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Primate Lentiviruses and AIDS Research

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Abstract

A number of African non-human primates are the natural hosts of a diverse subfamily of lentiviruses. Two primate species, the chimpanzee and the sooty mangabey are natural hosts to SIV_{cpz} and SIV_{sm}, which are found in the phylogenetic clades together with HIV-1 and HIV-2, respectively. Molecular epidemiological evidence suggests that the AIDS epidemic in humans may have arisen from several cross-species transmission events from these two nonhuman primate species to humans. Importantly, the cross-species transmission of SIV_{sm} from African sooty mangabeys to various Asian macaque species results in an AIDS-like disease. This observation has provided a very valuable animal model which has proven critical for addressing specific problems in AIDS research. The development of a variety of molecular clones of SIV_{sm} or SIV_{mac} has provided powerful tools to greatly further our insight into the pathogenesis of AIDS. Molecular clones of SIV have been extensively used in developing vaccine strategies for the prevention of AIDS. Chimeric molecular clones of HIV-1 and SIV_{mac} (SHIVs) have provided more refined macaque models and helped accelerate the development and evaluation of HIV-1 vaccines and novel therapeutics for the clinic.

Key words

HIV. SIV. Cross-species transmission. Pathogenesis. Vaccines

Introduction

In the mid-eighties it became apparent that a human retrovirus of the lentivirus subfamily[1], later designated human immunodeficiency virus type 1 (HIV-1), was the etiological agent of the acquired immune deficiency syndrome (AIDS)[2]. This disease was characterised by unusual opportunistic infections, neurologic abnormalities, gastrointestinal disorders and malignancies, such as Kaposi sarcoma and lymphomas, which were later found to be due to an insidious decay of the immune system[3]. The urgent need to gain insight in certain aspects of the pathobiology of this infection demanded relevant animal models. Similar lentiviruses, which had the potential to cause AIDS-like disease in their hosts, were identified in several other animal species[4]. However, only simian immunodeficiency virus of sooty mangabeys (SIV_{sm}) and feline immunodeficiency virus (FIV), which cause AIDS-like symptoms in macaques and cats, respectively, are commonly used as animal models[5].

An overview of the molecular biology of SIV and the contribution and applications of the SIV-macaque model for AIDS research is given. In particular the use of molecular clones of SIV to elucidate the pathogenesis of AIDS, as well as to evaluate both candidate vaccines and new therapeutics, is illustrated.

Origins of Simian and Human Immunodeficiency Viruses

In 1969, at the California Regional Primate Research Centre a large number of rhesus monkeys

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were diagnosed with a high incidence of lymphoma. Animals having this type of lymphoma had unusual opportunistic infections such as *Mycobacterium avium*. Studies suggested a viral etiology of this disease, especially when lymphocyte-associated herpesviruses, adenoviruses and retroviruses could be detected in cultured tumour tissue[6]. In the early 1980's comparable cases began occurring at the Tulane and New England regional primate centres as well[7,8]. First, type D retroviruses (known as simian retroviruses, SRV) were associated with this simian AIDS-like syndrome (SAIDS) in several species of macaques *(Macaca mulatta, M. cyclopis, M. nemestrina, M. fascicularis, M. fuscata and M. nigra)*. However, SRV was excluded as the etiologic agent for the observed disease in the rhesus macaques since, 1) experimental infection with a cell-free type D retrovirus stock failed to induce disease in healthy animals, 2) it was impossible to isolate type D retroviruses from the majority of the monkeys with AIDS upon cocultivation and, 3) T-cell lines producing SIV did not yield a type D retrovirus when cocultured on cell lines[6,9,10]. It was not until 1985 that lymphoma transmission studies, electron microscopic observations, and comparative pathology showed that the observed disease was associated with the presence of a lentivirus: STLV-III, later designated SIV, the simian counterpart of HTLV-III (HIV-1)[8]. Studies revealed that SIV_{mac}[11], the virus causing AIDS in rhesus macaques, and closely related isolates from various other Asian macaque species, were all derived from SIV_{sm}, the naturally occurring lentivirus of sooty mangabeys[6,12].

Natural lentivirus infections have not only been found in sooty mangabeys *(Cercocebus atys)*[13], but also occur in several other African nonhuman primates which appear to carry these virus infections asymptomatically[14] (Fig. 1). Based on sequence analysis these primate lentiviruses can be classified into 5 main lineages represented by; 1) SIV_{agm} from different subspecies of African green monkeys, 2) SIV_{lhoest} from l'hoest monkeys and SIV_{mnd} from mandrills, 3) SIV_{syk} from Sykes' monkeys and SIV_{tal} from talapoin monkeys[15], 4) SIV_{sm} from sooty mangabeys, SIV_{mac}, and HIV-2 of humans, and 5), SIV_{cpz} from chimpanzees and HIV-1 from humans[16-18] (Fig. 2). It is uncertain if SIVs recently isolated from drills[19] and red-capped mangabeys[20] should be classified in the SIV_{cpz}/HIV-1 lineage, or both represent novel SIV lineages.

SIV_{sm} does not commonly cause AIDS in its natural host[21-23]. However, inoculation of SIV_{sm} into rhesus macaques (Asian in origin) induces a disease remarkably similar to AIDS in humans[10]. Retrospective evidence suggests that SIV was introduced to the New England rhesus colony through a cohort of female rhesus macaques obtained from the California colony in 1970[6]. The high degree of genetic homology between SIV isolated from captive rhesus macaques (SIV_{mac}) and the virus found in naturally infected sooty mangabeys (SIV_{sm}) suggested that SIV_{mac} originated from SIV_{sm} by cross-species transmission while in captivity[6,12,13,24]. Similar molecular evidence suggests that HIV-1 may have been introduced into the human population by transmission of blood from common chimpanzees *(Pan troglodytes)*[25] (Fig. 1).

Genetic Organisation

The linear HIV-1/SIV_{cpz} or HIV-2/SIV_{sm} provirus is approximately 9.4-kb in length and contains structural genes (*gag* and *env*), non-structural genes *(pol, tat, rev)* and accessory genes *(vif, vpx or vpu, vpr and nef)*, and it is flanked by two long terminal repeats (LTR) (Fig. 3). The gag open reading frame encodes the core proteins p7, p9 (NC), p17 (MA) and p24/p27 (CA), whereas *pol* codes for the viral enzymes reverse transcriptase (RT) (which also has RNAse H activity), protease (PR) and integrase (IN). The envelope gene encodes the envelope glycoprotein (precursor) that is proteolytically cleaved into gp120/gp130 (SU) and gp41 (TM). The viral messenger RNAs code for Gag, Pol, Env, Tat and Rev proteins as well as for the accessory Vif, Vpr, Vpx or Vpu and Nef proteins. The latter are not required for viral growth *in vitro* but are important for viral replication, and may be required for virulence in susceptible species like humans and macaques[26]. The LTR flank either side of the SIV proviral genome and contain signals for initiation of viral transcription and regulatory elements.

Comparative Aspects of the Viral Replication Cycle

In general, the viral replication cycle of $SIV_{sm/mac}$ is very similar to that of HIV-1 and the reader is referred to several recent reviews on this subject[27,28]. Only aspects of the replication, which are known to significantly differ, will be considered further in this section.

HIV-1, HIV-2 and $SIV_{sm/mac}$ enter host cells by binding to their receptors followed by the fusion with the plasma membrane. In the case of HIV-1 this usually requires the interaction of virus envelope with CD4. This triggers a change in the envelope protein conformation thus enabling the virus to bind a seven-transmembrane (7TM) coreceptor. $SIV_{sm/mac}$ and HIV-2 also use CD4, but this appears not to be essential[29,30]. Interestingly, HIV-2 and $SIV_{sm/mac}$ probably have a different envelope protein conformation than HIV-1, and thus they may bind specific 7TM receptors in the absence of CD4 binding. The 7TM receptor chosen in the absence of CD4 appears to be either CXCR4 (X4) or CCR5 (R5) in the case of HIV-2, or predominantly CCR5 in the case of $SIV_{sm/mac}$[31,32]. To a lesser extent other TM receptors, such as GPR15/Bob, STRL33/Bonzo and GPR1, may be used[29,30,33,34].

During HIV-1 pathogenesis, the well-recognised phenomenon of phenotype switching from a non-syncytium inducing (NSI) phenotype (R5 or X4/R5) to a syncytium inducing (SI) phenotype with predominant X4 usage has been reported to occur in a large proportion of clade B infected persons[35-41].

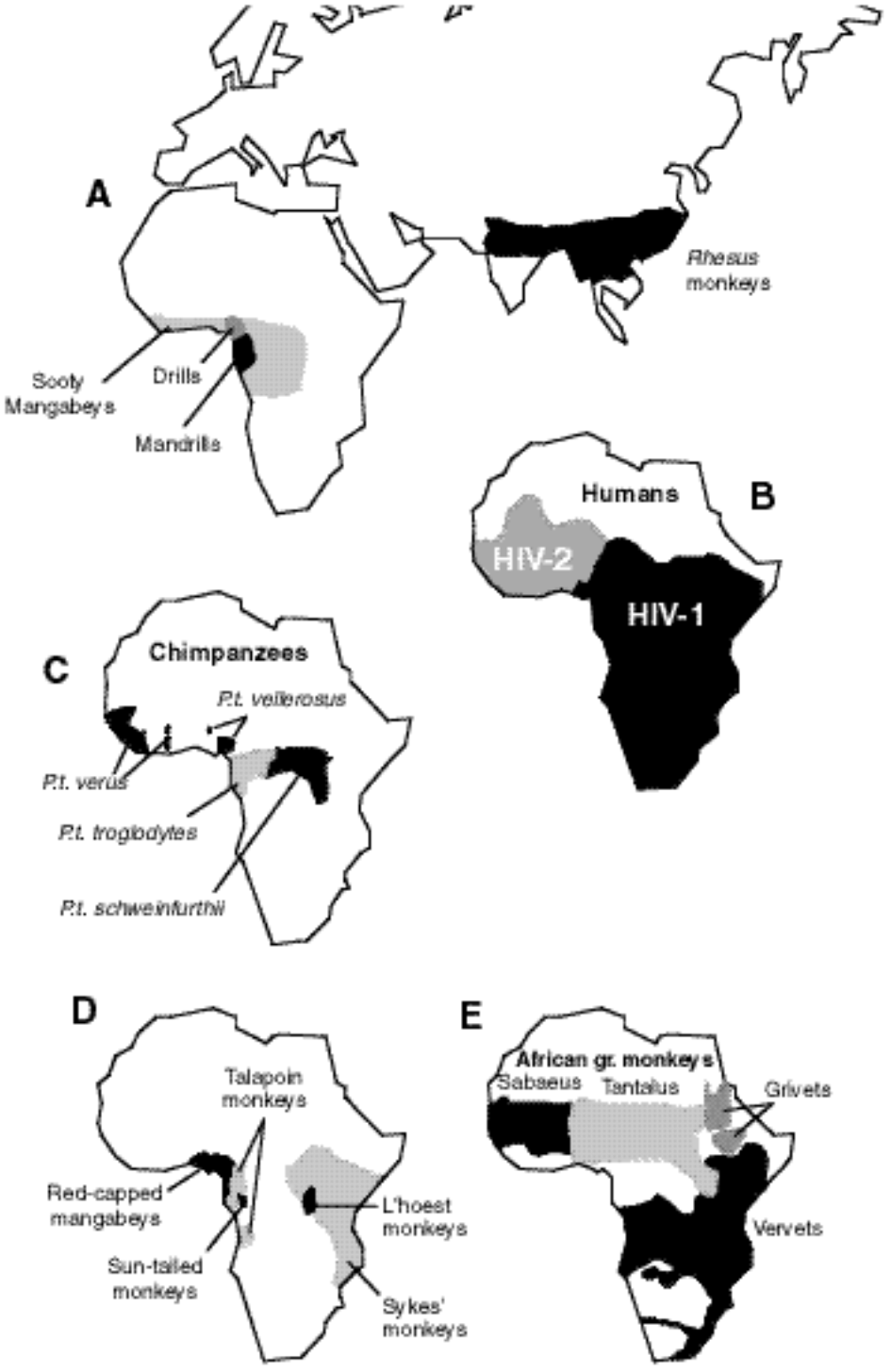

Fig.1. *Distribution of currently known African non-human primate species naturally infected with lentiviruses and which are resistant to disease on the African continent (A, C, D, and E).The geographic separation of Asian rhesus macaques which are susceptible to AIDS is illustrated in A.The approximate distribution of humans infected with HIV-1 versus HIV-2 early in the AIDS epidemic is depicted in B. The distribution of common chimpanzees and the location of currently designated subspecies, are depicted in C.*

Such a specific shift in co-receptor use and correlation with disease progression has not been commonly observed in HIV-2 infected persons or $SIV_{sm/mac}$ infected macaques. Interestingly, chimeric simian-human immunodeficiency viruses (SHIVs) have been constructed with either X4 or R5 or dual tropic (X4/R5) HIV-1 envelopes[42,43]. Data comparing $SHIV_{sf33p}$ (X4) and $SHIV_{sf162p}$ (R5) suggest that different HIV-1 envelopes with different co-receptor specificities may confer distinct differences in cell tropism with different pathogenic sequellae[43]. Although these results have yet to be confirmed they

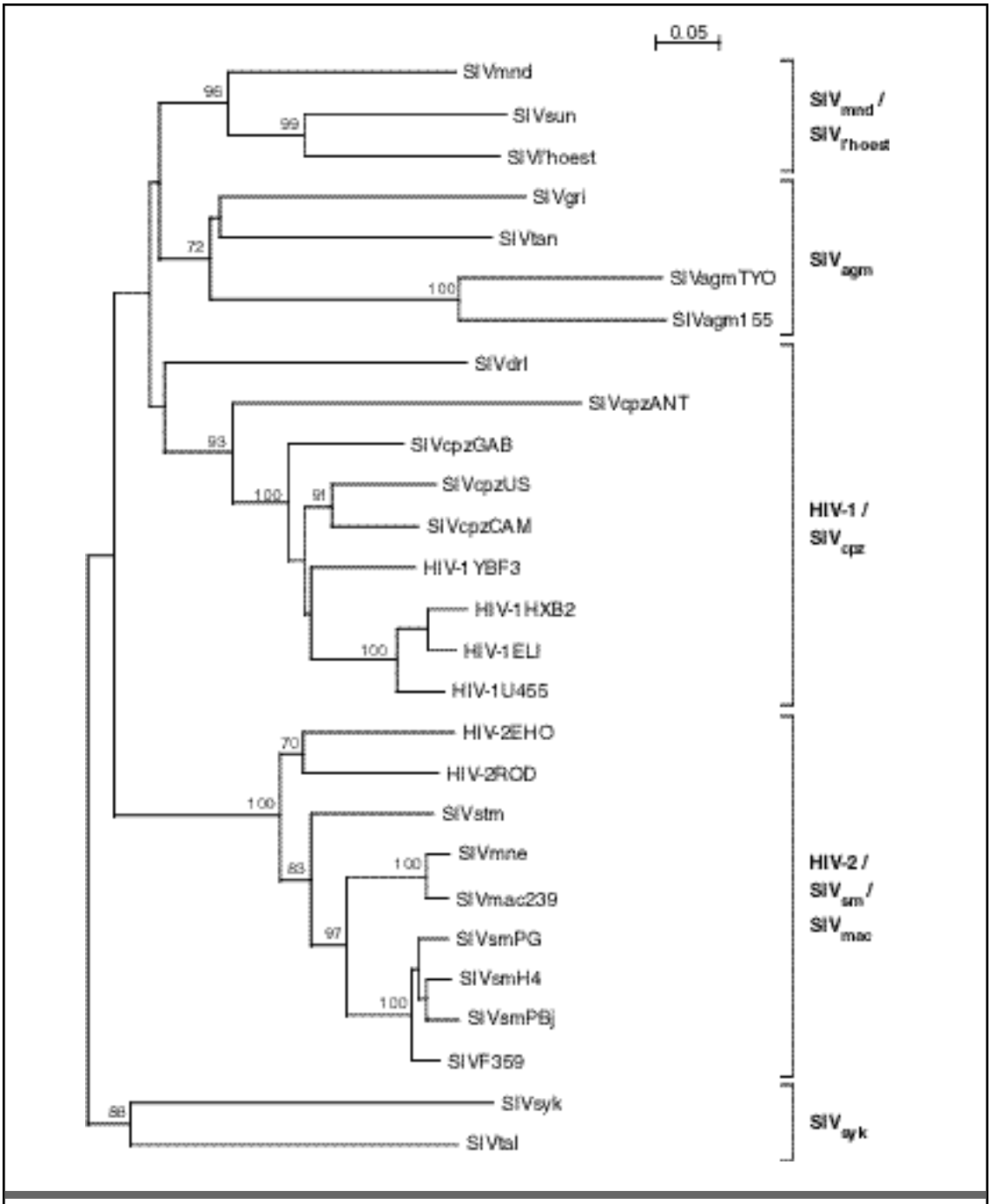

Fig.2. *The phylogenetic relatedness of the five currently identified lineages of primate lentiviruses[16-18]. The neighbour-joining tree was based on a 537 nt pol sequence and was constructed using PAUP[153]. Reliability of the tree was estimated using 1,000 bootstrap replicates. Values greater than 70% are shown in the tree.*

point to the important application of the SHIV model to delineate HIV-1 specific pathogenesis questions, which cannot be addressed in the $SIV_{sm/mac}$ model.

Another important difference between HIV-1 and $SIV_{sm/mac}$/HIV-2 is the use of the cellular chaperone cyclophilin A (CyPA) which is essential for the formation of infectious virus particles. CyPA is incorporated into HIV-1 virions by interacting with p24 Gag during assembly, and plays an essential role during early post-entry events in HIV-1. Interestingly, CyPA is not incorporated into $SIV_{sm/mac}$ virions during replication[44-50]. CyPA–Gag complex formation and, thus, CyPA incorporation into virions can be blocked by competition with CyPA-binding cyclosporins. Upon *de novo* HIV-1 infection in the presence of a CyPA-binding cyclosporin, formation of two-long terminal repeat (2-LTR) circles was found to be reduced, which indicated inhibition of nuclear localisation of the pre-integration complex[51,52]. Also, virus particles assembled in the presence of inhibitor showed reduced formation of 2-LTR circles after *de novo* infection[51]. Thus, lack of incorporation of CyPA into HIV-1 particles in the presence of cyclosporins, which compete with Gag protein for

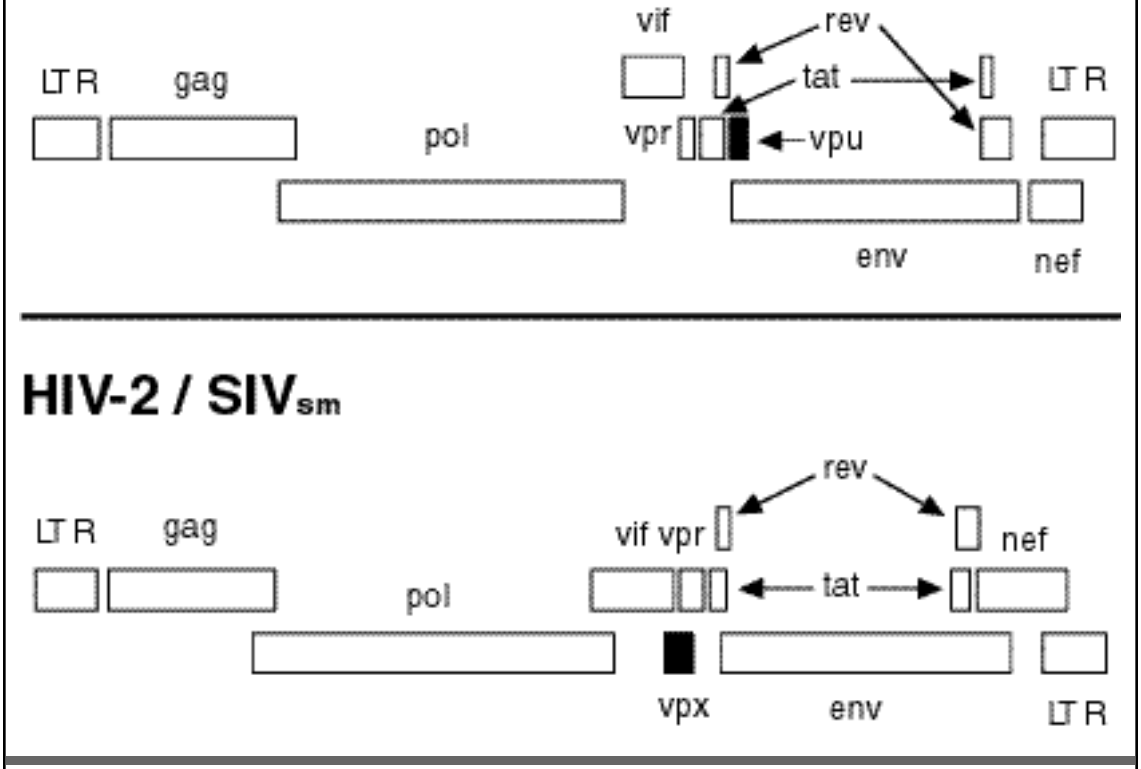

Fig.3. *Comparison of the genetic organisation of proviruses of the HIV-1/SIV_{cpz} lineage versus the $SIV_{mac/sm}$/HIV-2 lineage. The organisation of the structural and regulatory genes is highly similar with the exception of vpu and vpx, respectively.*

CyPA binding, leads to a defect in the post-entry process of infection, presumably during translocation of the pre-integration complex to the nucleus.

While HIV-1 virions, of all the M group and also the closely related $SIV_{cpz\ GAB}$ contain CyPA, and require this molecule for infectivity[53], HIV-2 and $SIV_{mac/sm}$ do not incorporate CyPA into their virions and do not need this compound for replication competence. These viruses were also found to be resistant against the inhibitory effect of cyclosporins[53,54]. Transfer of the CyPA binding HIV-1 amino acid stretch of p24 Gag protein to the corresponding position in $SIV_{mac/sm}$ resulted not only in efficient incorporation of CyPA into SIV virus particles[55] but in addition conferred HIV-1-like drug-sensitivity to this chimeric virus. Interestingly, HIV-1 clade O isolates package significant quantities of CyPA, and though this incorporation can be blocked by cyclosporin, the replication of these viruses is not sensitive to cyclosporin[53]. Thus, early post-entry events in replication of HIV-1 clade O are not dependent on CyPA function. Incorporation of another immunophilin into virions of SIV and such a requirement as reported for HIV-1 has not been detected so far.

The *vpr* gene of HIV-1 and the additional *vpx* gene of HIV-2/$SIV_{sm/mac}$ encode 15 kD proteins that are packaged into virions in quantities comparable to Gag proteins[56]. Both *vpr* and *vpx* genes are only present in members of the HIV-2/SIV_{sm} lineage of viruses while the HIV-1, SIV_{cpz}, SIV_{agm}, SIV_{mnd} and SIV_{syk} primate lentivirus lineages have only the *vpr* gene[57]. The Vpr protein of HIV-1 exerts two principal functions which are mediated by different domains of Vpr. First, HIV-1 Vpr enhances the nuclear import of pre-integration complexes in non-dividing target cells, and facilitates infection of differentiated cell types such as macrophages[58]. Secondly, HIV-1 Vpr alters the cell cycle and proliferation status of the infected host cell by restricting progression from G_2 to the M phase, resulting in increased virus production[59,60]. In contrast, in HIV-2 and $SIV_{sm/mac}$ these two properties are encoded by two independent genes, i.e. *vpr* and *vpx*[61]. $SIV_{sm/mac}$ Vpr causes cell cycle arrest, but is dispensable for nuclear localisation of viral DNA during infection of non-dividing macrophages. On the other hand, $SIV_{sm/mac}$ Vpx protein does not influence the cell cycle, but is necessary for efficient nuclear localisation of the pre-integration complex in non-dividing macrophages. Vpx is thus essential for efficient infection of macrophages by $SIV_{sm/mac}$. The $SIV_{sm/mac}$ model has played an essential role in determining if such *in vitro* observations with accessory gene mutants actually influence the pathogenesis of the viral infection *in vivo*. SIV clones mutated in either *vpr* or *vpx* genes caused disease in rhesus macaques, whereas the same clones mutated in both genes were highly attenuated. By careful *in situ* analysis it was determined that *vpx* of SIV_{smPBJ} is important for viral amplification in the rectal mucosa of Pig-tailed

macaques[62]. Evidence suggested that this event may be macrophage dependent and important for rapid dissemination *in vivo*[62]. The ability to modify infectious molecular clones to elucidate the role of specific viral genes in viral pathogenesis has been one of the most powerful aspects of the $SIV_{sm/mac}$ macaque model. This is best illustrated in the elucidation of the role of Nef in AIDS pathogenesis.

The *nef* gene in both the HIV and SIV genome is located at the 3' end of the *env* gene. *Nef* encodes a 27kD protein which is myristylated at the amino terminus and associates with the inner side of the plasma membrane where it interacts with the protein kinase PAK65[63,64]. A number of phenotypes have been associated with Nef *in vitro*: down regulation of cell surface CD4[65,66] and MHC class I[65,66] expression, enhancement of proviral DNA synthesis[67], enhancement of infection of primary lymphocytes and macrophages[68,69], and activation of CD4 cells[70]. In the rhesus monkey infection with model SIV_{mac} strains carrying a deletion in the *nef* gene was associated with low levels of virus replication, maintenance of normal levels of CD4 cells and a marked delay in disease development[71]. The interaction between the kinase PAK65 and Nef correlated with the ability of Nef to contribute to pathogenesis *in vivo*[72]. In fact specific sequence changes in Nef have a major impact on viral virulence resulting in increased lymphocyte activation and accumulation in the intestinal *lamina propria* resulting in acute hemorrhagic enteritis[73].

Another difference between HIV-1 and HIV-2/$SIV_{sm/mac}$ is Vpu, a 16kD transmembrane protein which is found only in HIV-1 and SIV_{cpz}. It down-regulates CD4 expression by blocking the transport of CD4 from the endoplasmic reticulum to the cell surface[74,75]. It also enhances release of virus particles from the infected cell[76] and it was recently demonstrated that it forms cation-selective ion channels in phospholipid membranes[77,78].

Non-human primate lentiviruses

Studies conducted on biological material from HIV-1 infected humans and AIDS patients have contributed enormously to the current knowledge of the biochemistry and molecular biology of the virus and about the pathology of the disease it induces. However, since it is difficult to identify humans at the time of early primary HIV infection, animal models have been invaluable in providing insights into these very early events of lentivirus infection. Primate models have enabled the detailed study of the target cells for viral infection within the first days of infection and have assisted in correlating this information with the biological phenotype of the virus[79-81]. These models have also been invaluable for elucidating the role of particular viral variants in pathogenesis[82], and have proved essential in experimental vaccine development and evaluation[83-85]. As a member of the retrovirus subfamily Lentiviridae, HIV-1 is morphologically and genetically related to other, non-human lentiviruses. Indeed, the morphological similarity of HIV-1 to equine infectious anaemia virus (EIAV) led to the identification of HIV-1 as a lentivirus. Since then lentiviruses have been discovered in cattle[86-88], cats[89] and a considerable number of African primate species (Fig.1).

As introduced earlier, lentiviruses naturally infect a number of African non-human primates (Fig. 1). All these viruses cause a persistent but asymptomatic infection in their natural hosts[21,90-92]. The pathogenic potential of these infections was not realised until molecular evidence revealed that Asian rhesus monkeys which had developed and died from an AIDS-like disease had been accidentally infected with material derived from a sooty mangabey[6-8,93]. Thus, SIV_{mac}, the virus isolated from rhesus macaques, was really SIV_{sm} which had undergone cross-species transmission to a species previously naïve to lentiviruses. A number of years later several lines of research based on compelling molecular similarities came to the same conclusion regarding HIV-2 infection in man[24,94]. The remarkable similarity between the sequences of HIV-2 and SIV_{sm} makes them almost indistinguishable[13]. Although direct proof of transfer from mangabeys to humans is still lacking, the circumstantial evidence is compelling. More recently, similar sequence evidence has suggested that SIV_{cpz} infection from the Central subspecies of common chimpanzees *(Pan troglodytes troglodytes)* is the source of HIV-1 infection in the human population (Fig. 2)[25,95]. Lentiviral infections have been found in two subspecies of the common chimpanzee (the central subspecies *P.t. troglodytes*, and the Eastern subspecies *P.t. schweinfurthii*). While the $SIV_{cpz\text{-}ant}$ isolate from a naturally infected *P.t. schweinfurthii* is more distantly related to HIV-1, SIV_{cpz} isolated from *P.t. troglodytes* (SIV_{cpzGAB}, SIV_{cpzUS}, SIV_{cpzCAM}) is closely related to the major M and N variants of HIV-1[14,95] (Fig. 2). However, the origin of HIV-1 in humans is still hotly debated, as is the subspecies nomenclature of chimpanzees. Alternatively, HIV-1 and SIV_{cpz} may have arisen from a yet unidentified common ancestor, in either another host species or from an isolated population of humans or great apes where it may have persisted for hundreds or thousands of years. Clearly, more data is needed to solve the mystery of the origin of HIV-1.

Non-human primate SIV models

When the magnitude of the evolving HIV-1 epidemic in the human population was realised, it became clear that animal models would be needed to develop vaccines, therapies and to better understand the pathogenesis of AIDS. HIV-1 proved to be very species specific and only great apes (chimpanzees and gibbon apes) could be successfully infected. Chimpanzees were, and still are, the model of choice for HIV-1 infection, vaccination and immune prophylactic studies[96,97]. This is largely because they are biologically and, most importantly, genetically more similar to humans than any other primate species. The majority of chimpanzees are, however,

resistant to the development of AIDS[21,23] making them of interest for comparative pathobiology and immunisation strategies[98], but limiting their utility when the development of AIDS or its treatment requires an animal model.

For the study of AIDS pathogenesis, therapy, and the development of vaccines the rhesus macaque model has been most widely used. In addition to rhesus macaques, cynomologus and pig-tailed macaques also develop AIDS when given SIV_{sm} and are also studied, but to a lesser extent. This is largely due to the fact that for rhesus monkeys the immunological reagents are best developed, and the immunogenetics are more extensively studied. The models using SIV_{sm}/SIV_{mac} or related strains provide important surrogate models for HIV-1 induced AIDS.

The use of molecular clones to study determinants of viral virulence

The early cases of reported disease in Asian macaques were caused by cross-species transmission from sooty mangabeys. The resulting viral isolates were designated based on the species they were isolated from; rhesus macaques (SIV_{mac}), stump-tailed macaques (SIV_{stm}) and pig-tailed macaques (SIV_{mne}). Several SIV isolates, varying in pathogenic potential, have been characterised over the years[6,99-103] and have been used in preclinical evaluation of antiviral agents, vaccines and in immunotherapeutic studies[85,100,104]. These well-characterised SIV isolates have been pivotal for mother-to-infant viral transmission studies[105-108], both in defining viral and host determinants involved in various stages of disease and in correlating certain emerging virus variants with disease progression[82,109].

Lentiviruses are highly variable RNA viruses. The high error rate of the viral reverse transcriptase results in the rapid evolution of genetic variants following initial infection of the host. These variants, or populations there of are commonly called a *quasi - species*, continue to accumulate mutations *in vivo*. This results in the maintenance of a genetically diverse population throughout the infection. In addition, template switching during reverse transcription can result in recombination contributing to the high levels of HIV-1/SIV genetic variability. The heterogeneity in SIV/HIV-1 inocula represents a drawback in studies that require a well-defined virus genotype. The use of molecular clones of SIV or HIV-1 has been important as starting material in such studies. HIV-1 develops the highest heterogeneity in the early asymptomatic stages of disease and the lowest levels at later stages (ARC/AIDS) of disease[110]. Virus variants, which emerge late in infection, display significantly different characteristics from variants evolving shortly after infection. Early variants are in general macrophage-tropic, the latter variants are T-cell-tropic and induce formation of syncytia (SI). These late variants are commonly believed to contribute significantly to the clinical outcome of infection[82]. As a consequence, the characteristics of a molecular clone derived from an infected animal greatly depends on the particular variant that was selected for the cloning procedure and this may be influenced by both the stage of disease and the types of cells from which the virus was isolated. Over the years several molecular clones for SIV have been generated[99,111-116]. The virulence of the molecular clones in macaques ranges from highly pathogenic[99] to non-pathogenic[116].

Molecular clones have been used to characterise determinants of pathogenicity. Studies have revealed that the determinants of viral virulence (i.e. macrophage tropism) are frequently complex and multiple and are dispersed throughout the genome[117,118]. Other studies have identified the *env* gene as the major determinant responsible for differences in biological properties such as cellular tropism and cytopathogenicity[119-121]. The importance of the *nef* gene on cellular activation *in vitro* and disease progression *in vivo* has been convincingly demonstrated using SIV_{mac} molecular clones[70,71,73,122-124]. Most recently studies in the SIV_{mac} model have clearly illustrated how the host's immune response, in particular cytotoxic T-cell responses (CTL) drive the selection of a predominant population of variants capable of escaping the immune responses[125]. Such observations have been confirmed by the study of molecular clones of an acute SIV_{sm} variant which remained relatively invariant despite *in vivo* passage when animals developed disease before immune responses could be mounted[126,127].

Overview of $SIV_{SM/MAC}$-strains and related molecular clones

A large number of molecular clones have been the basis of some of the most important contributions of the SIV macaque model. They have been used to fulfil Koch's postulates to firmly establish the lentivirus etiology of AIDS[128], and have been widely used to further the understanding of AIDS pathogenesis. One particular molecular clone, SIV_{mac239} developed by Desrosiers and colleagues[103,128-130] has been extensively used to evaluate live-attenuated vaccine strategies and to develop both Env and RT SHIV chimerics for the preclinical evaluation of HIV-1 vaccines or antiviral drugs in macaques[131-133]. The first SIV_{mac} isolates and molecular clones were generated at the New England Regional Primate Research Centre with the injection of rhesus monkey 251-79 with tissue of animal 78-72. The SIV isolate from this particular rhesus monkey (251) served as the source of several molecular clones. Since then the original virus isolates and clones have been somewhat inappropriately designated SIV_{mac} and some have undergone repeated passaged *in vitro* resulting in altered properties. For that reason comparisons between viral isolates or molecular clones can only be evaluated in their historical context. The origins and relationships of commonly used isolates and their derivatives are described below.

SIV_{mac251} molecular clones: The virus isolate derived from the rhesus monkey 251 was designated SIV_{mac251}. This was the source for several different molecular clones of SIV_{mac}. A first group of clones (SIV_{mac251}, $SIV_{macBK28}$, SIV_{mac102} and $SIV_{mac1A11}$) was derived after SIV_{mac251} was propagated on H9-cells, a human T-cell line. The second group of clones (SIV_{mac239} and $SIV_{mac316EM}$) was derived after SIV_{mac251} had been passaged in animals 239 and 316-85, respectively[134]. A third group of clones (SIV_{mac32H} (pJ5), SIV_{mac32H} (pB1) and SIV_{32H} (pC8)) was generated from the 251 isolate after inoculations into rhesus monkey 32H following in *vitro* culture[135].

$SIV_{smPBj14}$ molecular clones: The virus isolate SIV_{sm-9}, which has been used to inoculate the pig-tailed macaque (PBJ), had been passaged once on human PBMC following isolation from a seropositive sooty mangabey. After infection of animal PBJ with this isolate, virus was isolated by coculture with human PBMC. Similar virus preparations were made of spleen and lymph node tissues obtained after monkey PBJ died 8 days after infection. The biological clone $SIV_{smPBj14}$ had acquired increased virulence, which approached 100% mortality in *M. nemestrina* following intravenous inoculation[136]. Mortality rates of 33%, 75% and 33% were observed for *M. mulatta*, *C. atys* and *M. nemestrina*, respectively[136]. Animals inoculated with $SIV_{smPBj14}$ developed profuse hemorrhagic diarroea within five days and died within two weeks post-infection[137]. Several molecular clones ($SIV_{smPBj-1.9}$, $SIV_{smPBj-4.9}$ and $SIV_{smPBj4.14}$) have been derived from $SIV_{smPBj4.14}$ and induce relatively similar disease symptoms[99,130]. In rhesus macaques, however, these clones do not cause an acute, but rather a more chronic disease progression[137].

$SIV_{mac1A11}$: Virus derived from cells transfected with this clone are cytopathic for rhesus PBMC, replicates in cultures of rhesus macrophages, and infects rhesus macaques when inoculated intravenously. Six macaques inoculated with $SIV_{mac1A11}$ all became infected and produced antibodies to viral envelop glycoproteins that neutralise virus. This molecular clone is markedly attenuated[138,139]. No clinical evidence of disease was observed throughout a 7-month experimental period. Other reports described even longer disease-free periods[140].

$SIV_{smPGm5.3}$: Blood transfusion from a SIV/STLV positive sooty mangabey to rhesus and pig-tailed macaques resulted in induction of a neurologic disease and AIDS. Lesions in infected animals include extensive SIV-RNA positive giant cells in brain parenchyma and meninges. Based on morphology infected cells proved to be from the macrophage lineage. $SIV_{smPGm5.3}$ shows high levels of replication in pigtailed and rhesus macaque PBMC and macrophage cultures[111].

$SIV_{smE593-3}$: The biological isolate SIV_{smE543} was obtained late in disease from an immunodeficient rhesus macaque which suffered from SIV-induced encephalitis. The molecular clone $SIV^{smE543-3}$ replicates well in macaque PBMC and monocyte-derived macrophages and resists neutralisation by heterologous sera which broadly neutralise genetically diverse SIV variants *in vitro*[112].

SIV_{mne027}: Uncloned virus isolate SIV_{mne} was used to infect pig-tailed macaques and the molecular clone SIV_{mne027} was derived from mesenteric lymph node tissue. This molecular clone is minimally cytopathic and not syncytium-inducing, replicates well in non-stimulated PBMC and is highly cytopathic for CD4+ T-cell subpopulations in stimulated PBMC[113].

SIV_{F359}: The isolate SIV_{8980} was derived from SIV_{B670} by four subsequent *in vivo* end stage passages in Indian rhesus macaques[126]. Monkey 8980 rapidly progressed to AIDS following the last *in vivo* passage. Serum from this animal was, without culture, directly used to derive the SIV_{F359} molecular clone by RT-PCR[141]. This molecular clone causes marked cytopathic effect *in vitro* and replicates to very high levels in activated but not in resting rhesus PBMC[142]. It also infects, but does not replicate efficiently in rhesus monocyte-derived macrophages. Mature rhesus macaques infected with SIV_{F359} develop high virus loads, loss of $CD4^+$ T-cells, anemia, severe weight loss, and opportunistic infections characteristic of AIDS. Sequence and biological analysis confirmed that this clone is indeed a dominant end-stage variant of SIV_{8980} and is distinct from other molecular clones from the SIV_{sm}/SIV_{mac} group. It also represents the first end-stage pathogenic molecular clone of SIV derived from viral RNA in serum[141].

The chimeric simian-human immunodeficiency virus (SHIV) model

To develop an alternative model for the use of chimpanzees, chimeric viruses based on molecular clones of $SIV_{sm/mac}$ were constructed. Either HIV-1 RT-encoding sequences (for antiviral drug studies)[131] or the HIV-1 *env* gene (for HIV-1 envelope based vaccine studies)[132,133,143-145] were cloned into the SIV_{mac239} infectious molecular clone. The first generation of *env*-SHIV chimeras was not pathogenic (i.e. could infect but did not cause AIDS in rhesus monkeys), whereas the RT-SHIV proved to be moderately pathogenic[131]. *In vivo* passage of SHIV chimeras has resulted in certain mutations occurring in the SHIV genome, some of which have undoubtedly contributed to their ability to cause an AIDS-like disease in rhesus macaques[133,146-148]. The patterns of $CD4^+$ T cell loss with these *in vivo* passaged SHIV are, however, atypical. For example, infection with the env-$SHIV_{89.6p}$ variant results in an acute and profound loss of CD4+ T-cells[133]. In contrast, the chimeric RT-SHIV causes disease only after a long period of infection and with only slight decline in $CD4^+$ T-cells[131].

The SHIV model is very useful for addressing specific questions of HIV-1 pathogenesis. For example, in addition to CD4, HIV-1 utilises two major co-receptors, CXCR4 and CCR5 along with a number of different minor co-receptors[40,149,150]. To address the significance of HIV-1 co-receptor usage in AIDS pathogenesis, SHIV chimeras have been

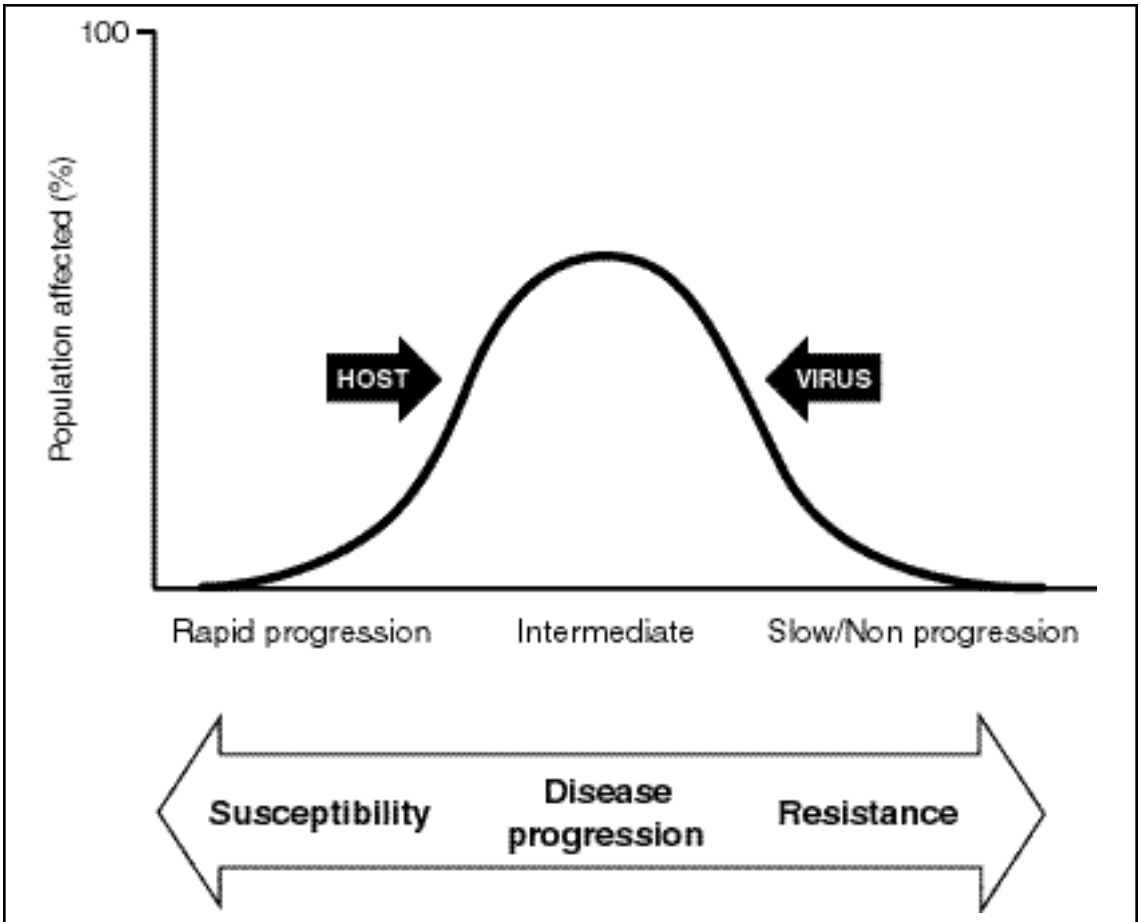

Fig.4. *A diagram depicting the different rates of progression to disease in an outbred population with survival being dependent on the interaction of both viral and host factors.*

constructed from HIV-1 molecular clones with distinct CCR5 or CXCR4 usage, designated R5 and X4 viruses, respectively. These SHIV chimeras cause quite distinct pathological lesions, suggesting infection of different CD4 T-cell subsets, when inoculated into rhesus monkeys[43].

Patterns of progression to AIDS in susceptible outbred populations

When HIV-1 infection is left untreated the mean survival time has been estimated to be approximately 10 to 12 years post-infection. However, the progression time to AIDS in different individuals is highly variable. The use of molecular clones of SIV in the macaque model have largely eliminated the issue of viral variability in the inoculum and have provided an important tool to better understand the host factors which influence the rate of progression to disease. Because of host differences and genetic polymorphisms of key immune regulatory genes such as MHC, certain individuals will progress slower or faster than the large majority of an infected population (Fig. 4). Similarly, in younger individuals such as juveniles or neonates or very old individuals such as geriatrics, the disease may progress more rapidly, in part due to an immature or debilitated immune system.

Similar to humans, in an outbred population of macaques there is a host dependent variation in the time to development to AIDS which, together with the virulence of the initial viral inoculum, greatly influences the mean rate of disease progression (Fig. 4). In rhesus macaques, a highly virulent strain of $SIV_{sm/mac}$ will likely cause a high and sustained viral load (Fig. 5A). In general, this is accompanied by a more rapid decline in $CD4^+$ T cell levels than in animals infected with a less virulent strain of SIV (Fig. 5B). Viral strains which have an intermediate virulence potential have a lower steady state level of plasma viral RNA than those of higher virulence. It is now clear from the SIV macaque model that the dose of initial inoculum does not affect steady state virus loads or the rate of disease progression, but rather this is determined the virulence of the initial inoculum as well as host factors[151].

Similar to HIV-1 in humans, the principal cell targets for $SIV_{sm/mac}$ are $CD4^+$ T cells and cells of the monocyte/macrophage lineage[81]. Infection of both cell types by HIV-1 is thought to be important for the pathogenesis of AIDS[39]. Cell tropism of HIV-1 has been correlated with the biological variants of HIV-1 and the co-receptors these viruses use to enter cells[39]. As mentioned earlier, HIV-1 utilises two major co-receptors and this co-receptor use also correlates with the two different biological variants of

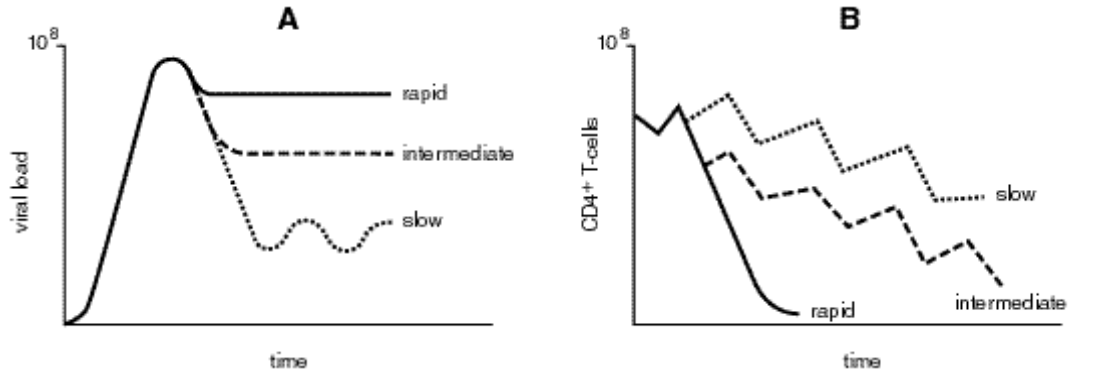

Fig.5. *Two schemes depicting the virus loads in plasma (concentration of viral RNA per ml plasma) and the influence of virus load on the rate of disease progression (A), which is inversely correlated with the rate of CD4⁺ T-cell decline (B).*

HIV-1 observed in patients[40]. In the early stages of infection in man, viral variant phenotypes are described as non-syncytium inducing (NSI). These viruses are usually macrophage-tropic (mø) and commonly use the CCR5 co-receptor[40]. In later stages towards the development of AIDS, syncytium inducing (SI) variants of HIV-1 emerge and become dominant[39]. SI variants are primarily T-cell-tropic and use the CXCR4[121] co-receptor, or they may be dual-tropic for both macrophages and T-cells (R5X4). The conversion from NSI to SI variants in many infected patients may signal the development of AIDS together with a change in CD4 T-cell subset tropism and a more rapid progression to AIDS[39,41]. In contrast to HIV-1, NSI and SI phenotypic variants of $SIV_{sm/mac}$ and their disease correlates in macaques are not commonly recognised. $SIV_{sm/mac}$ also infects both T-cells and macrophages. Interestingly, multi-nucleated giant cells are a frequent observation in the lymph nodes of SIV_{sm}-infected monkeys which develop AIDS[152]. $SIV_{sm/mac}$ isolates are also capable of infecting mature or naïve $CD4^+$ T cells, even resting T-cells in some cases[73]. In contrast to HIV-1, where the shift from R5 NSI to X4 SI variants is associated with a shift in cell tropism, phenotypic variants of $SIV_{sm/mac}$ have not been observed and coreceptor use remains relatively constant with regard to CCR5 use[31,32]. This may be a result of the lack of natural transmission studies. The vast majority of $SIV_{sm/mac}$ studies are carried out on animals which are experimentally infected (most often not mucosally) with viral variants which have already been highly selected. However, carefully carried out studies with molecular clones of SIV_{mac} have revealed a number of characteristics which are accumulated in end stage variants of $SIV_{sm/mac}$ infected animals with AIDS[82,109,142]. In fact, as previously described, $SIV_{sm/mac}$ isolates do not preferentially use the CXCR4 coreceptor.

In conclusion, there are a vast number of important similarities between the SIV-macaque model of AIDS and HIV-1 infection of humans, though in certain instances, important differences exist. The similarities are, however, overwhelming, especially with regard to the correlation of virus load patterns (Fig. 5A) with disease progression, and to the individual variation in susceptibility for the development of AIDS in outbred populations (Fig. 4 and 5). While the similarities may allow us to evaluate and develop vaccines and therapeutics in this model, the differences may reveal exploitable tools to further our understanding of the essential events, leading to AIDS.

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