[Frontiers in Bioscience 9, 521-539, January 1, 2004]

THE PARADOX OF SIMIAN IMMUNODEFICIENCY VIRUS INFECTION IN SOOTY MANGABEYS: ACTIVE VIRAL REPLICATION WITHOUT DISEASE PROGRESSION

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1. ABSTRACT

Simian immunodeficiency virus SIVsm causes an asymptomatic infection in its natural host, the sooty mangabey, but induces an immunodeficiency syndrome very similar to human AIDS when transferred to a new host species such as the rhesus macaque. Unexpectedly, SIVsm replication dynamics is comparable in the two species, with rapid accumulation of viral mutations and a high viral load detected in both mangabeys and macaques. In contrast, clear differences are observed in immune parameters. Pathogenic SIV infection in macaques is associated with decreased CD4+ T cell numbers and signs of generalized immune activation, such as increased numbers of cycling and apoptotic T cells, hyperplasic lymphoid tissues, and exacerbated immune responses. Mangabeys with asymptomatic SIV infection show normal T cell regeneration parameters and signs of a moderate immune response, appropriate in the setting of chronic viral infection. The comparative analysis of simian models thus suggests that viral load alone cannot account for progression to disease, and that the capacity of primate lentiviruses to induce abnormal immune activation underlies AIDS pathogenesis.

2. INTRODUCTION

Simian immunodeficiency viruses (SIVs) infect more than thirty species of Old World monkeys and apes of African origin (1-4). Though SIVs share many structural

and biological properties with human immunodeficiency virus (HIV), they do not seem to induce an immunodeficiency syndrome in their natural hosts. Studies of natural SIV infections thus provide unique opportunities to identify mechanisms of resistance to AIDS.

The study of natural SIVsm infection in the sooty mangabey (*Cercocebus atys atys*) is of particular interest, since this virus is thought to have crossed the species barrier and to be at the origin of the human AIDS virus HIV-2. In addition, SIVsm can induce a pathogenic infection very similar to AIDS when experimentally inoculated into macaque species of Asian origin (5-8), and thus provides a valuable simian model for AIDS.

This review will focus on insights gained from the comparative analysis of SIV infection in sooty mangabeys and rhesus macaques. Aspects pertaining to the emergence and evolution of human AIDS viruses will be treated in depth in the paper by P. Marx and colleagues (Front. Biosci., this issue) and will only be briefly mentioned here.

3. IDENTIFICATION OF SIVsm AS THE SOURCE OF THE SIMIAN AIDS VIRUS SIVmac

Simian immunodeficiency virus was first identified in 1985 at the New England Regional Primate Research Center (RPRC), in a colony of rhesus macaques (*Macaca mulatta*) with unexplained cases of immunodeficiency-associated pathologies and lymphomas (9). The isolated virus, designated SIVmac, was antigenically and genetically related to the recently identified human immunodeficiency virus type 2 (HIV-2) (10), but was clearly distinct from the type D retroviruses SRV and MPMV that were also responsible for immunodeficiency-associated pathologies in rhesus macaque colonies (11, 12). Inoculation of SIVmac in naive rhesus macaques induced an immunodeficiency syndrome remarkably similar to human AIDS, with a progressive depletetion of circulating CD4+ T lymphocytes, the occurrence of oportunistic infections by pathogens such as *Pneumocystis carinii*, mycobacteria, and cytomegalovirus, and an increased risk of developing lymphomas (13). Viruses genetically similar to SIVmac were isolated from pigtailed macaques (*M. nemestrina*) and stumptailed macaques (*M.* arctoides) in other primate centers (14). It was soon recognized that a lentivirus closely related to SIVmac infected sooty mangabeys kept at the Tulane RPRC in Lousiana (5). The virus, initially designated as STLV-III/Delta and later renamed as SIVsm, did not appear to cause disease in the mangabeys. SIVsm infection was also detected in sooty mangabey colonies at the Yerkes RPRC (Georgia) and the California RPRC, with seroprevalence rates reaching up to 80 % of adult animals (6, 15). Sooty mangabeys were found to be infected in the wild, suggesting that they were natural hosts for SIVsm (4, 16-18). Experimental transmission of viral isolates or blood from asymptomatic sooty mangabeys to macaques induced a pathogenic infection indistinguishable from that caused by SIVmac (7, 19, 20). Retrospective analysis indicated that accidental transmission of sooty mangabey tissues to macaques was the likely origin of some of the immunodeficiency disease outbreaks that struck macaque colonies during the seventies (11). In one documented case, sooty mangabeys were sent from the Yerkes RPRC to the Tulane RPRC, to be used in a primate model for leprosy. Inoculation of sooty mangabey tissues to rhesus macaques did transfer the leprosy bacillus but also SIVsm, which induced simian AIDS in recipient animals (21, 22).

SIVsm and SIVmac cannot be easily distinguished genetically and could be considered as the same virus. Both terms are indifferently used to designate viral isolates that induce disease in macaques, such as SIVsmB670 or SIVmac251. However, subtle tropism differences are observed between primary SIV isolates obtained from macaques and mangabeys, suggesting a degree of host-specific adaptation (see below). For this reason, macaque and mangabey viruses will be designated by separate terms (SIVmac and SIVsm, respectively) within this review.

4. ROLE OF SIVsm IN THE EMERGENCE OF HUMAN AIDS VIRUS HIV-2

Converging evidence supports the idea that cross-species transmission of the sooty mangabey lentivirus SIVsm to humans is at the origin of HIV-2.

4.1. Genetic evidence

SIVsm and HIV-2 are genetically related, with a mean of 75-80 % amino acid identity within the pol gene, and share a common genomic organization, characterized by the the presence of two accessory genes *vpr* and *vpx* in the central region of the genome (16-18, 23). SIVsm, SIVmac and HIV-2 form a distinct phylogenetic group among other primate lentiviruses. Within this group, HIV-2 and SIVsm show a high genetic diversity (up to 35% nucleotide divergence at the genome level) that is characteristic of primate lentiviruses (4, 24-28). Importantly, human and simian viruses do not cluster separately but rather occupy interspersed branches on phylogenetic trees. This tree topology can only be explained by the occurrence of multiple cross-species transmissions. Thus, the phylogeny of the HIV-2 / SIVsm group suggests that transmission of sooty mangabey viruses to humans occurred upon several occasions in the past.

4.2. Geographic coincidence

The distributions of SIVsm and HIV-2 infections are both centered in West Africa. The natural range of sooty mangabeys extends from Sierra Leone and Liberia to the Western half of Ivory Coast (29). The fragmented distribution of sooty mangabey populations suggests that their range was wider in the past and has been constricted by loss of forested habitat. The area of endemic HIV-2 infection clearly overlaps with the sooty mangabey range, even though it extends wider in West Africa. The two epidemic HIV-2 groups A and B have spread to the Western world and to other regions in Africa, but, importantly, all other HIV-2 groups (C to G) were identified within the range of the sooty mangabey. The most convincing evidence for cross-species transmission is

based on the phylogenetic clustering of SIVsm and HIV-2 isolated from the same geographic area, either in Sierra Leone or in Liberia (4, 17, 24).

4.3. Risk of cross-species transmission

SIVsm seroprevalence in the wild is variable, ranging from 0 to 40% depending on the sooty mangabey troop studied (4). A general seroprevalence of 22% suggests that ample opportunities exist for cross-species transmission. Sooty mangabeys inhabit primary and secondary forests, but are also found in gallery forests, swamp forests, and mosaic zones that are closer to human habitat (29). Sooty mangabeys are actively hunted in Western Africa, and tend to be considered as agricultural pests because they sometimes raid farms. A frequent scenario is that adult mangabeys are hunted for food while the orphaned youngs are kept as pets in the villages. SIVsm seroprevalence in pet monkeys is relatively low (4%), probably because most animals were captured at a young age, before they acquired SIVsm infection (4). Given the higher infection rate of adult feral sooty mangabeys, the highest risk of human exposure to SIVsm may be during the hunting and butchering of these monkeys.

Recent evidence indicates that SIV infects a plethora of African monkey species, and that SIV cross-reactive antibodies can be detected at high frequency in primate bushmeat sold in markets or near logging concessions (1, 2). The highest seroprevalence are found in guenons (Cercopithecus genus) and African green monkeys (Chlorocebus genus), for which the majority of adult animals carry SIV (3, 30). Given their widespread distribution and numerical importance, African green monkeys and guenons represent a major SIV reservoir. Mangabeys and phylogenetically related species such as mandrills and baboons have an intermediate prevalence of SIV infection. Both "drill-mangabeys" of the genus Cercocebus and "baboon-mangabeys" of the genus Lophocebus can be infected (1). Well-characterized viruses include SIVsm from sooty mangabeys but also SIVrcm from red-capped mangabeys (*Cercocebus torquatus torquatus*) in Cameroon and Nigeria (31-33). SIV also infects several species of colobus, which belong to a genetically distant group of Old World monkeys (34). Lastly, SIV infection has been detected at low seroprevalence in one ape species, the chimpanzee (*Pan troglodytes*). SIVcpz isolated from chimpanzee is phylogenetically related to HIV-1 and is the likely ancestor of the human virus (2, 35, 36). Considering the diversity of natural SIV infection in the wild, the risk of transmission of novel simian lentiviruses to humans is not negligible. A partial serological reactivity to SIV from mandrills was recently described in a person from Cameroon, suggesting that cross-species transmission of diverse SIVs does occur (37).

5. SIVsm PATHOGENIC POTENTIAL

5.1. Absence of pathogenesis in natural SIV infection

SIV infection has not been shown to cause disease in its natural hosts, with a few exceptions. This issue is obviously difficult to address in the wild, but has been addressed by epidemiological studies of sooty mangabey and African green monkey (AGM) populations bred in primate centers. For instance, SIVsm infection was not associated with a reduction of life expectancy or with particular diseases in the sooty mangabey population of the Yerkes RPRC (38). SIVsm seroprevalence is low in the young animals, but rises sharply in the juveniles and young adult mangabeys (between 2 and 4 years of age), suggesting that SIVsm is mainly transmitted through the sexual route. SIVsm infected sooty mangabeys can live up to three decades in captivity without developing simian AIDS, suggesting an absence of pathologic consequences (27, 38). Similarly, SIV infected AGM appear to remain asymptomatic in the long term (39). One possible case of pathology was reported in one naturally infected mandrill that showed CD4+ T cell depletion (40). Also, chimpanzees inoculated with the human virus HIV-1 can develop AIDS (one case reported in animal C499) or show signs of CD4+ T cell depletion (3 cases reported) (41-43). Transfer of blood from C499 to a naive chimpanzee readily induced CD4+ T cell depletion, suggesting that viral evolution toward a more pathogenic form had occurred (42). One "hyperpathogenic" strain of SIVsm, termed SIVsmPBj14, has been reported to induce an acute disease distinct from AIDS in sooty mangabeys and rhesus macaques (19, 44, 45). The PBj14 strain, which was isolated upon passage of a classic SIVsm strain into macaques, causes profuse diarrhea, dehydration, and severe lymphopenia and is usually lethal within two weeks in pig-tailed macaques. The pathogenesis of this novel syndrome appears to be cytokine-dependent and to result from the massive activation and infiltration of lymphocytes, particularly within the gastrointestinal tract (46). Taken together, these findings suggest that host / virus equilibrium may occasionally break down in SIV natural hosts. However, the vast majority of naturally infected animals that were followed on the long term remained healthy.

5.2. Acquisition of virulence in new hosts

Primate lentiviruses clearly have the potential to become pathogenic when transferred to new host species. The evidence is experimental in the case of SIVsm transmission to macaques, and based on phylogenetic inference in the case of human AIDS viruses. It is noteworthy that macaques, which are monkeys of Asian origin, do not carry SIV infection in the wild , and thus represent new hosts for primate lentiviruses (15, 47). Similarly, humans appear to be recent hosts for lentiviruses since AIDS cases have not been detected prior to 1959 (48). A likely hypothesis is that lack of host / virus adaptation underlies HIV and SIV pathogenesis in primates.

SIVsm is not the only lentivirus capable of inducing an AIDS-like disease in monkeys. Experimental inoculation of the African green monkey virus SIVagm90 causes an immunodeficiency syndrome in pig tailed macaques (*M. nemestrina*) (49). Similarly, SIVl'hoest, the virus that naturally infect l'Hoest monkeys (*Cercopithecus lhoesti*), is pathogenic when inoculated intravenously into pig-tailed macaques (50). Relatively few host / virus combinations have been tested in SIV cross-species

transmission experiments, so that the spectrum of primate lentiviruses susceptible to become pathogenic is not fully known.

An important issue is whether cross-species transmission of SIVs to humans or monkeys readily generates AIDS viruses or whether further adaptation to the new host is required for the emergence of a virulent strain. Elements of response can be obtained from the animal models of AIDS: rhesus macaques infected with certain SIVsm primary isolates have been shown to develop AIDS upon the first passage. For instance, intravenous inoculation of the SIVsmB670 strain resulted in rapid CD4+ T cell decline and development of opportunistic infections in rhesus macaques (7). On the other hand, inoculation of the SIVsm strains SL92b obtained in feral sooty mangabeys did not cause CD4+ T cell decrease nor clinical signs in rhesus macaques over a 5 years observation period (P. Marx, unpublished data). It is also relevant that accidental SIVsm or SIVmac transmission to laboratory and animal workers has been documented in at least 3 instances, with no cases of disease reported so far. One case resulted in a transient seroconversion, while another led to a persistent infection with rising antibody titers (no details are available for the third case) (51, 52). The variable natural histories associated with HIV infection are also informative. Epidemiological data indicate that HIV2 infection is associated with a slower rate of disease progression than HIV-1 infection (53). Certain individuals are infected with rare HIV-2 strains that have not spread epidemically, harbor very low virus loads, and show no signs of disease progression, which has raised the possibility of nonpathogenic HIV-2 infection in humans (24). Taken together, these observations suggest that SIVsm has the potential to directly cause disease upon transmission to a new host, but that this may actually be a rare occurrence.

6. SIVsm TROPISM

SIVsm shows a tropism similar to that of most other SIVs, in that it infects preferentially cells that express the receptor CD4 and the coreceptor CCR5. However, subtle differences in tropism are observed between SIVsm and SIVmac, which may reflect the adaptation of SIVsm to a new host species.

6.1. Coreceptor usage

As noted by P. Marx and colleagues, it is important, when comparing tropism of two SIVs, to use viral isolates that have been propagated exclusively in cells of their species of origin. In a study based on such primary isolates, SIVsm and SIVmac were found to use the CCR5 coreceptor most efficiently (54). Both viruses could use CCR5 of macaque and mangabey origin, when expressed in indicator cell lines that co-expressed CD4. The CCR5 proteins from rhesus macaque and sooty mangabey differ by 1 to 2 %, with most amino acid changes corresponding to individual polymorphisms that do not appear to inhibit viral entry (54, 55).

Both SIVsm and SIVmac can use alternate coreceptors, as demonstrated by their capacity to replicate in human PBMC which do not express CCR5 because of the presence of a homozygous deletion of 32 base pairs (Delta32) in this gene (56, 57). Alternate coreceptors include GPR15/BOB and STRL-33/BONZO, though it should be noted that most studies have been performed with coreceptors molecules of human origin expressed in transformed cell lines (58). SIVmac appears unable to use macaque-derived STRL33/BONZO, but can use the homologous human and sooty mangabey genes, which emphasizes the need for additional studies with species-specific coreceptors (59). The importance of alternate coreceptor usage *in vivo* is still to be established, as an SIVmac mutant clone impaired in its ability to use GPR15/BOB still replicates to high levels and causes disease in rhesus macaques (60).

SIVsm and SIVmac do not appear to use the ubiquitous CXCR4 molecule as a coreceptor, even though closely related HIV-2 isolates do so (54). Lack of CXCR4 usage by SIVs is not due to a lack of CXCR4 expression on monkey cells. CXCR4 molecules from mangabey and macaque serve as efficient coreceptors for HIV, suggesting that they are structurally similar to human CXCR4. Therefore, the capacity to use CXCR4 that often characterizes late stage HIV-1 and HIV-2 isolates may depend on adaptations of the viral envelope glycoprotein that occur specifically in the human host.

6.2. Coreceptor polymorphism

CCR5 is a particularly polymorphic protein, both in humans and in monkeys (61-63). Individual polymorphism associated with punctual mutations are frequent and have been described in at least 8 positions in the sooty mangabey CCR5 protein, out of a total of 352 amino acids (54, 55). The most striking example is the occurrence of a 24 base pair deletion (noted Delta24) that is detected at low allelic frequency (4 % of CCR5 alleles) in the sooty mangabey and at high frequency (86 %) in a closely related species, the red-capped mangabey (*Cercocebus torquatus torquatus*) (32). Homozygosity for this mutation is observed in the majority of red-capped mangabeys but has yet to be reported in sooty mangabeys. Monkeys homozygous for the Delta24 deletion are negative for CCR5 expression, since the mutation causes the retention of CCR5 within an intracellular compartment, prevents CCR5 surface expression, and abolishes functional responses to β-chemokines (32, 64). Remarkably, the SIVrcm virus that naturally infects red-capped mangabeys uses CCR2b rather than CCR5 as its coreceptor, a characteristic unique among SIVs described so far. The most likely explanation for the unusual tropism of SIVrcm is that this virus adapted to a CCR5-deficient host by switching coreceptors. This case illustrates the remarkable capacity of primate lentiviruses to adapt to new hosts and emphasizes the risks for emergence of new AIDS viruses with variant properties.

Sooty mangabeys heterozygous for the CCR5 Delta24 allele have a reduced CCR5 expression at the surface of activated lymphocytes (64). However, the presence of the mutation does not appear to diminish the risk of SIVsm transmission. Indeed, among captive mangabeys at the Yerkes RPRC, SIV seroprevalence and

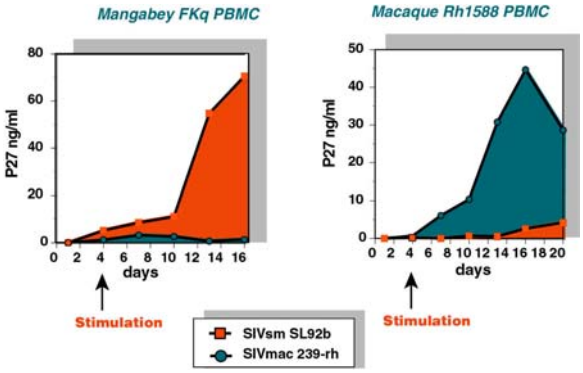

Figure 1. Primary SIVsm and SIVmac isolates replicate preferentially in cells of their species of origin. PBMC from a sooty mangabey and a rhesus macaque were infected with SIV in unstimulated conditions. Cultures were activated 4 days post-infection by the addition of interleukin-2 and the mitogen PHA. The primary isolates SIVsmSL92b and SIVmac239 had been propagated exclusively in cells from their species of origin prior to the experiment. SIVmac239-rh corresponds to an *in vivo* passaged viral isolate derived from the SIVmac239 molecular clone.

viral load were comparable in CCR5 heterozygotes and wild-type animals (64). Therefore, CCR5 expression levels do not appear to be a major limiting factor in natural SIV infection.

The Delta24 deletion in mangabey CCR5 is distinct from the Delta32 deletion detected in human CCR5, which is found at an allelic frequency of up to 15 % in Caucasians (63). It has been speculated that the CCR5 Delta32 allele was selected in humans for its ability to confer resistance to a widespread pathogen. Candidate agents include the plague (65) or poxviruses, some of which use chemokine receptors to enter their target cells (66). It is also likely that the CCR5 delta24 allele was selected in mangabeys to counter infection by a pathogenic agent. The independent occurrence of CCR5 deletion in both humans and mangabeys constitutes a case of convergent evolution, and suggests that negative selection against CCR5 must be a relatively frequent event, possibly mediated by different pathogens.

CCR5 appears to be highly polymorphic in several species, including African green monkeys, which are frequent carriers of the lentivirus SIVagm (67). Of note, an unexpectedly high number of CCR5 non-synonymous nucleotide changes identified in African green monkeys inhibit infection by SIVagm. Therefore, CCR5 may be under selection pressure to confer a degree of resistance to SIV itself. This would suggest that natural SIV infection has a cost in evolutionary terms and is not entirely benign.

6.3. CD4 usage

SIVsm and SIVmac use the CD4 molecule as their primary receptor. A difference between the two viruses concerns their capacity to infect target cells independently of CD4. Many SIVmac isolates, including in particular brain-derived and/or macrophage tropic isolates, have the capacity to infect CD4- CCR5+ cells in culture (68). Though the number of isolates studied is small, this property has not been reported for primary SIVsm, suggesting that infection in natural host species may be more strongly dependent on CD4 (54). In this respect, it is interesting that the sooty mangabey CCR5 molecule frequently carries a proline at position 180, a residue of the second extracellular loop, which has been associated with restriction of CD4-independent infection (69). In contrast, macaque CCR5, which has a serine at position 180, is particularly efficient at allowing SIV infection in the absence of CD4. The capacity to infect cells that express little or no CD4 has been associated with expanded tropism and in particular with the capacity to infect macrophages in vitro (70). One hypothesis is that the strict CD4-dependence characteristic of sooty mangabey isolates results in a narrow spectrum of target cells *in vivo*, a property that would limit virus-induced damage and favor host / virus adaptation. This hypothesis remains to be tested, however, since sooty mangabey macrophages can be infected by SIVsm in vitro (71), and since the nature of SIVsm target cells in vivo remains incompletely characterized.

6.4. CD4 polymorphism

Two major CD4 alleles have been identified in sooty mangabeys (72). The sm1 allele shares 96% amino acid identity with rhesus macaque CD4. The second allele, sm2, has 6 amino acid changes as compared to sm1, including the deletion of one lysine in the CDR-1 region and a threonine to isoleucine substitution the CDR-2 region. Since these last two mutations overlap with the putative HIV envelope glycoprotein binding site in the distal domain of CD4, it is possible that the sm2 allele is not optimal for SIV entry. Genetic screening of sooty mangabeys at the Tulane RPRC showed that the sm2 allele was present at relatively high frequency (0.26) but did not reveal any effect of this allele on the rate of natural SIVsm infection (L. Chakrabarti and P. Marx, unpublished observations). Thus, there was no evidence for natural selection of CD4 alleles that would restrict SIVsm infection *in vivo*.

6.5. Cellular tropism

Primary SIVsm and SIVmac can be distinguished on the basis of cellular tropism *in vitro*. An initial observation was that SIVmac readily infected the human cell line CEMx174, but that primary SIVsm required a period of adaptation before replicating efficiently in this cell line (54). No such differences were observed in the human PM1 cell line that expresses CCR5. Since CEMx174 expresses GPR15/BOB but not CCR5, a possible interpretation was that primary SIVsm did not use human GPR15/BOB efficiently. However, we did not detect differences in GPR15/BOB usage between SIVsm and SIVmac, when assayed on HOS-derived indicator cell-lines (unpublished data). Therefore, other differences, for instance in the use of as yet uncharacterized co-receptors, may account for the restricted replication of SIVsm in the CEMx174 cell line.

Differential tropism of SIVmac and SIVsm is also apparent in PBMC cultures. SIVsm will replicate preferentially in PBMC of sooty mangabey origin, while SIVmac will replicate preferentially in rhesus macaque PBMC. This difference is marked when PBMC are infected in unstimulated conditions and are activated 3-4 days later (Figure 1). This species-specific tropism suggests that, upon cross-species transfer to macaques, SIVsm underwent an adaptation that

optimized its replication into the new host. This adaptation does not revert readily, since experimental inoculation of the highly pathogenic SIVmac239 clone in sooty mangabeys results in low levels of viral replication *in vivo* (73).

6.6. Species-specific restriction

Factors that control the capacity of SIV to replicate in heterologous species have still to be fully characterized. This issue is of importance, since it conditions the emergence of new lentiviruses, and potentially of new AIDS viruses in humans.

In the case of SIVsm, no major blocks in the transmission of this virus to humans have been identified, since the virus replicates to high levels in human PBMC cultures. This is in agreement with the notion that SIVsm is at the origin of HIV-2. In contrast, the African green monkey virus, SIVagm, does not always replicate efficiently in human PBMC. The capacity of SIVagm to infect human cells, including PBMC and macrophages, varies depending on the viral isolate and the human cell donor (74, 75). The function of several SIVagm accessory proteins is impaired in human cells. For instance, the Vif protein of SIVagm/TAN does not enhance viral infectivity in human cells (76), possibly because interaction with the Vif cellular cofactor is defective (77). SIVagm Vpr function may also be impaired, since it does not induce G2/M arrest in human cells (78). These defects may account for the fact that SIVagm-derived lentiviruses have not been identified in humans, even though the risk of zoonotic transmission must be high, due to the widespread range of African green monkeys throughout Subsaharan Africa and the high prevalence of SIVagm infection in these hosts.

Species-specific restriction of primate lentiviruses also occurs at the entry or early post-entry stages of the viral lifecycle. For instance, HIV will not infect cells of New World monkey species such as squirrel monkeys and marmosets, because CD4 and CCR5 in these divergent species are not functional for HIV entry (79). HIV infection is blocked at an early post-entry stage in African green monkey and rhesus macaque cells, due to the presence of a species-specific restriction factor that prevents incoming viral particles from undergoing retrotranscription (80, 81). The nature of these factors, which confer an innate resistance to lentiviruses, is in the process of being unraveled. Recent studies indicate that this restriction may be similar to that of the Fv-1 restriction system in murine cells, in that they target the capsid protein of incoming retroviruses (80, 82).

It should be noted that, in spite of these diverse mechanisms of resistance, several primate lentiviruses have the capacity to replicate efficiently in stimulated human PBMC. These include not only the viruses phylogenetically linked to HIV, namely SIVsm and SIVcpz, but also recently identified SIVs from red-capped mangabeys, L'Hoest monkeys, and mandrills (1, 33, 37, 74). Therefore, multiple divergent lentiviruses have the potential to be transmitted to humans.

7. VIRAL REPLICATION IN NATURAL SIVsm INFECTION

7.1. Viral load in peripheral blood

An unexpected finding is that the level of viral replication and persistence is high in natural SIV infection. It had long been recognized that SIVsm could easily be isolated from cultures of SIV positive sooty mangabeys and that SIVsm Gag p27 antigen could be detected in the plasma of these animals (19). The plasma viral load in naturally infected sooty mangabeys ranges between 10^3 and 10^7 copies of viral RNA per ml plasma, with mean values between 10^4 to 10^6 for the majority of animals (27, 73, 83, 84). Such viral loads are as high as those observed in experimentally infected macaques that progress to disease, though they remain lower than the extremely high viral loads (10^8 to 10^9 RNA copies / ml) seen in macaques categorized as fast progressors. The cellular viral load is also elevated in feral and captive sooty mangabeys, with up to 5 x 10^3 copies of viral DNA per million PBMC (4, 27). SIVsm viral load may have actually been underestimated in previous studies, since it has recently been shown that SIVsm strains that infect sooty mangabeys at the Tulane and Yerkes RPRC colonies are unexpectedly diverse (84). Viral load measurements by PCR or the bDNA method (85) rely on the use of primers and probes designed from consensus SIVsm sequences, and may not optimally detect the most divergent SIVsm strains. Taken together, the evidence point to the presence of constitutively high levels of virus in natural SIVsm infection.

The viral load measured in plasma is a dynamic parameter that depends both on the amount of virus produced by the organism and on the rate at which the virus is cleared (86, 87). The viral load thus reflects the interplay between viral replication and host immune response, and is a critical determinant of the outcome of pathogenic lentiviral infections. Indeed, the level of viremia established after the primary infection stage, called the viral "setpoint", is one of the most accurate predictor of the risk of progression to disease in both HIV and SIVmac infections. Viral loads higher than 10^5 viral RNA copies per ml plasma are above the threshold values associated with disease progression in macaques (88, 89) and are associated with rapid progression to AIDS in humans (90). It is striking that such viral loads are commonly found in sooty mangabeys without detectable pathogenic consequences.

Data on plasma viral loads in other models of natural SIV infection suggests that the viral load is particularly high in sooty mangabeys. Values observed in African green monkeys of the vervet and sabaeus species are somewhat lower, ranging from less than 10^3 to 10^6 RNA copies / ml plasma (91, 92). In mandrills experimentally infected with SIVmnd1, mean viral load values center around 10^5 copies / ml (93). It is interesting that pig-tailed macaques inoculated with the pathogenic strain SIVagm90 developed higher viremia levels than sabaeus monkeys inoculated with the same strain, suggesting that containment of viral replication was more efficient in SIVagm natural hosts (49). The observation of

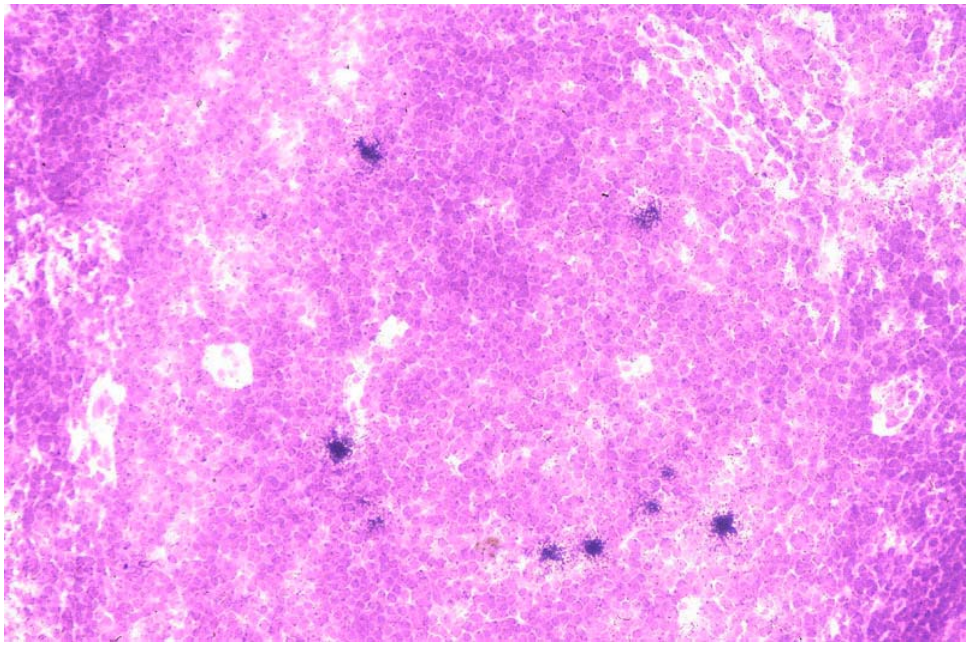

Figure 2. Productive SIVsm infection in lymphoid tissue from a naturally infected sooty mangabey. SIV viral RNA was detected by *in situ* hybridization in lymph node sections, using a ^{35}S-labeled riboprobe. The intensity of the hybridization signal, consisting in the accumulation of black silver grains, indicates productive infection of lymph node cells. (original magnification: x250). Adapted from (27).

low viral loads in a substantial number of naturally infected African green monkeys initially led to the hypothesis that natural hosts of SIV could efficiently control viral replication. Adult Africa green monkeys have naturally low numbers of CD4+ T cells, even in the absence of SIV infection, which suggests that the number of target cells could be limiting in this species (94). However, more recent analyses have revealed that African green monkeys can sustain stable viral loads above 10^5 copies / ml for long periods of times, which does not support the containment hypothesis (75, 91, 92).

7.2. Viral load in lymphoid organs

Assessing SIV infection in lymphoid organs is essential, since these organs constitute the major reservoir of potential CD4+ target cells for the virus. The viral load does not appear particularly low in SIVsm infected lymphoid tissue, since up to 2 x 10^4 copies of viral DNA were detected per million lymph node cells (27). Productively infected cells can be detected by in situ hybridization in peripheral lymph nodes (Figure 2), indicating that SIVsm actively replicate in these organs.

A hallmark of pathogenic HIV an SIV infection is the proportionally higher viral load in CD4+ T cells from lymphoid organs than from peripheral blood, hence the notion that lymphoid organs constitute a major reservoir of the virus (95). In contrast, equivalent cellular viral loads in blood and lymph nodes have been reported in SIVagm infection, raising the possibility that nonpathogenic infection does not preferentially target lymphoid organs (96, 97). In a limited study of sooty mangabey lymphoid tissues, we found results closer to those obtained in HIV infection, with a ratio of 2 to 4 between viral DNA in lymph node cells and viral DNA in PBMC (27). Further studies are needed to determine whether SIV infection targets lymphoid tissue differentially depending on the host species.

7.3. Viral replication rates

The high viremia detected in natural SIVsm infection could result from the accumulation of virus produced at a low rate and that would not be efficiently cleared from the blood, or from a dynamic equilibrium between active viral production and active viral clearance. It is important to determine the rate at which the virus is produced to distinguish between these two scenarios. This parameter can be inferred from the rate at which the virus accumulates mutations, since the number of mutations increases with the number of consecutive viral replication cycles.

The rate of mutation fixation has been extensively studied in the rhesus macaque / SIVmac model. It is known to be high, with the occurrence of 10^{-2} to 10^{-3} substitution per site per year in hypervariable regions of the env gene (98-100). About ten times less mutations (10^{-3} to 10^{-4}) are fixed in highly conserved regions of the genome, because of negative selection against deleterious mutations (101). We have evaluated the minimal mutation rate of SIVsm by comparing viral sequences in three naturally infected sooty mangabeys that were part of the same transmission chain. Rates of 4 x 10^{-3} to 6 x 10^{-3} substitution per site per year were estimated in relatively conserved regions (p17 Gag and TM), which was comparable to the mutation rates found for SIVmac (27). Experimental

inoculation of the SIVsmm9 isolate in rhesus macaques and sooty mangabeys even showed a slightly higher rate of mutation fixation in the mangabey species (102). These findings suggest that SIVsm replicates continuously and at a high rate in its natural host. This notion is further supported by the high genetic variability observed between diverse SIVsm phylogenetic groups. Analysis of phylogenetic trees shows that branch length for SIVsm lineages are comparable to those of HIV-2 lineages (4, 25, 84), which implies that the two viruses evolve at similar rates.

Taken together, the above studies indicate that SIVsm replicate actively and to high levels in its natural host. Therefore, the intrinsic resistance of sooty mangabeys to the pathogenic potential of SIVsm does not result from a low rate of viral production, or from a particularly efficient clearance of produced virus. It follows that SIV pathogenicity does not depend exclusively on the dynamics of viral replication and viral clearance.

7.4. Viral cytopathic effect

A key issue is whether SIVsm is able to kill its target cells. A possibility is that SIVsm replicates in sooty mangabey cells without inducing major cytopathic effects, which would account for the maintenance of elevated numbers of CD4+ T lymphocytes in the face of active viral replication. It is difficult to address this issue satisfactorily *in vitro*. In some studies, SIVsm was reported to be less cytopathic for mangabey cells than for macaque cells, while in others the difference was not apparent (19, 71). Comparisons of death rates in cultured CD4+ T cells of different origin are not straightforward, since primary SIVsm replicates preferentially in CD4+ T cells of the sooty mangabey species. For these reasons, in vivo studies are warranted. A preliminary analysis of the dynamics of viral replication in sooty mangabeys subjected to short-term antiretroviral treatment has been reported (103). The viral load in mangabeys treated with the reverse transcriptase inhibitor PMPA dropped rapidly, indicating that cells infected prior to treatment did not continue to produce virus for a long period of time. This study suggests that the half-life of infected cells is short, and thus that SIVsm may be cytopathic for its target cells in vivo. Thus, sooty mangabeys would be able to sustain an active and cytopathic SIV infection without developing major CD4+ T cell depletion. It is likely that the limited CD4+ T lymphocyte decrease observed in natural SIVsm infection results only from the direct but not indirect cytopathic effect of the virus. Since infected rhesus macaques clearly undergo a more severe CD4+ T cell depletion, both direct and indirect cytopathic effects have to be postulated in the latter species.

8. T CELL HOMEOSTASIS IN NATURAL SIVsm INFECTION

8.1. T cell numbers

Rhesus macaques show signs of CD4+ T cell depletion (numbers below 500 / mm3 blood) as SIVmac infection progresses to disease. In the case of sooty mangabeys, CD4+ T cell numbers are preserved or slightly decreased by SIVsm infection. For instance, we found that naturally infected mangabeys at the Tulane and Yerkes RPRC had a mean value of 1051 CD4+ T cells / mm3, while this number was 1618 / mm3 in uninfected mangabeys (83). A caveat is that several monkey species, including macaques and mangabeys, show an age-dependent decrease in the number of peripheral CD4+ T cells, independently of their infection status. After controlling for age-related changes, we confirmed that SIVsm infection indeed caused a moderate CD4+ T cell loss in sooty mangabeys (104). It was interesting to note that SIVsm viral load also decreased significantly with age, one possible explanation being the reduced availability of CD4+ target cell in older mangabeys. Thus, the number of susceptible target cells may be a limiting factor in natural SIVsm infection. Since CD4+ T cell numbers still remain relatively high, the limiting factor may actually be the number of properly activated T cells that can sustain viral replication rather than the sheer number of CD4+ T cells.

8.2. T cell death

The propensity of CD4+ and CD8+ T lymphocytes to die, as evidenced by spontaneous apoptosis, is increased in HIV-1-infected patients and in SIV-infected macaques (105-107). The susceptibility to Fas-mediated apotosis is equally increased (108). The fact that both T cell subsets are affected indicates that cell death is not only due to direct infection by the virus. Importantly, T cells from SIV-positive sooty mangabeys do not have an increased susceptibility to apoptosis, as measured as the percentage of cell death after overnight incubation with anti-CD3 antibody (109, 110). The intensity of spontaneous T cell apoptosis is also low in HIV infected chimpanzees, and is not inducible by various TCR-dependent activators (108). In a study of African green monkeys, CD4+ T cell apoptosis was unchanged by SIVagm infection, while CD8+ T cell apoptosis was increased in a subset of the animals (107). Therefore, low level of T cell activation-induced apoptosis appears to be a general finding in nonpathogenic lentiviral infections.

8.3. T cell activation

The basis for the differential susceptibility to apoptosis in pathogenic / nonpathogenic lentiviral infections is only imperfectly understood. A prevalent model is that apoptosis corresponds to activation-induced cell death, and that it is increased in pathogenic lentiviral infections because of increased T cell activation. This notion is supported by multiple lines of evidence for abnormal T cell activation in HIV and SIVmac infections, including abnormal expression of cellular activation markers in CD4 + T cells (increased HLA-DR and reduced CD25 expression) and in CD8+ T cells (increases in HLA-DR, CD38, CD57, and CD71 expression), elevated levels of pro-inflammatory cytokines, chemokines and soluble activation markers (neopterin, sCD25, sTNF-RII, ß2-microglobulin), and hyperplasia followed by involution of lymphoid organs (111-114).

Studies of T cell activation status in natural SIVsm infection have been few. One limiting factor is that comparative analyses of activation markers in monkeys

should be performed with SIV+ and SIV- populations that live in similar conditions, since environment can influence the degree of T cell activation. For instance, sooty mangabeys housed outdoors tend to express higher levels of CD69 and HLA-DR markers on CD8+ T cells than those housed indoors. Nevertheless, histopathologic analyses indicate that signs of abnormal T cell activation are not prominent in natural SIVsm infection. Lymph nodes from infected sooty mangabeys show a preserved architecture and normal numbers of lymphoid follicles (27). In contrast, lymph nodes from rhesus macaques collected during the asymptomatic stage of SIVmac infection show signs of paracortical hyperplasia (enlarged T cell zones), follicular hyperplasia (increase in the size and numbers of secondary follicles within the B cell zone), and CD8 + T cell infiltration within germinal centers (115-118). These findings suggest that generalized lymphoid activation is a feature characteristic of pathogenic SIV infection.

The particular disease caused by the hyper-pathogenic strain SIVsmPBj14 in macaques and mangabeys is associated with massive activation of T lymphocytes and overproduction of inflammatory cytokines (46, 119). Interestingly, SIVsmPBj14 has acquired the capacity to replicate in unstimulated PBMC cultures, while classical SIV and HIV cannot do so (44). This property is determined by the duplication of a YXXL motif in the SIV Nef protein, which generates an ITAM-like motif similar to those involved in lymphocyte activation through the T cell receptor (120, 121). The duplication of an NF-kappaB site in the enhancer region of the SIVsmPBj14 LTR may also play a role (122). Introduction of the duplicated YXXL motif in SIVmac or SIVagm molecular clones is sufficient to generate viruses that induce gastrointestinal pathology, but does not entirely reproduce the lethal SIVsmPBj14 phenotype in pigtailed macaques (123). This example shows that acquisition of a single activation motif through viral mimicry is sufficient to alter considerably SIV induced pathology. It is possible that classical SIV and HIV clones code for as yet unidentified activation motifs, given that these viruses do induce an abnormal immune activation *in vivo*.

Chronic T cell activation is not detected in HIV or SIVcpz infected chimpanzees (108, 124) and has not been reported in SIVagm infected African green monkeys (75). The degree of T cell activation thus appears as a key parameter in several models and could play a central role determining the outcome of SIV / HIV infections. The molecular basis for abnormal T cell activation in pathogenic lentiviral infection remains unknown. It is possible that species-specific differences in T cell susceptibility to activation and apoptosis play a role. For instance, sooty mangabey T cells have a better viability in culture than macaque T cells and show a low susceptibility to spontaneous apoptosis (110). In addition, sooty mangabey T cells possess and intrinsic resistance to anergy induced by anti-CD3 antibodies, which may result from their capacity to synthesize IL-2 upon stimulation with anti-CD3 alone (110). Therefore, sooty mangabey T cells may remain functional when the costimulatory molecules, such as CD4, are downregulated or sequestered by the virus. Species-specific differences in the transcriptional induction of kinases have been reported between the CD4+ T cells of SIV-infected mangabeys and macaques (125), which provides support to the view that different signaling pathways are activated in the two species.

8.4. T cell regeneration

Pathogenic HIV and SIV infection are known to perturb T cell regeneration. HIV-1 infected patients undergo a progressive depletion of all CD4+ T cell subsets and of naive CD8+ T cells in the periphery (126), indicating that T cell production fails to compensate for T cell loss. T cell regeneration failure may result from two major mechanisms, whose contributions are still debated: exhaustion of T cell proliferative capacity, which would fail to balance massive and chronic T cell loss, or blocking of T cell production in primary lymphoid organs, including bone marrow and thymus (127-130). It is important to assess these parameters in natural SIV infection, to determine whether resistance to disease is associated with specific T cell regeneration mechanisms.

8.4.1. Peripheral T cell proliferation

Increased T cell proliferation is a consistent feature of pathogenic SIV and HIV infections. Measurements of virus and T lymphocyte dynamics during antiretroviral therapy have revealed a massive production of HIV particles that is paralleled by a rapid turnover rate of CD4+ T lymphocytes (86, 87). The fraction of proliferating T lymphocytes, as measured by expression of the proliferation marker Ki-67, or by the incorporation of ^{2}H-glucose or of BrdU into T cell DNA, is significantly increased in pathogenic HIV and SIV infection (127, 131-134). For instance, Sachsensberg *et al.* reported that the Ki-67+ fractions, which are naturally low in human peripheral CD4+ and CD8+ T cells (mean 1%), increased to a mean of 4-6% in HIV-positive individuals, with values up to 20% in patients with low CD4+ T cell counts (131). Rhesus macaques have an intrinsically higher Ki-67+ fraction in T cells (7.0 %) than humans. SIVmac infection increases macaque T cell proliferation by a factor of 2 in CD4+ T cells and by a factor of 3 in CD8+ T cells. In contrast, sooty mangabey have a relatively low fraction of proliferating T cells (3 to 4% in both the CD4+ and CD8+ compartments), independently of their infection status (Figure 3) (83). Thus, SIV infection does not change the rate of T cell proliferation in mangabeys, while it leads to an increased T cell turnover in macaques.

The absolute number of proliferating T cells can be calculated by multiplying the Ki-67+ fraction by the number of CD4+ or of CD8+ T lymphocytes per mm^3 of blood. Using this measurement, the number of proliferating CD4+ cells is not changed by SIV infection in mangabeys (54 vs 41 Ki67+ cells / mm3 in SIV- and SIV+ animals, respectively), but is significantly decreased in macaques (99 vs 56 Ki67+ cells / mm3 in SIV- and SIV+ animals). Thus, while the fraction of proliferating cells is increased in macaques, this does not translate into an actual increase in the number of proliferating CD4+ T cells, due to the already low CD4+ T cell numbers in infected animals. These findings can be viewed as an indication that the T

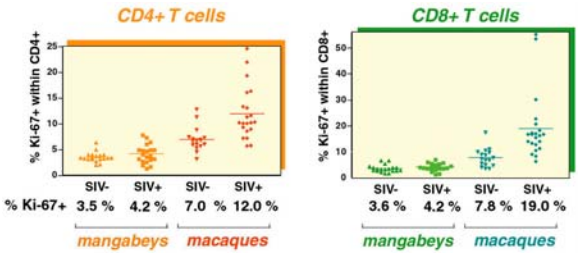

Figure 3. Limited peripheral T cell proliferation in natural SIVsm infection. The percentage of expression of the proliferation marker Ki-67 was measured in monkey CD4+ and CD8+ T cell subsets by cytofluorometry. Four populations were compared: uninfected sooty mangabeys (SIV-), sooty mangabeys naturally infected by SIVsm (SIV+), uninfected rhesus macaques (SIV-), and rhesus macaques experimentally infected by pathogenic SIVmac (SIV+). The mean percentage of Ki-67+ T cells is indicated by a horizontal bar. Adapted from (83).

cell regeneration system is under strain and fails to compensate for CD4+ T cell loss. This phenomenon appears specific to the CD4+ T cell compartment in susceptible species, and is not observed in SIV natural hosts (83).

The dichotomy between proliferation rate and proliferating cell numbers is not observed for CD8+ T cells. In SIV-positive macaques, increase in the proliferating fraction results in more than a doubling of the number of proliferating CD8+ T cells (from 69 to 167 Ki-67+ cells/mm^3). The maintenance of high CD8+ T cell numbers, at least during the early stages of SIVmac infection, may be due to an intrinsically higher proliferation capacity of CD8+ T cells as compared to that of CD4+ T cells. Interestingly, SIV infection also causes an increase in the number of proliferating CD8+ T cells in mangabeys (from 57 to 92 Ki-67+ cells / mm^3). Though the increase of the proliferating fraction is limited, SIV infection still has a detectable impact on proliferating CD8+ lymphocyte numbers in mangabeys and thus cannot be considered as immunologically silent. It is possible that this limited induction of CD8+ T cell proliferation reflects the normal response of the mangabey immune system to chronic viral infection.

A central issue is to identify mechanisms that drive T cell proliferation in pathogenic HIV and SIV infections. It has been recently recognized that proliferation rates measured in T cell labeling studies reflect the dynamics of activated T cells rather than that of the whole T cell population (129, 130). Elevated proliferation and death rates measured in HIV and SIVmac infections are thus indicative of successive bursts of T cells being activated, undergoing activation-induced proliferation, followed by activation-induced cell death. However, the homeostatic response to lymphopenia, that aims at restoring normal T cell numbers, can also lead to peripheral T cell proliferation. This mechanism may come into play in humans or monkeys that experience CD4+ T cell depletion (135). In both cases, proliferation concerns mainly cells with a memory phenotype, so that the two processes are not readily distinguished. However, since increased proliferation rates can be detected in SIV infected macaques that still have preserved peripheral T cell numbers, it is likely that activation induced T cell proliferation is the major mechanism at work in pathogenic SIV infection. The limited proliferation rates in SIV-infected sooty mangabeys would then be consistent with the absence of abnormal T cell activation.

8.4.2. Thymic function

There have been few direct measurements of thymic output in simian models. However, it is possible to indirectly assess this parameter through the detection of T cell receptor excisional circles (TRECS) (136). These DNA episomes are produced during αβ T cell maturation within the thymus and are enriched in recent thymic emigrants. Since TRECs are not replicated during mitosis, they are progressively diluted upon successive cell divisions, and become rare within activated or memory T cells that have actively proliferated. Thus, the quantitation of TRECS is highly dependent on the proportion of recent thymic emigrants within peripheral blood, and can be used to assess thymic activity. Since levels ofTREC also depend on variations in the rates of peripheral T cell proliferation and T cell death, TREC data have to be interpreted in conjunction with these two parameters (137, 138).

Real-time PCR was used to measure TRECs formed by the excision of the V-δ region during rearrangement of the TCRα locus (139). This episome is formed in more than 70% of maturing αβ T cells and thus can be used to monitor maturation within this population. Quantification of this episome by real-time PCR showed that TRECS decreased with age in both rhesus macaques and sooty mangabey populations, which was consistent with an age-dependent decrease of thymic activity. The impact of SIV infection on TREC numbers was moderate, with a limited decrease observed in both rhesus macaques and sooty mangabeys (83). In a longitudinal analysis of 6 chronically infected macaques over a two year period, only 3 of the animals showed a detectable decrease in TREC numbers, which reveals considerable individual variability, as seen in HIV infected humans (137). Decreases in TREC numbers may result from impaired thymic function, from increases in T cell proliferation, which causes TREC dilution, or from preferential death of TREC-rich cells, e.g. naive T cells. The last point is unlikely, given that apoptosis is observed mainly in the activated or memory T cell population. We observed an inverse correlation between TREC numbers and T cell proliferation rates in SIV-infected macaques and mangabeys (83). Similar observations were made for HIV-infected humans, which underscores the impact of T cell proliferation on TREC levels (138). Even if they depend on proliferation levels, TREC measurements can still be informative on thymic function. The notable finding in the monkey study was that TREC numbers did not vary much, even though T cell proliferation was intense in the macaque species. If thymic function was impaired, one would have expected a dramatic decrease of TRECs, that would be further amplified by the rapid proliferation rates. Thus, TREC data

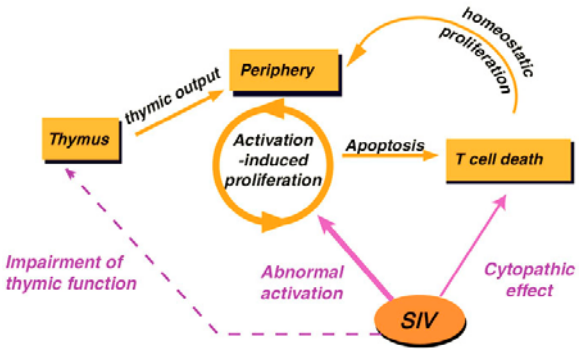

Figure 4. Pathogenic SIV infection perturbs T cell regeneration. The pathways involved in the dynamics of T cell production and destruction are indicated by orange arrows. The deleterious effects of pathogenic SIV infection on T cell regeneration are indicated by purple arrows.

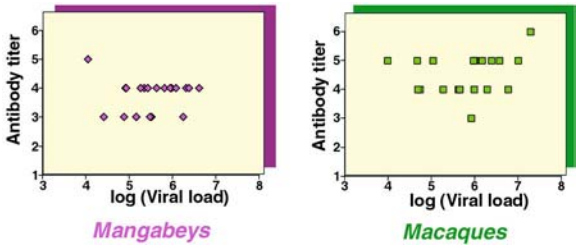

Figure 5. Comparison of antibody responses in SIV infected sooty mangabey and rhesus macaques. Anti-SIV antibody titers were measured in plasma from sooty mangabeys naturally infected with SIVsm and rhesus macaques experimentally infected with SIVmac. Titers correspond to the log of the inverse of the last dilution that gives a positive reading by ELISA assay. Antibody titers (y axis) are plotted in function of the log of the viral load (x axis). The viral load corresponds to the number of SIV RNA copies per ml plasma, as measured by the bDNA assay (85).

suggest that pathogenic SIV infection does not induce a major block in thymic function.

Signs of thymic dysfunction are not prominent in SIV infected adult monkeys, but have been observed in other settings. Histopathologic lesions have been observed in the thymus of AIDS patients. In this case, impairment of thymic function may result from direct infection of thymic progenitors by X4 viral isolates that emerge in late stage disease. Also, signs similar to those of severe congenital thymic defect have been observed in HIV infected infants that progress rapidly to disease (140), suggesting that the mechanism of T cell depletion is in part thymus-dependent in young hosts.

To summarize (Figure 4), pathogenic SIVmac infection perturbs T cell regeneration through several mechanisms: (1) substantial increase in T cell activation, which leads to increased T cell proliferation and apoptosis (2) direct killing of infected CD4+ T cells, which likely contributes to CD4+ T cell depletion given the high rates of viral replication (3) induction of a homeostatic response to decreased T cell numbers, which further drives peripheral proliferation (4) impairment of thymic function, which appears to be limited in adult animals. The respective contributions of these mechanisms need further evaluation. If direct cell killing by SIVmac indeed causes a moderate CD4+ T cell decrease, like that seen in natural SIVsm infection, then most of the CD4+ T cell loss can be attributed to indirect mechanisms that target uninfected cells.

9. ANTIVIRAL IMMUNE RESPONSE

9.1. Humoral responses:

Anti-SIV antibodies are detected in experimentally infected rhesus macaques and naturally infected sooty mangabeys. A difference lies in the intensity of the response, which appears to be lower in the natural host. Neutralizing antibodies are rarely detected in SIV infected sooty mangabeys (141). Analysis of antibody titers in function of viral load reveals that for equivalent viral loads, sooty mangabeys have antibody titers that are about one log lower than rhesus macaques (Figure 5). Measurement of antibody titers were based on ELISA kits containing whole inactivated HIV-2 particles (Elavia-II, Sanofi-Pasteur), so that cross-reactivity issues were minimized, the HIV-2 strain used being approximately equidistant from the SIVmac and SIVsm strainsanalyzed. Studies at the Yerkes RPRC colony show that sooty mangabeys that acquire natural SIVsm infection seroconvert slowly and occasionally remain seronegative for years when tested by ELISA assay (142). Low antibody response may be a general feature in natural SIV infection, since limited or undetectable levels of anti-SIV gag antibodies are frequently noted in infected African green monkeys (143).

9.2. Cellular responses

There is evidence that cellular antiviral immune responses are induced in natural SIVsm infection. CD4+ T cell dependent proliferative responses can be detected in SIV-positive sooty mangabeys, using monocytes as antigen presenting cells (144, 145). In addition, CD8+ T cells from infected sooty mangabeys secrete soluble factors that efficiently control viral replication in vitro (55, 109). Low but detectable CTL responses can be detected in naturally infected sooty mangabeys, which contrasts with the strong CTL responses observed in the early stages of SIVmac infection in rhesus macaques(146) (L. N. Martin, personal communication). Interestingly, CTL responses appear stronger upon experimental infection of sooty mangabey with the SIVmac239 molecular clone, a virus that is adapted to macaques rather than to mangabeys, and that replicates at low levels in the latter species (73). The intracellular cytokine expression profile during primary SIVmac239 infection is marked by increased IL-2 and IFN-γ expression in macaques and, interestingly, by increased IL-10 expression in sooty mangabeys, raising the possibility of a predominant Th2 type of response in the latter species (73). Taken together, these findings suggest that host / virus adaptation in natural SIV infection contributes to dampen the antiviral immune response. The finding of a high synonymous to non-synonymous mutation ratio in SIVsm infected sooty mangabeys suggests that

Table 1. Comparison of SIV infection in sooty mangabeys and rhesus macaques

	SIVsm+ Sooty mangabeys	SIVmac+ Rhesus macaques
Viral parameters		
Viral load (viral RNA copies / ml)	10^3 to 10^7	10^3 to 10^8-10^9
Viral mutation rate	high	high
Viral entry	CD4-dependent	CD4-dependent or independent
Major viral coreceptor	CCR5	CCR5
Host response		
Antibody response	moderate	strong
CTL response	moderate	strong
TREC numbers	limited decrease	limited decrease
number of cycling CD4+ T cells	normal	increased
number of cycling CD8+ T cells	limited increase	increased
T cell apoptosis	normal	increased
CD4 T cell numbers	limited decrease	marked decrease
Lymph node histology	normal	hyperplasia or involution
Pathology	none	progression to AIDS

selection pressure for amino acid changes is limited, and thus that selection pressure exerted by the immune system is of low intensity (102).

The fact that both the humoral and CTL responses are of relatively low intensities does not mean, however, that immunological tolerance to the virus has been achieved. Antiviral immune responses are detectable rather than completely absent, and must contribute to the clearance of infected cells and viral particles. Given the high rate of SIVsm replication in sooty mangabeys, the number of viral particles would increase exponentially and soon reach extremely large numbers if immune-mediated viral clearance did not occur.

How are we, then, to interpret the moderate immune responses characteristic of natural SIVsm infection? These antiviral responses are compatible with prolonged survival, contribute to viral clearance, and do not seem to impair T cell regeneration capacity on the long term. They can be viewed as a balanced and appropriate response to chronic viral infection. In contrast, CTL and antibody responses observed in the early stages of pathogenic SIV and HIV infection are of remarkably high intensity, if not of optimal efficacy. It is striking for instance that activated CTLs can be detected directly in the blood of HIV infected patients, without prior stimulation, a finding that is not common in other viral infections (147). The frequency of CD8+ T cells specific for a single HIV peptide / MHC-I complex can reach up to 5 % in the blood of infected patients, as measured by the utilization of MHC-class I peptide bearing tetramer reagents. Studies in the SIVmac model demonstrate that CD8+ T cell play an essential role in the rapid control of viremia that occurs in the primary infection stage, even though these responses are not sufficient to prevent the establishment of chronic viral replication (148, 149). Several mechanisms can limit the efficacy of the CD8+ T cell response, such as incomplete maturation of CD8+ effector T cells and the lack of CD4+ T cell help. Nevertheless, the breadth of the response indicates that the cellular arm of the immune system is massively activated. The humoral arm of the immune response is similarly activated in pathogenic HIV and SIV infections, as indicated by high antibody titers, generalized follicular hyperplasia in lymphoid organs, and hypergammaglobulinemia (111, 112). Again, several factors limit the efficacy of this humoral response, such as the predominance of antibodies directed to soluble viral antigens rather than to native viral particles, and the slow maturation of neutralizing antibodies (150). The picture that emerges is that of a hyperactive immune response of suboptimal efficacy. Comparisons with models of natural SIV infection indicate that the degree of immune activation observed for a given viral load is abnormal in pathogenic SIV infection. Excessive immune activation is known to be deleterious in the long term (151) and is likely to drive the process that leads to T cell regeneration failure and immunodeficiency.

10. CONCLUSION AND PERSPECTIVE

To recapitulate, SIVsm infection in sooty mangabeys is characterized by active viral replication, persistently high viral loads, a limited decrease in the number of peripheral CD4+ T cells, moderate antiviral immune responses, and generally normal T cell regeneration parameters (Table 1). Pathogenic SIV infection in rhesus macaques does not differ markedly in terms of virological parameters, indicating that viral load alone does not determine the risk of progression to simian AIDS. Rather, key differences lie in signs of abnormal immune activation in the susceptible species, such as high numbers of cycling and apoptotic T cells, high frequency of activated effector CD8+ T cells, and hyperplasia of lymphoid organs. Thus, study of simian models suggests that AIDS pathogenesis is driven by chronic, sustained immune activation in the absence of appropriate CD4+ T cell help. This notion opens the possibility of devising novel therapeutic strategies against AIDS that would target the abnormal immune activation process rather than viral replication. Identifying cellular signaling pathways deregulated by HIV and SIV would be essential to this goal.

11. ACKNOWLEDGEMENTS

This work was supported by the Pasteur Institute (Paris, France) and by the Aaron Diamond Foundation

(New York, NY, USA). Specimen used in these studies were obtained from the Tulane Regional Primate Research Center (base grant RR-00164) and Yerkes Regional Primate Research Center (base grant RR-00165).

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Note Added In Proof: A recent study by Silvestri and coll. (Immunity 18: 441-452, 2003) supports the idea that naturally infected sooty mangabeys manifest far lower levels of aberrant immune activation and apoptosis than are seen in pathogenic SIV and HIV infections.

Key Words: AIDS pathogenesis, simian immunodeficiency virus, SIVsm, sooty mangabey, Cercocebus torquatus atys, natural host, T lymphocyte regeneration, Review

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