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SIVagm: GENETIC AND BIOLOGICAL FEATURES ASSOCIATED WITH REPLICATION

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1. ABSTRACT

African green monkeys (AGMs) belong to a superspecies that include the following four species: vervet (Chlorocebus pygerythrus), grivet (C. aethiops), sabaeus (C. sabaeus) and tantalus monkeys (C. tantalus). Each species carries a distinct SIVagm subtype, named SIVagm.ver, SIVagm.gri, SIVagm.sab and SIVagm.tan but remain clinically asymtomatic throughout their life. SIVagm needs the CD4 molecule and a chemokine-receptor, usually CCR5, Bonzo and/or Bob, for cell entry. Molecular and functional analyses of AGM CD4 and CCR5 revealed evidence of a true co-evolution between SIVagm and their natural hosts. The V3 loop of the SIVagm external glycoprotein is relatively conserved compared to that of HIV-1, but equally a determinant for coreceptor usage. SIVagm are able to replicate in stimulated peripheral blood mononuclear cells (PBMC) and macrophages. Efficient replication of SIVagm *in vitro* is associated with a cytopathic effect. SIVagm regulatory proteins show low amino acid identities with that of HIV-1, but many functions are conserved. The Tat protein of SIVagm is a transactivator of SIVagm LTR, albeit to a relatively lesser extent than Tat of HIV-1. Vif of SIVagm increases virus infectivity in AGM PBMC, demonstrating that SIVagm Vif is a positive regulator of virion infectivity. Vpr of SIVagm shares with HIV-1 Vpr its ability to transactivate the viral LTR, causes cell cycle arrest in AGM cells and facilitates nuclear import of pre-integration complexes and infection of non-dividing cells. AGM Vpr has been reported to also induce apoptosis, but the pathways involved in the mechanisms leading to apoptosis seem to be divergent compared to HIV-1 Vpr. SIVagm.ver3delta nef shows limited replicative capacity *in vitro* and *in vivo*, which suggests that Nef of SIVagm provides activation signals to the cells that facilitate SIV replication. The levels of plasma viral RNA in naturally infected AGMs are in a wide range ($<10^3$ to $>6.10^6$ RNA copies/ml), with many animals displaying steady-state levels of $>10^6$ RNA copies/ml levels , which in humans are associated with progression towards AIDS. Viral load per se therefore is not directly linked with pathogenicity . SIVagm-infections differ, however, from pathogenic HIV/SIVmac infections by a low viral load in peripheral lymph nodes. These relatively low localized tissue viral load levels are associated with lack of signs of detectable immunopathology. More detailed studies on the precise immunological environment during natural SIV infections, especially during primary infection, and on the interactions between SIVagm and host-specific cellular proteins are needed to better understand the

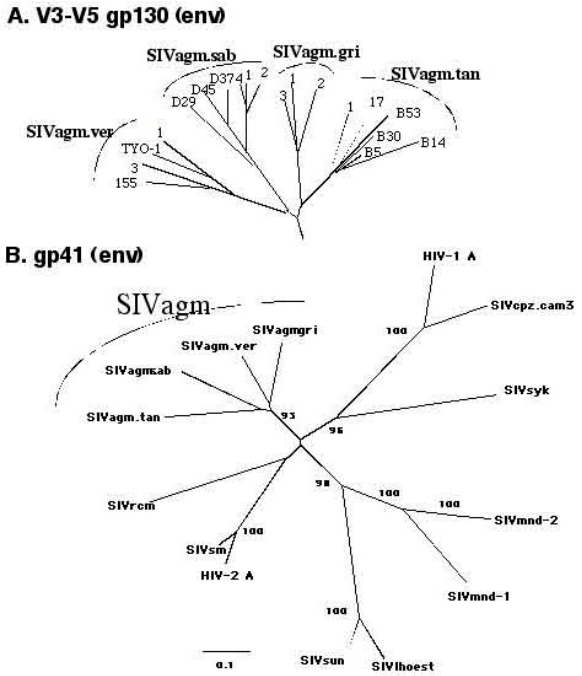

Figure 1. The four SIVagm subtypes.

mechanisms by which these naturally SIV infected nonhuman primate species are protected against progression towards AIDS.

2. INTRODUCTION

African non human primates are natural hosts of simian immunodeficiency viruses (SIV). The first species identified as natural carriers of SIVs were sooty mangabeys, African Green monkeys (AGMs), mandrills and chimpanzees. Today, SIVs from 30 distinct African non human primate species have been reported (1, 2). Full-length genome sequences have been described for SIVs from the following species: African Green monkeys (SIVagm), chimpanzees (SIVcpz), sooty mangabeys (SIVsm), red-capped mangabeys (SIVrcm), mandrills (SIVmnd), Sykes monkeys (SIVsyk), greater spot-nosed monkeys (SIVgsn), l'Hoest monkeys (SIVlhoest), sun-tailed monkeys (SIVsun) and colobus monkeys (SIVcol) (1, 2). Among these SIV isolates, SIVsm was found to be genetically the most similar to the SIV from macaques (SIVmac) (3, 4). Epidemiological studies indicate that Asian monkeys, such as macaques, are not infected in their natural habitat (5, 6). It was therefore reasoned that macaques most likely acquired SIV from naturally infected sooty mangabeys housed in primate centers (4).

SIV infection in macaques and in African non-human primates differ in one fundamental aspect: their clinical outcome. While macaques experimentally or accidentally infected by SIVsm/mac progress to AIDS similar to humans infected by HIV, African non human primates naturally or experimentally infected with their species-specific SIV do not develop AIDS (7). This has been demonstrated for species such as African Green monkeys (AGMs), sooty mangabeys and mandrills, for which long term studies in high numbers of SIV-infected animals had been possible. Naturally occurring primate lentiviruses are, however, not inherently non-pathogenic as experimental inoculation of macaques with SIVsm is associated with disease progression. Experimental infection of macaques with SIVagm results in most cases in transient or chronic asymptomatic infections. However, it has been shown that infection of pigtailed-macaques with the virus isolate termed SIVagm.ver90 induces AIDS (8). It has been suggested that a long-term co-evolution between the virus and its natural host might underlie the non-pathogenic outcome of natural SIV infection of nonhuman primates. Several studies indicate indeed that SIV infection in African non-human primates is ancient. The first data that suggested a co-evolution between SIV and their hosts were obtained from studies on SIVagm-infected African green monkeys.

3. PHYLOGENY OF SIVagm

African green monkeys (AGMs) belong to a superspecies comprising the following four species that are the following: vervet (Chlorocebus pygerythrus), grivet (C. aethiops), sabaeus (C. sabaeus) and tantalus monkeys (C. tantalus). The natural habitat of AGMs corresponds to subsaharan Africa with the exception of the tropical forests and deserts (9). The four species evolved approximatively 10,000 years ago. They are geographically separated today, vervets living in East- and South-Africa, grivets in East-Africa, sabaeus monkeys in West-Africa, and tantalus monkeys in Central-Africa. Still, an overlapping of the geographic locales is not totally excluded, such as for vervets and grivets in Ethiopia and vervets and tantalus monkeys in Uganda (9, 10)

The first SIVagm was isolated from vervets originating from East-Africa (6). Subsequently, SIVagms were identified in the other AGM species, i.e. grivets (11), sabaeus (12) and tantalus monkeys (13). SIVagm viruses display high genetic diversity, similar to HIV (14-17). The RT of SIVagm is as error prone as the homologous HIV-1 enzyme (18). Sequence analyses in the *env* gene of SIVagm viruses isolated from the four species revealed four distinct SIVagm subtypes, each specific for its host-species (Figure 1) (13). The subtypes are indeed divided according to their host species and not according to their geographic distribution as SIVagm.tan viruses originating from two separated countries (Uganda and Central African Republic) cluster together, whereas SIVagm.ver and SIVagm.gri from Ethiopia do not (19, 20). The four subtypes were therefore named SIVagm.ver, SIVagm.gri, SIVagm.sab and SIVagm.tan. The existence of four species-specific subtypes are most likely explained by evolution of SIVagm viruses coincident with the evolution and divergence of their respective hosts (12, 13).

Full-length genome sequences have been obtained for molecularly cloned SIVagm viruses, at least for one of each subtype (Table 1). The analysis of the SIVagm.sab genome provided additional data in support of an ancient infection of AGMs by SIV. Thus, SIVagm.sab viruses currently infecting wild sabaeus monkeys display a

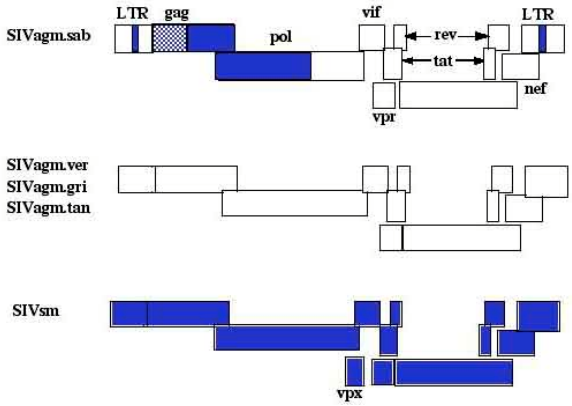

Figure 2. The mosaic genome of SIVagm.sab.

Table 1. Molecularly cloned SIVagm viruses

VERVET	GRIVET	SABAEUS	TANTALUS
TYO-1 [1]	gri-1 [5]	sab1 [6]	tan1 [7]
90.63-2 [2]			tanB14 [8]
3 [3]			
155 [4]			

[1]Fukasawa *et al* (87); [2]Hirsch *et al.* (8), this clone is pathogenic in pig-tailed macaques; [3] Baier *et al* (88); Fomsgaard *et al* [4](89); Johnson *et al* [5](11); Jin *et al* [6](21); [7]Soares *et al* (90); [8] Corbet *et al.* (unpublished results).

mosaic genome (Figure 2) (21). With the exception of the 3' part of pol, all regulatory genes as well as the env gene cluster within the SIVagm lineage, while the 3'gag-5'pol part of the genome groups with the SIVsm/SIVmac/HIV-2 lineage. Furthermore, the LTR of SIVagm.sab contains a duplication of the trans-activation response element (TAR), only found in SIVsm/SIVmac/HIV-2 (22). Sooty mangabeys, the natural hosts of SIVsm, reside in overlapping habitats with sabaeus monkeys. The mosaic genome most likely resulted from a recombination event between the ancestors of SIVagm and SIVsm (21). This recombinant SIVagm genome became fixed in the wild sabaeus population, indicating that the viral proteins that originate from two distinct SIV lineages are capable to interact efficiently.

Finally, our studies on intra-individual diversity in V3-V5 env of SIVagm have revealed low ratios of non-synonymous versus synonymous mutation frequencies (23). This has been confirmed by analysis of V2-C4 env of SIVagm present in tissues (24). These low ratios indicate that SIVagm has reached some equilibrium in AGMs outweighting the positive selection for change in contrast to HIV/SIVmac infections (25-27), further supporting the ancient infection of SIVagm in AGMs.

4. SEROPREVALENCE AND MODES OF TRANSMISSION OF SIVagm

The discovery of a mosaic SIVagm.sab genome suggested that the evolution of SIV viruses is complex and associated with cross-species transmission in their natural habitat. That SIVagm is transmitted from AGMs to other species in the wild has indeed been confirmed by the subsequent isolation in baboons and patas monkeys of SIVagm related viruses (20, 28, 29). The SIVs isolated from patas monkeys originating from West-Africa were related to SIVagm.sab and the virus from baboons living in East and South-Africa to SIVagm.ver, showing that the monkeys were infected by the specific SIVagm subtypes circulating within AGMs of the same geographic locales.

SIVagm infection in patas monkeys seems to be non-pathogenic (23). After experimental intravenous transmission of SIVagm into patas monkeys, the viral heterogeneity is lower than in AGMs that received the same viral inoculum (23). This is not due to a lower viral load in the patas monkeys (unpublished results). It rather suggests a stronger selection of single viral variants during or soon after transmission, similar to the bottleneck described for HIV-1 infected adults and children (30-32). These studies are consistent with an adaptation of SIVagm to its natural host.

The existence of cross-species transmissions of SIVagm correlates with their high seroprevalence in AGMs. The seroprevalence rates have been studied in several hundreds of free-ranging and wild born AGMs and revealed to be high (30-60%) (33). No difference according to the AGM species was observed (13). The distribution is however not uniform and varies significantly between distinct troops of animals (34). Furthermore, the distribution differs also significantly according to the age and the sex of the animals. SIV seropositivity is virtually absent in very young animals (35). Neonatal AGMs born from captive SIV-positive mothers have not to date been found to be virus isolation-positive after birth (36). AGMs generally become seropositive when they enter the breeding age (sub-adult females and adult males). The seroprevalence rate then gradually increases with age and can exceed 90% in adult females (35). These studies indicate that SIVagm is transmitted mainly horizontally and only rarely if ever maternally. Although sexual transmission appears to be the predominant horizontal mode of transmission according to these data, nonsexual avenues for transmission of SIVagm, including bites and allogrooming of open wounds by mouth, cannot be excluded. Such asexual transmission has been reported for other viruses, such as SIVmac and FIV (37).

The modes of SIVagm cross-species transmission in natura are not exactly known. Agressive as well as sexual contacts between animals of sympatric species have been observed (9, 38). Transmission through predation is also not excluded (39), especially as it has been recorded that baboons kill and eat vervets (40).

Seroprevalence rates as high as in AGMs may also exist in other African primate species such as mandrills, sooty mangabeys and guereza colobus monkeys. However, the number of free-ranging or wild born adults studied so far is too low to allow for meaningful conclusions (39, 41, 42). In chimpanzees, the infection rates seem to vary according to the subspecies (43-46). Among nonhuman primates known to be naturally infected by SIV, AGMs are thus the biggest reservoir for SIV as

Table 2. Cellular tropism of SIVagm compared to that of other SIV/HIV viruses

Cell Type	SIVagm	SIVsm	SIVmac	HIV-1
AGM PBMC	+	+	+	-
rhesus macaque PBMC	+	++	++	-
pigtailed macaque PBMC	+	++	++	-
human PBMC	+	++	++	++
macaque macrophages	+	+	+	-
human macrophages	+	+	+	+
MOLT4cl8	++	+	+	++
SupT1	++	+	+	++
MT4	+	++	++	++
CEM	+	+	-/+	+
CEM-ss	++	+	++	++
CEMx174	-	++	++	++
H9/HUT78	+/-	++	++	+
MT2	+/-	-	++	++
U937	-	-	-	++
Jurkat-T	-	nd	+	++

+: replication ++: efficient replication -: generally no productive infection detected

they are the most numerous and most geographically dispersed.

5. CELLULAR TROPISM OF SIVagm

The non-pathogenic outcome of SIV infection in their natural hosts in contrast to disease progression in macaques infected by SIVsm or SIVagm indicates that lentiviral virulence is dictated by a complex interplay between viral and host determinants. Understanding the molecular biology of these viruses, their replicative capacities and the specific interactions with the host cells are important to better understand the mechanisms underlying AIDS pathogenesis in macaques and humans.

5.1. PBMC and T lymphocytes

SIVagm replicates in AGM peripheral blood mononuclear cells (PBMC), but also in PBMC from humans (47, 48) and macaques (Table 2) (49-53). Similar to other HIV/SIV, SIVagm preferentially infects T $CD4^+$ cells when they are in an activated state and generally does not grow in unstimulated PBMC (54). A stimulation, such as that provided by a mitogen (PHA, ConA) is a prerequisite for a productive infection with wild-type SIVagm (52, 55, 56). A significant growth is associated with a cytopathic effect *in vitro* (57).

Isolation of SIV from primary $CD4^+$ cells of seropositive green monkeys has, however, revealed to be difficult due in part to low proliferation of AGM $CD4^+$ T cells *in vitro* (6, 24, 58). The most efficient way to isolate SIVagm is by coculture of AGM mononuclear cells with specific human T cell lines. The first isolation of SIVagm was thus performed by culture of mitogen (ConA) stimulated PBMC with MT4 cells (6). Others succeeded in efficient SIVagm isolation by co-cultivating unstimulated adherent mononuclear cells with MOLT4cl8 cells vitro (58), or by infection of MOLT4cl8 or CEMss cells with cell-free filtrates of tissue homogenates (24, 59, 60).

SIVagm replicates efficiently in human T cell lines, but not in all of them (Table 2). MOLT4cl8, SupT-1 and CEMss cells are highly permissive for SIVagm (6, 47, 48, 58, 61). SIVagm replication is associated with syncitium formation in these cells, that is especially dramatic in MOLT4cl8 cells (6, 19, 51, 58, 61). In contrast, SIVagm does not replicate efficiently in CEMx174 and HUT78 cells, as compared with the relatively efficient replication of SIVmac, SIVsm, HIV-2 and HIV-1 in these cell lines. (61). SIVagm replication in H9, MOLT4 and CEM cells is also less productive comparative to SIVmac (6, 16, 47, 58, 61). Moderate replication if any of SIVagm has been observed in U937 and Jurkat T cells (58, 61). On the other hand, SIVmac does not readily infect SupT1 cells in contrast to SIVagm, and induces less syncytia in these cells than SIVagm (61). It has been shown that determinant(s) in the V3 loop as well as within the gag-pol-vif-vpr region govern the tropism of SIVagm for human T cell lines (55, 62).

5.2. Macrophages

SIVagm is able to replicate in macrophages. Thus, SIVagm was shown to be successfully isolated from monocyte derived macrophages of two naturally infected AGMs (59). Furthermore, alveolar macrophages from the lung of one naturally infected AGM were found positive for SIV by *in situ* hybridization (59). *In vitro* infection studies have been performed with human and macaque macrophages as their culture is technically more feasible than with AGM macrophages (24). The molecularly cloned SIVagm9063-2 is capable to replicate in macaque macrophages (53, 56). Human macrophages can also be productively infected by SIVagm (24). The level of replication varied according to the virus used. Whereas no detectable replication could be observed with a SIVagm strain derived from lymph nodes of a naturally infected AGM, a rapid and productive replication was observed with SIVagm isolates derived from brain and CSF of the same animal (24). Molecularly cloned virus SIVagm-gri1, derived from chronically infected CEMss cells and primarily T cell tropic, was also able to replicate in these cells, albeit to a lesser extent. The SIVagm isolates were cytopathic for the human macrophages (24). Whether SIVagm viruses can also replicate in other cells, such as dendritic cells, remains to be elucidated.

Table 3. Peripheral blood $CD4^+$ and $CD8^+$ T lymphocyte numbers and percentages in AGMs

Age of the animals	Absolute Numbers		Percentages	
	$CD3^+CD4^+$	$CD3^+CD8^+$	$CD3^+CD4^+$	$CD3^+CD8^+$
neonates (2 days)	nd	nd	62 (61-64)	52 (37-67)
juveniles and subadults (1.4-3.5 years)	1500 (800-3400)	2100 (1100-4000)	30 (17-44)	40 (33-70)
adults (4-16 years)	550 (100-1300)	1200 (680-2800)	18 (2-36)	47 (35-67)

The mean values are indicated, followed by the range in parenthesis. The values were compiled from different studies (57, 67-70). nd = not determined.

6. CIS, CO- AND TRANS-RECEPTORS FOR SIVagm

As for other HIV/SIVs, SIVagm needs CD4, the major cellular receptor molecule, as well as chemokine-receptors as co-receptors for cell entry (47, 63). SIVagm apparently also uses attachment factors to optimize infection.

6.1. AGM T $CD4^+$ cells and $CD4^+$ usage by SIVagm

The CD4 molecule belongs to the immunoglobulin supergene family and contains several variable regions, comprising the V1 loop implicated in HIV/SIV envelope binding. The AGM CD4 molecule shares only 90% amino acid identity with the human homolog (64). The CD4 protein sequences from distinct AGM species show 98-100% identity with each other (65). It was shown that despite the genetic distance between the human and AGM CD4 molecule, syncitia were induced by SIVagm in cell-lines positive for either the AGM or the human CD4 (66). Macaque cells expressing the human CD4 protein were susceptible to SIVagm infection *in vitro*, but not to HIV-1. A human cell line expressing the AGM CD4 supported infection by HIV-1, but not by SIVagm (66). Whether the virus could infect the cells was therefore independent of the origin of the CD4 molecule.

The numbers and percentages of $CD3^+CD4^+$ SIVagm target cells in AGMs are highly variable according to the individual animal (Table 3). The absolute $CD3^+CD4^+$ cell numbers range from 0.1 - 3.4 x 10^3cells/microl, and the percentages from 2 to 64 % (mean: 26%) (57, 67-69). As in other primates, the $CD4^+$ T cells decrease with age (Tab.3) (69). The CD4/CD8 ratio is lower than in humans (0.4 versus 1.5). According to the animal, absolute numbers of $CD3^+CD4^+$ cells in AGMs are, however, not systemically lower than in humans, but can be similar or even higher due to the fact that total $CD3^+$ cells are on average two times higher in AGMs than in humans (68-70).

$CD4^+$ lymphocytes of AGM often weakly co-express CD8 (57), which explains why SIVagm could be isolated from $CD8^+$ positively-selected cells (59). No significant differences in the percentages of total $CD4^+$ lymphocytes or of $CD4^{bright}CD8^{low}$ were observed between infected and uninfected animals (57, 67). No difference in the replication kinetics of SIVagm in PBMC from adults and neonates were reported, despite the higher frequences and absolute numbers of $CD4^+$ lymphocytes in the neonates (67).

Activation by a mitogen (ConA) *in vitro* apparently leads to a gradual decrease of CD4 mRNA synthesis in AGM $CD4^+$ cells, culminating with almost no detectable levels of mRNA at day 12 of culture (57). This finding correlates with low levels of replication of SIVagm when infection was performed following cultivation of the PBMC for one week prior to infection (57). It has been suggested that these findings might explain, at least partially, difficulties of SIVagm growth in AGM PBMC *in vitro*.

6.2. CCR5 usage by SIVagm

SIVagm uses CCR5 as a major coreceptor for entry, similar to many other SIVs (71-74). AGM CCR5 is active in chemokine-mediated signaling (74). AGM kidney cells (CV1) become permissive to SIVagm when transduced to express CD4 and CCR5, whereas these cells do not support HIV-1 replication, the block of HIV-1 replication being at a post-entry level (75).

We analyzed the open reading frame of CCR5 from 30 animals belonging to the four AGM species. No deletion has yet been detected in any animal studied (76). However, the AGM ccr5 gene is highly polymorphic (74). The analysis of non-synonymous and synonymous mutations suggests that the AGM ccr5 gene evolved under relatively intense selection pressures (74, 76). Functional studies revealed that SIVagm apparently has become adapted to specific mutations in their respective AGM hosts species, since SIVagm strains can efficiently use wild-type AGM CCR5, whereas the latter is only a poor co-receptor for HIV-1 (74, 77).

In contrast to HIV, but similar to many other SIVs, most SIVagm viruses do not use CXCR4 (71, 74) or at least not as efficiently (78). This is striking, since CXCR4 derived from AGMs (as well as CXCR4 from macaques and sooty-mangabeys) facilitate infection by HIV (73, 79). The hypervariable V3 region of HIV-1 is an important domain in determining coreceptor binding and cellular tropism. We have shown that in contrast to HIV-1, the V3 loop of SIVagm viruses is highly conserved (Figure 3) (48). The replacement of the V3 loop sequence of a CCR5, Bonzo and Bob using SIVagm (SIVagm.ver3mc) with that of a X4 HIV-1 (BH10) resulted in a SIVagm strain which exclusively utilized CXCR4 for viral entry demonstrating that the V3 of SIVagm is equally implicated as a determinant for coreceptor usage (55). SIVagm.ver3mc is capable of replication in unstimulated AGM and pigtailed macaque PBMC, which may suggest that the SIVagm.ver3mc-X4 triggers an intracellular signal, which leads to cell activation that enables virus replication (55). However, the acquirement of CXCR4 usage was not sufficient to induce higher plasma viral load in pigtailed macaques compared to wild-type virus, and no signs of disease progression were evident (55). So far, only the

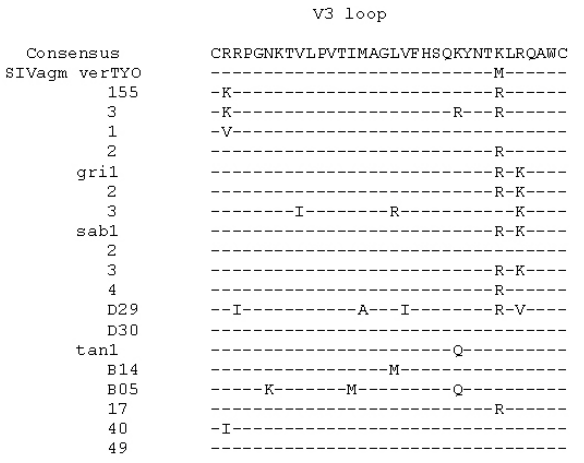

Figure 3. The V3 loop amino acid sequences of SIVagm surface glycoprotein are highly conserved.

CCR5 using SIVagm.ver90 was capable of inducing AIDS in pigtailed macaques (8).

6.3. Bonzo and Bob usage by SIVagm

The ability of SIVs to replicate in human T cell lines without using CXCR4 has lead to the discovery of additional co-receptors, in particular Bonzo/STLR33 and Bob/GPR15 (71, 80). Most SIVagm viruses are capable to use Bonzo and Bob in addition to CCR5. The usage of Bonzo *in vitro* seems to be more efficient in comparison to SIVmac, whereas SIVmac more readily uses Bob in comparison to SIVagm (71). The preferential usage of Bonzo and Bob by, respectively, SIVagm and SIVmac correlates with the expression patterns of these two coreceptors on human T cell lines. Thus, Bonzo is expressed on cells highly permissive for SIVagm (SupT1 and MOLT4cl8), whereas high levels of Bob, but not of Bonzo, are expressed on cells that preferentially replicate SIVmac (CEMx174 and HUT78) (71).

Functional analysis of simian Bonzo and Bob molecules are limited. It has been shown that Bonzo from sooty mangabeys is used efficiently by SIVsm, whereas SIVmac does not efficiently utilizes rhesus Bonzo (81). Strikingly, the utilization of Bob or Bonzo does not seem to provide a major advantage for SIVsm/SIVmac replication *in vivo* (73, 82). Functional analysis of Bonzo and Bob from AGMs have not been performed yet. Since it is known that minor species-specific sequence changes of these molecules affect the ability to act as coreceptors for HIV/SIV, it is important to ascertain whether the relevant receptors function in the animal model of choice. Finally, it is not excluded that SIVagm, and may be other SIVs, use not as yet identified molecules as additional coreceptors.

6.4. DC-SIGN usage by SIVagm

The human DC-SIGN molecule (CD209) has been shown to be an efficient attachment factor for HIV-1. It allows productive virus transmission *in trans* to activated T-cells and also facilitates infection when expressed *in cis* (83). AGMs, as well as other Old world monkeys, possess orthologues of the human *dc-sign* gene (84). *In vitro* studies have been performed in order to analyze whether SIVs from African non-human primates are able to use this mechanism of viral transmission. However, only interactions between DC-SIGN from human and rhesus macaques, expressed on THP-1 cells, with HIV-luc pseudotyped with SIVagm Env have been analyzed so far (85, 86). Interactions of SIV with human molecules do not necessarily reflect interactions of the virus with the homologous host-specific molecule. As mentioned above, SIVmac, although able to use human Bonzo, can not efficiently use the macaque homolog molecule for entry (81). Similarly, HIV-1 uses AGM CCR5 relatively inefficiently due to an amino acid difference (G163R) between the human and AGM protein, whereas SIVagm is adapted to efficiently employ this AGM CCR5 isoform (74). Analyses of the interactions of viruses with receptors derived from the same species are therefore needed. We have recently shown that DC-SIGN from AGMs is capable to efficiently transmit primary SIVagm isolates *in trans* to T cells *in vitro* (Ploquin *et al*, submitted). In order to better assess the relevance of transmission facilitated by DC-SIGN *in vivo*, it is important to obtain more insights into the regulation of the levels of expression of this molecule in primary cells and tissues. Furthermore, it is not excluded that additional trans-receptors on non-human and human primate cells will be identified in the future that may be relevant for virus dissemination (85).

7. ROLE OF SIVagm ACCESSORY PROTEINS IN REPLICATION

In addition to the three structural genes gag, pol and env, SIVagm genome codes for five regulatory genes: *vif*, *vpr*, *tat*, *rev* and *nef*. (8, 11, 21, 87-90). This structure also applies to SIVsyk, SIVlhoest and SIVmnd-1 (17). The *vpr* gene of SIVagm had initially been called *vpx* because it clusters with *vpx* of SIVsm/HIV2/SIVmac in phylogenetic trees. Subsequent detailed genetic comparisons with many other primate lentiviruses revealed however a significant sequence similarity with *vpr* (91). Tristem *et al* suggested to reclassify the SIVagm vpx gene as a vpr gene. The *vpx* gene of SIVsm/HIV-2/SIVmac was suggested to originate by gene acquisition of the SIVagm vpr gene (92). This re-classification of a *vpr* gene in all HIV/SIV created a simpler and more uniform picture for the genomic organization of the primate lentiviruses.

Regulatory open reading frames are generally equally maintained in SIVagm as in other HIV/SIVs, which is a sign of an important function of these proteins during the virus life cycle. Although amino acid identities are often very low between SIVagm and HIV-1 regulatory proteins, many functions seem to be conserved. The rev gene product for example seems to play a similar role in SIVagm expression as done by Rev in HIV-1 expression (93, 94). The studies on the functional roles of Tat, Vif, Vpr and Nef from SIVagm are listed below.

7.1. The trans-activation potential of SIVagm Tat

The Tat protein of SIVagm is a transactivator of SIVagm LTR (94). However, the LTR of SIVagm, as studied by the analysis of the LTR from four SIVagm

Table 4. Function of SIVagm Vpr relative to Vpr/Vpx functions of other HIV/SIVs

	Nuclear transport	LTR transcription activity	Cell cycle arrest	Apoptosis
HIV-1 vpr	+	+	+	+
SIVagm vpr	+	+	+[1]	+[2]
HIV-2 vpr	-	nd	+	nd
HIV-2 vpx	+	nd	-	nd
SIVmac vpr	-	nd	+	nd
SIVmac vpx	+	nd	-	nd

[1] in AGM cells (105-107), [2] by a distinct mechanism than for Vpr of HIV-1 (107).

viruses (TYO-1, TYO-2, TYO-5, TYO-7), have been reported to be only relatively poor responders to Tat of SIVagm.verTYO, and also to the Tat proteins of SIVmnd-1, HIV-2 and HIV-1 (95). In contrast, in the absence of viral Tat, LTRs of SIVagms acted as more efficient promoters than LTRs of HIV-1, HIV-2 and SIVmac239 (95). It has been suggested that the lack of negative elements in the AGM LTR may contribute to this high basal promotor activity relative to the HIV-1 LTR (96).

Tat of SIVagm.verTYO activates the LTRs from SIVagm, HIV-2 and SIVmac, but fails to efficiently trans-activate HIV-1 LTR (95). These data indicate that SIVagm and SIVsm HIV-2 Tat proteins are interchangeable with respect to their trans-activation potential of SIVagm.ver on SIVsm/HIV-2 LTRs, despite the fact that the structure to which Tat binds (TAR) is distinct between SIVagm.ver and SIVsm/HIV-2 (22). These findings are consistent with results suggesting that the number of TAR hairpins does not determine the relative specificities of HIV and SIV Tat proteins.

Viruses from the SIVagm.sab subtype show the peculiarity by the presence of stop codons within the *tat* gene (21, 97). Due to these mutations, the putative Tat protein of SIVagm.sab consists of only the first coding exon product. Such mutations are generally not observed for the three other SIVagm subtypes. Despite the differences in TAR and Tat structure, no detectable difference in viral load according to the SIVagm subtype could be observed by us and others (24, 59, 98, 99). Altogether, these findings suggests that the particular TAR and Tat structures of SIVagm.sab play no significant role in the lack of virulence in AGMs.

7.2. Interactions of SIVagm Vif with host-specific cellular factors

The Vif proteins of SIVagm and HIV-1 share between 26 and 29% amino acid identity. HIV-1 Vif is essential for the production of infectious progeny viruses in primary cells and certain human T cell lines, and, presumably, for the establishment of productive infections *in vivo*. It has been shown that Vif of SIVagm (tan1) increases virus infectivity by 16 fold in AGM PBMC, demonstrating that SIVagm Vif is also a positive regulator of virion infectivity (100).

In human cells, however, SIVagm Vif does not increase infectivity. Thus, although the infectivity of SIVagm is 10 fold higher when produced in permissive (CEMss) than non-permissive (H9) cells, the wild type and vif-deficient SIVagm viruses produced in both human cell lines show similar titers of infectious progeny (100). Furthermore, the SIVagm Vif can not complement for HIV-1, HIV-2 and SIVmac Vif in human cells, whereas Vif of HIV-1 significantly increase the infectivity of vif-deficient SIVagm in human cells (100). The SIVagm Vif protein being active in AGM cells but not in humans cells, together with other studies on HIV-1 Vif (101), indicates that the functional consequences of a given Vif protein are governed by cellular factors. Recently, several cellular partners as well as a cellular factor with anti-HIV activity (CEM15), have been identified in human cells (102a and b). Whether AGM cells express homologous proteins is possible, but needs to be demonstrated.

7.3. Vpr of SIVagm shares multiple functions with Vpr of HIV-1

Vpr of SIVagm bears around 30% amino acid identity with the HIV-1 protein. At a functional level, it shares its abilities to transactivate viral LTR (Table 4) (103). Vpr, as shown with studies using SIVagm9063-2, also becomes incorporated into the virion particles and facilitates subsequent nuclear import of pre-integration complexes (53, 104). It is required for efficient SIVagm replication in non-dividing cells, such as macaque monocyte-derived macrophages (53, 104). The ability to cause cell cycle arrest is also conserved for Vpr of SIVagm, as well as among many other primate lentiviruses (HIV-2, SIVmac, SIVsm, SIVsyk) (105, 106). This property is cell-type specific and depends on the particular primate species from which the cells are derived. SIVagm Vpr is capable of cell cycle arrest within AGM cells but is completely inactive in human cells. By contrast, HIV-1, HIV-2 and SIVmac Vpr proteins function in both AGM (CV1) and human (HeLa) cells. This indicates that Vpr exerts its G2 arrest function by interacting with cellular factors. Vpr of SIVagm also induces apoptosis, but the induction is cell cycle dependent in contrast to the induction of apoptosis by HIV-1 Vpr that requires G2 cell cycle arrest (107). These studies show that while multiple functions of Vpr are conserved between HIV-1 and SIVagm, the mechanisms leading to the execution of such functions can be divergent.

7.4. SIVagm Nef

SIVagm.ver3delta*nef* (displaying a 125bp deletion in the nef reading frame) showed delayed replication *in vitro* in MOLT4cl8, AGM PBMC and pigtailed PBMC compared with wild type SIVagm.ver3 (52). The *in vivo* replicative capacity of SIVagm.ver3

Table 5. SIVagm viral load during acute and chronic phase of infection

Study	Number of Animals	Species	Phase of Infection	Blood		Lymph Nodes	
				RNA plasma (ml)	DNA copies	DNA copies	RNA (copies or positive cells)
Diop *et al* (98)	4	sabaeus	primary	$2.0x10^7$	70000	70000	10^6 copies
Holzammer *et al* (99)	8	ns	primary	$9.3x10^6$	3729	nd	nd
Beer *et al* (109)	1	ns	primary	nd	nd	nd	25 cells #
Hartung *et al* (54)	9	vervet, grivet	chronic	nd	144	nd	nd
Beer *et al* (109)	16	vervet, grivet,sabaeus	chronic	nd	28	40	0 cells #
Diop *et al* (98)	4	sabaeus	chronic	$5.0x10^4$	700	325	10^4 copies
Holzammer *et al* (99)	8	ns	chronic	$7.4x10^5$	429	nd	nd
	26	ns	chronic	$1.7x10^6$	nd	nd	nd
Goldstein *et al* (59)	12	vervet	chronic	$8.2x10^4$	nd	nd	0-2 cells #
Broussard *et al* (24)	5 + 2	sabaeus, vervet	chronic	$3.8x10^6$	153[1]	2800[2]	nd
Müller-Trutwin *et al*[3]	10	tantalus	chronic	nd	76	21	1-8 cells #

RNA and DNA copy numbers are expressed as copy numbers per 10^6 cells. Only the mean values are indicated. #RNA positive cells per $500mm^2$ (129) or per analyzed section (59, 109). [1]DNA copy numbers in PBMC were studied in 5 animals (24). [2]DNA copy numbers in LNC were studied in 2 animals that were distinct from those 5 animals analyzed for proviral load in PBMC (24). [3]unpublished results and Dias-Tavares *et al.* (129). ns: not specified, nd: non determined.

deltanef was very limited, with only one out of four animals showing signs of transient infection (52). Although the biological properties of SIVagm Nef have not been studied yet, this suggests that Nef of SIVagm might provide activation signals to the cells that facilitate SIV replication.

SIVagm nef mutants have been used to better identify the pathogenic determinants in the nef gene of acutely pathogenic SIVs. The introduction of the immunoreceptor tyrosine-based activation motif (ITAM), a pathogenic determinant of the acutely pathogenic virus SIVsmPBj, into the *nef* gene of SIVagm.ver9063-2, allowed replication in unstimulated pigtailed-macaque PBMC *in vitro* (56). The *nef* variant induces a relatively more profound lymphopenia during acute infection in pig-tailed macaques than the SIVagm wild type. However, it was not associated with higher viral load compared to infections with the wild-type virus, and the SIVagm *nef* variant did not fully recapitulate the virulence of SIVsmPBj. The insertion of the whole *nef* gene from pSIVsmPBj into SIVagm (SIVagm.ver3) was also not sufficient to induce high viral load *in vivo* and to convert an apathogenic SIV into a virus pathogenic for pigtailed macaques (108), indicating that specific virus-host interactions and/or factors in addition to Nef are necessary for acute pathogenicity.

8. VIRAL LOAD OF SIVagm *IN VIVO*

Studies on the evolution of intra-animal SIVagm diversity indicated a continuous and rapid replication of SIVagm *in vivo* (23). However, for a long time, the studies on SIVagm viral load were limited to the analysis of cell-associated virus loads during the chronic phase. These studies suggested either similar or lower levels than that in HIV-infected humans progressing towards AIDS (109). Only recently, plasma virus levels have been determined that allow to compare viral set point levels in non-pathogenic SIVagm infection with that in pathogenic HIV-1/SIVmac infections.

8.1. Plasma viral load

Our studies on primary experimental infection revealed high levels of antigenemia in AGMs infected by SIVagm.sab92018, with peak values up to 5ng of P27/ml of plasma between 7 and 14 days after intravenous infection (98). P27 levels then decline below detection level in the chronic phase (59, 98). RNA copy numbers at the peak analyzed so far in two studies, including 12 animals in total, ranged from $3x10^5$-$2x10^8$ per ml (Table 5) (98, 99). The peak was followed by a 1-3 log drop in magnitude of viral copy numbers and stabilized at set points of $2x10^3$-$3x10^6$ RNA copies per ml (Table 5). These values are in the same range as those reported in naturally infected AGMs ($<10^3$ to $>6.10^6$ RNA copies/ml) (24, 59, 98, 99). The levels appeared stable over time, with a steady-state level that was characteristic for each individual monkey (59, 98).

The pattern as measured by RNA copy numbers correlate with data on infectious virus in plasma. We observed significantly high titers ($7x10^4$ TCID50/ml of plasma) at the peak of primary infection (98), and considerably lower and highly variable titers according to the animal were reported during the chronic phase (0-20 TCID50/ml of plasma) (54, 59, 98).

The viral RNA pattern observed in AGMs are comparable to that described in other natural hosts of SIV. Four mandrills experimentally infected with SIVmnd-1 thus displayed 10^6-10^8 RNA copies per ml of plasma at the peak and around 10^5 copies during the chronic phase (110). One naturally and two experimentally SIVcpz-infected chimpanzees showed values peaking at 6.10^6 RNA copies in primary infection and fluctuating between 10^2 and 10^5 RNA copies during chronic infection (111). Initial studies on SIVsm-infected sooty mangabeys (SM) suggested a consistently high RNA load in blood in contrast to AGMs (112, 113). However, more recent studies on chronically SIVsm-infected sooty mangabeys revealed a wide range

(<$5x10^2$-10^6 RNA copies/ml) comparable to that in AGMs (114).

Altogether, these studies revealed that in many SIVagm-infected AGMs, as well as in many other natural hosts for SIV, the levels of plasma viremia are similar to that in HIV-infected humans and SIVmac/sm-infected macaques (115, 116), but without pathologic consequences for the natural hosts, whereas HIV and SIV infected humans and macaques showing similar levels of plasma viremia respectively progress towards AIDS.

8.2. Cellular associated viral load in peripheral blood

The infectious titers of PBMC in chronically infected AGMs are relatively low and vary between 0 and 20 TCID50/10^6 PBMC (54, 98). This correlates with the observation that virus isolation from PBMC of chronically infected animals is not consistently positive. Nonetheless, virus isolation from PBMC of chronically infected animals is generally more successful than from plasma (60-100% versus 24-70%) (24, 54, 61, 98). The difference might be related to the fact that coculture (exposure to infected PBMC) is a more efficient way of infection than exposure to free viral particles (plasma). The AGM species does not influence the success rate of virus isolation (24).

The DNA viral load in PBMC reaches $0.5x10^2$-$1.4x10^5$ copies/10^6 cells at the peak (98, 99) and between 1-10^3 copies per 10^6 cells during the chronic phase (Table 5) (24, 54, 98, 99, 109). These levels are not significantly different from those reported in chronically infected sooty mangabeys ($4x10^1$-$6x10^3$ proviral copies per 10^6 PBMC) (41, 112). The number of productively infected cells, as measured by coculture of AGM PBMC with human cell lines, was obtained to correspond to 10^1- $7x10^3$ cells per 10^6 PBMC at the peak (99). Values thereafter were very variable, with the number of infected cells dropping frequently below the detection limit in chronically infected AGMs (1 per 10^6 PBMC) (99).

SIVagm-infected AGMs thus show relatively low levels of viral DNA in PBMC compared to SIV-infected macaques or HIV-1 infected, untreated humans despite similar levels of plasma viremia (117). In untreated humans, 20 infectious cells / 10^6 PBMC have been measured during the chronic phase (118). The DNA copy numbers recorded ranged from 10 to $5x10^5$ per 10^6 PBMC, the mean proviral load being on average one log higher than that observed in AGMs (119-121).

8.3. Viral burden in tissues

8.3.1. SIVagm distribution in tissues

Lymphoid tissues, such as lymph nodes (LN) and those found in the gut, are considered to be the primary sites for lentiviral replication in humans and macaques both in early and chronic stages of infection (122-125). So far, most studies on viral load in tissues during SIVagm infection have been performed during the chronic phase of infection. During the chronic phase, virus could be isolated, by coculture with CEMss or MOLT4cl8 cells, from many lymphoid tissues such as LNs (inguinal, axillary, mesenteric), spleen, thymus and intestinal tissues (duodenum, jejunum, ileum, cecum, esophagus, ...) (59, 60). Virus could also be occasionally isolated from bone marrow, kidneys and liver (59). Attempts of virus isolation from skin and heart have so far been unsuccessful (59).

Tissue specific virus distribution as measured by *in situ* hybridization (ISH) was similar. SIV-expressing cells could be observed predominantly in lymphoid tissues (axillary and mesenteric LNs, the lamina propria and submucosa of the gastrointestinal tract, the bronchus-associated lymphoid tissues) and not in non-lymphoid tissues such as the skin (59). Some non-lymphoid tissues that were negative by ISH or virus isolation, were positive by a more sensitive method (PCR). This was the case for cerebrellum for example (24).

However, the majority of naturally infected AGMs show restricted virus expression. In addition, the tissue distribution varies significantly between the animals. Thus, in a study of 7 naturally infected animals, SIV expressing cells were detectable only in the LNs of three out of the seven AGMs, although at least one of the animals negative for SIV in the LN showed moderate to high viremia (8.10^5 RNA copies/ml) (59). Furthermore, SIV expressing cells were not consistently detected in mesenteric lymph nodes, even if peripheral LNs were positive (59). The viral load is also variable in the brain. While the brain of two naturally infected AGMs displayed a low viral load and were consistently negative for virus isolation, the cerebellum of another naturally infected AGM displayed greater numbers of infected cells than PBMC (24). Furthermore, the levels of viral RNA in the CSF of some animals were as high (>10^5 copies/ml) as that found in the CSF of HIV-infected humans with AIDS associated dementia (126).

8.3.2. SIVagm burden in lymph nodes

The viral load in tissues has been quantified for lymph nodes. A wide range of viral DNA load in lymph nodes has been recorded in accordance with the wide range of viral burden in blood (Table 5). In chronically infected AGMs, it ranged from 2.10^0 -3.10^3 copies of DNA per 10^6 total LN cells (24, 59, 98). The DNA copy numbers are thus significantly lower than those reported for HIV-1 and SIVmac infections (>10^4 copies) (127, 128).

The numbers of infectious cells in lymphoid tissues, as estimated by limiting-dilution coculture, corresponded to 10-100 cells / 10^6 total lymphocytes (59). By *in situ* hybridization, 1 to 8 cells positive for viral RNA were detected per tissue section (2-500mm^2) (59, 109, 129), whereas up to 200 positive cells / 2 mm^2 are observed during pathogenic SIV/HIV infections (130). The SIVagm expressing cells are generally detected within the paracortical regions of the lymph nodes (59, 109). No signs of virus trapping by follicular cells in germinal centers could be detected in contrast to tissues from SIVmac/HIV-1 infections (59, 109, 130).

In another model of natural infection (SIVsm infected sooty mangabeys), the numbers of RNA positive cells in the LNs were similar to those found in macaques,

that were slow or intermediate progressors (112). The DNA levels reached $5x10^3$-$2x10^4$ DNA copies per 10^6 total lymphocytes (112). However, so far, only two mangabeys have been studied, which corresponded to animals also showing a relatively high proviral load in PBMC. Such relatively high infection levels in LNs can also be occasionally observed in AGMs (Table 5) (24). It is possible, that these high levels are not representative for all SIVsm infections in mangabeys. Furthermore, mangabeys infected by SIVsm (and also chimpanzees infected by SIVcpz), similar to AGMs infected by SIVagm, do not show signs of trapping of SIV by follicular dendritic cells (112).

Altogether, these data indicate moderate viral burden in the peripheral lymph nodes (59) of SIVagm-infected AGMs in comparison to HIV-1 and SIVmac infections. It is particularly striking that SIVagm-infected AGMs do not show a dichotomy in viral load between PBMC and LN lymphocytes (Table 5). This contrasts with human and macaques infected with pathogenic viruses showing considerably higher numbers of RNA positive cells and 5 to 10 fold more extensive proviral burden in their LNs than in peripheral cells (120, 127, 130). In contrast, SIVagm-infected AGMs, as shown by analyzing the same individual monkeys in blood and LNs, display viral DNA levels that are very close in both compartments (98, 109) (unpublished results). The average titers of infectious virus in PBMC and LNMC were also not statistically different (17 TCID50 per 10^6PBMC versus 21 TCID per 10^6 LNMC) (109). It did not matter whether the animals were infected experimentally or naturally (109).

The relatively low viral burden in LNs contrasts with the high levels of plasma viremia seen in several AGMs. The high levels of free virus particles in the blood might be in part a result of the lack of virus trapping in germinal centers. Furthermore, a clear dichotomy was observed when comparing the viral DNA copy numbers in peripheral lymph nodes and in the gut of a naturally infected AGM (24). The proviral burden was 1 to 3 log higher in the gut than in LNs of this animal. Such a dichotomy was not observed in two macaques studied at end-stage disease (24). One major source of high levels of cell-free virus in the blood of AGMs may therefore be the gut and only to a lesser extent peripheral secondary lymphoid tissues (24) (Gueye *et al*, submitted). This hypothesis needs to be further investigated. Furthermore, more studies are needed on the viral load in tissues during primary infection in the natural host, in particular in gut-associated lymphoid tissues, for a better comprehension of the mechanisms underlying the lack of immunodeficiency. So far, only lymph nodes have been studied for viral load during primary infection. The viral RNA and DNA loads in LNs peak during the second week of infection, concomitant to the peak in blood (98). Again, no dichotomy between PBMC and LN DNA viral load is observed (Tab. 5) (98).

9. CONCLUSIONS and PERSPECTIVES

We and others have described a wide range of viremia levels in SIVagm-infected AGMs. Many naturally and experimentally infected AGMs display high steady-state levels of viral RNA. Plasma RNA levels such as those observed in AGMs are usually associated with disease progression towards AIDS in HIV-1 infected humans. However, no significant depletion of T $CD4^+$ cells in the blood or LNs, or significant pathological changes of the brain seem to be associated with high SIVagm RNA levels in blood, CSF and/or brain (24). These data demonstrate that viral loads *per se* are not directly linked with pathogenicity. They rather indicate that the consequences of persistent viremia are totally different between SIVagm infections in the natural host and HIV-1 infections in humans. Interestingly, this suggests that the virus-host interactions are distinct during SIVagm and HIV-1 infections in AGMs and humans, respectively. Although SIVagm, as HIV, is cytopathic *in vitro*, the continuous replication of SIVagm *in vivo* seems to be associated with a limited bystander immunopathology. Thus, blood T $CD4^+$ cells from SIVagm-infected animals do not manifest increased susceptibility to cell-death by apoptosis as compared with uninfected animals (131). The LN architecture is not disrupted and the FDC network remains intact (109, 129). Furthermore, several data are in support of a lack of chronic hyperactivation of lymphocytes in infected AGMs despite high steady-state levels of viral RNA in blood. Indeed, LNs from AGMs display no signs of follicular hyperplasia specific for SIVagm infection in either early or the chronic phase of infection (98, 109). No infiltrations of $CD8^+$ cells are observed in germinal centers of LN from acutely and chronically infected AGMs (129). However, more detailed studies on the phenotype of T cells are needed to better understand the virus-host interactions during non-pathogenic infection. Together with comparative analyses on interactions of SIVagm with host-specific cellular proteins, these studies will be crucial to delineate the mechanistic basis for the avirulence of SIVagm infection. This might lead to the development of new prophylactic and therapeutic strategies that aim to protect against AIDS.

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11. REFERENCES

1. Hahn B. H., G. M. Shaw, K. M. De Cock & P. M. Sharp. AIDS as a zoonosis: scientific and public health implications. *Science* 287,607-614. (2000)

2. Peeters M., V. Courgnaud, B. Abela, P. Auzel, X. Pourrut, F. Bibollet-Ruche, S. Loul, F. Liegeois, C. Butel, D. Koulagna, E. Mpoudi-Ngole, G. M. Shaw, B. H. Hahn & E. Delaporte. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. *Emerg Infect Dis* 8,451-457. (2002)

3. Chakrabarti L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais & P. Sonigo. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. *Nature* (London) 328,543-547 (1987)

4. Hirsch V. M., R. A. Olmsted, M. Murphey-Corb, R. H. Purcell & P. R. Johnson. An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 339,389-392 (1989)
5. Lowenstine M. L., N. C. Pederson & J. Higgins. Seroepidemiologic survey of captive old world primates for antibodies to human and simian retroviruses and isolation of a lentivirus from Sooty-Mangabeys (Cercocebus atys). *Int. J. Cancer* 38,563-575 (1986)
6. Ohta Y., T. Masuda, H. Tsujimoto, K. Ishikawa, T. Kodama, S. Morikawa, M. Nakai, S. Honjo & M. Hayami. Isolation of simian immunodeficiency virus from African green monkeys and seroepidemiological survey of the virus in various non-human primates. *Int. J. Cancer* 41 (1988)
7. Müller-Trutwin M. C. & F. Barre-Sinoussi. Les infections non pathogènes par les virus de l'immunodéficience simienne (SIV) chez leurs hôtes naturels, les singes d'Afrique. *Primatologie* 2,325-356 (1999)
8. Hirsch V., G. Dapolito, P. R. Johnson, W. R. Elkins, W. T. London, R. J. Montali, S. Goldstein & C. Brown. Induction of AIDS by simian immunodeficiency virus from an African green monkey: species-specific variation in pathogenicity correlates with the extent of *in vivo* replication. *J. Virol*. 69,955-967 (1995)
9. Lernould J. M. Classification and geographical distribution of guenons: a review. In: A primate radiation: evolutionary biology of the African guenons. Edited by Gautier-Hion A, Bourliere F, Gautier J-P. Cambridge: Cambridge University Press; 1988:54-35.
10. Oates J. F. The distribution of cercopithecus monkeys in West Africal forests. In: A primate radiation: evolutionary biology of the African guenons. Edited by Gautier-Hion A, Bourliere F, Gautier J-P. Cambridge: Cambridge University Press; 1988:79-103.
11. Johnson P. R., A. Fomsgaard, J. Allan, M. Gravell, W. T. London, R. A. Olmstead & V. M. Hirsch. Simian immunodeficiency viruses from African Green monkeys display unusual genetic diversity. *J. Virol*. 64,1068-1092 (1990)
12. Allan J. S., M. Short, M. E. Taylor, S. Su, V. M. Hirsch, P. R. Johnson, G. M. Shaw & B. H. Hahn. Species-specific diversity among simian immunodeficiency viruses from African green monkeys. *J. Virol*. 65,2816-2828 (1991)
13. Müller M. C., N. K. Saksena, E. Nerrienet, C. Chappey, V. M. A. Hervé, J.-P. Durand, P. Legal-Campodonico, M.-C. Lang, J.-P. Digoutte, A. J. Georges, M.-C. Georges-Courbot, P. Sonigo & F. Barré-Sinoussi. Simian immunodeficiency viruses from Central and Western Africa: evidence for a new species-specific lentivirus in tantalus monkeys. *J. Virol*. 67,1227-1235 (1993)
14. Li Y., Y. M. Naidu, M. D. Daniel & R. C. Desrosiers. Extensive genetic variability of simian immunodeficiency virus from African green monkeys. *J. Virol*. 63,1800-1802 (1989)
15. Baier M., C. Garber, C. Müller, K. Cichutek & R. Kurth. Complete nucleotide sequence of a simian immunodeficiency virus from African Green Monkeys : a novel type of intergroup divergence. *Virology* 176,216-212 (1990)
16. Johnson P. R., M. Gravell, J. Allan, S. Goldstein, R. A. Olmsted & V. M. Hirsch. Genetic diversity among simian immunodeficiency virus isolates from African green monkeys. *J. Med. Primatol*. 18,271-277 (1989)
17. Diop O., Gueye A , A. Ayouba, E. Nerrienet, S. Corbet, P. Mauclere, F. Simon, F. Barre-Sinoussi & M. C. Müller-Trutwin. SIVs and the Origin of HIVs. in: AIDS in Africa 2nd Edition,104-120 (2002)
18. Manns A., H. König, M. Baier, R. Kurth & F. Grosse. Fidelity of reverse transcriptase of the simian immunodeficiency virus from African Green Monkey. *Nucl. Acids Res*. 19,533-537 (1991)
19. Hirsch V. M. H., C. McGann, G. Dapolito, S. Goldstein, A. Ogen-Odoi, B. Biryawaho, T. Lakwo & P. R. Johnson. Identification of a new subgroup of SIVagm in tantalus monkeys. *Virology* 197,426-430 (1993)
20. Jin M. J., J. Rogers, J. E. Philips-Conroy, J. S. Allan, R. Desrosiers, G. M. Shaw, P. M. Sharp & B. H. Hahn. Infection of a yellow baboon with simian immunodeficiency virus from African green monkeys: evidence for cross-species transmission in the wild. *J. Virol*. 68,8454-8460 (1994)
21.Jin M. J., H. Hui, D. L. Robertson, M. C. Müller, F. Barré-Sinoussi, V. M. Hirsch, J. S. Allan, G. M. Shaw, P. M. Sharp & B. H. Hahn. Mosaic genome structure of simian immunodeficiency virus from West African monkeys. *EMBO J*. 13,2935-2947 (1994)
22. Berkhout B. Structural features in TAR RNA of human and simian immunodeficiency viruses : a phylogenetic analysis. *Nucl. Acid Res*. 20,27-31 (1992)
23. Müller-Trutwin M. C., S. Corbet, M. Dias Tavares, V. M. A. Hervé, E. Nerrienet, M.-C. Georges-Courbot, W. Saurin, P. Sonigo & F. Barré-Sinoussi. The evolutionary rate of non-pathogenic Simian Immunodeficiency Viruses (SIVagm) is in agreement with a rapid and continuous replication *in vivo*. *Virology* 223,89-102 (1996)
24. Broussard S. R., S. I. Staprans, R. White, E. M. Whitehead, M. B. Feinberg & J. S. Allan. Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease. *J Virol 75*,2262-2275. (2001)
25. Balfe P., P. Simmonds, C. A. Ludlam, J. O. Bishop & A. J. Leigh-Brown. Concurrent evolution of human immunodeficiency virus type I in patients infected from the same source : rate of sequence change and low frequency of inactivating mutations. *J. Virol*. 64,6221-6223 (1990)
26. Burns D. P. W. & R. C. Desrosiers. Selection of genetic variants of simian immunodeficiency virus in persistently infected rhesus monkeys. *J. Virol*. 65,1843-1854 (1991)
27. Johnson P. R., T. E. Hamm, S. Goldstein, S. Kitov & V. M. Hirsch. The genetic fate of molecularly cloned simian immunodeficiency virus in experimentally infected macaques. *Virology* 185,217-228 (1991)
28. Bibollet-Ruche F., A. Galat-Luong, G. Cuny, P. Sarni-Manchado, G. Galat, J.-P. Durand, X. Pourrut & F. Veas. Simian Immunodeficiency virus infection in a patas monkey (Erythrocebus patas): evidence for cross-species transmission from African green monkeys (Cercopithecus aethiops sabaeus) in the wild. *J. Gen. Vir*. 77,773-781 (1996)

29. van Rensburg E. J., S. Engelbrecht, J. Mwenda, J. D. Laten, B. A. Robson, T. Stander & G. K. Chege. Simian immunodeficiency viruses (SIVs) from eastern and southern Africa: detection of a SIVagm variant from a chacma baboon. *J Gen Virol* 79,1809-1814. (1998)
30. Wolinsky S. M., C. M. Wike, B. T. M. Korber, C. Hutto, W. P. Parks, L. L. Rosenblum, K. J. Kunstman, M. R. Furtado & J. L. Munoz. Selective transmission of human immunodeficieny virus type-1 variants from mothers to infants. *Science* 255,1134-1137 (1992)
31. Zhang L. Q., P. McKenzie, A. Cleland, E. C. Holmes, A. J. Leigh Brown & P. Simmonds. Selection for specific sequences in the external envelope protein of human immunodeficiency virus type 2 upon primary infection. J. Virol. 67,3345-3356 (1993)
32. Zhu T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup & D. D. Ho. Genotypic and phenotypic characterization of HIV-1 in patients with primary infection. *Science* 261,1179-1181 (1993)
33. Phillips-Conroy J. E., C. J. Jolly, B. Petros, J. S. Allan & R. C. Desrosiers. Sexual transmission of SIVagm in wild grivet monkeys. *J. Med. Primat*. 23,1-7 (1994)
34. Galat-Luong A., G. Galat, F. Bibollet-Ruche, X. Pourrut, O. Diop, J. P. Durand, P. Sarni-Manchado, M. Senzani & G. Pichon. Social structure and SIVagm prevalence in two groups of green monkeys, cercopithecus aethiops sabaeus, in Senegal. in: Current Primatology 3,259-262 (1994)
35. Jolly C., J. E. Phillips-Conroy, T. R. Turner, S. Broussard & J. S. Allan. SIVagm incidence over two decades in a natural population of Ethiopian grivet monkeys (Cercopithecus aethiops aethiops). *J Med Primatol* 25,78-83. (1996)
36. Ostyula M., A. Gettie, M. Sulemane, R. Tarara, I. Mohamed & P. A. Marx. Apparent lack of vertical transmission of SIV in naturally infected African green monkeys. *Ann. Trop. Med. Parasitol*. 89,573-576 (1995)
37. Grindem C. B., W. T. Corbett, B. E. Ammerman & M. T. Tomkins. Seroepidemiologic survey of feline immunodeficiency virus infections in cats of Wake County, North Carolina. *J Am Vet Med Assoc* 194,226-228 (1989)
38. Galat-Luong A. Observation de contacts interindividuels interspecifiques avec échanges de fluides corporels entre singes verts, Cercopithecus aethiops, et patas, Erythrocebus patas. 6th International Conference on AIDS in Africa. Dakar, Sénégal,T.A.124 (1991)
39. Courgnaud V., X. Pourrut, F. Bibollet-Ruche, E. Mpoudi-Ngole, A. Bourgeois, E. Delaporte & M. Peeters. Characterization of a novel simian immunodeficiency virus from guereza colobus monkeys (Colobus guereza) in Cameroon: a new lineage in the nonhuman primate lentivirus family. *J Virol* 75,857-866. (2001)
40. Altmann S. A. & J. Altmann. Baboon ecology: African field research. Karger, Basel. (1970)
41. Chen Z., P. Telfier, A. Gettie, P. Reed, L. Zhang, D. D. Ho & P. A. Marx. Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. *J Virol* 70,3617-3627 (1996)
42. Souquiere S., F. Bibollet-Ruche, D. L. Robertson, M. Makuwa, C. Apetrei, R. Onanga, C. Kornfeld, J. C. Plantier, F. Gao, K. Abernethy, L. J. White, W. Karesh, P. Telfer, E. J. Wickings, P. Mauclere, P. A. Marx, F. Barre-Sinoussi, B. H. Hahn, M. C. Muller-Trutwin & F. Simon. Wild Mandrillus sphinx are carriers of two types of lentivirus. *J Virol* 75,7086-7096. (2001)
43. Corbet S., M. C. Muller-Trutwin, P. Versmisse, S. Delarue, A. Ayouba, J. Lewis, S. Brunak, P. Martin, F. Brun-Vezinet, F. Simon, F. Barre-Sinoussi & P. Mauclere. env sequences of simian immunodeficiency viruses from chimpanzees in Cameroon are strongly related to those of human immunodeficiency virus group N from the same geographic area. *J Virol* 74,529-534. (2000)
44. Santiago M. L., C. M. Rodenburg, S. Kamenya, F. Bibollet-Ruche, F. Gao, E. Bailes, S. Meleth, S. J. Soong, J. M. Kilby, Z. Moldoveanu, B. Fahey, M. N. Muller, A. Ayouba, E. Nerrienet, H. M. McClure, J. L. Heeney, A. E. Pusey, D. A. Collins, C. Boesch, R. W. Wrangham, J. Goodall, P. M. Sharp, G. M. Shaw & B. H. Hahn. SIVcpz in wild chimpanzees. *Science* 295,465. (2002)
45. Prince A. M., B. Brotman, D. H. Lee, L. Andrus, J. Valinsky & P. Marx. Lack of evidence for HIV type 1-related SIVcpz infection in captive and wild chimpanzees (Pan troglodytes verus) in West Africa. *AIDS Res Hum Retroviruses* 18,657-660. (2002)
46. Müller-Trutwin M. C., S. Corbet, S. Souquiere, P. Roques, P. Versmisse, A. Ayouba, S. Delarue, E. Nerrienet, J. Lewis, P. Martin, F. Simon, F. Barre-Sinoussi & P. Mauclere. SIVcpz from a naturally infected Cameroonian chimpanzee: biological and genetic comparison with HIV-1 N. *J Med Primatol* 29,166-172. (2000)
47. Kraus G., A. Werner, M. Baier, D. Binninger, F. J. Ferdinand, S. Norley & R. Kurth. Isolation of human immunodeficiency viruses from African Green monkeys. *Proc. Natl. Acad. Sci. USA* 86,2892-2896 (1989)
48. Müller M. C., N. K. Saksena, E. Nerrienet, C. Chappey, V. M. Herve, J. P. Durand, P. Legal-Campodonico, M. C. Lang, J. P. Digoutte, A. J. Georges, M. C. Georges-Courbot, P. Sonigo & F. Barré-Sinoussi:. Simian immunodeficiency viruses from central and western Africa: evidence for a new species-specific lentivirus in tantalus monkeys. *J Virol* 67,1227-1235. (1993)
49. Fomsgaard A., P. R. Johnson, W. T. London & V. M. Hirsch. Genetic variation of the SIVagm transmembrane glycoprotein in naturally and experimentally infected primates. *AIDS* 7,1041-1047 (1993)
50. Johnson P. R., S. Goldstein, W. T. London, A. Fomsgaard & V. M. Hirsch. Molecular clones of SIVsm and SIVagm: Experimental infection of macaques and African green monkeys. *J. Med. Primatol*. 19,279-286 (1990)
51. Gravell M., W. T. London, R. S. Hamilton, G. Stone & M. Monzon. Infection of macaque monkeys with simian immunodeficiency virus from African green monkeys: virulence and activation of latent infection. *J. Med. Primatol*. 18,247-254 (1989)
52. Beer B., M. Baier, J. zur Megede, S. Norley & R. Kurth. Vaccine effect using a live attenuated nef-deficient simian immunodeficiency virus of African green monkeys in the absence of detectable vaccine virus replication *in vivo*. *Proc. Natl. Acad. Sci*. 94,4062-4067 (1997)
53. Campbell B. F. & V. M. Hirsch. Vpr of simian immunodeficiency virus of African green monkeys is required for replication in macaque macrophages and lymphocytes. *J. Virol*.,5593-5602 (1997)

54. Hartung S., K. Boller, K. Cichutek, S. G. Norley & R. Kurth. Quantitation of a lentivirus in its natural host : simian immunodeficiency virus in Afrian Green Monkeys. *J. Virol*. 66,2143-2149 (1992)
55. König R. R., E. Flory, S. Steidl, J. Neumann, C. Coulibaly, E. Holznagel, S. Holzammer, S. Norley & K. Cichutek. Engineered CD4- and CXCR4-using simian immunodeficiency virus from African green monkeys is neutralization sensitive and replicates in nonstimulated lymphocytes. *J Virol* 76,10627-10636. (2002)
56. Dehghani H., C. R. Brown, R. Plishka, A. Buckler-White & V. M. Hirsch. The ITAM in Nef influences acute pathogenesis of AIDS-inducing simian immunodeficiency viruses SIVsm and SIVagm without altering kinetics or extent of viremia. *J Virol* 76,4379-4389. (2002)
57. Murayama Y., A. Amano, R. Mukai, H. Shibata, S. Matsunaga, H. Takahashi, Y. Yoshikawa, M. Hayami & A. Noguchi. CD4 and CD8 expressions in African green monkey helper T lymphocytes: implication for resistance to SIV infection. *Int. Immunol*. 9,843-851 (1997)
58. Daniel M. D., Y. Li, Y. Naidu, P. J. Durda, D. K. Schmidt, C. D. Troup, D. P. Silva, J. J. MacKey, H. W. KestlerIII, P. K. Seghal, N. W. King, Y. Ohta & M. Hayami. Simian immunodeficiency virus from African Green Monkeys. *J. Virol* 62,4123-4128 (1988)
59. Goldstein S., I. Ourmanov, C. R. Brown, B. E. Beer, W. R. Elkins, R. Plishka, A. Buckler-White & V. M. Hirsch. Wide range of viral load in healthy african green monkeys naturally infected with simian immunodeficiency virus: *J Virol* 74,11744-11753. (2000)
60. Honjo S., T. Narita, R. Kobayashi, A. Hiyaoka, K. Fujimoto, M. Takasaka, I. Sakakibara, R. Mukai, K. Ishikawa, Y. Ohta & M. Hayami. Experimental infection of African Green Monkeys and cynomolgus monkeys with a SIVagm strain isolated from a healthy African Green Monkey. *J. Med. Primatol*. 19,9-20 (1990)
61. Allan J. S., P. Kanda, R. C. Kennedy, E. K. Cobb, M. Anthony & J. W. Eichberg. Isolation and characterization of simian immunodeficiency viruses from two subspecies of African Green Monkeys. *AIDS Res. Hum. Retroviruses* 6,275-285 (1990)
62. Shimano R., R. Inubushi, K. Amano, T. Ogasawa & A. Adachi. Gag-Pol region determines the tropism of SIVagm for human cells. *Virus Genes* 16,137-139. (1998)
63. Allan J. S., J. Strauss & D. W. Buck. Enhancement of SIV infection with soluble receptor molecules. *Science* 247,1084-1087 (1990)
64. Fomsgaard A., V. M. Hirsch & P. R. Johnson. Cloning and sequences of primate CD4 molecules: diversity of the cellular receptor for simian immunodeficiency virus/human immunodeficiency virus. *Eur. J. Immunol*. 22,2973-2981 (1992)
65. Fomsgaard A., M. C. Müller-Trutwin, O. Diop, J. Hansen, C. Mathiot, S. Corbet, F. Barré-Sinoussi & J. S. Allan. Relation between phylogeny of African Green Monkey CD4 genes and their respective simian immunodeficiency virus. *J. Med. Primatol*. 26,120-128 (1997)
66. Fomsgaard A., Johnson, P R., Nielsen C, Novembre, F J, Hansen, J, Goldstein S, & Hirsch, V M. Receptor function of CD4 structures from African green monkey and pig-tail macaque for simian immunodeficiency virus, SIVsm, SIVagm, and human immunodeficiency virus type 1. *Viral Immunol*. 8,121-133 (1995)
67. Beer B., J. Denner, C. R. Brown, S. Norley, J. zur Megede, C. Coulibaly, R. Plesker, S. Holzammer, M. Baier, V. M. Hirsch & R. Kurth. Simian immunodeficiency virus of African green monkeys is apathogenic in the newborn natural host. *J Acquir Immune Defic Syndr Hum Retrovirol* 18,210-220. (1998)
68. Buijs L., W. M. Bogers, J. W. Eichberg & J. L. Heeney. CD8+ cell-mediated immune responses: relation to disease resistance and susceptibility in lentivirus-infected primates. *J Med Primatol* 26,129-138. (1997)
69. Holznagel E., S. Norley, S. Holzammer, C. Coulibaly & R. Kurth. Immunological changes in simian immunodeficiency virus (SIV(agm))-infected African green monkeys (AGM): expanded cytotoxic T lymphocyte, natural killer and B cell subsets in the natural host of SIV(agm). *J Gen Virol* 83,631-640. (2002)
70. Rappocciolo G., J. S. Allan, J. W. Eichberg & T. C. Chanh. A comparative study of natural killer cell activity, lymphoproliferation, and cell phenotypes in nonhuman primates. *Vet Pathol* 29,53-59. (1992)
71. Deng H., D. Unutmaz, V. N. KewalRamani & D. R. Littman. Expression cloning of new receptors used by simian and human immunodeficiency viruses. *Nature* 388,296-300 (1997)
72. Edinger A. L., A. Amedee, K. Miller, B. J. Doranz, M. Endres, M. Sharron, M. Samson, Z.-H. Lu, J. E. Clements, M. Murphey-Corb, S. C. Peiper, M. Parmentier, C. Broder & R. W. Doms. Differential utilization of CCR5 by macrophage and T cell tropic simian immunodeficiency virus strains. *Proc. Natl. Acad. Sci*. 94,4005-4010 (1997)
73. Chen Z., A. Gettie, D. D. Ho & P. A. Marx. Primary SIVsm isolates use the CCR5 coreceptor from sooty mangabeys naturally infected in West Africa: A comparison of coreceptor usage of primary SIVsm, HIV-2, and SIVmac. *Virology* 246,113-124 (1998)
74. Kuhmann S. E., N. Madani, O. M. Diop, E. J. Platt, J. Morvan, M. C. Muller-Trutwin, F. Barre-Sinoussi & D. Kabat. Frequent substitution polymorphisms in African green monkey CCR5 cluster at critical sites for infections by simian immunodeficiency virus SIVagm, implying ancient virus-host coevolution. *J Virol* 75,8449-8460. (2001)
75. Stoye J. P. An intracellular block to primate lentivirus replication. PNAS 99,11549-11551 (2002)
76. Müller-Trutwin M. C., S. Corbet, J. Hansen, M. C. Georges-Courbot, O. Diop, J. Rigoulet, F. Barre-Sinoussi & A. Fomsgaard. Mutations in CCR5-coding sequences are not associated with SIV carrier status in African nonhuman primates. *AIDS Res Hum Retroviruses* 15,931-939. (1999)
77. Kuhmann S. E., E. J. Platt, S. L. Kozak & D. Kabat. Cooperation of multiple CCR5 coreceptors is required for infections by human immunodeficiency virus type 1. *J Virol* 74,7005-7015. (2000)
78. Owen S. M., S. Masciotra, F. Novembre, J. Yee, W. M. Switzer, M. Ostyula & R. B. Lal. Simian immunodeficiency viruses of diverse origin can use CXCR4 as a coreceptor for entry into human cells. *J Virol* 74,5702-5708. (2000)
79. Dumonceaux J., C. Goujon, V. Joliot, P. Briand & U. Hazan. Determination of essential amino acids involved in

the CD4-independent tropism of the X4 human immunodeficiency virus type 1 m7NDK isolate: role of potential N glycosylations in the C2 and V3 regions of gp120. *J Virol* 75,5425-5428. (2001)
80. Liao F., G. Alkhatib, K. W. C. Peden, G. Sharma, E. A. Berger & J. Farber. STRL33, a novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J. Exp. Med*. 185,2015-2023 (1997)
81. Pohlmann S., B. Lee, S. Meister, M. Krumbiegel, G. Leslie, R. W. Doms & F. Kirchhoff. Simian immunodeficiency virus utilizes human and sooty mangabey but not rhesus macaque STRL33 for efficient entry. *J Virol* 74,5075-5082. (2000)
82. Pohlmann S., N. Stolte, J. Munch, P. Ten Haaft, J. L. Heeney, C. Stahl-Hennig & F. Kirchhoff. Co-receptor usage of BOB/GPR15 in addition to CCR5 has no significant effect on replication of simian immunodeficiency virus *in vivo*. *J Infect Dis* 180,1494-1502. (1999)
83. Geijtenbeek T. B., D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor & Y. van Kooyk. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* 100,587-597. (2000)
84. Bashirova A. A., L. Wu, J. Cheng, T. D. Martin, M. P. Martin, R. E. Benveniste, J. D. Lifson, V. N. KewalRamani, A. Hughes & M. Carrington. Novel member of the CD209 (DC-SIGN) gene family in primates. *J Virol* 77,217-227. (2003)
85. Wu L., A. A. Bashirova, T. D. Martin, L. Villamide, E. Mehlhop, A. O. Chertov, D. Unutmaz, M. Pope, M. Carrington & V. N. KewalRamani. Rhesus macaque dendritic cells efficiently transmit primate lentiviruses independently of DC-SIGN. *Proc Natl Acad Sci U S A* 99,1568-1573. (2002)
86. Pohlmann S., F. Baribaud, B. Lee, G. J. Leslie, M. D. Sanchez, K. Hiebenthal-Millow, J. Munch, F. Kirchhoff & R. W. Doms. DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. *J Virol* 75,4664-4672. (2001)
87. Fukasawa M., T. Miura, A. Hasegawa, S. Morikawa, H. Tsujimoto, K. Miki, T. Kitamura & M. Hayami. Sequence of simian immunodeficiency virus from African Green Monkey, a new member of the HIV/SIV group. *Nature* 333,457-461 (1988)
88. Baier M., A. Werner, K. Cichutek, C. Gerber, C. Müller, G. Kraus, F. J. Ferdinand, S. Hartung, T. S. Papas & R. Kurth. Molecularly cloned simian immunodeficiency virus SIVagm3 is highly divergent from other SIVagm isolates and is biologically active *in vitro* and *in vivo*. *J. Virol*. 63,5119-5123 (1989)
89. Fomsgaard A., V. M. Hirsch, J. S. Allan & P. R. Johnson. A highly divergent proviral DNA clone of SIV from a distinct species of African Green Monkey. *Virology* 182,397-402 (1991)
90. Soares M. A., D. L. Robertson, H. Hui, J. S. Allan, G. M. Shaw & B. H. Hahn. A full-length and replication-competent proviral clone of SIVAGM from tantalus monkeys. *Virology* 228,394-399. (1997)
91. Tristem M., C. Marshall, A. Karpas & F. Hill. Evolution of the primate lentiviruses: evidence from vpx and vpr. *EMBO J*. 11,3405-3412 (1992)
92. Sharp P. M., E. Bailes, M. Stevenson, M. Emerman & B. H. Hahn. Gene acquisition in HIV and SIV. *Nature* 383,586-587. (1996)
93. Sakai H., R. Shibata, J. Sakuragi, T. Kiyomasu, M. Kawamura, M. Hayami, A. Ishimoto & A. Adachi. Compatibility of rev gene activity in the four groups of primate lentiviruses. *Virology* 184,513-520. (1991)
94. Sakai H., J. Sakuragi, S. Sakuragi, M. Kawamura & A. Adachi. Compatibility of Tat and Rev transactivators in the primate lentiviruses. *Arch Virol* 129,1-10. (1993)
95. Sakuragi J.-I., M. Fukasawa, R. Shibata, H. Sakai, M. Kawamura, H. Akari, T. Kiyomasu, A. Ishimoto, M. Hayami & A. Adachi. Functional anlaysis of long terminal repeats derived from four strains of simian immunodeficiency virus SIVagm in relation to other primate lentiviruses. *Virology* 185,455-459 (1991)
96. Sakuragi J., S. Sakuragi, S. Ueda & A. Adachi. Functional analysis of SIVagm Long terminal repeat. *Virus Genes* 12,21-25 (1996)
97. Jubier-Maurin V., P. Sarni-Manchado, F. Veas, N. Vidal, F. Bibollet-Ruche, J.-P. Durand, A. Galat-Luong & G. Cuny. Regulatory genes of Simian Immunodeficiency viruses from West African Green Monkeys. *J. Virol*. 69 (1995)
98. Diop O. M., A. Gueye, M. Dias-Tavares, C. Kornfeld, A. Faye, P. Ave, M. Huerre, S. Corbet, F. Barre-Sinoussi & M. C. Muller-Trutwin. High levels of viral replication during primary simian immunodeficiency virus SIVagm infection are rapidly and strongly controlled in African green monkeys *J. Virol* 74, 7538-7547 (2000)
99. Holzammer S., E. Holznagel, A. Kaul, R. Kurth & S. Norley. High virus loads in naturally and experimentally SIVagm-infected African green monkeys. *Virology* 283,324-331. (2001)
100. Simon J. H., D. L. Miller, R. A. Fouchier, M. A. Soares, K. W. Peden & M. H. Malim. The regulation of primate immunodeficiency virus infectivity by Vif is cell species restricted: a role for Vif in determining virus host range and cross-species transmission. *EMBOo J* 17,1259-1267. (1998)
101. Madani N. & D. Kabat. An endogenous inhibitor of human immunodeficiency virus in human lymphocytes is overcome by the viral Vif protein. *J Virol* 72,10251-10255. (1998)
102a. Madani, N., R. Millette, E.J. Platt, M. Marin, S.L. Kozak, D.D. Bloch & D. Kabat. Implication of the lymphocyte-specific nuclear body protein Sp140 in an innate response to HIV-1. *J. Virol*. 76:11133-11138. (2002).
102b. Sheehy A. M., N. C. Gaddis, J. D. Choi & M. H. Malim. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 418,646-650. (2002)
103. Philippon V., Z. Matsuda & M. Essex. Transactivation is a conserved function among primate lentivirus Vpr proteins but is not shared by Vpx. *J Hum Virol* 2,167-174. (1999)
104. Accola M. A., A. A. Bukovsky, M. S. Jones & H. G. Gottlinger. A conserved dileucine-containing motif in

p6(gag) governs the particle association of Vpx and Vpr of simian immunodeficiency viruses SIV(mac) and SIV(agm). *J Virol* 73, 9992-9999. (1999)
105. Planelles V., J. B. Jowett, Q. X. Li, Y. Xie, B. Hahn & I. S. Chen. Vpr-induced cell cycle arrest is conserved among primate lentiviruses. *J Virol* 70,2516-2524. (1996)
106. Stivahtis G. L., M. A. Soares, M. A. Vodicka, B. H. Hahn & M. Emerman. Conservation and host specificity of Vpr-mediated cell cycle arrest suggest a fundamental role in primate lentivirus evolution and biology. *J Virol* 71,4331-4338. (1997)
107. Zhu Y., H. A. Gelbard, M. Roshal, S. Pursell, B. D. Jamieson & V. Planelles. Comparison of cell cycle arrest, transactivation, and apoptosis induced by the simian immunodeficiency virus SIVagm and human immunodeficiency virus type 1 vpr genes. *J Virol* 75,3791-3801. (2001)
108. Wagener S., M. T. Dittmar, B. Beer, R. König, R. Plesker, S. Norley, R. Kurth & K. Cichutek. The U3 Promotor and the nef Gene of SIVsmmPBj1.9 do not confer acute pathogenicity upon SIVagm. *J. Virol*. 72,3446-3450 (1998)
109. Beer B., J. Scherer, J. zur Megede, S. Norley, M. Baier & R. Kurth. Lack of Dichotomy between Virus Load of Peripheral Blood and Lymph Nodes during Long-Term Simian Immunodeficiency Virus Infection of African Green Monkey. *Virology* 219,367-375 (1996)
110. Onanga R., C. Kornfeld, I. Pandrea, J. Estaquier, S. Souquiere, P. Rouquet, V. P. Mavoungou, O. Bourry, S. M'Boup, F. Barre-Sinoussi, F. Simon, C. Apetrei, P. Roques & M. C. Muller-Trutwin. High levels of viral replication contrast with only transient changes in CD4(+) and CD8(+) cell numbers during the early phase of experimental infection with simian immunodeficiency virus SIVmnd-1 in Mandrillus sphinx. *J Virol* 76,10256-10263. (2002)
111. ten Haaft P., K. Murthy, M. Salas, H. McClure, R. Dubbes, W. Koornstra, H. Niphuis, D. Davis, G. van Der Groen & J. Heeney. Differences in early virus loads with different phenotypic variants of HIV-1 and SIVcpz in chimpanzees. *AIDS* 15,2085-2092. (2001)
112. Rey-Cuille M. A., J. L. Berthier, M. C. Bomsel-Demontoy, Y. Chaduc, L. Montagnier, A. G. Hovanessian & L. A. Chakrabarti. Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. *J Virol* 72,3872-3886. (1998)
113. Chakrabarti L. A., S. R. Lewin, L. Zhang, A. Gettie, A. Luckay, L. N. Martin, E. Skulsky, D. D. Ho, C. Cheng-Mayer & P. A. Marx. Normal T-cell turnover in sooty mangabeys harboring active simian immunodeficiency virus infection. *J Virol* 74,1209-1223. (2000)
114. Ling B., M. L. Santiago, S. Meleth, B. Gormus, H. M. McClure, C. Apetrei, B. H. Hahn & P. A. Marx. Noninvasive detection of new simian immunodeficiency virus lineages in captive sooty mangabeys: ability to amplify virion RNA from fecal samples correlates with viral load in plasma. *J Virol* 77,2214-2226. (2003)
115. Mellors J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd & L. A. Kingsley. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 272,1167-1170 (1996)
116. Ten Haaft P., B. Verstrepen, K. Uberla, B. Rosenwirth & J. Heeney. A pathogenic threshold of virus load defined in simian immunodeficiency virus- or simian-human immunodeficiency virus-infected macaques. *J. Virol.* 72,10281-10285 (1998)
117. Cao Y., L. Qin, L. Zhang, J. Safrit & D. Ho. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. *N Engl J Med* 332,201-208 (1995)
118. Ho D. D., T. Moudgil & M. Alam. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *New Engl. J. Med*. 321,1621-1625 (1989)
119. Cone R. W., P. Gowland, M. Opravil, P. Grob & B. Ledergerber. Levels of HIV-infected peripheral blood cells remain stable throughout the natural history of HIV-1 infection. Swiss HIV Cohort Study. *AIDS* 12,2253-2260. (1998)
120. Pantaleo G., C. Graziosi, J. F. Demarest & e. al. HIV infection is active and progressive in lymphoid tissue during the clinically latent state of disease. *Nature* 362,355-358 (1993)
121. Simmonds P., P. Balfe, J. F. Peutherer, C. A. Ludlam, J. O. Bishop & A. J. Brown. Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers. *J Virol* 64,864-872. (1990)
122. Batman P. A., A. R. Miller, S. M. Forster, J. R. Harris, A. J. Pinching & G. E. Griffin. Jejunal enteropathy associated with human immunodeficiency virus infection: quantitative histology. *J Clin Pathol* 42,275-281. (1989)
123. Veazey R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers & A. A. Lackner. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science* 280,427-431 (1998)
124. Heise C., S. Dandekar, P. Kumar, R. Duplantier, R. M. Donovan & C. H. Halsted. Human immunodeficiency virus infection of enterocytes and mononuclear cells in human jejunal mucosa. *Gastroenterology* 100,1521-1527. (1991)
125. Heise C., C. J. Miller, A. Lackner & S. Dandekar. Primary acute simian immunodeficiency virus infection of intestinal lymphoid tissue is associated with gastrointestinal dysfunction. *J Infect Dis* 169,1116-1120. (1994)
126. Brew B. J., L. Pemberton, P. Cunningham & M. G. Law. Levels of human immunodeficiency virus type 1 RNA in cerebrospinal fluid correlate with AIDS dementia stage. *J Infect Dis* 175,963-966. (1997)
127. Pantaleo G., S. Menzo, M. Vaccarezza & e. al. Studies in subjects with long term non progressive human immunodeficiency virus infection. *N. Engl. J. Med*. 332,209-216 (1995)
128. Chun T. W., L. Carruth, D. Finzi, X. Shen, J. A. DiGiuseppe, H. Taylor, M. Hermankova, K. Chadwick, J. Margolick, T. C. Quinn, Y. Kuo, R. Brookmeyer, M. A. Zeiger, P. Barditch-Crovo & R. F. Siliciano. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 387,183-188 (1997)
129. Dias Tavares M., M. C. Müller-Trutwin, P. Avé, M. Huerre, S. Camelo, V. Hervé, M. J, F. Barré-Sinoussi & S. Corbet. Non-pathogenic SIVagm infections of African mokeys correlate with a low viral burden associated with a

lack of alterations in lymphoid tissues. 14th Annual Symposium on nonhuman primate models for AIDS Portland, OR, USA,141 (1996)
130. Chakrabarti L., M.-C. Cumont, L. Montagnier & B. Hurtrel. Variable course of primary simian immunodeficiency virus infection in lymph nodes: relation to disease progression. *J. Virol*. 68,6634-6643 (1994)
131. Estaquier J., T. Idziorek, F. De Bels, F. Barré-Sinoussi, B. Hurtrel, A.-M. Aubertin, A. Venet, M. Mehtali, E. Muchmore, P. Michel, Y. Mouton, M. Girard & J.-C. Ameisen. Programmed cell death and AIDS : significance of T-cell apoptosis in pathogenic and nonpathogenic primate lentiviral infections. *Proc. Natl. Acad. Sci*. USA 91,9431-9435 (1994)

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