DEVELOPMENT OF A NON-HUMAN PRIMATE MODEL

FOR HUMAN HIV/AIDS

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BY

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This thesis is submitted in partial fulfilment for the degree of Master of Science in Biochemistry at the University of Nairobi
DECLARATION

I, David Maina Menge hereby declare that this is my original work and has not been presented for award of a degree in any other University.

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<th>Abbreviation</th>
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<tr>
<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>CAPS</td>
<td>Cyclohexylamino-1-propane sulphonic acid</td>
</tr>
<tr>
<td>CAV</td>
<td>Caprine arthritis virus</td>
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<tr>
<td>CBC</td>
<td>Complete blood count</td>
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<tr>
<td>CD</td>
<td>Cluster differentiation antigen</td>
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<tr>
<td>cDNA</td>
<td>copy DNA</td>
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<tr>
<td>CPE</td>
<td>Cytopathic effect</td>
</tr>
<tr>
<td>dNTPs</td>
<td>deoxynucleotide triphosphates</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetraacetate</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>FACS</td>
<td>Flourescent activated cell sorter</td>
</tr>
<tr>
<td>FIV</td>
<td>Feline immunodeficiency virus</td>
</tr>
<tr>
<td>gp</td>
<td>Glycoprotein</td>
</tr>
<tr>
<td>HIV-1</td>
<td>Human immunodeficiency type 1</td>
</tr>
<tr>
<td>HIV-2</td>
<td>Human immunodeficiency type 2</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>Human T-lymphotropic virus type 1</td>
</tr>
<tr>
<td>HTLV-2</td>
<td>Human T-lymphotropic virus type 2</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>LSM</td>
<td>Lymphocyte separating media</td>
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<tr>
<td>LTRs</td>
<td>Long terminal repeats</td>
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<tr>
<td>MTP</td>
<td>Microtitre plate</td>
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<tr>
<td>MCHC</td>
<td>Mean corpuscular haemoglobin concentration</td>
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<tr>
<td>OPD</td>
<td>Ortho-phenylenedianjine</td>
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PCR  |  Polymerase chain reaction
PBMCs |  Peripheral blood mononuclear cells
PTA  |  Phosphotungstic acid
rev  |  Regulator of expression of virion proteins
rpm  |  Revolutions per minute
RT   |  Reverse transcriptase
SDS  |  Sodium dodecyl-sulphate
SIV  |  Simian immunodeficiency virus
SIVmac |  Simian immunodeficiency virus from Rhesus macaques
SIVsmm |  Simian immunodeficiency virus from sooty mangabeys
SIVagm |  Simian immunodeficiency virus from African green monkeys
SIVcpz |  Simian immunodeficiency virus from chimpanzee
SIVsyk |  Simian immunodeficiency virus from sykes
SIVmnd |  Simian immunodeficiency virus from mandrils
SIVoeb |  Simian immunodeficiency virus from De Brazza's monkey
ssRNA |  Single stranded RNA
STLV-1 |  Simian T-lymphotropic virus type 1
STLV-2 |  Simian T-lymphotropic virus type 2
TAE  |  Tris acetate EDTA
tat  |  Transactivator
TBS  |  Tris buffured saline
TCID_{50} |  Tissue culture infectious dose 50%
TE   |  Tris EDTA
UA   |  Uranyl acetate
vif  |  Virus infectivity 'factor
vpr  virus protein R
vpu  virus protein U
vpx  virus protein X

**UNITS**

jaL  Microlitre
JM   Micromolar
g    Gram
mM   Millimolar
M    Molar
bp   Base pairs
OD   Optical density
SD   Standard deviation
°C   Degree Celsius/Centigrade
ng   Nanogram
%    Per cent
vpr  virus protein R
vpu  virus protein U
vpx  virus protein X

UNITS

\( \mu L \)        Microlitre
jaM           Micromolar
g           Gram
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SUMMARY

HIV infection and the resultant disease, AIDS poses a big threat to humans worldwide and an enormous challenge to scientists and healthcare workers. The causative agent of AIDS has been isolated and molecularly characterised. In addition reliable diagnostic procedures have been developed. Inspite of these, events leading to disease have not been well understood. What is responsible for the long latency of the virus in some individuals and rapid disease progression in others upon infection is still an enigma that has to be elucidated. The mechanism of immunosupression and tissue damage following lentiviral infection in a host are complex. Investigation of HIV pathogenesis in vivo in humans is hampered by important practical and ethical constraints.

The discovery of HIV-like viruses in non-human primates has opened up new avenues in research geared towards the understanding of the biology and pathologenesis of HIV/AIDS using these animals as models for studying HIV pathogenesis. The precise identity of infected cells, factors that increase virus production and mechanisms of virus induced immunodeficiency in vivo can effectively be elucidated in such experimental animal models and the results can then be extrapolated to address key and unanswered aspects of human disease.

In the recent past, a lot of efforts have been directed towards development of suitable animal models that can be used in HIV studies. In addition, such models can be used in testing the efficacy of vaccine candidates and chemotherapeutic agents. Studies using non-human primates naturally infected with an HIV-like lentivirus have shown that such animals could give the answer to the long sought model. This project was aimed at developing De Brazza's monkeys (Cercopithecus neglectus) naturally infected with an HIV-like virus as a model for HIV/AIDS in humans. To achieve this, wild caught or colony born De Brazza's monkeys Cercopithecus neglectus in-housed in a semi-breeding colony at the Institute of
Primate Research (IPR), Kenya were tested for anti-SIV antibodies using antibody ELISA. Virus was isolated from positive animals by end point titration using PM-1, CEMx174, SupT1, HUT and Molt-4-clone-8-cell lines. The isolated virus was further characterised by Western blots, electron microscopy and HIV-1 pol nested PCR. The pathological effects on infected animals was elucidated by CBCs, CD4:CD8 FACS analysis and lymph-node pathology by immunohistochemistry.

Antibody ELISA results indicated that over 50% of De Brazza's monkeys Cercopithecus neglectus at the IPR colony are SIV positive. SIV virus was isolated from three animals that had a TCID$_{50}$ of between $2.12 \times 10^5$ to $3 \times 10^3$ in PBMCs and plasma, a range that is comparable to that of HIV infected humans. Electron microscopy, Western blotting and HIV-1 nested pol PCR confirmed the isolated virus to be an HIV-like lentivirus. All the infected animals had persistently high antibody titres and CD4:CD8 ratio below 0.1. Lymph-node pathology revealed a destruction of the lymph-node architecture.

The observations made in this study indicate that De Brazza's monkeys (Cercopithecus neglectus) is naturally infected with a HIV-like lentivirus called SIV$_{Deb}$. This is confirmed by cross reactive antibodies in SIV$_{Deb}$ infected animals with SIVmac peptide, HIV-2 western blots, electron microscopy showing HIV like particles, HIV reverse transcriptase activity of virus isolates and HIV-1 pol based nested PCR. The AIDS-like parameters tested like the lowering of CD4:CD8 ratio and destruction of lymph-node architecture in infected animals is parallel to those of HIV/AIDS observed in human. This qualifies De Brazza's monkeys as one of the possible candidates of the long sought animal model systems for human HIV/AIDS.
ACKNOWLEDGEMENT

I am indebted to many people without whose support this thesis would not have been possible.

I am especially grateful to Dr. Moses Otsyula, the leader of virology programme at the Institute of Primate Research (IPR) for suggesting and financing the project and tireless criticism and moral support that always put me within the frame of this thesis.

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I would like to thank Mr. D. Langat for training and advice in reverse transcriptase assays and western blots. I am most grateful to the staff of Virus Research Programme at IPR Dr G. Chege, E. Munene and D. Odoyo without whose support and encouragement, this thesis would have never been completed.
DEDICATION

This thesis is dedicated to my father and mother Mr. Samuel Menge and Mrs. Joyce Menge for their priceless effort in sustaining my schooling.
The main roles of models is not so much to explain and to predict though ultimately these are the functions of science: - as to polarise thinking, to establish dialects, to pose sharp questions and above all, to lead to some radical, undreamed of unifying concept.
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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1. General Introduction

Research work aimed at the understanding of human immunodeficiency virus (HIV) with respect to the management of the resultant disease, acquired immunodeficiency syndrome (AIDS) is hindered due to (i) the nature of HIV, the causative agent and (ii) lack of an appropriate animal model necessary to facilitate research in understanding the biology, pathology and transmission patterns of HIV. Since the discovery of HIV/AIDS in 1981, research work directed in addressing this problem has been geared towards development of diagnostic procedures, vaccines, chemotherapeutic agents and animal models. So far, a number of reliable diagnostic procedures have been developed. However, reliable vaccines and chemotherapeutic agents and their prognostic procedures are yet to be developed.

An appropriate animal model is urgently required for understanding HIV pathogenesis and for development and testing vaccine candidates, drugs and immunomodulators. So far, efforts to develop an animal model for human HIV/AIDS fall into three categories (i) development of transgenic animals for example mice with CD4+ and other co-receptors on their T-helper cells, for example CCR4 and CCR5, to facilitate HIV infection (Cohen, 1997) (ii) comparative studies in non-human primates using simian immunodeficiency virus (SIV) (Tsujimoto et al, 1988, Tsujimoto et al, 1989, Otsyula et al, 1996 and Kurthc et al, 1997) and (iii) infection of non-human primates with HIV isolates (Alter et al, 1984, Markam et al, 1988 and Agy et al, 1992). Although transgenic mice are likely to be infected with HIV, it is not clear whether they will develop a disease whose pathology is comparable to human HIV/AIDS (Lohen, 1997). Furthermore, it has been observed that HIV has a great difficulty in replicating because some viral genes do not work well in murine cells. Experiments that involve infection of non-human primates with HIV are also yet to be perfected because the animals so far
infected with HIV do not actually develop a classical disease similar to that induced by HIV infection in human (Alter et al, 1984, Barnett et al, 1993 and Cohen, 1997).

1.2. Discovery of the AIDS Virus

The first indication that AIDS could be caused by a retrovirus came from the Pasteur Institute (Barre-Sinoussi et al, 1983) when a reverse transcriptase containing virus was recovered from the lymph node of a man with persistent lymphadenopathy syndrome (LAS) (Barre-Sinoussi et al, 1983 and Levy et al, 1984). At that time, some physicians suspected that this syndrome was associated with AIDS but there was no conclusive evidence (Abrams et al, 1984). Since enlarged lymph nodes are observed during several viral infections, many physicians believed initially that LAS resulted from a known human virus such as Epstein-Barr virus (EBV) or cytomegalovirus (CMV). In addition, the characteristics described for the retrovirus recovered by the Pasteur Institute group included some reported for the human T-cell leukemia virus (HTLV) (Barre-Sinoussi et al, 1983). Thus many investigators decided initially that the lymph node isolate was a member of this already recognised human retrovirus group. In support of this conclusion was the concomitant publication by Gallo et al, 1983, in the same issue of Science reporting the isolation of HTLV from individuals with AIDS (Barre-Sinoussi et al, 1983 and Gallo et al, 1983).

HTLV as an etiologic agent for AIDS, however, seemed unlikely because of its close cell association and its known poor replication in culture (Miyoshi et al, 1981, Poiesz et al, 1980 and Sagamura and Hinuma, 1993). Since haemophiliacs with AIDS had been reported (Baba et al, 1988), it was hard to visualise how such a virus could be transmitted by cell-free plasma products such as factor VIII. Moreover, HTLV does not lyse lymphocytes. In contrast, it often transforms them to continuous cell lines (Miyoshi et al, 1981). Thus the characteristic loss of CD4+ lymphocytes floserved in AIDS patients (Gotlieb et al, 1981, Mildvan et al, 1982 and Stahl et al, 1982) could not be explained by an HTLV infection.
Further studies by Montagnier and coworkers clarified these questions in relation to the LAS agent. The results indicated that this human retrovirus, although similar to HTLV in infecting the CD4+ lymphocytes, had quite distinct properties. The virus isolated by Montagnier's laboratory, latter called lymphadenopathy-associated virus (LAV), grew to substantial titres in CD4+ cells and killed them (Montagnier et al., 1984) instead of maintaining them in culture as does HTLV. These observations on LAV provided important evidence supporting the etiological role of the new retrovirus and causative agent for AIDS.

Several other laboratories were also searching for the agent responsible for this immune deficiency syndrome reported the characterisation of another human retrovirus distinct from HTLV they named HTLV-III (Gallo et al., 1984, Popovic et al., 1984, Sarngandharan et al., 1984 and Schaupbach et al., 1984). It was isolated from the peripheral blood mononuclear cells (PBMCs) of AIDS patients. The lymphotropic and cytopathic properties of the virus and its cross reactivity with some proteins of HTLV-I and HTLV-II, particularly the core p24 protein (Gallo et al., 1984).

Levy et al., (1984) also reported at that time the identification of retroviruses they named the AIDS- associated retroviruses (ARV). These viruses were recovered from AIDS patients from different risk groups, as well as from symptomatic and some healthy people. Finding ARV in asymptomatic individuals indicated for the first time a carrier state for the AIDS virus. This retrovirus, ARV, showed some cross-reactivity with LAV when examined by immunoflourescence techniques (Levy et al., 1984). Moreover, it grew substantially in PBMCs, killed CD4+ lymphocytes, and did not immortolise them. Thus, the three newly identified retroviruses had similar characteristics. Most importantly, infection by these retroviruses was not limited to AIDS patients. They were also recovered from individuals with other clinical conditions, including lymphadenopathy hence supporting the conclusion that LAS was part of the disease syndrome.

The three prototype viruses (LAV, HTLV-III and ARV) were recognised as members
of the same group of retroviruses, and their properties identified them as retrovirinae. Their proteins were all distinct from those of HTLV and their genomes showed only remote similarities to the genome of this agent, no more than that of the chicken virus (Rabson and Martin, 1985). Thus, the initial cross-reactivity of HTLV-III with HTLV proved incorrect. For all these reasons, the International Committee on Taxonomy of Viruses recommended giving the AIDS virus a separate name, the human immunodeficiency virus (HIV) (Coffin et al, 1986).

HIV isolates were subsequently recovered from the blood of many AIDS patients, AIDS-related complex, and neurologic syndromes, as well as from the PBMC of several clinically healthy individuals (Levy et al, 1985 and Salahuddin et al, 1985). Thus the wide spread of this agent was appreciated. Soon after the discovery of HIV-1, a separate subtype, HIV-2 was identified in Western Africa (Clavel et al, 1986). Both HIV sub-types can lead to AIDS, although the pathogenic course with HIV-2 might be different. Soon afterwards, an AIDS-like condition was observed in captive primates in various regional primate centres in the USA and subsequent isolation of an infectious agent later named simian immunodeficiency virus (SIV) from captive rhesus monkey, Macaca mulata (Daniel et al, 1986). Since then, several other SIVs have been isolated and characterised. These isolates are from sooty mangabey, Cercopithecus atys (Murphy-corb et al, 1986, Loweinstein et al, 1986 and Kodama et al, 1989), mandrils, Mandrillus sphinx (Tsujimoto et al, 1986), African green monkey, Cercopithecus aethiops (Ohta et al, 1988), and Sykes monkey, Cercopithecus mitis (Emau et al, 1991). However, except in rhesus monkeys, no AIDS-like disease has been reported in other monkey hosts. Diagnostic kits and procedures have since then been developed to facilitate detection, isolation and characterisation of novel isolates.
1.3. Significance of comparative studies of lentiviruses in non-human primates

The primate lentiviruses so far isolated and characterised have been classified into HIV and SIV. These two groups belong to a group of single stranded RNA viruses known as retroviruses. Retroviruses can be divided into two groups; transforming and cytopathic retroviruses. The transforming retroviruses induce changes in cell growth that lead to cancer. These viruses often carry genes called oncogenes that influence cellular growth. Included in this group are bovine leukemia virus, equine infectious leukemia virus, feline leukemia virus, avian type C virus, mammalian type C virus and human and simian T-lymphotropic virus type I and II (HTLV-I, HTLV-II, STLV-I and STLV-II) which cause T-cell leukemia. (Shrewn and Todaro, 1979, Desrosiers et al, 1989 and Emau et al, 1991).

The cytopathic retroviruses are members of the lentivirus family. One branch of this group include visna virus, caprine arthritis virus (CAV) and feline immunodeficiency virus (FIV). The other branch of this group includes HIV-1 and HIV-2 and SIVs (Desrosiers et al, 1989). Comparative molecular, immunological, and pathological studies of HIV and SIV have generated useful information that has been used to speculate on the origins and evolutionary relationship of HIV and SIV. The HIV and SIV studied to date form four genetically distinct subgroups of lentiviruses; SIVsmm/SIVmac/HIV-2, SIVagm, SIVmnd and HIV-1/SIVcpz whereas SIVsyk has not yet been well classified. (Loweinstine, 1988 and Emau et al, .1991). At the molecular level, SIVmac and SIVsmm are 70-90% related to each other overall, 70-80% related to HIV-2 isolates but only 40-50% related to HIV-1, SIVagm and SIVmnd (Chakrabati et al, 1987, Franchini et al, 1987, Fusakawa et al, 1988, Hirsch et al, 1986 and Tsujimbto'e/ al, 1989), indicating that SIVsmm, SIVmac and HIV-2 possibly share a common ancestry. Further studies have shown that SIVsmm does not induce immunodeficiency in the natural host but induces AIDS in rhesus macaques. Seroepidemiologic studies have identified macaque
monkeys as being SIV negative in their natural habitats (Ohta et al, 1988), but some indigenous sooty mangabeys in West Africa where HIV-2 was first identified have been found to be seropositive. This has led to the hypothesis that sooty mangabeys may be the natural hosts of SIVsmm, SIVmac and HIV-2 (Murphy-Corb et al, 1986, Hirsch et al, 1989 and Desrosiers, 1989). Comparative studies have demonstrated that SIVsmm, SIVmac and HIV-2 are genetically equidistant from each other but differ significantly from SIVagm, SIVmnd and HIV-1. The use of naturally occurring lentiviruses in non-human primates in modeling human HIV/AIDS has the advantage of inducing an HIV-like disease similar to AIDS in humans whereas HIV infection in chimpanzees and pigtailed macaques, the two non-human primates susceptible to HIV-1 infection is not associated with disease (Marnix et al, 1997, Agy et al, 1992 and Morrow et al, 1989).

So far, no known SIV in a non-human primate that is closely related to HIV-1 has been identified. However, a lentivirus with 65% amino acid sequence similarity to HIV-1 has been isolated from a chimpanzee in Gabon (Peters et al, 1990 and Emau et al, 1991). Seroepidemiologic studies show that several other non-human primates are infected with HIV or SIV related lentiviruses (Ohta et al, 1988, Huet et al, 1990 and Otsyula et al, 1996). There is need to isolate, identify and characterise these viruses facilitate a better understanding of the origin, evolutionary relationships and biology of SIV and HIV.

1.4. Morphology of HIV and SIV

HIVs and SIVs are composed of a dense cylindrical core surrounded by a lipid envelope (Figure 1). Viral glycoproteins emerge from the core and jut through the lipid envelope. The core is made of structural viral proteins that encase the two identical strands of RNA (genome) and viral enzyme (reverse transcriptase). Surrounding the genome are two ^yers of proteins forming ^"cylindrical core. The proteins composing the core are designated ^ and P24. Outside the core is a lipid bilayer envelope that the virus acquires from an
Fig. 1 Schematic diagram of atypical lentivirus (HIV or SIV) the causative agent of AIDS in human and non-human primates respectively. The virus consists of two identical strands of RNA (the viral genome) with associated reverse transcriptase and core proteins p24 and p17 all enclosed in a phospholipid membrane envelope derived from the host cell. Virally encoded proteins (gpl20 and gp41) are bound to the membrane. (Redrawn from Baltimore et al 1990)
The protein encoded by rev promotes transcription of the structural viral proteins that are necessary for viral assembly in the lytic stage. The vif, vpu and vpx genes encode proteins required for virion maturation. The remaining HIV gene vpr encodes a regulatory protein that is a weak transcriptional activator. The proviral genes are flanked by repetitive sequences called long terminal repeats (LTRs) which contain the essential regulatory sequences controlling viral expression and integration.

HIV and SIV have a limited sequence identity and have potentially important differences in their structure (Franchini et al, 1987, Charkrabarti et al, 1989, Hirsh et al, 1987 and Franchini and Bosch 1989). The genomes of HIV and SIV differ in that HIV has a vpu gene that is absent in SIV, whereas SIV, and not HIV-1 has a vpx gene that has probably arisen as a duplication of vpr gene (Tristem et al, 1990). Such genetic differences could impact cellular tropisms. It has been demonstrated that vpx gene is involved in determining the efficiency of virus replication in primary cells, especially monocytes (Xu et al, 1991 and Park and Sodroski 1995). Other important determinants of cell tropisms are found on envelope glycoproteins of these viruses. For HIV-1, the determinant for cell tropism appears to be the V3 region of the SU glycoprotein gp120 (Chesbro et al, 1991, Chesbro et al, 1992 and Westervelt et al, 1992) consistent with the findings that the V3 sequence may in part determine preference for co-receptor usage (Cocchi et al, 1995). For SIV the cell tropism determinants are primarily outside the V3 region and may involve both the SU and Tm glycoproteins (Sakai et al, 1993, Mori et al, 1992 and Sakuragi et al, 1995).
FIG. 2 Genetic Map of the DNA form of the genome of human immunodeficiency virus (HIV).
(Redrawn from Ahl et al. 1991)
1.6. Natural History of HIV and SIV

The entry of HIV or SIV into target involves two steps; binding of virions to receptors on target cells followed by fusion of the viral envelope with the plasma membrane of the target cell. Once inside the target cells, the viral genome is released from the viral core and with the use of reverse transcriptase, a viral cDNA is synthesised using the viral template. The synthesised DNA then integrates into the host genome, forming a provirus which may remain in the latent state or be activated, transcribed and translated into proteins (Fig 3).

T-helper cells are the major target cells for HIV and SIV infection because of the presence of CD4 molecules on their surface membranes. Macrophages, monocytes, dentritic cells, Langerhan's cells and microglial cells also express low levels of CD4+ and thus support binding of virions. After binding of virions, the viral envelope fuses with the target cell's plasma membrane. The fusion event appears to be mediated by the hydrophobic region near the amino terminus of gp41 (fusogenic domain). Following fusion, the viral nucleocapsid is internalised and the viral RNA is uncoated and transcribed into DNA by the viral reverse transcriptase. Uncoating in this case involves the removal of PI7 and P24 hence releasing single stranded RNA (ssRNA). Reverse transcriptase copies ssRNA into RNA-DNA hybrid Second strand DNA synthesis begins after the action of ribonuclease-H, which partially degrades the original RNA template. The viral DNA duplex is then translocated to the nucleus and randomly integrated into the host chromosomal DNA by viral integrase enzyme. At this point, the integrated HIV or SIV is referred to as a provirus. The provirus can remain in the latent state for months or years. During this time, cell division passes the proviral DNA onto the progeny cells. Activation of the provirus results in the transcription of the proviral 'DNA an subsequent translation into viral structural proteins. The virus leaves infected cells by budding out of the plasma membrane. In some cells, this process goes on at low levels, referred
**Fig 3.** Diagrammatic representation of steps involved in the lentivirus life cycle (HIV or SIV) (Redrawn from Abul et al. 1991)
proivirus results in rapid viral assembly leading to massive membrane damage upon viral budding so that the host cell is lysed in the process.

The infection of macrophages and monocytes does not lead to cell death, and there is a speculation that the virus bearing macrophages may serve as a major reservoir of the virus carrying it to various organs and even the brain blood barrier. Considerable evidence indicate that a retrovirus infected cell must be activated before the provirus can replicate. Stimulation of HIV infected T-cells with various antigens or mitogens for example has been shown to induce viral replication (Gallo et al, 1978, Tsujimoto et al, 1988 and Emau et al, 1991). It has been shown that the proviral genes are flanked by sequences called LTRs (long terminal repeats) which play a role in regulating viral gene expression. Because these LTRs exhibit some sequence homology with the cellular genes involved in T-cell activation, it has been speculated that the signals that activate the T-cells may also activate the provirus (Gallo, 1990).

1.7. Gene Regulation of HIV and SIV

The transcription of the genes of the integrated DNA provirus is regulated by long terminal repeats (LTR) sequences, which flank either side of the viral structural genes. The LTRs contain polyadenylation signal sequences, TATA box promoter sequence, and cis-acting regulators of transcription of the viral genes. These m-acting sequences include tandemly repeated enhancer sequences that are known to bind at least two nuclear regulatory factors NFκ-B and SP1. NFκ-B-like nuclear regulatory proteins, which bind to sequences in regulatory regions of interleukin 1 (IL-1) and interleukin 2 (IL-2) receptor genes, can also bind to similar sequences in HIV and SIV LTRs and activate proviral transcription.
Early HIV-I Gene Expression

LTR

- W - H H

NRE TAR A A

e

Transcription

Full Length Viral RNA

Splicing Transport

Full Length Viral RNA

gag pol

tat

env

Net

Rev

(Structural proteins produced in low amounts)

(Regulatory proteins)

Late HIV-I Gene Expression

LTR

NRE TAR

Transcription

Full Length Viral RNA

Splicing Transport

Virus maturation

Production of virions

Lysis of infected cell

Fig. 4 Diagrammatic representation of the events leading to gene expression in HIV-I which is also similar to the situation in HIV-2 and SIV. This shows the early and late expression of HIV. (Redrawn from Green 1990)
Initiation of HIV and SIV gene transcription in T-cells is probably linked to physiologic activation of the T-cell by antigen or cytokine stimulation. The LTRs of HIV and SIV are influenced by T-cell receptor (TCR) and cytokine stimulation of the host cells. For example, TCR binding lectins, tumour necrosis factor (TNF) and interleukin-6 (IL-6), all stimulate HIV and SIV LTR-directed expression of linked genes and increased viral production. TCR and cytokine stimulation of HIV and SIV gene transcription probably involves the induction of nuclear factors that bind to the NF-κB binding sequences in the viral LTR. This phenomenon may be significant in the pathogenesis of AIDS in two ways. First, physiological activation of a latently infected T-cell may be the way in which latency is ended and virus production begins. Second, the multiple infections that AIDS patients acquire lead to elevated TNF production. This, in turn, may stimulate virus production and the infection of additional cells. Thus viral replication is stimulated by the same mechanism that promotes the growth of the host T-cell. In addition to the enhancer elements, there are also silencer sequences in the viral LTR that are involved in the negative regulation of transcription. The activation state of the T-cell probably determines which nuclear binding proteins are available for binding to these enhancer or silencer sequences, which in turn influences the transcriptional activity of the proviral genome and new viral production. It is interesting that when the regulatory sequences of HIV and SIV LTR are linked to other genes and transfected into various cell types, they work efficiently. Therefore, the tissue specificity of productive viral infection is not a function of these regulators but, rather, reflects the specificity of virus binding and internalisation by CD4+ cell types.

Synthesis of mature, infectious viral particles begins after the various viral genes are expressed as proteins, and full length genomic RNA transcripts are produced (Fig 4). The pol 8 gene product is a precursor protein that is sequentially cleaved to form reverse transcriptase, Protease and integrase entries.
1.8. Diagnosis and treatment

A diagnosis of HIV or SIV is usually made on the basis of ELISA test and confirmed by a western blot. However in later stages of AIDS, the level of antibodies against the virus might be very low beyond detection due to the impairment of T-helper cells. In paediatric AIDS this diagnosis will also not be reliable as it depends on antibodies which might be maternal and hence lead to a wrong diagnosis. At the same time, there is lag phase between the entry of the virus and the expression of appreciable quantities of detectable antibodies. In the case of SIV cross reactivity may not occur if one is dealing with a novel strain of the virus. Vims isolation from body fluids is therefore the most highly specific means to make a definitive diagnosis whereas PCR using the correct primers is the most highly sensitive.

Currently, no cure or vaccine against HIV or SIV infection and AIDS has been found. However, a number of antiviral agents and immunomodulators are in their experimental stages. 3'-thymidine (AZT) has for example been used in delaying the clinical disease in infected individuals, in cases where AIDS has been diagnosed, standard therapy consists of antiviral therapy, prophylaxis using reverse and non-reverse transcriptase inhibitors and treatment of opportunistic infections as aggressively as they occur. Currently, a new dimension of HIV therapy that is based on the principle of gene therapy is emerging (Donald et al, 1996). Here, the goal is not replacement of a congenitally deficient gene as in the case of replacement of the gene for adenosine deaminase to correct severe combined immune deficiency (SCID). In the contrary, this involves the engineering of cells so that they do not support the replication of HIV. Numerous synthetic genes have been developed which are capable of inhibiting replication of HIV including antisense ribozymes, dormant-negative mutant versions of HIV regulatory genes, and intracellular antibodies which can prevent intracellular processing of HIV viral proteins.

The mechanisms by which SIV and HIV causes loss of immune response is the major mystery of AIDS. Several studies suggest that immune abnormalities can be observed in T-cells, B-cells, and macrophages early in infection even before loss of CD4+ cells begins. Besides the effects of viral proteins, induced apoptosis and HIV or SIV superantigens, several other factors have been shown to induce immunodeficiency. The principal immunological feature of AIDS is the elimination of CD4+ lymphocytes. This phenomenon was at first attributed to the induction of cytopathic effect (CPE) on the CD4+ lymphocytes. However this has been disapproved by the following observations: (i). Human herpes virus-6 (HHV-6) kills T-cells but an infection with this virus is not associated with AIDS. (ii). Direct cytopathicity is incompatible with the long latency period of eight to ten years observed in AIDS. (iii). HIV infected chimpanzees and pigtailed macaques do not develop AIDS although HIV isolates passed through the non-human primates are cytopathic to CD4+ lymphocytes from the same animals. (iv). There is a rapid loss of CD4+ lymphocytes in hu-PBL-SCID (human immunodeficiency virus severe combined immunodeficiency mice grafted with human peripheral blood lymphocytes) mice induce noncytopathic isolates, (v). There is no correlation between the cytopathicity of HIV strains in vitro and AIDS pathogenesis in vivo. In SIV or HIV induced disease, there is a strong antiviral immune response that apparently does not protect the host. It has been suggested that anti-HIV antibodies may enhance the HIV infection lymphocytes and monocytes (Robinson et al, 1988). Furthermore, there is increasing evidence that in concert with cytopathic effect, a disturbance of self-nonself recognition plays an important role in the immunopathogenesis of AIDS. The progression to AIDS is usually associated with HIV induced autoimmunity (Susal et al, 1992). Lentiviruses are associated with a disease involving autoreactive immunopathology (Westby et al, 1996). This depends on the genetic background of the host since symptomatic infection occurs in some strains but not
in others for example visna-maedi infection in sheep. Other well characterised lentiviruses are slow at inducing disease and it is possible that HIV and SIV employ similar mechanisms of pathogenesis. Many features of HIV and SIV infection in human and non-human primate populations are consistent with this, particularly the rate of progression to disease with the degree of immune activation and antigen specificity and the level of apoptosis. Conversely, all these features are in HIV infected chimpanzees and pigtailed macaques which can be infected with HIV but do not progress to AIDS (Heeney, 1995). Although recent data have revealed that high viral load in lymph node and spleen shortly after infection (Pantaleo et al, 1993 and Cheynier et al, 1994), followed by high rates of virus replication and mutation during the clinically asymptomatic phase (Ho et al, 1995 and Wei et al, 1995), susceptibility to disease progress cannot yet be explained solely on the basis of infecting HIV or SIV species.

Classical studies have also shown that effects of HIV and SIV infection on the immune system are largely due to the specific tropism of HIV and SIV gpl20 for the CD4 molecule. CD4 is expressed on helper T-cells, and these cells play a central role in the induction of most immune responses. HIV infection can lead to lysis of CD4+ T-cells or functional inactivation of these cells without cytolysis. In either case, the diminished helper T-cell activity results in impairment of all types of immune responses.

The cytolytic effect of HIV on CD4+ T-cells is reflected by the marked reduction of these cells in AIDS patients. The ratio of CD4+ to CD8+ T-cells in the peripheral blood is approximately 2:1 in normal individuals but is often reduced to as low as 0.5 in AIDS patients. CD4+ T-cells are also reduced in lymphoid tissues and at sites of inflammation. This profound depletion of helper T-cells occurs despite the fact that in these patients, probably less than 1 % of CD4+ T-cells are directly infected with HIV and more than 99 % of these infected cells are latently infected without detectable viral transcripts. Furthermore, various immunologic abnormalities are present in HIV-infected individuals even before their CD4+ T-cells are reduced in numbers. Therefore, in order to understand the HIV-induced immunosuppression, it
is crucial to elucidate why the number of CD4+ T-cells destroyed is far greater than the number infected, and why those immune cells that are not killed still do not function normally.

Although the answer is not yet known, it is likely that the depletion of CD4+ T-cells is due to a combination of both direct lysis of infected cells, and indirect mechanisms involving both infected and uninfected cells. At least three mechanisms of direct SIV or HIV-induced cytolysis of infected CD4+ T-cells have been described.

1. The process of virus production leads to lysis of infected T-cells because of increased plasma membrane permeability resulting from env glycoprotein insertion and/or virion budding. The increased permeability results in influx of ions and water, leading to osmotic lysis or an influx of a lethal amount of calcium.

2. A large amount of cytoplasmic viral DNA remains in the cytoplasm and is not incorporated into the cellular genome of the HIV-infected cells. This may be toxic to the infected cells. Similarly, high levels of non-functional viral mRNA transcripts in the cytoplasm may also interfere with the normal cellular functions.

3. Env gene products, such as gp120 may bind newly synthesised or recycled CD4 molecules within the cytoplasm, and this intracellular interaction may be lethal to the cell.

Although HIV can infect macrophages, these cells are relatively resistant to cytolytic effects of the virus. This may reflect the fact that high levels of CD4 expression is required not only for viral entry into the cells but also for virus-induced cytotoxicity. The lower expression of CD4 m the macrophages in comparison with the helper T-cells may protect the former from virus-induced killing. Many macrophages, in fact, may become infected by a CD4 independent route, such as phagocytosis of other infected cells or Fc receptor-mediated endocytosis of antibody-coated HIV. Since macrophages can become infected but generally not killed by the virus, they are probably a major reservoir for the virus during the long clinical course of HIV-related disease. In fact, the quantity of macrophage-associated SIV or HIV far exceeds T-cell associated virus in most tissues from AIDS patients, including brain, lung and lymph node.
At least three mechanisms are hypothesised to account for indirect HIV-induced depletion of uninfected CD4+ T-cells:

1. HIV may block maturation of CD4+ T cells by infecting cells that produce cytokines required for T cell maturation.

9. HIV infected cells, expressing cell surface gp120, can bind to and fuse with uninfected T cells. This can lead to formation of fused cells, multinucleated giant cells or syncitia \textit{in vitro}. The life span of these giant cells is short. It is possible that this phenomenon may contribute to the CD4+ T cell depletion \textit{in vivo}, although there is no direct evidence for this.

3. HIV infection of some CD4+ cells may lead to autoimmune destruction of both infected and uninfected CD4+ T cells. Soluble gp120 released from infected cells can bind to CD4 on the surface of infected cells. Many patients have circulating anti-gp120 antibodies that could mediate destruction of these gp120-coated T cells, by antibody-dependent cell mediated cytotoxicity (ADCC) or by complement fixation. It is also possible that antibodies specific for viral proteins may cross-react with normal T-cell surface proteins, leading to their destruction. For example, the viral protein gp41 molecule has a region of homology with the β1 domain of class II MHC molecules. Since activated human T-cells express class II MHC, antibodies against gp41 may cross react with and mediate killing of these T cells. Similarly, gp120 and IL-2 share regions of homology and anti-gp120 antibodies in AIDS patients may bind to IL-2 and interfere with IL-2-dependent growth of T-cells. Alternatively, gp120 binding to uninfected CD4+ T-cells can render them susceptible to lysis by gp120-specific CTLs. This has been demonstrated \textit{in vitro} and occurs when uninfected CD4+ T-cells internalize and process the gp120 protein and present a peptide derivative in association with class II MHC molecules. Class II-restricted, gp120-specific CTLs have been isolated and cloned from Peripheral blood of HIV-infected patients, and these CTLs can lyse other CD4+ T-cells that have processed and presented the gp120 molecule.
In addition to the depletion of CD4+ T cells, which becomes most significant late in the course of HIV infection, the virus also causes functional impairment of CD4+ T cells in ways not directly related to cytotoxic effects of T cell depletion. Uninfected T cells in HIV infected patients have a decreased expression of IL-2 in response to soluble antigens both in vivo and in vitro. Humoral response to certain viruses is also impaired, probably as a consequence of failure of CD4+ helper T cells to secrete adequate amounts of appropriate cytokines required for functional differentiation of B-cells and CTLs.

As with cytolytic effects, these functional abnormalities of CD4+ T cells are most likely due to the binding of HIV or free gp120 to CD4 molecules on helper T cells. Such binding could have numerous consequences. CD4 that has bound gp120 may not be available to interact with class II molecules on the antigen presenting cells (APCs), and thus T cell responses to soluble antigens would be inhibited. Alternatively, gp120 binding to CD4 may down-regulate surface expression of a variety of molecules required for T cell activation, including CD3 and CD4 itself. This down-regulation may be due to gp120 induced modulation of CD4, and perhaps associated with CD3, as well as inhibition of transcription of CD4 and CD3 genes. In addition, the HIV tat protein can also block antigen induced responses of T cells, presumably by interfering with intracellular T cell activation pathways.

The immunosuppressive effects of HIV may also be partly related to the effects the virus has on cells other than CD4+ cells. Abnormalities of B-lymphocyte activation are frequently observed in HIV-infected individuals. Paradoxically, these patients typically have elevated serum immunoglobulin levels resulting from polyclonal activation of B cells. This may be a result of a polyclonal activating effect of HIV or gp120 itself. Polyclonal activation of B cells may also be caused by EPV infection, which can be largely controlled in HIV-infected individuals as a result of poor T-cell immune responses. Despite this generalised B hyperactivity, humoral immune responses to newly introduced antigens are greatly paired. Diminished T cell help, as well as refractoriness of the B cells themselves, may both
play a role in this deficit.

Macrophages are frequently infected with the virus, and there are reports that various macrophage functions are impaired in HIV infected patients. These impairments include decreased chemotaxis, IL-1 production, and oxygen metabolite-dependent killing of microbes. In addition, antigen-presenting capabilities of monocytes and macrophages are reduced in AIDS patients. One possible mechanism for this effect is the down-regulation of class II MHC expression, which is reported to occur in HIV infected macrophages.

NK cells from AIDS patients are also reported to be functionally impaired by an unknown mechanism, and this may be a reason for inadequate anti-retroviral immunity in these individuals.

It is apparent from the discussion above that many abnormalities in the cells of the immune system have been shown to result from HIV infection. It is, however not clear which defects contribute most significantly to the immunodeficiency. Continued comparative studies on retrovirus in different host systems is therefore required to establish this.

### 1.10. Use of Animal Models

Although there have been great advances in the understanding of events occurring during acute and chronic HIV infection recently, the precise mechanisms of disease development remain unknown. SIV infection of primates results in rapid development of AIDS in some primates and in long term inapparent infection in others (Kurth et al, 1997). By comparing such models it may be possible to identify possible mechanisms of disease in uction. About one third of African green monkeys and sooty mangabeys in the wild are infected with the HIV-related viruses SIVagm and SIVsmm respectively (Otsyula et al, 1996).

Despite infection lasting most of the animal's adult lives, these natural hosts do not develop disease. However th uses'themselves have the potential to cause AIDS in susceptible hosts with SIVsmm appearing to be the ancestor of SIV and HIV-2 (Murphy-Corb et al, 1986,
peeters et al, 1989 and Kurth et al, 1997). It appears that the host plays a major role in determining the outcome of immunodeficiency virus infection. Efforts to elucidate the reasons for the natural host's apparent resistance to disease have revealed a number of interesting similarities to primates where SIV infection becomes pathogenic. There is so far no evidence that a particularly rigorous immune response controls the virus and indeed, the plasma virus loads appear to be as high as seen during HIV infection in humans (Kurth et al, 1997). In vivo, virus variability is similar to HIV as is the cellular host range. The fact that trapping of viral antigen in the lymph nodes with resulting destruction of lymph node architecture does not occur in SIVagm infected African green monkeys suggests that immunologic mechanisms which may result in the breakdown of the immune system during AIDS are not active in the natural hosts (Kurth et al, 1997). The understanding of what appears to be selective pathogenicity of SIV will be enhanced by discovering more SIVs in as many primates as possible and doing comparative studies.

The most important feature of SIV is its ability to model AIDS in animals. Its usefulness stems from, (i). The extensive similarity of HIV to SIV in genetic make up and biological properties and (ii). the ability of SIV to induce AIDS in rhesus monkeys in a time frame suitable for laboratory investigation (Desrosiers et al, 1990). Infection of macaques with some strains of SIV most notably those obtained from macaques and sooty mangabeys can induce a fatal disease most remarkably similar to AIDS in humans. Some early passage of SIV has been shown to induce AIDS and death within 3-5 months (Daniel et al, 1985, and Benevenste, 1988). One isolate of SIV, PBJ, kills macaques in 1-2 weeks (Fultz et al, 1989). In cases where death is not rapid, macaques remain persistently infected and eventually succumb 1-3 years or even later following initial infection (Daniel et al, 1987).

The similar genomic organisation, the extensive sequence homology and the similarity biological properties both An vitro and in vivo suggest a certain commonality between SIVs and HIVs. Most fundamental observations made in SIV systems are likely to be extrapolable to
jjIV in humans and vice versa. One of the most common clinical features in macaques infected with SIV is diarrhoea which is often associated with pathogenic bacteria or protozoa (Baskin et al, 1988). The diarrhoea is often associated with a wasting disease similar to the pattern seen in humans infected with HIV. In these cases a nonspecific enteropathy consisting of blunting of small intestinal villi, shortening of the crypts of Lieberkuhn, a predominantly mononuclear inflammatory infiltrate within the lamina propria and the attenuation or immaturity of the epithelium has been seen. SIV RNA can be localised to mononuclear cells within the lamina propria in these cases. Similar changes have been described in human patients with HIV (Kotler et al, 1984).

SIV infected monkeys also develop immunological abnormalities including decrease in CD4 cell numbers (Letivin et al, 1985 and Benevenste et al, 1988), decreased responsiveness of peripheral blood lymphocyte to mitogen stimulation (Letivin et al, 1985 and Benevenste et al, 1988) and thymic atrophy (Letivin et al, 1987). Immunologic abnormalities result in a range of opportunistic infections, many of which are seen in HIV-1 infected patients. These include pneumocystis carinii pneumonia, disseminated cytomegalovirus (cmv) infection oral and oesophageal candidiasis, intestinal cryptosporidiosis, mycobacterium avium/intracellular infection, giadiasis, adenoviral pancreatitis, and multiple bacterial infections of the intestinal tract and the skin (King et al, 1989, Baskin et al, 1988 and Baskin et al, 1987). In addition, the lymphadenopathy seen in SIV infected macaques has been well described and is similar in most respects to that observed in HIV infected humans (Ringler et al, 1989 and Wyand et al, 1989).

One of the key features of SIV and HIV induced disease is the nature of the persistent infection (Daniel et al, 1987). SIV and HIV like other lentiviruses, have a remarkable ability to persist eventually to induce a chronic, debilitating disease in spite of an apparently 8°st immune response to the virus. Due to such parallel similarities, SIV systems in non-human nri P mates have a potential in the elucidation of viral and host factors that will be
important in the management of HIV/AIDS.

1.11. Rationale

Since the onset of HIV/AIDS, scientists and health workers have been puzzled by the nature of the causative agent and the resultant disease. The response of both scientists and health workers to this new disease has been the development of accurate and reliable diagnostic methods. However, vaccines and drugs need to be developed and hence the need for animal models to facilitate research geared towards the understanding HIV pathogenesis and testing of vaccines and drugs before use in humans. This study describes the elucidation of features comparable to the human AIDS that results from HIV-like infection in De Brazza's monkeys *Cercopithecus neglectus* naturally infected with an HIV-like virus called simian immunodeficiency virus (SIVo,b). This model can be applied to key and unanswered aspects of human disease. The model is also critical in understanding pathogenesis and immune responses to HIV infection. Further, this model will useful in testing vaccine candidates and chemotherapeutic agents. Lastly, such a model will shed light on the evolutionary relationships of this new retrovirus and host-virus relationships in the lentivirus family.

1.12. The overall Objective and Specific Aims

112.1. The Overall Objective

The aim of this project is to develop an SIV_{DEB} animal model for HIV/AIDS using De Brazza monkeys (*Cercopithecus neglectus*). This will be achieved by isolating and Arising simian immunodeficiency virus from wild and captive De Brazza's monkeys *C. neglectus* in Kenya and determining their relationship to human and simian "deficiency viruses*. 

T_0 clearly understand the definition, origin, natural history and evolutionary
relationship of HIV and SIV groups, it will be necessary to isolate more lentiviruses from naturally infected non-human primates and to study genetic relationships and their biology in their respective hosts. This thesis describes the isolation an HIV-like virus from De Brazza's monkeys *Cercopithecus neglectus*, its biology, biochemical characterisation, and comparison with other viruses of the HIV and SIV groups.

### 1.12.2. Specific Aims

1. To determine SIV\textsubscript{Deb} and quantitate antibodies in De Brazza's monkeys.

2. To isolate SIV\textsubscript{Deb} from De Brazza's monkeys and determine the appropriate cell lines for isolation of SIV\textsubscript{Deb}

3. To characterise SIV\textsubscript{Deb} by reverse transcriptase, EM, Western blotting and PCR

4. To study the pathogenic effect of SIV\textsubscript{Deb} on the De Brazza's monkeys haematopoietic system and lymph node architecture.
relationship of HIV and SIV groups, it will be necessary to isolate more lentiviruses from naturally infected non-human primates and to study genetic relationships and their biology in their respective hosts. This thesis describes the isolation an HIV-like virus from De Brazza's monkeys *Cercopithecus neglectus*, its biology, biochemical characterisation, and comparison with other viruses of the HIV and SIV groups.

### 1.12.2. Specific Aims

1. To determine $SIV_{De,b}$ and quantitate antibodies in De Brazza's monkeys.

2. To isolate $SIV_{De,b}$ from De Brazza's monkeys and determine the appropriate cell lines for isolation of $SIV_{De,b}$

3. To characterise $SIV_{De,b}$ by reverse transcriptase, EM, Western blotting and PCR

4. To study the pathogenic effect of $SIV_{De,b}$ on the De Brazza's monkeys haematopoietic system and lymph node architecture.
CHAPTER TWO

MATERIALS AND METHODS

2.1 Enzyme-Linked Immunosorbent Assay (ELISA)

Heparinized blood (15ml) was obtained from 8 De Brazza's monkeys (*Cercopithecus neglectus*) either wild caught or colony born and housed in a semi-breeding colony at the Institute of Primate Research (IPR). Plasma was obtained by centrifugation at 2000 rpm for 25 minutes. The plasma obtained was then kept in plasma vials at 4°C while PBMCs were processed by separating on Lymphocyte Separation Media (LSM) [Organon Teknika Corporation, Durham, NC] and frozen in liquid nitrogen until they were required (Otsyula *et al.*, 1996). An optimal antigen-coating concentration was determined by checker-board titration at different antigen and anti-sera dilutions following overnight incubation in microtitre plates (Falcon 3911, Becton Dickinson USA) at 4°C with 0.4^g per well of SIV antigen in CAPS (3-[cyclohexylamino]-l-propanesulfonic acid (Sigma chemical co. USA). The antigen used was a 21- amino acid synthetic peptide derived from a conserved immunodominant region of SIVmac transmembrane glycoprotein (H-NAWUGCAFQVRCHTVPWPMAS-OH, Research Genetics, Huntsville, AL). The wells were washed three times with dilution buffer pH 7.4 containing 0.05% Tween 20. The plates were washed again and incubated at 37°C for 3 hours with 1:100 sera dilution. The plates were taken out and washed three times with wash buffer and incubated with 100^1 of peroxidase-conjugated goat anti-monkey immunoglobulin G (IgG) (Sigma Chemical Co. USA) diluted 1:2000 in dilution buffer without Tween 20. The wells were then washed with buffer and 100^1 substrate added (0.4mg/ml ortho-phenylenediamine [OPD] Sigma, USA) freshly prepared 0.1M dilution buffer pH 7.4 With 0.13% H2O2 as a substrate) (Appendices II). The colour change was allowed to proceed for 30 s in the dark after which the reaction in all the wells was stopped by adding 50^1 of 4M "H2SO4 and the rmt" 1 a 

optical density (OD) values read at 450 nm with multiscan ELISA reader
To quantitate the SIV antibodies in the positive animals, positive plasma was serially diluted two fold up to 1:25600 and ELISAs performed as described above. SIV control and test plasma were assayed in duplicate and samples were considered positive if the mean OD for the same wells was greater than the mean OD for the wells of SIV negative control plasma plus 3SD of the SIV negative control plasma. The value of the last serial dilution for which the plasma was positive was taken as the antibody titre for the animal in question.

2.2. Virus isolation and quantitation

Virus isolation was attempted from PBMCs and plasma of all De-Brazza's monkeys whose sera tested positive on antibody ELISA. Briefly, 15 ml heparinized blood was obtained from each of these De-Brazza's, plasma was separated by spinning the blood at 2,000 rpm for 25 minutes. The cellular component obtained was mixed well with RPMI-1640 media equal to the volume of removed plasma, and layered onto 5ml of lymphocyte separation media (LSM) [Organon Teknika Corporation, Duham, NC]. This was then centrifuged at 2,000 rpm for 25 minutes and then the PBMCs band at the interface of LSM and RPMI-1640 media was pipetted out. The PBMCs obtained were suspended in 10 ml RPMI-1640 media and an aliquot taken for cell counting. The PBMCs were pelleted by centrifugation at 1,000 rpm for 10 minutes. The BMCs were stored in aliquots of 1 x 10⁷ cells. T-supresser lymphocytes were removed by tamuno-magnetic depletion. The isolated lymphocytes were co-cultured with SupT1, Molt-4 P, HUT, PM-1 and CEM X174 cell lines. The cultures were examined daily for cytopathic Cl (CPE) and supernatant aliquots taken at 4 days intervals for antigen capture ELISA. rVira quantitation assays PBMCs obtained were serially diluted at the following concentrations: 10⁷ in 6 in* wU , 10 , 10² and 10¹, while the plasma was diluted at 1:2, 1:10, •80 and 1:160. The 10⁷ and 10⁶ PBMCs were co-cultured with 10⁶ of PM-1 cells in
T-75 and T-25 tissue culture flasks respectively while lOMO¹ PBMC dilutions were cultured with 10⁶ of PM-1 cells in replicas of four in 24-well microtitre plates. For plasma, the 1:2 plasma dilution was co-cultured with 10⁶ PM-1 cells in T-25 culture flasks while 1:10 - 1:160 plasma dilutions were cultured with the same number of PM-1 cells in replicas of four in 24-well microtitre plates. All cultures were maintained in RPMI-1640 media supplemented with L-glutamine, penistrap and fetal-calf serum. The cultures were incubated at 5% CO₂, 37°C and monitored for CPE. The cultures' supernatants were sampled every week and tested by antigen capture ELISA. Data based on CPE and antigen capture ELISA was used to calculate TCID₅₀ using the Reed and Muench method.

2.3. Reverse transcriptase assay

Cultures of the isolated virus were expanded and retroviral particles concentrated by centrifugation at 35,000 rpm for 3 hours on a 20% sucrose gradient cushion. The concentrated retroviral particles were assayed for specific reverse transcriptase (RT) activity using reverse transcriptase assay (non-radioactive) kit (Boehringer Mannheim, Germany). Briefly, the pellets were resuspended in 40|il lysis buffer (50 mM Tris buffer, containing 80 mM KCl, 2.5 mM Dithiothreitol (DTT), 0.75 mM EDTA and 0.5 % Triton X-100) and the suspension transferred to a fresh reaction tube. This was incubated at room temperature for 30 minutes to completely solubilize the viral particles.

HIV-1 reverse transcriptase working dilutions used to prepare a calibration curve were prepared as per manufacturer's instructions. Briefly, 10 fI of HIV-1 reverse transcriptase stock solution (2 ng/|il, provided with the kit) was serially diluted in 1:2 dilution steps as described in Table 1.
Table 1: Summary of contents of RT assay tubes for standard curve

<table>
<thead>
<tr>
<th>HIV-1 RT</th>
<th>Lysis buffer</th>
<th>HIV-1 RT conc (ng/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>150 nl</td>
</tr>
<tr>
<td>1</td>
<td>10 fil (stock)</td>
<td>390 jil</td>
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<tr>
<td>2</td>
<td>150 fil of step 1</td>
<td>150 jil</td>
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<tr>
<td>3</td>
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<td>6</td>
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<td>150 jil</td>
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</tbody>
</table>

40 μl of each working dilution was transferred into a sterile reaction tube. The sample containing no HIV-1 RT (step 0) served as a negative control. The dilutions were prepared in duplicates. 20 μl of the reaction mixture (50 mM Tris-HCl, 290 mM/litre KC1, 30 mM MgCl2, 10 mM DTT, 10 μM dUTP, and 750 A260nm/ml template/primer hybrid, poly (A)01igo(dT)15) was then added to each reaction tube containing the viral lysates or HIV-1 RT standards. They were then incubated for 1 hour at 37°C.

After the reverse transcriptase reaction, the samples (60 fil) and the HIV-1 RT working dilutions for establishing the calibration curve (60 fil) were transferred into the wells of the micro-titre plate (MTP) modules pre-coated with streptoavidin and post-coated with blocking reagent. The modules were then covered with tin foil and incubated for 1 hour at 37°C. The e then completely removed from the wells and the wells rinsed 5 times for 30 seconds each time with 250 fil of washing buffer per well. 200 jil of anti-digoxigenin-working dilution (200 mU/ml) was added to each well. The MTP modules were
then covered again with cover foil and incubated at 37°C for 1 hour.

The solutions were then removed completely and the wells rinsed 5 times with 250 of washing buffer per well for 30 seconds each time. The washing buffer was then carefully removed and 200 μl of ABTS® substrate solution was pipetted into each well. The MTP modules were then incubated at room temperature until the colour development (green colour) was sufficient for photometric detection (10 to 30 minutes). The absorbance was measured at 405 nm with a reference wavelength of 490 nm using a micro-plate ELISA reader (Dynatech, Germany). A plot of the absorbance of the standard working dilutions as a function of the HIV-1 RT concentrations was constructed to obtain the calibration curve. The RT activity in the unknowns was estimated by interpolation from the curve and confirmed by linear regression analysis. SIVagm infected and uninfected Molt-4 Clg cell preparation were used as positive and negative controls, respectively.

2.4. Electron Microscopy

Concentrated viral cultures were washed in PBS buffer, pH 7.4 and the cells fixed using aldehyde fixative (2% glutaraldehyde and 1% paraformaldehyde in 0.1 M PBS, pH 7.4). The cells were dehydrated using acetone with increasing concentrations and post-fixed with 1% Osmium tetraoxide for 1 hour before being embedded in epoxy resin. Ultra-thin sections were cut using a Philips 300 ultra-microtome. The sections were stained with 0.05M lead citrate and saturated uranyl acetate prior to examination. Isolated retroviral particles were first pre-fixed in 2% buffered glutaraldehyde while pelleting at 35,000rpm at 4°C (SW41 rotor, Beckman instruments, USA). After rinsing, the pellets were post-fixed in 1% Osmium tetraoxide and hydrated in series of graded ethanol containing uranyl acetate, soaked in propylene oxide embedded in epoxy. Ultra-thin sections were then cut using a Philips 300 ultra-embedded on copper grids, stained with uranyl acetate/lead citrate and examined.

° characterise the type of cell free viral particles from infected cell cultures electron
microscopy (EM) negative staining methods adopted by Lyden et al was used (1994). In brief, this procedure involved staining cells with 1% Phosphotungstic acid (PTA) and 0.5% uranyl acetate (UA). Sample fractions (50) were placed on carbon/formvar-coated 200 mesh grids. They were incubated for 30 seconds at 20°C and the liquid portion withdrawn from the surface using Whatman #5 filter paper (Clifton, NJ). The grids were allowed to air-dry for 30 seconds, then 50 \% stain was applied (1 % PTA for 45-60 seconds, 0.5 % UA for 30 seconds). The excess stain was removed by filter paper-blotting and the grids air-dried for 20 minutes before examination.

2.5. Polymerase Chain Reaction

DNA was extracted from cell pellets using the phenol/chloroform/proteinase-K protocol as described by Hirt, 1967. Briefly, 5 ml of TNE buffer, 500 of 10 % SDS and 50 ul of RNase were added to the pellet and incubated for 1 hour at 30°C. Then 250 \% of Proteinase-K was added and incubated for 5 hours at 55°C. This was followed by addition of 5ml phenol and spinning for 10 minutes at 3,000 rpm. Five millilitres of phenol/chloroform at a ratio of 1:1 was then added and centrifuged for 10 minutes, and then the resultant supernatant was transferred to a new Falcon tube. The procedure was repeated twice. Five millilitres of chloroform was then added to the supernatant, centrifuged for 10 minutes, and the supernatant collected in a new tube. Three volumes (15 ml) of 100 % ethanol and 500 ml of sodium acetate was then added and stored at -20°C overnight. The following morning, the tubes were centrifuged for 30 minutes at 3000 rpm. The ethanol was then removed without disturbing the DNA pellet. The pellet was washed in 70 % ethanol by centrifuging for 15 minutes for each wash. The 70 \% ethanol was removed and the remaining ethanol blotted off by inverting the tube onto blotting paper. The DNA pellet was then resuspended with 500 ml of TE buffer. A nested \begin{array}{l}
\text{polymerase chain reaction (PCR)} \\
\text{was then performed according to the method developed by Katrien et al, (1994).}
\end{array}
The primers used were;

Outer 1 • HPOL 4235  d(CCCTACAATCCCCAAAGTCAAGG)
2. HPOL 4538  d(TACTGCCCCTTCACCTTTCCA)
Inner 3. HPOL 4327  d(TAAGACAGCAGTACAAATTGGCAT)
4. HPOL 4481  d(GCTGTCCCTGTAATAACCG)

The reaction mix was loaded into the DNA Thermocycler and nested PCR of 32 cycles run as follows:

(i) 94°C for 30 seconds (Denaturation)
(ii) 60°C for 30 seconds (Annealing)
(iii) 72°C for 60 seconds (Elongation)

After the first round of 32 cycles, 5 μL first round of the PCR product was taken and a second round of 32 cycles were run using

HIV-1 primer 3 3. HPOL 4327
HIV-1 primer 4 4. HPOL 4481

The PCR Products at the end of second round were then resolved on 3% non-reducing Nu-sieve agarose gel with 1% Ethidium bromide for band visualisation against UV background.
<table>
<thead>
<tr>
<th>Component</th>
<th>Addition order</th>
<th>Volume (μL)</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;piitiUed sterile water</td>
<td>1</td>
<td>57.5</td>
<td></td>
</tr>
<tr>
<td>10X PCR Buffer with MgCl</td>
<td>2</td>
<td>10</td>
<td>IX</td>
</tr>
<tr>
<td>dATP</td>
<td>2</td>
<td></td>
<td>200 nM</td>
</tr>
<tr>
<td>dCTP</td>
<td>3</td>
<td>10</td>
<td>200 μM</td>
</tr>
<tr>
<td>dGTP</td>
<td>2</td>
<td></td>
<td>200 μM</td>
</tr>
<tr>
<td>dTTP</td>
<td>2</td>
<td></td>
<td>200 μM</td>
</tr>
<tr>
<td>HIV-1 Primer 1 HPOL 4235</td>
<td>4</td>
<td>2</td>
<td>0.5 nM</td>
</tr>
<tr>
<td>HIV-1 Primer 2 HPOL 4538</td>
<td>5</td>
<td></td>
<td>0.5 μM</td>
</tr>
<tr>
<td>AmpliTaq DNA</td>
<td>6</td>
<td>0.5</td>
<td>2.5 U/100 μL</td>
</tr>
<tr>
<td>+ve Control DNA or 8 copies/100 μL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ve Control DNA or DNA from Uninfected Molt-4-clone-8 cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental DNA sample</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>TOTAL MIX</td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
2.6. Western Blotting

positive sera was confirmed by western blot technique using New LAV Blot II (Sanofi Diagnostics Pasteur, France) which is specific for HIV-2 viral proteins. Nitro-cellulose strips containing HIV-2 retroviral antigens (provided with the kit) were incubated for two hours with the serum samples diluted 1:1000 washing buffer at room temperature. The strips were washed 3 times, incubated with 2 ml of conjugate (Alkaline phosphatase-labelled anti-human IgG goat antibodies) for 1 hour. Antigenic reactivities were detected using 5-Bromo-4-Chrolo-3-Indolyl phosphate(BCIP) substrate and NitroBlue Tetrazolium as developing buffer, then bands were evaluated. The samples which showed at least one band, were taken to be positive.

2.7. Effect on haematopoietic system and lymph-node pathology

The effect of SlVoeb on the haematopoietic system was elucidated by performing CBCs, FACS analysis and pathological examination of lymph nodes.

The lymph nodes which had been stored at -70°C in TissueTek OCT compound (Miles Diagnostics, Elkhart, USA) were thawed to about -10°C in running water, fixed onto specimen chucks using OCT. The specimen chucks were then placed in the cryocabinet of the cryostat (2800 Frigocut N, Cambridge Instruments, UK) for 1 hour so that its temperature decreased to that of the cryostat (-20°C). The chucks were then fixed onto the microtome and 10 fim thick sections were then cut and removed from the cryostat blade by flash condensation onto previously prepared microscope slides. The sections were air-dried for 1 hour, fixed in cold acetone (at 4°C) for 10 minutes. The sections were wrapped in aluminium foil and parafilm nd stored at -20°C until required.

The sections were stained using the Zymed Streptoavidin-Biotin peroxidase .method fHict

\text{
\text{\textit{\textsuperscript{1}old stain-SP Kit, Zymed Laboratories, USA). The primary polyclonal antibodies were \textsuperscript{1}uted 1:500 in 5% fat-free'skimmed milk (Marvel, Cadbury's, UK) in phosphate-buffered S\textsubscript{d} (PBS), pH 7.4. The commercial antibodies were diluted as per instructions provided.}}}

The sections were then incubated with 100 \( \mu l \) of the serum blocking solution (10 % non-immune goat serum, provided with the kit) per slide for 10 minutes. The solution was then blotted off and 100 \( \mu l \) of the diluted primary antibodies were added to the corresponding marked slides (enough to cover the section). They were then incubated in a moist box at room temperature for 30 minutes. The sections were then washed in PBS, (pH 7.4) three times. Subsequently, 100 \( \mu l \) of the relevant biotinylated secondary antibody (goat anti-mouse, anti-monkey, anti-human or anti-rabbit IgG) was added to each section (enough to cover the section). The sections were then incubated at room temperature for 10 minutes in moist box, and later rinsed three times with PBS. One hundred microlitres of the enzyme conjugate (streptavidin peroxidase) was added to each section and incubated for 10 minutes prior to rinsing three times with PBS. One hundred microlitres of the substrate-chromogen mixture (Amino-F.thyl Carbazole (AEC) solution with 0.6 % hydrogen peroxide) was then added to each section and incubated for 10 minutes and subsequently rinsed with distilled water. The sections were counter-stained with 100 \( \mu l \) haematoxylin for 3 minutes, then rinsed with tap water. Two drops of aqueous GVA mountant (Glycerol-polyVinyl Alcohol) were then added to each slide and mounted with a cover slip. The slides were then left to dry overnight, before examination under a light microscope.
CHAPTER THREE

RESULTS

3.1 Enzyme linked immunosorbent assay (ELISA)

Five out of eight animals used in this study had anti-SIV IgG antibodies during the study period as represented by the solid bars whereas uninfected animals did not elicit any anti-SIV antibody responses (Fig 5).

Figure 5: Antibody titres for SIV<sub>Deb</sub> of Animals in a Semi-breeding colony at IPR
3.2 Virus isolation and quantitation

SIVoeb was isolated from three animals PBMCs, that is Deb44, Deb 45 and Deb50 whereas it was only isolated from the plasma of Deb45 and Deb50. The isolation was successful on Molt-4-clone-8, SupT1 and PM-1 cell lines but possible on HUT and CEMx174 cell lines. The isolates were confirmed by positive antigen capture ELISA and the induction of HIV-like CPE on the cell lines used (Plate 1 and 2).

Quantitation of virus from virus positive animals revealed a high viral load in both cell associated virus in PBMCs and cell free virus in plasma (Table 3).

Plate 1: A photomicrograph of virus infected cells showing syncytia formation by molt-4 clone-8 cells due to the induction of fusogenic proteins by SIV_{Deb}. Cells showing syncytia are shown by the arrow.
**Plate 2:** A photomicrograph of normal molt-4-clone-8 cells in culture that are not infected by SIVΔeb. These cells have a normal shape as compared with the balloon like infected cells infected with virus as shown in plate 1 above.
Table 3: TCID50 for PBMC and plasma associated virus for SIV positive animals

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>TCID50 per 10^6 PBMC</th>
<th>TCID50 per ml of plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deb 44</td>
<td>3.19 x 10^-6</td>
<td>3.2 x 10^3</td>
</tr>
<tr>
<td>Deb 45</td>
<td>2.12 x 10^5</td>
<td>5.9 x 10^-4</td>
</tr>
<tr>
<td>Deb 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deb 54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deb 56</td>
<td>1.8 x10^-4</td>
<td>3.2 x10^3</td>
</tr>
</tbody>
</table>

**Key:** - means virus isolation was not successful

**These results** show a TCID50 for both plasma and cell associated virus with has ranges that comparable to the titres reported for humans infected with HIV.
The TCID₅₀ P⁵⁷ PBMCs or per ml of plasma for each positive animal was calculated using CPE data from 50% end-point viral titration and isolation cultures using the Reed and Muench method as illustrated below with the example of Deb 44;

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Virus Tissue culture CPE data</th>
<th>Accumulated values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPE</td>
<td>No CPE</td>
</tr>
<tr>
<td></td>
<td>CPE ratio</td>
<td>No CPE ratio</td>
</tr>
<tr>
<td>10⁻³</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

The proportionate distance (PD) from the dilution just above the 50% endpoint to the endpoint itself was calculated as follows;

Proportionate distance (PD) = (%CPE - 50%)/ (%CPEₐ - CPEₜₜ) x log dilution factor.

\[
PD = \frac{(80 - 50)}{(80 - 20)} \times \log 10
\]

PD = 0.5

Where

\[\text{%CPE}_a = \text{%CPE at dilution above 50%} \quad > \quad \text{%CPE}_b = \text{%CPE at dilution below 50%}\]

\[
\text{TCID}_{50} \text{ titre} = \log \text{dilution factor above 50%} - \text{PD} = (-5) - 0.5 = -5.5
\]
Therefore, \(\text{TCID}_{50} = \frac{\ln 5}{n} = \frac{1}{n} \ln 10 \times 10^{-5}\) PBMC.

3.3 Reverse transcriptase activity

Significant reverse transcriptase activity was detected in the SLVoeb supernatant tested as shown by Fig. 6 and Table 3. The positive control used was SIVagm infected Molt-4 cell. The negative control was uninfected Molt-4 cytosolic cell preparation. The activity of this enzyme is a testament that the virus in question is a retrovirus. The reverse transcriptase enzyme is a key enzyme in the life cycle of lentiviruses as it catalyses the first and committed step that determines the productive infection by lentiviruses (Fig 3). This enzyme is involved in the conversion of viral RNA into cDNA which is then integrated into the host genome hence leading to a productive infection.

3.4 Electron microscopy

Electron microscopy studies of test samples revealed budding viral particles and inclusions with HIV-like budding characteristics and shape (Plate 3 and 4) unlike the control samples (Plate 5). Negative staining revealed viral particles with spikes comparable to HIV or known SIV. The viral particles observed are type D particles which is consistent with characteristic shape of lentiviral particles.

3.5. Polymerase chain reaction (PCR)

The \(\text{Pol}\) PCR of both PBMCs and co-cultures of PBMCs and plasma from seropositive animals gave a product of about 200 bp (Plate 6 and 7). However, \(\text{gag}\) and \(\text{env}\) PCR did not produce any product. The PCR product was obtained from PBMCs of all the animals that were \(\text{SIV}_{\text{Deb}}\) positive ELISA despite the fact that successful virus isolation was only achieved in three animals. This could be due to the fact that proviral sequences were not being expressed (the virus not replicating) like in the case of the latent stage in human HIV infection. These results show that SIV\(_{\text{Deb}}\) is a lentivirus and shares a sequence homology in the
pol gene of HIV-1 given that HIV-1 pal primers used were highly sensitive with an annealing temperature of 55°C.

3.6. Western blotting

Western blots of isolated viral particles revealed positive cross reactions with sera from five animals (Plate 8) that were also seropositive by antibody ELISA. The cross reactivity of the sera tested with HIV-2 proteins confirms SIVDeb to be an HIV related lentivirus. Given that the antibody ELISA used in this case is SIVmac peptide specific whereas the western blot was founded on HIV-2 specific proteins, the results obtained imply that SIVDeb has sequence homology to both known SIVs and HIV-1 and 2.
Fig 6* standard curve obtained for non-radioactive reverse transcriptase activity

Standard Curve for RTase assay

0.3 ,

0.2

0.1

0.2  0.4  0.6  0.8
HIV-1 RT conc (1:2 dilution)(ng/well)
**Table 4: Results of RT assay**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Absorbance (405 nm)</th>
<th>RT Concentration (nm/well)</th>
<th>RT Concentration (mU/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SIVoeb sample</strong></td>
<td>0.387</td>
<td>0.222</td>
<td>1.11</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive control (SIVagm)</td>
<td>1.736</td>
<td>1.782</td>
<td>5.86</td>
</tr>
<tr>
<td>Negative control</td>
<td><strong>0.262</strong></td>
<td>0.0</td>
<td><strong>0.00</strong></td>
</tr>
</tbody>
</table>

**Key**

1. One unit (U) is the amount of enzyme required for incorporation of 1mMol of labelled dNTP in 10 minutes at 37°C using poly(A) oligo(dT)$_5$

2. 2ng/μg is approximately equal to 10mU/μl
Plate 3: An electron micrograph showing SIV<sub>deb</sub> budding from infected Molt-4-clone-8, in culture. The arrow points to a budding viral particle.

Represents 0.5 μm

The budding particle shown is type D viral particle. This confirms that SIV<sub>deb</sub> is a lentiviral like the known SIVs and HIV-1 and two which have type D viral shape.
Plate 4: An electron micrograph of molt-4 clone-8 cells with viral inclusions. The at type D budding viral particles which are characteristic of lentiviral shapes.

Represents 1 urn
Plate 5: An electron micrograph of molt-4 clone-8 cells which is uninfected with SIV.,

Represents 0.25 fim
Plate 6: SIV<sub>1cb</sub> pol PCR from De Brazza's monkeys (Cercopithecus neglectus) PBMCs. The product is a band of about 200bp DNA.

Key:

M : Molecular weight marker
1. Deb 62
2. Deb 60
3. Deb 56
4. Deb 53
5. Deb 50
6. Deb 54
7. Negative control
8. Negative control
9. Deb 45
10. Negative control
11. Negative control
12. Deb 44
13. Blank
Plate 7: SIVoeb pol PCR from De Brazza's monkeys *Cercopithecus neglectus* PBMCs cultured with molt-4-clone-8 cells for up to 30 days. The product is a band of about 200^DNA.

Key:

M : Molecular weight marker

1. Deb 62
2. Deb 56
3. Deb 60
4. Deb 50
5. Deb 53
6. Deb 54
7. Negative control
8. Deb 45
9. Negative control
10. Deb 44
Plate 8: Western blot of SIV\(^1\) isolate with sera from De Brazza's monkeys *Cercopithecus neglectus* in a semi-breeding colony at IPR. Five out of the eight animals in the study shown to be SIV\(_{reb}\) positive.

Lane M: Molecular weight marker
Lane 1: SHIV-infected animal
Lane 2: Deb 62
Lane 3: Deb 60
Lane 4: Negative control
Lane 5: Deb 56
Lane 6: Deb 54
Lane 7: Deb 53
Lane 8: Deb 50
Lane 9: Deb 45
Lane 10: Deb 44
Lane 11: Negative control
3.7. Effect on haematopoietic system and lymph-node pathology

Table 5. Effect of SIVcharger on Haematopoietic system

<table>
<thead>
<tr>
<th>Animal #</th>
<th>CD4:CD8 Ratio</th>
<th>SIV status</th>
<th>Antibody titres (IgC.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deb 44</td>
<td>0.0659</td>
<td>+ve</td>
<td>400</td>
</tr>
<tr>
<td>Deb 45</td>
<td>0.0127</td>
<td>+ve</td>
<td>1600</td>
</tr>
<tr>
<td>Deb 50</td>
<td>0.0865</td>
<td>+ve</td>
<td>800</td>
</tr>
<tr>
<td>Deb 53</td>
<td>0.170</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>Deb 54</td>
<td>0.225</td>
<td>+ve</td>
<td>800</td>
</tr>
<tr>
<td>Deb 56</td>
<td>0.0225</td>
<td>+ve</td>
<td>400</td>
</tr>
<tr>
<td>Deb 60</td>
<td>0.311</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>Deb 62</td>
<td>0.290</td>
<td>-ve</td>
<td></td>
</tr>
</tbody>
</table>

These results show that the SIVcharger infected animals have a low CD4:CD8 cells which is a consequence of viral infection just as is the case in HIV infected humans.
**Plate 9.** Photomicrograph showing collapsed lymph-node architecture in SIV\(^\text{infect}\) animal. This could be due to the trapping of virus infected cells in the lymph node prompts the dissemination of an immune response leading to the destruction of the lymph node. The arrow points to the site of destruction of the lymph node.

**KEY**

A: Capsule  
B: Cortex  
C: Medulla
Plate 10. Photomicrograph showing lymph-node architecture in SIV\textsuperscript{uninfected} animal.

KEY

A: Capsule
B: Cortex
C: Medulla
CHAPTER FOUR

DISCUSSION

The results obtained here reveal that De Brazza's monkeys (*Cercopithecus neglectus*) are naturally infected with an HIV-like virus here called SIV$_{Db}$- The results obtained by IgG specific antibody ELISA using a conserved region of SIVmac peptide (H-NAWUGCAFROVCFTTVPMAS-OH) and successful isolation of virus on CD4 bearing cell lines is an indication that the virus in question is related to HIV and SIV. This agrees with the hypothesis that non-human primates are natural hosts of a continuum of closely related retroviruses (Deinhardt, 1980 and Gardener et al, 1988) some of which are endogenous while others are exogenous. Some of these cytopathic retroviruses that have been isolated have been shown to induce AIDS in Asian macaques (Lerche et al, 1984, Daniel et al, 1985 and Desrosiers et al, 1989). These retroviruses have been shown to be closely related to human retroviruses associated with adult T-cell leukaemia disease and acquired immune deficiency syndrome (AIDS) (Nicholas et al, 1984). The relationship between these non-human primate retroviruses and human immunodeficiency virus (HIV) are that both have similarity in morphological and molecular structure. That SIV$_{Db}$ subscribes to this similarity in morphology and molecular structure is implied by the cross reactivity in antibody ELISA and antigen capture ELISA using SIVmac peptides and western blot using HIV-2 and SIVmac antigen. In addition, they both cause CPE in CD4+ bearing cells (Lowenstine and Nicholas, 1988). It is for this reason that non-human primate retroviruses have become a cause of public health concern (Bryant et al, 1985, Lowenstine and Nicholas, 1988 and Otsyula et al, 1996). Although the immune response to SIV and HIV infection may not be adequate for eliminating infection or eventual disease, it does probably play a crucial role in delaying the onset of disease. Several lines of evidence from other monkey sytems suggest this: (a) The ability of macaques to survive SIV infection correlates directly with the strength of the immune response (Daniel et al...
1987, Kanagi et al 1986 and Zhang et al 1988). This is not only true for antibody responses but also true for cytolytic T lymphocyte responses (Desrosiers et al 1990). (b). Antibody levels fluctuate reciprocally with antigenemia (Zhang et al 1988) and persistent antigenemia has poor prognosis (c). The CD8+ lymphocytes, probably CTLs from infected individuals can inhibit outgrowth of SIV or HIV from their PBMCs and suppress SIV replication in vitro (Miller et al 1989, Kanagi et al 1988 and Tsubota et al 1989). (d). Prior vaccination can provide a protective effect against SIV induced AIDS (Desrosiers et al 1990).

According to the virus isolation results, SIV_{Deb} is specific to CD4 bearing cell lines and induces HIV-like syncytia. This is attributed to the ability of these class of viruses to induce the expression of fusogenic proteins that are responsible for the fusion of infected cells. This event leads to balloon-like cells (plate 1) with aggregated nuclei and eventual cell death. This is one of the mechanisms of destruction of CD4 cells and hence reduction of these important cells of the immune system resulting in the break down of the immune system. Other mechanisms that lead to CD4+ cell destruction include cell lysis due to incorporation of its membrane coat into nascent budding viral particles and specific recognition and destruction of virus infected cells due to conformational change in cell membrane. It is such destruction of CD4 cells that leads to the change in CD4:CD8 ratios in infected animals. Uninfected cells normally live for several years but infected cells have a highly reduced longevity (about 2 months). Depending on the kinetics of viral multiplication, at first, the body's immune system is able to cope with the destruction of CD4 cells as the viral load is low. This period corresponds with the quiescent period of virus infection. However, with time, the body's immune system is outpaced by virus multiplication and hence increased CD4 cell destruction. From the CD4:CD8 ratios obtained in this study, the higher the viral load, the lower is the CD4:CD8 ratio (Table 4).
The CD4:CD8 ratio is an indication of the extent of the damage of the immune system due to infection. The De Brazza's monkeys, *Cercopithecus neglectus* SIV\textsubscript{De} system shows a reversed CD4:CD8 ratio. This system is therefore parallel to that of human HIV/AIDS and therefore suitable as a model with respect to this parameter. Much of the research with SIV on the pathogenesis of disease has sought to define the system and to demonstrate its similarity to HIV infection in humans. In only a few cases have the observations been novel and not preceded by similar observations with HIV in humans. Examples of novel contributions include; (a). Evidence for antigen trapping by follicular dendritic cells in hyoperplastic lymph nodes (Ringler et al 1989 and Wyand et al 1989). (b). The association of poor immune responses with rapid death (Ringler et al 1989 and Wyand et al 1989). (c). Selective depletion of CD4+ cells as is the case in this study and (d). Morphological evidence for cytolytic response against macrophage-related dendritic cells in the inflammatory response associated with SIV/HIV infection. Therefore, the definition of SIV systems is expected to make important contributions to the understanding of the basic mechanisms underlying the disease course.

Limited characterisation of the SIV\textsubscript{De} isolated in this study indicated that this is likely to be a retrovirus. EM results show a dense viral particle with budding characteristics similar to those of HIV and known SIVs. Negative staining EM revealed a viral particle with HIV or SIV-like spikes with an electron dense centre. SIV\textsubscript{De} is further shown to be a retrovirus by the cross reactivity of sera from positive monkeys and HIV-2 proteins on western blots and by RT activity using Mg dependent RT assay. Despite the failure of PCR based on SIVmac env and gag primers. SIV\textsubscript{De} is further authenticated by HIV-1 pol primers. Given that the annealing temperature used in this PCR was 60\(^\circ\)C, there is no doubt that the primers used were highly specific for SIVoeb-
Virus isolation and quantitation results of SIVDeb is comparable to that of humans Deb50 and Deb54 are virus positive by antibody ELISA, western blot and PCR but negative by tissue culture. This demonstrates the carrier phenomenon and long latency or persistent infection period which is one of the key features of SIV and HIV disease (Daniel et al., 1987). SIV and HIV like lentiviruses, have a remarkable ability to persist and eventually to induce a chronic and debilitating disease in spite of a strong host immune response to the virus. Infected individuals may remain clinically well for years as in DeBrazza's monkeys (Cercopithecus neglectus) in this study while maintaining easily detectable humoral and cellular immune responses, only to eventually succumb to the virus. This type of persistence is somewhat unique to lentiviruses (Desrosiers et al., 1990) since adequate immune reponses to other types of retroviruses like feline leukemia virus provide long term protection against non-oncogenic viruses very often in the abscence-of undetectable viruses unlike what has been observed in this study.

Just like HIV, SIVDeb infected animals manifest viral mediated destruction of the immune system as exemplified' by low CD4:CD8 ratios and disrupted lymph node architecture in infected animals. Neutralisating antibodies appear in the serum not long after primary infection with HIV or SIV, but despite high antibody levels in serum, the antibody response is unable to clear the virus from the lymphoid tissues hence leading to the destruction of the lymph node. Virus coated antibody is trapped in the germinal centres of the lymph nodes when it binds to the complement receptor on the follicular dendritic cells. Though many HIV infected patients and or SIV infected animals experience a long clinical latency with very low levels of viremia in early years of infection, the virus is actively replicating in the lymphoid tissue throughout the course of the disease. Over time, the viral infection* destroys the architecture of the germinal centres (plate 9) as opposed to plate 10 from uninfected animal. The destruction of the lymph node germinal centres impacts negatively on T-cell populations
which heralds the collapse of the immune system. In this regard, SIV$_{Deb}$ destroys the De Brazza's immune system much the same way as HIV destroys the human's immune system and hence pointing out the potential of SIV$_{Deb}$ and the De Brazza's monkey as a model for human HIV/AIDS.

CONCLUSION AND RECOMMENDATIONS

This study shows that;

(i) De Brazza's monkeys, *Cercopithecus neglectus* are naturally infected with an HIV-like lentivirus called SIV$_{Deb}$ as shown by antibody and antigen presence, virus isolation and proviral sequence.

(ii) It is possible to isolate SIV$_{Deb}$ on CD4 bearing cell lines.

(iii) Since SIV$_{Deb}$ isolate has all the characteristics of a lentivirus, it is a retrovirus that can be used in studies modelling HIV infection by inference.

(iv) Because of the effects of the effects of SIV$_{Deb}$ on the De Brazza's haematopoietic system, particularly WBC counts and CD4:CD8 ratios and lymph-node pathology, SIV$_{Deb}$ in the De Brazza's monkeys system is a potential animal model for human HIV/AIDS.

Further characterisation of SIV$_{Deb}$ is required in order to qualify SIV$_{Deb}$ De Brazza's system as a choice amongst the animal models being developed for Human HIV/AIDS. It would be an interesting and rewarding venture to clone and sequence SIV$_{Deb}$ and design both antibody and antigen capture ELISAs, western blot and PCR systems that are specific to SIV$_{Deb}$. This will facilitate more refined studies with SIV$_{Deb}$ in both its natural host De Brazza's monkeys *Cercopithecus neglectus* and other non-human primates. The study is also useful contributing to the evolutionary relationships among the retroviruses. Study of SIV systems has already resulted in contributions regarding the origins of HIVs, AIDS pathogenesis and vaccine
therapy research. Continued use of these SIV systems is expected to further advance the progress in these areas. Better understanding of the viral and host factors contributing to the pathogenesis of AIDS in particular will fuel efforts to develop effective therapies and vaccines.
REFERENCES


Daniel, M.D., Letvin, N.L., Sehgal, P.K., Hunsmann, G., Schmidt, D.K., King, N.W. and


Douglas, N.W., Munro, G.H. and Daniels, R.S. (1997). HIV/SIV glycoproteins: Structure-
function relationships. *Journal of Molecular Biology* 273, 122-149.


Gallo, R.C., Gallagher, R.E., Wong-Staal, F., Aoki, T., Markharm, P.D., Schetters, H.,
Ruscetti, F., Valerio, M., Walling, M.J., O'Keefle, R.T., Saxinger, W.C., Smith, R.G.,
components from a gibbon ape (Hyalobates lar) with lymphocytic leukemia. *Virology* 84, 359-
373.

Gallo, R.C., Salahuddin, S.Z., Popovic, M., Shearere, G.M., Kaplan, M., Haynes, B.F., Palker,
T.J., Redfield, R., Oleske, J. And Safai, B. (1984). Frequent detection and isolation of
cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science*
224, 500-503.

and AIDS. In perk K(ed). *Immunodeficiency Disorders and Retroviruses*. Academic Press,
New York 171-226.


Green, W.C. (1990). Regulation of HIV-1 gene expression. *Annual Review of Immunology* 8,
453-476.

Synthetic peptide immunoassay distinguishes HIV type 1 HIV type 2 infections. *Science* 237,
1346-1349.

Gottlieb, M.S., Schroff, R.JSchanker, S., Weisman, J.D., Fan, P.T., Wolf, R.A. and Saxon, A.
(1981). Pneumonocystis carinii pneumonia and mucosal candidiasis in previously healthy


*Review of Veterinary Pathology* 23, 345-353.


Macaca nigra) with immune deficiency and retroperitoneal fibromatosis. Journal of Virology 56, 571-578.


