1. Introduction

1.1 Retroviruses

The family of *Retroviridae* is divided into seven genera (Table 1). The virus-host interaction of most retroviruses is restricted to mammalians causing a variety of symptoms including oncological, neurological and immunodeficient deseases as well as inapparent courses of infection. Retroviruses have a single stranded RNA genome and a double stranded DNA genome serving as an intermediate product during virus replication and integration into the host cell genome. In 1970 H.M. Temin, S. Mituzami and D.Baltimore discovered the retroviral enzyme reverse transcriptase that allows the transcription of single stranded RNA into double stranded DNA. In 1980 Robert C. Gallo described for the first time a retrovirus inducing T-cell leukaemia in humans (HTLV) and shortly after the human immunodeficiency viruses HIV-1 and HIV-2 causing the aquired immune deficiency syndrom (AIDS) in infected humans (for review see Karpas 2004).

Table 1 Retrovirus genera. Adapted from Overbaugh et al., 2001

Genus	Morphology	Examples[a]
Alpharetrovirus	C type	RSV, ASLV
Betaretrovirus	B and D type	MMTV, SRV-1 to SRV-5, BaEV, JSRV, ENTV
Gammaretrovirus	C type	MoMLV, A-MLV, 10A1 MLV, X-MLV, P-MLV, AKV, GALV, MDEV, FeLV, PERV, RD-114, SNV, REV
Deltaretrovirus		HTLV-1, HTLV-2, STLV-1 to STLV-3, BLV
Epsilonretrovirus		WDSV
Lentivirus		HIV-1, HIV-2, SIV
Spumavirus		HFV, SFV

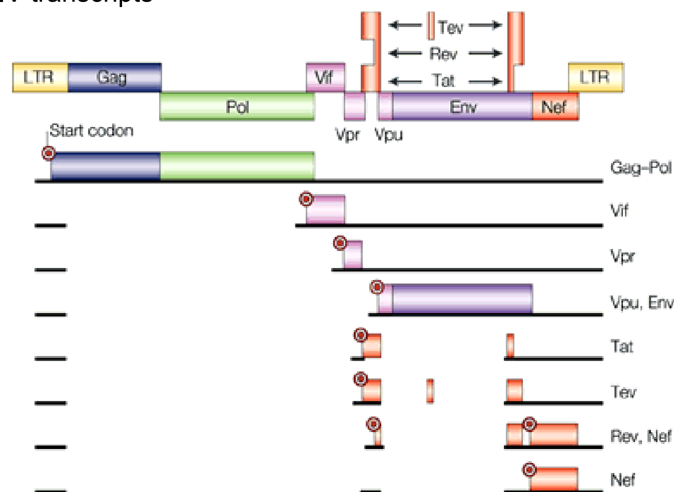
a RSV, Rous sarcoma virus; MMTV, mouse mammary tumor virus; ENTV, enzootic nasal tumor virus; MoMLV, Moloney MLV; X;MLV, xenotropic MLV; P-MLV, polytropic MLV; AKV, AKV MLV; MDEV, M. dunni endogenous virus; REV, reticuloendotheliosis virus; STVL, simian T-lymphotropic virus; BLV, bovine leukemia virus; WDSV, walleye dermal sarcoma virus; HFV, human foamy virus; SFV, simian foamy virus.

Retroviruses exist in two different forms: (i) Exogenous viruses bear genetic information necessary for the generation of replication competent viral particles and are able to be transferred from one organism to another. (ii) Endogenous viruses are vertically transmitted through the germline of its host and most of them have lost the genetic information to produce viral particles. For example retrotransposons are present in about one percent of the human genome, only existing of the genome flanking sequences of ancient retroviruses. However, under certain circumstances the function of endogenous viruses can be restored, e.g. by the infection of an exogenous virus showing a certain degree of homology with the endogenous one. For example subgroup B FeLVs evolve by recombination with portions of endogenous FeLV-like envelope sequences, which have a high degree (~80%) of homology to FeLV-A (Anderson et al., 2001).

All infectious retroviral particles show a similar structure with a diameter of about 100nm. The viral membrane is built up mainly by its host cell cytoplasm membrane, associated with the viral envelope proteins.

The matrix proteins (MA) are associated with the viral membrane by amino terminal myristyl acids forming an isometric structure of the particles. The virus capsid is encased by the viral membrane and contains the group specific antigen proteins (Gag). It contains two unattached copies of the single stranded RNA virus genome, which is associated with nucleocapsid proteins (NC). The capsid membrane is connected to the capsid core by the p6 Link protein (in HIV-1) and contains also the enzymatic reactive gag proteins reverse transcriptase, integrase and protease.

The retroviral genome is differing in sizes from 7000 to 12000 base pairs depending on the type of virus (HIV: 9000bp, FeLV-A 8500bp), encoded by a single stranded RNA consisting of a 5`cap structure and a 3´ polyadenylation signal. Retroviral genomes encode for proteins Gag (group specific antigens), Pol (enzymatic activities) and Env (envelope glycoproteins). More complex retroviruses such as lentiviruses, spumaviruses and HTLV encode additional regulatory and accessory proteins. The coding regions are flanked by regulatory control sequences termed long terminal repeats (LTR) (Fig.1A). These sequences of repetitions contain three different regions termed U3, R and U5 which are located at the 3´end and the 5´end in the provirus genome at the same orientation. The LTR bears all *cis* active sequences as well as elements of the promoter and enhancer sequences controlling the gene expression. In addition the LTR´s are essential for the process of reverse transcription and integration of the provirus DNA into the host cell genome. Cellular proteins transactivate the transcription of viral protein sequences mainly by binding to the U3 region. For example the nuclear factor kappa B (NFκB) and cytokines IL-1 or TNF-α bind to their specific sequence within the HIV-1 U3 region and activates viral transcription. This mechanism plays an important role in the pathogenic course of an HIV infection due to the fact that any stimulation of the immune system resulting in an increasing activity of NFκB may lead to the expression of HIV in its host.



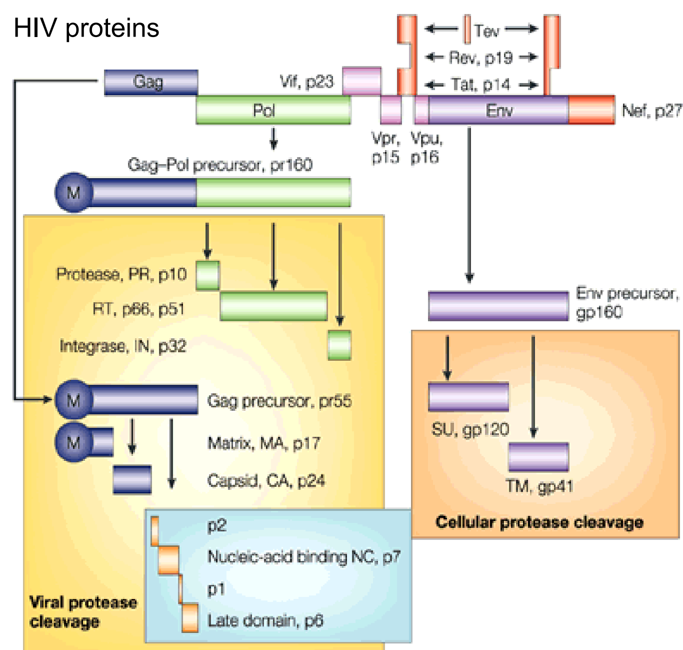


Figure 1 The HIV genome, transcripts and proteins. (**A**) HIV transcripts. Integrated into the host chromosome, the 10-kb viral genome contains open reading frames for 16 proteins that are synthesized from at least ten transcripts. Black lines denote unspliced and spliced transcripts, above which coding sequences are given, with the start codons indicated. Of these transcripts, all singly spliced and unspliced transcripts shown above those encoding the transcriptional transactivator (Tat) require regulator of virion gene expression (Rev) for their export from the nucleus to the cytoplasm. The RNA target for Rev, the Rev response element (RRE), is contained in the gene encoding envelope protein (Env). (**B**) HIV proteins. Group-specific antigen (Gag) and Gag–Pol (polymerase) polyprotein precursors are processed by the viral protease into nine subunits: protease (PR), reverse transcriptase (RT), which contains RNAse H, integrase (IN), matrix (MA), capsid (CA), p2, nucleocapsid (NC), p1 and p6 (shown in the yellow box). Env is cleaved by cellular proteases, such as furin, into surface (SU) gp120 and transmembrane (TM) gp41 moieties (shown in the orange box). Tat is the main transcriptional regulator of the long terminal repeat (LTR). Its RNA target, the transactivation response (TAR) element, is present at the 5' end of all viral transcripts. Rev is the main nuclear-export protein and it regulates the shift between early and late viral gene expression. The viral-infectivity factor (Vif), viral protein r (Vpr), viral protein u (Vpu) and negative effector (Nef) proteins are known as accessory proteins because they are dispensable for viral growth in some cell-culture systems. Nevertheless, they have essential roles in viral replication and progression to AIDS *in vivo*. Arrows below polyprotein precursors point in the direction of their processing to mature proteins. Tev contains Tat, Env and Rev sequences and functions as Tat and Rev. Adapted from Peterlin & Trono 2003.

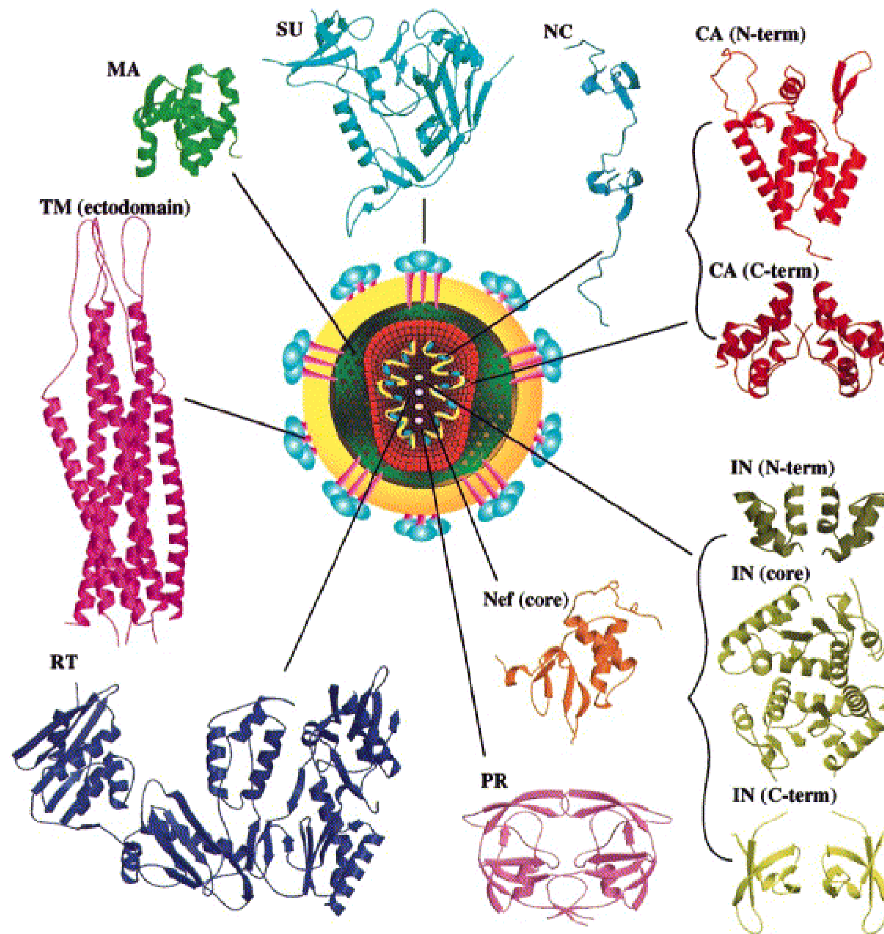


Figure 2 Drawing of the mature HIV virion surrounded by ribbon representations of the structurally characterised viral proteins and protein fragments. The protein structures have been drawn to the same scale. The TM ectodomain shown is that determined for the closely related SIV. Adapted from Turner and Summers 1999.

1.1.1 Gag (group specific antigens)

The retroviral *gag* gene encodes for a precursor protein with a size of 55kDa in case of HIV and 65kDa in case of FeLV, synthesized on ribosomes in the cytoplasm of the host cell. During maturation of the virus the protein is being processed by the viral protease into the matrix protein (MA), the capsid protein (CA), the nucleocapsid protein (NC) and in case of HIV also into the link protein (p6). The sequential position of the gag proteins located in the retroviral genome (Fig.1) is equal for all retroviruses. The gag precursor proteins are essential for particular structures during virus morphogenesis. The nucleocapsid protein forms together with the viral RNA a ribonucleo-protein complex by the interaction with a leader sequence termed ψ- region on the RNA genome. By this mechanism it supports the assembly of the RNA genome and of the t^{Lys}-RNA primer in newly generated viral particles (De Rocguigny et al., 1992; Gorelik et al., 1993) and initiates the reverse transcription. In HIV this protein-nucleotide interaction is mediated by motifs similar to zinc finger

domains. The matrix proteins aggregate in trimeric complexes and are associated by their N-terminal myristylations with the interior of the viral membrane. It mediates the transport of the DNA provirus genome to the cellular nucleus. Unlike all other retroviruses, in case of HIV the matrix protein allows the infection of non-proliferating host cells. In HIV the p6 link protein interacts with the vacuolar protein sorting (Vps) machinery (Strack et al., 2003) and is essential for the budding of the virus from the cellular membrane.

1.1.2 Pol (enzymatic activities)

Genes encoding for the viral protease, the reverse transcriptase and the integrase (*pol* genes) are processed as a single precursor protein together with the gag proteins located N-terminal. This fusion protein has a size of 160 kDa in HIV-1 and 140kDa in FeLV-A. Its synthesis is mediated by a ribosomal frame shift within a uridine rich region of the mRNA also serving for the translation of the gag proteins. Due to a hairpin conformation of the mRNA protein synthesis is slowed down and an occasional frame shift (of -1 in HIV-1) leads to the expression of the precursor protein in about five percent of translations. The gag/pol precursors are myristilated at their N-terminus and are processed sequentially into their enzymatic components by the protease during the maturation of the virus.
The protease is similar to aspartyl proteases and is organised as a homodimer with a molecular weight of 9 to 10kDa. The enzymatic center consists of two asparagin acids essential for its enzymatic activity.
The HIV-1 reverse transcriptase (RT) is magnesium (Mg^{2+}) dependant and catalyzes the transcription of viral RNA into proviral DNA. In addition to that it bears an RNase H activity allowing degradation of RNA from DNA-RNA hybrid strands. It is arranged as a heterodimer with molecular masses of 66kDa and 51kDa. The enzyme does not have a proof reading function. Thus it increases the probability of wrong base pair incorporation to 10^{-3} to 10^{-4} into newly transcribed DNA.
The integrase is part of the preintegration complex and cleaves the 5´and the 3´LTR of the double stranded proviral DNA at their 3´ends. By the resulting ends the proviral DNA is integrated into the host cell genome followed by padding the free 3´ends of cellular DNA.

1.1.3 Env (envelope glycoproteins)

In general the surface envelope proteins mediate the absorption of the viral particle to their cellular receptor and therefore are the primary determinants of the viral host range. The interaction of the SU portion of the retroviral envelope to its receptor induces a conformational change that exposes a viral fusion peptide, present in the ectodomain of TM, allowing the viral membrane to fuse with the host cell membrane. For most retroviruses, fusion occurs at neutral pH. The process of fusion is not energetically advantageous and requires bypassing of steric and electrostatic barriers (Patel, M. et al.,

1993). Until today the mechanism of membrane fusion is not resolved completely and different fusion models exist (Fig.3).

The Env membrane precursor protein is translated from one mRNA spliced once with a size of 160kDa in HIV-1 and 85kDa in FeLV-A. An amino terminal leader sequence directs the precursor mRNA towards the endoplasmatic reticulum (ER) and chaperones mediate the translation of the protein into the lumen of the ER. The hydrophobic transmembrane domain in the TM protein anchors the precursor protein in the ER membrane (Fig.4). The proteins are arranged in trimeric complexes organised in six helix bundles. Three surface envelope proteins and three transmembrane envelope proteins form the so called knob. During the transport of Golgi vesicles from the ER towards the cellular membrane, the precursor protein becomes glycosylated and is finally cleaved by a cellular protease into the transmembrane and the surface protein component. The HIV transmembrane envelope protein gp41, as well as the surface protein gp120 contain glycosylation motifs. In FeLV only the surface envelope protein gp70 is glycosylated while the transmembrane envelope protein p15E is not.

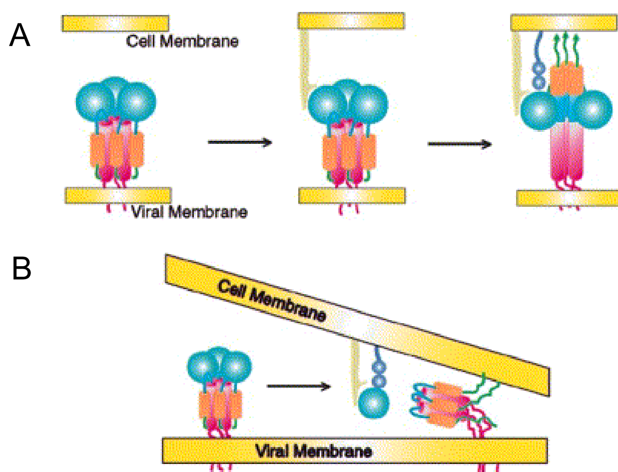


Figure 3 Potential mechanisms for CD4 and chemokine receptor induced fusion of the viral and cellular membranes. (**A**) Spring-loaded mechanism similar to that proposed for hemagglutinin, where conformational changes in the TM ectodomain lead to a major displacement of the N-terminal fusogenic peptide toward the cellular membrane.
(**B**) Shedding mechanism, where CD4 and chemokine binding result in the loss of SU proteins, enabling reorientation of the TM and membrane fusion.
Adapted from Turner and Summers 1999.

The surface envelope protein of HIV gp120 is highly glycosylated and is divided into an outer and an inner domain, connected by a β-bridging sheet. The inner domain consists of two α helices, a five stranded β-sheet and several loops. The outer domain has a double barrel structure, while one of them is built up from a six stranded β-sheet and one α-helix and the other one is organised from a seven stranded β-sheet orientated in an anti parallel form. The protein contains five highly variable regions termed V1-V5 stabilised by disulfide bindings and six conserved regions termed C1-C6. Within the variable regions amino acid deletions, insertions and changes of glycosylation sites occur during the course of infection in different isolates from a single patient. During the attachment process HIV gp120 mediates the contact to the host cell by binding the CD4 receptor followed by an interaction

with a secondary cellular co-receptor. The binding of CD4 by gp120 induces a change of conformation within the trimeric envelope protein structures (Salzwedel and Berger, 2000) and leads to the exposition of the viral co-receptor binding site (Kwong et al., 1998). The co-receptor binding is essential for the infection of a $CD4^+$ host cell (McDoughal et al., 1986) and permits either the infection of T-cells by binding to CXCR4 (Feng et al., 1996) or of monocytes and macrophages by binding to CCR5 (Alkhatib et al., 1996). Both co-receptors belong to the family of seven helix chemokine receptors. Ongoing conformational changes finally induce the loosing of gp120 from the trimeric complexes (shedding) and to the exposition of the gp41 protein towards the cellular membrane (Fig 3B).

The transmembrane enevelope protein has a size of 41kDa in HIV-1 and a size of 15kDa in FeLV-A. The extracellular components of the TM proteins within the family of retroviruses show structural homologies (Gallaher et al., 1989; Benit et al., 2001). In general retroviral transmembrane proteins are organised in three domains. From the N-terminus towards the C-terminus, the extraviral domain (ectodomain) is followed by the transmembrane domain and the cytoplasmatic part of the protein. The ectodomain is subdivided into an N-terminal fusion peptide, an N-terminal helix region (NHR) including the immunosuppressive domain (Denner et al., 1994) and a cysteine-loop connecting the N-terminal part with the C-terminal helix region (CHR). The fusion peptide consists of approximately 20 hydrophobic amino acids (Fig.4). During the process of attachment of a viral particle and its host cell, it fixes the viral membrane with the host cell membrane and thus initiates membrane fusion. Retroviral transmembrane proteins show a higher degree of conserved amino acid sequences than the surface proteins.

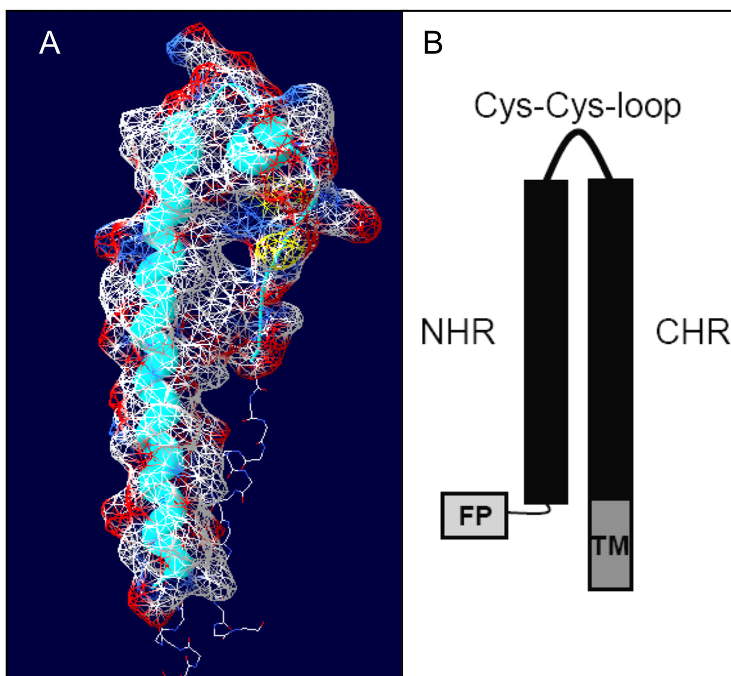


Figure 4 Models of monomeric retroviral transmembrane proteins (TM). (**A**) Theoretical model of FeLV-A p15E ectodomain generated by using Swiss model database (aa 467-583).
(**B**) Schematic model of FeLV-A p15E ectodomain inclusive the transmembrane domain. Indicated are the cysteine-loop (Cys-Cys-loop), the N- and C-terminal helix regions (NHR, CHR), the fusion peptide (FP) and the transmembrane domain (TM).

In HIV six helix bundles of the gp41 proteins are arranged by coiled coil structures of N-terminal helices stabilised by the interaction of hydrophobic conserved amino acids (Weissendorn et al., 1997). Similar molecular structures are found for gammaretroviruses (Fig.5). In contrast to other retroviruses HIV gp41 consists of a greater intraviral portion shown to play an important role in virus replication (Dubay et al., 1992, Freed and Martin, 1995).

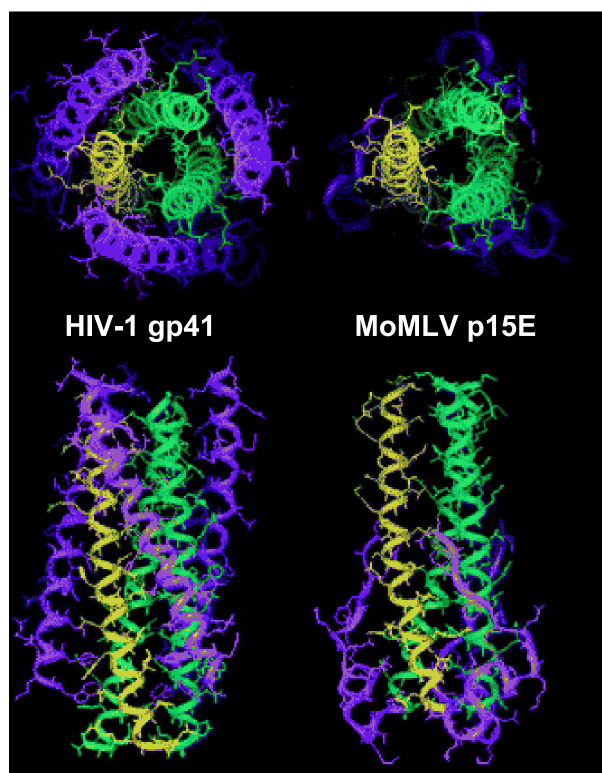


Figure 5 Comparison of HIV-1 gp41 with the gammaretroviral Mo-MLV p15E structures. The top panel shows an end-on view of the two structures from the top. The bottom panel shows a side view. The three monomers forming the central coiled coil of each structure are colored yellow, green, and blue. Supporting structures are colored purple. The figure was generated using the program Insight (Biosym). Adapted from Chan 1997.

1.1.4 Regulatory proteins

Until today the regulatory Tat protein was described for lentiviruses only. However, i.e. for HTLV and for HSRV proteins with functional regulation of transcription are also known. In the early phase of the HIV-1 infection cycle transcription of proviral DNA is incomplete due to the suppression of the elongation. In later stages of the infection cycle the Tat protein accumulates and binds to the TAR (trans-activating response) element on the newly synthesized mRNA. Thus the resulting tat-cyclin T complex can activate the cyclin-dependant protein kinase 9 (Cdk9) which leads to the phosphorylation of the RNA-polymerase II and finally to the stabilisation of the transcriptional elongation.

A post transcriptional transactivator was firstly discovered for HIV-1. This protein is essential for the time dependant regulation of gene expression during the replication cycle. In case of HIV-1 it is the Rev protein that participates in the transport mechanism of viral mRNA from the nucleus to the cytoplasm. Therefore it binds to the Rev-response element (RRE) located on the mRNA encoding for the env gene and introduces the nucleus shuttle protein exportin-1 and the nucleus factor Ran-

Guanosine-Triphosphate-GTP. By these mechanisms Rev serves as a regulator separating the transcription of early genes, which depend on mRNA spliced intensively (i.e. Tat, Rev and Nef), from the transcription of late genes (Gag, Pol, Env or accessory proteins) which depend on mRNA unspliced or spliced only once.

1.1.5 Accesory proteins

The majority of accessory proteins has been described for more complex retroviruses such as lentiviruses. In HIV-1 at least five different accessory proteins are known with distinct impacts on pathogenicity, evasion from the host immune response and influence on viral replication.

The Nef protein (negative factor protein) is an early phase protein that accumulates in the cytosol of the infected host cell. It increases viral replication and at the same time it decreases expression of CD4 molecules on the host cell membrane by clathrine mediated endocytosis. Thus CD4-Env complexes at the cell surface are prevented and the probability of a secondary HIV-1 infection of that particular host cell is reduced. Further more Nef downregulates the major histo-compatibility complex II (MHC-II) presentation on infected cells and interacts with a variety of different cellular proteins.

The Vif (viral infectiosity factor) is encoded in a reading frame between the *pol* and the *env* gene. It was supposed that the protein participates in the uncoating of the virus and in the initiation of the reverse transcription. It is assumed by that Vif mediates the proteasome depending degradation of a cellular deoxycytidine deaminase (APOBEC3G) and thus allows the production of infectious viral particles in non-permissive cells (Mehle et al., 2003).

The Vpr (virion associated protein r) binds to the nucleus import complex composed of nucleoporin and importin-α and by this it participates in the transport of the preintegration complex towards the nucleus. In addition vpr has an influence on the host cell cycle by arresting it in the G2 phase. The Vpu (viral protein U) interacts with CD4 molecules in the ER and triggers its degradation in the ubiquitine proteasome pathway, preventing generation of CD4-env complexes in the ER.

1.1.6 The retroviral cycle of replication

The retroviral cycle of infection can be divided into several distinct phases. The early phase is marked by the recognition and adsorption of the virus to the host cell membrane mediated by membrane protein complexes. In general different cellular receptors for retroviruses are known belonging to the classes of multiple- and / or single- transmembrane proteins with a variety of functions in uninfected cells (Overbaugh et al., 2001). In case of HIV-1 the cellular receptor CD4 as well as the two possible co-recpetors CXCR4 and CCR5 are well described (Markovic et al., 2004). In case of FeLV-A the receptor still remains unidentified while the multiple membrane protein Pit2 was defined as the cellular receptor for subgroup B (Anderson et al., 2001). After fusion of virus membrane and host cell

membrane the virus enters the cell by a fusion pore followed by uncoating of viral RNA. The viral RNA genome is released into the cytoplasm of the host cell and transcribed by the reverse transcriptase into viral DNA. The viral DNA associates with components of the preintegration complex (IN, MA, RT and in case of HIV with vpr and cellular HMG-I(Y)) and is transported to the nucleus where the viral integrase promotes the integration of provirus DNA into the host cell genome. In case of HIV the late phase of the infection cycle starts with the transcription of the regulatory genes *rev* and *tat* and the accessory genes. The Tat protein stabilises the elongation of transcription of viral proteins by indirect phosphorylation of the mRNA polymerase II. In this phase the gp160 protein is translated into the ER and post translational processes are initiated i.e. glycosylation and cleavage of gp160. The resulting trimer of non-covalent bound gp120 and gp41 is transported to the cell surface by Golgi vesicles. The gag polyprotein is translated from an unspliced mRNA and attaches to the Gag/Pol fusion proteins. The virus assembly is followed by budding of the virus from the cellular membrane or as described recently into endosomes of infected cells (Kramer et al., 2005). The final maturation of the infectious particle occurs outside the host cell (Fig.6).

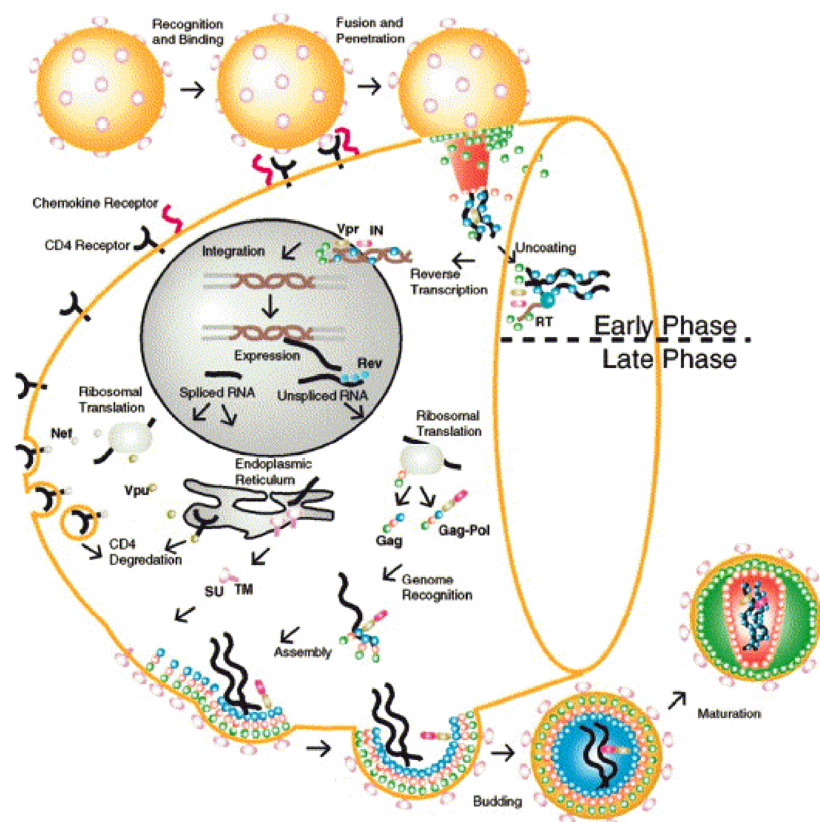


Figure 6 General features of the HIV-1 replication cycle. The early phase (upper portion of the diagram) begins with CD4 recognition and involves events up to and including integration of the proviral DNA, and the late phase includes all events from transcription of the integrated DNA to virus budding and maturation. Adapted from Turner and Summers 1999.

1.2 The interaction of the immune system with retroviral infections

1.2.1 The immune system is based on different ways of reaction specific for the type of pathogen

In general the immune response against viral infections and other pathogens can be separated in two distinct complexes of immunological reactions. The so called first line of defense is mainly based on the unspecific innate immune system. In the evolutionary progression it is the anterior system which leaded to the development of a secondary complex, the adaptive immune system (Medzhitov and Janeway, 1999). After a virus has overcome the outer barriers of the organism components of the innate immune system recognise the possible pathogen as a non-self contaminant and may be able to eliminate it. In this process monocytes, granulocytes, macrophages and natural killer cells are involved. Further more the innate immune response is supported by acute phase proteins, the complement system as well as cytokines and interferons.

The adaptive immune system is an additional response system with the capability of highly specific interactions with the pathogen. It can be subdivided into the humeral immune system consisting of antibody producing B-cells and the cellular immune system consisting of T-helper cells and cytotoxic T-cells. In a repeated infection or exposition of the pathogen the adaptive immune response is able to eliminate the contaminant faster and more effective. This adaptation is based on the clonal production of the most specific B- and T-cell response due to the mechanism of selective maturation as well as due to the generation of a specific B- and T-memory cell repertoire.

1.2.2 The innate immune system displays the first line of defense

The innate immunity uses a broad spectrum of unspecific cellular and soluble components to react on a retroviral infection. The cellular components comprise B1-cells, plasmacytoids dendritic cells (PDCs) and non-cytotoxic, antiviral $CD8^+$ cells. The soluble factors include cytokines, chemokines, α-defensines, complement, collectines, pentraxines and cathelicidines (Chang TL, et al., 2005; Levy et al., 2003). For example, the importance of the innate immunity in controlling HIV infection is becoming increasingly appreciated (Lehner 2003, Levy 2003a). The inverse correlation between the level of viremia and the ability of NK cells to inhibit HIV replication is predominantly mediated through secretion of CC chemokines, including macrophage inflammatory protein-1α (MIP-1α), MIP-1β, and RANTES, that inhibit HIV-1 entry via CCR5 (Kottilil et al. 2003). Furthermore the PDC´s and non-cytotoxic, antiviral CD8+ have an important role in the innate immune response against HIV. PDC´s produce type1-interferones. HIV-1 positive patients with an interferon-α concentration above 300U per millilitre blood are less often affected by opportunistic infections than these which show levels below that value (Lopez et al., 1983, Siegal et al., 1986). The antiviral impact of non-cytotoxic, antiviral CD8+ cells is based on the suppression of the retroviral transcription (Copeland et al., 1995;

Levy et al., 1996). The antiviral activity of soluble factor(s) from these cells is known as CD8+ antiviral factor(s) (CAF). It is found very early in primary infection before the presence of antibodies against HIV (Mackewicz et al., 1994) and correlates with delayed disease progression in HIV-1–infected people (Walker et al., 1990; Carmichael et al., 1993; Mackewicz et al., 1991).

1.2.3 The adaptive cellular immune response is important in the defense of intra-cellular pathogens

The adaptive cellular immune response is predominantly involved into the immunological reactions against intra-cellular and phagocyted pathogens as well as into the recognition and elimination of tumor cells. The mediation of the cellular immune response is based on cytotoxic T-lymphocytes capable for the induction of apoptosis and lysis in the infected or transformed target cell. The CTL response is initiated by the MHC-I complex, presenting pathogen specific antigens on target cells.

In case of many retroviral infections, the CTL response is essential for the immunological control of the course of infection. For example in HIV infected patients it was shown that a strong CTL response correlates with a decelerated progression of infection (Rinaldo et al., 1995; Harrer et al., 1996, Greenough et al., 1997), even if a complete clearance of HIV has not been observed. In patients previously exposed to HIV-1 but not being infected a specific CTL response was shown to maintain a protection against infection to some degree (Goh et al., 1998). However, the high genetic variability of HIV-1 promotes the induction of escape mutants being resistant towards any kind of an adaptive immune response (Peeters and Sharp, 2000). In contrast to HIV-1, FeLV infected cats frequently clear circulating virus and show a correlation between clearance and the appearance of FeLV specific CTL´s (Flynn et al., 2002).

1.2.4 The B-cell mediated adaptive humoral immune response is important in the defense of extra-cellular pathogens

The B-cell mediated immune response is predominantly involved into the immunological reactions against extra-cellular pathogens and toxins. Contaminants are being bound by immunoglobulins attached to or secreted from antigen specific B-lymphocytes. Different kinds of reactions are leading either to the absorbance of the pathogen or toxin by macrophages, to its opsonisation introducing the complement system or even to its neutralisation by neutralising antibodies. In addition antibodies can recognise infected cells and initiate an antibody dependant cellular cytotoxicity (ADCC) reaction towards them. An antibody secreting B-cell specific for protein antigens previously has to be activated by an antigen specific T-helper cell (thymus-dependant reaction). In contrast B-cells secreting antibodies specific for certain ingredients of microbes, i.e. bacterial polysachharids, are not dependant on T-helper cells (thymus-independent reaction). For the effective prevention of a viral infection the

titre of neutralising immunoglobulins is of particular interest. The capability of virus neutralisation is dependant on the affinity and avidity of immunoglobulins for viral proteins critical in the mechanism of infection. In retroviruses the envelope proteins display an effective target for virus neutralisation mediated by immunoglobulins. Animals or humans infected with retroviruses show humoral immune responses against the viral envelope proteins with different capacities of virus neutralisation. However, in many cases the immunologic control of retroviral infections by these antibodies is not sufficient. For example in HIV-1 infected patients neutralising antibodies against gp120 are unable to provide virus clearance (Ruppach et al., 2000) due to high genetic variability of HIV-1 depending on the imprecisely transcribing reverse transcriptase (Preston et al., 1988, Roberts et al., 1988). In addition to that the double stranded RNA genome of HIV allows an efficient rate of recombination in host cells infected with two different subtypes. Both mechanisms of genetic variability allow HIV-1 to evade the immune response and medical therapy by the generation of escape mutants (Evans and Desroisiers, 2001; Kijak et al., 2002; Kwong et al., 2002; Wei et al., 2003).

1.2.5 Antibody characteristics

Antibodies are glycoproteins organised from two light and two heavy chains, building a molecular Y-structure. The light chains have an N-terminal variable region and a C-terminal constant region. The complementary determining regions (CDR) defining the antibody specifity and the affinity towards its epitope are located within the variable sequences. The corresponding region on the antigen is the antigenic determinant or epitope. Intra-molecular disulfide bindings stabilize the different domains and an inter-molecular disulfide binding connects the light and the heavy chains with each other (Fig7).

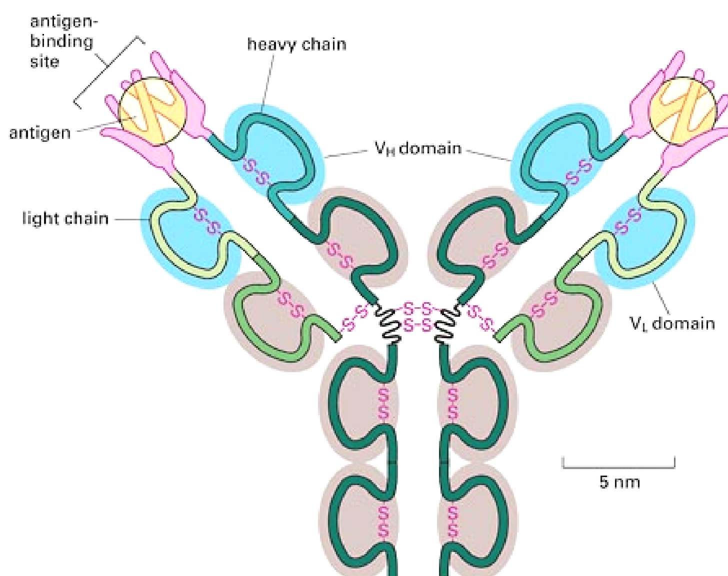
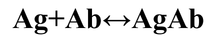


Figure 7 Schematic drawing of a typical antibody molecule. The protein is composed of four polypeptide chains (two identical heavy chains and two identical and smaller light chains) held together by disulfide bonds. Each chain is made up of several different domains, here shaded either blue or gray. The antigen-binding site (CDR) is formed where a heavy chain variable domain (VH) and a light chain variable domain (VL) come close together. Modified from Alberts et al., 1988

The binding of an antibody to its antigen is reversible and mediated by weak non-covalent forces, including hydrogen bonds, hydrophobic van der Waals forces, and ionic interactions. For an antibody-antigen interaction molecules have to be close enough allowing the antigen to interact with the complementary recesses on the surface of the antibody. Multivalent antigenic macromolecules are characterised by many different epitopes while polyvalent antigens consist of at least two or more identical epitopes, i.e. in a polymer with a repeating structure. The antigen (Ag)- antibody (Ab) interaction can be expressed as:



$$\mathbf{Ag+Ab \leftrightarrow AgAb}$$

The equilibrium point depends both on the concentrations of Ab and Ag and on the strength of their interaction. Thus a larger fraction of Ab will become associated with Ag as the concentration of Ag increases. The strength of the interaction is generally expressed as the affinity constant (K_a):

$$\mathbf{K_a = [AgAb] / [Ag][Ab]}$$

The affinity of an antibody for an antigenic determinant describes the strength of binding of a single copy of the antigenic determinant to a single antigen-binding site, and it is independent of the number of sites. The avidity describes the total binding strength of a polyvalent antibody with a polyvalent antigen.

Immunoglobulins can be subdivided into the classes of IgM, IgG, IgA, IgD and IgE depending on their molecular size and their immunological function:
IgM antibodies are secreted in the early phase of immunoglobulin production in infection and mediate the activation of the complement system. Membrane anchored IgM molecules are localized in the cytoplasm membrane of precursor B-cells functioning as antigen receptors. Secreted IgM antibodies are organised in a pentamer structure stabilized by a J-peptide connecting the Fc parts of the molecules. They show a relatively weak affinity towards its epitopes compensated by a high avidity based on its multimerised structure.

IgD antibodies are similar to IgM produced in the early phase of infection and have a membrane anchored counterpart. It is supposed that IgD antibodies are involved in the differentiation of precursor B-cells to plasma cells.

IgG antibodies represent the main population of antibodies in the serum. They have a key function in protective immunity after repeated exposition of an antigen. The IgG antibody class is the most specific one and can be subdivided into several IgG-subclasses. Due to distinct immunological functions, the induction of IgG subclasses is based on the type of contaminant being exposed in the organism. Viral infections predominantly lead to the production of IgG_1 and IgG_3, capable of activating the complement system. IgG_1 is mainly induced by the exposition of bacterial

polysaccharids. IgG specific Fc receptors can be found on macrophages, monocytes and neutrophile granulocytes binding the antibody-antigen complexes in order to phagocyte them. Further more the ADCC reaction is mediated by IgG antibodies.

IgA antibodies are produced in secretory organs and are predominantly involved into the humoral immune response in mucosal tissue. IgA antibodies are organised in a dimer structure stabilised by a J-peptide connecting the Fc parts of the molecules.

IgE antibodies are the major component of the humoral immune response against parasites. With the Fc receptor IgE antibodies can bind to basophile granulocytes and mast cells inducing the secretion of histamines. IgE molecules display a critical component of allergetic reactions and thus can mediate an allergetic shock reaction.

B-cells develop from pluripotent stem cells of the bone marrow and differentiate under the influence of cytokines to precursor B-cells. An ongoing differentiation towards pre-B-cells is induced by IL4, IL5 and IL6. During this process a somatic recombination occurs in the B-cells. This recombination leads to the reorganisation of genes encoding for V, D and J segments displaying the variable domains of the immunoglobulins (VDJ recombination). By that mechanism several million possibilities of VDJ combinations lead to a specifity for almost any antigen. In that phase, membrane anchored IgM molecules serve as antigen receptors and initiate the endocytosis of antigen-antibody complexes into B-cells. The phagocyted antigen is processed in the cellular proteasome and resulting peptides bound to MHC-II complexes are presented on the cell surface. Specific T-cells bind the MHC-II presented antigen by their T-cell receptor and initiate the differentiation of B-cells to antibody secreting plasma cells by releasing different cytokines. A change of the predominant immunoglobulin class occurs depending on cytokine signalling (switch). Alternatively processes of mRNA splicing rearrange the variable domains of the heavy chains and lead to the translation of antibody classes with higher affinities for the antigen, i.e. IgG classes. Within the V-segments encoding for the highly variable CDR domains hypermutations occur. These result in the production of highly specific antibodies with high affinities to their specific antigens. The selection process of affinity maturation of antibodies is mediated by repeated hypermutations as a result of ongoing antigen exposition. Thus antibodies evolve increased affinities for their antigens during the course of infection.

1.2.6 Neutralising antibodies

Neutralising antibodies are able to prevent the absorption of viral particles to their cellular receptor blocking the entry of the virus. It has been shown that in lentiviral replication cycles neutralising antibodies can decelerate the infection (Haynes 1992). It is supposed that neutralising antibodies limit

the replication of lentiviruses by the observation that escape mutants are generated resistant to neutralisation (Albert et al., 1990). In HIV-1 infected patients high titres of these antibodies appear to decelerate HIV-pathogenesis (Reitz et al., 1988, Nara et al., 1990, Watkins et al., 1996, Parren et al., 1999, Dianzani et al., 2002). Until today only four broadly neutralising monoclonal antibodies have been isolated from HIV-1 infected patients. IgG_1 2G12 and IgG_1 b12 bind to gp120, IgG_1 2F5 and IgG_1 4E10 bind to the ectodomain of gp41. Others have been described with a less effective capacity in broad neutralisation of HIV-1 subtypes (fab fragment X5, IgG447-52D and 17b against gp120 and Z13 against gp41).

1.2.7 The HIV-1 neutralising monoclonal antibodies 2F5 and 4E10

The mAb2F5 (Muster et al., 1993) and the mAb4E10 (Zwick et al., 2001a) are produced in hybridoma cell lines generated by fusion of B-lymphocytes of an HIV-1 positive donor with a human tumor cell line (Buchacher et al., 1994). The encoding gene for 2F5 shows an abnormality within its D-segment for the heavy immunoglobulin chain by a length of 52 nucleotides (Kunert et al. 1998). This unusual length is supposed to result from recombination processes and leads to a relatively long CDR3 loop of the 2F5 immunoglobulin. 2F5 is capable of neutralising laboratory as well as primary isolates of group M and shows an in *vitro* neutralisation at a $TCID_{50}$ of $<1\mu g/ml$ in dependence of the subtype being neutralised (Conley et al., 1994, Muster et al., 1994, Purtscher et al., 1994, Trkola et al., 1995, Purtscher et al., 1996). 2F5 binds to the linear sequence ELDKWA (AS 662-667, HIV- reference genome HXB2, NCBI K03455, Ratner et al., 1985). The epitope is located within the C-terminal region of gp41, displaying a domain being highly conserved in subtypes of group M (Zwick et al., 2001) and thus explaining the broad spectrum of neutralisation by 2F5. The core of the epitope (L)DKWA is supposed to be essential for neutralisation (Muster et al., 1993, McGaughy et al., 2003) due to the fact that amino acid deletions therein, i.e. isolates of group O, result in a loss of function (Trkola et al., 1995, Parren et al., 1998).

The 4E10 epitope NWFN/DIT is located three amino acids downstream of the 2F5 epitope and was original produced as an IgG_3 antibody. An exchange of the subclass specific constant immunoglobulin regions to an IgG_1 molecule (Kunert et al., 2000) resulted in an increased capacity of neutralisation and subtype specifity (Stiegler et al., 2001). Similar to 2F5 also 4E10 neutralises HIV-1 laboratory and primary subtypes of group M.

The combination of 2F5 and 4E10 with other neutralising antibodies showed a synergistic effect increasing neutralising efficacy (Li et al., 1997, Mascola et al., 1997, Zwick et al., 2001, Kitabwalla et al., 2003). The injection of high dosis of different neutralising antibodies into HIV-1 infected patients showed a decline in virus burden (Stiegler et al., 2002, Armbruster et al.2002). Despite numerous studies focussing on the induction of 2F5-like antibodies the induction of broadly neutralising antibodies towards the gp41 C-terminal domain was not successful until today. In addition to the

ELDKWA and the NWFN/DIT epitopes it was recently published that 2F5 as well as 4E10 should bind to cardiolipin suggesting a polyspecific autoreactivity for these antibodies (Haynes et al., 2005). Thus the question arose if an autoantigen mimicry of the conserved membrane-proximal epitopes of the virus prevents the induction of 2F5- and 4E10-like antibodies.

1.2.8 Neutralising antibodies in the course of FeLV infection in cats

In case of FeLV virus neutralising antibodies appear to correlate with immunity to FeLV (Russel & Jarrett 1978, Hoover et al., 1978). They are considered being mediated mainly by the induction through the FeLV surface glycoprotein gp70. Cats that resist infection also develop antibodies to the so-called feline oncornavirus-associated cell membrane antigen (FOCMA). Anti-FOCMA antibodies have been associated with protection from FeLV related neoplastic disease, but their role in protection from persistent viraemia is less clear (Rojko & Hardy 1994). Of 21 cats that did not develop virus neutralising antibodies after challenge in one study, 20 (95%) became persistently viraemic compared to only three of the 24 (13%) that did develop virus neutralising antibodies (Grant et al., 1980). The presence of neutralising antibodies was shown to be clearly associated with resistance to infection since passive transfer of antibodies either naturally through the colostrum (Hoover et al., 1977, Jarrett et al., 1977), or experimentally by infusion (Haley et al., 1985) protects cats against FeLV challenge.

1.3 Vaccine development against retroviruses

1.3.1 Vaccination strategies

At least four different main vaccination strategies exist based on the immunisation with (i) attenuated viruses, (ii) inactivated viruses, (iii) virus derived protein and peptide constructs and (vi) DNA constructs encoding viral genes. (i) Attenuated viruses differ from the wild type virus by a decreased replication efficacy and pathogenicity. They are able to activate the humoral as well as the cellular immune response and lead to the induction of neutralising antibodies. The success of attenuated virus vaccines against diseases such as smallpox, measles and mumps has dramatically reduced morbidity and mortality worldwide and has led to the eradication of smallpox (Arita, 1979). However the use of an attenuated virus vaccine against SIVmac in neonatal macaques, deleted for the *nef* gene (SIVmacΔ*nef*), induced a persistent infection resulting in AIDS symptoms (Baba et al., 1995, Cohen et al., 1997). This data questioned the safety of an attenuated HIV-1 vaccine. Further more an attenuated HIV-1 vaccine also bears the possibility of gain of function mutations or insertion mutations by an integrated retroviral genome in vaccinated patients. (ii) Inactivated virus particles are used in vaccines against influenza and polyomeyelitis. They are based on wild type viruses inactivated by chemicals

maintaining conformational structures of viral proteins but degenerating viral nucleotides. (iii) Usage of virus derived protein or peptide components in vaccines eliminate a possible contamination with infectious particles. In general viral surface proteins display critical targets for vaccination. In case of the hepatitis B-virus its recombinant surface protein HbsAg is used inducing a neutralising antibody and a CTL response in vaccinated humans. (iv) DNA vaccines primarily initiate a CTL response (Lemieux, 2002). Applicated DNA is absorbed by cells and encoded proteins are expressed and presented by MHC-I molecules on the cell surface.

1.3.2 Vaccination with recombinant proteins can provide protection from retroviral infections

Immunisation is the most effective method to prevent diseases caused by infectious agents. It is of interest that most, if not all, successful vaccines are based on the induction of neutralising antibodies. Although the development of vaccines against HIV, the retrovirus that causes AIDS, is one of the major task of present vaccinology, all attempts have until now failed. Approximately 40 million individuals worldwide were living with HIV in 2004 and more than 28 million have died since the pandemic began. Although there are therapies available that prevent or delay the onset of AIDS, there is at present no cure for the infection. Present therapies are based mainly on combinations of inhibitors of the viral reverse transcriptase and of the protease. In addition, fusion inhibitors such as T20, that corresponds to a conserved domain of the transmembrane envelope protein gp41 of HIV-1 and can prevent infection, have been included in combination therapies (Turpin, 2003).
In contrast, vaccines against feline leukaemia virus (FeLV) not only exist but are also commercially available, showing that antiretroviral vaccines are not impossible. The number of cases of FeLV-induced disease has been reduced by the development and use of several vaccines against FeLV-A. However, none of the seven commercial FeLV vaccines currently available in the USA and Europe provide 100% protection against infection. Three vaccines are composed of inactivated whole virus, two are gp70 subunit vaccines and two are recombinant vaccines (Sparkes, 1997). The Leucogen (Virbac) vaccine contains the recombinant unglycosylated p45 of the surface envelope glycoprotein gp70 and is one of the most effective (Jarrett & Ganiere, 1996).

Table 2 Marketed vaccines against FeLV-A

Vaccine	Company	Multiple Vaccine	FeLV component	Laboratory Animals in Studies	PF* (%)	Reference
Eurifel FeLV	Merial	-	canarypox virus expressing *env, gag of FeLV-A*	20	93.2%	(Hoover et al., 1996)
Eurifel RCP / FeLV	Merial	+	canarypox virus expressing *env, gag of FeLV-A*	-	-	-
Fevaxyn FeLV	Fort Dodge	-	subunit filtrate containing inactivated feline leukaemia virus (strain 61 E)	144	90.4%	(Hines et al., 1991)
Fel-O-Vax	Fort Dodge	-	inactivated feline leukaemia virus (strain FeLV-61E-A)	12	86%	(Jarrett & Ganiere 1996)
Purevax Leucat	Merial	-	inactivated feline leukaemia virus	12	14.3%	(Jarrett & Ganiere 1996)
Leucocine	SmithKline	-	mixed subunit filtrate containing feline leukaemia virus antigen gp 70 (1330ng)	-	-	-
Leukocell 2	Pfizer	-	mixed subunit filtrate containing feline leukaemia virus antigen gp 70 (1330ng)	148	74,8%	(Pollock & Haffer 1991)
Leucogen	Virbac	-	purified recombinant p45	12	52,4%	(Jarrett & Ganiere 1996)

*preventable fraction

1.3.3 FeLV is a useful model to explore the factors that should be taken into account in developing a successful HIV vaccine

Feline leukaemia virus (FeLV) is a gammaretrovirus comprising three subtypes A, B and C (Jarrett 1975) of which FeLV-A is of veterinary importance, being the predominant serotype in cats (Jarrett & Hardy 1978). FeLV infection was, until recently, the most common fatal disease of cats.

Cats exposed to FeLV may either become persistently viremic or recover from infection. Viremic cats show little evidence of an immune response to the virus and are at high risk of developing a fatal disease within 2 to 4 years. In contrast, recovered animals produce virus neutralising antibodies and FeLV-specific cytotoxic T-cells (CTLs), are resistant to reinfection, and do not develop FeLV-related diseases (Hardy et al., 1980). A third possible outcome is the establishment of a latent infection, in which cats are not viremic but have a covert infection of bone marrow cells and, like fully recovered cats, have virus-neutralising antibodies (Madewell & Jarrett 1983). In most cats with latent infection the virus is eventually eliminated, but occasionally the infection persists for several years (Pacitti & Jarrett 1985) and may be reactivated at a later date so that cats become viremic. Co-infection with the immunosuppressive feline immunodeficiency virus (FIV) has been shown to reactivate FeLV (Hofmann-Lehmann et al., 1997).

FeLV causes different fatal diseases, among them leukaemia. Leukaemia was the first disease associated with FeLV and, thus, the source of its name. However, more cats die from immunosuppression induced by FeLV than from leukaemia and these immunodeficiencies resemble in

many parameters AIDS induced by HIV in humans or by FIV in cats. This includes a decrease in the number of CD4+ cells (Hofmann-Lehmann et al., 1997) and numerous opportunistic infections (Hardy 1993, Hardy et al., 1976). Although all three viruses are retroviruses, they belong to a different genus (FeLV is a gammaretrovirus, HIV and FIV are lentiviruses) and differ in their morphology and genetic composition. Nevertheless, the experience of successful vaccination against FeLV is of great interest when developing strategies for an AIDS vaccine. FeLV and FIV (which is more closely related to HIV) provide useful systems to explore the factors that should be taken into account in developing successful retroviral vaccines for an outbred population.

1.4 Goals of this study-Targeting at FeLV-A and HIV-1

The transmembrane envelope proteins of retroviruses are very similar in their structure due to their specific functions during infection. Conformational changes include an intramolecular interaction between a N-terminal and a C-terminal helix region (Follis et al*.,* 2002). Although the surface envelope proteins gp70 of gammaretroviruses are not as variable as the surface envelope protein gp120 of HIV-1, the transmembrane envelope proteins of all retroviruses are more highly conserved and therefore possibly represent a better antigen for vaccination.

The aim of this study was to analyse the humoral immune response against the ectodomain of FeLV-A p15E in different species in order to establish a model for the immunological reaction against retroviral transmembrane proteins. Eventually induced neutralising antibodies should be determined in order to evaluate the effectiveness of a transmembrane protein based vaccine against retroviruses. Further more a potential vaccine candidate should be analysed in comparison to commercial vaccines against FeLV. The FeLV-A model also allows to test the effectiveness of a possible vaccine candidate in an *in vivo* analysis by immunisation and virus challenge of cats. In comparison to previously published data (Fiebig et al., 2003) describing the immune response against the p15E of the porcine endogenous retrovirus firstly possible similarities in the humoral immune reactions within the genus of gammaretroviruses should be determined. Based on the data obtained from these immunisation attempts a concept should be worked out for the construction of a recombinant gp41-derived protein inducing neutralising antibodies against HIV-1. This concept should consider main aspects found for the model of the immune response against gammaretroviruses.