Secretion of \(\beta\)-chemokines by bronchoalveolar lavage cells during primary infection of macaques inoculated with attenuated nef-deleted or pathogenic simian immunodeficiency virus strain mac251

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Primary infection of macaques with simian immunodeficiency virus (SIV) as a model of human immunodeficiency virus (HIV) infection represents a unique opportunity to investigate early lentivirus–host interactions. In order to gain insight into immunopathogenic events taking place in the lung during lentiviral infection, we analysed lymphocyte expansion in the lung and chemokine secretion by mononuclear cells obtained by bronchoalveolar lavage (BALMCs) during primary infection by a pathogenic and a non-pathogenic SIV. Two groups of cynomolgus macaques were inoculated intravenously with a fully pathogenic isolate of SIVmac251 or with an attenuated, nef-deleted, molecular clone of SIVmac251. Spontaneous MIP-1\(\alpha\), MIP-1\(\beta\) and RANTES production was assessed by ELISA in supernatants of short-term cultured BALMCs. Kinetics of haematological, virological and immunological parameters were investigated simultaneously. All 11 inoculated animals became infected. Monkeys inoculated with the nef-deleted SIV clone exhibited a significantly reduced plasma virus load and a less pronounced accumulation of lymphocytes in the lung compared to monkeys infected with the pathogenic SIVmac251 isolate. Compared to pre-infection levels, we observed an increase in the levels of RANTES, MIP1-\(\alpha\) and MIP1-\(\beta\) production in the two groups of monkeys, by the time of peak viraemia. Strikingly, a greater enhancement of RANTES and MIP-1\(\alpha\) production was detected in monkeys infected with the attenuated virus. Given the potential influence of \(\beta\)-chemokines on the immune response and virus replication, such results suggest that RANTES, MIP1-\(\alpha\) and MIP1-\(\beta\) could contribute to the singular features of the immune response elicited during infection of macaques with an attenuated SIV.

Introduction

Chemoattractant cytokines or chemokines are low molecular mass proteins that mediate inflammation via the recruitment of phagocytic and other immune cells (Luster, 1998). They are involved in several non-infectious inflammatory diseases and are implicated in the immune response to several infectious agents (Luster, 1998). During human immunodeficiency virus (HIV) infection, enhanced chemokine expression has been demonstrated, concomitant with accumulation of leukocytes in lymphoid and non-lymphoid tissues (Denis & Ghadirian, 1994; Sasseville et al., 1996; Schmidtmayerova et al., 1996; Tedla et al., 1996), which strongly supports a role for chemokines in the regulation of leukocyte recruitment to tissues of HIV-infected patients. Moreover, the \(\beta\)-chemokines MIP-1\(\alpha\), MIP-1\(\beta\) and RANTES were found to contribute \textit{in vitro} to the non-cytolytic inhibition of HIV replication by CD8\(^+\) lymphocytes (Cocchi et al., 1995). This suppressive effect is likely to be due to inhibition of the coreceptor function for virus entry of their natural shared ligand: the seven-transmembrane-spanning receptor CCR5 (Moore et al., 1997). In addition, the chemokines may also be involved in inhibition of HIV/SIV (simian immunodeficiency virus) replication through cytolytic pathways (Wagner et al., 1998). Nevertheless, to date, the role of \(\beta\)-chemokines in the natural history of HIV infection remains to be clearly elucidated.
The in vivo biological relevance of these chemokines in HIV infection can be investigated using the experimental infection of macaques with SIV, which is the most relevant animal model of HIV infection in humans (Daniel et al., 1985). SIV is a primate (non-human) lentivirus that has a similar genomic organization to and shares biological properties with HIV-1 and HIV-2. Some SIV strains, like pathogenic isolates of SIVmac251, induce AIDS in macaques (Daniel et al., 1985). Interestingly, SIVmac and the monocyte/macrophage-tropic strains of HIV-1 that predominate early after infection share CCR5 as a coreceptor of the CD4 molecule for entry into target cells (Moore et al., 1997) and MIP-1x, MIP-1β and RANTES inhibit SIV infection in vitro (Cocchi et al., 1995; Wang et al., 1998).

The availability of molecular clones of SIV that vary in cellular tropism and virulence makes this experimental model particularly useful. Live attenuated SIV provide good insights into HIV pathogenesis and represent, to date, the most effective vaccines for protection from SIV infection (Almond et al., 1996; Moore et al., 1996; Clements et al., 1995; Daniel et al., 1992; Norley et al., 1996; Sharpe et al., 1997; Stahl-Hennig et al., 1996; Wyand et al., 1996). However, principally because of safety concerns (Baba et al., 1995; Cohen, 1997; Ruprecht et al., 1996), it is unlikely that a live attenuated vaccine will be used in humans in the foreseeable future.

In previous studies of primary infection with a pathogenic SIV, we highlighted a significant early increase in the proportion of CD8+ lymphocytes among mononuclear cells obtained by bronchoalveolar lavage (BALMC) (Cheret et al., 1996). This enhancement was especially marked in the lung tissue and was not encountered with the same intensity in the other body compartments analysed (peripheral blood and lymph nodes). Given that CD8+ lymphocytes, as well as resident macrophages which remained the predominant cell population among BALMC, represent well-known cellular sources of β-chemokines (Luster, 1998), studying chemokines synthesized in the lung during primary infection could provide a good insight into HIV immunopathogenic events occurring in the lung. Thus, we investigated the lymphocyte expansion in lung tissue and β-chemokine production by BALMC during primary infection of macaques with a pathogenic primary isolate and a non-pathogenic, nef-deleted SIV molecular clone.

Methods

Animals and viruses. Eleven adult cynomolgus monkeys (Macaca fascicularis), weighing 2.5 to 3.5 kg, were imported from Mauritius Island. The sera of these monkeys tested negative for the following viruses: SIV and herpesvirus B, simian T-lymphotropic virus, Ebola virus (strains Reston, Sou, Zaïre), Marburg virus, Lassa virus, measles virus and simian retrovirus (SRV) types 1 and 2. Animals were housed in single cages within level 3 biosafety facilities in accordance with EC guidelines for primate experiments (Journal Officiel des Communautés Européennes, L358, 18 December 1986). During handling, the monkeys were always anaesthetized with ketamine (Imalgène, Rhône-Mérieux, France).

A cell-free pathogenic SIVmac251 stock for in vitro experiments was kindly provided by A. M. Aubertin (Laboratoire de Virologie, Université Louis Pasteur, Strasbourg, France). Virions were obtained from a cell-free supernatant of infected rhesus macaque peripheral blood mononuclear cells (PBMCs). These cells were infected in vitro with a culture supernatant obtained from a coculture of rhesus macaque PBMCs and a spleen homogenate from a rhesus macaque infected with SIVmac251 (provided by R. C. Desrosiers, New England Regional Primate Center, Southborough, MA, USA). The in vitro titre of this stock after intravenous inoculation was 40 000 AID50 (50% animal infectious dose)/ml. Monkeys were inoculated in the saphenous vein with 4 AID50.

The attenuated SIVmac251 ΔNef virus was provided by A. M. Aubertin. The SIVmac 251 ΔNef clone was derived from the BK28 clone (Kornfeld et al., 1987) by deletion of nucleotides 9226 to 9400 in the nef gene, mutation of the nef initiation codon AGT to AGG at 9059, and mutation of the env in-frame stop codon TAG to CAG at 8785. The parental clone BK28 is partially attenuated, as it induces AIDS after a long incubation period and only in a fraction of the inoculated macaques (Chakrabarti et al., 1995). Nef mutations were introduced in the BK28 clone to ensure attenuation. The animals were inoculated intravenously with 1 ml of viral supernatant corresponding to approximately 70 000 c.p.m. in a reverse transcriptase assay.

Blood and bronchoalveolar lavage (BAL) sample collection. PBMC were separated by standard density-gradient centrifugation (MSL, Eurobio, Les Ulis, France). Bronchoalveolar lavages were performed after local anaesthesia with lignocaine (Xylovet, Sanofi, France), by instilling four to five aliquots of 20 ml of warm 0-9% sterile saline solution through an endotracheal tube. The BAL fluids were collected by immediate gentle aspiration after each aliquot and pooled in a sterile heparinate lithium container. Five bronchoalveolar lavages were performed at weekly intervals, before SIV inoculation. After SIV inoculation, lavages were done on days 7, 14, 21, 28, 35 and 49 post-inoculation (p.i.). BAL samples were centrifuged at 350 g for 10 min. The cells were washed with PBS and then mononuclear cells were separated by standard density-gradient centrifugation.

The percentage of BAL fluid recovered was approximately 75% of the instilled fluid. The alveolar cellularity ranged from 50 000 to 200 000 cells per ml of fluid recovered. No statistically significant difference in cellularity was noticed between baseline and post-infection bronchoalveolar lavage (data not shown). To rule out potential side-effects, we have verified that repeated bronchoalveolar lavages in uninfected animals do not affect the percentages of CD8+ and CD4+ lymphocytes among BALMC if weekly lavages were followed by bi-weekly lavages (Cheret et al., 1996) (data not shown). During the experiment, the majority of BALMC that were recovered were macrophages (more than 80%).

T-Lymphocyte subset determination. PBMC and BALMC were analysed by flow cytometry using a direct immunofluorescence assay to determine the percentages of CD4+ and CD8+ lymphocytes. About 300 000 cells were maintained at 4 °C for 30 min with anti-CD4 monoclonal antibody (CD4 Leu-3a PE; Becton Dickinson) plus anti-CD8 monoclonal antibody (CD8 Leu-2a FITC; Becton Dickinson) in the same assay to determine the percentages of CD4+ and CD8+ lymphocytes among BALMC. In some experiments, a FACScan cytomter/CellQuest software (Becton Dickinson) was used.

PCR amplification of integrated viral genome in BALMCs and PBMCs. DNA was extracted from BALMCs and PBMCs. SIVmac251 DNA was amplified using a nested set of primers specific for the env and gag genes. The env outer and inner primer pairs were as follows: 6597 N (TCAGCTGCTTATCGCCATCTTGC), 8185 C (AGACTAGTCTTCGGTCTTCC), 6597 N (TCAGCTGCTTATCGCCATCTTGC), 8185 C (AGACTAGTCTTCGGTCTTCC)
ACCTGCCGTTCCGAAAACC; 6847 N (GTATGGCAAACCTCTTTCAGACCTGCAATA); 7756 C (TCTCCTGCAATTTTGCCACAT).

The gag outer and inner primer pairs were as follows: 1096 N (TTAGGCTAGCCAAAACCGGAAAAGA), 1592 C (AAAGATCATGTGGAGAGCATGTAGT); 1121 N (ATAAGGGGTCACCCCTGACAG), 1592 C (AAAGATCATGTGGAGAGCATGTAGT). The first round reaction mixture contained total genomic DNA (1 µg), the outer pair of primers (25 pmol each), dNTPs (10 µM each), 10 µl of Taq polymerase buffer (10 mM Tris–HCl, pH 8.3, 1.5 mM MgCl₂, 50 mM KCl, 0.1% Triton X-100) and 0.5 units of Taq polymerase (Appligen). Two µl of the first amplification mixture was used as template in a second round of amplification with the inner primer pair. Amplification products were separated by electrophoresis on a 1.5% agarose gel and detected by ethidium bromide staining.

- Determination of viral load. SIV RNA levels in plasma samples were measured using the Chiron branched-(b)DNA assay.
- Production of RANTES, MIP-1α and MIP-1β by BALMC. Evaluation of chemokine production in BAL fluids was performed using BALMCs separated on a Ficoll gradient. Spontaneous chemokine production was assessed in culture fluids collected 72 h after plating 0.5 × 10⁶ nonstimulated cells/ml in 48-well flat-bottomed plastic microplates (Becton Dickinson). The culture medium consisted of RPMI 1640 (Boehringer), 10% complement-depleted FCS (Boehringer), 1% trinitrobenzene sulfonic acid (Triton X-100) and 0.5 units of Taq polymerase (Appligen). Two µl of the first amplification mixture was used as template in a second round of amplification with the inner primer pair. Amplification products were separated by electrophoresis on a 1.5% agarose gel and detected by ethidium bromide staining.

- Statistical analysis. Wilcoxon rank analysis, ANOVA and correlation coefficients were determined using STATVIEW 4.5 software (Abacus Concept, Berkeley, CA, USA).

Results

Virological follow-up

All 11 animals, in both groups, became infected. Viral RNA was detected for the first time in plasma between days 7 and 14 p.i. (Fig. 1a) from the monkeys inoculated with the pathogenic isolate; the peripheral viral load then peaked between days 14 and 21 p.i. Monkey S73B exhibited the highest viral burden (5 × 10⁸ Eq. copies/ml at day 21 p.i.). Viral RNA remained detectable in plasma at day 49 p.i. for all animals of this group.

In contrast, primary infection with the attenuated clone was characterized by a significantly lower viral burden (Fig. 1b). Indeed, in this group, SIV RNA became detectable between days 7 (three of the four animals) and 14 p.i. (monkey B670D); the viral load peaked between days 7 and 21 p.i. Peak values were at least 2 logs lower than those measured for animals infected with the pathogenic isolate. Monkey S1124 exhibited the highest viral load at day 21 p.i. (8.4 × 10⁶ Eq. copies/ml). By day 28 p.i., plasma viral load was below the cut-off value of the Chiron bDNA assay (10⁶ Eq. copies/ml) in all the animals of this group.

The presence of the virus in the lung was assessed by PCR analysis. Proviral DNA in BALMC was evident in all the animals as soon as day 14 p.i. in both groups.

Changes in lymphocytes subsets

Circulating lymphocytes. The seven animals infected with the pathogenic isolate exhibited a decrease of their number of circulating CD4⁺ T lymphocytes at day 14 p.i. (Fig. 2a). Two animals (J495 and J116) later displayed a significant enhancement of CD4⁺ lymphocytes at, respectively, days 21 and 28 p.i. Finally, for six monkeys the numbers of circulating CD4⁺ T lymphocytes had recovered to the basal level by day 35 p.i.; in monkey J116 the number of circulating CD4⁺ T lymphocytes at day 35 p.i. remained higher than the basal level.

In the monkeys infected with the pathogenic isolate we also detected a decrease of circulating CD8⁺ T lymphocytes at day 14 p.i. (Fig. 2a). Thereafter, an enhancement was evident in three animals (S73B, S62A and J116) at days 28 and 35 p.i. Five monkeys (all except S62A and S73B) recovered their normal basal level of circulating CD8⁺ T lymphocytes by day 35 p.i.

A decrease of circulating CD4⁺ T lymphocyte numbers occurred at day 7 p.i. in the animals infected with the attenuated clone (Fig. 2b). CD4⁺ T lymphocyte numbers were again within the normal range at day 35 p.i. for all the monkeys. The same trend was evident for circulating CD8⁺ T...
lymphocytes: a decrease at day 7 p.i. was observed (Fig. 2b). Moreover, when analysing the changing CD8+ T lymphocyte numbers at day 28 p.i., a general upward tendency was observed, but at day 35 p.i. all the animals had normal basal levels of circulating CD8+ lymphocytes.

**BAL lymphocytes.** The percentages of CD4+ lymphocytes in BALMC remained stable during our study for all monkeys inoculated with the pathogenic isolate, except for S73B, in which a slight decrease was observed (Fig. 3a). In contrast, the percentages of CD8+ lymphocytes in BALMCs increased substantially in the monkeys infected with the pathogenic isolate (Fig. 3a). It is noticeable that monkeys S73B, S62A and J116, which exhibited the greatest enhancement of CD8+ T lymphocytes in BALMC, also exhibited the greatest enhancement of CD8+ cells in the peripheral blood. Moreover, monkey S73B, which had the highest peripheral viral burden, also had the greatest increase in CD8+ T lymphocytes among BALMC. From week 3 after SIV inoculation, the majority of BAL lymphocytes were CD8+ (up to 90% for monkey S73B).

The percentages of CD4+ lymphocytes in BALMCs were stable during our study for all monkeys inoculated with the attenuated viral clone, except for B670D which displayed a slight increase (Fig. 3b). The percentages of CD8+ lymphocytes in BALMCs increased in two out of four animals (BrIIIa and B670D) (Fig. 3b) in which the CD4+/CD8+ ratio in BAL lymphocytes was markedly altered in favour of CD8+ cells. It is noticeable that these two animals also had the lowest peripheral viral burden.

**Production of RANTES, MIP-1α and MIP-1β by BALMC**

In order to study β-chemokine production by BALMC during primary infection, bronchoalveolar lavages were performed sequentially in both groups of monkeys, at weekly intervals, after SIV inoculation. At each time-point, the freshly isolated mononuclear BAL cells were cultured for 72 h without in vitro stimulation, and spontaneous β-chemokine production was assessed in culture supernatant fluids by ELISA.

During primary SIV infection, production of all β-chemokines tested was increased in BALMC supernatants (non-parametric Wilcoxon rank test) at day 14 p.i. in both groups of animals (Figs 4, 5 and 6). Thereafter, this increase was evident at several time-points for all animals, with the exception of MIP-1β in monkeys inoculated with the pathogenic virus (Fig. 5a). Secretion peaks of RANTES by BALMC were observed by the time of maximal viral load: a highly significant correlation was demonstrated with plasma SIV RNA copies at day 14 p.i. in the group of animals inoculated with the pathogenic isolate ($r = 0.99$) or the attenuated SIV ($r = 0.97$).
In addition, for MIP-1α and MIP-1β, this correlation was only observed in animals infected with the nef-deleted virus (respectively \( r = 0.95 \) and \( r = 0.99 \)). BALMC of animals inoculated with the attenuated virus secreted significantly higher amounts of MIP-1α (\( p = 0.02 \)) and RANTES (\( p < 0.01 \)). Finally, no correlation could be established between chemokine production and accumulation of lymphocytes in the lung.

**Discussion**

The lung is a key organ in HIV-induced pathophysiology, and becomes infected during the early stages of infection (Agostini et al., 1996). Among the multiple lines of host reaction elicited by the virus invasion, CD8\(^+\) T lymphocyte expansion in the lung micro-environment constitutes one of the most impressive (Chéret et al., 1996; Rosenberg et al., 1997). The exact mechanisms responsible for this accumulation of lymphocytes are at the moment poorly understood. CD8\(^+\) T lymphocyte expansion may result from *in situ* proliferation of CD8\(^+\) T cells in response to local antigens (Spain et al., 1995; Zambello et al., 1992) and/or from preferential extravasation of circulating CD8\(^+\) T lymphocytes (Saukkonen et al., 1997). Among the candidates that potentially contribute to T cell recruitment, the chemokines RANTES, MIP-1α and MIP-1β, locally elaborated, might play a pivotal role. Owing to their suppressive activity on HIV replication (Barker et al., 1998; Cocchi et al., 1995; Zagury et al., 1984, 1998), these chemokines could be critically involved in the course of HIV infection. Nevertheless, despite the growing body of results in the chemokine field, their *in vivo* relevance in HIV pathogenesis remains a controversial issue.

Given that the course of viral infection in tissues of SIV-infected macaques shares many similarities with HIV infection (Simon et al., 1992), experimental infection of macaques with SIV is a very relevant model to assess *in vivo* the involvement of β-chemokines in the immunopathogenic mechanisms taking place in the lung. In the present study, we investigated lymphocyte recruitment in lung tissues and β-chemokine production by BAL cells during primary infection with a pathogenic primary SIV isolate and a non-pathogenic nef-deleted SIV molecular clone.

Our results indicate that, during primary infection, animals infected with the pathogenic isolate exhibited a high peripheral viral burden and an early and marked enhancement of CD8\(^+\) T lymphocytes in BALMC. We have verified that repeated bronchoalveolar lavages in uninfected animals do not affect the percentages of CD8\(^+\) and CD4\(^+\) lymphocytes among BALMC.
Fig. 4. Secretion of MIP-1α by BALMC during primary SIV infection. (a) Monkeys infected with pathogenic SIVmac251. (b) Monkeys infected with attenuated SIVmac/∆Nef. The top and bottom of the boxes represent the 75th and 25th percentiles, respectively, whereas the median is represented by the horizontal line between the box limits. Upper and lower bars of the diagram represent, respectively, the 90th and 10th percentiles. The open circles represent individual values which are not included between the 90th and 10th percentiles. The Day 0 value represents the mean of three time-points tested before SIV inoculation. p, Wilcoxon rank test (days post-infection values compared to pre-inoculation values).

Fig. 5. Secretion of MIP-1β by BALMC during primary SIV infection. (a) Monkeys infected with pathogenic SIVmac251. (b) Monkeys infected with attenuated SIVmac/∆Nef. The top and bottom of the boxes represent the 75th and 25th percentiles, respectively, whereas the median is represented by the horizontal line between the box limits. Upper and lower bars of the diagram represent, respectively, the 90th and 10th percentiles. The open circles represent individual values which are not included between the 90th and 10th percentiles. The Day 0 value represents the mean of three time-points tested before SIV inoculation. p, Wilcoxon rank test (days post-infection values compared to pre-inoculation values).

even if weekly bronchoalveolar lavages are followed by bi-weekly lavages (Chéret et al., 1996) (data not shown). The pattern of β-chemokine production by BALMC was characterized by increased secretion of MIP-1α, MIP-1β and RANTES. A different pattern characterizes the primary infection of macaques inoculated with the attenuated clone. These animals had a lower peripheral viral burden and exhibited a transient decrease of peripheral T lymphocytes at the time of peak viraemia. Immunological phenomena could contribute to such a decrease; however, it may also be attributed to a residual pathogenicity of the attenuated viral clone used, although, to date, this particular strain has not been found to induce AIDS in adult macaques. Nevertheless, it is of primary importance to undertake long-term follow-up studies of such infected macaques in order to discard any doubt about the potential pathogenicity of nef-deleted SIV clones. In two of the four monkeys that we studied, we also found an increase in T CD8+ BAL lymphocytes. Nevertheless, enhanced MIP-1α, MIP-1β and RANTES secretion by BALMCs was detected. Strikingly, the increase in RANTES and MIP-1α production was greater in these monkeys than in those inoculated with the pathogenic isolate. Thus, consistent with results obtained from previous investigations in other body compartments (Benveniste et al., 1996; Chakrabarti et al., 1995; Lackner et al., 1994; Zou et al., 1997), the early events taking place in the lung also distinguish strikingly between the two infections.

These results raise several questions. Is there a link between viral load and lung leukocytic expansion? Do RANTES, MIP1-α, MIP1-β have a regulatory role in lymphocytic recruitment? What are the consequences of their enhancement on the immune response elicited during the primary SIV infection? Is there a correlation between viral load and β-chemokine production?

In HIV (Bach, 1987) and in other lentivirus infections (Brodie et al., 1992), an association was demonstrated between
had the highest viral burden, had no enhancement in T CD8+ cytolytic percentage in BALMC, whereas monkey S1124A, which peripheral viral burden, had the greatest increase in lymphocytes among BALMC. Monkey B670D, which had the lowest different pattern was observed in animals infected with the 1996) and supports the postulate of a role for viral load in determining the lung lymphocytic expansion. Interestingly, a previous study (Che et al., 1995; Clements et al., 1995; Titti et al., 1997) immune responses in animals infected with attenuated SIV viruses compared to animals infected with pathogenic isolates. Therefore, it may be hypothesized that β-chemokines, in particular RANTES, could actively favour a stronger induction of the immune response during primary infection of macaques with the pathogenic isolate or with the deleted viral clone.

While chemokines primarily promote leukocyte chemotaxis, they also modulate a number of other leukocyte activities such as helper and cytolytic T cell functions during genesis of the immune response (Dairaghi et al., 1998; Schrum et al., 1996; Taub et al., 1996b). MIP-1α, MIP-1β and RANTES seem to regulate lymphocyte-mediated cytolytic responses, antigen-specific T cell activation, T cell costimulation and antigen-presenting cell costimulatory functions. Thus, the enhanced production of all three chemokines highlighted herein might stimulate the specific antiviral immune response during primary infection of macaques with the pathogenic isolate or with the deleted viral clone.

Given that RANTES has the most potent immunostimulatory effect (Taub et al., 1996a), it is noteworthy that its production is more prominent in BALMC cultures of macaques infected with the attenuated SIV. Several reports have pointed out significant T cell activation (Veazey & Lackner, 1998) and increased cellular (Dittmer et al., 1995; Johnson et al., 1997) and humoral (Chakrabarti et al., 1995; Clements et al., 1995; Titti et al., 1997) immune responses in animals infected with attenuated SIV viruses compared to animals infected with pathogenic isolates. Therefore, it may be hypothesized that β-chemokines, in particular RANTES, could actively favour a stronger induction of the immune response during the non-pathogenic infection. This higher β-chemokine production induced by the ΔNef virus infection would tend to support the view that chemokines do not participate solely in the inflammatory process but also actively contribute to the onset and maintenance of an efficient immune response.

Our findings for the lung compartment differ from those of Zou et al. (1997) who analysed early chemokine gene expression in lymph nodes of macaques infected with SIV and found strong induction of chemokine gene expression only in virus replication and leukocyte recruitment. Our present results seem to indicate a correlation between the peripheral viral load and the accumulation of lymphocytes during primary infection with the pathogenic SIV isolate. This correlation extends the observations of a previous study (Cheret et al., 1996) and supports the postulate of a role for viral load in determining the lung lymphocytic expansion. Interestingly, a different pattern was observed in animals infected with the nef-deleted viral clone. Monkey B670D, which had the lowest peripheral viral burden, had the greatest increase in lymphocytic percentage in BALMC, whereas monkey S1124A, which had the highest viral burden, had no enhancement in T CD8+ lymphocytes among BALMCs. These results could stress that, in addition to the viral burden, several other factors determine accumulation of lymphocytes in the lung.

An altered pattern of leukocyte trafficking appears to be critical in HIV pathogenesis, owing to its importance for the

### Fig. 6. Secretion of RANTES by BALMC during primary SIV infection. (a) Monkeys infected with pathogenic SIVmac251. (b) Monkeys infected with attenuated SIVmac/ΔNef. The top and bottom of the boxes represent the 75th and 25th percentiles, respectively, whereas the median is represented by the horizontal line between the box limits. Upper and lower bars of the diagram represent, respectively, the 90th and 10th percentiles. The open circles represent individual values which are not included between the 90th and 10th percentiles. The Day 0 value represents the mean of three time-points tested before SIV inoculation. p, Wilcoxon rank test (days post-infection values compared to pre-inoculation values).
macaques infected with the pathogenic isolate. Although a different attenuated molecular clone of SIV was used, their results indicate the differences that may exist between distinct body compartments of infected animals.

In our study, there was a clear upward tendency of chemokine production at day 14 p.i., by the time of peak viraemia. Moreover, by this time-point, provirus became detectable in BALMCs by PCR assay. Based upon previous in vitro studies (Canque et al., 1996; Triozzi et al., 1998), these early increases in chemokine productions during SIV infection could support the view that virus replication induces chemokine production. Furthermore, since the enhanced chemokine production is maintained until late time-points in primary SIV infection, it may also be hypothesized that chemokines could participate in the control of virus replication, as previously suggested (Cocchi et al., 1995; Paxton et al., 1998; Ullum et al., 1998; Zagury et al., 1998). Interestingly, a recent study on macaques chronically infected with live attenuated SIV strains (Gauduin et al., 1998) indicated that unidentified soluble factors, distinct from RANTES, MIP-1α and MIP-1β, might play a dominant role in mediating suppression of SIV replication. Although not excluding the involvement of β-chemokines, such results underscore the likelihood that suppression of the antiviral response may be a more complex and multifactorial phenomenon than initially thought.

Finally, the enhanced production of all three chemokines during infection with a ΔNef viral clone is very appealing since prevention of SIV infection of macaques has been achieved with vaccine protocols able to induce increased chemokine secretion (Lehner et al., 1996; Wang et al., 1998). In our study, the greater induction of RANTES in animals infected with the attenuated clone is particularly interesting since RANTES has, in vitro, the most potent inhibitory effect on virus replication (Cocchi et al., 1995; Coffey et al., 1997; Trkola et al., 1998; Wang et al., 1998). In so far as this chemokine has a suppressive effect in vivo on virus replication, a comprehensive analysis of the fine mechanisms governing chemokine modulation would help in the design of new, safe and efficient antiviral strategies.

Further investigations are needed to identify the exact cellular source of β-chemokines. It is critical that such a question should be addressed because the cellular source could be a determinant of the potential suppressive effect on virus replication (Saha & Volsky, 1998). It has been suggested that, since spread of HIV occurs more efficiently through cell–cell contact (Levy, 1993), only chemokine release in immediate proximity to the virus target, or endogenous chemokine production by the target cell, would be effective in blocking virus entry.

In conclusion, our findings characterize the very early immune events that occur in the lung during pathogenic or non-pathogenic primary infection. This study provides evidence that, in addition to the differences demonstrated in other body compartments (Benveniste et al., 1996; Chakrabarti et al., 1995; Lackner et al., 1994; Zou et al., 1997), attenuated and pathogenic SIV differ also in the immune processes elicited in the lung during primary infection.

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References


β-Chemokines in the lung during SIV infection


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