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Construction, Replication, and Immunogenic Properties of a Simian Immunodeficiency Virus Expressing Interleukin-2

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To study the effect of interleukin-2 (IL-2) on simian immunodeficiency virus (SIV) replication, pathogenesis, and immunogenicity, we replaced the *nef* gene of SIVmac239 by the IL-2 coding region. The virus, designated SIV-IL2, stably expressed high levels of IL-2 in cell culture. In comparison to SIVmac239, SIV-IL2 replicated more efficiently in peripheral blood mononuclear cells in the absence of exogenously added IL-2. To determine whether this growth advantage would be of relevance in vivo, four juvenile rhesus monkeys were infected with SIV-IL2 and four monkeys were infected with a *nef* deletion mutant of SIV (SIVΔNU). After a peak in the cell-associated viral load 2 weeks postinfection, the viruses could barely be isolated 3 to 7 months postinfection. Mean capsid antigen levels were higher in the SIV-IL2 group than in the *nef* deletion group 2 weeks postinfection. Viruses reisolated from the SIV-IL2-infected animals expressed high levels of IL-2 during the acute phase of infection. Deletions in the IL-2 coding region of SIV-IL2 were observed in two of the SIV-IL2-infected macaques 3 months postinfection. Urinary neopterin levels, a marker for unspecific immune stimulation, were higher in the SIV-IL2-infected macaques than in SIVΔNU-infected animals during the acute phase of infection. The SIV-specific T-cell-proliferative response and antibody titers were similar in both groups. Cytotoxic T cells directed against viral antigens were detected in all SIV-IL2-infected macaques and in two of the SIVΔNU-infected animals. Expression of IL-2 did not seem to alter the attenuated phenotype of *nef* deletion mutants fundamentally, although there might have been a slight increase in virus replication and immune stimulation during the acute phase of infection. Deletion of the viral IL-2 gene 3 months postinfection could be a consequence of a selective disadvantage due to local coexpression of viral antigen and IL-2 in the presence of an antiviral immune response.

Cytokines might play an important role in the pathogenesis of AIDS. A dysregulation of cytokine levels can already be observed during the asymptomatic phase of infection. In human immunodeficiency virus type 1 (HIV-1)-infected patients, interleukin-6 (IL-6) and tumor necrosis factor alpha levels are increased (41), while in peripheral blood mononuclear cells (PBMCs) from these patients, IL-2, IL-12, and gamma interferon (IFN-γ) production after stimulation in cell culture is reduced (5). Different cytokines were proposed for the treatment of AIDS, and a number of clinical trials were performed with IL-2 and interferons (4, 26, 27, 35, 42–44, 48, 49, 54). By enhancing immune responses or the regeneration of $CD4^+$ cells, cytokines could be of therapeutic use. On the other hand, cytokines could also increase replication of immunodeficiency virus by a T-cell-stimulatory effect. Activation of T cells is required for efficient viral replication (32, 56). A T-cell-stimulatory signal could therefore increase the target population of the virus. Expression of cytokine genes from recombinant poxviruses revealed that cytokines can modulate the antiviral immune response (29, 38). An IL-2-expressing vaccinia virus was severely attenuated in comparison to the nonproducing parental virus in an immunocompromised host (14, 40). Systemic IL-2 treatment did not prevent disease in this model (39). To study the effect of local cytokine production on simian immunodeficiency virus (SIV) replication, pathogenesis, and immunogenicity, we developed a replication-competent SIV vector that allows expression of heterologous genes. In the present report we show that inserting the IL-2 coding region into this

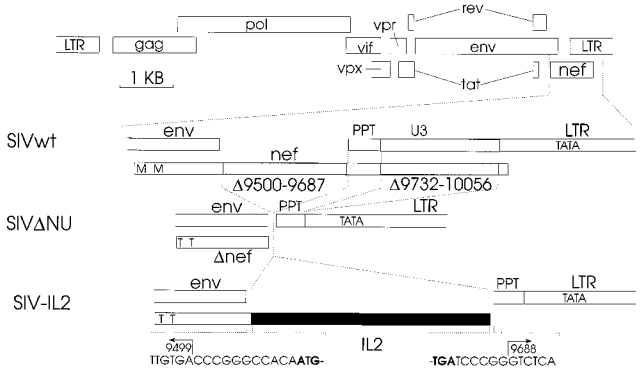

FIG. 1. Structure of SIVmac239 (SIVwt), SIVΔNU, and SIV-IL2. Deleted areas are shaded, and the inserted IL-2 coding region is marked by a black box. The first two methionine residues (M) of Nef were mutated to threonine (T). The nucleotide sequence of the IL-2 flanking region is given below the drawing of SIV-IL2. Base numbering is according to SIVmac239 GenBank entry M33262. PPT, polypurine tract.

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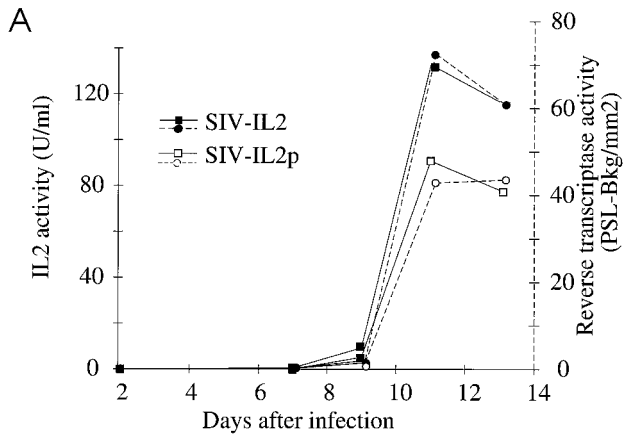


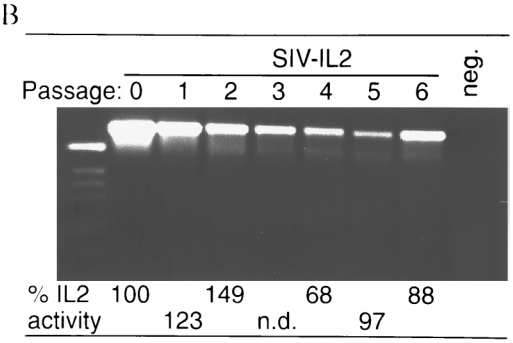
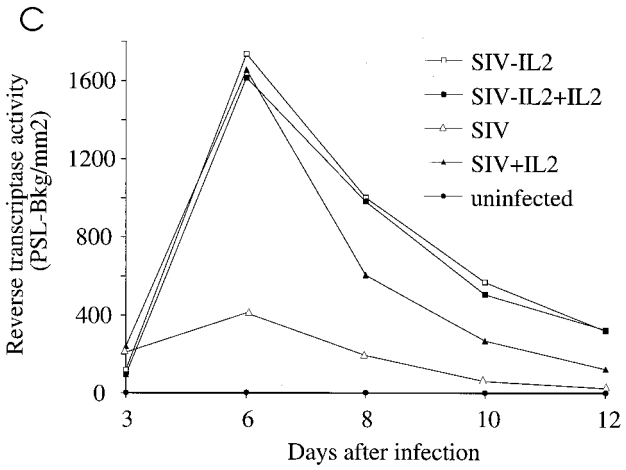


FIG. 2. Replication and stability of SIV-IL2. (A) CEMx174 cells were infected with unpassaged SIV-IL2 and SIV-IL2 passaged six times (SIV-IL2p). PSL-Bkg, photostimulated luminescence minus background. (B) The presence of the IL-2 coding region in the viral genome after each passage was analyzed by PCR using primers flanking the inserted IL-2 cDNA. Below each lane, IL-2 activity in the supernatant of CEMx174 cells infected with passaged SIV-IL2 is given as the percentage of IL-2 activity of the unpassaged SIV-IL2 stock after normalization of the supernatants for reverse transcriptase activity. n.d., not done. neg., DNA from uninfected CEMx174 cells. (C) Rhesus monkey PBMCs were stimulated with concanavalin A for 3 days and infected with SIV-IL2 or SIVmac239 (SIV) in the presence or absence of 100 U of recombinant IL-2.



SIV vector allows stable, high-level expression of IL-2 in cell culture and during the acute phase of infection in macaques. IL-2 expression seemed to confer a slight growth advantage to SIV during the acute phase of infection. Once an effective antiviral immune response has been raised, IL-2 expression might favor destruction of infected cells, leading to selection of viruses with deletions in the IL-2 coding region.

MATERIALS AND METHODS

Construction of SIV-IL2. With the primers Δ*nef*/U3 (5′-TT*GAGCTC*ACTCT CTTGTGA*CCCGGG*TCTCATTTTATAAAAGAAAAGGGGGGACTGGAA GGGATTTATAAGAGGTTAGAAGAAGGCT-3′) and S10535α (5′-G*GAAT TC*TGCTAGGGATTTCCTGCT-3′; relevant restriction sites are in italics), a PCR fragment was generated from SIVmac239 proviral DNA that spanned sequences from the 3′ end of *env* to the 3′ end of the 3′ long terminal repeat (LTR). The primers were designed so that the PCR fragment contained deletions in *nef* from 9500 to 9688 and in U3 from 9732 to 10056 (all numbering according to GenBank entry M33262). The *Sac*I- and *Eco*RI-digested PCR fragment was inserted in the *Sac*I (9486) and *Eco*RI (introduced at the 3′ end of the genome) sites of plasmid pBRmac239 (kindly provided by T. Kodama), which contained the full-length SIVmac239 genome. To inactivate the two methionine residues of *nef* located upstream of the deleted region, a *Bsu*36I (9305)-*Bgl*II (9374) fragment was replaced by annealed synthetic oligonucleotides S9305Ms (5′-TCAGGACTGAACTGACCTACCTACAATA**C**GGGTGGAGCTATTTC CA**C**GAGGCGGTCCAGGCCGTCTGGA-3′; mutated nucleotides are in bold type) and S9374Mα (5′-GATCTCCAGACGGCCTGGACCGCCTC**G**TGGAA ATAGCTCCACCC**G**TATTGTAGGTAGGTCAGTTCAGTCC-3′), which contained the ATG-to-ACG mutation at amino acids 1 and 7 of Nef. The amino acid sequence of the overlapping *env* reading frame remained unchanged. This construct, designated pBRmacΔNU, contained a unique *Xma*I site 3′ to the stop codon of *env*. The IL-2 coding region was PCR amplified from the cDNA of phytohemagglutinin-stimulated human PBMCs with the primers IL2s (5′-CCG GGCCACAATGTACAGGATGCA-3′) and IL2α (5′-CCGGGATCAAGTCA GTGTTGAGATGA-3′) and inserted in the *Xma*I site after partial exonuclease III digestion of the PCR product (22), resulting in pBRmac-IL2. All nucleotide sequences derived from PCR or synthetic oligonucleotides were confirmed by sequence analysis as described previously (20).

Virus cultures. To generate biologically active SIV, pBRmac239, pBRmacΔ NU, or pBRmac-IL2 plasmid DNA was transfected into CEMx174 cells by the DEAE-dextran method. Reverse transcriptase activity of the supernatant of SIVmac239-, SIVΔNU-, and SIV-IL2-infected cells was determined as described previously (37). As a readout for reverse transcriptase activity, photostimulated luminescence was quantitated in a phosphorimager. SIV-IL2 was passaged six times by infecting 5×10^5 CEMx174 cells with 500 μl of filtered supernatant of SIV-IL2-infected cultures. Viral supernatants were harvested every 5 to 8 days as soon as peak syncytium formation was observed. For the in vivo experiments, viral stocks were prepared on rhesus monkey PBMCs (51). The 50% tissue culture infectious dose ($TCID_{50}$) of the PBMC-grown virus stocks was determined on CEMx174 cells as described previously (21). The minimal number of PBMCs of infected macaques required for virus isolation in cocultures with CEMx174 cells was determined as a measure of cell-associated viral load (20).

Infection of rhesus monkeys. Animals were housed at the German Primate Center in Göttingen, Germany. Handling of the monkeys and collection of specimens were performed according to institutional guidelines as described previously (46). Four rhesus monkeys of Indian origin (seronegative for SIV, D-type retroviruses, and simian T-cell leukemia virus type 1) were infected intravenously with 300 $TCID_{50}$ of SIVΔNU, and four animals received 300 $TCID_{50}$ of SIV-IL2.

PCR. To analyze the stability of SIV-IL2, CEMx174 cells infected with different SIV variants were lysed in buffer K (50 mM KCl, 15 mM Tris, 2.5 mM $MgCl_2$, 0.5% Tween 20, 100 μg of proteinase K per ml). By using the primers SL16 and SL17, which flank the inserted IL-2 cDNA and the U3 deletion, fragments were amplified from the lysates under conditions previously described (28).

Immunological methods. Capsid antigen levels in the plasma of infected macaques were determined with an HIV-1 and HIV-2 antigen test (Innogenetics, Zwijnaarde, Belgium) as described by the manufacturer. IL-2 levels in the plasma of infected macaques were determined using a commercially available Quantikine human IL-2 kit from R&D Systems Europe (Abbington, United Kingdom). IL-2 bioactivity was measured in CTLL-2 cells essentially as described previously (18). One unit of IL-2 was defined as the amount that gave the same [^{3}H]thymidine incorporation as one unit of a recombinant IL-2 standard (specific activity, 2,000 U/μg) (catalog no. 1011456; Boehringer Mannheim, Mannheim, Germany). PBMCs were phenotypically characterized by three-color fluorescence analysis on a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany). $CD4^+$ (OKT4; Ortho, Neckargemuend, Germany) T cells were further differentiated into "memory" and "naive" T-helper cells according to high ($CD29^+$) or low levels of expression of CD29 (4B4; Coulter, Krefeld, Germany), respectively. The humoral immune response of infected monkeys against viral antigens was determined by enzyme-linked immunosorbent assay using pelleted whole SIVmac251 as an antigen as described previously (47). For the T-cell-proliferation assay, PBMCs were stimulated with 0.25 μg of purified heat-inactivated SIVmac251 for 7 days, and [^{3}H]thymidine incorporation was measured after a 6-h incubation period (20). The stimulation index was calculated from the mean counts of triplicate wells by dividing the mean counts of antigen-containing

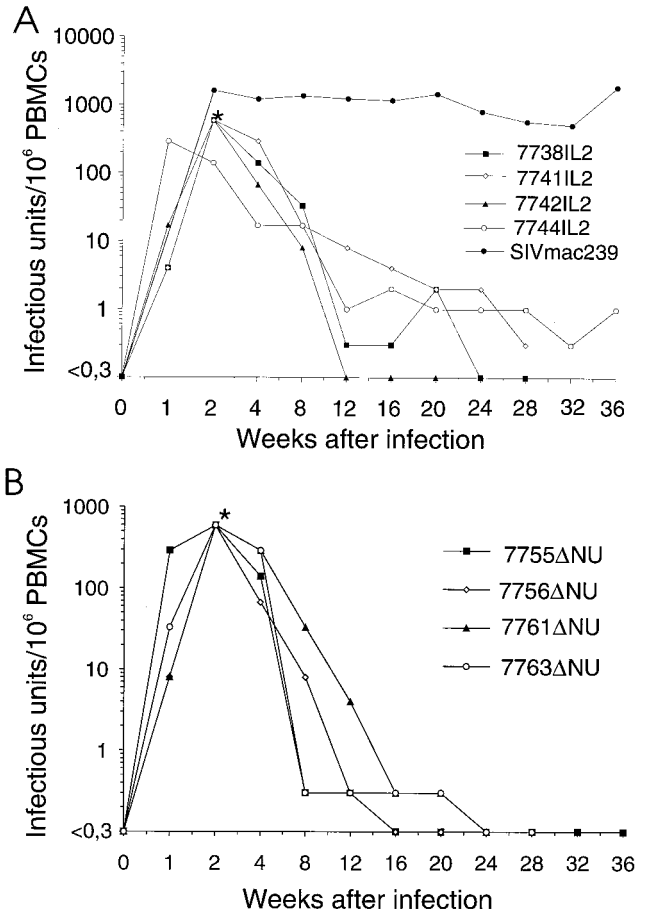


FIG. 3. Cell-associated viral load in macaques infected with SIV-IL2 (A) and SIVΔNU (B). The minimal number of PBMCs required for virus reisolation was determined in cocultures with CEMx174 cells. The four-digit numbers are monkey designations and are followed by the stock used for infection. The mean cell-associated viral load of seven SIVmac239-infected macaques is also shown in panel A. Asterisks indicate levels of ≥500 infectious units/10^6 PBMCs; due to the unexpectedly high cell-associated viral load, the endpoint of the limiting dilution coculture had not been reached at this time.

cultures by the mean counts of cultures without antigen. Stimulation indices over 2 were considered positive. The cytotoxic T-cell response was determined as described previously (10). Briefly, PBMCs were stimulated with concanavalin A and IL-2 prior to incubation with autologous B-lymphoblastoid cells as target cells. The target cells were infected with wild-type vaccinia virus strain Copenhagen or recombinant vaccinia viruses expressing *gag-pol* or *env* of SIVmac C8 virus. Lysis was measured with the CytoTox96TM assay (Promega, Madison, Wis.). Urinary neopterin levels were determined and normalized for urinary creatinine concentration as previously described (13, 28).

RESULTS

Construction and in vitro characterization of a SIV vector expressing IL-2. To analyze the effect of IL-2 on SIV replication, pathogenesis, and immunogenicity, we replaced the *nef* gene of SIVmac239 by the IL-2 coding region. To avoid an increase in the overall length of the viral genome, 513 bp were first deleted from the *nef* gene and the U3 region of SIVmac239 (Fig. 1). The deletions introduced mimic deletions which spontaneously arose in macaques infected with a 181-bp *nef* deletion mutant of SIV (24). The polypurine tract and the 5′ end of the LTR were left intact. The start codon of *nef* and a second methionine residue located upstream of the deleted region were mutated without changing the overlapping *env* reading frame, resulting in SIVΔNU. The IL-2 coding region was inserted downstream of the *env* gene of SIVΔNU. IL-2 should be expressed from mRNA species which initiate in the 5′ LTR and which use the *nef* splice sites. After transfection of proviral SIV-IL2 DNA into susceptible CEMxl74 cells, replication-competent SIV-IL2 was recovered. IL-2 activities of more than 100 U/ml were detected in the supernatant of SIV-IL2-infected CEMx174 cells (Fig. 2A). To analyze whether IL-2 is stably expressed, SIV-IL2 was passaged six times by infecting fresh cells with the supernatant of infected cultures. PCR analysis of the passaged virus did not give any evidence for truncation of the inserted IL-2 coding region (Fig. 2B). IL-2 production of the passaged viruses was not significantly reduced in comparison to the unpassaged virus (Fig. 2). To analyze whether biologically significant amounts of IL-2 were produced, PBMCs were stimulated with concanavalin A and were then infected with SIV-IL2 or SIVmac239 in either the presence or absence of exogenous IL-2. In comparison to SIVmac239, replication of SIV-IL2 was more efficient in the absence of exogenous IL-2 (Fig. 2C). Replication of SIV-IL2 was not enhanced by exogenous IL-2, indicating that saturating amounts of IL-2 were produced by SIV-IL2.

Infection of macaques with SIV-IL2. To analyze whether the growth advantage of SIV-IL2 in PBMCs in the absence of exogenously added IL-2 would be relevant in vivo, four juvenile rhesus monkeys were intravenously infected with 300 $TCID_{50}$ of SIV-IL2. As a control, four monkeys were infected with the same dose of SIVΔNU. Cell-associated viral loads were similar in both groups and reached peak values of more than 500 infected cells/10^6 PBMCs 2 weeks postinfection (Fig. 3). At 12 to 16 weeks postinfection, the cell-associated viral load declined to barely detectable levels. The mean of the cell-associated viral load in seven macaques previously infected with SIVmac239 was higher than that in the SIV-IL2-infected macaques during the acute phase of the infection and remained high throughout the course of infection (Fig. 3A). Two weeks postinfection, we were able to detect viral capsid antigen in the plasma of all SIV-IL2-infected macaques and in three of four SIVΔNU-infected macaques (Table 1). The mean capsid antigen levels were threefold higher in the SIV-IL2 group than

TABLE 1. Capsid antigen levels in SIV-IL2- and SIVΔNU-infected macaques

Stock and animal	Capsid antigen level (pg/ml) in plasma at week postinfection				
	0	1	2	4	8
SIV-IL2					
7738	–[a]	–	398	–	–
7741	–	–	108	–	–
7742	–	–	106	–	–
7744	–	–	311	–	–
Mean			231		
SIVΔNU					
7755	–	–	58	–	–
7756	–	–	–	–	–
7761	–	–	86	–	–
7763	–	–	118	–	–
Mean	–	–	73	–	–

[a] –, <30 pg/ml. For values below the detection limit, 30 pg/ml was used for statistical analyses with SPSS software.

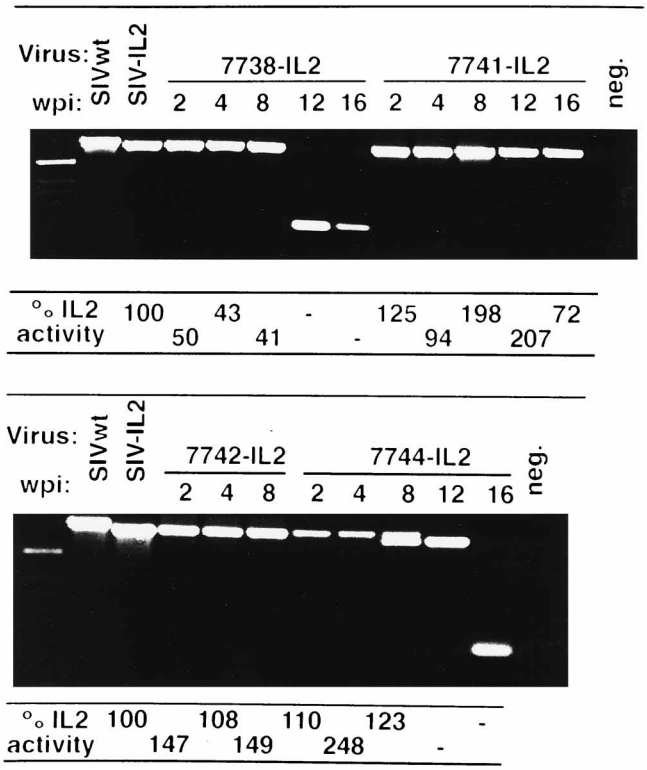


FIG. 4. Stability of SIV-IL2 in rhesus monkeys. The presence of the IL-2 coding region in isolates recovered at different time points after infection from SIV-IL2-infected macaques 7738-IL2, 7741-IL2, 7742-IL2, and 7744-IL2 was analyzed by PCR with primers flanking the inserted IL-2 cDNA. Below each lane, IL-2 activity in the supernatant of CEMx174 cells infected with the reisolates is given as the percentage of IL-2 activity of the SIV-IL2 stock after normalization of the supernatants for reverse transcriptase activity. wpi, weeks postinfection; SIVwt, SIVmac239; neg., DNA from uninfected CEMx174 cells, −, no IL-2-activity detected.

in the SIVΔNU group. However, this difference was not statistically significant due to variations within the small groups ($P = 0.083$; two-tailed Mann-Whitney test). In SIVmac239-infected macaques, antigen levels were higher 2 weeks postinfection and ranged from 2.9 to 11.7 ng/ml (unpublished observations). The SIV-IL2-infected macaque with the highest capsid antigen level had the only detectable amounts of IL-2, 44 pg/ml of plasma, 2 weeks postinfection, indicating expression of IL-2 in vivo. All other animals had plasma IL-2 levels below 30 pg/ml at 0, 1, 2, or 4 weeks postinfection. To analyze the stability of SIV-IL2, viruses were reisolated from the SIV-IL2-infected macaques at different time points after infection. The IL-2 coding region was amplified by PCR using SIV primers flanking the IL-2 insertion side. Until 8 weeks postinfection, a band of the expected size was obtained in all animals and reisolated viruses still expressed biologically active IL-2 (Fig. 4). The virus reisolated from one animal (7741-IL2) produced IL-2 until at least 16 weeks postinfection. In two other macaques, additional smaller bands appeared, probably representing deletions in the IL-2 gene. In parallel with the detection of deletions in the PCR analysis, IL-2 production by the reisolated viruses was not detectable anymore. Therefore, IL-2 expression is stable during the acute phase of infection but can be lost at later times. Although the viral load determination did not give any evidence of pathogenicity of the SIV-IL2 virus, the percentage of $CD29^+$ $CD4^+$ cells was determined, since a drop in this population is an early prognostic marker for a beginning immunodeficiency in humans (3, 11) and macaques (25, 34). However, the $CD29^+$ $CD4^+$-cell levels remained stable in both groups throughout the observation period (Fig. 5).

Follow-up of immunological parameters. Local coexpression of viral antigen and IL-2 may stimulate antiviral immune responses. Therefore, the effect of IL-2 expression on humoral and cellular immune responses was analyzed. Seroconversion was observed in both groups 4 to 8 weeks postinfection. Antibody titers were similar in both groups (Fig. 6). These antibody titers were up to 100-fold higher than the titers in SIVmac239-infected macaques that progressed to disease rapidly, but they were in a range similar to the antibody titers in slowly progressing SIVmac-infected macaques (reference 20 and unpublished observations). During infection with pathogenic immunodeficiency viruses, a gradual loss of T-cell-proliferative responses can be observed (6, 7, 19, 30, 31, 33). Macaques infected with pathogenic SIV strains fail to raise a SIV antigen-specific T-cell-proliferative response and loose proliferation in response to recall antigens (9, 10, 52). After infection with SIV-IL2 or SIVΔNU, SIV antigen-specific T-cell proliferation was detected repeatedly in all macaques throughout the obser-

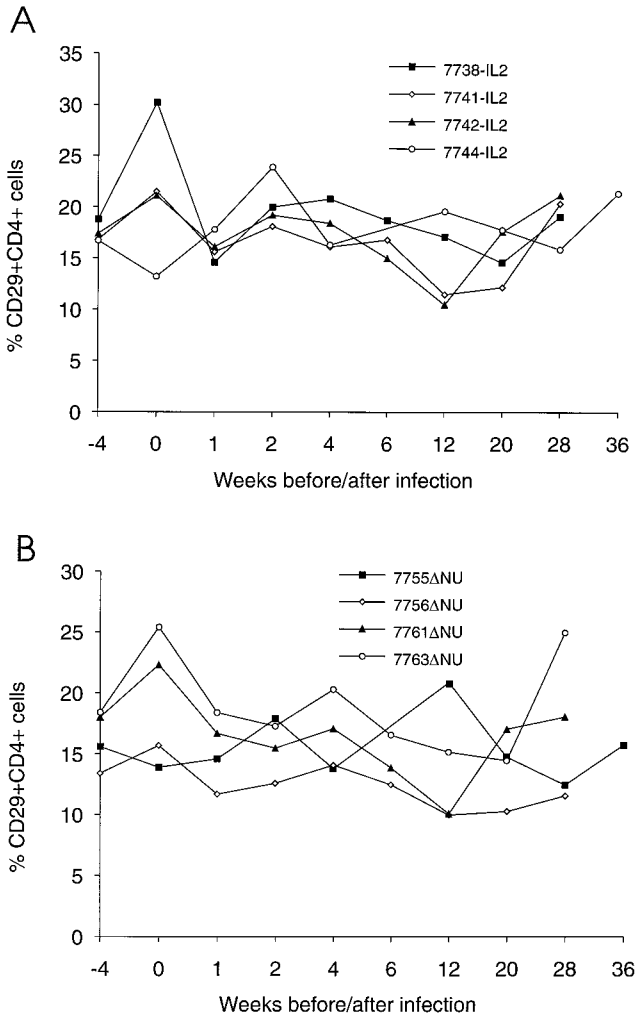


FIG. 5. Percentage of $CD29^+$ $CD4^+$ cells in the peripheral blood lymphocytes of macaques infected with SIV-IL2 (A) and SIVΔNU (B). Numbers of $CD29^+$ $CD4^+$ cells are expressed as a percentage of total lymphocytes. The four-digit numbers are monkey designations and are followed by the stock used for infection.

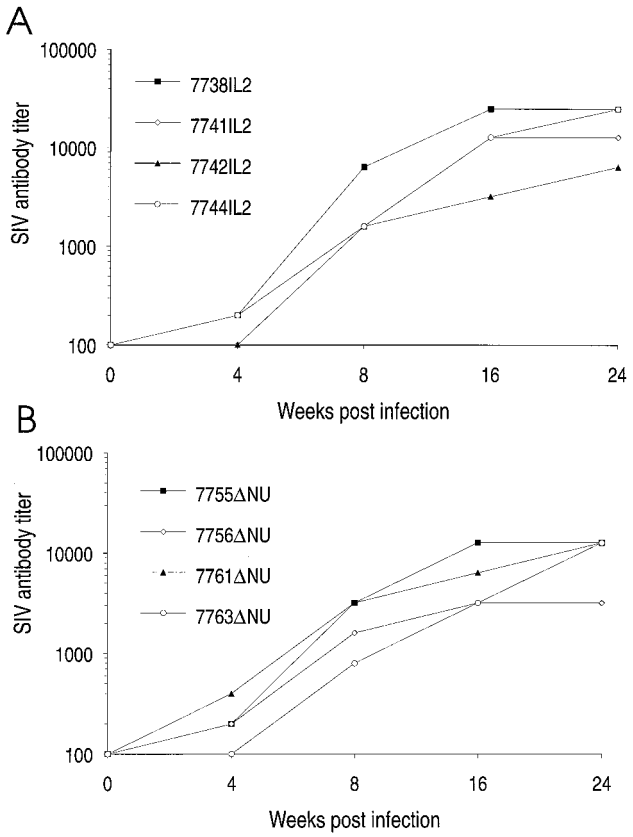


FIG. 6. SIV antibody titers in macaques infected with SIV-IL2 (A) and SIVΔNU (B). The reciprocals of the highest dilutions giving absorption values which were double the background value at different times after infection are shown. The four-digit numbers are monkey designations and are followed by the stock used for infection.

vation period (Fig. 7). Proliferation in response to phytohemagglutinin or a recall antigen (keyhole limpet hemocyanin) was not impaired in either group (data not shown). Therefore, SIV-IL2-infected macaques show a pattern of T-cell-proliferative responses, which is typical for macaques infected with *nef* deletion mutants of SIV. SIV-specific cytotoxic T cells were detected in all SIV-IL2-infected macaques and in two of three SIVΔNU-infected macaques (Table 2). In addition to the SIV-specific immune response, neopterin levels were determined as a marker for unspecific immune stimulation. Neopterin is released from activated T cells and macrophages. During the acute phase of infection with immunodeficiency viruses and progression to disease, neopterin levels are elevated (13, 15, 16, 20, 28). After infection with both viruses, SIV-IL2 and SIVΔNU, an increase in urinary neopterin levels was observed. However, peak neopterin levels were significantly higher in the SIV-IL2-infected macaques 2 weeks postinfection ($P = 0.02$; two-tailed Mann-Whitney test) (Fig. 8). One to two months postinfection, neopterin levels were back to baseline values in both groups.

DISCUSSION

This report describes the construction and properties of a replication-competent SIV vector that stably expresses high levels of IL-2 in cell culture and in macaques during the acute phase of infection. The SIV vector contains deletions in *nef* and in the U3 region that total 513 bp. Since similar-sized deletions spontaneously arose in macaques infected with a 181-bp *nef* deletion mutant of SIV (24), these deletions should not impair replication of the virus more than a small *nef* deletion does. However, the large deletions allowed insertion of the IL-2 coding region without increasing the overall length of the viral genome. Giavedoni and Yilma observed rapid deletion of IFN-γ cDNA inserted in a SIV that contained a 186-bp deletion of *nef*, already in cell culture (17). Introducing an additional promoter 5′ to the IFN-γ cDNA further reduced stability. The larger deletion introduced in the SIV vector described in this report might therefore contribute to increased stability. Alternatively, IFN-γ expression might inhibit virus replication, or IL-2 expression could confer a selective advantage. In CEMx174 cells no difference was observed in the replication kinetics of SIVmac239 and SIV-IL2 (data not shown), suggesting that selection is not the reason for the stability of SIV-IL2 in this cell line.

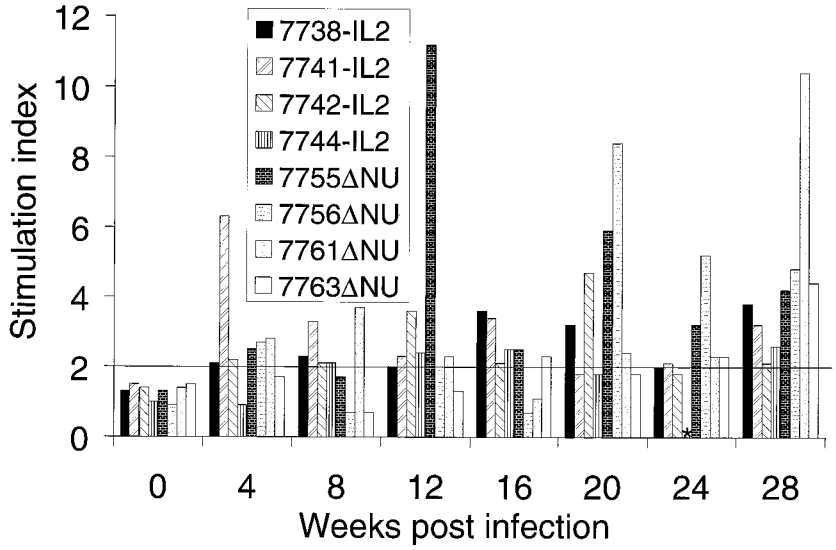


FIG. 7. SIV antigen-specific T-cell proliferation. [^{3}H]thymidine incorporation was determined in PBMC cultures in the presence or absence of whole inactivated SIV. Stimulation indices over 2 were considered positive. The four-digit numbers are monkey designations and are followed by the stock used for infection. *, not done.

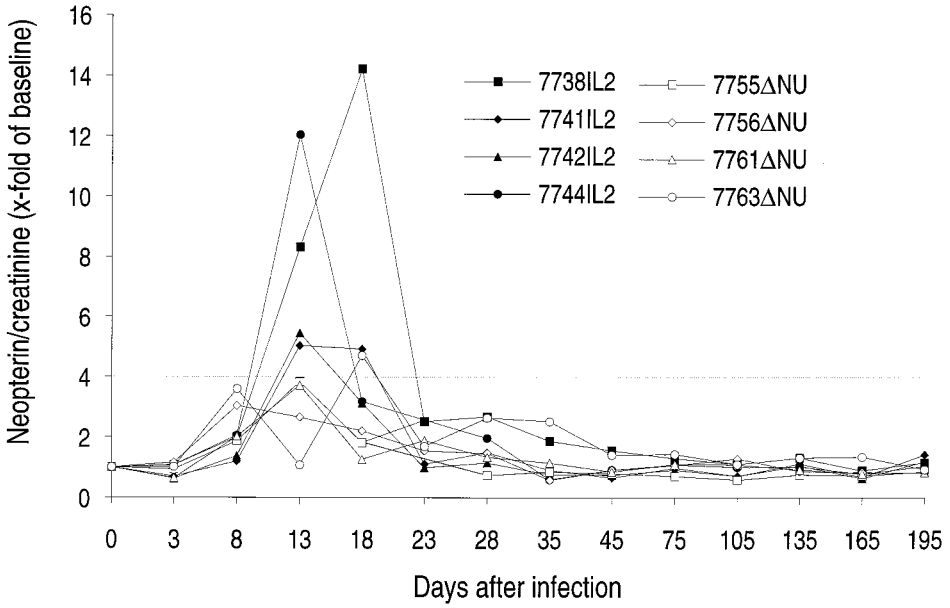


FIG. 8. Urinary neopterin levels in SIV-IL2- and SIVΔNU-infected macaques. The neopterin/creatinine ratio is expressed for each animal as the fold increase over the mean of six to eight independent neopterin/creatinine ratios determined before infection. For the first 30 days, the means of 2 or 3 values for 5-day intervals are shown; for days 31 to 40, the means of at least 3 values for a 10-day interval are shown; for days 41 to 60, mean values for a 20-day interval are shown; and for days 61 to 210, mean values for a 30-day interval are shown. The four-digit numbers are monkey designations and are followed by the stock used for infection.

In PBMCs, however, replication of SIV-IL2 was more efficient than replication of SIVmac239 in the absence of exogenous IL-2. This growth advantage of SIV-IL2 was not observed in vivo, since the viral load in SIV-IL2-infected macaques is greatly reduced in comparison to the viral load in macaques infected with SIVmac239. The unimpaired T-cell-proliferative response and the stable percentage of $CD29^+$ $CD4^+$ cells in SIV-IL2-infected macaques also strongly suggest that SIV-IL2 is attenuated. Since SIVΔNU-infected macaques show a similar course of infection, the low viral load seems to be due not to IL-2 expression but to deletions in the *nef* gene. Infection of juvenile and adult macaques with *nef* deletion mutants of SIV results in an asymptomatic persistent infection (23). In comparison to SIVΔNU, SIV-IL2 might have a slight growth advantage during the acute phase of infection as indicated by higher capsid antigen levels in the plasma 2 weeks postinfection. This is in agreement with the observation that systemic IL-2 treatment of HIV-1-infected individuals also increased viral load (26).

IL-2 expression from recombinant vaccinia viruses led to attenuation of the virus even in an immunocompromised host (14, 40). In contrast to SIV, vaccinia virus replication itself is probably not influenced by IL-2. Therefore, the immunostimulating capacity of IL-2 seems to prevail, leading to the control of vaccinia virus infection by the host. In the SIV model, we could not find a clear enhancement of SIV-specific antibody titers, T-cell proliferation, or cytotoxic T cells by local coexpression of SIV antigen and IL-2. However, more subtle changes could have escaped detection. The significantly higher neopterin levels in the SIV-IL2-infected macaques during the second week of infection indicate that IL-2 expression resulted in a more general immune stimulation. As a consequence, activation of T cells might favor viral replication, leading to the increased capsid antigen levels observed in SIV-IL2-infected macaques 2 weeks postinfection. However, an effective antiviral immune response at later times might result in rapid destruction of SIV-IL2-infected cells. IL-2 expression by SIV-IL2

TABLE 2. Cytotoxic T-lymphocyte response in SIV-IL2- or SIVΔNU-infected macaques

Stock and animal	VV[a]	% Specific lysis at week postinfection				
		0	6	10	14	18
SIV-IL2						
7738	WT	0	35		2	
	GP	0	**51**[b]	ND[c]	9	ND
	ENV	7	**47**		0	
7741	WT	5	3	5	10	7
	GP	0	**28**	0	7	5
	ENV	2	10	8	13	4
7742	WT		5			
	GP	ND	3	ND	ND	ND
	ENV		**21**			
7744	WT	1	20	5	2	15
	GP	1	20	6	4	14
	ENV	0	5	1	**13**	12
SIVΔNU						
7755	WT	22	4	1	0	0
	GP	20	1	0	8	4
	ENV	28	6	0	9	0
7756	WT	0	0	0	0	
	GP	4	0	0	0	ND
	ENV	8	**10**	0	**16**	
7761	WT	5	14		16	5
	GP	3	**27**	ND	16	13
	ENV	9	**35**		13	**19**

[a] VV, vaccinia virus. Autologous target cells were infected with vaccinia viruses expressing *gag-pol* (GP) or *env* (ENV) or with wild-type vaccinia virus (WT) and incubated with an effector-to-target-cell ratio of 5:1.

[b] Boldface type indicates lysis which was 10% above the unspecific lysis of wild-type-infected target cells; this degree of lysis was regarded as SIV specific (53).

[c] ND, not done.

might then reduce virus spread, as suggested by the outgrowth of SIV-IL2 variants containing deletions in the viral IL-2 gene 3 to 4 months postinfection. However, the possibility that SIV-IL2 deletion mutants have a growth advantage due to the reduced genome size cannot be excluded, although additional deletions in viruses from macaques infected with a *nef* deletion mutant of SIV generally did not dominate the virus population during the first 17 months postinfection (24).

Macaques previously infected with *nef* deletion mutants of SIV are protected from high-dose challenges of cell-free and cell-associated pathogenic virus strains (1, 8, 36, 55). The mechanisms of the protection induced by live attenuated SIV vaccines are not known. It will be interesting to analyze the effect of local coexpression of viral antigen and IL-2 on protective immunity.

One of the functions proposed for Nef is to cause lymphocyte activation (2, 12, 45, 50). The results presented here show that the T-cell growth factor IL-2 cannot functionally replace Nef. While IL-2 exerts its growth-stimulatory effect on previously activated T cells, Nef might affect T-cell activation at an earlier step. Since SIV-IL2 seems to have a slight growth advantage in comparison to SIVΔNU during the first 2 weeks postinfection but not at later times, as indicated by deletion of the viral IL-2 gene, IL-2 seems to be a double-edged sword for the treatment of immunodeficiency virus infection. Its therapeutic benefit might depend on the immune status of the infected individual.

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