Simian immunodeficiency virus types 1 and 2 (SIV mnd 1 and 2) have different pathogenic potentials in rhesus macaques upon experimental cross-species transmission

Sandrine Souquière, 1 Richard Onanga, 1 Maria Makuwa, 1 Ivona Pandrea, 1,2 Paul Ngari, 1 Pierre Rouquet, 3 Olivier Bourry, 3 Mirdad Kazanjii, 1 Cristian Apetrei, 1,2 François Simon, 1,4 and Pierre Roques 1,5

1Laboratoire de Rétrovirologie, Centre International de Recherches Médicales (CIRMF), Franceville, Gabon
2Tulane National Primate Research Center, Covington, LA 70433, USA
3Centre de Primatologie, Centre International de Recherches Médicales (CIRMF), Franceville, Gabon
4Laboratoire de Virologie, Hôpital St Louis, Paris, France
5Service de Neurovirologie, CEA iMETI, 92265 Fontenay aux Roses, France

The mandrill (Mandrillus sphinx) is naturally infected by two types of simian immunodeficiency virus (SIV): SIVmnd types 1 and 2. Both of these viruses cause long-term, non-progressive infections in their natural host despite high plasma viral loads. This study assessed the susceptibility of rhesus macaques to infection by these two types of SIVmnd and compared the virological and basic immunological characteristics of the resulting infections with those observed in natural infection in mandrills. Whilst both SIVmnd types induced similar levels of virus replication during acute infection in both mandrills and macaques, they produced a more pronounced CD4+ T-cell depletion in rhesus macaques that persisted longer during the initial stage of infection. Pro-inflammatory cytokine responses were also induced at higher levels in rhesus macaques early in the infection. During the chronic phase of infection in mandrills, which in this case was followed for up to 2 years after infection, high levels of chronic virus replication did not induce significant changes in CD4+ or CD8+ T-cell counts. In rhesus macaques, the overall chronic virus replication level was lower than in mandrills. At the end of the follow-up period, although the viral loads of SIVmnd-1 and SIVmnd-2 were relatively similar in rhesus macaques, only SIVmnd-1-infected rhesus macaques showed significant CD4+ T-cell depletion, in the context of higher levels of CD4+ and CD8+ T-cell activation, compared with SIVmnd-infected mandrills. The demonstration of the ability of both SIVmnd types to induce persistent infections in rhesus macaques calls for a careful assessment of the potential of these two viruses to emerge as new human pathogens.

INTRODUCTION

African non-human primates (NHPs) are the natural hosts of simian immunodeficiency viruses (SIVs) (Apetrei et al., 2004; Hahn et al., 2000; VandeWoude & Apetrei, 2006). To date, more than 40 different SIVs have been described and they infect different African species of monkeys and apes at high levels of prevalence (Apetrei et al., 2004; Hahn et al., 2000; VandeWoude & Apetrei, 2006). Unlike pathogenic human immunodeficiency virus (HIV) in humans and SIV in macaques, natural SIV infections generally do not progress to acquired immune deficiency syndrome (AIDS) (Chakrabarti, 2004; Hirsch, 2004; Muller & Barré-Sinoussi, 2003; Norley et al., 1999; Onanga et al., 2002, 2006; Pandrea et al., 2008b; Silvestri, 2005). In fact, only a handful of cases of immunodeficiency have been described to date in African NHP species, all of which have occurred in captive animals (Ling et al., 2004; Pandrea et al., 2001; Traina-Dorge et al., 1992). This lack of SIV-related disease progression in natural NHP hosts does not appear to be due to better infection control, as natural SIV infections are characterized by high levels of virus replication and...
immune responses that are not necessarily stronger than those observed in pathogenic infections (Dunham et al., 2006; Wang et al., 2006). It is currently believed that this observed lack of pathogenicity is due to the host's ability to control the deleterious side effects of the SIV infection. Supporting this view is the observation that natural infections are characterized by only a transient depletion of peripheral CD4+ T cells during primary infection and CD4+ T-cell restoration during the chronic infection to near-baseline levels (Kaur et al., 1998; Kornfeld et al., 2005; Pandrea et al., 2005, 2006; Silvestri et al., 2005). Also, natural SIV infections are characterized by only transient and moderate increases in immune activation and proliferation during acute infection, with a return to baseline levels during the chronic infection (Kaur et al., 1998; Kornfeld et al., 2005; Onanga et al., 2006; Pandrea et al., 2006, 2007; Silvestri et al., 2005). Finally, normal levels of apoptosis are observed throughout natural SIV infections (Estaquier et al., 1994; Pandrea et al., 2007; Silvestri et al., 2003). However, at mucosal sites, CD4+ T-cell depletion is more pronounced and prolonged, and restoration is only partial throughout the chronic infection (Gordon et al., 2007; Pandrea et al., 2007).

In striking contrast to the general lack of disease progression of SIV infection in natural hosts, pathogenic HIV and SIVmac infections in humans and Asian macaques, respectively, lead to CD4+ T-cell depletion and invariable progression to AIDS (Hirsch & Johnson, 1994). HIV-1 and HIV-2 originated from independent cross-species transmissions of SIVcpz, a virus that naturally infects chimpanzees in central Africa (Keele et al., 2006; Peeters et al., 2002), and SIVsmm, a virus that naturally infects sooty mangabeys in western Africa (Hirsch et al., 1989; Marx et al., 1991), respectively. The emergence of SIVmac occurred by accidental transmission of SIVsmm from sooty mangabeys to various macaque species housed in US-based primate centres (Apetrei et al., 2005, 2006). Although it is not yet clear what mechanisms led to this dramatic increase in the pathogenicity of SIVs in these new hosts, serial passage may have been involved in this transformation (Apetrei et al., 2006; Marx et al., 2001).

Experimental infections involving the cross-species transmission of various SIVs have resulted in widely variable outcomes. In addition to the pathogenic infections described previously, rhesus macaques infected with SIVagm from African green monkeys (genus Chlorocebus) (Pandrea et al., 2007), SIVrcm from red-capped mangabeys (Cercopithecus torquatus) (Smith et al., 1998), SIVhoest from l’Hoesti monkeys (Cercopithecus lhoesti lhoesti) (Hirsch et al., 1999), SIVsyk from Sykes’ monkeys (Cercopithecus albogularis) (Hirsch et al., 1993) and SIVtal from talapoin monkeys (Miopithecus talapoin) (Osterhaus et al., 1999) developed transient infections characterized by an active SIV replication during the acute infection and complete control of virus replication during the chronic infection. Conversely, cross-species SIV transmission to pig-tailed macaques resulted in progressive disease in most circumstances, with cases of AIDS in pig-tailed macaques being described following experimental infections with SIVsun from solatus monkeys (Cercopithecus lhoesti solatus) (Beer et al., 2005), SIVhoest (Beer et al., 2005), SIVagm from vervet (Hirsch et al., 1995) and sabaëus monkeys (I. Pandrea, unpublished observation) and SIVrcm (C. Apetrei, unpublished observation).

Mandrills (Mandrillus sphinx) are endemic in Gabon, central Africa, and are to date the only NHP species shown to be infected with two types of SIV: SIVmnd type 1 (SIVmnd-1) (Souquiere et al., 2001; Tsujimoto et al., 1988, 1989) and SIVmnd type 2 (SIVmnd-2) (Hu et al., 2003; Souquiere et al., 2001; Takehisa et al., 2001). These two SIVmnd types have different origins, with SIVmnd-1 belonging to the SIVhoest lineage and clustering together with SIVhoest from l’Hoesti monkeys and SIVsun from solatus monkeys (Beer et al., 1999; Hirsch et al., 1999; VandeWoude & Apetrei, 2006), and SIVmnd-2 being closer to other Papionini viruses, such as SIVdrl from drills (Mandrillus leucophaeaus) and SIVrcm (Hu et al., 2003; Souquiere et al., 2001; Takehisa et al., 2001). SIVmnd-2 is a recombinant virus, harbouring gag, pol, vif, vpx and tat sequences that are closely related to SIVrcm sequences, whereas its env and nef sequences are closely related to SIVmnd-1 (Hu et al., 2003; Souquiere et al., 2001).

Although SIVmnd-1 and SIVmnd-2 have different origins, these two viruses have identical virological characteristics in their natural hosts (Onanga et al., 2002, 2006). Thus, during experimental infection, high peak viral loads (VLs) and high levels of chronic virus replication are associated with only transient decreases in peripheral CD4+ T-cell counts.

The aim of this study was to investigate the dynamics of SIVmnd infections in rhesus macaques. We found that both SIVmnd-1 and SIVmnd-2 induced persistent infections in experimentally infected rhesus macaques. Although the levels of chronic SIVmnd-1 and SIVmnd-2 replication were comparable, a continuous CD4+ T-cell depletion was observed only in SIVmnd-1-infected rhesus macaques.

**METHODS**

**Animals and infections.** Four captive mandrills and six rhesus macaques of Chinese origins were included in this study. All of these animals, which tested negative for SIV and simian T-lymphotropic virus, were housed at the CIRMF Primate Center and handled under Biosafety level 3 conditions in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The mandrills comprised two females: 21 (9 years old) and 12D4 (7 years old), and two males: 5A4 (10 years old) and 2H (13 years old). All rhesus macaques were male: 93057 (8 years old), 93183A (3 years old), P9110A (6 years old), 93036 (8 years old), 93183B (3 years old) and 93046A (6 years old). All animal protocols and procedures were approved by the Gabonese Ethics Committee for Animal Experimentation.

The viral inocula used consisted of plasma obtained directly from acutely infected mandrills at peak viraemia following experimental
infection, to avoid potential problems due to virus selection through in vitro culture, as described previously (Onanga et al., 2002, 2006). Mandrills 21 and 5A4 and rhesus macaques 93057, 93183A and 99110A were inoculated with 500 μl plasma containing 7.5 × 10^6 RNA copies of SIVmnd-1 (strains 16C and 12A3) (Pandrea et al., 2008a). Mandrills 12D4 and 2H and rhesus macaques 93036, 93183B and 93046A were inoculated with 400 μl plasma containing 2.8 × 10^7 RNA copies of SIVmnd-2 (strain 10I) (Onanga et al., 2006). Animals were anaesthetized with ketamine/HCl for handling and were inoculated via the saphenous vein.

**Specimen collection.** Blood samples were collected on days 0, 4, 7, 11, 14, 28, 32, 60, 120, 180, 270, 360 and 750 days post-infection (p.i.) in EDTA K2 tubes for mandrills, and on days 0, 4, 7, 11, 28, 32, 60, 90, 120, 180, 330, 360 and 750 p.i. for rhesus macaques. Blood was used for flow cytometry, and peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll–Hypaque gradient centrifugation (Sigma–Aldrich), aliquotted in 10% DMSO (Sigma–Aldrich) in fetal bovine serum (Gibco–BRL) and frozen at –80 °C. Plasma was centrifuged at 3000 g for 10 min, dispensed into 1 ml aliquots and frozen at –80 °C.

Excisional inguinal lymph nodes (LN) were collected from mandrills and rhesus macaques on days 0, 7, 28, 60 and 360 p.i. Cells from LNs were separated by passage through a mesh screen. The cells obtained were washed in RPMI (Gibco–BRL), centrifuged for 10 min at 1500 g, aliquoted in 10% DMSO in bovine fetal serum and frozen at –80 °C.

**Kinetics of antibody production.** Anti-SIVmnd antibody dynamics were monitored using a peptide-based ELISA detecting antibodies against SIVmnd-specific peptides mapping the V3 region of the env glycoprotein (Simon et al., 2001). The same SIVmnd GB1 peptide was used to evaluate the presence of anti-SIVmnd antibodies in both types of infection, as the two types of SIVmnd share the same env sequences as a consequence of recombination events (Souquiere et al., 2001) and our preliminary studies showed a similar sensitivity of peptide-based ELISA for both SIVmnd types (Simon et al., 2001). The test was performed as described previously (Simon et al., 2001).

**SIVmnd RNA quantification.** RNA was extracted from 150 μl plasma using a QiaAmp Viral RNA Mini kit (Qiagen) and eluted in 50 μl TE buffer, as recommended by the manufacturer. For LNs, we used a QiaAmp RNA Isolation kit (Qiagen) to extract RNA from frozen aliquots of cells.

Real-time PCR assays specific for each virus type were developed for SIVmnd quantification. Briefly, quantification by real-time RT-PCR was performed with 5 μl extracted RNA, using a Quantitect SYBR Green RT-PCR kit (Qiagen) in capillary tubes, with the LightCycler System (Roche Diagnostics). For both viral strains, quantification was based on amplification of a 230 bp fragment located in the integrase region. Specific sets of primers were designed for each viral strain. The primers used for SIVmnd-1 were M17IF: 5'-AACAGATTGTGGCA- AAAGTGCA-3' and M17IR: 5'-CTTATCTGTGTTGTTTTACTAATA-3'. The primers used for SIVmnd-2 quantification were M26IF: 5'-GCAAAGGAGATAGTAGCTCAGTGTC-3' and M26IR: 5'-GCCATTATCTGTAGAAATGTTCACCT-3'. Primers were used at a final concentration of 1 μM and the final MgCl2 concentration was 2.5 mM. The amplification protocol for SIVmnd-1 quantification consisted of reverse transcription (20 min at 50 °C), followed by denaturation and activation of HotStart Tag DNA polymerase (15 min at 95 °C) and cDNA amplification (50 cycles of denaturation for 15 s at 95 °C, annealing for 15 s at 60 °C and elongation for 22 s at 72 °C). For SIVmnd-2 quantification, the conditions were the same except that the annealing temperature was 60 °C. Data were analysed using LightCycler Software version 3.5. The RNA copy number was determined by comparison with an external standard curve and was expressed as RNA copies ml⁻¹ for plasma and RNA copies per 10⁶ cells for LNs.

Standards, consisting of non-virus-specific tailored competitor RNA complementary to sequences specific for SIVmnd-1 or SIVmnd-2, were added at both the 5' and 3' ends. All molecular constructs were produced by Mobidab Molekularbiologie GmbH & Co. The MO4-01 RNA standard for SIVmnd-1 was 230 bp and the MO4-02 RNA standard for SIVmnd-2 was 233 bp. Each standard was subjected to 10-fold dilutions to generate solutions with 10⁻⁵ to 10⁹ RNA copies. The detection limit of the SIVmnd quantification assays was 250 RNA copies (ml plasma)⁻¹.

**Lymphocyte studies and flow cytometry.** Lymphocyte subsets were analysed by three-colour fluorescence-activated cell sorter analysis (FACScalibur; Becton Dickinson). For staining, 50 μl cell suspension (2 × 10⁵ PBMCs) was mixed with 5 μl of different combinations of the following monoclonal antibodies: fluorescein isothiocyanate-conjugated CD3 (clone SP-34), phycoerythrin (PE)-conjugated CD4 (clone M-T477), PE-conjugated CD8 (clone Leu-2a) and peridinin–chlorophyll–protein-conjugated HLA-DR (clone L-243) (all from BD Biosciences). The cells were incubated for 20 min at 4 °C in the dark and then washed twice with PBS and resuspended in 300 μl CellFix (Becton Dickinson). An irrelevant anti-mouse IgG1 mAb (MOPC-31C; BD Biosciences) was used as a negative control. Analysis was performed using FlowJo software version 7.2.2 (TreeStar).

**Gamma interferon (IFN-γ) assay.** Quantification of IFN-γ in plasma at days 0, 3, 7, 10, 14 and 28 p.i. was evaluated using a commercial ELISA kit (Monkey IFN-γ ELISA kit; Cell Sciences) according to the manufacturer’s instructions. The sensitivity of the kit was 2 pg (ml plasma)⁻¹.

**Statistical analysis.** Comparisons between macaques and mandrills were performed using a non-parametric Mann–Whitney U-test with Statistica software (StatSoft France, version 7.1; www.statsoft.fr).

## RESULTS

**Antibody response profile in infected mandrills and rhesus macaques**

V3 ELISA testing showed that all SIVmnd-1- and SIVmnd-2-infected rhesus macaques and the four mandrills had seroconverted by day 28 p.i. (Fig. 1a, b). However, anti-V3 antibody titres were significantly higher for rhesus macaques than for mandrills at day 360 (P<0.02) (Fig. 1c).

**Plasma SIVmnd-1 and SIVmnd-2 dynamics in rhesus macaques and mandrills**

Acute infection showed common replication patterns for both viruses in mandrills and rhesus macaques. In mandrills, plasma VLs peaked between days 7 and 11 and ranged from 1.5 × 10⁹ to 2.6 × 10⁹ RNA copies ml⁻¹ (Fig. 2a). In rhesus macaques, plasma VLs ranged from 5.9 × 10⁹ to 3.8 × 10⁹ RNA copies ml⁻¹ between days 7 and 14 (Fig. 2b). No significant VL difference was found during acute infection between mandrills and rhesus macaques. Interestingly, in three rhesus macaques (93057, 93183A and 93183B), plasma VLs decreased just before reaching the
peak (Fig. 2b). This biphasic profile was confirmed in several independent plasma VL determinations.

The post-acute and chronic replication patterns were very different between mandrills and rhesus macaques. In mandrills, plasma VLs reached a set point by day 28 p.i., which was maintained during the follow-up period. The set-point VLs were remarkably stable with the exception of some points around day 180 p.i., when VLs decreased to reach $10^4$ RNA copies ml$^{-1}$. At the 2-year follow-up, plasma VLs were $8.4 \times 10^4$ to $9.9 \times 10^5$ RNA copies ml$^{-1}$ (mean: $3.95 \times 10^5$) for both SIVmnd-1- and SIVmnd-2-infected mandrills (Fig. 2a). In rhesus macaques, plasma VLs decreased below the threshold of detection ($250$ SIVmnd RNA copies ml$^{-1}$) between days 90 and 330. However, this control of SIVmnd-1 and SIVmnd-2 replication in rhesus macaques was only transient. Blips of detectable VL were observed for both viruses in rhesus macaques. At the end of the follow-up period, 2 years after infection, all rhesus macaques but one (93036) showed detectable VLs that ranged between $9.2 \times 10^3$ and $2.4 \times 10^5$ RNA copies ml$^{-1}$ (mean: $8.9 \times 10^4$) (Fig. 2b). Plasma VL dynamics in rhesus macaques showed no significant differences for SIVmnd-1 and SIVmnd-2.

**LN RNA VL dynamics**

SIVmnd RNA loads in LNs generally paralleled that of plasma VL dynamics (Fig. 2c, d). At day 7 p.i., RNA copies ranged from $3.5 \times 10^5$ to $1.5 \times 10^6$ per $10^6$ cells for mandrills and $1.8 \times 10^3$ to $1.3 \times 10^6$ per $10^6$ cells for rhesus macaques. At day 60 p.i., in all cases, LN RNA VL decreased moderately by around 1 log. After 1 year of infection, there was no significant difference between rhesus macaques and mandrills except for two rhesus macaques (93057 and 93183A) that had a very low LN VL ($5 \times 10^2$ RNA copies per $10^6$ cells).

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**Fig. 1.** Anti-V3 antibody titres in mandrills (a) and rhesus macaques (b) infected with SIVmnd-1 (red) and SIVmnd-2 (blue). (c) Comparison of Anti-V3 antibody titres between mandrills and rhesus macaques at day 360 using a Mann–Whitney U-test.
SIVmnd-1 and SIVmnd-2 have a different impact on CD4⁺ and CD8⁺ T cells in mandrills and rhesus macaques

In spite of the fact that acute virus replication patterns were similar between mandrills and rhesus macaques, their impact on CD4⁺ T cells was different for the two species. A moderate, transient increase in CD4⁺ T cells was observed in two mandrills just before the peak VL, followed by moderate decreases in CD4⁺ T cells occurring after the peak of virus replication in two of the four mandrills (Fig. 3a). During the chronic infection, CD4⁺ T-cell counts returned to close to the baseline levels in all mandrills (Fig. 3a). As previously reported, we did not observe any difference in CD4⁺ T-cell counts between mandrills infected with SIVmnd-1 and SIVmnd-2 (Fig. 3a).

Conversely, in both SIVmnd-1- and SIVmnd-2-infected rhesus macaques, a significant decrease in CD4⁺ T-cell counts occurred during week 1 p.i., which was followed by a partial CD4⁺ T-cell restoration. Moreover, differences in CD4⁺ T-cell counts were recorded between SIVmnd-1- and SIVmnd-2-infected rhesus macaques. Thus, during the acute infection, CD4⁺ T-cell depletion appeared to be more important in SIVmnd-2-infected macaques (93036, 93183B and 93046A), in which 56–80% of the CD4⁺ T cells were depleted (Table 1). In SIVmnd-1-infected rhesus macaques, only 39–64% of CD4⁺ T cells were depleted during the acute infection (Table 1). However, these differences were not significant ($P=0.12$). Conversely, during the chronic infection, after a 2-year follow-up period, the SIVmnd-2-infected rhesus macaques maintained their CD4⁺ T-cell counts, whereas the SIVmnd-1-infected rhesus macaques showed significant CD4⁺ T-cell depletion (Fig. 4). Thus, the mean decrease in SIVmnd-1-infected rhesus macaques was significantly higher (67%) than in SIVmnd-2-infected rhesus macaques (14%) ($P<0.05$).

Only moderate variations in CD8⁺ T-cell levels were observed in SIVmnd-infected mandrills during acute or chronic infection (Fig. 3c). Conversely, in four out of six macaques (both SIVmnd-1- and SIVmnd-2-infected), CD8⁺ T-cell counts increased considerably after the peak of virus replication, and frequent transient increases were noted during the follow-up period (Fig. 3d). At 2 years p.i., CD8⁺ T cells returned to baseline levels in all SIVmnd-1- and SIVmnd-2-infected rhesus macaques, except for P9110A. Interestingly, this decrease in CD8⁺ T-cell levels corresponded to an increase in virus replication, with plasma VLs being consistently detectable at 2 years p.i. (Fig. 2b).
Fig. 3. Variations in peripheral CD4^+ and CD8^+ T-cell counts in mandrills (a, c) and macaques (b, d) compared with baseline. Animals infected with SIVmnd-1 are indicated in red, whilst animals infected with SIVmnd-2 are indicated in blue. The means are shown as solid lines.

Table 1. Number of CD4^+ T cells in macaques infected by SIVmnd-1 and SIVmnd-2 at day 0 and on the day corresponding to the peak VL, and percentage decrease from baseline

<table>
<thead>
<tr>
<th>SIVmnd type</th>
<th>Macaque no.</th>
<th>No. CD4^+ T cells µl^{-1} (day 0)</th>
<th>No. CD4^+ T cells µl^{-1} (day p.i.)</th>
<th>Decrease from baseline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIVmnd-1</td>
<td>93057</td>
<td>691</td>
<td>425 (day 11)</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>93183A</td>
<td>509</td>
<td>261 (day 7)</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>P9110A</td>
<td>637</td>
<td>228 (day 7)</td>
<td>64</td>
</tr>
<tr>
<td>SIVmnd-2</td>
<td>93036</td>
<td>510</td>
<td>96 (day 14)</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>93183B</td>
<td>624</td>
<td>116 (day 4)</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>93046A</td>
<td>816</td>
<td>356 (day 7)</td>
<td>56</td>
</tr>
</tbody>
</table>
Fig. 6 shows significant increases (up to 10-fold) in the levels of IFN-γ were observed in rhesus macaques (Fig. 6b) (P=0.01). In both species, these increases corresponded to the peak of virus replication.

**DISCUSSION**

Systematic studies of cross-species-transmitted SIV infections are critical in order to understand the mechanisms responsible for the spectacular increase in pathogenicity that has been observed for some of these viruses. Here, we showed that SIVmnd-1 and SIVmnd-2, two viruses that naturally infect mandrills in the wild, induced persistent infections in rhesus macaques with relatively similar replication patterns. However, the pathogenic outcomes of these two infections were different: SIVmnd-2-infected rhesus macaques maintained their CD4+ T-cell levels during a 2-year follow-up period, whilst significant CD4+ T-cell depletion occurred in SIVmnd-1-infected rhesus macaques, in the context of a higher T-cell immune activation, suggesting a more pathogenic outcome.

Data published prior to this study on the outcome of cross-species-transmitted infections in Asian primates identified some interesting patterns. The first was a general tendency towards controlled infection in rhesus macaques infected with SIVs from African primates, which includes Sykes’ monkeys, African green monkeys, talapoin monkeys and mangabeyes. The second was a general tendency towards persistent, progressive infection in pig-tailed macaques after infection with various SIVs.

For example, earlier studies reported that exposure of rhesus macaques to SIVsmm, the virus naturally infecting sooty mangabeys, resulted in persistent infection and rapid progression to AIDS (Murphey-Corb et al., 1986). In fact, accidental transmission of SIVsmm to rhesus macaques led to the development of the current animal model for AIDS research (Apetrei et al., 2005, 2006; Gormus et al., 2004). However, as shown in subsequent studies, the current reference strains for use in macaques resulted from serial passage of SIVsmm, a factor that contributed to this increased pathogenicity (Apetrei et al., 2006; Mansfield et al., 1995). In fact, the intrinsic pathogenic potential of primary SIVsmm isolates in rhesus macaques is significantly lower than initially believed (C. Apetrei, unpublished data), similar to other studies that have reported that SIVsyk (Hirsch et al., 1993), SIVagm (Pandrea et al., 2007), SIVtal (Osterhaus et al., 1999) and SIVrcm (Smith et al., 1998) replicate only transiently in rhesus macaques. In all of these cases, replication only occurred during acute infection, and both VLs and viral cultures were generally negative starting from relatively early time points of chronic infection.

As mentioned above, pig-tailed macaques seem to be more susceptible to cross-species-transmitted viruses than rhesus macaques. Progression to AIDS has been reported to occur...
Fig. 5. Dynamics of immune activation, as illustrated by changes in HLA-DR expression in peripheral CD4⁺ and CD8⁺ T cells from mandrills (a, b) and macaques (c, d). Animals infected with SIVmnd-1 are indicated in red, whilst animals infected with SIVmnd-2 are indicated in blue. Comparison of HLA-DR expression on CD4⁺ and CD8⁺ T cells between mandrills and rhesus macaques at day 750 was carried out using a Mann–Whitney U-test. *, P < 0.01; **, P < 0.001.

Fig. 6. Quantification of IFN-γ in the plasma of SIVmnd-1- and SIVmnd-2-infected mandrills (a) and rhesus macaques (b). Monkeys infected with SIVmnd-1 are indicated in red, whilst monkeys infected with SIVmnd-2 are indicated in blue. The detection limit was 2 pg ml⁻¹.
in pig-tailed macaques after exposure to SIVsmm (Fultz, 1991), SIVagm (Hirsch et al., 1995), SIVhoest and SIVsun (Beer et al., 2005). However, it is important to note that disease progression in pig-tailed macaques upon SIV cross-species transmission is not a constant feature: in the same experiment, some animals may progress to AIDS, whereas others may control the infection, for example in SIVagm infection (Hirsch et al., 1995).

Interestingly, rhesus macaques infected with viruses that are known to naturally infect monkey species phylogenetically close to rhesus macaques (within the Papionini tribe) have generated discordant data. For example, SIVsmm produced a persistent infection (McClure et al., 1989; Murphey-Corb et al., 1986), whilst SIVrcm from red-capped mangabeys resulted in a controlled infection (Smith et al., 1998)

Mandrills are also phylogenetically close to rhesus macaques, as both belong to the Papionini tribe (Harris & Disotell, 1998). Mandrills are naturally infected by two SIV types, SIVmnd-1 and SIVmnd-2, which have different origins. SIVmnd-2 is closely related phylogenetically to other Papionini SIVs, such as SIVrcm and SIVdrl from drills (Clewley et al., 1998; Hu et al., 2003; Souquiere et al., 2001). In its current form, SIVmnd-2 emerged following recombination of the ancestral SIVrcm-related virus with the SIVmnd-1 ancestor. This recombination was probably ancient, as the recombinant virus was also transmitted to the related drill monkey (Clewley et al., 1998; Hu et al., 2003). In contrast, it is considered that SIVmnd-1 emerged following cross-species transmission from the sympatric l’Hoesti supergroup. The cross-species transmission event resulting in the emergence of SIVmnd-1 was probably ancient, before the subspeciation of mandrills, which occurred about 1 million years ago (Telfer et al., 2003).

Previously, we demonstrated that the pathogenic potential of SIVmnd-1 in mandrills is not different from that of other species-specific SIVs (Muller & Barré-Sinoussi, 2003; Pandrea et al., 2006; Silvestri et al., 2003) and we reported that there was no pathogenic difference between SIVmnd-1 and SIVmnd-2 in mandrills (Onanga et al., 2002, 2006). In the present study, we first confirmed these findings and showed again, although in a limited number of mandrills, that there was no discernible difference in biological or clinical outcome between SIVmnd-1 and SIVmnd-2. The main objective of this study, however, was to compare the outcome of the cross-species transmission of these viruses in rhesus macaques.

We have shown here that both SIVmnd-1 and SIVmnd-2 induce persistent infections in rhesus macaques. We found different patterns of T-cell activation and antibody production between SIVmnd-infected rhesus macaques and mandrills. In addition, we observed differences in the secretion of pro-inflammatory cytokines, as illustrated by the production of IFN-γ, which was significantly higher in rhesus macaques than in mandrills. These findings confirm previously reported divergent host responses between pathogenic and non-pathogenic SIV infections (Silvestri et al., 2005).

Moreover, during the follow-up period, a significant and persistent CD4+ T-cell depletion was observed in SIVmnd-1-infected rhesus macaques. This result was somewhat unexpected, as SIVmnd-1 is a virus acquired by mandrills through cross-species transmission (Beer et al., 1999; Souquiere et al., 2001) and is more closely related to Cercopithecus-specific SIVs. Based on the results of previous studies, we would have expected Cercopithecus-specific SIVs to be associated with lower pathogenicity in rhesus macaques. However, SIVmnd-1-infected rhesus macaques showed immunological signs of a more pathogenic infection, as supported by continuous CD4+ T-cell decline. Together with the similar pathogenicity of SIVmnd-1 and SIVmnd-2 in mandrills, these results suggest that SIVmnd-1 is currently well adapted for replication and persistence in Papionini hosts. SIVmnd-1 adaptation to its ‘new’ mandrill host is probably the best explanation for the relatively high pathogenicity of this virus in rhesus macaques, as illustrated by persistent infection and CD4+ T-cell depletion.

Immune activation of T cells and plasma IFN-γ secretion were identical for both types of SIVmnd in rhesus macaques, which is apparently in disagreement with the different pathogenic outcomes of the two infections. Nevertheless, our results did not exclude the possibility that SIVmnd-2-infected rhesus macaques may progress to AIDS after a longer incubation period beyond the follow-up period in this study. Alternatively, it is possible that the pathogenicity of these two viruses differs because of different mechanisms of CD4+ T-cell depletion in SIVmnd-1 and SIVmnd-2 infections. A previous study reported CXCR4 co-receptor usage for the SIVmnd-1 strain GB1, from which the virus strains used in the present study are derived (Schols & De Clercq, 1998), and CXCR4-tropic SIVs are more prone to induce CD4+ T-cell depletion at extra-intestinal mucosal sites. In contrast, CCR5-tropic SIVs, such as SIVmnd-2, are more prone to induce intestinal depletion of effector memory CD4+ T cells, as described previously for SIVsmm strains (Brown et al., 2007; Nishimura et al., 2007). In order to address this question, investigation of the mucosal pathogenicity of these two viruses is needed.

The difference in pathogenic potential for rhesus macaques between SIVmnd-2, which induced persistent infection, and SIVrcm, which is controlled in rhesus macaques, may also appear somewhat surprising in the context of the close phylogenetic relationship between these two viruses. However, this difference could also be associated with the fact that these viruses utilize different receptors, specifically CCR2b in the case of SIVrcm (Chen et al., 1998) and CCR5 in the case of SIVmnd-2 (Hu et al., 2003). In addition, this difference may be related to the recombinant nature of SIVmnd-2, which has thus gained an evolutionary advantage compared with SIVrcm. Similar recombination
events have been described for SIVcpz, which evolved as a chimpanzee virus following recombination of viruses related to SIVrcm and SIVgsm/mon/mus (Bailes et al., 2003). This relatively high propensity for successful cross-species transmission of the SIVmnd viruses, one already cross-species transmitted and the second a recombinant virus, suggests that host species barriers can be overcome relatively easily by cross-species-transmitted SIVs through recombination and adaptation. As SIVcpz is the direct ancestor of HIV-1, this observation calls for effective measures for prevention of cross-species transmission of SIVs to humans in central Africa, the area of endemicity of most SIVs.

ACKNOWLEDGEMENTS

This work was funded by the Centre International de Recherches Médicales de Franceville (CIRMF), Gabon. CIRMF is supported by the Government of Gabon, Total-Elf Gabon and the Ministère de la Coopération Française. We would also like to thank the staff of the CIRMF Primate Center. We thank Patricia Reed for critical reading of the manuscript. I. P. and C. A. are supported by grants RO1 AI064066 and R21AI069935 (I. P.) and RO1 AI065325 (C. A.) from the National Institute of Health.

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