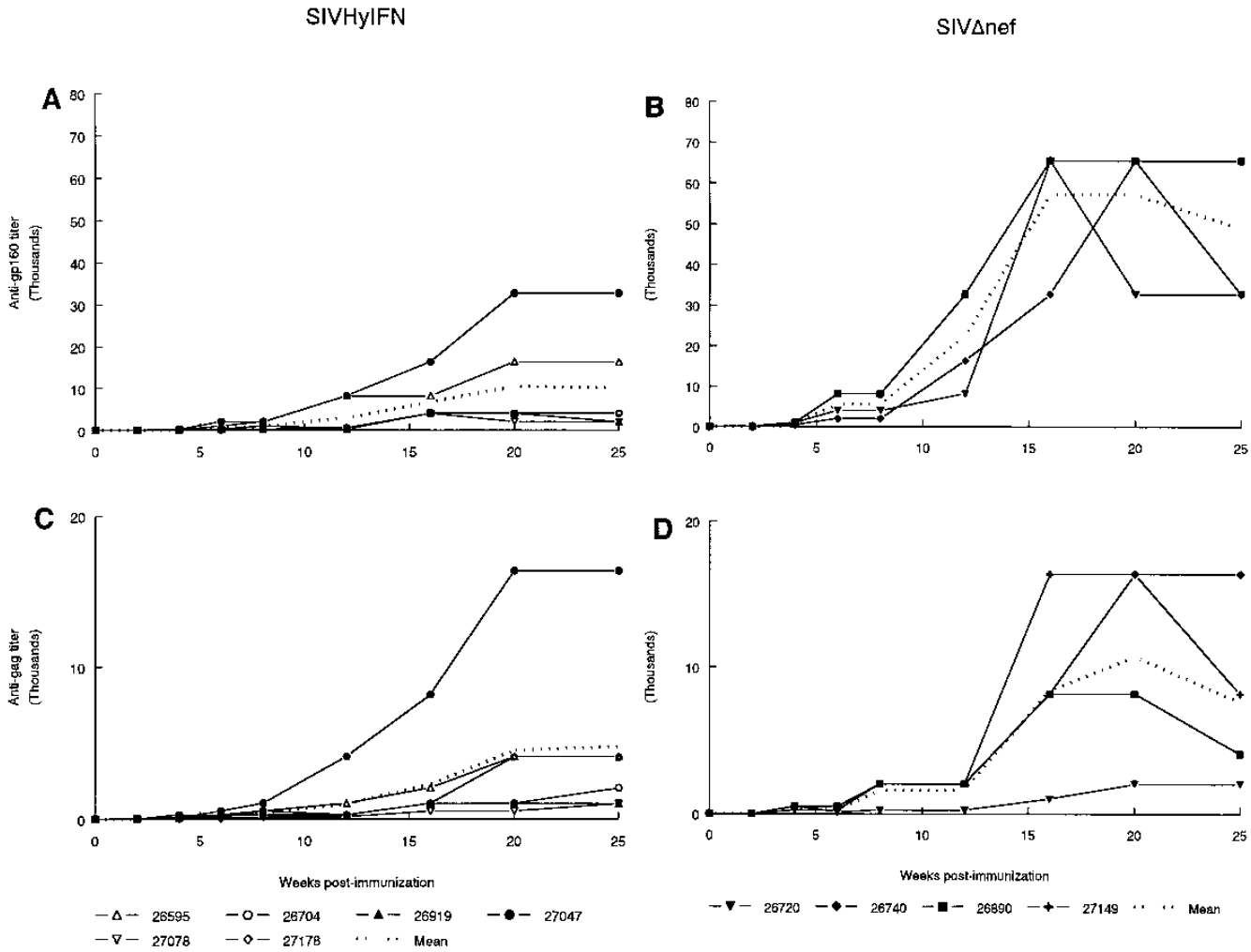

FIG. 3. Prechallenge humoral immune responses in macaques vaccinated with $SIV_{\Delta nef}$ and SIV_{HyIFN}. The titers of anti-gp160 (A and B) and anti-Gag (C and D) were determined in plasma of macaques immunized with SIV_{HyIFN} (A and C) and $SIV_{\Delta nef}$ (B and D) by ELISA (1). Results are expressed as the reciprocal to the dilution that gives an optical density twice that of negative control plasma.

TABLE 2. Cell-associated virus load in PBMCs of rhesus macaques after challenge with 100 MID_{50} of SIV_{mac251}

Animal	Vaccine	$TCID_{50}/10^6$ cells[a]											
		0 WPC	1 WPC	2 WPC	4 WPC	6 WPC	8 WPC	12 WPC	16 WPC	20 WPC	24 WPC	28 WPC	32 WPC
26595	SIV_{HyIFN}	<1	<1	10	<1	32	100	215	316	316	3,162	316	316
26704	SIV_{HyIFN}	316	<1	32	3	100	32	215	1,000	32	46	316	316
26919	SIV_{HyIFN}	<1	47	1,000	1,479	1,000	3,162	171	316	316	32	215	32
27047	SIV_{HyIFN}	10	21	<1	316	32	100	1,000	46	22	32	316	<10
27078	SIV_{HyIFN}	<1	<1	<1	3	3	3	46	10	5	32	<10	5
27178	SIV_{HyIFN}	18	3	316	316	32	1,000	3,162	3,162	316	316	316	3,162
26720	$SIV_{\Delta nef}$	1	<1	32	100	215	215	32	316	316	215	170	170
26740	$SIV_{\Delta nef}$	10	32	32	2,153	3,162	316	316	3,162	3,162	46,451	31,623	3,162
26890	$SIV_{\Delta nef}$	316	171	31,622	1,000	1,000	31,623	3,162	31,623	3,162	4,645	3,162	2,153
27149	$SIV_{\Delta nef}$	316	1,795	3,162	316	316	2,153	4,645	31,623	2,153	31,623	3,162	3,162
26658	Naive	<1	215	2,153	316	316	3,162	ND	316				
26905	Naive	<1	316	14,791	3,162	3,162	3,162	464					

[a] Single underline, vaccine virus was isolated; no underline, challenge virus was isolated; double underline, both vaccine and challenge viruses were isolated. Macaque 26658 died 18 WPC; macaque 26905 died 12 WPC. ND, not determined.

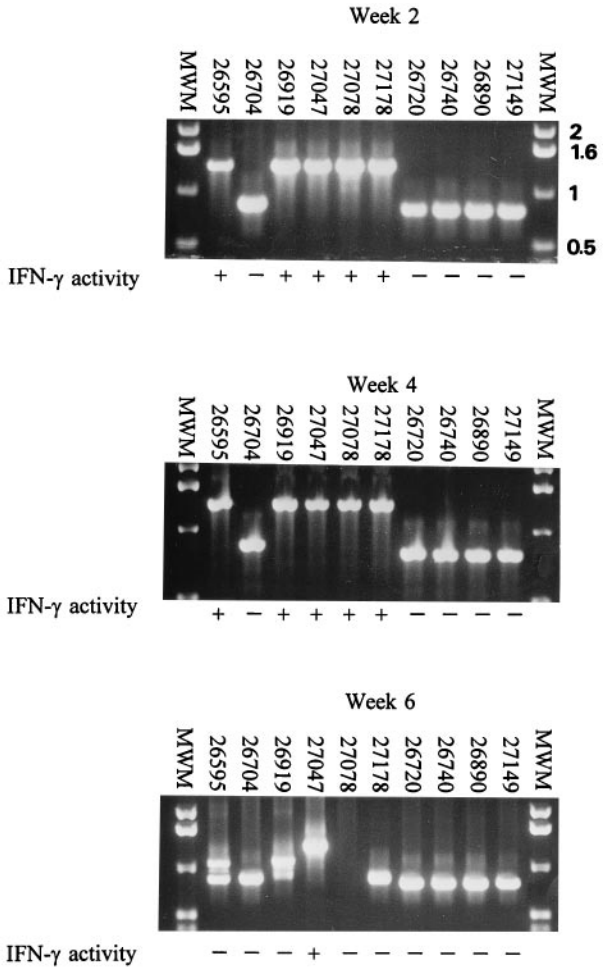

FIG. 4. Genetic analysis of virus isolated from immunized macaques. DNA was isolated from cocultures of CEM-x-174 and LNCs (weeks 2 and 4) or PBMCs (week 6) and analyzed by PCR (14). The full-length PCR-generated fragments are 800 bp for $SIV_{\Delta nef}$ and 1,300 bp for SIV_{HyIFN}. IFN-γ levels in the supernatants of the same cultures used for DNA analysis were measured by antiviral assays. Sizes of molecular weight markers (MWM) are indicated in kilobases.

venously with 10^4 $TCID_{50}$ of $SIV_{\Delta nef}$ or SIV_{HyIFN} (Fig. 1). Samples consisting of LNCs and PBMCs were tested for infectious virus (Table 1). Virus was isolated from PBMCs of $SIV_{\Delta nef}$-vaccinated macaques in samples from the first week, and titers remained generally high through week 12. Although the viral titers varied from animal to animal, all animals were positive for virus isolation at all time points from either PBMCs or LNCs. In contrast, virus was detected for the first time in SIV_{HyIFN}-vaccinated macaques in samples taken on week 2, and these animals maintained very low viral titers. After 12 WPV, virus isolation from 1 million cells was sometimes unsuccessful, and one animal (27078) had detectable virus only sporadically and only in LNCs. The differences in viral loads between the two groups, measured as the AUC of viral load versus time during the first 12 WPV, were statistically significant ($P < 0.01$). Plasma antigenemia (p27) could not be detected in SIV_{HyIFN}-vaccinated animals at any time point, whereas three of four animals in the $SIV_{\Delta nef}$ group (26740, 26890, and 27149) had less than 100 pg/ml on week 2. Variations in $CD4^+$ and $CD8^+$ cells were determined by fluorocytometry. Both $SIV_{\Delta nef}$- and SIV_{HyIFN}-inoculated macaques had a slight increase in their ratios by 1 WPV, but CD4/CD8 ratios remained within normal limits throughout the 25-week prechallenge period (Fig. 2). Antibody responses were assessed by ELISA, using baculovirus-expressed SIV gp160 and SIV Gag as coating antigens. In general, antibody titers to Gag and gp160 correlated with the virus load in both groups (Table 1). Macaques infected with $SIV_{\Delta nef}$ and SIV_{HyIFN} developed anti-Gag and anti-gp160 antibodies that peaked by 20 WPV (Fig. 3). Throughout the 25 weeks, the anti-gp160 and anti-Gag mean titers were higher in macaques vaccinated with $SIV_{\Delta nef}$ than in those vaccinated with SIV_{HyIFN}. These differences were more pronounced between 12 and 20 WPV. Three of four animals in the $SIV_{\Delta nef}$ group had anti-Gag titers greater than 8,000 by 20 WPV. Anti-gp160 antibody titers were similarly higher among animals in the $SIV_{\Delta nef}$ group. Animals inoculated with SIV_{HyIFN} had a delayed anti-Gag antibody response, with five of six animals reaching titers of ≤4,096 by 20 WPV; macaque 27047 was the exception (Fig. 3C). Anti-gp160 antibodies were induced in a similar delayed fashion, and only macaque 27047 reached titers over 20,000 by 25 WPV (Fig. 3A).

The in vivo stability of SIV_{HyIFN} was also studied by PCR

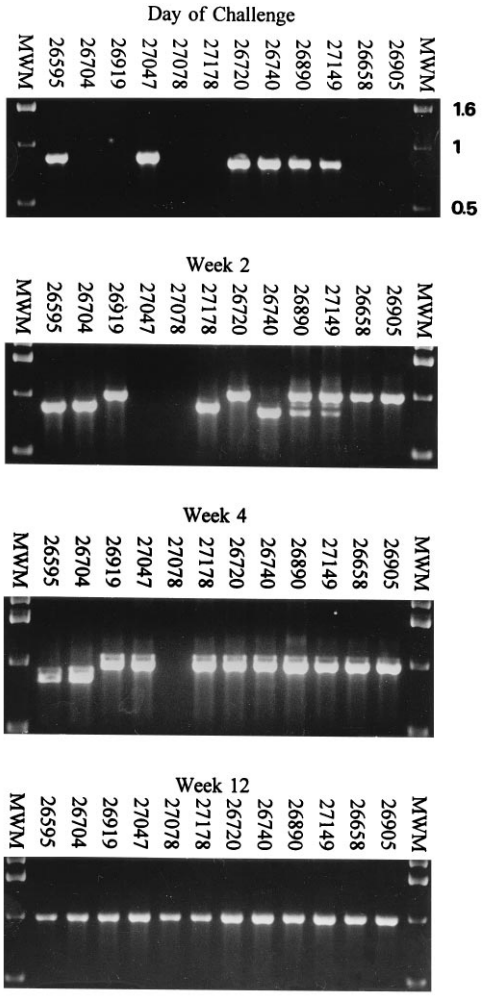

FIG. 5. Characterization of virus isolated from LNCs of macaques after challenge with SIV_{mac251}. DNA was isolated from cocultures of CEM-x-174 and LNCs and was analyzed by PCR as described in the legend to Fig. 4. The PCR-generated fragments are 800 bp for $SIV_{\Delta nef}$, 850 bp for SIV_{HyIFN}, and 1,000 bp for SIV_{mac251}. Sizes of molecular weight markers (MWM) are indicated in kilobases.

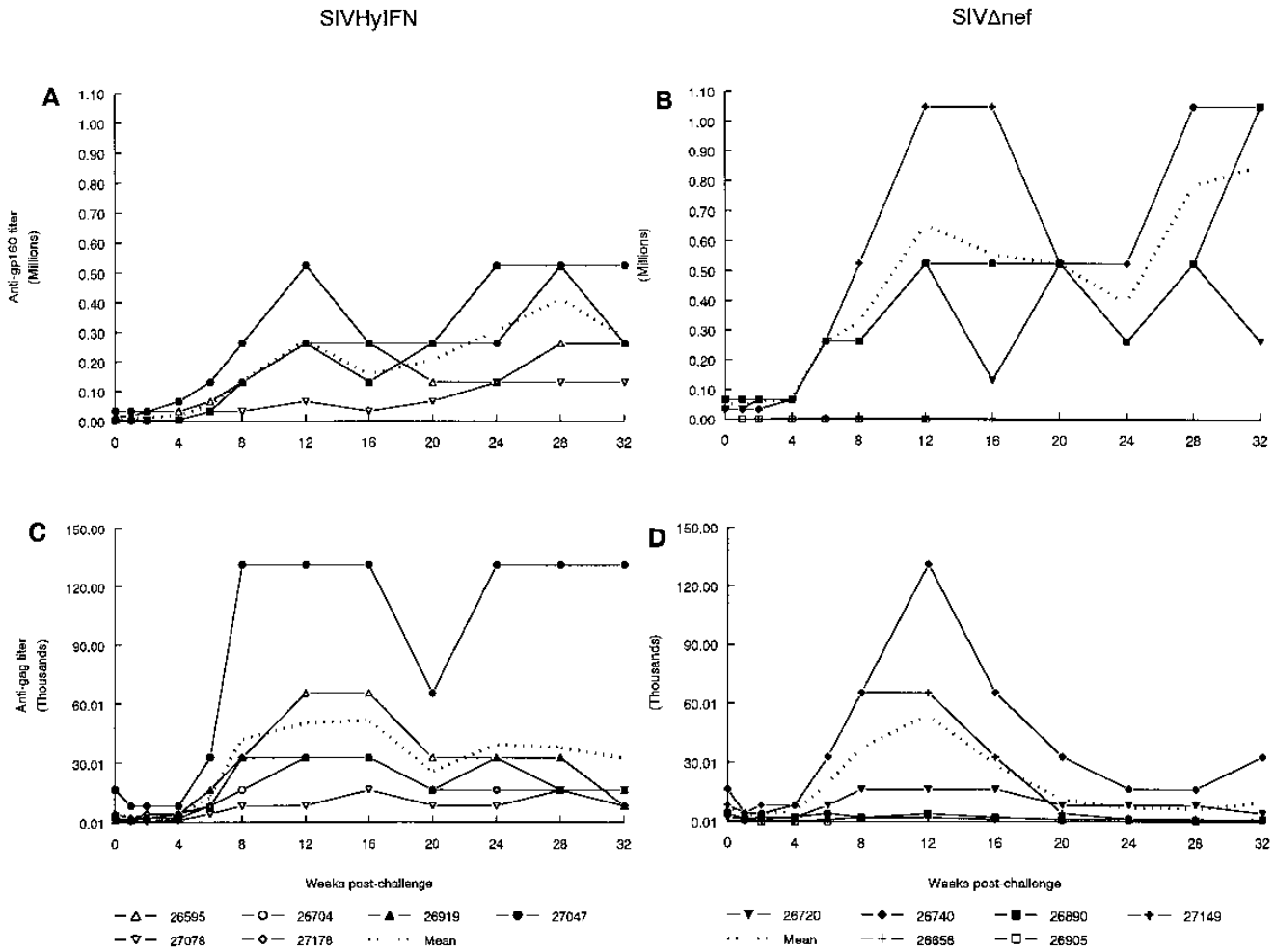

FIG. 6. Humoral immune responses of macaques after challenge with SIV_{mac251}. Anti-gp160 (A and B) and anti-Gag (C and D) antibodies in macaques immunized with SIV_{HyIFN} (A and C) and $SIV_{\Delta nef}$ (B and D) were measured as described in the legend to Fig. 3.

analysis of the 3′-end region of virus isolated from vaccinated macaques (Fig. 4). On weeks 2 and 4, the virus isolated from five of the six SIV_{HyIFN}-vaccinated animals still retained the IFN-γ insert, and antiviral activity was present in the cultures from which the virus was isolated. By week 6, only virus isolated from lymphocytes of macaque 27047 retained both the IFN-γ gene and antiviral activity in culture. By week 12, no virus with a full-length IFN-γ gene could be isolated, and no antiviral activity was detected in cultures infected with these viruses. In addition, a very sensitive ELISA kit, specific for human IFN-γ, failed to detect this cytokine in the plasma of SIV_{HyIFN}-vaccinated macaques at any point after vaccination.

Effectiveness of live attenuated vaccines. Vaccinated animals along with two naive controls were challenged at 25 WPV with 100 MID_{50} of SIV_{mac251} propagated in rhesus PBMCs. Viral loads for all 12 animals were measured at various times postchallenge (Table 2). Although all animals were infected by the challenge virus, the viral load for SIV_{HyIFN}-vaccinated animals, calculated as the AUC (viral load versus time) for each animal, was significantly lower than for those vaccinated with $SIV_{\Delta nef}$ ($P = 0.03$). Of the two naive controls, 26905 produced very low levels of antibodies against SIV and died at 12 weeks postchallenge (WPC) from severe AIDS-related complications. The second unimmunized control (26658) generated an antibody response to SIV; however, the animal developed similar complications and was euthanized at 18 WPC.

Virus isolated from PBMCs and LNCs postchallenge was characterized by PCR (Fig. 5). Challenge virus was isolated from both naive control animals at 1 WPC. The ratio of virus types isolated from vaccinated macaques after challenge gradually shifted from vaccine virus alone, to a mixture of vaccine and challenge virus, and finally to challenge virus only. However, virus replacement progressed more slowly in the SIV_{HyIFN}-vaccinated groups of macaques. In the $SIV_{\Delta nef}$ group, only challenge virus was isolated from all animals by 4 WPC. In contrast, this conversion to challenge virus was completed at 6 WPC in the SIV_{HyIFN}.

In contrast to our previous studies with recombinant and subunit vaccines, where antibody titers to SIV gp160 or Gag increased sharply after challenge (1, 15), no anamnestic antibody response was observed in SIV_{HyIFN}- and $SIV_{\Delta nef}$-vaccinated animals until 6 WPC (Fig. 6). The replacement of vaccine virus with challenge virus was followed by a rise in antibody titer in both groups of vaccinated animals. For the $SIV_{\Delta nef}$-inoculated macaques, three of four animals showed a rise in anti-Gag titers that reached a peak by 12 WPC, after which there was a gradual decline (Fig. 6D). In SIV_{HyIFN}-inoculated macaques, similarly, anti-Gag titers increased by 4

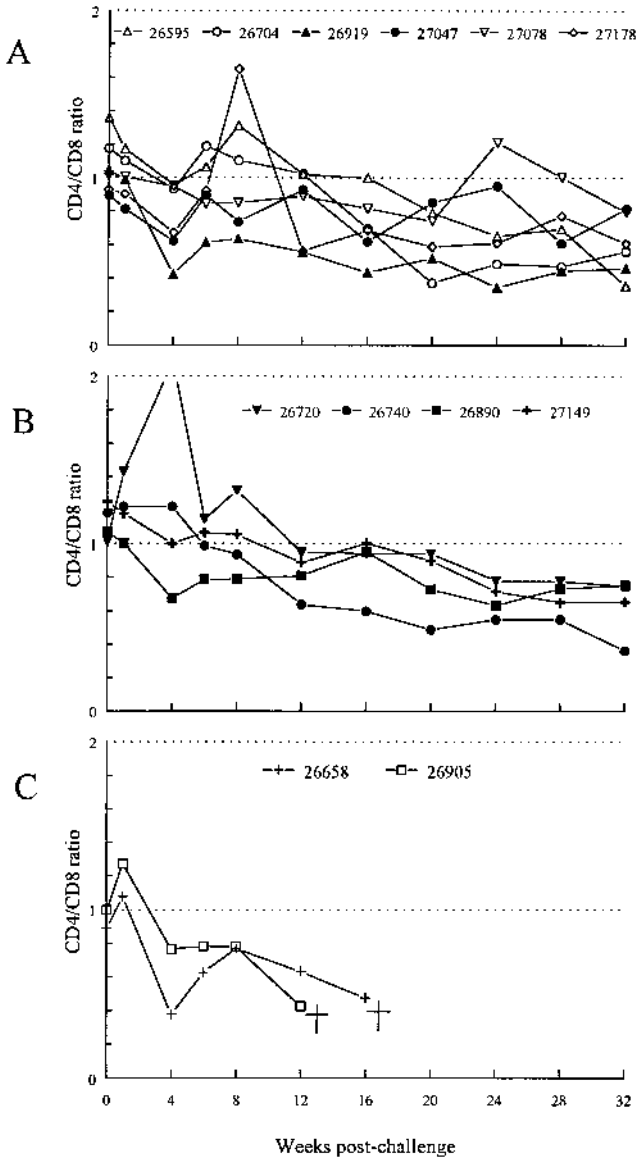

FIG. 7. Variation in CD4/CD8 ratios after challenge with SIV_{mac251}. The data represent the ratio between the percentage of $CD4^+$ versus $CD8^+$ cells for animals vaccinated with SIV_{HyIFN} (A) or with $SIV_{\Delta nef}$ (B), or naive animals (C). The symbol + indicates the time when the animal had to be euthanized.

to 6 WPC and reached their maximum by 12 WPC; however, the decline in anti-Gag titers was not as pronounced as the one observed for the $SIV_{\Delta nef}$-inoculated macaques (Fig. 6C). Antibodies to SIV gp160 in vaccinated animals of both groups had different profiles, but in general, mean titers for both groups increased and did not decline throughout the 32-week post-challenge period. In contrast to the anti-Gag response, the mean anti-gp160 titers remained two- to fivefold higher in $SIV_{\Delta nef}$-vaccinated macaques than SIV_{HyIFN}-vaccinated macaques.

Infection with SIV_{mac251} was accompanied by a decline in CD4/CD8 ratios in all animals, with naive macaques experiencing the most dramatic reduction (Fig. 7). For all vaccinated animals, a slower decrease in CD4 counts was observed starting at 12 WPC. An exception for this was macaque 26919, which had a sharper drop in its ratios; however, this animal had no clinical manifestations during 32 WPC.

DISCUSSION

We have previously reported the construction and in vitro characterization of SIV_{HyIFN}, a modified $SIV_{\Delta nef}$ that expresses high levels of HuIFN-γ. HuIFN-γ is fully active in monkey cells, induces an antiviral state in Vero cells, and enhances major histocompatibility complex class II expression in macaque PBMCs (25, 26). SIV_{HyIFN} was unstable and sustained deletions in the IFN-γ gene after serial passage in CEM-x-174 cells; however, antiviral activity was present through the sixth passage even though IFN-γ sequences were barely detectable at this point by PCR amplification (14). Here, we report that macaques vaccinated with SIV_{HyIFN} have much lower viral loads than those vaccinated with $SIV_{\Delta nef}$. No SIV_{HyIFN} with a full-length IFN-γ gene was detected past 6 WPV, but remarkably, this deletion did not lead to an increase in virus load. The progressive deletion of the IFN-γ gene in vivo appears to have a positive cumulative effect in inducing a stepwise immune response. The immune system is activated initially by a highly attenuated virus (SIV_{HyIFN}) expressing IFN-γ. As virulence increases to the level of $SIV_{\Delta nef}$ (through loss of the IFN-γ gene), the emerging virus is met by an immune system primed for the encounter by earlier exposure to SIV_{HyIFN}. The fact that the IFN-γ gene is deleted and is present only during the early phase of the immune response to SIV eliminates the possibility of any untoward effects from continuous expression of the lymphokine. It is significant that at no time during the course of the experiment was HuIFN-γ detected in the plasma of SIV_{HyIFN}-vaccinated macaques.

The level of immunity induced by SIV_{HyIFN} and $SIV_{\Delta nef}$ in vaccinated macaques was tested by challenge with a heavy dose of pathogenic SIV_{mac251} (100 MID_{50}). Confirming the severity of the challenge, the two naive controls died by 12 and 18 WPC. However, this study demonstrates that although SIV_{HyIFN} is more attenuated than $SIV_{\Delta nef}$, it induces a significant level of immunity manifested by reduced virus load post-challenge. This is in sharp contrast to results obtained from other attenuated SIV vaccine studies, in which the degree of protection directly correlated with the virulence of the vaccine virus (24) or with vaccine virus loads after immunization (32). The type of immunity that correlates with protection remains to be elucidated, although the level of anti-SIV antibodies does not appear to predict the ability of the macaques to limit SIV replication. It has also been observed that $SIV_{\Delta nef}$-based vaccines require a maturation period of at least 1 year to induce sterilizing immunity (32). Although the mechanism of protection cannot yet be postulated, viral interference does not appear to be involved since the vaccine virus in our study groups was replaced by challenge virus in a relatively short period of time.

There is an urgent need for a safe and efficacious vaccine for AIDS. Several possible vaccine candidates have been tested in animal model systems. Until now, only $SIV_{\Delta nef}$ and $SIV_{\Delta 3}$ have provided significant protection against challenge with pathogenic SIV_{mac251}. The importance of *nef* in the pathogenesis of both SIV and HIV has been further reinforced by two recent reports of studies using macaques and humans, respectively. In macaques, the in vivo restoration of a 12-bp deletion in *nef* resulted in the onset of clinical disease in the affected macaques (31). Moreover, genetic analysis of HIV isolated from some long-term nonprogressors individuals showed the presence of a nonfunctional *nef* gene (10). However, as indicated earlier, there are a number of concerns about the use of

$SIV_{\Delta nef}$, including its reported pathogenicity for neonates (3). We show in this report that SIV_{HyIFN} induces a transient, low-titer viremia in juvenile macaques and that the deletion of the IFN-γ gene soon after infection does not correlate with reversion to virulence. Although we have not demonstrated sterilizing immunity with SIV_{HyIFN} at 6 months postvaccination, we have shown that this vaccine does delay the onset of disease similarly to what was seen with $SIV_{\Delta nef}$. Additionally, SIV_{HyIFN} provides a higher level of efficacy, as demonstrated by a significant reduction in viral load compared to $SIV_{\Delta nef}$. The higher humoral responses to viral antigens (both gp160 and Gag) correlate with the higher virus loads in the $SIV_{\Delta nef}$-vaccinated group (Fig. 3 and 6; Tables 1 and 2). Furthermore, the $SIV_{\Delta nef}$-vaccinated animals exhibit a more rapid decline in anti-Gag antibody titers than the SIV_{HyIFN} group (Fig. 6D); such a decline in titer is used as a predictor for progression to disease in AIDS (4). The importance of this finding becomes clearer if we accept the premise that our goal in AIDS vaccine research may well have to be prevention of disease rather than of infection (6). In conclusion, we propose that incorporation of IFN-γ or other lymphokines provides an approach that will lead to the development of safer and efficacious vaccines for AIDS and other infectious diseases.

ACKNOWLEDGMENTS

We thank A. Hendrickx for support and encouragement, S. Owens for the critical review of the manuscript, and L. Parodi and J. Imhoof for excellent technical assistance. We also thank H. Louie, L. Antipa, and D. Bennet for additional assistance.

L.G. is a scholar of the American Foundation for AIDS Research. L.J. received support from the National Institute of General Medical Sciences Graduate Training Program in Biotechnology (GM08343-01A1) and training grant AI07398. This work was supported by National Institutes of Health grant UO1-A129207 to T.Y. Other support was provided by Center for AIDS research grant AI27732.

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