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SIV envelope evolution and virus virulence
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Chapter 1

SIV and HIV: A General Introduction
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The Start of the AIDS Epidemic and the Discovery of HIV:
In the early 1980's an epidemic of Kaposi's Sarcoma and other opportunistic infections emerged among homosexual men in the United States (13). Originally considered a rare syndrome, AIDS subsequently spread among intravenous drug users, haemophiliacs, partners of infected individuals, blood product recipients, and also the infants of mothers with AIDS. The epidemiology of AIDS suggested an infectious etiology. In 1983 a French group led by Montagnier first isolated a lentivirus from patient material which then became known as LAV (now known as Human Immunodeficiency Virus 1 or HIV-1 (5)). First recognized in the United States, reports of HIV-1 seronegative AIDS later came from West-Africa, and in 1986 the same French team isolated another HIV-1 related virus designated HIV-2 (18). The gene order as well as phylogenetic relationship of HIV-1 and HIV-2 (Figure #1) made it clear that the two viruses were of the same family. Phenotypic differences were observed with HIV-2 infection appearing to be much less virulent, and less infectious according to lower transmission rates.

AIDS in Non-Human Primates:
In the 1980's an increase in the incidence of lymphoma in rhesus macaques in the California Regional Primate Research Center and subsequently in the New England and Tulane primate centers were observed. Unlike the first increase in lymphomas in the 1960's and 1970's these tumors were not consistently positive for SRV (Simian Retrovirus (typeD)(53)), certain serotypes of which cause retroperitoneal fibromas in rhesus monkeys. Years earlier leprosy in African sooty mangabeys was being studied at the Tulane Primate Research Center (6). As this species was endangered there was an effort to develop another animal model for leprosy using rhesus macaques. Tissue from lepromatous leprosy lesions from sooty mangabeys was used to inoculate Asian rhesus macaques. It is highly likely that these transmission experiments led to the transmission of Simian Immunodeficiency Virus (SIV) from these sooty mangabeys to rhesus macaques. Unaware of the asymptomatic infection of sooty mangabeys with SIVsm (the small letters denote the species specific strain of the virus) the housing of these animals with rhesus macaques likely led to the spread of the infection. Simultaneously, similar outbreaks in rhesus macaques were observed at the California Regional Primate Research Center (35). Evidence suggests that asymptomatic of SIVsm carriers were transferred from Tulane and then from California to other research centers, precipitating the outbreaks of lymphomas at Tulane, California, and the New England Primate Research Center. Antibodies of affected animals reacted with some antigens of HIV-1, and a subsequent study revealed a significant disease association with transmission of tissues from animals which developed lymphoid neoplasms (42,55). The first virus isolations (21,44,54,60), and their use in experimental infections, revealed that an immunodeficiency syndrome could be reproduced in Asian nonhuman primates with an etiologic agent related to HIV-2 (subsequently called SIV). Certain variants of SIVsm were found that were extremely pathogenic (31), and were studied extensively although the disease characteristics were not very characteristic of those of AIDS in humans. Designated SIVsmPBj14 this isolate caused enormous immune activation (23) characterized by extensive lymphoid hyperplasia and haemorrhagic enteritis (32), displaying unique lentivirus-host interactions resembling superantigen-like activity (67). The linking of the epidemic of lymphomas, opportunistic infections, and AIDS in macaques with a lentivirus recovered from asymptomatic sooty mangabeys (30,60) allowed investigations to trace the origin and spread of the primate
epidemic in the major primate centres. The viruses isolated from sooty mangabeys (6,30,60) were later found to be natural asymptomatic infections in this species. These findings sparked the search for the origin of the lineage of primate lentiviruses and lead to the discovery of more and more primate specific lentiviruses causing asymptomatic infections in African nonhuman primates.

Figure #1: The genomic organization of the primate lentiviruses HIV-1/SIVcpz and HIV-2/SIVsm. Both have similar patterns of genomic organization and length (between 9 and 10 kb) of linear double-stranded proviral DNA. Structural genes are shaded; regulatory and accessory genes are are represented by black squares. Vpu is found exclusively in HIV-1 and SIVcpz.

Natural Asymptomatic Infection in Africa:
The five major lineages of currently known primate lentiviruses are designated SIVcpz from chimpanzees, SIVagm from African green monkeys, SIVsm from sooty mangabeys, SIVsun from sun-tailed monkeys, and SIV1'hooest from L'hoest monkeys (together with SIVmnd from mandrills). Based upon genetic analysis the origin of HIV-2 was traced to SIVsm transmitted to humans (34,40). Virus characterization studies revealed the geographic clustering of HIV-2 strains in humans with SIVsm in feral sooty mangabeys, supporting the hypothesis that each HIV-2 subtype originated from widely divergent SIVsm strains (16,37). This led to an extensive, and still ongoing, search of the African continent for new virus strains from all of the nonhuman primate species. Knowledge of the ability or inability to infect other species has added to the understanding of the transmission of lentiviruses between man and the nonhuman primates. The ability to efficiently infect other species is essential to the spread from one species to another. Of the 92 species of monkeys in Africa currently only seven (possibly eight, SIVrcm from a Red capped mangabey has an original env but appears to be a chimaera (36,69)) are known to be infected with distinct viruses of the HIV/SIV family. The greatest problem is the difference between viral phylogenies and the actual distribution history of the virus itself. The extinction of lineages during history and the fragmentary recollection of the development of the pandemic from the information available only shows a fraction of what may have taken place. The recent discovery of a new SIVcpz isolate (SIVcpzUS) which clusters closer to HIV-1 than another isolate from chimpanzees (SIVcpzant) is important as it is most related to a new group of human isolates (type N (33)).
Phylogenetic analysis has provided data supporting the relationship of the human viruses HIV-1 with SIVcpz, and HIV-2 with SIVsm (Chapter 2, Figure 2 (38,61)). The nonhuman primate viruses being nonvirulent in their natural hosts and the human viruses being highly pathogenic.

**Evidence for Cross-species Transmission between Primates:**

Aside from the experimental or nosocomial transmission of SIVsm from African sooty mangabeys to Asian rhesus macaques, there are cases of transmission of SIVagm from sympatric African green monkeys to a yellow baboon, a chacma baboon, and a patas monkey (7). Studies in West Africa had shown a distinct clustering of the different HIV-2 strains in humans to be related to several sooty mangabey strains (34). Marx et al studied 197 captive and wild sooty mangabeys, and local inhabitants in West Africa. Each separate subtype of HIV-2 found in villagers clustered geographically with the SIV strain found in sooty mangabeys from that locale (16). There was greater variation between the viruses found within a feral troupe of sooty mangabeys than there was between selected variants with individual sooty mangabeys and the HIV-2 found in the human inhabitants. Based on this observation they proposed that multiple transmissions had occurred between man and sooty mangabeys. More recently evidence for cross species transmission of SIVcpzUS a virus of Pan troglodytes troglodytes has been proposed as the closest lentivirus relative to HIV-1 in man, and a likely source for the current HIV-1 epidemic in humans ((33,37) Figure #2).

**Figure #2:** A phylogenetic tree of the complete outer membrane or envelope gene (env or gp160) of representative isolates of the HIV/SIV lentiviral family. Env is approximately 3.5 kb in length and contains the regions of the virus which interact with the cellular membrane during binding, entry and infection, and is one of the main targets of the immune response. The relationship of the various genes is shown by the length of the branches of the tree, with a longer branch denoting a less related relative. Representative isolates of the virus, and their relationship to HIV-1 types M, N, and O, SIVcpz, HIV-2, and SIVsm are observed. The closely related viruses are believed to be the result of the cross species transmission from chimpanzee (SIVcpz) and sooty mangabey (SIVsm) to man (HIV-1 and HIV-2 respectively).
Assessment of the in vitro capabilities for transmission into cells of other species led to several set backs in models for HIV-1 infection. The known isolates from chimpanzees resemble that of HIV-1 but are of little experimental value as they do not infect lower species of primates (8). The only strains of the virus that do grow in human cells are SIVcpz, SIVsm, SIVI’hoest, and SIVsun. The cross species barriers of infection became even more complicated. This coupled with the species barriers between the African nonhuman primates (8) and their species specific strains (2) points to these four viruses as the existing relatives of the progenitors of the human lentiviruses HIV-1, and HIV-2.

**Molecular Evolution of SIV:**

The finding that variation of only nine of the thousand amino acids in the envelope of the virus (Env, or gp160 (the translated protein), or env (the viral gene) could lead to a one hundred fold difference in replication between SIV variants (3) revealed the importance of small numbers of amino acid changes encoded in env. Large scale sequencing and analysis of env clones demonstrated that the hydrophilic and externalized regions were highly variable (V regions). The internalized and hydrophobic regions were found to be less variable (constant or C regions (24)) and have altered codon usage (70). The first variable regions of the SIV env were suggested to play a role in mucosal transmission (V1 and V2 (4,57)). The effect of glycosylation on the tertiary structure of env became apparent when the Ab binding profiles to successive Envs during infection were found to vary according to the addition or deletion of glycosylation sites in the V1 and V4 regions (64,65). The spontaneous changes brought about by the infidelity of the reverse transcriptase of the virus was thought to inadvertently allow the selection of escape mutants (63) which Abs could no longer bind. The complexity of the folded structure of the HIV and SIV env and its biological function in binding and infection showed that various nonadjacent areas of the nascent Env participate in the conformation and binding to CD4, accessory coreceptors and to facilitate entry (12). The study of the Env structure showed that the cooperation of numerous amino acids scattered throughout the internal and external areas of the folded protein cooperate to define its biological function (43,48,49,56,58,59). Antibody-antigen interactions followed during in vivo infection indicated that mutations in the env occur in a site specific manner, and these Ab sensitive sites were mapped to the V1,2,3,4 and V5 (virtually all the externalized areas (11,47)). Using mutagenesis and studying variants generated in vivo, two independent groups demonstrated that the V3 region of the SIV env did play a definite role (39,50,71) in SIV pathogenesis. Several mutations were mapped which markedly affected cell entry, and phenotype (from monocyte-derived macrophage tropic to infectious in peripheral blood mononuclear cells (PBMCs) or the complete abrogation of either (39,71)).

**Biological Variation of SIV:**

Cellular tropism of HIV and SIV is largely controlled by the env gene and is currently one of the most intensely studied areas of AIDS research. HIV and SIV infection is initiated by viruses with a macrophage tropic phenotype (Mφ). Frequently during progression to AIDS HIV strains will switch to T cell tropism (TT). This TT phenotype characterized in vitro by growth in MT-2 cells and the formation of syncytia in these cells (SI for syncytium inducing, and NSI for nonsyncytium inducing viruses (20,22,51)). The cloning of the first coreceptors which HIV-1 utilizes during binding and entry (CCR5 and CXCR4), in conjunction with the
Figure #3: Different patterns of HIV and SIV infection in vivo. Shown (upper) is the typical picture observed of a progressor of HIV-1 infection in man. An initial peak in virus load is seen, followed by containment of viral replication, and then decreasing CD4 T-cell counts, eventual rise in viral load, and progression to AIDS. In long term nonprogressors of HIV-1 infection in man (middle) the initial virus load peak is contained for an indeterminate amount of time and no or minimal loss of CD4 T-cells is seen. The natural infection of nonhuman primates is characterized by to moderate high virus loads, and sustained normal amounts of CD4 T-cell counts with no progression to AIDS (lower).
CD4 T-cell receptor, was a major breakthrough in AIDS research (1,25,26,29). The family of G-protein-coupled seven-transmembrane-domain proteins (19,29) are the mediators of chemokine activity. The majority of Mφ isolates appear to specifically use CCR5, a receptor for the CC chemokines RANTES, MIP-1α, and MIP-1β (19). The TT viruses predominantly use CXCR4, a receptor for the CXC chemokine SDF-1 (9). Subsequently the V3 mediated SI and NSI virus infections were shown to be mediated by a TT coreceptor (1,25), and a Mφ coreceptor respectively (29). More recently coreceptors for HIV and SIV have been cloned (27). HIV and some SIV isolates use a myriad of coreceptors with which to bind to a cell and gain entry, and some isolates can do so even without the use of CD4, using only the coreceptor to gain cellular fusion (28). Human coreceptors allow cell entrance to SIV (14,17) without any loss of infection efficiency. In the case of SIVsm Mφ and TT viruses can both use CCR5. Usage of the same coreceptor by the two types SIVsm virus is differential with two different domains of the coreceptor being utilized (26). HIV-2/SIVsm viruses generally prefer the CCR5 coreceptor, although they can utilize numerous other coreceptors (15,17,27,66). HIV-1 generally uses CCR5 in early infection, and CXCR4 exclusively or as well as CCR-5 (dual tropism) in late symptomatic infection during progression to AIDS. Broadening of coreceptor usage and its importance to disease progression in humans is still heavily debated (20,22). Progression to AIDS in SIV infection is neither related to broadening nor switching of coreceptor use (45). In fact CXCR4 is never used by SIV strains early or late in infection (14,45,46).

Determinants of Transmission of SIV:
The entry of SIV through non-abrasive mucosal oral, vaginal or rectal surfaces results in the selection of a small number of genomes (bottleneck transmission) and results in the decrease in genomic heterogeneity (10,57,62). Even with intravenous, mucosal or infected microgolial cell inoculation of virus the resulting effect is the same (52,62). Regardless of the route of infection, or the size of the inoculum, there is an initial selection that results in the decreased heterogeneity of virus. Later in the infection variants found in the primary inoculum but not predominant early in infection are frequently re-emerge (4,57). Transmission studies with SIVsmB670 have shown genotypic and phenotypic selection of Mφ variants in the initial infection in neonates (4,57). The genomes present in the blood as measured by genotypic and phenotypic characteristics are influenced by humoral and cellular immune responses and the stage of infection.

The SIV Model Used in the Present Study:
Holterman and coworkers from the Biomedical Primate Research Centre (Rijswijk, The Netherlands) performed a passage experiment in rhesus macaques that was recently published with the department of Human Retrovirology (AMC, Amsterdam, The Netherlands (41)). Initially ten Asian rhesus macaques of an average age of 18 months were inoculated with a SIV B670 stock (6,60) originating from a virus infecting sooty mangabey. It took seven months before the first of these rhesus macaques became ill, or showed signs of progression to AIDS. During a total of five passages of large inoculum the time to death decreased from seven to thirty three months during the first passage to one to two months during passages four and five respectively. A correlation was shown in this study between the plasma viral load and monkey survival. Plasma load as indicated by this study is clearly related to the virulence of the virus and/or the susceptibility of the host (68). The present thesis includes a genetic analysis of the env of the in vivo serial passage of SIVsmB670.
Scope of this Thesis:
Chapter two describes the use of Likelihood Mapping and Quartet Puzzling to unveil the family tree of SIVs isolated from different species of African monkeys. The evolution of viral genes having different roles in the asymptomatic infection of African feral nonhuman primates is analysed. The next chapters three to six describe the SIV env evolution during serial in vivo passage of SIVsmB670 in rhesus macaques. Chapter three describes the amino acid changes in the SIV env gene related to increased virulence and changes (or the absence thereof) in coreceptor usage. Chapter Four describes the shortening of the asymptomatic period during serial population passage and how it is related to changes in the variable versus the constant env regions. The phylogenetic methods used to determine the course of natural SIV infections were subsequently applied to the SIV data set in Chapter Five. Chapter Six compares the viral load data and env evolution during serial passage and the relationship between viral load and genetic diversity. Finally in chapter seven the relationship between SIV virulence and env variation is discussed.

References:


