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Comparative interleukin (IL)-2/interferon (IFN)-γ and IL-4/IL-10 responses during acute infection of macaques inoculated with attenuated *nef*-truncated or pathogenic SIVmac251 virus

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ABSTRACT **Comparison of immune responses to infection by a pathogenic or a nonpathogenic immunodeficiency virus in macaques may provide insights into pathogenetic events leading to simian AIDS. This work is aimed at exploring cytokine expression during infection by simian immunodeficiency virus (SIV). We used semiquantitative reverse transcription–PCR to monitor interleukin (IL)-2/interferon (IFN)-γ (Th1-like), and IL-4/IL-10 (Th2-like) expression in unmanipulated peripheral blood mononuclear cells (PBMCs), during the acute phase of infection of eight cynomolgus macaques (*Macaca fascicularis*) with a pathogenic primary isolate of SIVmac251 (full-length *nef*), and of four other cynomolgus macaques by an attenuated molecular clone of SIVmac251 (*nef*-truncated). All the monkeys became infected, as clearly shown by the presence of infected PBMCs and by seroconversion. Nevertheless, PBMC-associated virus loads and p27 antigenemia in monkeys infected by the attenuated virus clone remained lower than those observed in animals infected with the pathogenic SIVmac251 isolate. A rise of IL-10 mRNA expression occurred in both groups of monkeys coincident with the peak of viral replication. In monkeys infected with the pathogenic SIVmac251, IL-2, IL-4, and IFN-γ mRNAs were either weakly detectable or undetectable. On the contrary, animals infected by the attenuated virus exhibited an overexpression of these cytokine mRNAs during the first weeks after inoculation. The lack of expression of these cytokines in monkeys infected with the pathogenic primary isolate may reflect early immunodeficiency.**

The role of cytokine network dysregulation in the pathogenesis of infectious diseases has been reported recently in a variety of experimental studies. A growing body of literature documents associations between T helper cell 1 or 2 (Th1 or Th2) cytokine patterns (1) and the quality of the immune response, not only in experimental animal models (1) but also in humans (2). Evidence that cytokine production undergoes changes during human immunodeficiency virus (HIV) disease progression has been documented (3–7). Nevertheless, the pattern of Th1/Th2 cytokines and its role and/or association with HIV-1-induced disease progression remains controversial (6, 8–11).

Of particular interest are the early events following exposure to the virus for two main reasons: (*i*) This period coincides with the initiation of the immune responses (humoral and/or cellular). Although immune responses seem at first to efficiently control the virus load, they fail subsequently to prevent the establishment of a chronic infection and disease progression. (*ii*) The expression of cytokines during this short period of time may be directly under the influence of the virus replication rather than other physiopathogenic events such as infection by opportunistic pathogens. Since studies performed with samples from HIV-1-infected humans depend on uncontrollable variables (e.g., the availability of samples) that limit objective prospective analysis of the role of the cytokines, animal models are helpful.

Experimental infection of macaque monkeys with the attenuated simian immunodeficiency virus SIVmac32H pC8 clone, which has a 12-bp deletion in the *nef* open reading frame, leads to persistent infection associated with a low virus burden (12, 13). Furthermore, prior infection with this attenuated virus clone protects macaques against superinfection with the pathogenic J5 clone (13). Indeed, protection of rhesus macaques against SIVmac251, a strongly pathogenic isolate, was achieved by Desrosiers and coworkers (14) by using prior infection with an attenuated SIV partly deleted in the *nef* gene. Despite not-inconsiderable safety concerns, this strategy has been considered for the development of a human AIDS vaccine (15). Whether or not this goal can be achieved directly, the SIV model offers the opportunity to determine the basis of the apathogenic infection and to explore the mechanisms that induce the state of superinfection resistance, information that may aid greatly the development of a vaccine. The study reported here focuses on the immune response in cynomolgus macaques infected with the attenuated SIVmac32H(pC8) or the pathogenic primary isolate of SIVmac251, in terms of T-cell cytokine responses. To this end, we have quantified the expression of interleukin (IL)-2/interferon (IFN)-γ (Th1-like), and IL-4/IL-10 (Th2-like) mRNAs in unmanipulated (*ex vivo*) peripheral blood mononuclear cells (PBMCs) by using a semiquantitative reverse transcriptase (RT)-PCR procedure.

MATERIALS AND METHODS

Animals. Twelve cynomolgus monkeys (*Macaca fascicularis*) from Mauritius, weighing 2.8–7 kg, were housed in single cages in biosafety level 3 facilities. Animals were examined clinically while they were under ketamine hydrochloride anesthesia (Mérieux, France). Blood samples were obtained weekly (always in the morning before food distribution) for hematological parameter determination and for virological and immunological assays. All experimental procedures were performed according to European guidelines for animal care (*Journal Officiel des Communautés Européennes*, L358, December 18, 1986).

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Abbreviations: Th1 and Th2, T helper cell 1 and 2; HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; IL, interleukin; IFN, interferon; PBMC, peripheral blood mononuclear cell; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; p.i., post inoculation.

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Viruses. The pathogenic SIVmac251 viral stock utilized for infecting the macaques had been prepared by A. M. Aubertin (Laboratoire de Virologie, Université Louis Pasteur, Strasbourg, France) as a cell-free supernatant of infected rhesus PBMCs. This isolate was originally obtained by coculture of spleen cells from an SIVmac251-infected rhesus macaque with PBMCs from a noninfected rhesus monkey, and it has never been passaged in cells of human origin (16, 17). A first group of eight monkeys were given four 50% animal infectious dose (AID_{50}) units by injection in the right saphenous vein.

SIVmac32H was reisolated from rhesus macaque 32H, which was inoculated with a SIVmac251 virus pool (18). The sequence analysis of the SIVmac32H clones has been described (19) and, as expected, showed 98% nucleotide sequence identity with SIVmac251. Essentially, the SIVmac32H(pC8) virus clone differs from the clone SIVmac32H(pJ5) by a 12-bp deletion (encoding amino acids 143–146), and two nonsynonymous nucleotide changes resulting in conservative amino acid changes (Thr-149 to Ala and Arg-191 to Lys) (19) in the *nef* open reading frame. A second group of four animals were given 10^4 tissue culture 50% infectious dose ($TCID_{50}$) units of the pC8 clone by injection in the right saphenous vein, as has been described previously (13).

Detection of Circulating p27 Antigenemia. The Gag p27 SIV protein was detected as described previously (17) in undiluted plasma by using the Coulter SIV core antigen assay (Coulter). The cut-off value was 0.05 ng/ml.

Detection of Circulating SIV-Infected Cells. SIV-infected cells were detected as described previously (17). Briefly, 3-fold dilutions of fresh Ficoll-isolated monkey PBMCs were cocultivated with CEMX174 cells. On day 14 of culture, microplates were examined under an inverted microscope for syncytia induction, and the cell suspension was lysed. The lysate was used to coat ELISA microplates. Plates were then incubated with anti-SIV serum, which was detected by using peroxidase-labeled goat IgG directed against monkey IgG (Organon, Teknika, The Netherlands) and *ortho*-phenylenediamine substrate. Plates were analyzed for absorbance at 490 nm (cut-off = average A_{490} from 12 noninfected control wells of the same culture microplate ± SD).

Detection of SIV-Specific Antibodies. Since there is substantial sequence homology between HIV-2 and SIV, specific anti-SIV serum reactivity was determined by using an HIV-2 antigen detection assay (ELAVIA II kit, Diagnostics Pasteur, France), as described previously (16). The anti-human IgG supplied in the kit for use as the secondary reagent was replaced by the goat anti-monkey IgG described above. Tenfold dilutions were assayed, starting from the 1:100 dilution.

T-Lymphocyte Subset Determination. $CD4^+$ and $CD8^+$ PBMC counts were determined by using a direct immunofluorescence assay as described previously (20). Briefly, blood cell suspensions were stained with monoclonal antibodies: leu 3a-PE anti-CD4 or leu 2a-FITC anti-CD8 (Becton Dickinson; PE = phycoerythrin, FITC = fluorescein isothiocyanate). Cell percentages were evaluated using the LYSIS II software on a FACScan (Becton Dickinson).

Detection of Cytokine mRNA by Semiquantitative RT-PCR. The assay was performed using 10^6 freshly isolated monkey PBMCs, as described previously (20, 21). In brief, total cellular RNA was subjected to first-strand cDNA synthesis using 150 units of Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL). The PCR mixture contained each specific primer, buffer as supplied by the manufacturer, and 1.25 units of *Taq* DNA polymerase (Boehringer Mannheim). Primer sequences of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), IL-2, IL-4, IL-10, and IFN-γ were used as published (20–22). The optimal number of PCR cycles was determined initially by using a variable number of cycles to identify a linear range of amplification for each transcript. A portion of the PCR product was labeled with the fluorescent intercalating agent thiazole orange dimer (TOTO-1, Molecular Probes), then subjected to electrophoresis through an acrylamide/bisacrylamide gel on an automated sequencer (373A DNA Sequencer, Applied Biosystems). PCR products were quantitated by measuring the intensity of fluorescence of the specific band on the gel, using GENESCAN software (Applied Biosystems). All cytokine PCR levels were normalized for the amount of mRNA encoding GAPDH, a housekeeping gene, detected in the same sample. The data are given as the cytokine mRNA/GAPDH mRNA ratio.

Baseline Expression of Cytokines Before Virus Inoculation and Statistical Analysis. Four of the 12 monkeys (514, 141, 351, and 947) were bled eight times for cytokine mRNA determinations at days −128, −122, −116, −98, −45, −14, −7, and 0 before virus inoculation. The 8 remaining monkeys were bled three times (days −14, −7, and 0) before infection. The mean (±SD) of the values obtained represents the baseline level for each monkey separately (see Tables 1, 2, 3, and 4).

To compare the values obtained for different monkeys, we expressed each value as a percentage of the mean expression obtained before infection for each monkey separately (referred to as 100%). These values were then pooled as 56 preinfection data points, and the overall distribution was analyzed. When cytokine mRNAs were undetectable, we attributed the value 0.01, which is the detection limit of our method. For each cytokine, we checked the distribution by using percentile analysis. Differences between baseline values and values obtained after virus inoculation were examined with the distribution of the 56 values obtained before the SIV infection and considered significant when the values exceeded the 97th percentile.

Differences between the two groups of monkeys with respect to viremia or level of cytokine expression were characterized by using analysis of variance.

RESULTS

Clinical Follow-up. All eight animals inoculated with the pathogenic SIVmac (full-length nef) developed characteristic clinical manifestations of primary SIV infection: persistent generalized lymphadenopathy starting on day 7 post inoculation (p.i.) and fever approximately 2 weeks p.i. One of the animals (D351) died 4 weeks p.i. from a disease not related to SIV infection.

None of the animals inoculated with the attenuated SIVmac (nef-truncated) developed characteristic clinical manifestations of primary SIV infection.

Hematological Follow-up. White blood cell counts decreased transiently starting on day 14 p.i. and returned to normal within 1 week in animals infected by the pathogenic SIVmac. Animals infected by the attenuated SIVmac exhibited a transient hyperlymphocytosis on day 21 p.i. (1.8-fold on average). There were no major change in other hematological parameters (red blood cell counts, hemoglobin, hematocrit, and platelet counts) in all animals (data not shown).

Changes in T-Lymphocyte Subsets After SIV Infection. The 10th and 90th percentiles were defined by drawing the cell distribution of 157 samples of 55 healthy uninfected monkeys (20). The values obtained were 790 to 2800 $CD4^+$ cells per μl and 1010 to 3060 $CD8^+$ cells per μl.

As shown in Fig. 1, significant decreases in circulating $CD4^+$-lymphocyte counts were detected in four (46713, 46880, 141, and 351) of the eight monkeys infected by the pathogenic SIVmac between day 7 and day 14 p.i. During the same period, a decrease (assessed by the fall of the absolute cell count) in $CD8^+$ cells was also observed in four (46880, 383, 141, and 351) of the eight monkeys. Note that the increasing absolute count of $CD4^+$ or $CD8^+$ cells observed in monkeys 514 and 947 at day 44 p.i. was due to a parallel enhancement of the total lymphocyte count, since the cell percentages remained stable over time. As shown in Fig. 2, a transient decrease in circulating

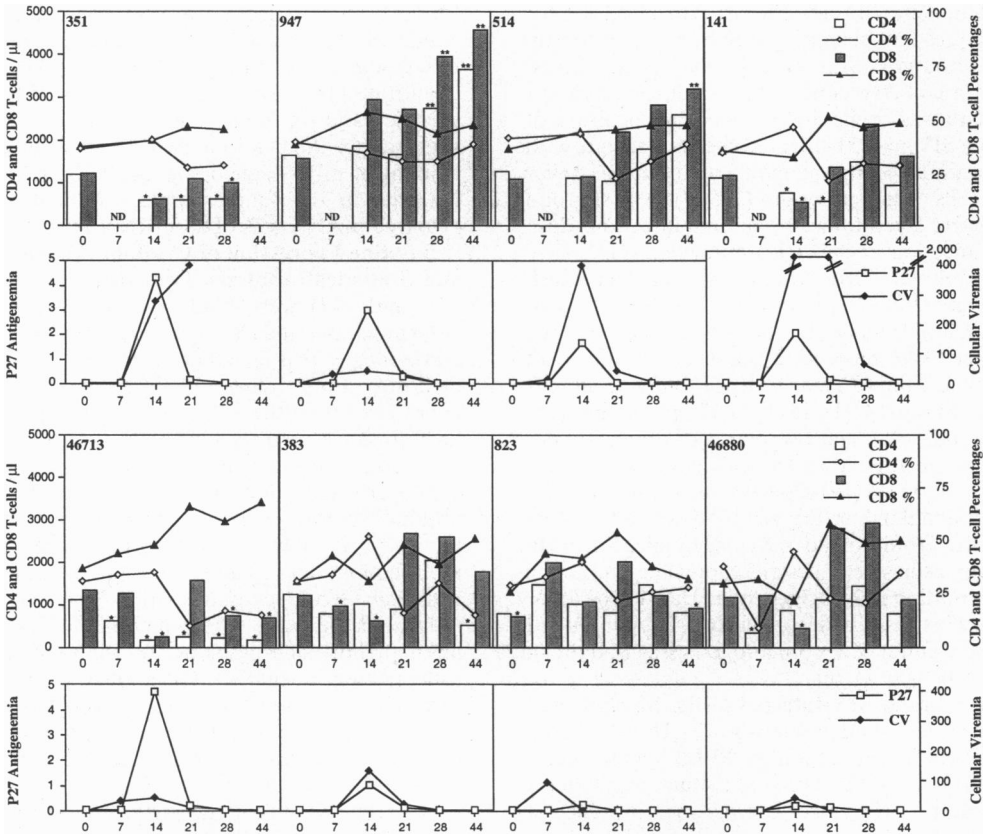

FIG. 1. Evaluation of absolute numbers of circulating $CD4^+$ and $CD8^+$ PBMCs per µl, relative numbers of $CD4^+$ and $CD8^+$ PBMCs (CD4 %, CD8 %), p27 antigenemia (P27, ng/ml), and cellular viremia (CV, $TCID_{50}/10^6$ PBMCs) in blood of eight SIVmac251-infected macaques as a function of time (days p.i.). *, <10th percentile; **, >90th percentile.

$CD4^+$-lymphocyte counts was also detected in two (371 and 392) of the four monkeys infected by the attenuated SIVmac during the course of the experiment, whereas an increase in $CD8^+$ cells (absolute cell count and percentage) was observed in two monkeys (371 and 559) between days 14 and 28 p.i. Furthermore, in all animals infected by the attenuated virus, no significant decrease in the $CD4^+$ blood cell count was evident 12 months p.i. (on average), whereas among the eight SIVmac251-infected monkeys, three displayed significant drops of CD4 blood cell counts and four did not (data not shown). The last one (monkey 351) died 1 month p.i.

Virological Follow-up. All eight animals infected by the pathogenic SIVmac had detectable levels of p27 antigen in their serum at day 14 p.i. (Fig. 1). The values peaked from 4.72 ng/ml for monkey 46713 to 0.27 ng/ml for monkey 823, and became undetectable by day 28 p.i. None of the animals infected by the attenuated SIVmac had detectable levels of p27 antigen in their serum between days 0 and 125 p.i. (Fig. 2). The difference between the two groups is significant ($P = 0.017$) by analysis of variance.

Virus-infected PBMCs from the animals inoculated with the pathogenic SIVmac were detected by coculture as early as day 7, and infected PBMC counts peaked between days 14 and 21 p.i. (Fig. 1). Cellular viremia persisted thereafter at low levels. Infectious PBMCs were also detected by coculture in the animals infected by the attenuated SIVmac as early as day 7, and titers peaked between days 14 and 21 p.i. (Fig. 2). The peak of viremia was, on average, 1/70 of that in the animals infected by the pathogenic SIVmac. Cellular viremia persisted thereafter at low levels until day 44 and became undetectable in 3 of the 4 animals at day 125 (Fig. 2). Nevertheless, the difference between the two groups is not significant by analysis of variance.

Detection of Anti-SIV Serum Antibodies. The animals infected by the pathogenic SIVmac seroconverted during the acute phase of infection, between days 14 and 28 p.i., and their anti-SIV IgG antibody titers ranged from 1:100 to 1:1000 on day 28 p.i.. All the animals infected by the attenuated SIVmac seroconverted sooner after the virus inoculation. Indeed, on day 7 p.i., these monkeys had detectable anti-SIV IgG antibodies (titers ranging from 1:100 to 1:1000). Nevertheless these titers remained stable thereafter, in contrast to SIVmac251-infected monkeys, which raised their antibody titers thereafter (1:10,000 to 1:100,000 3 months p.i.).

Patterns of Cytokine mRNA Expression in PBMCs. The constitutive levels of IL-2 mRNA were at the limit of detection or undetectable (Table 1) before the injection of SIV, except for monkey 823. After the infection by pathogenic SIVmac, IL-2 mRNA remained undetectable in the main in 7 animals, and it became undetectable at day 21 p.i. in monkey 823. In contrast, 1–2 weeks p.i. the expression of IL-2 increased to become detectable in every monkey infected by the attenuated SIVmac. This enhancement of mRNA level persisted for 3 or 4 weeks in monkeys 371 and 559 and became undetectable thereafter, and it appeared to be more lasting for the other two animals. The difference observed between the two groups was significant ($P = 0.008$) by analysis of variance.

Similarly, low to undetectable levels of IFN-γ and IL-4 mRNA levels (Tables 2 and 3) were found to be expressed constitutively by PBMC samples from the uninfected monkeys except animals 823 for both IFN-γ and IL-4 and 46880 and 371 for IFN-γ. After the injection of the pathogenic SIVmac, IFN-γ and IL-4 mRNA remained mostly undetectable. They became undetectable at 21 days p.i. for monkey 823. The inoculation of the attenuated SIVmac induced a marked increase in IFN-γ and IL-4 mRNA levels as soon as 1 week p.i. in three of the four monkeys. The remaining animals (respectively 392 for IFN-γ and 559 for IL-4) did not show a detectable expression of these cytokines after virus inoculation. Never-

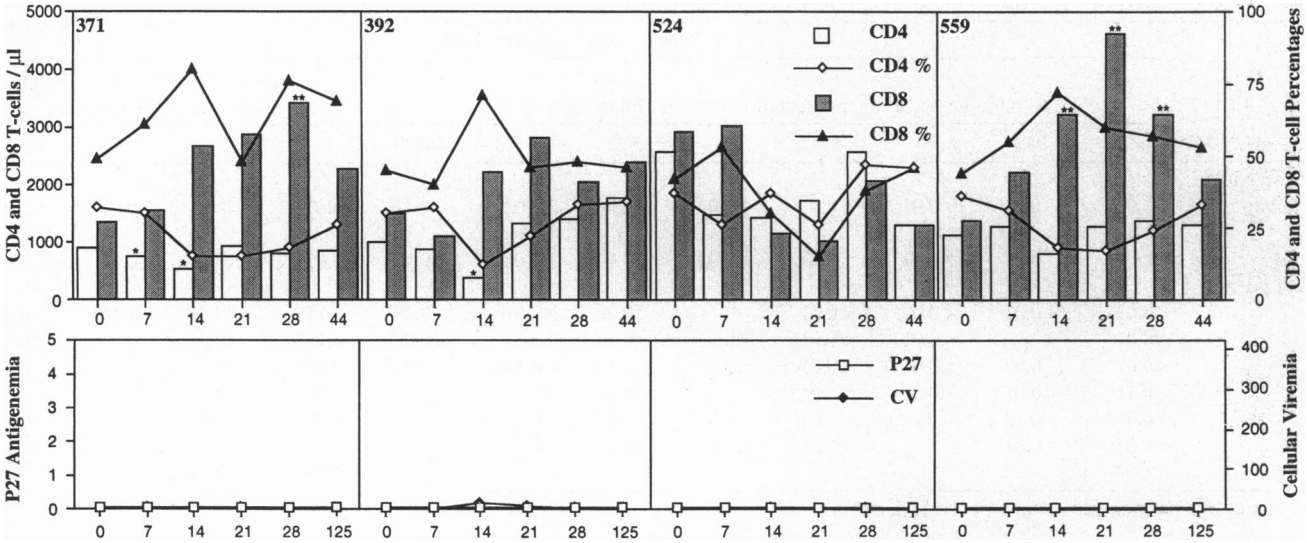

FIG. 2. Evaluation of absolute numbers of circulating $CD4^+$ and $CD8^+$ PBMCs per µl, relative numbers of $CD4^+$ and $CD8^+$ PBMCs (CD4 %, CD8 %), p27 antigenemia (P27, ng/ml), and cellular viremia (CV, $TCID_{50}/10^6$ PBMCs) in blood of four attenuated SIV32H(pC8)-infected macaques as a function of time (days p.i.) *, <10th percentile; **, >90th percentile.

theless, the differences observed between the two groups were significant ($P = 0.028$ for IFN-γ and $P = 0.007$ for IL-4) by analysis of variance.

The levels of IL-10 (Table 4) were significantly increased after the injection of the pathogenic SIVmac for monkeys 141, 947, 351, 514, 383, and 46713 between days 7 and 21 p.i. The remaining two animals (823 and 46880) did not show any increase in the expression of IL-10 mRNA after SIV inoculation. As seen in Table 4, every monkey infected by the attenuated SIVmac started to overexpress IL-10 mRNA as early as the first week p.i. for monkeys 392 and 559, and by the second to the fourth weeks p.i. for the two other animals. Subsequently, IL-10 mRNA expression decreased to baseline levels. No significant differences in IL-10 mRNA levels between the two groups were seen by analysis of variance.

DISCUSSION

The infection of macaques with SIVmac provides a unique model to analyze kinetically the relationships between virus load, disease progression, and spectrum of cytokines secreted (17, 20–25). The pC8 clone of SIVmac251 is known to be attenuated as assessed by low virus burden after infection, lack of disease progression in the animals chronically infected, and persistence of normal $CD4^+$ cell numbers (12). Accordingly, all four of the monkeys infected by this strain exhibited no detectable serum p27 antigenemia and a low cell-associated virus load. In contrast, pathogenic SIVmac251-infected monkeys always exhibited a spike of serum antigenemia 14 ± 3 days p.i., and a higher virus burden in PBMCs (17, 23, 24). Clinically, all four monkeys infected by the attenuated SIVmac remained healthy during the period of observation. Except during the first weeks p.i., they also maintained normal $CD4^+$ lymphocyte counts after, to date, 12 months of follow-up. Again, these observations are dramatically different from those after an infection with the pathogenic SIVmac251, which always leads to simian AIDS with $CD4^+$ cell loss between 6 and 36 months (24). Indeed, in the group of the SIVmac251-infected monkeys, 1 year p.i., the $CD4^+$ blood cell count dropped significantly in three monkeys of eight. These results are also in accordance with those obtained by Desrosiers and coworkers (14, 23).

Mosmann et al. (1) defined distinct patterns of cytokine produced by cloned T-helper cells in mice, called Th1 (producing IL-2 and IFN-γ), Th2 (producing IL-4, IL-5, IL-10, and IL-13), and their precursor Th0 (producing both types) (26).

Table 1. IL-2 mRNA levels (as a ratio of IL-2 mRNA/GAPDH mRNA)

	IL-2 mRNA relative level											
	In pathogenic SIVmac-infected macaques (full-length *nef*)								In attenuated SIVmac-infected macaques (*nef*-truncated)			
	141	947	351	514	383	823	46713	46880	371	392	524	559
Preinfection												
Mean	<0.01	0.02	<0.01	0.02	<0.01	0.16	0.03	0.03	<0.01	<0.01	<0.01	<0.01
n	8	8	8	8	3	3	3	3	3	3	3	3
SD	<0.01	0.03	<0.01	0.04	<0.01	0.02	0.06	0.06	<0.01	<0.01	<0.01	<0.01
Days p.i.												
7	<0.01	<0.01	<0.01	<0.01		0.18	<0.01	0.05	**0.32***	**0.18***	<0.01	**0.14***
14	<0.01		<0.01	<0.01	<0.01	0.18	**0.17***	<0.01	**0.23***	**0.30***	0.06	**0.10***
21	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.36***	0.03	**0.30***	**0.36***
28	<0.01	<0.01	<0.01	**0.10***		<0.01	<0.01	<0.01	<0.01	0.06		0.04
37	<0.01	<0.01		0.05	<0.01	<0.01	<0.01	**0.12***	<0.01	0.03	**0.15***	<0.01
44	<0.01	<0.01		<0.01	<0.01	<0.01	0.08	<0.01				
128									<0.01	**0.12***	0.06	<0.01

n represents the number of time points tested before SIV inoculation. * and boldface indicate >97th percentile.

Table 2. IFN-γ mRNA levels (as a ratio of IFN-γ mRNA/GAPDH mRNA)

	IFN-γ mRNA relative level											
	In pathogenic SIVmac-infected macaques (full-length *nef*)								In attenuated SIVmac-infected macaques (*nef*-truncated)			
	141	947	351	514	383	823	46713	46880	371	392	524	559
Preinfection												
Mean	0.02	0.05	<0.01	0.02	0.04	0.06	0.03	0.12	0.07	<0.01	0.01	<0.01
n	8	8	8	8	3	3	3	3	3	3	3	3
SD	0.04	0.14	<0.01	0.02	0.04	0.04	0.06	0.16	0.01	<0.01	0.01	<0.01
Days p.i.												
7	<0.01	<0.01	<0.01	<0.01		0.20	<0.01	0.13	**0.17***	<0.01	**0.10***	**0.04***
14	<0.01		<0.01	<0.01	<0.01	0.10	<0.01	<0.01	**0.13***	<0.01	0.04	<0.01
21	<0.01	0.02	<0.01	<0.01	<0.01	0.10	**0.13***	<0.01	**0.37***	<0.01	0.02	**0.06***
28	**0.11***	<0.01	<0.01	0.01		<0.01	0.01	0.29	**0.10***	<0.01		**0.12***
37	**0.08***	0.10		<0.01	<0.01	<0.01	**0.18***	<0.01	**0.24***	<0.01	**0.06***	**0.24***
44	<0.01	<0.01		<0.01	<0.01	0.02	**0.19***	0.11				
128									**0.30***	<0.01	**0.08***	**0.10***

n represents the number of time points tested before SIV inoculation. * and boldface indicate >97th percentile.

More recently, the use of type 1 or type 2 terminology was applied to more-physiological polyclonal T-cell responses independent of the cellular origin of the cytokines (9). Following this terminology, since our approach is not clonal, no type 1 or 2 T-helper response was seen clearly after inoculation of the pathogenic virus. On the other hand, infection with the attenuated clone led to an overexpression of both IL-2/IFN-γ and IL-4/IL-10. As a consequence, the cytokine profile observed may thus be type 0 in character. Finally, in both cases no clear Th1 or Th2 dichotomy was observed.

The main finding of this study is that monkeys infected by the attenuated *nef*-truncated virus showed enhanced levels of IL-2, IL-4, and IFN-γ mRNAs early during infection, whereas monkeys infected by the pathogenic SIVmac251 isolate did not. It is of concern that mRNA levels may not totally reflect cytokine protein levels, a factor which may change the interpretation of the data. Nevertheless, the lack of expression of these cytokines in monkeys infected with the pathogenic primary isolate may reflect early T-cell immunodeficiency, since IL-2 and IL-4 are essentially secreted by T-helper cells, whilst IFN-γ is secreted by these and other cell types (1).

The first possible explanation of these results is that attenuated infection leads to a better T-cell activation than does infection with pathogenic virus. This observation may be related to the viral load, since in some cases the weakest antigenic burden is capable of eliciting the strongest immune responses (in term of proliferation or IL-2 production) (27, 28). Nevertheless, in monkey 823, which exhibited a quantifiable baseline level of IL-2, IL-4, and IFN-γ mRNA, the expression of these cytokines became undetectable at the time of intense viral replication, strongly suggesting their down-expression after the infection by the pathogenic virus rather than a weak activation of the T-cell response.

The second hypothesis is that this observation may reflect early induction of immune response paralysis. The dichotomy observed in the expression of IL-2, IL-4,and IFN-γ suggests that the infection with the pathogenic lentivirus leads to a paralytic status in the T-helper cells shortly after infection. In contrast, the infection with the attenuated clone leads to T-cell activation. Accordingly, other authors have demonstrated that in HIV infection, T cells loose their capacity to proliferate in the presence of recall antigens (29). The same phenomenon is also described in macaques infected by SIVmac but not when they are infected by HIV-2, which is predominantly avirulent in this species (30). The origin of this paralysis is not fully understood. It may be due to one of the following: (*i*) An accessory dysfunction of the antigen-presenting cells (as macrophages). Indeed, *in vitro*, decreased accessory cell function has been demonstrated in peripheral blood monocytes (31) after HIV infection. (*ii*) The suppressive role of a soluble factor secreted during this period. For instance, an immunosuppressive effect of HIV-1 envelope glycoprotein gp120 on $CD4^+$ cells *in vitro* has been shown (32), as well as the effects of gp120 or gp41 oligopeptides (32). (*iii*) The direct effect of virus-associated suppression on the T-helper cells, such as induction of anergy (30) preceding their programmed cell

Table 3. IL-4 mRNA levels (as a ratio of IL-4 mRNA/GAPDH mRNA)

	IL-4 mRNA relative level											
	In pathogenic SIVmac-infected macaques (full-length *nef*)								In attenuated SIVmac-infected macaques (*nef*-truncated)			
	141	947	351	514	383	823	46713	46880	371	392	524	559
Preinfection												
Mean	<0.01	<0.01	<0.01	<0.01	0.01	0.18	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
n	8	8	8	8	3	3	3	3	3	3	3	3
SD	<0.01	<0.01	<0.01	<0.01	0.02	0.03	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
Days p.i.												
7	<0.01	<0.01	<0.01	<0.01		0.18	<0.01	<0.01	**0.12***	**0.10***	**0.27***	<0.01
14	<0.01		<0.01	<0.01	**0.06***	0.06	<0.01	<0.01	<0.01	**0.20***	**0.07***	<0.01
21	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.20***	<0.01
28	<0.01	<0.01	<0.01	<0.01		<0.01	**0.06***	<0.01	**0.20***	0.04		<0.01
37	<0.01	<0.01		<0.01	<0.01	<0.01	<0.01	<0.01	**0.10***	<0.01	0.02	<0.01
44	<0.01	<0.01		<0.01	<0.01	<0.01	<0.01	<0.01				
128									**0.12***	<0.01	<0.01	<0.01

n represents the number of time points tested before SIV inoculation. * and boldface indicate >97th percentile.

Table 4. IL-10 mRNA levels (as a ratio of IL-10 mRNA/GAPDH mRNA)

	IL-10 mRNA relative level											
	In pathogenic SIVmac-infected macaques (full-length *nef*)								In attenuated SIVmac-infected macaques (*nef*-truncated)			
	141	947	351	514	383	823	46713	46880	371	392	524	559
Preinfection												
Mean	0.03	0.22	0.06	0.22	0.13	0.15	0.11	0.28	0.43	0.12	0.55	0.37
n	8	8	8	8	3	3	3	3	3	3	3	3
SD	0.02	0.05	0.03	0.02	0.04	0.06	0.10	0.02	0.03	0.04	0.06	0.03
Days p.i.												
7	**0.18***	0.08	**0.24***	0.22		0.14	0.07	0.33	0.38	**0.53***	0.38	0.49
14	0.03		0.13	**0.57***	0.12	0.13	0.13	0.04	0.38	**0.31***	0.69	**0.74***
21	0.03	**0.54***	**0.53***	0.16	**0.36***	0.07	0.06	0.05	0.25	**0.38***	**1.15***	0.41
28	**0.44***	**0.60***	0.02	0.27		0.03	0.19	0.35	0.70	0.21		0.31
37	**0.37***	**0.23***		0.18	0.17	0.10	**0.30***	0.23	0.40	0.22	0.79	0.42
44	**0.21***	0.10		0.08	0.04	0.02	0.22	0.14				
128									0.31	0.21	0.31	0.38

n represents the number of time points tested before SIV inoculation. * and boldface indicate >97th percentile.

death (PCD) (33). (*iv*) Preferential infection and/or depletion of memory T cells.

Thus, our study suggests that an immune response dysfunction may occur early during pathogenic SIV infection in macaques, in a high virus burden context, before clinical events. However, we cannot reach a conclusion on the mechanism involved in the lack of T-cell cytokine expression. Nevertheless, the results may reflect that weak responsiveness, immune paralysis or anergy, well known to be implicated in the ultimate stage of AIDS pathogenesis, may also be present shortly after infection by pathogenic virus and therefore may govern the quality of the subsequent immune responses. One of the striking resemblances between the early phase after infection and the ultimate state in the HIV (or SIV) disease is that the viral loads are elevated in both cases. One may then hypothesize that the immune unresponsiveness observed may be linked to the presence of high viral burdens rather than being secondary to the dramatic loss of $CD4^+$ T cells or loss of various immune functions. Accordingly, the infection by the *nef*-truncated clone leads to lower viral loads, which may also explain its attenuated phenotype.

This study outlines the need of further evaluation of the immune responses (e.g., the mechanisms involved in the lack of immune response paralysis) elicited by infection with the attenuated SIV. The use of this model has indeed two major goals: (*i*) comprehension of physiopathogenetic events leading to AIDS; and (*ii*) provision of clues for vaccine development.

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