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HIV-2 GENETIC EVOLUTION
AND VIRAL DYNAMICS

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Till minne av min Mormor

- som själv aldrig fick studera.
Mormor uppmuntrade mig att läsa
och det slutade med att jag gick hela vägen.

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LIST OF ABBREVIATIONS

aa	Amino acid
AIDS	Acquired immunodeficiency syndrome
APOBEC	Apolipoprotein B editing complex protein
ART	Antiretroviral treatment
CA	Capsid
CCR	CC chemokine receptor
CD4 cells	Helper T lymphocytes
CD8 cells	Cytotoxic T- lymphocytes
CXCR	CXC chemokine receptor
DNA	Deoxyribonucleic acid
dNTP	Deoxy nucleotide triphosphate
env	Envelope gene
FI	Fusion inhibitors
gag	Group specific antigen gene
HAART	Highly active antiretroviral treatment
HBV	Hepatits B virus
HIV	Human immunodeficiency virus
HTLV	Human T cell lymphotropic virus
LAV	Lymphadenopathy associated virus
LTNP	Long-term nonprogressors
LTR	Long terminal repeats
MA	Matrix
MRCA	Most recent common ancestor
NC	Nucleocapsid
NNRTI	Non nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
pfu	Plaque forming units
PI	Protease inhibitors
pol	Polymerase gene
PR	Protease
RNA	Ribonucleic acid
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase polymerase chain reaction
RVA	Recombinant virus assay
SIV	Simian immunodeficiency virus
SIVcpz	SIV chimpanzee
SIVsm	SIV sooty mangabey monkey
SU	Outer surface protein
TM	Transmembrane protein
tRNA	Transfer RNA
V1 – V5	Variable region 1 – 5

LIST OF PUBLICATIONS

I. **Eleonor Brandin**, Lindborg L, Gyllensten K, Broström C, Hagberg L, Gisslén M, Tuvesson B, Blaxhult A and Albert J. 2003
    Pol gene sequence variation in Swedish HIV-2 patients failing antiretroviral therapy. AIDS Res Hum Retroviruses. 19:543-550

II. Shi Y, **Eleonor Brandin**, Vincic E, Jansson M, Blaxhult A, Gyllensten K, Moberg L, Broström C, Fenyö E and Albert J. 2005
    Evolution of HIV-2 coreceptor usage, autologous neutralization, envelope sequence and glycosylation. J Gen Virology. 86:3385-3396

III. **Eleonor Brandin**, Broström C, Gille E, Bonhoeffer S and Albert J. 2005
    HIV-2 dynamics. AIDS Res Hum Retroviruses. 21:608-610

IV. **Eleonor Brandin**, Thorstensson R, Bonhoeffer S and Albert J. 2006
    Rapid viral decay in SIV infected macaques receiving quadruple antiretroviral therapy. Submitted

ABSTRACT

HIV-2 is less pathogenic and less transmissible compared to HIV-1. This is believed to be due to the lower viral load that is seen in HIV-2 infected patients. However, why this lower viral load is seen is not known. In this thesis we have investigated different aspects of HIV-2 in order to understand the pathogenesis of HIV-2 better.

In paper I genetic variation and evolution in the *pol* gene was studied, with special interests in resistance-associated mutations in HIV-2 infected patients failing antiretroviral treatment. We observed some mutations known to cause resistance in HIV-1 such as M184V, Q151M and E219D; but most resistance mutations appeared to differ from what is known in HIV-1. The main conclusion from this work is that specific algorithms for HIV-2 resistance interpretation are needed, because the algorithms for HIV-1 cannot be used.

In paper II we studied the evolution of co-receptor usage, neutralization escape and *env* gene sequence. We report a switch from CCR5 to CXCR4 using virus during disease progression. We did not see a clear pattern of neutralization escape as is seen in HIV-1. We saw a pattern of changes in the number of potential N-linked glycosylation sites during disease progression. HIV-2 has fewer potential N-linked glycosylation sites in V3. We propose that HIV-2 V3 is more open and accessible to neutralizing antibodies.

In paper III and IV we have studied the rate of viral clearance in HIV-2 infected patients as well as in experimentally SIV infected macaques. In paper III we conclude that the clearance of actively virus producing cells appears to be similar in HIV-1 and HIV-2 infection. Thus, we estimated a t½ for virus producing cells of 2.2 and 2.0 days respectively in two HIV-2 patients who started antiretroviral therapy. These findings indicate that differences in pathogenesis between HIV-1 and HIV-2 are not due to differences in viral clearance or life span of virus producing cells. In paper IV we studied the decay of plasma virus in SIV-infected macaques that received aggressive antiretroviral therapy. In these animals we observed a very fast half-life of virus producing cells, 0.5 days, which is the shortest ever reported for any lentivirus. This might be due to the high potency of the antiretroviral therapy or differences between in SIV-infected monkeys and HIV-infected humans.

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AIMS OF THIS THESIS

The general aims of this thesis were to understand more about HIV-2. To better understand the differences that exist between HIV-1 and HIV-2 could help us to answer basic questions about HIV pathogenicity and virulence.

The specific the aims were:

- ✓ To study the genetic variation in the HIV-2 pol gene in Swedish HIV-2 infected patients.

- ✓ To characterize mutations associated with drug resistance in HIV-2 infected patients experiencing virological failure.

- ✓ To study the HIV-2 co-receptor use over time in HIV-2 infected patients.

- ✓ To study how the neutralizing antibody response evolves in HIV-2 infected.

- ✓ To study the rate of HIV-2 production and clearance *in vivo*.

- ✓ To study the rate of SIV clearance in SIV infected macaques *in vivo*.

INTRODUCTION

DISCOVERY AND ORIGIN OF HUMAN IMMUNODEFICIENCY VIRUS

Almost 25 years ago, on June 5th 1981, Centers for Disease Control and Prevention (CDC) published a note, *Pneumocystis carinii* in Los Angeles (CDC 1981b), in their paper Morbidity and Mortality Weekly Report (MMWR). This was the first note of the new pandemic that we now know as the HIV/AIDS pandemic. This first report was followed by many and today a search on the web on HIV/AIDS gives 103 000 000 hits!

Already on June 6th San Francisco Chronicle had picked up the story and wrote the first news article about the fact that young healthy gay men in the city were becoming ill. A month later MMWR published another note, Kaposis sarcoma and Pneumocystis pneumonia among homosexual men – New York City and California (CDC 1981a). Opportunistic infections such as pneumonia caused by *Pnemocystis carinii*, candida infections, cytomegalovirus and herpes simplex virus infections and a rare form of cancer, Kaposis sarcoma, were observed in formerly healthy, young homosexual men. The first reports were only focused on the patients that were gay, but it was not long before similar symptoms were observed in other groups such as hemophiliacs, transfusion recipients, intravenous drug users and sexual partners of individuals in these groups. The fact that the first reports of the new disease were discovered in California among young gay men has two sides. It helped to get attention and focus on this new disease. In a highly educated group who are used to fight for their rights the search for answers as to what caused this new disease had a tremendous speed. Nothing had previously gone so fast from complete unawareness to knowing which virus (Barre-Sinoussi et al. 1983; Popovic et al. 1984) caused this deterioration of the immune system, how it infects and development of screening tests to be able to stop the use on contaminated blood products (Sarngadharan et al. 1984; Schupbach et al. 1984). Today the SARS epidemic is another example of research with tremendous speed. On the other hand the fact that it was the gay community in the Western world that first were known to be infected leads to the fact that a lot of people still, 25 years later, think that HIV/AIDS has nothing to do with them. The president of South Africa, Tabo Mbeki, denies that HIV causes AIDS and questions if AIDS really does exist. South Africa is

one of the countries in Africa with the highest number of HIV/AIDS cases (Kallings 2005).

The causative agent, a new retrovirus, was isolated in 1983 by a French group (Barre-Sinoussi et al. 1983) and later an American group verified the finding that the virus isolated by the French did cause AIDS (Popovic et al. 1984). These groups called their virus isolates lymphadenopathy associated virus (LAV) and human T cell lymphotropic virus 3 (HTLV-3), respectively and later the virus was named human immunodeficiency virus (HIV) (Coffin et al. 1986). In order to understand how important this new disease was one can think about the fact that it was turned into a political issue and the Prime minister of France and the President of the United States had to make an agreement over patent rights and that the two scientists Montagnier and Gallo are both co discoverer of this new virus (Gallo 2002; Gallo et al. 2002; Montagnier 2002; Prusiner 2002).

In 1986 a new second virus was isolated from individuals in West Africa who had acquired immunodeficiency syndrome (AIDS), but had no detectable antibodies against HIV (Clavel et al. 1986). This new virus was called HIV-2 and short thereafter the virus was also isolated in Sweden from a patient with immunodeficiency (Albert et al. 1987). Thus, HIV-2 is the second causative agent of AIDS.

So where did this new virus come from? There are many speculations. There has been, and still is, a big controversy as to where the virus originated. Was it man made spread in Africa on purpose, as a few has suggested, or was it spread in Africa by man but as a coincidence, as others has proposed.

The Nobel Peace prize laureate in 2004, Wangari Maathai claimed in a speech given short after she was announced, that HIV/AIDS was created as a biological weapon in order to control the African population. This is what many in Africa believe and that makes the work in Africa against HIV/AIDS and prejudices concerning HIV/AIDS difficult. There are so many hinders to overcome to stop the HIV pandemic in Africa and Wangari Maathai had a golden opportunity to help spread the facts on how to protect yourself, how to protect your children and to make the leaders in several African countries take responsibility and act against the spread of HIV. Later she has taken back her statement but the harm is already done (Kallings 2005).

In the book The River, Edward Hooper claims that HIV was spread with the polio vaccine given in Africa. The vaccine, according to Hooper, was contaminated when poliovirus was grown on chimpanzee cells infected with SIV and thereby the virus infected many that received the polio vaccination (Hooper 1999). This has been debated and tested. Vaccine batches have been tested for SIV as well as tested for mitochondria DNA from chimpanzee. All samples were negative both when searching for virus and the traces of chimpanzee cells. The scientists involved in the mass vaccination against polio in Africa has also discounted Hooper's story (Koprowski 2001).
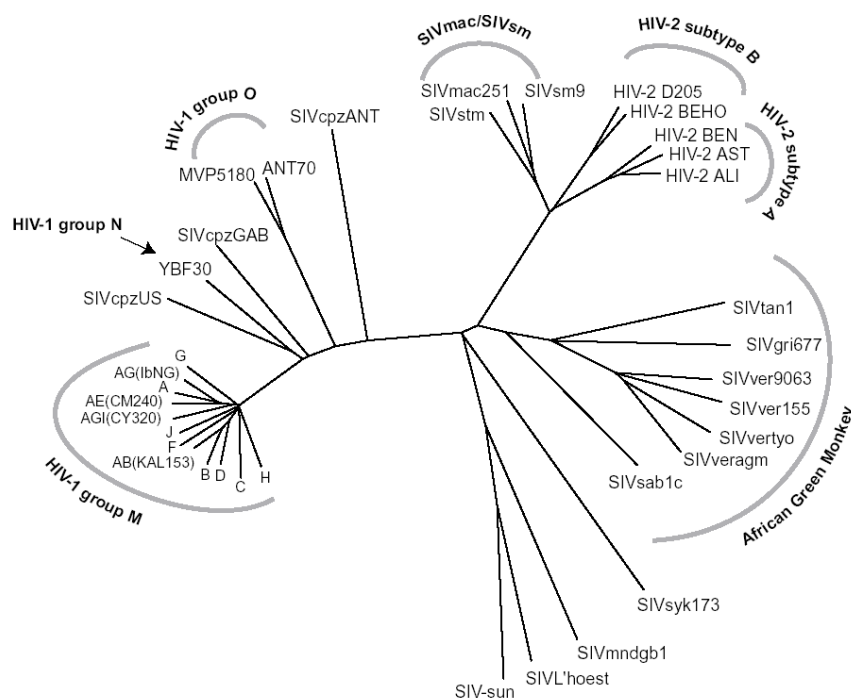

Figure 1. Phylogenetic tree illustrating the relationship between primate lentiviruses. From Human Retroviruses and AIDS 1999, Los Alamos national Laboratory (Kuiken 1999).

Scientists and many others believe without debate that HIV was transferred from monkeys to man as a cross species transmission. This has happened on several different occasions (Kanki et al. 1986; Hahn et al. 2000; Holmes 2001). HIV-1 has been transferred as far as we know today at least three times which has given rise to three genetic groups of HIV-1 (HIV-1 group M, N and O). Chimpanzees are the carrier of SIVcpz, which is the closest relative to HIV-1 (Figure 1) (Gao et al. 1999; Hahn et al. 2000). Also the chimpanzees have been infected through a cross species transmission from lower monkeys (Bailes et al. 2003), chimpanzees hunt and eat small monkeys. The sooty mangabey monkey is infected with the SIVsm, which is the close relative to

HIV-2 (Figure 1) (Hirsch et al. 1989; Gao et al. 1992). HIV-2 is thought to have been transferred to humans on eight known occasions (HIV-2 subtype A-H).

The most likely explanation on how the transfer of the viruses HIV-1 and HIV-2 to humans has occurred is during hunting and butchering (Peeters et al. 2002). Bush meat is a large market in Africa and during butchering of monkeys one can get exposed to SIV infected blood. Monkeys are also kept as pets and infections could probably also occur through bites from SIV infected animals.

THE HIV PANDEMIC

Why a pandemic spread has been seen involving HIV-1 group M and not HIV-1 group N we do not know. Increasing travel over the African continent as well as changes in sexual behavior and the use of unsterile needles in the hospital care can spread the virus to several people. Large vaccinations campaigns may help in spreading HIV to large amounts of people due to the reuse of needles. This of course happened when we knew nothing about the virus and it was difficult to foresee the future. The common knowledge used to be that blood was a clean product. Several lab technicians were hepatitis B infected (HBV) due to working with HBV infected blood samples (Levy et al. 1977). Today routines for working with patients' samples are very rigid, gloves and lab coats are worn at all times.

What is more alarming is the continuous use of unsterile needles and low hygiene during surgery in some parts of the world. In China HIV was spread among blood plasma donors in 1994 – 1995 when tests for HIV were available. Village people were convinced by small financial compensations to donate blood plasma and many were infected with HIV in the process (Wu et al. 2001). The infection occurred because the blood cells were pooled before given back to the individual's donating blood plasma, this in a time when we had the knowledge and the methods to prevent this.

Today we, in the western part of the world, see very few HIV infected newborns due to mother-to-child transmission. Treatment is offered to the mother and child and caesarean section and no breast-feeding are recommended. The goal would be that these simple strategies could be used all over the world to limit the spread among the youngest and most vulnerable. In some parts of Africa one dose nevirapine is given before birth to the mothers but it is difficult to reach the most remote settings. There are

also controversies regarding giving one dose nevirapine to mothers as studies have shown high numbers of resistance associated mutations occurring after the treatment. How this will affect future treatment options for the patients is not known (McIntyre 2006). Stigmatization is a problem; if HIV diagnoses are revealed you will not be included in the social life, which is important to be able to lead a normal life. If the children are not breast-feed, the mothers reveal their HIV diagnosis. It is a very difficult task to overcome the prejudices about HIV/AIDS all over the world.

The WHO summary of the AIDS pandemic in December 2005 shows 40.3 million people living with HIV; 4.9 million were infected during 2005. This means that more than 9 people per minute get infected with HIV worldwide.

In contrast to HIV-1, HIV-2 has not caused a pandemic. Instead HIV-2 infections are confined mainly to West Africa and the highest prevalence is seen in Guinea-Bissau. In Europe, Portugal, France and Spain have the largest numbers of HIV-2 infected due to colonial contacts with West Africa. In Portugal 4.5% of all AIDS cases are due to HIV-2 infection (Soriano et al. 2000).

HIV-2 SUBTYPES OR GROUPS

HIV-1 has been transferred to humans on three different occasions from chimpanzees resulting in three genetic groups of HIV-1, group M, N and O. Group M is causing the pandemic while group N and O is not as widely spread. In HIV-1 group M extensive evolution has occurred over the years and today a large number of subtypes and recombinant forms are seen (Figure 1).

There are eight subtypes described for HIV-2 and every known subtype (A-H) is thought to be an independent transfer from sooty mangabeys to humans. The subtypes are as genetically similar as are the HIV-1 groups, and would be better referred to as HIV-2 groups A-H to have a consistency in the nomenclature of HIV (Damond et al. 2004).

HIV-2 subtypes A and B are the only two subtypes that have been shown to transmit between humans. The other six subtypes (C-H) have only been reported in a few cases and probably represent dead-end infections (Gao et al. 1994; Chen et al. 1997; Yamaguchi et al. 2000; Damond et al. 2004). Subtype C and D were isolated from

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healthy adults from Liberia and subtype E from an asymtomatic male from Sierra Leon (Gao et al. 1994). Subtype F represent a divergent HIV-2 virus and has been isolated from an individual from Sierra Leon (Chen et al. 1997). Subtype G was isolated from an asymptomatic blood donor from Ivory Coast (Yamaguchi et al. 2000). One of the few subtypes isolated from a symptomatic patient is subtype H, isolated from an individual from Ivory Coast after arrival in France (Damond et al. 2004). Recombination of different subtypes has been reported, but does not appear to be common (Gao et al. 1994). Recombination between HIV-1 and HIV-2 has never been seen, even though one person can be infected with both viruses (Curlin et al. 2004).

The most recent common ancestor (MRCA) of HIV-2 subtype A has been estimated to have existed around 1940 by back-calculations based on genetic distances (Lemey et al. 2003). HIV-2 has its epicenter in West Africa were also sooty mangabeys are common (Hahn et al. 2000).

THE HIV GENOME, PROTEINS AND REPLICATION CYCLE

HIV belongs to the *Lentivirus* subfamily of the *Retroviridae* family and carries its genetic information as two single strands of RNA in an enveloped virus particle. The particle also contains a reverse transcriptase and an integrase, as well as two cellular transfer RNA (tRNA) that are base paired to the genome and act as primers for the reverse transcriptase once the viral particle has entered into the cell (Murray 2002).
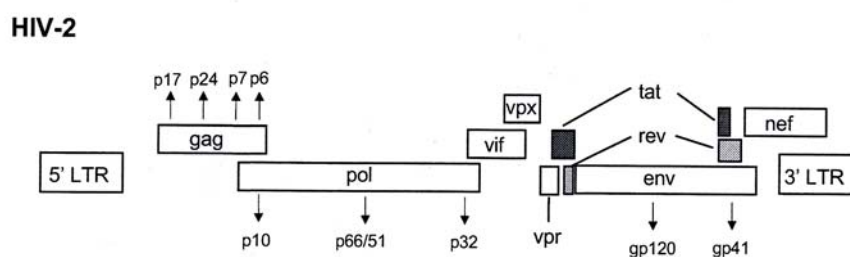
Figure 2. Schematic picture of HIV-2s genome. Envelope gp160 is cleaved to gp125 and gp36 in HIV-2.

The genome is organized as in Figure 2. Long terminal repeats (LTR) are non-coding regions and include regulatory sites such as promoters and enhancers. The group specific antigen (*gag*) gene codes for the matrix (MA), capsid (CA), and nucleocapsid (NC) proteins, the polymerase (*pol*) gene codes for the enzymes reverse transcriptase, protease and integrase and the envelope (*env*) gene codes for the glycosylated envelope

proteins, gp125 and gp36. Gp125 is the outer surface protein (SU), which is anchored to the virus membrane via the gp36 transmembrane protein (TM). The size of the glycoprotein varies between the different viruses; in HIV-1 gp160 is cleaved to gp120 and gp41.

In addition there are six other genes, which encode regulatory and accessory proteins: *tat*, *rev*, *nef, vif, vpr* and *vpx/vpu* (Table 1). HIV-2 and SIV has the regulatory protein *vpx*, which is not seen in HIV-1, but HIV-2 instead lacks *vpu*. The *gag* and *pol* genes are relatively conserved between different viral strains while the *env* gene has five highly variable regions, which are called V1 – V5. The HIV-2 genome consists of around 9,700 nucleotides, which is slightly longer than the HIV-1 genome. This difference is mainly due to larger LTRs in HIV-2 (Fields et al. 1996).

Table 1. Regulatory and accessory genes and their function.

Gene	Function
Tat	Transactivation of viral and cellular genes
Rev	Regulation of RNA splicing, arrests cell cycle
Nef	Down regulates CD4, MHC I, virion infectivity, alter cell activation
Vif	Viral infectivity, promotes assembly, inhibits APOBEC
Vpx / Vpu	Release of virus, decrease of cell surface CD4
Vpr	Transportation into the nucleus, arrest cell cycle

The envelope of HIV is a glycoprotein cleaved into two parts, gp 120 and gp 41 for HIV-1 and gp125 and gp36 for HIV-2. The envelope of HIV-1 is more studied and one can assume that the envelope of HIV-1 and HIV-2 are similar. The gp120 as well as the gp125 consists of five variable regions (V1-V5) interspersed with conserved regions; the gp41 is more conserved (Fields et al. 1996). The envelope gp120 is a major target for the immune responses in the infected individual while the gp41 is the transmembrane part covered until the fusion step. This region can be highly conserved whereas the gp120 has to be able to change to avoid the immune system. The variable regions V1-V4 of the envelope appears as loops on the surface with disulfide bonds at the base. The V3 loop is the most studied and consists of 35 amino acids both in HIV-1 and HIV-2. It has been shown to be important in the viral cell tropism where only one amino acid change is needed to change cell tropism. V3 is also an important antigen for neutralizing antibodies (Ivanoff et al. 1992; Verrier et al. 1999). Gp120 is highly glycosylated; 50% of the molecular mass is due to glycosylation of the protein. It has

been proposed that the glycans shield the viral protein and the virus appears as self in the infected individual and thereby avoids neutralizing antibodies. Movement of the N-linked glycosylation sites has been seen and this movement might further help the virus to escape the immune system (Wei et al. 2003). The glycosylation of the protein, in particular the N-linked glycans influence the structural folding of the envelope. It has been shown that elimination of glycans within or adjacent to the V3 loop makes the virus more susceptible to neutralizing antibodies (McCaffrey et al. 2004).

Table 2. Human chemokine receptors used by HIV as coreceptors. ** Major co-receptors for HIV the others are minor co-receptors.

Receptor	Cellular distribution
CXCR4**	T-lymphocyte (preferentially on unactivated CD4+ T cells), B-lymphocyte, epithelial cells, endothelial cells, dendritic cells
CXCR6	T-cells, dendritic cells, epithelial cells
CCR1	Neutrophil, monocyte, macrophages, T-lymphocytes, natural killer cells, B-lymphocytes, mast cells, astrocytes, neurone
CCR2	Monocyte/macrophages, T-lymphocytes, B- lymphocyte, basophil
CCR3	T-lymphocyte, basophil, eosinophil
CCR5**	T-lymphocyte (expressed on activated T cells and memory cells), monocyte/macrophage, dendritic cells
CCR8	Monocyte/macrophages, thymus

Modified from C. Murdoch 2000 (Murdoch et al. 2000)

The lifecycle of HIV-2 is not as carefully studied as that of HIV-1, but the two viruses can be assumed to have similar replication strategy. HIV-2, like HIV-1, uses CD4 as its major receptor (Dalgleish et al. 1984; Klatzmann et al. 1984). The CD4 receptor is found on CD4+ T-lymphocytes, monocytes, macrophages and dendritic cells. In addition, HIV-1 as well as HIV-2 requires co-receptors for infection. The co-receptors used by HIV are chemokine receptors and are members of 7-transmembrane G-protein-coupled receptor family. The receptors used by HIV are found on many different cells (Table 2) and their normal function varies. CCR5 and CXCR4 are the major co-receptors for both HIV-1 and HIV-2, but HIV-2 has been shown to have a more promiscuous use of alternative co-receptor (CCR1, CCR2b, CCR3, CXCR6, etc.) *in vitro* (Morner et al. 1999; Blaak et al. 2005). In HIV-1 the co-receptor usage is

correlated to disease progression. Viruses that use CXCR4 receptor are usually found in patients with advanced immunodeficiency. The same appears to be true for HIV-2, as shown in this thesis and earlier studies (Albert et al. 1990b; Shi et al. 2005).

The HIV replication (Figure 3) starts with the binding of the glycoprotein (gp125 for HIV-2 gp120 for HIV-1) on the virus to the CD4 receptor on the cell. This is followed by extensive conformational changes in the glycoprotein, which enables the gp125 to bind the co-receptor (commonly CCR5). After the binding of CD4 and co-receptor the fusion peptide on gp36 can assist in the fusion of the virus and the cell membrane. Once in the cytoplasm the reverse transcriptase uses the tRNA as primer and synthesizes DNA. The double stranded DNA is transported into the nucleus and integrated into the host chromosome; it is then called a provirus. Once integrated the viral DNA is transcribed as a cellular gene. The HIV replication is then dependent on the cells machinery but HIV also carries several genes (Table 1) that regulate the replication. The new viral particles are released by budding and thereby become an enveloped virus. After the release of the viral particle the viral protease cleaves the gag and gag-pol polyproteins, this is required to make a mature infectious viral particle.
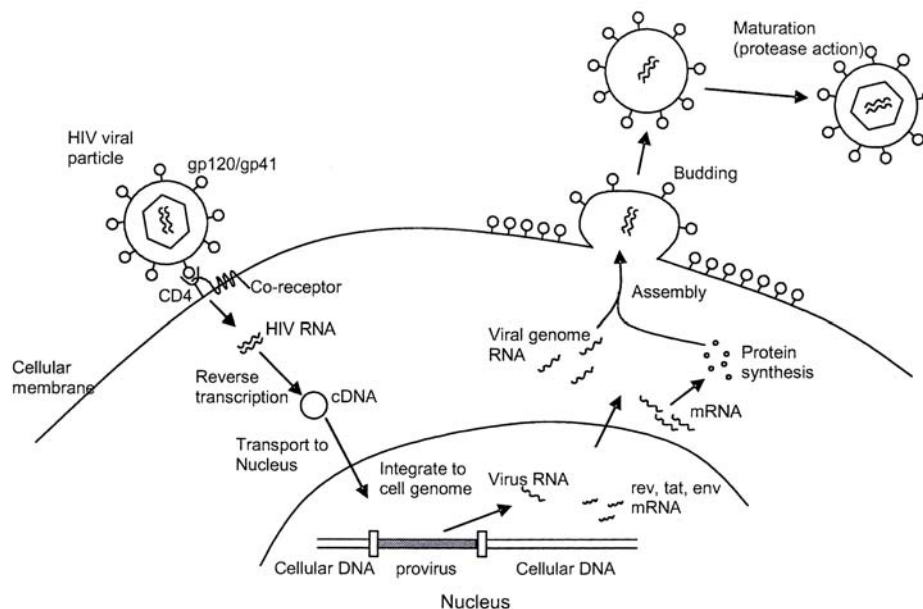

Figure 3. Schematic drawing of the life cycle of HIV. For HIV-2 gp160 is cleaved into gp125 and gp36.

HIV GENETIC VARIATION

HIV is characterized by very high genetic variability, due to several factors. The virus itself contributes with the error prone process of the reverse transcription. The RT

enzyme lacks proofreading ability and thereby mutations occur frequently, approximately one mutation per replication cycle (Mansky et al. 1995). The cellular RNA polymerase II also lacks proof reading ability and will increase the mutations in the new virus genome. It is believed that the viral RT enzyme has the most importance of the two enzymes in the genetic variability of the virus (Roberts et al. 1988).

Members of the cellular apolipoprotein B editing complex protein (APOBEC) family of cytidine deaminases have been described to contribute to genetic variability. APOBEC is mostly expressed in human T lymphocytes and the effect on the virus is due to an ability to trigger G-A mutation (Mangeat et al. 2003). However, normally the viral protein Vif protects the virus by degrading APOBEC (Yu et al. 2003). *In vitro* studies made with HIV defect vif (HIV Δvif) show that APOBEC can cause mutations but how important this is *in vivo* with a normal vif protein, which will hinder APOBEC of being included in the virion, is not proven. Deletion, insertion and recombination frequently occur in HIV replication. This high genetic variability contributes to the intra-host variability and the inter-host variability (Nowak 1992). The immune system of the host as well as antiretroviral therapy will provide high pressure and selection on the viral population.

The high mutation rate of HIV causes a patient to carry a population of genetic virus variants which is referred to as a quasispecies (Goodenow et al. 1989; Meyerhans et al. 1989; Eigen 1993). As a result, HIV can rapidly adapt to environmental changes by selection of pre-existing variants with advantages in the environment, such as reduced susceptibility to immune control or antiretroviral drugs.

IMMUNE RESPONSES TOWARDS HIV

The immune responses against HIV have been extensively studied. These studies show that HIV is the winner and that the virus out competes the human immune response. The final stage is deterioration of the immune system followed by opportunistic infections and death. Immune responses towards HIV is an important field to learn more about to be able to provide a vaccine towards HIV infection. An HIV-2 infection seems to be more controlled by the immune system than the HIV-1 infection. In settings where medications are not available HIV-2 infections, in contrast to HIV-1 infections, do not always ultimately lead to death in AIDS (Poulsen et al. 1997). Many HIV-2 infected individuals lead a normal life and die of natural causes, which is not

commonly seen in HIV-1 infected where AIDS is expected after an average of 10 years. HIV-2 infected individuals have normal CD4 counts and undetectable viral load during the asymptomatic phase; this is also seen in long-term nonprogressors (LTNP) in HIV-1 infected.

At infection, HIV probably infects cells of the macrophage lineage, macrophages, monocytes, dendritic cells as well as CD4 + T-cells. At infection through the sexual route most likely the Langerhans dentritic cells in the epithelium are infected. The Langerhans cells are thought to transport the virus to the lymph nodes where activated CD4+ T-cells will be infected, which of course is to the advantage of the virus. Once in the lymph node CD4+ T-cells will constantly be around to be infected by HIV and new viral particles can infect new cells. A virus-producing cell will eventually die either the viral replication in it self is being fatal for the cell or by apoptosis or a cytotoxic cell will kill the infected cell. The immune system is beating itself.

One might assume that the HIV-2 infection is less pathogenic due to the fact that the immune system is strong against the infection. In HIV-2 infected a broader autologous as well as heterologous neutralization is seen and a stronger CTL response (Andersson 2001; Reeves et al. 2002). This is also to some extent seen in LTNP in HIV-1 infected.

HIV DISEASE AND TRANSMISSION

HIV-2 is transmitted through sexual contact, through blood products and vertical from mother to child. Breast-feeding should also be avoided. The rate of disease progression and transmission is much lower for HIV-2 than for HIV-1 (Andreasson et al. 1993; De Cock et al. 1994; Kanki et al. 1994; Marlink et al. 1994; Norrgren et al. 2003). Gomes *et al.* proposes that the most important route of infection for HIV-2 is the parental exposure (injections, vaccination campaigns, blood products and traditional practices). This would also explain why there is no HIV-2 pandemic in the world (Gomes et al. 2003). The risk of vertical transmission is <5 % in HIV-2 compared to 15 % – 40 % in HIV-1 (in absence of antiretroviral prophylaxis). Primary infection in HIV-1 is associated with a burst of plasma viremia followed by a latency period. One would assume that HIV-2 follow the same pattern, although a lower viral set-point is reached followed by a longer clinical latency period (Andersson 2001). We have seen a latency period of more than 20 years in one of the patients we follow. This patient was infected in the mid-80s and still has an asymptomatic HIV-2 infection. A normal HIV-2 infected

patient has undetectable HIV-2 RNA viral load, normal CD4 count and is completely asymptomatic. It is more common do die from other causes than from the HIV-2 infection (Poulsen et al. 1997). Some HIV-2 patients experience progressive immunodeficiency. When such patients develop AIDS there is no longer any differences seen between the two HIV infections.

TREATMENT AND DRUG RESISTANCE IN HIV INFECTION

In 1987 the first HIV drug, zidovudine, was available on the market and it seemed very promising (Fischl et al. 1987). However it did only take a few months for resistance mutations to develop towards this one drug (Larder et al. 1989). In 1996 the picture changed dramatically when a new group of drugs, protease inhibitors, was introduced and treatment with a combination of at least three drugs was started, so called highly active antiretroviral therapy (HAART), today the simpler term antiretroviral therapy (ART) is more often used.

There are four classes of antiretroviral drugs available for treatment of HIV-1 infected patients: nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) and entry inhibitors (Table 3). New drugs towards other targets are under development and the entry inhibitor was fairly recently licensed. The different groups inhibit different steps in the virus life cycle and thereby stop new infectious viral particles from infecting new cells. NRTI mimic dNTPs, which are the normal substrates for RT, and terminate DNA chain elongation upon incorporation. NNRTI binds directly to the RT and thereby blocks the enzymatic activity of the protein. PIs are active in a later stage of the virus lifecycle, where they inhibit the proteolytic cleavage of polyproteins and thereby lead to the production of immature and non-infectious virions. The only entry inhibitor on the market (T-20) is a fusion inhibitor, which binds to the gp41 thus inhibiting the entry of the viral particle into the cell. New entry inhibitors not yet on the market has different actions, one example is a CCR5 entry inhibitor, which binds to the CCR5 receptor thereby blocking the virus from binding. Another example of entry inhibitor is where the active drug binds to the virus gp120 mimicking the CCR5 receptor, however the virus will faster mutate to avoid this drug, a drug which binds to the cell, blocking the viral binding might be better out of this perspective.

HIV infection is commonly treated with a combination of two NRTI and one PI, or two NRTI and one NNRTI. The protease inhibitor lopinavir are always given with ritonavir. Ritonavir at a very low dose can inhibit a metabolic pathway (Kageyama et al. 2005); thereby the plasma levels of the protease inhibitor given will be higher and more constant in the patient. In Kaletra a fixed combination of lopinavir and a low-dose of ritonavir in a single capsule limits the number of tablets the patients needs to take. There are combinations of NRTI as well, such as Combivir (zidovudine and lamivudine) and Trizivir (abacavir, zidovudine and lamivudine).

Table 3. Antiretroviral drugs used for treating HIV-1 infection (RAV 2005). For HIV-2 treatment only NRTI and PI can be used. * Tenofovir is a nucleotideanalog. ** Amprenavir and Fosamprenavir cannot be used for treatment of HIV-2 infection.

Nucleoside RT inhibitors (**NRTI**)	Non nucleoside RT inhibitors (NNRTI)	Protease inhibitors (**PI**)	Entry inhibitors
Zidovudine (AZT)	Nevirapin (NVP)	Ritonavir (RTV)	Enfuvirtid, T-20
Stavudine (d4T)	Efavirenz (EFV)	Indinavir (IDV)	
Lamivudine (3TC)		Saquinavir (SQV)	
Emtricitabine (FTC)		Nelfinavir (NFV)	
Didanosine (ddI)		Lopinavir (LPV)	
Abacavir (ABC)		Tipranavir (TPV)	
Tenofovir*		Atazanavir (ATV)	
		Amprenavir(APV)**	
		Fosamprenavir **	

Current available drugs are customized for treatment of HIV-1 infection and therefore some of the available drugs do not work efficiently on HIV-2. NNRTI are not recommended for treatment of HIV-2 infection because several studies show that HIV-2 has an inherent resistance to NNRTIs (Witvrouw et al. 1999; Isaka et al. 2000; Isaka et al. 2001; Smith et al. 2001). It is also discussed if some PIs may be less effective against HIV-2 than HIV-1, amprenavir has been reported to be less effective against HIV-2 (Witvrouw et al. 2004). HIV-2 protease is highly polymorphic. Domains that are functionally important in HIV-1 harbors polymorphic sites in HIV-2; this could prevent the PIs to work as efficiently for HIV-2 (Colson et al. 2004; Pieniazek et al. 2004; Damond et al. 2005; Rodes et al. 2006a; Rodes et al. 2006b). Also the newer

drugs on the market are developed for HIV-1 group M and may not be as efficient against HIV-2. The licensed entry inhibitor T-20 does not work efficiently in HIV-2 therapy (Poveda et al. 2004).

Mutations constantly arise during HIV replication. The high mutation rate means that drug resistance easily develops during monotherapy. Wild-type virus strains are overgrown by new, drug resistant, viral strains that are more fit under drug selective pressure. For some drugs, like lamivudine, only one mutation is required for development of high-level resistance. For other drugs, like zidovudine, multiple mutations are needed. Resistance develops more quickly to the former type of drugs. Some of the mutations have a fitness cost for the virus. This means that the wild type virus often will replace the virus with drug resistant mutations if treatment is terminated. Sub-optimal treatment that allows viral replication is associated with the greatest risk for development of drug resistance.

In the newest version of the European Resistance Guidelines (discussed on the 4th European HIV drug resistance workshop, Monte Carlo, March, 2006) it is planned that a part about HIV-2 resistance will be added:

- Therapy failure should be judged on both viral load measurements and CD4 counts.
- Resistance testing should be performed when the patient has therapy failure.
- Both genotyping and phenotyping should be considered, since genotyping is difficult to interpret.

Current guidelines recommend that resistance testing is routinely used today as a tool before treatments is started in HIV-1 infected individual. If virological failure is seen resistance testing should be performed before therapy switch. The available tests are based on two different principles, the genotypic test and the phenotypic test. The genotypic test is based on RT-PCR and sequencing of the plasma RNA virus. The sequence is interpreted using one or several of the available algorithms (ANRS, REGA, Stanford). This is the most commonly used test; it is comparably cheap, fast and can be performed in an ordinary lab. The databases for interpreting the relevance of mutations found have gathered large quantities of data and the clinical relevance is good. The phenotypic test is time consuming and expensive. Today it is based on recombinant

virus assay (RVA) where the sample virus is PCR amplified and recombined *in vitro* in a HIV-1 vector. The recombinant virus carrying the patient's specific pol sequence is then grown in the presence of drugs in different concentrations. However the interpretation of this test is not simple, the *in vitro* results are not always comparable to *in vivo* drug efficacy. The test most widely used and recommended is the genotypic test. The drawback is when a new drug becomes available and the specific resistance-associated mutations are poorly known. For HIV-2 resistance testing there are no standardized algorithms for interpretation of genotypic resistance assays. The only laboratory doing phenotypic testing for HIV-2 is located in Portugal.

SUMMARY OF SIMILARITIES AND DIFFERENCES OF HIV-1 VS. HIV-2

Table 4. Similarities and differences between HIV-1 and HIV-2.

	HIV-1	**HIV-2**
Geographic distribution	Global pandemic	Endemic in West Africa
Transmission		
Transmission routs	Sexual, vertical and blood	
Transmission incidence, sexual	Significantly higher for HIV-1	
Transmission incidence, vertical	15-35 %	< 5%
Age specific prevalence	Peak at 20-40 years of age	Increases with age
Immunology		
Progression to AIDS	10-12 years	Slow, >20 years
CD4 decline	Faster	Slower
Co-receptor usage	Narrow, CXCR4 and CCR5	Broad *in vitro*
Apoptosis	Higher	Lower
Neutralizing antibody	Neutralization escape	Efficient, broad specificity
CTL response	Escape mutations are common	Stronger
Virology		
Proviral load	Equal proviral load	
Plasma viral load, in asymptomatic stage	Detectable	Undetectable
Genetic similarity	40 – 60 % homology	
Genome structure	Vpu gene	Vpx gene
Virus isolation in asymptomatic stage	Possible	Difficult

Modified from H.C Whittle 1998 (Whittle et al. 1998)

MATERIALS AND METHODS

PATIENTS

All known HIV-2 infected living in Sweden have been included in this thesis. Within this thesis RNA quantification and to some extent experimental genotyped resistance testing has been provided to the clinics treating HIV-2 infected individuals. Thereby it has been easier to perform the research in this thesis.

To date we have follow-up on 24 patients. This number has varied over the years as this thesis work has progressed, a few patients have moved from the country and others have been diagnosed and included. All patients asked, except one, has been positive to take part in the studies we have performed. The follow-up varies from 20 years in one single patient, to only a few years on some of the more recently diagnosed patients. Several of the patients have good follow up; they attend a clinic on a regular basis. The visits to the clinic vary from once a year to every three months depending on the HIV-2 status. When the patient is on treatment a closer follow up is needed, however if it is just monitoring a HIV-2 infection during the asymptomatic phase once a year is sufficient. Almost all of the HIV-2 infected are of West African origin and are immigrants in Sweden and most of the patients live in the cities of Stockholm and Göteborg (18 out of 24). Several of them are healthy with low or undetectable viral load and normal CD4 counts. A few of the patients have advanced HIV-2 infection and three of the patients have AIDS diagnosis.

Antiretroviral therapy has been given to several of the patients at different time intervals. A few of the females have had treatment during pregnancies according to the recommendations for HIV-1(Connor et al. 1994). The antiretroviral treatment used for the patients has been following the recommendations for treatment of HIV-1 infection. A few of the patients were started on therapy at diagnosis of HIV-2 and have had therapy since; one patient has been on ART with few short interruptions for 16 years. In paper I and II the subtype of the virus has been investigated for the patients by DNA sequencing and they were all subtype A.

Table 5. Twenty-two of the HIV-2 infected patients followed within the frame of this thesis work.

Patient study code	Sex	Year of birth	Year of HIV diagnosis	HIV status	Treatment	Included in papers
1	F	1963	1985	Symptomatic	ART	I II
2	F	1963	1992	AIDS	ART	I II
3	F	1959	1992	Asymptomatic	ART during pregnancies	I II
4	F	1969	1990	AIDS	ART	I II III
5	M	1969	2000	Unknown	Unknown	I
7	F	1978	1993	Asymptomatic	Naïve	I II
8	F	1966	1995	AIDS	ART	I II
9	M	1939	1991	Asymptomatic	Naïve	I II
10	M	1941	1997	Unknown	ART	I II
11	F	1965	1993	Unknown	Unknown	I
12	F	1972	1997	Unknown	ART during pregnancies	I II
13	M	1965	1999	Unknown	Naïve	I
14	M	1960	1990	Asymptomatic	Naïve	I II
16	F	1952	1988	Asymptomatic	Naïve	I II
18	F	1969	1994	Unknown	ART	I
19	M	1962	1993	Unknown	ART	I
20	M	1963	1993	Asymptomatic	Naïve	I
21	M	1984	1993	Asymptomatic	Naïve	I
23	M	1969	2001	Asymptomatic	Naïve	I II
24	F	1960	2001	Asymptomatic	Naïve	I II
25	M	1965	1994	Unknown	Unknown	
26	M	1982	2003	Symptomatic	ART	II III

Naïve has never had antiretroviral treatment.

METHODS

RNA quantification

(Paper I, II, III, IV)

Two different systems to quantify RNA levels have been used: one experimental test from Roche and a commercially available test from Cavidi Tech. In brief, the Roche (Alameda, CA, USA) test was based on plasma HIV-2 RNA levels measured by extracting RNA and performing a RT PCR and analyzed with an ELISA detection set-up (Andersson et al. 2000). RNA was extracted from 200 µl plasma; the volume was chosen to save patient material. With our setup the assay had a lower detection limit of 125 HIV-2 RNA copies/ml. In the RNA extraction step an oligonucleotide (quantification standard, QS) was added in a known amount as an

internal control. The QS had the same primer binding sites as HIV-2 with a unique intervening region. A 202 base pairs fragment of the HIV-2 LTR region was amplified in a RT -PCR along with the QS fragment that had the same size as the HIV-2 fragment. The PCR product was denatured with denaturation solution immediately after the final elongation step in the PCR. An ELISA-like method was used for detection; wells coated with a HIV-2 specific probe or with a QS specific probe were used. The PCR product was titered out in a 96-well plate and the optical density was read in a spectrophotometer. The values were then converted to HIV-2 copies /ml using the following formula:

HIV-2 RNA copies/ml = (total HIV-2 OD) x Input QS x 10 / (total QS OD)

The ExaVir$^{®}$ (CavidiTech, Uppsala, Sweden) (Greengrass et al. 2005) test is based on measuring RT activity. This test could therefore be used on HIV-2 as well as on SIV and HIV-1 M, N and O. Briefly, 1ml plasma was used and RT was extracted with the use of a column where the viral particle would bind and in a later step were denatured and RT was released. The detection was ELISA-like and the wells were coated with a RNA strand and a primer (Figure 4). If the added sample contained RT a complementary DNA strand was made. A specific antibody detected the DNA. The results were measured in a spectrometer at several different time intervals to be able to see both high and low levels of RT. A standard was used in the test from the start and the result was calculated from the standard using software provided by the company and converted into RNA copies/ml.
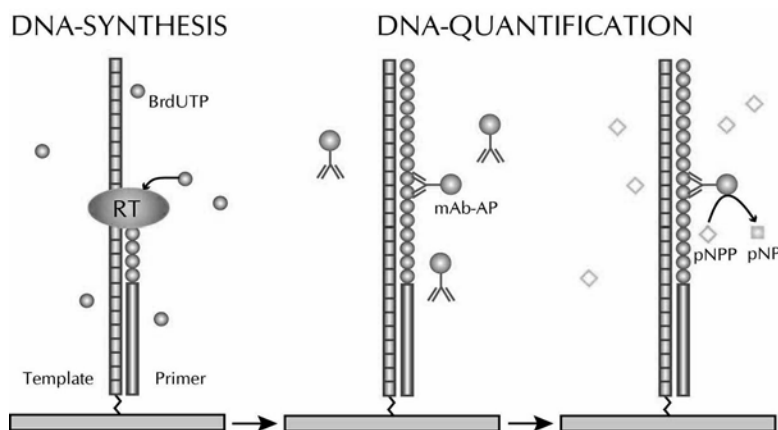

Figure 4. Shows the detection of RT in the well of the ELISA plate in ExaVir$^{®}$. BrdUTP RT substrate, mAb-AP AP conjugated antibody, pNpp colorimetric substrate for AP (From Corrigan, CavidiTech).

Direct sequencing of *pol* and *env* genes

(Paper I and II)

A 1305 base pair region corresponding to protease and the first half of the reverse transcriptase were amplified by nested PCR from plasma samples and were directly sequenced. RT-PCR was first attempted from RNA isolated for RNA quantification by the Roche assay. If amplification was unsuccessful, new RNA isolations were performed with the Nuclisense method or RNA extraction with the QIAamp® metod and if that failed PBMC, if available, were used for DNA extraction with the QIAmp® method.

A 1588 base region covering gp125 in the envelope gene was amplified from supernatants from virus cultures. RNA isolation was performed from 200μl supernatant with the Nuclisense method. Primers and detailed set up of the PCR are well described in Paper I (Brandin et al. 2003) and Paper II (Shi et al. 2005).

The PCR products were purified using the QIAquick® PCR Purification Kit. Cycle sequencing was carried out with the ABI Prism Big Dye according to the manufacturers recommendations and the sequences were run on an automated DNA sequencer model 310 ABI Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The sequences were analyzed using the software program Sequencer (Genecode Corporation, Ann Arbor, MI, USA). Alignments were made using reference sequences from the Los Alamos database and with the use of the software program BioEdit (Hall 1999). Neighbor-joining phylogenetic trees were constructed using the Tree Con (Van de Peer et al. 1994) or MEGA v2.1 software.

Virus stock preparation

(Paper II)

Virus stocks were prepared from patients PBMC grown on blood donors PBMC pre-stimulated with PHA for three days. After seven days a cross-reactive ELISA for both HIV-2 p24 and HIV-2 p27 was used to test the cultures for positive virus. When the cultures were positive supernatants were collected and stored in -70°C.

Coreceptor usage determination

(Paper II)

Coreceptor usage was determined by using U87.CD4 cells expressing CCR1, CCR2, CCR3, CCR5, CXCR4 and GHOST(3) cells expressing CCR3, CCR5, CXCR4, CXCR6 or BOB. Parental U87.CD4 and GHOST(3) without expression of any coreceptors were also included. Presence of syncytia and positive p27 ag test were scored as usage of a specific coreceptor.

Neutralization assay

(Paper II)

Neutralization was tested with the use of a test developed by Shi *et al*. (Shi et al. 2002). The principle of the neutralization test is plaque formation in U87.CD4 cells. IgG was separated from the plasma samples before the neutralization test to avoid any interference of antiretroviral therapy. The virus stocks were diluted to contain 20 – 50 p.f.u. / well (plaque forming units per well) this was an appropriate number to handle when analyzing the result. The purified IgG was mixed with diluted virus for an hour in 37 °C and then added in dilutions in triplicate or if lack of material duplicate wells to U87.CD4-CXCR4 or U87.CD4-CCR5 cells grown in micro titer plates. Results were read as plaques after haematoxylin staining in a light microscopy after three to four days. The neutralizing capacity of the serum was calculated by the formula:

[1-(p.f.u. with serum/p.f.u. without serum) x 100]

Which expresses the degree of reduction of p.f.u. in the presence of serum compared to wells without serum.

Mathematical calculations

(Paper III and IV)

We performed calculations on the viral dynamics in two of the papers; we used two different mathematical models to do this.

In paper III the log RNA viral load corresponded to exponential decay; due to this a linear regression could be used. The slope (S) was used to calculate the half-life for HIV-2 with the formula $t\frac{1}{2} = -\ln(2)/S$. Similar calculations have been used to estimate the dynamics for HIV-1 by Ho *et al*. and Wei *et al*.(Ho et al. 1995; Wei et al. 1995).

Patient 26 in paper III was sampled frequently during the first week on therapy and we saw an expected pattern of a lag phase and a decline in two parts as has been described for HIV-1(Perelson et al. 1996). We performed a piecewise linear regression on the data from the first week (day 1-7) and also a separate linear regression on the data from days 16 - 29.

The expected pattern of clearance of virus producing cells is three to four phases of decay. First there is delay in viral decline, a shoulder or a lag phase representing the time it takes for the drugs to be administer in the body, then the virus decline in two or three phases depending on the follow-up time. A rapid decay followed by a slower decay and if follow-up is long enough a fourth even slower decay can be seen (Stevenson 2003).

In paper IV we used a piecewise linear regression calculation as in paper III and we also used a more sophisticated non-linear method. The SIV infected macaques were sampled more frequently than the patients infected with HIV-2 included in paper III. The more sophisticated model does not have a clear distinction between the first two decay phases. The non-linear model that we used had the following expression:

$$v(t) = \begin{cases} v_a + v_b & for \quad t \leq 1 \\ v_a \exp(a(t-1)) + v_b \exp(b(t-1)) & for \quad 1 < t \leq 7 \\ v(7) \exp(c(t-7) & for \quad 7 < t \end{cases}$$

where v(t) is the virus load at time t (days), v_a and v_b are the initial cell loads in the two different compartments with different decay rates.

ETHICAL CONSIDERATIONS

Ethical approvals for the studies were received from the Ethics Committee of Karolinska Institute (96-189, 99-462, papers I - III) and from the Swedish Animal Committee on Animal Experiments (N73/03, paper IV).

RESULTS AND DISCUSSION

HIV-2 has not been as extensively studied as HIV-1, mainly because HIV-1 has caused the pandemic while HIV-2 is confined to West Africa. Although many similarities are seen between the two viruses there are extensive differences as well. HIV-2 is less pathogenic, less transmissible and gives rise to a stronger immune response. Broader neutralization, more CTL response and more efficient T helper response has been seen in HIV-2 infected as well as higher CD4 counts and lower HIV-2 viral loads in plasma (Andersson 2001; Reeves et al. 2002). All these different aspects could give very important answers to understand the pathogenesis of HIV.

In this thesis we have focused on understanding more about HIV-2 and learn more of how the HIV-2 virus affects the human being as well as how the virus changes during disease progression. We have studied the evolution of the virus longitudinally in both the *pol* and *env* genes. We have also tried to characterize resistance-associated mutations occurring during virological failure in HIV-2 infected patients on antiretroviral therapy. We have calculated the viral dynamics in HIV-2 infected patients as well as in a SIV model. We have tried to investigate the co-receptor usage during disease progression and the longitudinal autologous neutralization response.

POL GENE EVOLUTION IN HIV-2 INFECTED INDIVIDUALS

When combining our data to what other research groups (van der Ende et al. 1996; Rodes et al. 2000; van der Ende et al. 2000; Smith et al. 2001; Colson et al. 2004; Damond et al. 2004; Pieniazek et al. 2004; Rodes et al. 2006a; Rodes et al. 2006b) have reported it is clear that interpretation of HIV-2 resistance tests cannot be performed with the same algorithms that are used for HIV-1. HIV-2 shows a different pattern of resistance associated mutations and has different polymorphic sites as compared to HIV-1.

Several of the available drugs on the market cannot be used for HIV-2. This includes the whole NNRTI group (Witvrouw et al. 1999; Isaka et al. 2001; Witvrouw et al. 2004) and the entry inhibitor (T-20) as well as a few protease inhibitors which likely have reduced virulence against HIV-2 (Parkin et al. 2004; Witvrouw et al. 2004). With this knowledge it is obvious that we need to learn more about how to care for the

HIV-2 patients, which drugs to use and we also need to set up algorithms for resistance testing for HIV-2.

There is a newly started European HIV-2 collaboration, ACHIeV$_2$E, which will first focus on HIV-2 RNA quantification and standardizing the quantification. ACHIeV$_2$E can continue to work with guidelines for treatment as well as standards for resistance testing. Today many labs run their own in-house quantification test and comparing results are thereby difficult. This European collaboration has strength in combining the knowledge from many groups around Europe, hence increasing the number of patients conclusions are drawn from.

In paper 1, 20 of the known HIV-2 patients in Sweden were included. Viral loads, CD4 count, treatment and *pol* gene sequences were studied, virus from 12 of the patients were sequenced in the *pol* gene. Sequencing failed in eight patients who all had CD4 >500 and low viral load. All sequenced viruses were of HIV-2 subtype A. Five of the patients experienced virological treatment failure and were followed longitudinally to investigate the possible evolution of drug resistance-associated mutations. Protease and part of RT was sequenced and the sequences were aligned to each other as well as to reference HIV-2 sequences from the Los Alamos database. We were looking for mutations known to be involved in resistance in HIV-1 as well as novel mutations occurring over time in the HIV-2 infected individuals on antiretroviral treatment. One of the strengths of our study compared to earlier studies was that we had longitudinally followed our patients, a few of the patients were followed before treatment were started, thus we could compare wild-type virus to virus which has evolved during antiretroviral therapy. In our study we observed mutations at a few well-described sites that confer resistance in HIV-1 and in one case also in HBV, i.e. the lamivudine resistance mutation M184V in RT (Allen et al. 1998; Johnson et al. 2005). One resistance mutation that is common in HIV-1 infected treated with zidovudine is the T215F/Y substitution, which was not seen in our patients even though they had received zidovudine. Instead we saw E219D, which is a secondary mutation in HIV-1. All of our HIV-2 viruses sequenced had mutations at one or both of position 181 and 188 in RT, these mutations are known to lead to resistance towards NNRTI in HIV-1. This was expected since HIV-2 has inherent resistance towards the NNRTI group and this is likely due to differences at the positions 181 and 188 seen in RT between HIV-1 and HIV-2. We saw also mutations occurring at position K65R, N69S, K70S, and

Q151M in RT, all major resistance associated sites in HIV-1. The mutation at position 151 in RT is rare in HIV-1, but is associated with multi-drug resistance. However in HIV-2 the Q151M appears to be more common. Whether or not it cause multi-drug resistance is not completely clear. In one of our patients with the Q151M mutation we saw a large increase in viral load after treatment interruption, suggesting the NRTI had sustained antiviral effect we also saw a reversion back to wild type virus suggesting that there was a fitness cost for the virus.

When we have sequenced our samples to investigate resistance-associated mutations protease (99 aa) and a part of RT (250 aa) was covered. This is the part of *pol* that is known to hold almost all resistance-associated mutations in HIV-1, however we cannot be certain that this is true also for HIV-2. Thus it cannot be excluded that mutations in gag cleavage sites and the carboxy-terminus of RT may be more important in HIV-2. If sequencing should be performed for the complete *gag-pol* this would be more cumbersome since it is a large fragment to PCR amplify and sequence.

One of the patients (patient 8) whom we followed closely in paper 1 has been on continuous follow-up and additional resistance testing was performed. Patient 8 has had HIV-2 diagnosis since 1995, antiretroviral treatment since 1996 and AIDS diagnosis since 2001. She has an advanced HIV-2 infection. Several different treatment combinations including NRTI and PI have been given over the years, however the adherence has been low at times, but nevertheless she has been adherent enough to generate extensive evolution in the protease gene. RT gene has not shown such extensive evolution although mutations have been observed at a few sites, which, most probably is resistance, associated mutations, i.e. K70S, M184V, W219K as well as some rare mutations I10V, R20K, R35K, K40R and T163A.

```
Pat8-95       PQFSLWKRPV VTAYIEEQPV EVLLDTGADD SIVAGIELGN NYTPKIVGGI GGFINTKEYK DVEIKVLNKR VRATIMTGDT PINIFGRNIL TALGMSLNL
Pat8-97       ---------- ---------- ---------- ---------- ---------- ---------- N--------- ---------- ---------- ---------
Pat8-00       ---------- ---------- ---------- ---------- --I-R----- ---M------ N--------- I--------- ---------- -T------F
Pat8-01-apr-1 ---------- ---------- ---------- --------S --I-R----- ---M------ N---E----- I--------- ---------- ------F
Pat8-01-apr-2 ---------- ---------- ---------- --------S --I-R----- ---M------ N---E----- I--------- ---------- -T------F
Pat8-01-aug   ---------- ---------- ---------- ---------- --I-R----- ---M------ N--V------ I--------- ---V------ -T------F
Pat8-01-nov   ---------- ---------- ---------- ---------- --I-R--A-V ---M------ N--V------ I--------- ---------- -T------F
Pat8-03-sep   ---------- ------G--- ---------- --I------- --I-R----V ---M------ N--V------ I--------- ----L---V- -T--V---F
```
Figure 5. Alignment of protease from patient 8 from 1995 until 2003. Several unique mutations are seen.

The alignment for protease is shown in figure 5 with the wild type virus from 1995 and the latest virus from 2003. More recent samples have been tried for sequencing but have failed on several occasions from several different samples. It is possible that more

evolution has occurred in the virus so that the primers used in the PCR are mismatched. We are currently investigating this. This is one of the most evolved HIV-2 protease gene described, apparently the HIV-2 protease gene can harbor a number of mutations without losing function.

As a clinician caring for HIV-2 patients it is necessary to search for information on how to use the available (NRTI and a few PI) antiretroviral drugs. The knowledge from treating HIV-1 patients can only partly be of help. In Sweden most of the HIV-2 infected live in the bigger cities and thereof the clinics they attend has more experiences.

One of the new topics in the European Guidelines mentioned earlier were that all HIV-2 patients experiencing treatment failure should be resistance tested before any changes in therapy. This is a very good vision but today there are few laboratories performing genotypic resistance testing for HIV-2 and there is only one laboratory, in Portugal, performing phenotypic testing for HIV-2. We have not gathered enough information to analyze the results correctly. On the other hand if testing was centralized to one lab, there would be a good opportunity to collect data and perform genotypic and phenotypic testing as well as to be able to set the standards for HIV-2 treatment and resistance testing.

Today the collected knowledge on HIV-2 treatment and resistance-associated mutations is essentially based on case reports from few scientific groups. Mostly genotypic data is performed but also a few studies are based on phenotypic data. I will try to gather the information on the treatment options based on available phenotypic data we have for HIV-2 infected individuals. The NNRTI group does not work for HIV-2, phenotypic data from Witvrouw *et al*. shows decreased susceptibility to nevirapine, efavirenz and delavirdine (Witvrouw et al. 2004). The same study also shows reduced susceptibility to amprenavir, Rodés *et al.* also show this (Rodes et al. 2006a). In the study by Rodés *et al.* they showed decreased susceptibility for nelfinavir as well. If this is true this leaves the drugs in Table 6 for HIV-2 antiretroviral treatment. When discussing treatment options with experienced scientists, mainly from Portugal, Spain or France, where there are many HIV-2 cases, they all agree to wait longer with the treatment since the disease progress more slowly and since the treatment options are fewer and the treatment fails more easily.

The combinations given to achieve optimal therapy should include two NRTIs (van der Ende et al. 2003) and a double boosted protease (personal communication Dr R. Camacho). The double boosted protease should contain ritonavir-boosted lopinavir in combination with indinavir or saquinavir.

Table 6. Drugs to use for HIV-2 antiretroviral therapy based on published phenotypic susceptibility data along with resistance mutations associated with reduced phenotypic susceptibility.

Nucleoside RT inhibitors (NRTI)	Probable resistance associated mutations, RT	Protease inhibitors (PI)	Resistance associated mutations, PI
Zidovudine (AZT)	M151Q	Ritonavir (RTV)	I54V V82F*
Stavudine (d4T)		Indinavir (IDV)	V47A, I54V V82F *
Lamivudine (3TC)	M184V	Saquinavir (SQV)	
Emtricitabine (FTC)		Lopinavir (LPV)	V47A, I54V V82F *
Didanosine (ddI)	M151Q	Tipranavir (TPV)	I54V V82F *
Abacavir (ABC)		Atazanavir (ATV)	I54V V82F *
Tenofovir*			

PI, * I54V together with V82F cause multi-resistance in all PI except SQV.

Interpretation of genotypic resistance testing is very difficult but there are a few phenotypic studies which one can draw a few conclusions from. In RT the M184V mutations also known in HIV-1 and HBV does cause resistance to 3TC (Adje-Toure et al. 2003). The Q151M leads to multi-resistance in HIV-1. However the mutation seems to occur more often in HIV-2 infected and correspond to low level drug resistance as shown by Adjé-Touré *et al.* where two patients acquired the M151Q and had resistance to didanosine and zidovudine (Adje-Toure et al. 2003). The V47A mutation in protease has been described to lead to high level resistance to indinavir and lopinavir (Rodes et al. 2006a; Rodes et al. 2006b). They also showed that mutation I54M and V82F along with natural occurring changes in protease resulted in a multi-PI-resistant virus where only saquinavir retained full activity.

CO-RECEPTOR USAGE, NEUTRALIZATION, GLYCOSYLATION AND EVOLUTION IN HIV-2

In this paper II we try to investigate why HIV-2 is less virulent than HIV-1. We have studied the evolution of co-receptor usage, autologous and heterologous neutralization, envelope sequence and glycosylation in longitudinal samples.

It is well known that HIV-2 has a more promiscuous use of co-receptors as compared to HIV-1, *in vitro*, but the importance of this *in vivo* is not clear (Morner et al. 1999; Morner et al. 2002). However the major co-receptors used in both HIV-1 and HIV-2 are the same i.e. CCR5 and CXCR4 (Blaak et al. 2005). In HIV-1, it is known that a switch from CCR5 to CXCR4 use often occurs during disease progression (Åsjö et al. 1986). This has not been investigated for HIV-2 even though is has been reported that CXCR4 are usually found in patients with advanced disease. In this study we have shown that HIV-2 can switch from CCR5 to CXCR4 usage during disease progression.

In HIV-1 is has been shown that escape from neutralizing antibodies is rapid early in infection and autologous neutralization is low or absent later in infection. Heterologous neutralization in HIV-1 is often absent or low (Albert et al. 1990a; Scarlatti et al. 1993; Fenyö et al. 1996; Wei et al. 2003). It is also known that in HIV-2 it is more common with autologous neutralization response than in HIV-1 infection (Bjorling et al. 1993). However, it has never been studied longitudinally in HIV-2 before. In our study we have four HIV-2 infected individuals selected on the basis that we had matched sera and virus isolate over time to be able to do the autologous neutralization evolution studies. We also selected 11 patients' sera for heterologous neutralization tests. We saw no clear pattern of neutralization escape. The sera could neutralize the autologous viruses but also viruses from other HIV-2 infected Swedish patients virus as well as a subtype B HIV-2 virus. Thus, HIV-2 appears less able than HIV-1 to escape neutralization.

In the variable region 3 (V3) in the SU protein we found a correlation between the overall charge and the co-receptor usage. All isolates that could use CCR5 had a net charge of +5 or +6 and all isolates that used CXCR4 had a net charge of +7. All isolates that lacked the ability to use CCR5 displayed a positively charged amino acid (arginine or lysine) at position 323 in the V3 loop, while all isolates that could used the CCR5 displayed a neutral amino acid (valin or isoleucine) at the same position. This is in

agreement with what has been described for HIV-2 in earlier studies as well as in agreement with data on HIV-1 (Fouchier et al. 1992; Albert et al. 1996; Isaka et al. 1999).

The enveloped gene from the viral isolates used in this study was sequenced. We saw that the variable regions 1 (V1) and 2 (V2) of the surface protein (SU) varied in length and that this was associated with the rate of disease progression in the patients. Thus, V1 and V2 increased in length in the healthier patients, while the length was stable in the two patients with more advanced HIV-2 infection. Similar changes in simian immunodeficiency virus have been described and was reported to correlate with neutralization escape (Chackerian et al. 1997; Rybarczyk et al. 2004). It is known that the V1/V2 of HIV-2 contains neutralizing epitopes (McKnight et al. 1996). It is surprising that despite the elongation we see in two of our patients of V1/V2 we did not see any pattern of neutralizing escape in these two patients.

We also investigated the potential glycosylation sites of the isolates and found that the changes in the glycosylation pattern were associated with disease progression. The isolates that had a longer V1/V2 region also had an increased number of glycosylation sites and had a less advanced HIV-2 infection. The number of potential N-linked glycosylation sites varied between 23 and 29. HIV-2 shows fewer potential glycosylation sites in V3 than reported for HIV-1. HIV-2 had 2-3 sites compared to HIV-1 that has reported 4-5 potential glycosylation sites (Figure 3, paper II). In HIV-1 it has been shown that a de-glycosylation of V3 leads to a broader co-receptor usage (Pollakis et al. 2001). It has also been shown that if glycans are removed, HIV-1 show an increased sensitivity to neutralizing antibodies (Benjouad et al. 1992; Derdeyn et al. 2004; McCaffrey et al. 2004; Nabatov et al. 2004). It has also been shown for HIV-1 that fewer glycosylation sites in V1/V2 leads to a lower viral set point and thereby has an impact on disease progression (Chohan et al. 2005). This is in agreement with HIV-2 since HIV-2 infection generally has a lower set point, lower viral load and a slower disease progression.

In light of this we propose that HIV-2 has fewer glycosylation sites, thereby leaving HIV-2 with a more open and accessible V3 loop, which will be more easily neutralized by neutralizing antibodies.

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VIRAL DYNAMICS IN HIV-2 INFECTED INDIVIDUALS

To understand why HIV-2 is less pathogenic than HIV-1 we decided to see if there were a difference in the viral dynamics of the two viruses. We hypothesized that we would see a difference. HIV-2 is less pathogenic and we believed we would find a slower viral turn over of actively virus producing cells *in vivo* in HIV-2 as compared to HIV-1.

The viral dynamics of HIV-1 were studied more than 10 years ago (Ho et al. 1995; Wei et al. 1995) but the viral dynamics of HIV-2 has never been studied before. Two of the HIV-2 patients in our cohort in Sweden who were starting treatment agreed to be sampled for this investigation. Patient 4 has been followed for several years; she was diagnosed as HIV-2 positive in 1990 and has had treatment since, she has reached AIDS. After a treatment interruption, when she was resuming therapy, she agreed to be sampled every week for a period of five weeks. Patient 26 was diagnosed in 2003 and started treatment a year later due to immunodeficiency. He agreed to be sampled frequently for a period of one week as well as a follow-up period with sampling once a week for five weeks.

The plasma HIV-2 RNA viral load was measured and the values plotted in a graph. For patient 4 we saw a decline and then a rebound after one month (Figure 1 in paper III). In-patient 26, who was sampled more frequently, we saw a lag phase, as expected, and then a rapid decline followed by a slower decline (Fig.1 in paper III) (Perelson et al. 1996; Perelson et al. 1997). The decline for both patients were exponential, this made it possible to use a piecewise linear regression analysis to calculate the rate of virus decline.

The slope for patient 4 was -0.32 ± 0.03 corresponding to a half-life of 2.2 days and for patient 26 the slope was -0.35 ± 0.03 corresponding to a half-life of 2.0 days. Our results are very similar to data reported for HIV-1using the same mathematical model, which was 2.1 and 2.0 respectively (Ho et al. 1995; Wei et al. 1995).

As patient 26 had been sampled more frequently, we could distinguish the different phases in the viral decline where a lag phase of approximately a day was followed by a rapid decline and then a slower second decline (Perelson et al. 1997; Stevenson 2003). Therefore we split the data from patient 26 so we could calculate both the rapid first

phase decline as well as the slower second decline. A piecewise linear regression was performed on viral loads from days 1-7 and days 16-29 (Figure 1 paper III). We saw that the change between the first and second phase occurred between days 7 and 16 but our patient was not sampled on the day of transition. The slope calculated for days 1-7 was -0.53 ± 0.07 corresponding to a half-life of 1.3 days and the slope calculated for days 16-29 was -0.046 ± 0.006 which corresponds to a half-life of 15 days. These estimates done on relatively infrequent sampling and simple calculations are surprisingly similar to those of Perelson *et al*. for HIV-1 based on sophisticated mathematics and extensive sampling. They saw a first phase decline of 1.55 ± 0.57 days and a second phase corresponding to 14.1 ± 7.5 days (Perelson et al. 1997).

The unexpected similarity seen between the HIV-1 and HIV-2 clearance *in vivo* indicate that the clearance of infected virus producing cells from the body occurs with the same rate regardless of the infection. After giving it some thought is not so surprising, HIV-1 and HIV-2 probably has the same replication cycle rate in vivo and the virus infected cells die at the same rate. However it does not give us any answers as to why HIV-2 is less pathogenic that HIV-1.

VIRAL DYNAMICS IN SIV INFECTED MACAQUES

We have continued to study the viral dynamics to understand more about the HIV pathogenesis. In paper IV macaques with a chronic experimental SIVsm infection received a quadruple therapy regimen and viral load was measured. Nowak *et al*. has studied the dynamics in macaques before and estimated a half-life for SIV clearance to 0.7 – 1.4 days (Nowak et al. 1997). This is likely an underestimation since the macaques in their experiments received monotherapy, which we know today, is not the appropriate treatment for HIV/SIV infection.

In this study we followed four experimentally infected macaques during a two-week period. Quadruple treatment was given subcutaneous and was terminated on the 7th day due to injection site reactions. Blood samples were drawn every day and SIV plasma viral load was measured. The values were plotted in graphs and we saw as expected a lag phase and then a rapid decline. The four monkeys reacted very differently after the first phase thereby no conclusions can be drawn from the later time points. We calculated the rate of the rapid decline using two different mathematical calculations, a

piecewise linear regression model as well as a non-linear model. The two models gave similar results and the four different animals showed similar results, thus the estimated half-life was 0.5 days. This is the first realistic estimate of viral dynamics in experimental SIV infection in macaques reported. It is also the fastest decay reported for any lentivirus.

This faster half-life could be due to differences between the two viruses of hosts or, more simply, the fact that it was a very potent treatment administered in a very controlled fashion.

The quantification test used in this paper IV is based on RT activity measurement, which is quite different from the standard method used for HIV-1 quantification in the routine setting. One important issue to consider is that the drugs given should not interfere with the test results. This however has been tested, samples has been run on standard RNA quantification tests as well as with the ExaVir® and the results has not been significantly different (personal communication G. Corrigan).

All studies in which the dynamics of HIV or SIV has been investigated has assumed a complete stop of new infection by the virus. Otherwise other mathematical models would have had to be used. When very strong antiretroviral therapy is given we come closer to the actual truth of stopping all new infection. The data from Markowitz *et al*. indicate that this assumption is not completely fulfilled with most standard regimens. Markowitz *et al*. did calculations on HIV-1 dynamics using a quadruple regimen and they showed that the earlier measurement of HIV-1 half-life were underestimations, the half-life they reported was 0.7 days for HIV-1, this is considerably shorter than what has previously been reported (Markowitz et al. 2003). One of their conclusions is that the standard antiviral therapies used at that time are not as effective as the quadruple therapy they have used in their experiment. This is interesting in view of the available antiviral drugs for HIV-2; it would be interesting to do similar studies to see the potency of the drugs towards HIV-2.

CONCLUDING REMARKS

My specific aims in this thesis were:

- ✓ To study the genetic variation in the HIV-2 *pol* gene in Swedish HIV-2 infected patients.
- ✓ To characterize mutations associated with drug resistance in HIV-2 infected patients experiencing virological failure.
- ✓ To study the HIV-2 co-receptor use over time in HIV-2 infected patients.
- ✓ To study how the neutralizing antibody response evolves in HIV-2 infected.
- ✓ To study the rate of HIV-2 production and clearance *in vivo*.
- ✓ To study the rate of SIV clearance in SIV infected macaques *in vivo*.

The general conclusions drawn from this thesis are:

- ✓ Resistance testing cannot be performed in HIV-2 infected with the use of the same algorithms that are used for HIV-1. Additional studies are needed and a new and specific algorithm has to be made for HIV-2.
- ✓ It is important to note which drugs are effective when treatment is started in a HIV-2 infected individual.
- ✓ The HIV-2 protease gene can accommodate a large number of mutations without losing its function.

- ✓ HIV-2 can switch from CCR5 to CXCR4 co-receptor usage during disease progression.
- ✓ Neutralization escape is rare in HIV-2 infected.
- ✓ The V1/V2 region of the envelope gene varies in length in HIV-2 infected and an increase in length over time was seen in healthier patients.
- ✓ The charge of the V3 loop correlate's with the co-receptor use of the virus.
- ✓ Changes in the number of potential N- linked glycosylation sites were seen during disease progression and seemed to correlate with disease progression where the number of changes decreased as disease progressed.
- ✓ HIV-2 harbour fewer potential glycosylation sites in V3, which may leave HIV-2 with a more open and accessible V3 loop.

- ✓ HIV-1 and HIV-2 has the same clearance rate and production rate *in vivo*.
- ✓ Half-life of HIV-1 and HIV-2 represent the clearance of virus producing cells and this occurs with the same rate regardless of the virus.
- ✓ The first realistic estimate of SIV viral decay is 0.5 days. This is faster than what has been reported for HIV-1, if this is due to differences in the monkey model compared to humans is not known.
- ✓ The fastest decay reported for any lentivirus infection is 0.5 days.

There are still many questions unanswered regarding HIV-2 pathogenesis. However, I hope that the conclusion we have been able to draw from my thesis will help others to continue to gather information, which in the end is what science is all about.

SAMMANFATTNING FÖR LEKMÄN

> Vi är vana vid att epidemier har en början och ett slut. Kommer AIDS- epidemin att ha ett slut? Jag vet inte. Ingen vet det, för den är inte lik någon annan farsot som drabbat mänskligheten. Dess omfattning är redan större än någon annan känd epidemi i vår historia men det är inte bara det som gör den speciell; smittan är bunden till människans mest basala drift, den till fortplantningen, och den överförs till nästa generation i födelseögonblicket från mor till barn och genom den livgivande modersmjölken. Smittämnet införlivas livslångt i vår arvsmassa. Det selekterar de unga, starka samhällsbärarna. Det decimerar de åldrar som ska föra överlevnadskunskap vidare till nästa generation. Smittämnet är diaboliskt, det angriper hjärtat av vårt infektionsförsvar och är ogripbart genom en föränderlighet som är större än hos något annat känt infektionsämne. Det är dödligt.
>
> Ur Den yttersta plågan Boken om AIDS av Lars O. Kallings 2005

I juni i år är det 25 år sedan den första korta rapporten publicerades om det som blivit den världsomspännande HIV/AIDS epidemi som drabbat världen. Den första rapporten rörde en oro som flera läkare i USA kände då de hade sett flera unga starka män med mycket ovanliga sjukdomar. Snart insåg man att flera grupper i samhället var drabbade av denna nya sjukdom, inte bara de homosexuella männen som varit de första att uppmärksammas. Tyvärr finns det två sidor av att en specifik grupp pekades ut mycket tidigt i HIV/AIDS debatten. Vi har aldrig tidigare lärt oss så mycket på så kort tid om ett nytt virus och en ny sjukdom, detta tack vare att det var en grupp välutbildade, välsituerade män som drabbades först. Baksidan är att ännu idag tror många människor, även de som är lika välutbildade och välsituerade, att det bara drabbar homosexuella. Detta är ett stort problem i många delar av världen där HIV/AIDS inte tas på allvar av ledare vilket leder till att befolkningen drabbas mycket hårt.

Viruset som orsakade detta nya syndrom upptäcktes redan 1983 av en fransk grupp, ledd av Luc Montagnier, som döpte sitt virus till LAV. En amerikansk grupp, ledd av Robert Gallo, kunde snart verifiera deras fynd och döpte viruset till HTLV-3. För att förstå hur viktigt detta nya fynd ansågs vara kan man fundera över det faktum att det hela blev en politisk fråga där Frankrikes premiär minister och USA: s president deltog för att sluta en överenskommelse om patenträttigheter och vem som var den första att upptäcka viruset. Man överenskom om att Montagnier upptäckte HIV och Gallo fastställde att HIV orsakar AIDS. Så småningom (1986) fick viruset namnet human immunodeficiency virus, HIV.

1986 isolerades ytterligare ett HIV virus som kom att kallas HIV-2. HIV-2 har visats sig vara ett "snällare" virus som inte har samma smittsamhet och inte är lika

sjukdomsframkallande som HIV-1. Varför det är så vet man fortfarande inte, vilket är bakgrunden till mitt doktorandarbete.

Varifrån har dessa nya virus kommit? Det har skett ett hopp mellan arter. Många sorters apor i den gamla världen har sitt artspecifika virus (SIV) och viruset har spridits från apa till människa under jakt, slakt och möjligen också via bett där blodvite uppstått. De två HIV virus som är kända har spridits från olika apor till människan vid flera tillfällen. HIV-1 är orsaken till HIV epidemin som sprids över världen. HIV-2 är mest spritt i västra Afrika och i Europa finns flest fall i Portugal.

HIV är ett svårbehandlat virus. 1987 kom den första medicinen ut på marknaden och sedan dess har en 20 tal nya tillkommit och nya är hela tiden under utvecklande, men det räcker inte. Viruset har en fantastisk förmåga till att förändra sig snabbt och mycket lätt uppstår en variant som är resistent mot medicinerna. För att undvika detta i möjligaste mån ges tre till fyra mediciner tillsammans. För HIV-2 infekterade individer fungerar inte medicinerna lika bra då alla kända mediciner är utvecklade för HIV-1.

WHO summerade HIV/AIDS epidemin i december 2005 och visade att 40,3 miljoner människor lever med HIV och under 2005 smittades 4,9 miljoner. För att förstå dessa siffror så är det 9 människor i minuten som smittas med HIV!

I denna doktorsavhandling har jag arbetat med HIV-2. Vi vill försöka förstå varför HIV-1 och HIV-2 är så skilda i den sjukdom de orsakar. Om man kan förstå vad som gör HIV-2 mindre smittsamt, mindre sjukdomsframkallande och långsammare i sin sjukdom så kanske vi kan komma en bit på vägen om hur vi ska få bukt med HIV-1 epidemin. Vi har försökt identifiera hur resistensmönstret ser ut hos HIV-2 infekterade som får behandling men ändå inte har en kontrollerad sjukdom (artikel 1). Vi har studerat hur HIV-2 tar sig in i cellen som den infekterar. Vi har dessutom tittat på om HIV-2 infekterade individer bildar antikroppar som kan vara till hjälp och kanske är en av orsakerna till att HIV-2 är en "snällare" infektion. Vi har också sett att HIV-2 ser lite annorlunda ut och kanske inte kan skydda sig lika bra mot dessa antikroppar som vi bildar vid en infektion (artikel 2). Dessutom har vi beräknat hur snabbt HIV och SIV infekterade celler dör i samband med att de producerar virus i människa respektive apa (artikel 3 och 4).

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