

# Specific passage of simian immunodeficiency virus from end-stage disease results in accelerated progression to AIDS in rhesus macaques

Lennart Holterman,<sup>1</sup> Henk Niphuis,<sup>1</sup> Peter J. F. ten Haaft,<sup>1</sup> Jaap Goudsmit,<sup>2</sup> Gary Baskin<sup>3</sup> and Jonathan L. Heeney<sup>1</sup>

<sup>1</sup> Department of Virology, Biomedical Primate Research Centre, Lange Kleiweg 157, 2288 GJ, Rijswijk, The Netherlands

<sup>2</sup> Department of Human Retrovirology, Academic Medical Centre, Amsterdam, The Netherlands

<sup>3</sup> Department of Pathology, Tulane Regional Primate Research Centre, Tulane University, Covington, LA 70433, USA

To determine whether passage of late-stage variants of simian immunodeficiency virus (SIV) would lead to a more virulent infection and rapid disease progression, a study was designed to examine the effects of selective transmission of SIV from late-stage cases of AIDS in *Macaca mulatta*. In a uniform group of 10 age-matched animals from the same genetic breeding stock infected with SIV<sub>B670</sub>, it took 7 months before one of the ten animals developed AIDS. Passage of virus taken from this animal immediately prior to death resulted in death of the recipient due to AIDS within 4 months. Again, subsequent passage of virus taken late in disease resulted in an accelerated disease course, with AIDS developing within 2·5 and 1·8 months in two recipients. The fourth passage of virus taken late in disease from the most rapid progressor (1·8 months) resulted in AIDS developing in this recipient within 1 month of infection. During each consecutive passage *in vivo*, the loss of memory T cells became more acute. Evidence that the virus became more virulent with selective passage of late-stage variants was provided by the markedly increased levels of both plasma antigen and viral RNA. Subsequent *in vivo* passage from end-stage AIDS selected for a strain of SIV capable of causing the acute development of AIDS as rapidly as 1 month post-infection. The pathology of acute AIDS in these cases closely resembled that seen after a chronic disease course.

## Introduction

The rate of progression to AIDS is highly variable, influenced by both virus and host factors. The median time to AIDS if left untreated has been estimated to be between 11 and 14 years, dependent on the cohort studied (Munoz *et al.*, 1989). At each end of the survival spectrum in a population infected with human immunodeficiency virus type 1 (HIV-1), individuals can be identified who either develop the disease more rapidly (rapid progressors, disease within 3 years of infection) or become long-term survivors or long-term non-progressors (Munoz *et al.*, 1995). In addition to host factors (i.e. co-receptor mutations) (Anzala *et al.*, 1998; Dean *et al.*, 1996; Kaslow *et al.*, 1996; Kostrikis *et al.*, 1998; Smith *et al.*, 1997; Winkler *et al.*, 1998), virus factors have been identified (i.e. deletions in *nef*)

that contribute to a less pathogenic disease course (Kirchhoff *et al.*, 1995; Salvi *et al.*, 1998). However, despite the well-documented host factors that may contribute to a more rapid disease course, the virus virulence factors responsible for rapid progression to AIDS have not been identified. In contrast to virus virulence factors associated with rapid progression, several virus virulence factors associated with chronic progression to AIDS in the simian immunodeficiency virus (SIV)–macaque model have been localized to particular regions of the genome (Edmonson *et al.*, 1998; Kestler *et al.*, 1991; Marthas *et al.*, 1993). To develop a model to evaluate the possible role of virus virulence on rapid progression to AIDS, we turned to the use of non-human primates. Rhesus monkeys (*Macaca mulatta*), when infected with certain strains of SIV, develop a disease that in almost all respects mimics AIDS in humans (Heeney, 1996).

The SIVs are a group of HIV-related, but distinct, lentiviruses (Li *et al.*, 1989) isolated from several different

**Author for correspondence:** Jonathan Heeney.  
Fax +31 15 284 3986. e-mail heeney@bprc.nl

African primates including chimpanzees ( $SIV_{cpz}$ ) (Peeters *et al.*, 1992), sooty mangabeys ( $SIV_{sm}$ ) (Fultz *et al.*, 1990; Murphey-Corb *et al.*, 1986), mandrills ( $SIV_{mnd}$ ) (Tsujimoto *et al.*, 1989) and African green monkeys ( $SIV_{agm}$ ) (Allan *et al.*, 1991; Johnson *et al.*, 1990). The infection of Asian macaques with different SIV strains derived from sooty mangabeys results in the development of an AIDS-like disease remarkably similar to AIDS in humans infected with HIV-1 (Simon *et al.*, 1994). The SIV-macaque model has proven important for investigating the pathogenesis of lentivirus-induced immunodeficiency (Baskin & Soike, 1989; Desrosiers & Ringler, 1989; Hirsch & Johnson, 1994), transmission (Miller, 1998; Trichel *et al.*, 1997) and virus-host interactions (Lang *et al.*, 1997; Sawai *et al.*, 1996) as well as the evaluation of antiviral therapy (Donahue *et al.*, 1998; Van Rompay *et al.*, 1998) and candidate vaccines (Heeney, 1996).

A number of different SIV strains have been described that cause AIDS in Asian macaques (Simon *et al.*, 1994), which have different disease-causing potential (P. Mooij, W. Bogers, H. Niphuis, W. Koornstra, R. Dubbes & J. Heeney, unpublished results). Certain commonly studied SIV strains such as  $SIV_{mac251}$  cause AIDS over a variable period of time in outbred macaques, with 50% mortality ranging from 1 to 2 years (Daniel *et al.*, 1985, 1987; Letvin *et al.*, 1985; P. Mooij, W. Bogers, H. Niphuis, W. Koornstra, R. Dubbes & J. Heeney, unpublished results). Other isolates that have been passaged more extensively in human T cell lines become highly attenuated (Daniel *et al.*, 1985; Kornfeld *et al.*, 1987) and some molecular clones, for instance, are non-pathogenic (Miller *et al.*, 1998). This attenuation is frequently attributed to passage of SIV on human T cell lines, which may result in truncations in the SIV transmembrane open reading frame (Hirsch *et al.*, 1989), and in a growth advantage of certain *in vitro*-adapted variants over others (Goodenow *et al.*, 1989; Kodama *et al.*, 1990; Meyerhans *et al.*, 1989).

In contrast to the more chronic AIDS-causing SIV isolates, a particular isolate designated  $SIV_{simmPBj14}$  has been described that causes rapid death of pigtail macaques.  $SIV_{simmPBj14}$  was derived from  $SIV_{sm}$  (Fultz *et al.*, 1989). The histopathology of the acute disease syndrome does not resemble AIDS, however.  $SIV_{simmPBj14}$  infection results in an acute haemorrhagic diarrhoea, culminating in metabolic acidosis and death in experimentally infected pigtailed macaques within 10–14 days (Dewhurst *et al.*, 1990; Fultz & Zack, 1994; Zacharias *et al.*, 1994). Since this syndrome is distinct from the AIDS-like illness observed in rhesus macaques, and since infection of pigtailed macaques does not result in long-term progressive disease, this  $SIV_{simmPBj14}$  isolate is considered an atypical lentivirus (Fultz & Zack, 1994). Interestingly, if the acute haemorrhagic diarrhoea is treated successfully, animals may recover and then, only after a protracted period, develop a chronic disease course resembling a more classic AIDS-like disease more than a year after infection (Rosenberg *et al.*, 1991).

The aim of this study was to determine whether primate lentiviruses become more virulent with *in vivo* evolution, as was suggested by the recent observations of Kimata *et al.* (1999) (see also Hirsch, 1999). We proposed that, by selective end-stage disease passage of SIV, a true AIDS-like disease may develop acutely in rhesus macaques. Using the strategy of serial *in vivo* passage, we attempted to increase the virulence of the SIV strain used and to determine how rapidly AIDS could develop in a uniform group of juvenile macaques. The result was an SIV strain, designated  $SIV_{8980}$ , that was capable of inducing a highly accelerated AIDS-like syndrome with extremely high virus loads and rapid loss of  $CD4^+$  T cells within weeks of infection. The histopathological lesions observed in all acute cases were indistinguishable from those found after a chronic disease course resulting in AIDS.

## Methods

■ **Animals and viruses.** The study population consisted of a uniform group of 16 captive-bred, retrovirus-free, male juvenile macaques (*Macaca mulatta*), which were approximately 18 months old at the time of infection. The animals were from a common outbred stock of Indian origin and bred in captivity at the Biomedical Primate Research Centre. All were raised maternally until 6 months old and then weaned and group-housed in preparation for the study. In the first *in vivo* passage (P1), 10 age-matched male juvenile *Macaca mulatta* were infected with  $5 \times 10^2$  monkey infectious doses ( $MID_{50}$ ) of the  $SIV_{sm}$ -derived strain  $SIV_{B670}$  (Baskin *et al.*, 1986; Murphey-Corb *et al.*, 1986). From the first animal that developed AIDS (7 months post-infection),  $2 \times 10^6$  peripheral blood mononuclear cells (PBMC) were used to passage the infection consecutively to the next group of animals in each subsequent passage. Animals were monitored haematologically to measure T helper/memory cell loss, as well as to determine levels of plasma viraemia for evidence of development of AIDS. On the basis of clinical and haematological evidence of AIDS, animals were euthanized, followed by comprehensive autopsy with complete histopathological and bacteriological assessment.

■ **Biochemical and haematological analysis.** Routine clinical biochemical and haematological analysis were performed at routine intervals to support the clinical diagnosis of AIDS. FACS analysis was performed by using Leu 3a (anti-CD4), Leu 2a (anti-CD8) and 4B4 (anti-CD29) to monitor changes in T helper/memory subsets. Staining of mononuclear cells for lymphocyte subsets was performed by double labelling by using FACS analysis with Leu 3a and 4B4 as described previously. Plasma antigen levels were determined by SIV p27 antigen-capture assay (Coulter) according to the manufacturer's recommendations.

■ **Viral RNA levels in plasma.** In order to determine SIV RNA levels in plasma of infected macaques, a highly sensitive and reproducible quantitative competitive RT-PCR assay has been developed (ten Haaf *et al.*, 1998). Briefly, 200  $\mu$ l plasma was added to 600  $\mu$ l guanidine isothiocyanate-based lysis solution containing 300 copies of an internal standard RNA. The RNA was precipitated with 2-propanol and was reverse-transcribed and amplified with *rTth* DNA polymerase. The amplification products were hybridized in six fivefold dilutions to a capture probe that was detected by a streptavidin-horseradish peroxidase-mediated colorimetric reaction. The amplified internal standard was hybridized to a rearranged 26 bp capture probe in separate microwells. The number of RNA copies in the plasma sample was calculated from the absorbance of the sample wells compared with that of the corresponding

**Table 1.** Time to death due to AIDS, plasma antigenaemia and principal findings at autopsy in sequential passages of SIV in macaques

Monkey	Time to death (months)	Plasma antigenaemia		Principal histopathological findings
		SIV p27 (ng/ml)*	Duration	
<b>Passage 1. Infected with SIV<sub>B670</sub> stock</b>				
P1a	7	< 10	Persistent	Pneumocystis, candidiasis, CMV, lymphoid atrophy
P1b	7	< 10	NP	Interstitial pneumonia, CMV, cryptosporidium, candidiasis
P1c	9.5	< 10	NP	Meningitis, candidiasis, pulmonary abscesses
P1d	13.5	< 10	NP	Interstitial pneumonia, cryptosporidium, candidiasis
P1e	16	< 10	NP	Interstitial pneumonia, meningitis, glomerular sclerosis
P1f	20.8	< 10	NP	Persistent encephalitis, interstitial pneumonia, CMV, candidiasis
P1g	23	< 10	NP	Peritonitis, cholangitis, interstitial pneumonia
P1h	32.7	< 10	NP	Disseminated CMV, interstitial pneumonia, glomerular sclerosis
P1i	32.8	< 10	NP	Interstitial pneumonia, candidiasis, giardiasis
P1j	Alive	< 10	NP	–
<b>Passage 2. Infected from P1a</b>				
P2	4.1	15.5	NP	Persistent interstitial pneumonia, CMV, glomerulonephritis, cryptosporidium
<b>Passage 3. Infected from P2</b>				
P3a	1.8	35.5	Persistent	Encephalitis/meningitis, cryptosporidium, interstitial pneumonia
P3b	2.5	39.9	Persistent	Enteritis/wasting, encephalitis/meningitis
<b>Passage 4. Infected from P3a</b>				
P4	1.0	104.8	Persistent	Interstitial pneumonia, CMV, diarrhoea/wasting, encephalitis/meningitis
<b>Passage 5. Infected from P4</b>				
P5a	2.0	45.3	Persistent	Interstitial pneumonia, CMV, encephalitis/meningitis
P5b	2.0	12.7	Persistent	Interstitial pneumonia, CMV/generalized lymphoid atrophy

\* Maximum level of plasma antigen measured during the course of the disease.  
NP, Not persistent; CMV, cytomegalovirus.

internal standard. Data were plotted for each individual animal involved in the sequential *in vivo* passage in RNA equivalents per ml (Fig. 3). Furthermore, to compare survival with virus load, the concentration of plasma viral RNA at the threshold achieved at the set point (defined for this model by ten Haaft *et al.*, 1998) was plotted against the number of weeks each animal survived post-infection (Fig. 4).

■ **Preparation of SIV<sub>8980</sub> virus stocks.** PBMC from rhesus monkey 8980 (P4) were isolated from EDTA-treated blood by banding over lymphocyte separation medium and washed twice with RPMI medium supplemented with 10% foetal calf serum. Cells were cultured in an autologous system using only the animal's own PBMC (Heeney *et al.*, 1996) to avoid culture bias or attenuation, as has been reported when SIV is propagated on human T cell lines. After 14 days of culture, cells were transferred to fresh feeder plates and grown for another 14 days. The supernatant from each well was collected and the amount of SIV Gag antigen was measured by using a p27 antigen-capture assay (Coulter). Supernatants from wells containing the largest amounts of p27 were pooled, clarified to remove cellular debris, filtered over a 0.22 µm sterile filter (Millipore) and aliquoted. Virus was administered intravenously to eight rhesus macaques as before. Animals were followed for disease progression for comparison of survival with animals (P1) infected with the pre-passage isolate, SIV<sub>B670</sub>.

■ **Statistical analysis.** The survival of animals infected with the two virus strains (SIV<sub>B670</sub> and SIV<sub>8980</sub>) was compared by plotting Kaplan–

Meier curves. The difference in the survival times between these two virus isolates in separate groups of monkeys was compared to demonstrate the increase in virulence following serial *in vivo* passage. The significance of this observation was tested by using log-rank analysis.

## Results

Each consecutive passage in age-matched juvenile rhesus monkeys resulted in a reduction of the asymptomatic period and a dramatic acceleration of the progression to AIDS, until the most rapid progression time to AIDS of 1 month was reached by the fourth passage. In the first passage (P1) in 10 monkeys, the time to death due to AIDS ranged from 7 months (P1a and P1b) to more than 3 years (P1j) (Table 1). The first animals to develop AIDS in P1 were asymptomatic for approximately 5.5 months. Disease development in the first animals to succumb to AIDS was characterized by loss of T helper/memory (CD4<sup>+</sup>/CD29<sup>+</sup>) cells and persistent plasma antigenaemia. Table 1 lists the AIDS-defining pathological diagnoses of all animals included in the study, based on complete histopathological and microbiological workup. Although the length of the asymptomatic period decreased, the clinical and pathological manifestations of the disease did

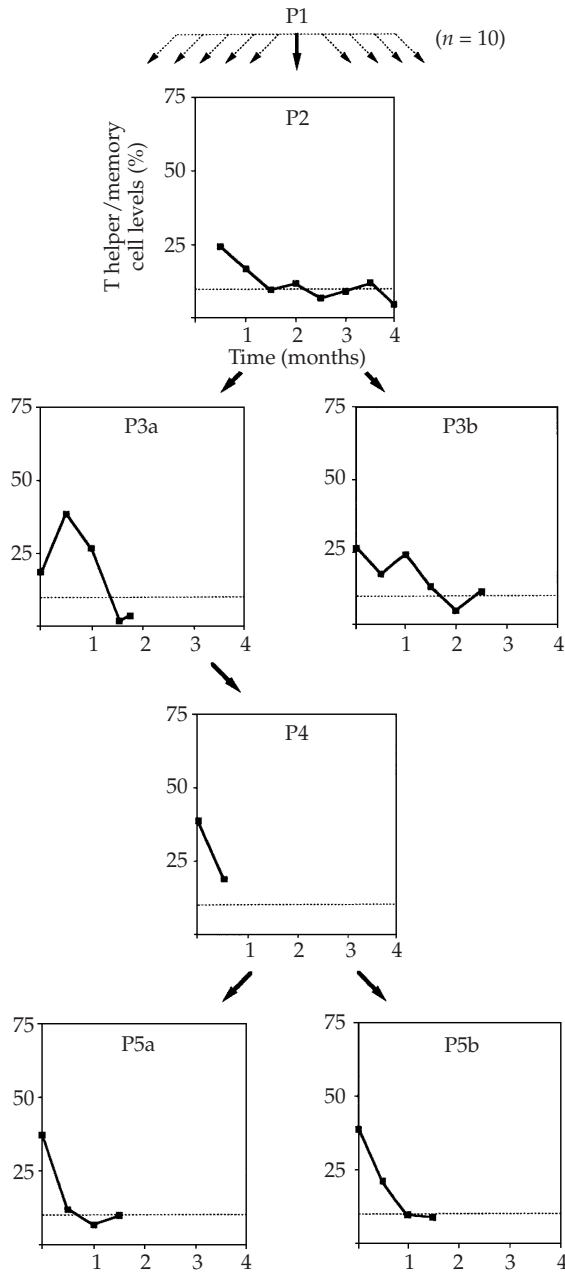


Fig. 1. Changes in the CD4<sup>+</sup>/CD29<sup>+</sup> T cell population during progression to AIDS in animals infected with each subsequent passage of virus.

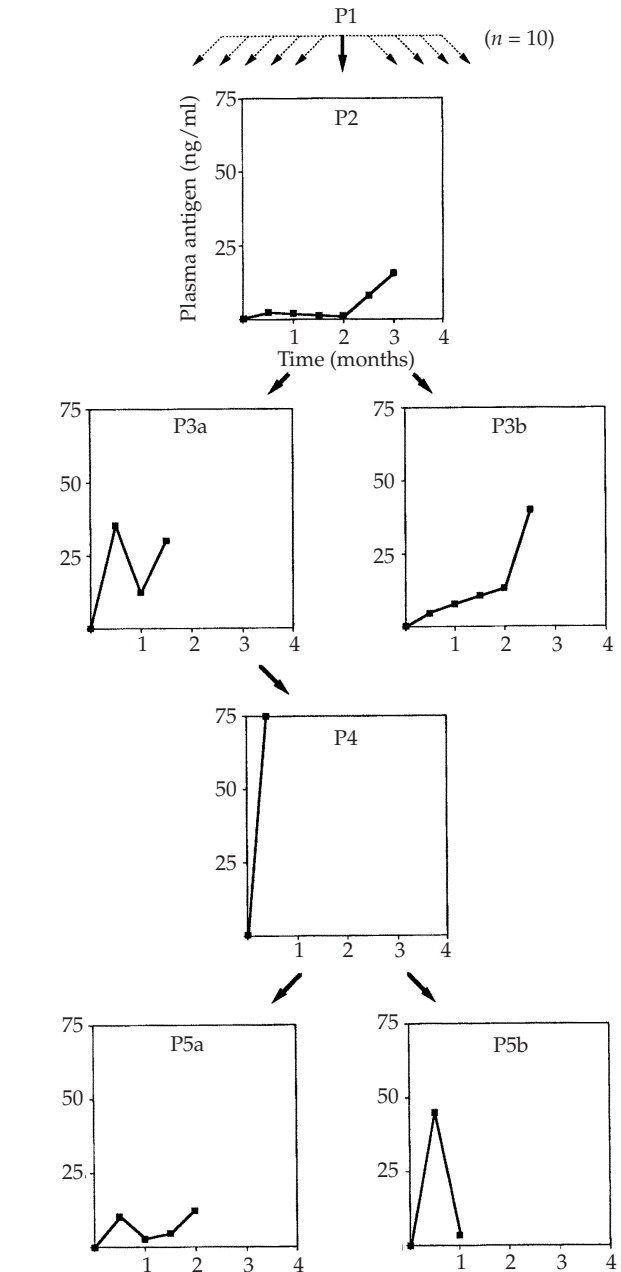


Fig. 2. Plasma antigen concentrations (ng/ml) during progression to AIDS in animals infected after subsequent *in vivo* passage of SIV.

not change. Surprisingly, the same spectrum of pathological lesions that developed after chronic SIV infection was also found after the more rapid disease course (Table 1). By the third and fourth passages (P3 and P4, respectively), only a brief asymptomatic period could be observed, for a period of days. In the 10 monkeys infected in passage 1, plasma antigen levels were predominantly below the detection level of the antigen capture assay (< 10 ng) (Table 1). T helper cell numbers, and most prominently T helper/memory cells, declined slowly but persistently after 4–5 months of infection in animal P1a, the first animal in passage 1 to develop AIDS (not shown).

The second passage (P2) consisted of a single animal inoculated with cryopreserved PBMC taken from the first animal to develop AIDS in P1 at 6.5 months post-infection. This animal, P2, was asymptomatic for only 3 months, during which time CD4<sup>+</sup>/CD29<sup>+</sup> T cells declined (Fig. 1) and plasma antigen levels increased slowly and persisted until AIDS developed at 17 weeks (4.1 months) (Fig. 2). PBMC taken from P2 at the last time-point before death were administered to two animals, P3a and P3b. These animals developed levels of plasma antigen that were persistent and rose to higher concentrations (Fig. 2) than the previous animals, P1 or P2,

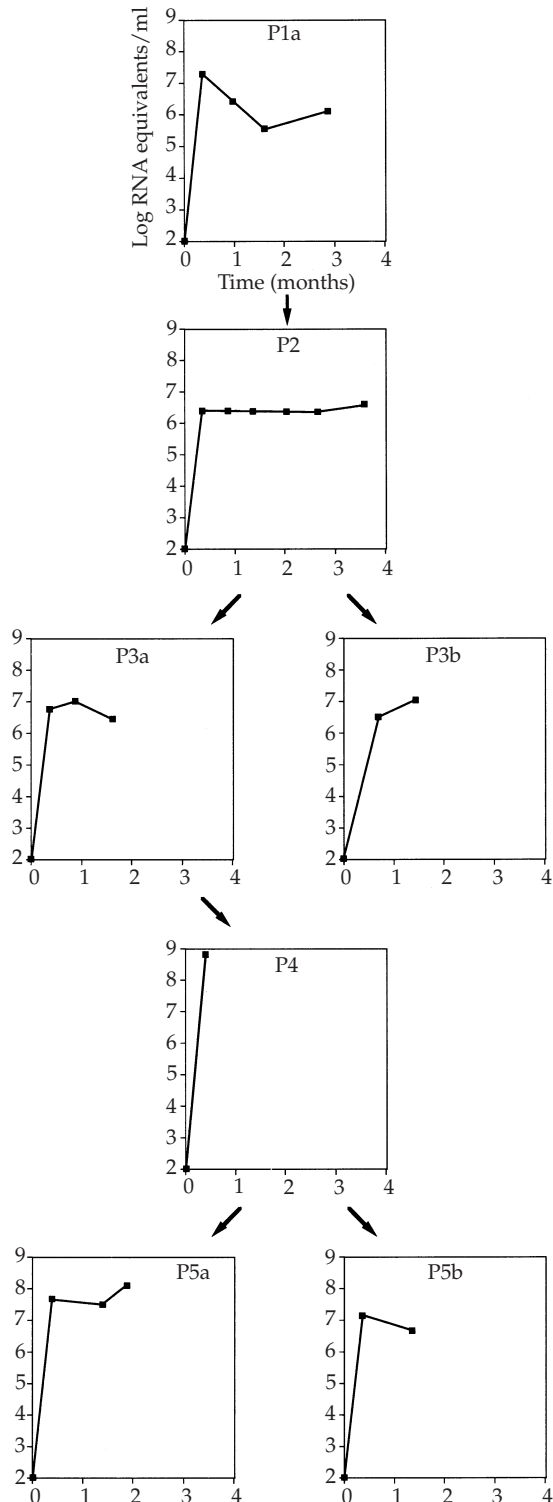


Fig. 3. Plasma viral RNA concentrations (RNA equivalents per ml) during progression to AIDS in animals infected after subsequent *in vivo* passage of SIV.

from which they had acquired the virus (35.5 and 39.9 ng p27 for P3a and P3b, respectively) (Table 1). During the asymptomatic period in these animals, which ranged from 4 to 6

weeks, T helper/memory cell numbers declined rapidly. AIDS developed in animals P3a and P3b by 1.8 and 2.5 months, respectively.

In the fourth passage, PBMCs were administered to animal P4 from animal P3a, which had developed AIDS the fastest in passage 3. Within 2 weeks, plasma antigen levels rose to levels greater than 100 ng/ml (Fig. 2), with an abrupt decline of CD4<sup>+</sup>/CD29<sup>+</sup> T cells (Fig. 1). This animal, P4, died with AIDS within 1 month of infection, with histopathological lesions characteristic of AIDS (Table 1). PBMC from P4 taken from the last time-point before death were administered to animals P5a and P5b. Progression to AIDS more rapid than 1 month post-infection was not observed. It appeared that a maximum virulence had been achieved by the fourth passage, since the approximate time to AIDS in the fifth passage was similar to that observed in the third passage. Both animals P5a and P5b progressed rather uniformly to AIDS within 2 months, with a characteristic rapid loss of T helper/memory cells (Fig. 1) as well as a very high and persistent plasma antigenaemia (Fig. 2). To rule out that other pathogens, opportunistic infections or cofactors were transmitted during the *in vivo* passage, all animals were carefully studied for seroconversion of herpesviruses after SIV infection. There was no evidence of transmission of herpesviruses with the SIV inoculum. Furthermore, all animals remained free of simian T-lymphotropic virus and type D retrovirus infections during the course of these studies. By all criteria, the accelerated disease course was due solely to an increase in virulence of SIV following *in vivo* passage.

One of the most important predictors of the rate of progression to AIDS in humans is the level of viral RNA in plasma (Mellors *et al.*, 1996). In the rhesus SIV and simian-human immunodeficiency virus models, we have also demonstrated that there is a critical threshold of viral RNA in plasma that is required for a pathogenic disease course. Furthermore, a correlation between the magnitude of plasma viral RNA maintained and virus virulence was observed (ten Haaf *et al.*, 1998). Retrospectively, we examined all available plasma samples from this passage study and compared them with the rate of disease progression and the level of passage (Fig. 3). A relationship was observed between the maximum or sustainable level of plasma viral RNA and the rate of progression to AIDS (Fig. 4), although statistically more animals would be required to strengthen this analysis. The animal that developed AIDS most rapidly had the highest overall level of plasma viral RNA (Fig. 3), whereas those that survived longer had lower RNA loads (Fig. 4). Not unexpectedly, there was a relationship between plasma RNA loads and plasma antigen levels (Table 1, Figs 2 and 3). An inverse relationship was found between the final level of plasma RNA and the CD4<sup>+</sup>/CD29<sup>+</sup> T cell levels in each animal. In general, the higher the sustainable plasma RNA loads obtained, the more rapidly T helper/memory T cells declined (Figs 1 and 3). Taken together, these data suggest that

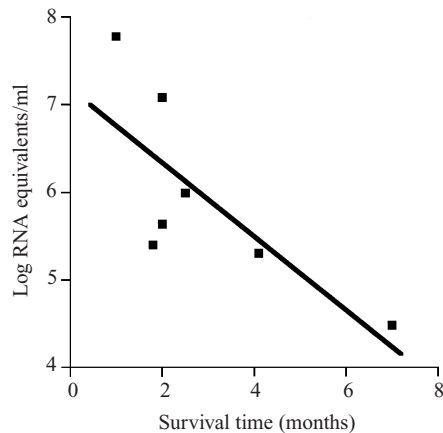


Fig. 4. Correlation of survival (months) with virus load (RNA equivalents per ml). Virus load was based on the value observed at the 'set point', previously documented in this model as being between 6 and 8 weeks (ten Haaf *et al.*, 1998). If animals died before 2 months, the last plasma load value before death was used.

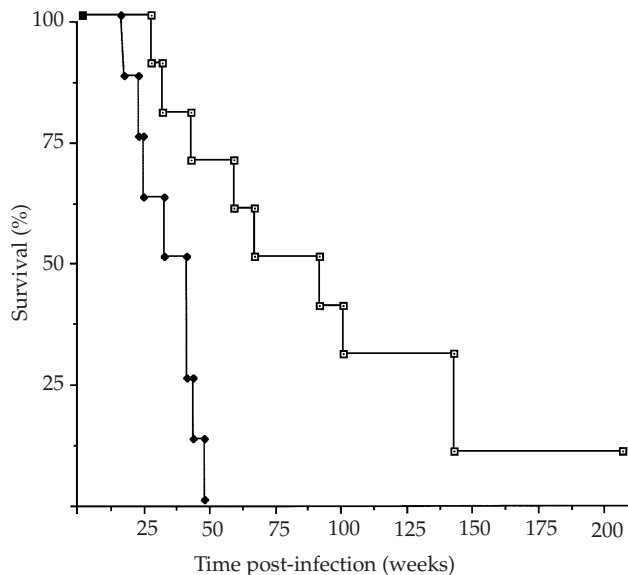


Fig. 5. Kaplan-Meier plot comparing the percentage survival over time of animals infected with the pre-passage isolate (SIV<sub>B670</sub>) (□;  $n = 10$ ) with that of animals infected with the post-passage isolate (SIV<sub>8980</sub>) (◆;  $n = 8$ ) derived from animal P4.

the virulence of the SIV strain increased substantially with each passage. Further proof of the increased virulence acquired by sequential end-stage disease passage was obtained by infecting eight more animals with SIV from the fourth-passage animal (P4). Comparison of the survival of macaques infected with the original pre-passage SIV<sub>B670</sub> strain with that of animals infected with the post-passage virus strain (SIV<sub>8980</sub>) isolated from the P4 acute AIDS case (animal 8980) (Fig. 5) revealed distinctly different Kaplan-Meier curves, with significantly poorer survival ( $P = 0.0036$ ) in the group of animals infected with the passaged SIV<sub>8980</sub> strain. Remarkably, the passage SIV<sub>8980</sub> strain

was even more pathogenic in mature rhesus monkeys than in the juvenile animals infected with the pre-passage SIV<sub>B670</sub> stock (Fig. 5). If it had been possible to infect more juvenile macaques with the passaged SIV<sub>8980</sub> stock derived from the P4 animal, then a more acute disease course similar to the P5 animals would have been expected.

## Discussion

This study demonstrated that serial *in vivo* passage of SIV from end-stage disease resulted in an accelerated disease course of AIDS in rhesus monkeys. The 10 animals infected with SIV<sub>B670</sub> in the first passage developed terminal AIDS in a period of time ranging from 7 months to more than 3 years. In contrast, during the passage of this virus, a marked reduction in the time to develop AIDS was observed, which ranged from 4.1 to 1 month post-infection. Our experiments also demonstrated that the accelerated development of AIDS following SIV<sub>sm</sub> passage in Asian monkeys was associated with the development of very high concentrations of plasma antigen and virus RNA in the host. These data strongly support the hypothesis that AIDS-causing lentiviruses become more virulent upon *in vivo* evolution and acquire the capacity to accelerate the progression to AIDS (Hirsch, 1999; Kimata *et al.*, 1999).

SIV infection is a naturally occurring, asymptomatic lentivirus infection restricted to several African primate species including sooty mangabeys (SIV<sub>sm</sub>) (Fultz *et al.*, 1990; Murphey-Corb *et al.*, 1986), African green monkeys (SIV<sub>agm</sub>) (Allan *et al.*, 1991; Johnson *et al.*, 1990) and chimpanzees (SIV<sub>cpz</sub>) (Peeters *et al.*, 1992). SIV infection does not occur naturally in Asian primate species such as macaques, yet, when infected experimentally with SIV strains derived from sooty mangabeys, they develop AIDS with remarkable similarity to the disease in man (Hirsch *et al.*, 1989; Simon *et al.*, 1994). This model has enabled the study of possible causes of rapid progression to AIDS in a uniformly susceptible host population. Certain host factors such as infection in the neonatal or geriatric period, immunosuppressive treatment for autoimmune disease or transplantation or patients with other concurrent chronic diseases are potential risk factors for rapid progression to AIDS. In the absence of host risk factors, some infected individuals still become rapid progressors to AIDS. We propose that such individuals who do not have host risk factors but become rapid progressors are infected with particularly virulent strains. Several reports suggest that more virulent HIV-1 variants emerge late in the course of the disease as AIDS develops (Koot *et al.*, 1993, 1999). The vast majority of HIV-1 transmissions occur unknowingly from apparently healthy individuals relatively early in infection before symptoms of AIDS develop. Occasionally, however, transmission of more virulent variants may occur from individuals with more advanced HIV infection, and infection with such variants may give rise to rapid progression in the absence of host

susceptibility factors (Tersmette *et al.*, 1988, 1989*a, b*). Although it can be expected that transmission from advanced AIDS patients is now unlikely, since the disease is now adequately diagnosed and treatment and counselling take place in developed countries, it may still occur in some instances. Indeed, certain individuals such as intravenous drug users and sex workers may continue to share needles or sell unprotected sex despite having developed AIDS. Infections acquired from individuals with advanced disease may result in a more virulent infection and rapid disease course. To model this scenario, we transferred blood selectively from animals with a late-stage infection to healthy, naive animals of the same age, sex and breeding stock. Although not inbred, they were from the same origin and bred in the same facility under the same conditions. By (i) selecting the first animal in a group to develop AIDS and (ii) selecting late-stage samples (2 weeks prior to AIDS) for passage, we eventually acquired a virus (SIV<sub>8980</sub>) that caused very rapid progression to AIDS. Although the number of rhesus monkeys used was relatively small, after several *in vivo* passages of SIV a remarkably accelerated progression time to AIDS was noted, which was not observed in the initial group of 10 animals. These results suggest that our experimental late-stage transmission from the most rapid progressors selected for particularly virulent virus variants. Finally, the importance of controlling for host variables became apparent when we examined the virulence of the derived SIV strain in older animals (data not shown). When the same virus inoculum was administered to animals of a different age group or geographical origin, the pattern of disease observed was somewhat different, emphasizing the importance of controlling for host variables in studies on disease progression.

With passage, there was a marked increase in maximum plasma antigen levels observed (from < 10 to > 100 ng/ml p24). Results were similar when plasma RNA levels were examined. In passage 3, virus loads rapidly reached levels of  $1 \times 10^7$  RNA equivalents per ml and tended to persist at these relatively high levels until death ensued shortly afterwards. In passage 4, a markedly high peak reaching almost  $1 \times 10^9$  RNA equivalents per ml was accompanied by death of the animal (Fig. 3). A plot of the time to AIDS against the last plasma virus load measured before death was also indicative of the relationship between very rapid disease course and high virus loads (Fig. 4). Further evidence of selection of more virulent variants was obtained at the molecular level. On studying the variation of envelope during each successive passage, there was a narrowing of the quasispecies over time, with the emergence of a dominant genotype. In the first two passages, there was a decrease in the ratio of synonymous to non-synonymous mutations, suggesting that immune responses played a role in selective pressure (Valli *et al.*, 1998). As the virulence of the inoculum increased and the progression time to AIDS decreased to less than 2 months, a dominant variant emerged in which only synonymous mutations were observed

(Valli *et al.*, 1998). This is probably due to the marked degree of virus replication, as shown by the very high levels of viral RNA reached in the most rapid progressors (Fig. 4). These animals progressed to AIDS before an effective immune response could be mounted (Table 1).

The pathology observed in these cases was relatively indistinguishable from AIDS observed after a chronic disease course. Importantly, the pathology observed in these cases was distinctly different from the rapid disease syndrome observed with SIV<sub>PBj</sub> (Dewhurst *et al.*, 1990) in pigtail macaques (Fultz & Zack, 1994; Zacharias *et al.*, 1994). The acute disease syndrome observed in SIV<sub>PBj</sub>-infected animals is characterized by haemorrhagic enteritis, with death at approximately 10–14 days post-infection. If these animals are treated symptomatically, they can survive the acute enteritis and later develop a chronic disease course resulting in AIDS 1 to 2 years later (Rosenberg *et al.*, 1991). That end-stage AIDS can develop as rapidly as 1 month post-infection after infection with this late-passage strain of SIV is itself important pathologically. The study of the pathogenic events that precipitate such an acute disease course may provide more fundamental understanding of the mechanisms of the disease itself. To understand the pathogenesis of acute AIDS further, a molecular clone has been derived from the most-rapidly progressing animal. Further studies are in progress with this molecular clone to define the virus virulence factors associated with rapid progression to AIDS.

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## References

- Allan, J. S., Short, M., Taylor, M. E., Su, S., Hirsch, V. M., Johnson, P. R., Shaw, G. M. & Hahn, B. H. (1991). Species-specific diversity among simian immunodeficiency viruses from African green monkeys. *Journal of Virology* **65**, 2816–2828.
- Anzala, A. O., Ball, T. B., Rostron, T., O'Brien, S. J., Plummer, F. A. & Rowland-Jones, S. L. (1998). CCR2–64I allele and genotype association with delayed AIDS progression in African women. University of Nairobi Collaboration for HIV Research. *Lancet* **351**, 1632–1633.
- Baskin, G. B. & Soike, K. F. (1989). Adenovirus enteritis in SIV-infected rhesus monkeys. *Journal of Infectious Diseases* **160**, 905–907.
- Baskin, G. B., Martin, L. N., Rangan, S. R., Gormus, B. J., Murphey-Corb, M., Wolf, R. H. & Soike, K. F. (1986). Transmissible lymphoma and simian acquired immunodeficiency syndrome in rhesus monkeys. *Journal of the National Cancer Institute* **77**, 127–139.
- Daniel, M. D., Letvin, N. L., King, N. W., Sehgal, P. K., Hunt, R. D., Kanki, P. J., Essex, M. & Desrosiers, R. C. (1985). Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* **228**, 1201–1204.

- Daniel, M. D., Letvin, N. L., Sehgal, P. K., Hunsmann, G., Schmidt, D. K., King, N. W. & Desrosiers, R. C. (1987). Long-term persistent infection of macaque monkeys with the simian immunodeficiency virus. *Journal of General Virology* **68**, 3183–3189.
- Dean, M., Carrington, M., Winkler, C., Huttley, G. A., Smith, M. W., Allikmets, R., Goedert, J. J., Buchbinder, S. P., Vittinghoff, E., Gomperts, E., Donfield, S., Vlahov, D., Kaslow, R., Saah, A., Rinaldo, C., Detels, R. & O'Brien, S. J. (1996). Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the *CKR5* structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* **273**, 1856–1862.
- Desrosiers, R. C. & Ringler, D. J. (1989). Use of simian immunodeficiency viruses for AIDS research. *Intervirology* **30**, 301–312.
- Dewhurst, S., Embretson, J. E., Anderson, D. C., Mullins, J. I. & Fultz, P. N. (1990). Sequence analysis and acute pathogenicity of molecularly cloned SIVSMM-PBj14. *Nature* **345**, 636–640.
- Donahue, R. E., Bunnell, B. A., Zink, M. C., Metzger, M. E., Westro, R. P., Kirby, M. R., Unangst, T., Clements, J. E. & Morgan, R. A. (1998). Reduction in SIV replication in rhesus macaques infused with autologous lymphocytes engineered with antiviral genes. *Nature Medicine* **4**, 181–186.
- Edmonson, P., Murphey-Corb, M., Martin, L. N., Delahunty, C., Heeney, J., Kornfeld, H., Donahue, P. R., Learn, G. H., Hood, L. & Mullins, J. I. (1998). Evolution of a simian immunodeficiency virus pathogen. *Journal of Virology* **72**, 405–414.
- Fultz, P. N. & Zack, P. M. (1994). Unique lentivirus–host interactions: SIVsmmPBj14 infection of macaques. *Virus Research* **32**, 205–225.
- Fultz, P. N., McClure, H. M., Anderson, D. C. & Switzer, W. M. (1989). Identification and biologic characterization of an acutely lethal variant of simian immunodeficiency virus from sooty mangabeys (SIV/SMM). *AIDS Research and Human Retroviruses* **5**, 397–409.
- Fultz, P. N., Anderson, D. C., McClure, H. M., Dewhurst, S. & Mullins, J. I. (1990). SIVsmm infection of macaque and mangabey monkeys: correlation between in vivo and in vitro properties of different isolates. *Developments in Biological Standardization* **72**, 253–258.
- Goodenow, M., Huet, T., Saurin, W., Kwok, S., Sninsky, J. & Wain-Hobson, S. (1989). HIV-1 isolates are rapidly evolving quasispecies: evidence for viral mixtures and preferred nucleotide substitutions. *Journal of Acquired Immune Deficiency Syndromes* **2**, 344–352.
- Heeney, J. L. (1996). Primate models for AIDS vaccine development. *AIDS* **10** (Suppl. A), S115–S122.
- Heeney, J., Bogers, W., Buijs, L., Dubbes, R., ten Haaf, P., Koornstra, W., Niphuis, H., Nara, P. & Teeuwssen, V. (1996). Immune strategies utilized by lentivirus infected chimpanzees to resist progression to AIDS. *Immunology Letters* **51**, 45–52.
- Hirsch, V. M. (1999). Evolution of the fittest ends in tragedy [comment]. *Nature Medicine* **5**, 488–489.
- Hirsch, V. M. & Johnson, P. R. (1994). Pathogenic diversity of simian immunodeficiency viruses. *Virus Research* **32**, 183–203.
- Hirsch, V. M., Edmondson, P., Murphey-Corb, M., Arbeille, B., Johnson, P. R. & Mullins, J. I. (1989). SIV adaptation to human cells. *Nature* **341**, 573–574.
- Johnson, P. R., Fomsgaard, A., Allan, J., Gravell, M., London, W. T., Olmsted, R. A. & Hirsch, V. M. (1990). Simian immunodeficiency viruses from African green monkeys display unusual genetic diversity. *Journal of Virology* **64**, 1086–1092.
- Kaslow, R. A., Carrington, M., Apple, R., Park, L., Munoz, A., Saah, A. J., Goedert, J. J., Winkler, C., O'Brien, S. J., Rinaldo, C., Detels, R., Blattner, W., Phair, J., Erlich, H. & Mann, D. L. (1996). Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. *Nature Medicine* **2**, 405–411.
- Kestler, H. W., III, Ringler, D. J., Mori, K., Panicali, D. L., Sehgal, P. K., Daniel, M. D. & Desrosiers, R. C. (1991). Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. *Cell* **65**, 651–662.
- Kimata, J. T., Kuller, L., Anderson, D. B., Dailey, P. & Overbaugh, J. (1999). Emerging cytopathic and antigenic simian immunodeficiency virus variants influence AIDS progression [see comments]. *Nature Medicine* **5**, 535–541.
- Kirchhoff, F., Greenough, T. C., Brettler, D. B., Sullivan, J. L. & Desrosiers, R. C. (1995). Brief report: absence of intact *nef* sequences in a long-term survivor with nonprogressive HIV-1 infection. *New England Journal of Medicine* **332**, 228–232.
- Kodama, T., Burns, D. P., Kestler, H. W., III, Daniel, M. D. & Desrosiers, R. C. (1990). Molecular changes associated with replication of simian immunodeficiency virus in human cells. *Journal of Medical Primatology* **19**, 431–437.
- Koot, M., Keet, I. P., Vos, A. H., de Goede, R. E., Roos, M. T., Coutinho, R. A., Miedema, F., Schellekens, P. T. & Tersmette, M. (1993). Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS [see comments]. *Annals of Internal Medicine* **118**, 681–688.
- Koot, M., van Leeuwen, R., de Goede, R. E., Keet, I. P., Danner, S., Eeftinck Schattenkerk, J. K., Reiss, P., Tersmette, M., Lange, J. M. & Schuitemaker, H. (1999). Conversion rate towards a syncytium-inducing (SI) phenotype during different stages of human immunodeficiency virus type 1 infection and prognostic value of SI phenotype for survival after AIDS diagnosis. *Journal of Infectious Diseases* **179**, 254–258.
- Kornfeld, H., Riedel, N., Viglianti, G. A., Hirsch, V. & Mullins, J. L. (1987). Cloning of HTLV-4 and its relation to simian and human immunodeficiency viruses. *Nature* **326**, 610–613.
- Kostrikis, L. G., Huang, Y., Moore, J. P., Wolinsky, S. M., Zhang, L., Guo, Y., Deutsch, L., Phair, J., Neumann, A. U. & Ho, D. D. (1998). A chemokine receptor CCR2 allele delays HIV-1 disease progression and is associated with a CCR5 promoter mutation [see comments]. *Nature Medicine* **4**, 350–353.
- Lang, S. M., lafrate, A. J., Stahl-Hennig, C., Kuhn, E. M., Nisslein, T., Kaup, F. J., Haupt, M., Hunsmann, G., Skowronski, J. & Kirchhoff, F. (1997). Association of simian immunodeficiency virus *Nef* with cellular serine/threonine kinases is dispensable for the development of AIDS in rhesus macaques. *Nature Medicine* **3**, 860–865.
- Letvin, N. L., Daniel, M. D., Sehgal, P. K., Desrosiers, R. C., Hunt, R. D., Waldron, L. M., MacKey, J. J., Schmidt, D. K., Chalifoux, L. V. & King, N. W. (1985). Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. *Science* **230**, 71–73.
- Li, Y., Naidu, Y., Fultz, P., Daniel, M. D. & Desrosiers, R. C. (1989). Genetic diversity of simian immunodeficiency virus. *Journal of Medical Primatology* **18**, 261–269.
- Marthas, M. L., Ramos, R. A., Lohman, B. L., Van Rompay, K. K., Unger, R. E., Miller, C. J., Banapour, B., Pedersen, N. C. & Luciw, P. A. (1993). Viral determinants of simian immunodeficiency virus (SIV) virulence in rhesus macaques assessed by using attenuated and pathogenic molecular clones of SIVmac. *Journal of Virology* **67**, 6047–6055.
- Mellors, J. W., Rinaldo, C. R., Jr, Gupta, P., White, R. M., Todd, J. A. & Kingsley, L. A. (1996). Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* **272**, 1167–1170.
- Meyerhans, A., Cheynier, R., Albert, J., Seth, M., Kwok, S., Sninsky, J.,

- Morfeldt-Manson, L., Asjo, B. & Wain-Hobson, S. (1989).** Temporal fluctuations in HIV quasispecies in vivo are not reflected by sequential HIV isolations. *Cell* **58**, 901–910.
- Miller, C. J. (1998).** Does viral tropism play a role in heterosexual transmission of HIV? Findings in the SIV–rhesus macaque model. *AIDS Research and Human Retroviruses* **14** (Suppl. 1), S79–S82.
- Miller, C. J., Marthas, M., Greenier, J., Lu, D., Dailey, P. J. & Lu, Y. (1998).** In vivo replication capacity rather than in vitro macrophage tropism predicts efficiency of vaginal transmission of simian immunodeficiency virus or simian/human immunodeficiency virus in rhesus macaques. *Journal of Virology* **72**, 3248–3258.
- Munoz, A., Wang, M. C., Bass, S., Taylor, J. M., Kingsley, L. A., Chmiel, J. S. & Polk, B. F. (1989).** Acquired immunodeficiency syndrome (AIDS)-free time after human immunodeficiency virus type 1 (HIV-1) seroconversion in homosexual men. Multicenter AIDS Cohort Study Group. *American Journal of Epidemiology* **130**, 530–539.
- Munoz, A., Kirby, A. J., He, Y. D., Margolick, J. B., Visscher, B. R., Rinaldo, C. R., Kaslow, R. A. & Phair, J. P. (1995).** Long-term survivors with HIV-1 infection: incubation period and longitudinal patterns of CD4+ lymphocytes. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* **8**, 496–505.
- Murphey-Corb, M., Martin, L. N., Rangan, S. R., Baskin, G. B., Gormus, B. J., Wolf, R. H., Andes, W. A., West, M. & Montelaro, R. C. (1986).** Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. *Nature* **321**, 435–437.
- Peeters, M., Fransen, K., Delaporte, E., Van der Haesevelde, M., Gershy-Damet, G. M., Kestens, L., van der Groen, G. & Piot, P. (1992).** Isolation and characterization of a new chimpanzee lentivirus (simian immunodeficiency virus isolate cpz-ant) from a wild-captured chimpanzee. *AIDS* **6**, 447–451.
- Rosenberg, Y. J., White, B. D., Papermaster, S. F., Zack, P., Jarling, P. B., Eddy, G. A., Burke, D. S. & Lewis, M. G. (1991).** Variation in T-lymphocyte activation and susceptibility to SIVPBj-14-induced acute death in macaques. *Journal of Medical Primatology* **20**, 206–210.
- Salvi, R., Garbuglia, A. R., Di Caro, A., Pulciani, S., Montella, F. & Benedetto, A. (1998).** Grossly defective nef gene sequences in a human immunodeficiency virus type 1-seropositive long-term nonprogressor. *Journal of Virology* **72**, 3646–3657.
- Sawai, E. T., Khan, I. H., Montbriand, P. M., Peterlin, B. M., Cheng-Mayer, C. & Luciw, P. A. (1996).** Activation of PAK by HIV and SIV Nef: importance for AIDS in rhesus macaques. *Current Biology* **6**, 1519–1527.
- Simon, M. A., Brodie, S. J., Sasseville, V. G., Chalifoux, L. V., Desrosiers, R. C. & Ringler, D. J. (1994).** Immunopathogenesis of SIVmac. *Virus Research* **32**, 227–251.
- Smith, M. W., Dean, M., Carrington, M., Huttley, G. A. & O'Brien, S. J. (1997).** CCR5-delta 32 gene deletion in HIV-1 infected patients. *Lancet* **350**, 741; discussion 742.
- ten Haaft, P., Verstrepen, B., Uberla, K., Rosenwirth, B. & Heeney, J. (1998).** A pathogenic threshold of virus load defined in simian immunodeficiency virus- or simian–human immunodeficiency virus-infected macaques. *Journal of Virology* **72**, 10281–10285.
- Termette, M., de Goede, R. E., Al, B. J., Winkel, I. N., Gruters, R. A., Cuypers, H. T., Huisman, H. G. & Miedema, F. (1988).** Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *Journal of Virology* **62**, 2026–2032.
- Termette, M., Gruters, R. A., de Wolf, F., de Goede, R. E., Lange, J. M., Schellekens, P. T., Goudsmit, J., Huisman, H. G. & Miedema, F. (1989a).** Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. *Journal of Virology* **63**, 2118–2125.
- Termette, M., Lange, J. M., de Goede, R. E., de Wolf, F., Eeftinck-Schattenkerk, J. K., Schellekens, P. T., Coutinho, R. A., Huisman, J. G., Goudsmit, J. & Miedema, F. (1989b).** Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. *Lancet* **i**, 983–985.
- Trichel, A. M., Roberts, E. D., Wilson, L. A., Martin, L. N., Ruprecht, R. M. & Murphey-Corb, M. (1997).** SIV/DeltaB670 transmission across oral, colonic, and vaginal mucosae in the macaque. *Journal of Medical Primatology* **26**, 3–10.
- Tsujimoto, H., Hasegawa, A., Maki, N., Fukasawa, M., Miura, T., Speidel, S., Cooper, R. W., Moriyama, E. N., Gojobori, T. & Hayami, M. (1989).** Sequence of a novel simian immunodeficiency virus from a wild-caught African mandrill. *Nature* **341**, 539–541.
- Valli, P. J., Lukashov, V. V., Heeney, J. L. & Goudsmit, J. (1998).** Shortening of the symptom-free period in rhesus macaques is associated with decreasing nonsynonymous variation in the env variable regions of simian immunodeficiency virus SIVsm during passage. *Journal of Virology* **72**, 7494–7500.
- Van Rompay, K. K., Berardi, C. J., Aguirre, N. L., Bischofberger, N., Lietman, P. S., Pedersen, N. C. & Marthas, M. L. (1998).** Two doses of PMPA protect newborn macaques against oral simian immunodeficiency virus infection. *AIDS* **12**, F79–F83.
- Winkler, C., Modi, W., Smith, M. W., Nelson, G. W., Wu, X., Carrington, M., Dean, M., Honjo, T., Tashiro, K., Yabe, D., Buchbinder, S., Vittinghoff, E., Goedert, J. J., O'Brien, T. R., Jacobson, L. P., Detels, R., Donfield, S., Willoughby, A., Gomperts, E., Vlahov, D., Phair, J. & O'Brien, S. J. (1998).** Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. ALIVE Study, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort. *Science* **279**, 389–393.
- Zacharias, D. A., Garamszegi, N. & Strehler, E. E. (1994).** Characterization of persistent artifacts resulting from RT–PCR of alternatively spliced mRNAs. *Biotechniques* **17**, 652–655.

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