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2007

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*Citation for published version (APA):*

Antonsson, L. (2007). *Recombinant CXCR4/CCR5 hybrid receptors as tools for studies of HIV-1 receptor usage*. [Doctoral Thesis (compilation), Department of Experimental Medical Science]. Department of Experimental Medical Science, Lund University.

*Total number of authors:*

1

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An academic dissertation regarding

# **Recombinant CXCR4/CCR5 hybrid receptors as tools for studies of HIV-1 receptor usage**

**Liselotte Antonsson**



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With the approval of the Faculty of Medicine, Lund University,  
the public defense of this thesis will take place in  
Segerfalksalen at the Biomedical Center, Lund, June 7, 2007 at 10 a.m.

Faculty Opponent:

**Professor Birgitta Åsjö**  
Center for Research in Virology  
The Gade Institute, University of Bergen  
Norway

Organization Lund University Department of Experimental Medical Science Faculty of Medicine		Document name DOCTORAL DISSERTATION
		Date of issue: June 7, 2007
Author: Liselotte Antonsson		Sponsoring organization
Title and subtitle: Recombinant CXCR4/CCR5 hybrid receptors as tools for studies of HIV-1 receptor usage		
Key words: GPCR, HIV-1, coreceptor, CCR5, CXCR4, RANTES, TAK-779, evolution, pathogenesis, cerebrospinal fluid		
<p>Abstract:</p> <p>The chemokine receptors CCR5 and CXCR4 are required, together with CD4, for the entry of HIV-1 into target cells. CCR5 using HIV-1 dominates during transmission and the asymptomatic phase of infection. During progression, virus phenotypes with the ability to use CXCR4 emerge in about 50% of the infected individuals. Individuals continuously harbouring CCR5-restricted isolates still progress to AIDS. Differences among CCR5 using isolates, has been found and an evolution towards an altered mode of CCR5 coreceptor use and a reduced sensitivity to inhibition by natural CCR5 ligands has also been described.</p> <p>With the aim to study interactions of natural ligands and HIV-1 isolates with these chemokine receptors, a set of hybrid CXCR4/CCR5 receptors were constructed. Signalling response to their respective natural ligands, SDF-1 and RANTES were studied and prototypic R5 and X4 isolates (HIV-1<sub>BaL</sub> and HIV-1<sub>IIIb</sub>) were tested for their ability to use these chimeric receptors. The results showed that ligands and virus isolates use different receptor epitopes which, in turn, vary between the two receptors.</p> <p>Further, the evolution of primary HIV-1 isolates was studied. A total of 246 sequential primary HIV-1 isolates were studied. Using our chimeric CXCR4/CCR5 receptors, we showed that R5 isolates from immunosuppressed individuals are distinct from those isolated from individuals with higher CD4<sup>+</sup> T-cell counts, with regards to coreceptor usage. The analysis also showed that the ability to utilize chimeric receptors correlated inversely with the sensitivity to RANTES inhibition of infection. The R5 isolates used receptor chimeras to various degrees. Based on these results, the R5 viruses could be subdivided into two groups: the R5<sup>narrow</sup> phenotype and the R5<sup>broad</sup> phenotype. The R5<sup>narrow</sup> phenotype is defined as viruses that use wt CCR5 but no chimeric receptors, whereas viruses using at least one chimeric receptor in addition to CCR5, are designated R5<sup>broad</sup> viruses.</p> <p>The mode of coreceptor use by paired plasma and CSF isolates from HIV-1 infected individuals with varying degree of immunodeficiency and neuropathology were studied. The R5 viral phenotypes predominated both in plasma and in CSF. We were able to identify discordant plasma/CSF wt coreceptor use but also, varying R5 viral phenotypes in the paired isolates within individual patients. There were no characteristic patterns of receptor use that could distinguish CSF from plasma isolates. R5 virus use of chimera FC-2 correlated highly with immunosuppression. Efficient chimeric receptor use also correlated, with an increased resistance to inhibition by the CCR5 antagonist TAK-779.</p> <p>In conclusion, our findings propose that alterations in the mode of CCR5 use may be a key event in R5 virus pathogenesis. We believe that R5 virus ability to utilize these CXCR4/CCR5 chimeric receptors reflects a more flexible and more efficient CCR5 usage, which may include a reduced dependency upon interactions with the N-terminal of the receptor for infection. The findings are important, not only with regards to R5-virus pathogenesis and optimization of emerging treatment with CCR5 antagonists, but also for HIV-infection within the CNS.</p>		
Classification system and/or index terms (if any):		
Supplementary bibliographical information:		Language: English
ISSN and key title: 1652-8220		ISBN 978-91-85559-92-3
Recipient's name:	Number of pagers: 105	Price:

Distribution by (name and address): Liselotte Antonsson, Department of Experimental Medical Science, BMC A12, Lund University, S-221 84 Lund, Sweden.

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Printed by KFS, Lund, Sweden  
© Liselotte Antonsson  
ISSN 1652-8220  
ISBN 978-91-85559-92-3  
Lund University, Faculty of Medicine Doctoral Dissertation Series 2007:114

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## ABBREVIATIONS

<b>7TM</b>	7-transmembrane	<b>IP<sub>3</sub></b>	Inositol triphosphate
<b>aa</b>	Amino acid	<b>kb</b>	Kilobases
<b>AC</b>	adenylyl cyclase	<b>kD</b>	KiloDalton
<b>ADC</b>	AIDS dementia complex	<b>mAb</b>	Monoclonal antibody
<b>AIDS</b>	Acquired immuno- deficiency syndrome	<b>MIP</b>	Macrophage inflammatory protein
<b>AZT</b>	3'-Azido-3'-deoxy- thymidine (zidovudine)	<b>NK-cell</b>	Natural killer cell
<b>BBB</b>	Blood-brain barrier	<b>NNRTI</b>	non-nucleoside reverse transcriptase inhibitor
<b>B-cell</b>	B-lymphocyte	<b>NRTI</b>	nucleoside reverse transcriptase inhibitor
<b>bp</b>	Base pair	<b>NSI</b>	Non-syncytium inducing
<b>CCL</b>	CC chemokine ligand	<b>PBMC</b>	Peripheral blood mononuclear cell
<b>CCR5</b>	CC chemokine receptor 5	<b>PCR</b>	Polymerase chain reaction
<b>CD</b>	Cluster of differentiation	<b>PHA</b>	Phytohemagglutinin
<b>CHO</b>	Chinese hamster ovary cells	<b>PI</b>	Protease Inhibitor
<b>CNS</b>	Central nervous system	<b>PKC</b>	Protein kinase C
<b>CSF</b>	Cerebrospinal fluid	<b>PLC</b>	Phospholipase C
<b>CTL</b>	Cytotoxic T-lymphocyte	<b>R5</b>	CCR5-using virus
<b>CXCR4</b>	CXC chemokine receptor 4	<b>RANTES</b>	Regulated on activation normal T-cell expressed and secreted
<b>DAG</b>	Diacyl glycerole	<b>RNA</b>	Ribonucleic acid
<b>DC</b>	Dendritic cells	<b>RT</b>	Reverse transcriptase
<b>DNA</b>	Deoxyribonucleic acid	<b>T-cell</b>	T-lymphocyte
<b>EC<sub>50</sub></b>	50% Effective concentration	<b>TM</b>	Transmembrane
<b>ECL</b>	Extracellular loop	<b>SDF</b>	Stromal derived factor
<b>ELISA</b>	Enzyme-linked immunosorbent assay	<b>SI</b>	Syncytium inducing
<b>GALT</b>	Gut associated lymphatic tissue	<b>SIV</b>	Simian immuno- deficiency virus
<b>GDP</b>	Guanosine diphosphate	<b>U87</b>	Astrogloma cell line
<b>gp</b>	Glycoprotein	<b>wt</b>	Wild type
<b>GPCR</b>	G protein-coupled receptor	<b>X4</b>	CXCR4-using virus
<b>G-protein</b>	GTP-binding protein		
<b>GTP</b>	Guanosine triphosphate		
<b>HAART</b>	Highly active anti- retroviral therapy		
<b>HIV</b>	Human immuno- deficiency virus		
<b>IC<sub>50</sub></b>	50% Inhibitory concentration		
<b>ICL</b>	Intracellular loop		
<b>IL</b>	Interleukin		

## LIST OF PUBLICATIONS

This thesis is based on the following papers.

### Paper I

**Molecular mapping of epitopes for interaction of HIV-1 as well as natural ligands with the chemokine receptors, CCR5 and CXCR4.** Antonsson, L., Boketoft, Å., Garzino-Demo, A., Olde, B., Owman, C. (2003) AIDS 17, 2571-9.

### Paper II

**Coevolution of RANTES sensitivity and mode of CCR5 receptor use by human immunodeficiency type 1 of the R5 phenotype.** Karlsson, I., Antonsson, L., Shi, Y., Öberg, M., Karlsson, A., Albert, J., Olde, B., Owman, C., Jansson, M., Fenyö E. M., (2004) J Virol. 78, 11807-15.

### Paper III

**Discordant HIV-1 phenotypes in paired plasma and cerebrospinal fluid samples - clinical implications of varying mode of coreceptor use.** Antonsson, L., Karlsson, U., Repits, J., Ljungberg, B., Kidd-Ljunggren, K., Hagberg, L., Svennerholm, B., Jansson, M., Gisslen, M., Owman, C. (2007) *Submitted manuscript*



## INTRODUCTION

### Brief history

In the early 1980's clusters of cases of *Pneumocystis carinii* pneumonia and Kaposi sarcoma, were observed among previously healthy homosexual men in the United States. These severely immunosuppressed patients were identified and reported by the Centers for Disease Control (CDC) and Gottlieb et al (2, 64) and were eventually recognized as the first cases of acquired immunodeficiency syndrome (AIDS). In 1983 the virus causing this disease was identified as a lentivirus, belonging to the *Retroviridae* family (14, 57). The virus was eventually named Human Immunodeficiency Virus (HIV) in 1986 (35).

In 1996 certain cell membrane receptors were shown to be necessary, in addition to the CD4 molecule, for the virus to enter its target cells (6, 30, 41, 43, 44, 52). These virus coreceptors were chemokine receptors, and members of the superfamily of G protein-coupled receptors (GPCRs). This discovery was the beginning of extensive studies, opening a whole new field of research. The understanding of HIV infection and the pathogenesis of AIDS is essential for the development of new antiretroviral therapies.

### G protein-coupled receptors

#### ***A family of cell surface receptors***

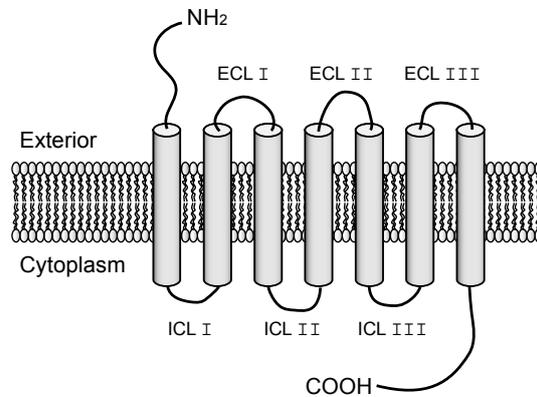
Cell surface receptors facilitate cell communication. Cells need to respond rapidly to changes in their environment. In multicellular organisms, cells also have to coordinate their actions.

The superfamily of GPCRs is the largest family of cell surface receptors and also the largest protein superfamily in the mammalian genome (56). About 2% of the human genome encodes GPCRs. There are about 800 identified human GPCR sequences. These receptors are involved in virtually all physiological processes and approximately 40% of the modern drugs act on GPCRs (21). These receptors transduce signals in response to a variety of stimuli such as peptides, ions, neurotransmitters, hormones, lipids, odorants or even photons. Additionally, the fact that these receptors are found in such diverse organisms as *e.g.* zebrafish, fruit fly (*Drosophila melanogaster*), a

nematode (*Caenorhabditis elegans*) and plants, further illustrates their crucial importance for most complex living organisms and also the evolutionary success of this signalling mechanism (75).

### **Common features of GPCRs**

GPCRs are highly conserved and recognized by their seven hydrophobic transmembrane spanning helices connected by three hydrophilic intracellular loops (ICL1-3) and three hydrophilic extracellular loops (ECL1-3). The receptor proteins, also called 7-transmembrane receptors (7TM) are composed of about 300-1000 amino acid (aa) residues. Bundle of helices are formed in the cell membrane, with an extracellular amino (N-) terminal and an intracellular carboxy (C-) terminal of variable length (Figure 1). Other common characteristics for these receptors, are their highly conserved cysteines in ECL1 and ECL2, allowing disulfide binding in order to stabilize the receptor structure, the DRY motif in the intracellular side of TM3 and the NPXXY motif in TM7. The C-terminal loop can form a fourth ICL with the cell membrane, through palmitoylation at C-terminal cysteine residues (137).



**Figure 1.** Schematic representation of a GPCR. The seven transmembrane spanning helices are connected by three intracellular loops (ICL1-3) and three extracellular loops (ECL1-3). The bundle of helices is placed in the plasma membrane, with an extracellular N- terminus and an intracellular C- terminus.

Several common characteristics are maintained in the GPCRs, but their ligands differ greatly in size and structure. The size and aa sequence composition in extracellular regions also vary between GPCRs.

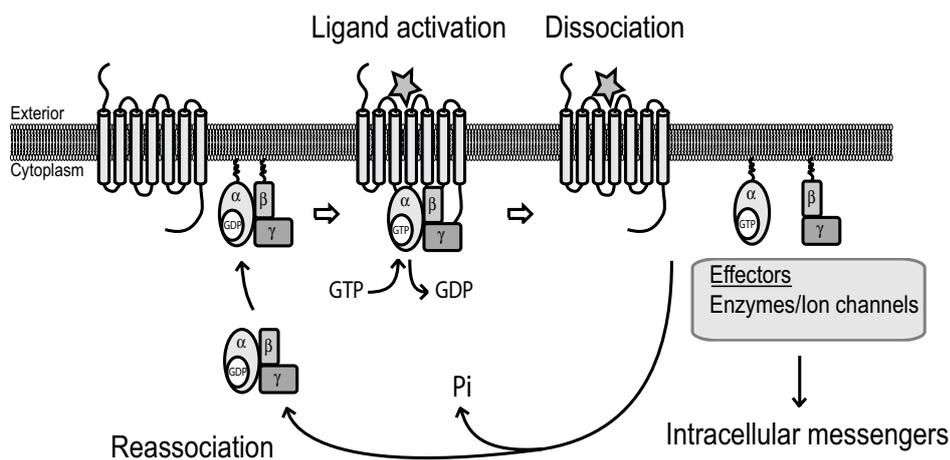
The human GPCRs were recently classified, based on phylogenetic analysis, into five different groups; glutamate, rhodopsin, adhesion, frizzled/taste2 and secretin, of which the rhodopsin family contains the most members (56). The receptors studied in this research project belong to the rhodopsin family.

## Signalling

GPCRs transmit extracellular stimuli into the cell. As their name implies, GPCRs induce their signal through interaction with a G-protein (GTP binding protein). The G-protein is composed of three subunits,  $\alpha$ ,  $\beta$  and  $\gamma$ . There are four classes of G-protein  $\alpha$ -subunit families,  $G\alpha_s$ ,  $G\alpha_{i/o}$ ,  $G\alpha_{q/11}$  and  $G\alpha_{12/13}$ . Each of these G-protein classes, regulate specific classes of effector molecules.  $G\alpha_s$  stimulates adenylyl cyclase (AC),  $G\alpha_{i/o}$  inhibits AC and activates  $K^+$  channels, and  $G\alpha_{q/11}$  stimulates phospholipase C (PLC).  $G\alpha_{12/13}$  are mainly involved in the small G-protein, Rho mediated responses (153).

The mechanism of signal transduction through a GPCR was originally described as a simple model (Figure 2) where ligand binding causes a conformational change in the receptor, which then recruits intracellular heterotrimeric G-proteins. As a result, the G-protein exchanges GDP for a GTP on the  $\alpha$ -subunit. This replacement induces dissociation of the subunits. Both the  $\alpha$  subunit and the  $\beta\gamma$  subunit regulate downstream intracellular effectors like enzymes or ion channels. The GTP is then hydrolyzed to GDP and the  $\alpha$  and  $\beta\gamma$  subunits re-associate (110).

The picture is now complicated by studies showing that the G-proteins are central but that other associated proteins also are important. The intracellular events following receptor activation, engagement of different pools of G-proteins, non-G-



**Figure 2.** GPCR activation and signalling.

protein effectors and multiple active receptor states gives the impression of a complex signal diversity (101). The existence of receptor dimers and oligomers, influencing receptor maturation, trafficking and ligand binding, further adds complexity to the system (55).

Fine-tuning of the receptor response occurs through different processes. Agonist binding to the GPCR starts a chain of events at both receptor level and further downstream, that regulate the ability of the receptor to respond. The receptor can be desensitized, internalized and also recycled again. The regulation of signalling can also occur through modulations of G-proteins or effectors (101).

## **Chemokines and their receptors**

Chemokines are small chemoattractant cytokines found in *e.g.* mammals, birds, amphibians and fish. They comprise a superfamily of structurally similar (20-50% homology), 8-15kD, (70-120 aa) polypeptides involved in immunity and inflammation (91, 115, 116, 135). Most chemokines are secreted and they act on GPCRs, induce chemotaxis and recruit cells into sites of inflammation. They are also involved in a variety of other activities such as maturation, homing, hematopoiesis and organogenesis (12, 84, 100, 114).

Chemokines are classified (CXC, CC, CX<sub>3</sub>C and XC) based on the number and spacing of the first two of four conserved cysteine residues in the N-terminal of the molecule. X stands for an aa residue other than cysteine. Most chemokines belong to the CC or CXC chemokines. There are now approximately 50 known human chemokines (24).

The chemokine receptors belong to the rhodopsin family of GPCRs. They are usually 340-380 aa in length with 25-80% sequence identity (19, 114). There are approximately 19 known chemokine receptors (24). Chemokine receptors have two conserved cysteine residues, in the N-terminal and in ECL3, which are believed to form a disulfide bridge, stabilizing the structure together with the putative bridge between ECL1 and ECL2, common in all GPCRs (18).

Homeostatic chemokines are constitutively expressed in specific organs, whereas inflammatory chemokines tend to be produced by many cell types in inflamed tissues. Chemokines induce chemotaxis in neutrophils, monocytes, lymphocytes, eosinophils, fibroblasts and keratinocytes. Dysfunction of the chemokine system has been

implicated in various conditions like multiple sclerosis, type-1 diabetes, rheumatoid arthritis, asthma and allergy (10, 19, 61, 122).

There is an overlap and redundancy in the chemokine system, since most chemokine receptors can bind more than one ligand and chemokines can often bind to and activate more than one receptor. Cells can also express multiple chemokine receptors (19).

In addition to their role as mediators of receptor signalling, many chemokines have also demonstrated *in vitro* antimicrobial potency, highlighting another property for the group of chemokines (165).

### **CCR5**

The CC-chemokine receptor CCR5 was cloned in 1996 (133) and is a receptor for the natural ligands, Regulated upon activation normal T-cell expressed and secreted (RANTES, CCL5), Macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ , CCL3) and MIP-1 $\beta$  (CCL4). CCR5 is involved in inflammation and is expressed on monocytes, macrophages, memory/effector T-cells, Natural Killer cells (NK-cells) and immature dendritic cells (DCs). The ligands are predicted to interact with the CCR5 N-terminal, but also with regions in ECL1 and ECL2. Like for most chemokine receptors, CCR5 activation engages G $\alpha_i$  proteins, which activates phospholipase C (PLC) producing the second messengers inositol triphosphate (IP<sub>3</sub>) and diacylglycerol (DAG), leading to intracellular calcium mobilisation and activation of protein kinase C (PKC). Other G-protein independent pathways may also be activated (94, 117, 133). This receptor is also the main coreceptor for entry of R5 strains of HIV-1 (44).

### **CXCR4**

The CXC-chemokine receptor CXCR4 was originally cloned in 1993 (51, 99). The receptor has only one known ligand, Stromal derived factor (SDF-1), which exists in at least two isoforms, SDF-1 $\alpha$  and  $\beta$ . CXCR4 is more of a house-keeping chemokine receptor, expressed on leukocytes, especially on naïve T-cells, B-cells, monocytes and also at lower levels in many other tissues (89, 106). SDF-1 is constitutively expressed in many tissues, which together with results from CXCR4/SDF-1 knockout mice show their essential role for different aspects of embryonic development (107). CXCR4 activation engages G $\alpha_i$  proteins. This receptor is also the main coreceptor for entry of X4 strains of HIV-1 (52).

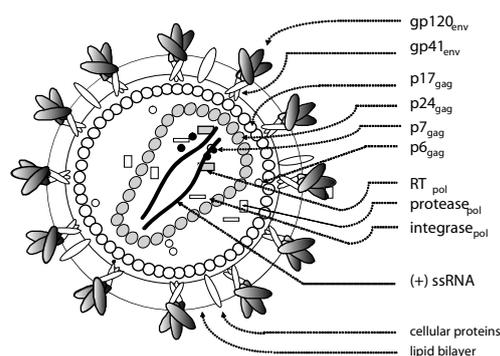
## HIV

### History

HIV, a lentivirus belonging to the *Retroviridae* family was isolated in 1983 (14, 57) and found to be the causative agent for AIDS. It was further divided into two types; HIV-1 and HIV-2, when a related virus was found in 1986 (32). HIV-1 is spread worldwide, but HIV-2 which is slightly less virulent, is mainly prevalent in certain regions of West Africa. Molecular phylogenetic studies have shown that HIV-1 originates from a strain of simian immunodeficiency virus (SIV) found in a subspecies of chimpanzees, a result of several occasions of cross-species transmission. HIV-2 originates from a SIV strain from sooty mangabeys. Based on sequence analyses, HIV-1 viruses are divided into three groups, M (most HIV-1 isolates), O (rare outliers) and group N. Group M has diverged to subtypes (clades) A-K. The subtypes have different geographic distribution with subtype C being most prevalent in the world and subtype B being most common in North America and Europe (69) ([www.unaids.org](http://www.unaids.org)).

### Virus structure

The mature virus is approximately 120 nm in diameter (Figure 3). The characteristic cone-shaped nucleocapsid composed by the viral capsid protein p24 in the core of the virus, contains the HIV-1 genome consisting of two copies of the plus stranded 10 kb RNA molecule, encoding nine genes. The RNA is closely associated with the stabilizing nucleocapsid protein p7 and the viral enzymes, reverse transcriptase (RT), integrase and cellular tRNA. The virus envelope consists of a lipid bilayer, originating



**Figure 3.** Structure of HIV-1.

from the host cell when the new virus buds off. The matrix protein, p17 lines the inside of the envelope. The viral glycoproteins, gp120 and gp41 are organized as trimers, forming spikes protruding the bilayer (145). Three of the nine genes, gag, pol and env encode structural proteins. The gag encodes a precursor protein, which among other things give rise to p24, p17 and p7 proteins. The env encodes the precursor gp160 which is cleaved to form gp120 and gp41 proteins. Pol

encodes three viral enzymes, RT, protease and integrase. The other six genes encode regulatory and accessory proteins involved in the virus replication cycle and infection (145, 156).

### **G protein-coupled receptors as coreceptors for HIV**

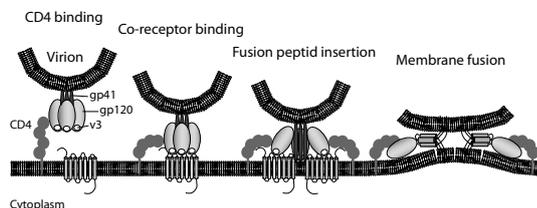
In 1995 the natural ligands of the receptor CCR5, were shown to inhibit HIV-1 cell entry (33). It was further shown, in 1996, that in addition to a CD4 molecule, virus interaction with either of the chemokine receptors CCR5 or CXCR4 was required for entry into target cells *in vitro* (6, 30, 41, 43, 44, 52).

The central role of these coreceptors *in vivo* was proven, when a mutant allele for CCR5, with a 32bp deletion within the coding region of CCR5, was identified. Homozygous individuals are strongly protected against infection of viruses using CCR5 for entry. This deletion causes a frame shift resulting in a truncated protein, not expressed on the cell surface. The allele is most common in northern Europe where about 15% of the population is heterozygous and approximately 1% is homozygous, for this mutation (40, 73, 98, 134).

In the years after the discovery of the major coreceptors of HIV-1, several other related GPCRs, were found to support fusion of different HIV-1 strains *in vitro*. These receptors are *e.g.* CCR2b (43), CCR3 (43), CCR8 (79), CXCR6 (46, 168), Gpr1 (46), Gpr15 (46), ChemR23 (132), BLT1 (118), RDC-1 (143), CX<sub>3</sub>CR1 (168) and APJ (168). The importance of these receptors *in vivo* is debated. CCR5 and CXCR4 seem to be the major coreceptors *in vivo* (166, 167, 169).

### **Viral entry and the replication cycle**

The HIV-1 enters its target cells by fusion with the cell membrane (Figure 4). The entry process begins with the interaction of trimeric viral gp120/gp41 with CD4 on the host cell. A conformational change is triggered in gp120 which allows a hidden



**Figure 4.** HIV binding and fusion with the target cell membrane.

region of gp41 to interact with one of the coreceptors, CCR5 or CXCR4. Further, a fusion peptide of gp41 is inserted in the target cell membrane and a six-helix bundle structure is formed, bringing the membranes together, leading to membrane fusion of viral envelope

and target cell membrane. Following fusion, the viral nucleocapsid is released into the cytoplasm (145). The RNA is uncoated and transcribed to double-stranded DNA by the virus encoded RT. The resulting proviral DNA is transported into the cell nucleus, where it is integrated into the host cell chromosomal DNA by the viral enzyme integrase. The integrated viral genome, the provirus, is transcribed by host cell RNA polymerase into new viral genomes and also mRNA transcripts which are translated into new viral proteins. Host cell transcription factors regulate the transcription. Viral RNA copies and proteins are transported to the cell membrane and assembled to immature virions. The budding triggers the virus encoded protease, to process precursor proteins, generating mature virus particles (145).

The coreceptor tropism is controlled by the *env* gene (74). The envelope protein gp120 contains five constant regions (C1-C5) and five variable regions (V1-V5). The V3 loop is suggested as the main determinant of biological phenotype (142, 144, 161) and small changes in the aa composition can alter the coreceptor use. However, other regions of gp120 have also been suggested as important for coreceptor usage (22, 77).

### ***Evolution of virus***

HIV is one of the fastest evolving organisms. The viral RT has no proofreading and as a consequence generates about 0.2 errors per genome in each replication cycle. There are also further errors done during transcription. The “HIV-1 viral generation time, defined as the time from release of a virion until it infects another cell and causes the release of a new generation of viral particles” (123) is about 2.5 days and approximately  $10^{10}$  virions are produced every day even in asymptomatic HIV-1 infected individuals. These properties together with recombination events and genetic selection create a huge antigenic variation and a challenge for the host immune system and for development of treatment strategies (123, 129).

### ***Biological phenotypes***

The biological phenotype of primary HIV isolates was originally described by their cell tropism, *in vitro* syncytium inducing capacity or cell culture replication kinetics. Isolates were defined as macrophage-tropic, non-syncytium inducing (NSI), slow/low virus and T-cell line tropic, syncytium inducing (SI) or rapid/high viruses (11, 53, 150). These classifications were only partly synonymous. When the coreceptors CCR5 and CXCR4 were discovered and shown to be in large responsible for different biological phenotypes, a new classification based on coreceptor use was established.

CCR5-using isolates were called R5 viruses, CXCR4-using isolates were described as X4 viruses and viruses able to use both receptors for entry were called R5X4 viruses (15).

R5 viruses are almost invariably isolated from patients with asymptomatic HIV-1 infection (36, 159, 172). During disease progression viruses able to use CXCR4 alone (X4-viruses) or in combination with CCR5 (R5X4) emerge in about 50% of infected individuals (16, 81, 150, 151). CXCR4-using viruses are considered more virulent and their appearance is linked to disease progression (11, 25, 53, 150, 151).

Individuals continuously harbouring CCR5-restricted isolates still progress to AIDS (39). Differences among primary R5-isolates have been described, as well as a progress towards a reduced sensitivity to C-C chemokines and especially RANTES inhibition of infection, during disease progression (76, 78). Increased cytopathicity of R5-isolates from individuals with disease progression, has also been observed (90, 138).

Why R5 viruses are transmitted and dominate during the asymptomatic phase, and why there is a subsequent switch from CCR5-using to CXCR4-using viruses is poorly understood. The switch is associated with declining CD4+ T-cell levels, but disease progression still occurs in individuals only harbouring CCR5-using viruses. Different hypothesis have been outlined trying to explain these events (105, 130).

In short, the dominance of R5 viruses early in infection is suggested to be a result of differences in coreceptor expression on target cells during pathogenesis (38) or the expression pattern of chemokines at sites of transmission (3). The switch of coreceptor use may also be explained by chance events of mutations in the predominated R5 viruses, changes of the viral fitness, further influenced by altered selection pressures occurring in immuno-compromized individuals (130).

### ***Transmission and pathogenesis***

In January 2006, UNAIDS (Joint United Nations Programme on HIV/AIDS) and WHO estimated that AIDS had killed more than 25 million people, since 1981. The total number of people living with HIV had reached 39.5 million ([www.unaids.org](http://www.unaids.org)).

The major routes of HIV transmission are through hetero and homosexual intercourse, sharing contaminated needles, vertical transmission from mother to child during pregnancy, breast-feeding, or receiving infected blood products (95).

### *Target cells*

The presence of CD4 and the coreceptors CCR5 or CXCR4 are the basis for making cells permissive for viral entry and infection. CD4+ T-cells, macrophages, microglial cells, and DCs are the main targets for HIV-1 infection.

### *Dendritic cells*

Immature DCs in the mucosa are believed to be important for the sexual transmission of HIV-1. These cells express low levels of CD4, CCR5 and CXCR4 and may be infected by HIV-1 but productive infection is restricted. Following virus binding to C-type lectins, like DC-SIGN and the mannose receptor, DCs migrate to lymphoid tissues and transmit HIV-1 to CD4+ T-cells. The DCs also present HIV-antigen and initiate immune responses (59, 163).

### *T-lymphocytes*

CD4+ T-cells can be divided in three subsets; (i) antigen naïve ( $T_N$ ) which circulate between blood and secondary lymphoid tissues and the memory cells, (ii) the antigen experienced, central memory ( $T_{CM}$ ) and (iii) effector memory cells ( $T_{EM}$ ).  $T_N$  express CXCR4, but little or no CCR5 whereas memory cells often express both CCR5 and CXCR4.  $T_{CM}$  are circulating in blood and lymphoid tissues and  $T_{EM}$  are found in large numbers in gut, liver and lung. There are numerous of CCR5 expressing  $T_{EM}$  cells. Located within and below genital and gastrointestinal tract epithelial layers this population is easily available at sexual transmission. The  $T_{EM}$  cells in gut-associated lymphatic tissues (GALT) seem to be responsible for the massive viral replication which can be detected in plasma analysis of HIV-1 RNA levels in acute HIV-1 infection. The loss of memory cells is followed by an increased differentiation of  $T_N$  cells and thus a suggested fatal reduction in the  $T_N$  pool (66, 67, 97, 102, 125).

### *Mononuclear phagocytes*

Macrophages are among the first cells to be infected by HIV-1. Although the number of infected macrophages are 10-100-fold lower, compared to CD4+ T-cells (47), they are considered as an important viral reservoir throughout infection and may contribute substantially to virus production in individuals with low CD4+ T-cells (AIDS) (149). Infection occurs primarily via the CCR5 receptor but CXCR4 facilitated macrophage infection also occurs (62, 86, 108). Macrophages express low levels of CD4, CXCR4 and CCR5 and virus isolates which replicate efficiently in macrophages may have adapted to the use of low receptor levels (152, 158). Monocytes have a low susceptibility to HIV-1 infection *in vitro*, but infection *in vivo*

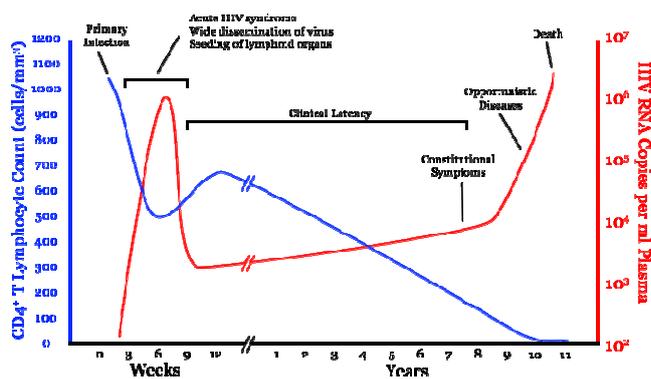
is evident from recently presented work (173). Macrophages and microglial cells are the major target cells for HIV-1 in the central nervous system (CNS) (65, 152).

### Stages of infection

The pathogenesis of HIV-1 infection is a slow process, with the average time from infection to symptomatic AIDS and death, without therapy, being about 10 years (119). Although considered slow, HIV-1 causes an active progressive disease.

The acute phase of infection in general lasting for a few weeks, is characterized by a massive viral replication reflected by high plasma levels of HIV-RNA, and a substantial loss of CD4+ memory T cells (Figure 5). Some people are asymptomatic, but many develop flue-like symptoms during this phase (31, 80, 125).

After a few weeks a partial control of infection is normally achieved, and the CD4+ T-cell count is partially restored. Simultaneously, the plasma viral load decreases to a steady level, the viral set point, which is a predictor for the future rate of disease progression, where a high set point predicts for a faster progression (103). This chronic, usually asymptomatic phase, where replication is partially controlled, often lasts for several years. The rate of memory T-cell proliferation is elevated, but the average life-span of the cells is shortened. The persistent loss of memory CD4+ T-cells requires a continuous differentiation of naïve CD4+ T-cells into memory CD4+ T-cells. The pool of naïve T-cells are eventually believed to be exhausted (111). After several years, the continuous CD4+ T-cell depletion, most often leads to the collapse of the immune system. At this point CD4+ T-cell counts rapidly decrease and plasma



**Figure 5.** General time-course of HIV-1 infection. The figure shows CD4+ T-cell count (blue) and plasma virus load (red).

viral load increase again (20, 125, 146). CD4+ T-cell counts <200 cells/ $\mu$ l and/or the occurrence of opportunistic infections, lymphomas and certain cancers are AIDS defining criteria (1).

There are a few HIV infected individuals, who remain asymptomatic and who have no

signs of immunological deprivation for several years without antiretroviral treatment. These long-term non-progressors, are clinically healthy after having been infected for >8 years, with CD4+ T-cell counts >500 cells/ $\mu$ l. Some of them eventually progress to AIDS but a few have remained healthy for more than 15 years. The explanation for their unique control of infection is incompletely understood, but it probably includes a mix of favourable host and viral factors (37, 120).

### **Central nervous system infection**

HIV-1 enters the CNS early in the course of infection (27, 28, 128). In the absence of anti-retroviral treatment, HIV-1 frequently causes neurological abnormalities such as AIDS dementia complex (ADC) (93, 162).

Symptoms of HIV-1 associated encephalitis are common and observed early in patients, suggesting that the neuropathological changes are a gradual process that may begin early. However severe ADC is more generally observed in later stages of infection (93, 162).

The blood-brain-barrier (BBB) separates the CNS from the circulatory system. A compartmentalization of HIV-1 infection seems to exist, since evidence of a separate viral evolution, differing from that in peripheral immune cells, has been found in phenotypic (26, 29) and genotypic studies (48, 58, 87, 112).

HIV-1 mainly invades the CNS via infected monocytes and/or T-cells. The HIV-1 infection in CNS persists at low levels until the onset of AIDS. Resident macrophages and microglial cells are the main HIV producing cells in the brain (88, 124). A viral adaption to replication in these target cells may include changes in coreceptor use. HIV-1 enter these cells mainly through the coreceptor CCR5 (5, 62, 68, 141, 147). Other cells in the CNS, such as astrocytes and neuronal cells, may also be infected and serve as a reservoirs for the virus even though infection seem to be non-productive (88).

The compartmentalization in CNS may reduce effectiveness and durability of drug treatment, as the BBB hampers the accessibility of antiretroviral agents. CNS may also serve as a sanctuary for drug resistant mutants (88). Compartmentalized differences in HIV-1 coreceptor use may affect the efficiency of emerging treatment with coreceptor antagonists.

Damage to the brain is suggested to be produced by the virus itself in combination with cellular factors released by activated and infected cells (88). Furthermore, viral gp120 cause apoptotic responses and neuronal damage through its interaction with chemokine receptors (23, 70, 170).

### ***Immune responses and escape***

The immune response against infections is made up of surface barriers, the innate and the adaptive immune response. The adaptive immune response is composed of the humoral and the cellular response.

The innate defence against HIV-1 mainly is composed of soluble factors *e.g.* cytokines, the complement system, and effector immune cells. TNF- $\alpha$  and IFN- $\gamma$  are examples of cytokines controlling viral replication. Effector cells of the innate immune system are macrophages, DCs, neutrophils and NK-cells. Chemokines recruit and affect cytotoxic function of NK-cells, T-cells and macrophages and can inhibit viral replication. The innate immune system is rapid and present at the major site of HIV-1 entry, the mucosal surface. It also activates the adaptive immune system against HIV-1 (96).

The humoral immune system is effectuated by HIV-specific antibodies produced by B-cell derived plasma cells. HIV specific antibodies (often non-functional) are detected soon after initial infection, within a few days or weeks. Eventually a small proportion of the antibodies are neutralizing with the ability to control infection of cells. There are epitopes for neutralization in both the variable and conserved regions of gp120 envelope protein. Broadly inhibiting antibodies recognize conserved epitopes and strain specific neutralization antibodies recognize variable loops (148, 164).

Within a few weeks after infection, HIV-specific Cytotoxic T-lymphocytes (CTLs) appear. Studies of SIV infection have shown that these cells partially control the viral load (136). CTLs are found in high numbers during the chronic phase and then decline in late stages of disease. These cells recognize and kill infected cells but also produce cytokines and chemokines, influencing and inhibiting infection (42, 139). Expression levels of CC-chemokines has been shown to affect risk of infection and disease progression (34, 157).

During the chronic phase of the disease, the viral replication continues at a level determined by the balance between immunological control and viral escape. HIV

neutralizing antibodies and CTL responses are important for the initial decrease in replication to the viral set-point. Viral escape occurs through evolution in and by concealing of neutralization epitopes, through heavy glycosylation and steric hindrance of conserved receptor binding sites. The gp120 tolerates a high level of escape mutations, and neutralization antibodies are often formed to an earlier virus strain (13, 42, 139).

### ***Treatment options***

In 1987 a nucleoside reverse transcriptase inhibitor (NRTI), zidovudine (AZT), became available for HIV infected individuals (49, 104). The drug appeared to prolong life and reduce the mortality (54), but the promising results were soon hampered by the invariable appearance of virus strains with a reduced sensibility to the drugs (92).

The development of other drugs, such as non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PIs) and the combinatorial use of drugs from different classes (Highly active anti-retroviral therapy, HAART), dramatically improved the prognosis for HIV infected patients with access to the drugs and have heavily reduced the morbidity and mortality (140). Though more successful than monotherapy, drug resistant virus still appear, and potentially serious side effects are a major concern.

Antiretroviral therapy is generally initiated when there is a high risk of opportunistic infections, usually at a CD4+ T-cell count level of about 200-300 cells/ $\mu$ l (U. Karlsson, MD personal communication).

In addition to several new approved NRTIs, NNRTIs, and PIs, new classes of drugs are under development, including integrase inhibitors and entry inhibitors. The only approved entry inhibitor, enfuvirtide (T-20), targets a gp41 region of the envelope and inhibits the fusion process (121, 146).

Also, chemokine receptor antagonists are currently developed for therapy. Several small molecule antagonists for CCR5 and CXCR4 have been tested for their ability to inhibit HIV-1 replication and a few have progressed to clinical trials. Since the drug target is a receptor, it will not undergo mutations in response to drug pressure, but the virus may evolve to change its use of CCR5. Developing drugs targeting CXCR4 has been difficult, since CXCR4 and its natural ligand SDF-1 seem essential for cellular processes (155).

A vaccine is probably the best strategy to prevent disease and the transmission of HIV. Several vaccines have been in clinical trials, but the work is challenging (45).

There are still no drugs available that are able to completely eliminate virus. HIV infection is chronic and life-long treatment is needed. New drugs and treatment strategies are, thus required (146).

## **AIMS OF THIS THESIS**

**Paper I** To construct CXCR4/CCR5 receptor chimeras (also called hybrid receptors) in order to characterize and compare epitopes used by the natural chemokine ligands, RANTES and SDF-1, and prototypic R5 and X4 HIV-1 isolates.

**Paper II** To study the evolution of HIV-1 biological R5 phenotypes in switch and non-switch patients, by the use of CXCR4/CCR5 receptor chimeras. Also, to investigate the correlation between mode of CCR5 use and disease progression. RANTES inhibition of infection in relation to chimeric coreceptor use was also included.

**Paper III** To evaluate wt and chimeric coreceptor use of paired plasma and cerebrospinal fluid HIV-1 isolates and further correlate these results with the degree of immunosuppression and the sensitivity to the CCR5 antagonist, TAK-779.

## **METHODOLOGY**

This section gives a short summary of the main methods used. For detailed information, see respective paper in the Appendix (Paper I-III).

### **Construction of chimeric receptors (Paper I, II)**

The receptor chimeras of CXCR4 and CCR5, were made by stepwise exchanging portions of CCR5 with corresponding regions of CXCR4. They were constructed using a variant of the single overlap and extension PCR method where the final sequence is made up of two pieces, each containing an overlapping sequence of the joint region of each receptor segment. The joint region was placed in conserved parts of TM regions, in order to avoid disturbance of protein conformation.

### **Cell lines and receptor expression (Paper I, II)**

Chinese Hamster Ovary cells (CHO-K1) were stably transfected with pCMV.IRES.AEQ plasmid using calcium phosphate precipitation. The clone with the highest luminescence function was chosen for further experiments. The IRES element of the vector ensured a high frequency of positive clones, since the gene of interest and the gene for selection are transcribed as one mRNA.

For signalling studies, CHO-K1.AEQ cells were transiently transfected using Lipofectamine. A pIRES-Puro vector containing the gene for the wild-type (wt) receptor or receptor chimeras was introduced into the cells. Flow cytometric analyses were used to evaluate cell surface expression of one cell aliquot. The other cell aliquot was used for calcium mobilisation assay. The high level of receptor expression and reproducibility allowed for transient transfections, as opposed to the more time-consuming stable transfections used for the infections experiments.

Human astrogloma U87.CD4 cells were stably transfected with receptor constructs and individual clones were tested for cell surface expression. Flow cytometric analyses were used to select a set of clones with similar receptor expression. Monoclonal cell lines were chosen for further experiments.

## **Generation of CXCR4 monoclonal antibody (Paper I)**

The monoclonal antibody (mAb) recognizing CXCR4, was generated by immunizing Balb/c mice with a synthetic peptide consisting of the N-terminal aa 2-16 of CXCR4. Positive clones were evaluated in cell-ELISA and the best clone, 5/5B5, was purified by protA affinity chromatography and tested on cell surface expressed CXCR4 receptors using flow cytometry. This antibody allowed for testing cell surface expression of all chimeric receptors and CXCR4, using one antibody.

## **Flow cytometry (Paper I, II, III)**

Flow cytometric analyses, were performed using the in-house CXCR4 antibody, or commercially available CCR5 and CD4 antibodies. The CD4 antibody was applied to evaluate the CD4 expression of all established U87 cell lines. A CCR5 antibody was used to evaluate the wt CCR5 expressing CHO-K1.AEQ or U87 cells.

## **Virus isolates (Paper I, II, III)**

Prototypic isolates HIV-1<sub>BaL</sub> and HIV-1<sub>IIIB</sub> used in Paper I, were a kind gift from professor Eva Maria Fenyö, Lund University, Sweden.

The 34 patients studied in Paper II were selected from a larger cohort of 53 HIV-1 infected individuals (76, 81). They were selected on basis of different rates of CD4+ T-cell decline and biological phenotypes, according to cell line assays. The study began in the mid-1980s and few were on antiretroviral therapy at onset of study.

The 28 HIV-1 infected patients in Paper III were retrospectively selected from a longitudinal study cohort at the Department of Infectious Diseases, Sahlgrenska University Hospital, Göteborg, Sweden (60). Fourteen patients were severely immunodeficient and seven patients were diagnosed with ADC. Twenty-five patients were anti-retroviral treatment naïve, and none had received anti-retroviral medication during at least nine months prior to virus isolation.

Virus was isolated from peripheral blood mononuclear cells (PBMCs) and cerebrospinal fluid (CSF), according to standard procedures (4, 7).

The prototypic HIV-1 isolates (Paper I) and the primary patient isolates (Paper II, III) were passaged in phytohemagglutinin-P (PHA-P) and interleukin-2 (IL-2)

stimulated PBMCs. Virus was grown and harvested at days 7 and 10. The virus stocks were stored at  $-140^{\circ}\text{C}$ . Virus content was evaluated in terms of p24 assays. Chosen isolates were also evaluated for RT activity.

### **Cell signalling (Paper I)**

Receptor-mediated cell function was analyzed using the aequorin assay. The photoprotein aequorin is stably expressed in CHO-K1 cells. Briefly, CHO-K1.AEQ cells transiently expressing receptor protein were incubated with coelenterazine and portions of the cells were stimulated with different concentrations of ligands. The calcium response was then evaluated in a luminometer, and 50% effective concentration ( $\text{EC}_{50}$ ) was calculated.

### **Infection assay (Paper I, II, III)**

U87.CD4 cell lines stably expressing wt or chimeric receptors were seeded in 48-well plates and after three days they were incubated with HIV-infected PBMC culture supernatants. The cells were washed and fresh medium added. Supernatants from the infected cell cultures were collected at day 0 (after washing) and day 5 or 7 of infection and assayed with p24 antigen ELISA. The cell cultures were inspected regularly and syncytia formation was also recorded.

### **Inhibition of infection (Paper II, III)**

PHA stimulated PBMCs were infected in the presence of RANTES. This was diluted in three-fold steps starting at a final concentration of 600 ng/ml. After three days the medium was changed and RANTES concentrations restored. The 50% inhibitory concentrations ( $\text{IC}_{50}$ ) were calculated seven days after infection, using results of p24 ELISA.

RT-normalized virus was used for the TAK-779 inhibition experiments. PHA-activated PBMCs were infected in triplicates with virus in the presence of TAK-779. TAK-779 was serially diluted in three-fold steps, starting at the concentration of 990 nM, and simultaneously added to the cells and virus. Infected PBMCs were washed on day 1 and fresh inhibitor at concentrations corresponding to the setup were added. Supernatants were harvested on day 7. The sensitivity to TAK-779 was evaluated as  $\text{IC}_{50}$  and  $\text{IC}_{90}$ , calculated from p24 antigen release in the control cultures.

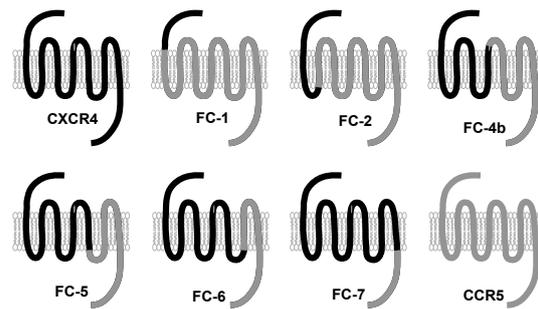
## SUMMARY OF RESULTS

For details, see respective paper in the Appendix (Paper I-III).

### Paper I

We set out to compare the epitopes of CCR5 and CXCR4 used by the natural ligands for signalling, and the epitopes used by prototypic R5 and X4 HIV-1 strains for entry (8). A set of seven hybrid CXCR4/CCR5 receptors were constructed, where increasing portions of CCR5 were replaced with the corresponding parts of CXCR4. The cell surface expression was analyzed by flow cytometry after the constructs were transfected into cell lines. An in-house mAb, recognizing the N-terminal of CXCR4 was generated and could be used to study the cell surface expression of all chimeras. Five of the chimeras, FC-1, FC-2, FC-5, FC-6 and FC-7, were successfully expressed. Two of them, FC-3 and FC-4, did not reach the cell surface. Three closely related variants, FC-3a, FC-3b and FC-4b, were constructed and one of them, chimera FC-4b showed cell surface expression. Thus, a set of six chimeras, FC-1, FC-2, FC-4b, FC-5, FC-6 and FC-7 (Figure 6), were used in the further experiments.

The aequorin-based assay was used for signalling analysis with the natural ligands, SDF-1 and RANTES. CHO-K1.AEQ cells, stably expressing the aequorin protein, were transiently transfected with the receptor constructs. Cell surface expression was verified in each experiment before stimulation with different concentrations of each ligand. The cells expressed similar amounts of receptors, and  $EC_{50}$  values of each receptor ligand-stimulation, were calculated.



**Figure 6.** Schematic representation of CXCR4 (black) and CCR5 (grey) and the CXCR4/CCR5 chimeric receptors FC-1, FC-2, FC-4b, FC-5, FC-6 and FC-7.

Both RANTES and SDF-1 stimulated their cognate wt receptor in a concentration-dependent manner, giving  $EC_{50}$  values in the normal physiological range. Only two of the chimeras, FC-1 and FC-2, showed signalling in response to RANTES. The  $EC_{50}$  values were 100-1000 times higher than for the wt CCR5 receptor. Only the chimera FC-7 signalled in response to SDF-1.

The same set of chimeric receptors, were stably transfected into U87.CD4 cells, and cell lines expressing similar amounts of receptors were selected for subsequent experiments. The set of U87.CD4 cell lines expressing wt and chimeric coreceptors, was tested for HIV-1 entry. Cells were infected with the laboratory strains, HIV-1<sub>BaL</sub> and HIV-1<sub>III<sub>B</sub></sub>. Infection with HIV-1<sub>BaL</sub>, but not HIV-1<sub>III<sub>B</sub></sub>, was strongly supported by wt CCR5. The ability of HIV-1<sub>BaL</sub> to infect the test cells was lost when the N-terminal of CCR5 had been exchanged with that of CXCR4 (chimera FC-1). However, when larger segments of CXCR4 were introduced (chimera FC-2 and FC-4b), infection was partly resumed. The other chimeras, as well as wt CXCR4, did not show coreceptor activity in the presence of HIV-1<sub>BaL</sub>.

Insertion of the N-terminal from CXCR4 into CCR5 (chimera FC-1) did not support infection with HIV-1<sub>III<sub>B</sub></sub>. In the remaining chimeras, with increasing contribution of CXCR4, a varying degree of HIV-1 infection was resumed. The chimera FC-5 differed somewhat from the others in that the p24 levels resulting from infection were quite variable. The coreceptor function of the chimera containing the CCR5 C-terminal in CXCR4 (chimera FC-7) was as efficient as for wt CXCR4. HIV-1<sub>III<sub>B</sub></sub> did not infect cells expressing wt CCR5.

There was a considerable difference in the way the chimeric constructs interacted with the natural ligands RANTES and SDF-1, compared to the interactions with the two HIV-1 strains. The results with RANTES and HIV-1<sub>BaL</sub> suggested that relatively small parts of the receptor are of critical importance, but that the epitopes involved differ. For RANTES there seemed to be a complementary use of both the N-terminal and the first two ECLs. SDF-1 required an essentially complete CXCR4, whereas HIV-1<sub>III<sub>B</sub></sub> was less demanding in its use of the receptor.

The results show that the two prototypic HIV-1 strains tested, interact with different epitopes on their respective coreceptors, and that the interaction of the respective ligands with corresponding receptors diverges from the viral interaction. These findings would provide a basis for tailoring future drugs that block viral entry through the two major coreceptors without interfering with their physiological function.

## **Paper II**

In this paper (83) the evolution of primary HIV-1 isolates was studied. A total of 246 isolates from 31 HIV-1 infected individuals were tested. The patients were selected based on their different rates of CD4<sup>+</sup> T-cell decline in the first five years of their

infections. The viral evolution was then further correlated to pathogenesis. Isolates were tested for wt coreceptor use. Virus isolations from 17 patients yielded R5 isolates throughout the study and 14 initially yielded R5 isolates but later switched to X4 isolates. The set of six CXCR4/CCR5 chimeras in U87.CD4 cells were infected with these sequentially isolates.

The R5 isolates used FC-1, FC-2, and FC-4b chimeras to various degrees, but did not use the other chimeric receptors, or CXCR4. Based on these results, the R5 viruses could be subdivided into two groups: those with the R5<sup>narrow</sup> phenotype and those with the R5<sup>broad</sup> phenotype. The R5<sup>narrow</sup> phenotype is defined as viruses that use wt CCR5 but no chimeric receptors, whereas viruses using at least one chimeric receptor in addition to CCR5 are designated R5<sup>broad</sup> viruses. Depending on the number of chimeric receptors used, the R5<sup>broad</sup> viruses were further divided into R5<sup>broad(1)</sup>, R5<sup>broad(2)</sup>, and R5<sup>broad(3)</sup> phenotypes.

The R5 phenotype and its relation to pathogenesis was evaluated. In short, R5 phenotypes from first and last R5 isolates, obtained from 26 patients and isolated at least 18 months apart, were tested. An evolution of the R5<sup>narrow</sup> phenotype towards a broader use of CCR5 was found. The R5 phenotype was also correlated with the degree of immunosuppression, as measured by the CD4+ T-cell counts. Broader R5 isolates were more often isolated from patients with low CD4+ T-cell counts than from patients with high CD4+ T-cell counts. The evolution of R5 phenotypes occurred in both the switch and non-switch group, but was more pronounced in the switch group.

Infectious titers of R5 isolates with different phenotypes were tested. The R5<sup>broad</sup> phenotypes showed an increased infectivity, compared to R5<sup>narrow</sup> isolates, in studies on CCR5 expressing cells.

Different R5 phenotypes were also evaluated for their sensitivity to inhibition by the CCR5 ligand RANTES. The analysis showed that the ability to utilize chimeric receptors correlated inversely with the sensitivity to RANTES inhibition.

The work indicates that various R5 phenotypes isolated from different stages of infection differ in their mode of coreceptor use. Phenotypic changes during viral evolution may reflect a more flexible or efficient use of CCR5. While some viruses require the N-terminal of CCR5 (R5<sup>narrow</sup>), others can utilize alternative receptor

epitopes (R5<sup>broad</sup>). Importantly, the mode of coreceptor use correlated with the sensitivity to inhibition by the CCR5 ligand, RANTES.

### **Paper III**

In this paper (9) we set out to study the mode of coreceptor use by paired plasma and CSF isolates from HIV-1 infected individuals with varying degree of immunodeficiency and neuropathology. Twenty-eight HIV-1 infected patients were selected from a longitudinal study cohort at the Department of Infectious Diseases, Sahlgrenska University Hospital, Göteborg, Sweden (60). Fourteen patients were severely immunodeficient and seven patients were diagnosed with ADC. Twenty-five patients were anti-retroviral treatment naïve, and none had received anti-retroviral medication during at least nine months prior to virus isolation.

The paired plasma and CSF isolates were tested for their ability to infect U87.CD4 cells expressing CCR5 or CXCR4. The R5 viral phenotypes predominated both in plasma and in CSF. CXCR4 using viruses were found in plasma samples from seven patients. In three of the corresponding CSF-isolates only R5 phenotypes were detected and one CSF isolate (R5X4) utilized CXCR4 30 times less efficiently as compared to the corresponding (X4) plasma isolate.

The mode of CCR5 use was also studied using the chimeric CXCR4/CCR5 receptors. The chimeras FC-1, FC-2 and FC-4b used by R5-isolates in Paper II were applied in the study. We were able to identify discordant plasma/CSF R5 viral phenotypes in six of 21 patients, but there were no characteristic patterns of chimeric receptor use that could distinguish CSF-isolates from plasma isolates. Further, R5 phenotypes ranging from R5<sup>narrow</sup> to R5<sup>broad(3)</sup> were represented in both compartments in a non-specific manner. In seven patients with ADC, no specific patterns of chimeric coreceptor use by CSF-isolates or plasma isolates were found. CSF neopterin levels did not correlate with the mode of coreceptor use.

To evaluate a possible relationship between mode of coreceptor use and susceptibility to inhibition by the CCR5 antagonist, TAK-779, we selected paired R5-virus isolates from seven patients with varying degree of immunodeficiency and chimeric receptor use, including three patients with ADC. Virus isolates with a moderate to high ability to utilize chimeric receptors were all relatively resistant to inhibition by TAK-779.

The mode of CXCR4/CCR5 chimeric receptor use was correlated with CD4+ T cell counts and viral load for each individual. Individuals harbouring plasma R5<sup>broad(2-3)</sup> phenotypes had significantly lower CD4+ T-cell counts as compared to individuals with R5<sup>narrow</sup> or R5<sup>broad(1)</sup> phenotypes. The strongest association with immune suppression was found when comparing individuals with FC-2 using (FC-2+) R5 plasma isolates to those with FC-2 negative phenotypes (FC-2-). The presence of X4 or R5X4 phenotypes was, as expected, linked to immunosuppression. Looking at viral phenotypes within the CSF, we found a significant correlation between the presence of FC-2+ R5 isolates and elevated CSF viral load.

The R5 virus dominance in CSF isolates may be explained by a lower capacity of X4/R5X4 variants to replicate in target cells within CNS. We further believe that the ability to utilize our CXCR4/CCR5 chimeric receptors reflects a different mode of CCR5 use. Efficient chimeric receptor use correlated to increased resistance to TAK-779 inhibition. The chimera FC-2, was shown to be a useful tool for studying the R5 phenotypes during disease progression.

## DISCUSSION AND PERSPECTIVES

CCR5-using isolates are transmitted and can be isolated at all stages of disease progression, but in approximately 50% of HIV-1 infected individuals CXCR4 using viruses emerge during progression to AIDS (16, 81, 150, 151). The CXCR4 using isolates are associated with disease progression and increased virulence (11, 25, 53, 150, 151).

With the aim of mapping the epitopes by which HIV-1 interacts with its major target cell receptors, CCR5 and CXCR4, and further to compare them with the epitopes used by the natural ligands SDF-1 and RANTES during receptor activation, hybrid (*i.e.* chimeric) CXCR4/CCR5 receptors were constructed (Paper I). We wanted to study the dynamics of the shift by virus and the natural ligands, respectively from one receptor to the other. Therefore, the two receptors were mixed in various proportions. We successively replaced larger segments of CCR5 with corresponding segments of CXCR4, and the junctional region was hence moved along the receptor structure in an unbiased manner to include an additional TM region in each new segment. A set of six receptor chimeras was used in the experiments. The chimeras were stimulated with the natural chemokine ligands SDF-1 and RANTES and also with prototypic R5 and X4 isolates, respectively. The ligands and virus were found to use different epitopes which, in turn, varied between the receptors. The results open up for a possibility to develop inhibitors of HIV-1 entry, without interfering with the physiological function of these receptors.

CCR5 and CXCR4 are differently targeted during disease progression and these receptor chimeras made it in addition possible to detect an unprecedented variation in coreceptor use during HIV-1 pathogenesis (82).

The evolution of HIV-1 coreceptor use during disease progression and the switch from CCR5 to CXCR4 use has been studied extensively. However, the mechanisms behind the disease progression in individuals that develop AIDS due to infection with exclusive R5 phenotypes are less understood. An increased sensitivity to RANTES inhibition, was shown to appear in R5-isolates from patients developing AIDS (76, 78). R5 viruses were also shown to evolve into more virulent phenotypes, in patients with progressive disease (90). In addition, evolution of CCR5 use has also been shown *in vitro*, during selection pressure of a small-molecule CCR5 antagonist (154). Also, Gorry et al have reported on HIV-1 variants with increased affinity for CCR5

and reduced dependence of CCR5 and CD4 (63). Together, these results have suggested evolutionary changes within the group of R5 viruses.

Using our chimeric CXCR4/CCR5 receptors, we could show that R5 isolates from immunosuppressed individuals are distinct from those isolated from individuals with higher CD4+ T-cell counts with regard to coreceptor usage (Paper II). Broad CCR5 usage, as measured by the use of chimeric coreceptors, was also associated with a decreased sensitivity to inhibition by the natural CC-chemokine, RANTES.

The CNS is invaded early in the course of HIV-1 infection, (27, 28, 128) and replication in macrophages and microglial cells eventually induces neuropathologic conditions. Due to the existence of a BBB the CNS is considered to be a restricted compartment, where a separate viral evolution has been described (26, 29, 48, 58, 87, 112). We set out to study coreceptor usage of paired plasma and CSF isolates (Paper III). The chimeras FC-1, FC-2 and FC-4b were applied in the study. Discordant CXCR4 and CCR5 use was found in paired isolates. In addition, the chimeras allowed us to detect discordance in chimeric receptor use between the two compartments. Furthermore, the ability of R5 isolates to use chimera FC-2 correlated with immunosuppression. Efficient chimeric receptor use also correlated with an increased resistance to the CCR5 antagonist, TAK-779.

The findings from Paper II and III are also in agreement with more recent studies by others that have demonstrated an increased viral resistance to entry inhibitors, including TAK-779 and natural CCR5 ligands, by R5 isolates from individuals with AIDS (65, 85, 113, 131). We have expanded upon these observations by showing, in more detail, how the mode of coreceptor use is linked to the sensitivity by these inhibitors.

We believe that the ability of R5 viruses to utilize these CXCR4/CCR5 chimeric receptors reflects a more flexible and more efficient CCR5 usage. This may include a reduced dependency upon interactions with the N-terminal residue of the receptor during infection. R5 variants with the ability to use CCR5 lacking the N-terminal has been described (126, 127). Also, the N-terminal of CCR5 has been shown to be important for signalling (Paper I) (17, 71, 109, 171), and this may be one explanation for the correlation between chimeric receptor use and viral sensitivity to inhibition by RANTES.

In our studies of R5 isolates we applied the CXCR4/CCR5 receptor constructs. Since both receptors are HIV-1 coreceptors, parts of CXCR4 may compensate for exchanged parts of CCR5. To address this issue, new chimeras were constructed, and a combination of CXCR2 and CCR5 was chosen. Unfortunately, these CXCR2/CCR5 hybrid receptors could not be expressed on the cell surface.

The evolution of R5 isolates possibly reflects changes in expression patterns of the natural ligands during disease progression. The viral envelope may be selected for changes in coreceptor density, conformation, or post-translational modifications. Naturally occurring R5 env variants, with distinct mode of CCR5 and CD4 interaction and varying sensitivity to inhibition by RANTES, has been reported (72). Moreover, glycosylation and sulfation patterns of receptors have also been shown to affect coreceptor function (50, 160). This viral selection possibly reflects changes in the access to target cells and their particular way of presenting the coreceptors. Late in disease progression the main available target cells, the T-cells, are limited and macrophages may well be the main source of virus production. This R5 virus evolution may be a consequence of a collapsed immune system which allows for the appearance of HIV-1 phenotypes with a specific mode of coreceptor use.

The CXCR4/CCR5 chimeric receptors have proven to be valuable tools in studying coreceptor use and especially R5 viral evolution. They made it possible to explore the existence of R5 phenotypes that were not possible to distinguish in studies using only wt CCR5 or CXCR4 receptors, respectively. The chimeras have thus provided the basis for a new nomenclature of HIV-1, where R5 viruses can be further subdivided, based on chimeric receptor use. According to which R5 isolates can be subdivided into the R5<sup>narrow</sup> and the R5<sup>broad(1-3)</sup> isolates.

In Paper III we found that R5 virus use of the chimeric receptor, FC-2, correlated highly with immunosuppression. The chimera FC-2 alone may be a useful tool in future studies on R5 pathogenesis, and also for the optimization of emerging treatment with CCR5 antagonists. The possible correlation between chimeric receptor use and cell tropism is a subject for future studies, as this would help to further clarify the mechanisms behind R5 virus pathogenesis. The correlation between chimeric receptor use and sensitivity to inhibition by CCR5 antagonists needs to be verified in larger studies with clinical isolates. It may well be that individuals harbouring R5 isolates can be tested and further subdivided for different treatment strategies. The possibility that CCR5 antagonists may give rise to viral escape mutants of the R5<sup>broad</sup> phenotype should also be considered. Our studies have

relevance not only with regards to R5-virus pathogenesis and optimization of treatment strategies, but also for HIV-infection within the CNS.

In conclusion, our findings propose alterations in the mode of CCR5 use that may be a key event in R5 virus pathogenesis. HIV-1 disease progression is paralleled by an increased capacity of R5 viral phenotypes to utilize CXCR4/CCR5 chimeras and a concomitant decreased viral sensitivity to inhibition by RANTES and TAK-779. Further studies of R5 isolates should be carried out to broaden the understanding of HIV-1 pathogenesis and to develop new treatment strategies.

## POPULÄRVETENSKAPLIG SVENSK SAMMANFATTNING

Människan är uppbyggd av ett stort antal celler. För att kroppens olika organ ska kunna samverka och för att kroppen ska kunna fungera som en helhet, måste de olika cellerna kommunicera med varandra.

Cellernas kommunikationssystem är uppbyggt av mottagare, s.k. receptorer, på de olika cellernas yta och signaler som skickas runt mellan cellerna. Receptorerna och signalerna fungerar som nyckel och lås, d.v.s. när rätt signal når rätt receptor så får just den cellen information från andra celler och omgivningen. På det sättet når en viss information bara de celler som behöver just den upplysningen. Olika delar av kroppen dirigeras för att fungera på ett visst sätt, vid ett tillfälle. Signalerna kan exempelvis bestå av luktsignaler, hormoner, ljus som lyser in i ögat eller ämnen som påverkar kroppens immunsystem. De flesta receptorer tillhör en familj som heter GPCR.

Man har uppskattat att det finns ungefär 800 olika GPCR-receptorer i vår kropp varav ungefär hälften bearbetar luktsignaler som kommer in via näsan. Ett flertal andra signaler och receptorer gör att vårt immunförsvar samarbetar. Immunförsvaret får signaler om främmande ämnen som kommit in i kroppen, eller från en skada som skett i något av kroppens organ. Med hjälp av olika signaler kan sedan immunsystemet agera för att ta bort ämnet eller för att reparera skadan i kroppen.

År 1981 började man lägga märke till att ett flertal människor i världen drabbades av en ny okänd sjukdom som bröt ner immunförsvaret. Dessa tidigare friska människors immunsystem fungerade inte längre och de blev sjuka och dog av olika infektionssjukdomar och cancersjukdomar. År 1983 fann man att viruset HIV (Human immunodeficiency virus) orsakade denna nya sjukdom, AIDS (Acquired immunodeficiency syndrome). Sedan dess har många människor i världen blivit infekterade och avlidit i AIDS.

Detta arbete handlar om två GPCR-receptorer, CCR5 och CXCR4, som normalt fungerar i kroppens immunförsvar. 1996 visade det sig att dessa två receptorer utnyttjas av HIV när det tar sig in och infekterar olika celler i kroppen. Utan dessa receptorer på cellytan kan cellerna inte infekteras. Denna nya kunskap ledde till att intresset för hur HIV använder sig av och kommer in i cellerna via dessa receptorer, blev mycket stort.

När en person blir infekterad använder HIV nästan alltid den ena receptorn CCR5. Hos personer som är svårt sjuka i AIDS har man kunnat visa att de virus som de har i kroppen då, ofta använder den andra receptorn, CXCR4. Det verkar alltså som att HIV förändras under den tid det tar från att man smittats, tills man är svårt sjuk i AIDS, något som ofta tar många år.

I det här arbetet har vi tagit reda på mer om hur det går till när HIV tar sig in i cellerna genom att använda dessa receptorer. För att kunna göra detta tillverkade jag blandreceptorer (hybrider), som består av delar från både CCR5 och den andra receptorn CXCR4.

I ett första arbete visade vi att HIV använder receptorerna på andra sätt än immunförsvarets naturliga signalämnen. Resultatet visade att man kan utveckla HIV-läkemedel som blockerar användandet av dessa receptorer utan att störa de naturliga signalerna som överförs via receptorerna.

Genom samarbeten med Lunds universitetssjukhus och Sahlgrenska universitetssjukhuset i Göteborg har vi kunnat ta fram HIV från ett flertal patienter. HIV från smittade, fortfarande relativt friska personer, samt från patienter som är svårt sjuka i AIDS, har isolerats. Med hjälp av de konstgjorda hybridreceptorerna har vi sedan kunnat visa att dessa olika HIV-isolat använder receptorerna på olika sätt för att ta sig in i cellerna. Det sker alltså en utveckling av viruset under sjukdomsprocessen. Viruset förändrar sitt sätt att använda receptorn, CCR5, när personen blir sjukare och det blir också svårare att blockera infektionen med vissa typer av HIV-läkemedel.

Vi har dessutom isolerat HIV från hjärnan för att jämföra med HIV som finns i kroppens blodbanor utanför hjärnan. Även här har vi kunnat se skillnader i hur viruset använder CCR5 och att viruset i hjärnan ibland skiljer sig från viruset i blodbanan, hos en och samma person. Detta ökar förståelsen för den speciella AIDS-demens som patienter kan drabbas av. Även i denna studie hittade vi virus med olika känslighet för blockering med läkemedel.

Hybridreceptorerna som har använts i de tre studier som avhandlingen bygger på, har visat sig vara mycket bra verktyg för att förstå infektionen och sjukdomsprocessen. Genom att lära sig mer om hur HIV fungerar och tar sig in i den infekterade personens olika celler i kroppen, kan man öka kunskapen om sjukdomen, optimera behandlingen, samt lättare utveckla nya läkemedel mot HIV och sjukdomen AIDS.

## ACKNOWLEDGEMENTS

I would like to thank all those who have taken part in the completion of this thesis:

Professor **Christer Owman**, my supervisor, for giving me the opportunity to work with this thesis and learn more about GPCRs. Thank you also for your support and for creating a positive atmosphere and for sharing your deep knowledge about molecular biology and medicine.

**Björn Olde**, for introducing me to all those different molecular biological techniques and for sharing your great knowledge about GPCRs.

**Joanna Daszkiewicz-Nilsson**, **Meta Pusch** and **Ulla-Britt Andersson**, for your valuable technical assistance at different time points and for being warm and friendly.

**Ulf Karlsson**, my co-author, for nice collaborations, for always having a positive attitude and also for valuable scientific discussions.

**Caroline Sandén**, for all those nice walks and talks.

For all other past and present members of Division of Molecular Neurobiology and Drug Target Discovery (in no particular order): **Annika Pettersson**, **Erik Flodgren**, **Al Sabirsh**, **Knut Kotarsky**, **Ulrika Mårtensson**, **Jesper Bristulf**, **Åke Boketoft**, **Niclas Nilsson**, **Ylva Tryselius**, **Jenny Eklund**, **Kristina Ryberg**, **Johan Enquist**, **Marie Ingemarsson**, **Katarina Danielson**, **Dong-Soo Kang** and professor **Fredrik Leeb-Lundberg**. For all the help and support on many different things and for kindness, coffee breaks and fun.

Professor **Eva Maria Fenyő** and her group, especially **Elzbieta Vincic**, for valuable technical assistance, and my co-authors **Ingrid Karlsson**, **Marianne Jansson** and **Johanna Repits**, for nice collaborations in fruitful projects but also in those interesting pilot-projects... **Anna Laurén** and **Mattias Mild** for nice collaborations and good company in the p3 lab.

Dr **Robert C. Gallo** for giving me the opportunity to work at IHV.

Dr **Alfredo Garzino-Demo** for teaching me infection experiments and at the same time sharing his sense of humour.

Collaborators at Sahlgrenska University Hospital in Gothenburg.

Co-authors.

My friends and family, my parents, my brother and his family. My father-in-law. For always believing in me.

**Peter** and **Simon**. You two are my love, my source of energy and my joy.  
Thank you Peter for endless support!

## REFERENCES

1. 1993. From the Centers for Disease Control and Prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *Jama* **269**:729-30.
2. 1981. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California. *MMWR Morb Mortal Wkly Rep* **30**:305-8.
3. **Agace, W. W., A. Amara, A. I. Roberts, J. L. Pablos, S. Thelen, M. Ugucioni, X. Y. Li, J. Marsal, F. Arenzana-Seisdedos, T. Delaunay, E. C. Ebert, B. Moser, and C. M. Parker.** 2000. Constitutive expression of stromal derived factor-1 by mucosal epithelia and its role in HIV transmission and propagation. *Curr Biol* **10**:325-8.
4. **Albert, J., and E. M. Fenyo.** 1990. Simple, sensitive, and specific detection of human immunodeficiency virus type 1 in clinical specimens by polymerase chain reaction with nested primers. *J Clin Microbiol* **28**:1560-4.
5. **Albright, A. V., J. T. Shieh, T. Itoh, B. Lee, D. Pleasure, M. J. O'Connor, R. W. Doms, and F. Gonzalez-Scarano.** 1999. Microglia express CCR5, CXCR4, and CCR3, but of these, CCR5 is the principal coreceptor for human immunodeficiency virus type 1 dementia isolates. *J Virol* **73**:205-13.
6. **Alkhatib, G., C. Combadiere, C. C. Broder, Y. Feng, P. E. Kennedy, P. M. Murphy, and E. A. Berger.** 1996. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272**:1955-8.
7. **Andersson, L. M., B. Svennerholm, L. Hagberg, and M. Gisslen.** 2000. Higher HIV-1 RNA cutoff level required in cerebrospinal fluid than in blood to predict positive HIV-1 isolation. *J Med Virol* **62**:9-13.
8. **Antonsson, L., A. Boketoft, A. Garzino-Demo, B. Olde, and C. Owman.** 2003. Molecular mapping of epitopes for interaction of HIV-1 as well as natural ligands with the chemokine receptors, CCR5 and CXCR4. *Aids* **17**:2571-9.
9. **Antonsson, L., U. Karlsson, J. Repits, B. Ljungberg, K. Kidd-Ljunggren, L. Hagberg, B. Svennerholm, M. Jansson, M. Gisslen, and C. Owman.** 2007. Discordant HIV-1 phenotypes in paired plasma and cerebrospinal fluid samples - clinical implications of varying mode of coreceptor use. Manuscript.
10. **Arimilli, S., W. Ferlin, N. Solvason, S. Deshpande, M. Howard, and S. Mocci.** 2000. Chemokines in autoimmune diseases. *Immunol Rev* **177**:43-51.

11. **Asjo, B., L. Morfeldt-Manson, J. Albert, G. Biberfeld, A. Karlsson, K. Lidman, and E. M. Fenyo.** 1986. Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. *Lancet* **2**:660-2.
12. **Baggiolini, M.** 1998. Chemokines and leukocyte traffic. *Nature* **392**:565-8.
13. **Bailey, J., J. N. Blankson, M. Wind-Rotolo, and R. F. Siliciano.** 2004. Mechanisms of HIV-1 escape from immune responses and antiretroviral drugs. *Curr Opin Immunol* **16**:470-6.
14. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dautet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220**:868-71.
15. **Berger, E. A., R. W. Doms, E. M. Fenyo, B. T. Korber, D. R. Littman, J. P. Moore, Q. J. Sattentau, H. Schuitemaker, J. Sodroski, and R. A. Weiss.** 1998. A new classification for HIV-1. *Nature* **391**:240.
16. **Bieniasz, P. D., and B. R. Cullen.** 1998. Chemokine receptors and human immunodeficiency virus infection. *Front Biosci* **3**:d44-58.
17. **Blanpain, C., B. J. Doranz, J. Vakili, J. Rucker, C. Govaerts, S. S. Baik, O. Lorthioir, I. Migeotte, F. Libert, F. Baleux, G. Vassart, R. W. Doms, and M. Parmentier.** 1999. Multiple charged and aromatic residues in CCR5 amino-terminal domain are involved in high affinity binding of both chemokines and HIV-1 Env protein. *J Biol Chem* **274**:34719-27.
18. **Blanpain, C., B. Lee, J. Vakili, B. J. Doranz, C. Govaerts, I. Migeotte, M. Sharron, V. Dupriez, G. Vassart, R. W. Doms, and M. Parmentier.** 1999. Extracellular cysteines of CCR5 are required for chemokine binding, but dispensable for HIV-1 coreceptor activity. *J Biol Chem* **274**:18902-8.
19. **Borish, L. C., and J. W. Steinke.** 2003. 2. Cytokines and chemokines. *J Allergy Clin Immunol* **111**:S460-75.
20. **Braun, M. M., W. L. Heyward, and J. W. Curran.** 1990. The global epidemiology of HIV infection and AIDS. *Annu Rev Microbiol* **44**:555-77.
21. **Brink, C. B., B. H. Harvey, J. Bodenstein, D. P. Venter, and D. W. Oliver.** 2004. Recent advances in drug action and therapeutics: relevance of novel concepts in G-protein-coupled receptor and signal transduction pharmacology. *Br J Clin Pharmacol* **57**:373-87.

22. **Carrillo, A., and L. Ratner.** 1996. Cooperative effects of the human immunodeficiency virus type 1 envelope variable loops V1 and V3 in mediating infectivity for T cells. *J Virol* **70**:1310-6.
23. **Catani, M. V., M. T. Corasaniti, M. Navarra, G. Nistico, A. Finazzi-Agro, and G. Melino.** 2000. gp120 induces cell death in human neuroblastoma cells through the CXCR4 and CCR5 chemokine receptors. *J Neurochem* **74**:2373-9.
24. **Charo, I. F., and R. M. Ransohoff.** 2006. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* **354**:610-21.
25. **Cheng-Mayer, C., D. Seto, M. Tateno, and J. A. Levy.** 1988. Biologic features of HIV-1 that correlate with virulence in the host. *Science* **240**:80-2.
26. **Cheng-Mayer, C., C. Weiss, D. Seto, and J. A. Levy.** 1989. Isolates of human immunodeficiency virus type 1 from the brain may constitute a special group of the AIDS virus. *Proc Natl Acad Sci U S A* **86**:8575-9.
27. **Chiodi, F., B. Asjo, E. M. Fenyo, G. Norkrans, L. Hagberg, and J. Albert.** 1986. Isolation of human immunodeficiency virus from cerebrospinal fluid of antibody-positive virus carrier without neurological symptoms. *Lancet* **2**:1276-7.
28. **Chiodi, F., A. Sonnerborg, J. Albert, H. Gaines, G. Norkrans, L. Hagberg, B. Asjo, O. Strannegard, and E. M. Fenyo.** 1988. Human immunodeficiency virus infection of the brain. I. Virus isolation and detection of HIV specific antibodies in the cerebrospinal fluid of patients with varying clinical conditions. *J Neurol Sci* **85**:245-57.
29. **Chiodi, F., A. Valentin, B. Keys, S. Schwartz, B. Asjo, S. Gartner, M. Popovic, J. Albert, V. A. Sundqvist, and E. M. Fenyo.** 1989. Biological characterization of paired human immunodeficiency virus type 1 isolates from blood and cerebrospinal fluid. *Virology* **173**:178-87.
30. **Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P. D. Ponath, L. Wu, C. R. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, and J. Sodroski.** 1996. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85**:1135-48.
31. **Clark, S. J., and G. M. Shaw.** 1993. The acute retroviral syndrome and the pathogenesis of HIV-1 infection. *Semin Immunol* **5**:149-55.
32. **Clavel, F., M. Guyader, D. Guetard, M. Salle, L. Montagnier, and M. Alizon.** 1986. Molecular cloning and polymorphism of the human immune deficiency virus type 2. *Nature* **324**:691-5.

33. **Cocchi, F., A. L. DeVico, A. Garzino-Demo, S. K. Arya, R. C. Gallo, and P. Lusso.** 1995. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* **270**:1811-5.
34. **Cocchi, F., A. L. DeVico, R. Yarchoan, R. Redfield, F. Cleghorn, W. A. Blattner, A. Garzino-Demo, S. Colombini-Hatch, D. Margolis, and R. C. Gallo.** 2000. Higher macrophage inflammatory protein (MIP)-1alpha and MIP-1beta levels from CD8+ T cells are associated with asymptomatic HIV-1 infection. *Proc Natl Acad Sci U S A* **97**:13812-7.
35. **Coffin, J., A. Haase, J. A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt, and et al.** 1986. Human immunodeficiency viruses. *Science* **232**:697.
36. **Connor, R. I., K. E. Sheridan, D. Ceradini, S. Choe, and N. R. Landau.** 1997. Change in coreceptor use coreceptor use correlates with disease progression in HIV-1--infected individuals. *J Exp Med* **185**:621-8.
37. **Crotti, A., F. Neri, D. Corti, S. Ghezzi, S. Heltai, A. Baur, G. Poli, E. Santagostino, and E. Vicenzi.** 2006. Nef alleles from human immunodeficiency virus type 1-infected long-term-nonprogressor hemophiliacs with or without late disease progression are defective in enhancing virus replication and CD4 down-regulation. *J Virol* **80**:10663-74.
38. **Davenport, M. P., J. J. Zaunders, M. D. Hazenberg, H. Schuitemaker, and R. P. van Rij.** 2002. Cell turnover and cell tropism in HIV-1 infection. *Trends Microbiol* **10**:275-8.
39. **de Roda Husman, A. M., R. P. van Rij, H. Blaak, S. Broersen, and H. Schuitemaker.** 1999. Adaptation to promiscuous usage of chemokine receptors is not a prerequisite for human immunodeficiency virus type 1 disease progression. *J Infect Dis* **180**:1106-15.
40. **Dean, M., M. Carrington, C. Winkler, G. A. Huttley, M. W. Smith, R. Allikmets, J. J. Goedert, S. P. Buchbinder, E. Vittinghoff, E. Gomperts, S. Donfield, D. Vlahov, R. Kaslow, A. Saah, C. Rinaldo, R. Detels, and S. J. O'Brien.** 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* **273**:1856-62.

41. **Deng, H., R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, and N. R. Landau.** 1996. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**:661-6.
42. **Derdeyn, C. A., and G. Silvestri.** 2005. Viral and host factors in the pathogenesis of HIV infection. *Curr Opin Immunol* **17**:366-73.
43. **Doranz, B. J., J. Rucker, Y. Yi, R. J. Smyth, M. Samson, S. C. Peiper, M. Parmentier, R. G. Collman, and R. W. Doms.** 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* **85**:1149-58.
44. **Dragic, T., V. Litwin, G. P. Allaway, S. R. Martin, Y. Huang, K. A. Nagashima, C. Cayanan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton.** 1996. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **381**:667-73.
45. **Duerr, A., J. N. Wasserheit, and L. Corey.** 2006. HIV vaccines: new frontiers in vaccine development. *Clin Infect Dis* **43**:500-11.
46. **Edinger, A. L., T. L. Hoffman, M. Sharron, B. Lee, B. O'Dowd, and R. W. Doms.** 1998. Use of GPR1, GPR15, and STRL33 as coreceptors by diverse human immunodeficiency virus type 1 and simian immunodeficiency virus envelope proteins. *Virology* **249**:367-78.
47. **Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase.** 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* **362**:359-62.
48. **Epstein, L. G., C. Kuiken, B. M. Blumberg, S. Hartman, L. R. Sharer, M. Clement, and J. Goudsmit.** 1991. HIV-1 V3 domain variation in brain and spleen of children with AIDS: tissue-specific evolution within host-determined quasispecies. *Virology* **180**:583-90.
49. **Ezzell, C.** 1987. AZT given the green light for clinical treatment of AIDS. *Nature* **326**:430.
50. **Farzan, M., T. Mirzabekov, P. Kolchinsky, R. Wyatt, M. Cayabyab, N. P. Gerard, C. Gerard, J. Sodroski, and H. Choe.** 1999. Tyrosine sulfation of the amino terminal of CCR5 facilitates HIV-1 entry. *Cell* **96**:667-76.

51. **Federspiel, B., I. G. Melhado, A. M. Duncan, A. Delaney, K. Schappert, I. Clark-Lewis, and F. R. Jirik.** 1993. Molecular cloning of the cDNA and chromosomal localization of the gene for a putative seven-transmembrane segment (7-TMS) receptor isolated from human spleen. *Genomics* **16**:707-12.
52. **Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger.** 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272**:872-7.
53. **Fenyo, E. M., J. Albert, and B. Asjo.** 1989. Replicative capacity, cytopathic effect and cell tropism of HIV. *Aids* **3 Suppl 1**:S5-12.
54. **Fischl, M. A., D. D. Richman, M. H. Grieco, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, R. T. Schooley, and et al.** 1987. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N Engl J Med* **317**:185-91.
55. **Fotiadis, D., B. Jastrzebska, A. Philippsen, D. J. Muller, K. Palczewski, and A. Engel.** 2006. Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. *Curr Opin Struct Biol* **16**:252-9.
56. **Fredriksson, R., M. C. Lagerstrom, L. G. Lundin, and H. B. Schiöth.** 2003. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* **63**:1256-72.
57. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, and et al.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* **224**:500-3.
58. **Gartner, S., R. A. McDonald, E. A. Hunter, F. Bouwman, Y. Liu, and M. Popovic.** 1997. Gp120 sequence variation in brain and in T-lymphocyte human immunodeficiency virus type 1 primary isolates. *J Hum Virol* **1**:3-18.
59. **Geijtenbeek, T. B., D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor, and Y. van Kooyk.** 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* **100**:587-97.
60. **Gisslén, M., L. Hagberg, B. Brew, P. Cinque, R. W. Price, and L. Rosengren.** 2007. Elevated Cerebrospinal Fluid Neurofilament Protein Predicts Development of AIDS Dementia Complex. . *J Infect Dis* **In press**.

61. **Godessart, N., and S. L. Kunkel.** 2001. Chemokines in autoimmune disease. *Curr Opin Immunol* **13**:670-5.
62. **Gorry, P. R., G. Bristol, J. A. Zack, K. Ritola, R. Swanstrom, C. J. Birch, J. E. Bell, N. Bannert, K. Crawford, H. Wang, D. Schols, E. De Clercq, K. Kunstman, S. M. Wolinsky, and D. Gabuzda.** 2001. Macrophage tropism of human immunodeficiency virus type 1 isolates from brain and lymphoid tissues predicts neurotropism independent of coreceptor specificity. *J Virol* **75**:10073-89.
63. **Gorry, P. R., J. Taylor, G. H. Holm, A. Mehle, T. Morgan, M. Cayabyab, M. Farzan, H. Wang, J. E. Bell, K. Kunstman, J. P. Moore, S. M. Wolinsky, and D. Gabuzda.** 2002. Increased CCR5 affinity and reduced CCR5/CD4 dependence of a neurovirulent primary human immunodeficiency virus type 1 isolate. *J Virol* **76**:6277-92.
64. **Gottlieb, M. S., R. Schroff, H. M. Schanker, J. D. Weisman, P. T. Fan, R. A. Wolf, and A. Saxon.** 1981. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* **305**:1425-31.
65. **Gray, L., J. Sterjovski, M. Churchill, P. Ellery, N. Nasr, S. R. Lewin, S. M. Crowe, S. L. Wesselingh, A. L. Cunningham, and P. R. Gorry.** 2005. Uncoupling coreceptor usage of human immunodeficiency virus type 1 (HIV-1) from macrophage tropism reveals biological properties of CCR5-restricted HIV-1 isolates from patients with acquired immunodeficiency syndrome. *Virology* **337**:384-98.
66. **Groot, F., T. M. van Capel, J. Schuitemaker, B. Berkhout, and E. C. de Jong.** 2006. Differential susceptibility of naive, central memory and effector memory T cells to dendritic cell-mediated HIV-1 transmission. *Retrovirology* **3**:52.
67. **Grossman, Z., M. Meier-Schellersheim, W. E. Paul, and L. J. Picker.** 2006. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nat Med* **12**:289-95.
68. **He, J., Y. Chen, M. Farzan, H. Choe, A. Ohagen, S. Gartner, J. Busciglio, X. Yang, W. Hofmann, W. Newman, C. R. Mackay, J. Sodroski, and D. Gabuzda.** 1997. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **385**:645-9.
69. **Heeney, J. L., A. G. Dalgleish, and R. A. Weiss.** 2006. Origins of HIV and the evolution of resistance to AIDS. *Science* **313**:462-6.

70. Hesselgesser, J., D. Taub, P. Baskar, M. Greenberg, J. Hoxie, D. L. Kolson, and R. Horuk. 1998. Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1 alpha is mediated by the chemokine receptor CXCR4. *Curr Biol* **8**:595-8.
71. Howard, O. M., A. K. Shirakawa, J. A. Turpin, A. Maynard, G. J. Tobin, M. Carrington, J. J. Oppenheim, and M. Dean. 1999. Naturally occurring CCR5 extracellular and transmembrane domain variants affect HIV-1 Co-receptor and ligand binding function. *J Biol Chem* **274**:16228-34.
72. Hu, Q., K. B. Napier, J. O. Trent, Z. Wang, S. Taylor, G. E. Griffin, S. C. Peiper, and R. J. Shattock. 2005. Restricted variable residues in the C-terminal segment of HIV-1 V3 loop regulate the molecular anatomy of CCR5 utilization. *J Mol Biol* **350**:699-712.
73. Huang, Y., W. A. Paxton, S. M. Wolinsky, A. U. Neumann, L. Zhang, T. He, S. Kang, D. Ceradini, Z. Jin, K. Yazdanbakhsh, K. Kunstman, D. Erickson, E. Dragon, N. R. Landau, J. Phair, D. D. Ho, and R. A. Koup. 1996. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nat Med* **2**:1240-3.
74. Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. R. Cullen. 1991. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. *Science* **253**:71-4.
75. Jacoby, E., R. Bouhelal, M. Gerspacher, and K. Seuwen. 2006. The 7 TM G-protein-coupled receptor target family. *ChemMedChem* **1**:761-82.
76. Jansson, M., E. Backstrom, A. Bjorndal, V. Holmberg, P. Rossi, E. M. Fenyo, M. Popovic, J. Albert, and H. Wigzell. 1999. Coreceptor usage and RANTES sensitivity of non-syncytium-inducing HIV-1 isolates obtained from patients with AIDS. *J Hum Virol* **2**:325-38.
77. Jansson, M., E. Backstrom, G. Scarlatti, A. Bjorndal, S. Matsuda, P. Rossi, J. Albert, and H. Wigzell. 2001. Length variation of glycoprotein 120 V2 region in relation to biological phenotypes and coreceptor usage of primary HIV type 1 isolates. *AIDS Res Hum Retroviruses* **17**:1405-14.
78. Jansson, M., M. Popovic, A. Karlsson, F. Cocchi, P. Rossi, J. Albert, and H. Wigzell. 1996. Sensitivity to inhibition by beta-chemokines correlates with biological phenotypes of primary HIV-1 isolates. *Proc Natl Acad Sci U S A* **93**:15382-7.
79. Jinno, A., N. Shimizu, Y. Soda, Y. Haraguchi, T. Kitamura, and H. Hoshino. 1998. Identification of the chemokine receptor TER1/CCR8 expressed in brain-derived cells and T cells as a new coreceptor for HIV-1 infection. *Biochem Biophys Res Commun* **243**:497-502.

80. **Kahn, J. O., and B. D. Walker.** 1998. Acute human immunodeficiency virus type 1 infection. *N Engl J Med* **339**:33-9.
81. **Karlsson, A., K. Parsmyr, E. Sandstrom, E. M. Fenyo, and J. Albert.** 1994. MT-2 cell tropism as prognostic marker for disease progression in human immunodeficiency virus type 1 infection. *J Clin Microbiol* **32**:364-70.
82. **Karlsson, I., L. Antonsson, Y. Shi, A. Karlsson, J. Albert, T. Leitner, B. Olde, C. Owman, and E. M. Fenyo.** 2003. HIV biological variability unveiled: frequent isolations and chimeric receptors reveal unprecedented variation of coreceptor use. *Aids* **17**:2561-9.
83. **Karlsson, I., L. Antonsson, Y. Shi, M. Oberg, A. Karlsson, J. Albert, B. Olde, C. Owman, M. Jansson, and E. M. Fenyo.** 2004. Coevolution of RANTES sensitivity and mode of CCR5 receptor use by human immunodeficiency virus type 1 of the R5 phenotype. *J Virol* **78**:11807-15.
84. **Kim, C. H., and H. E. Broxmeyer.** 1999. Chemokines: signal lamps for trafficking of T and B cells for development and effector function. *J Leukoc Biol* **65**:6-15.
85. **Koning, F. A., D. Kwa, B. Boeser-Nunnink, J. Dekker, J. Vingerhoed, H. Hiemstra, and H. Schuitemaker.** 2003. Decreasing sensitivity to RANTES (regulated on activation, normally T cell-expressed and -secreted) neutralization of CC chemokine receptor 5-using, non-syncytium-inducing virus variants in the course of human immunodeficiency virus type 1 infection. *J Infect Dis* **188**:864-72.
86. **Koning, F. A., D. Schols, and H. Schuitemaker.** 2001. No selection for CCR5 coreceptor usage during parenteral transmission of macrophagetropic syncytium-inducing human immunodeficiency virus type 1. *J Virol* **75**:8848-53.
87. **Korber, B. T., K. J. Kunstman, B. K. Patterson, M. Furtado, M. M. McEvilly, R. Levy, and S. M. Wolinsky.** 1994. Genetic differences between blood- and brain-derived viral sequences from human immunodeficiency virus type 1-infected patients: evidence of conserved elements in the V3 region of the envelope protein of brain-derived sequences. *J Virol* **68**:7467-81.
88. **Kramer-Hammerle, S., I. Rothenaigner, H. Wolff, J. E. Bell, and R. Brack-Werner.** 2005. Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. *Virus Res* **111**:194-213.
89. **Kucia, M., K. Jankowski, R. Reza, M. Wysoczynski, L. Bandura, D. J. Allendorf, J. Zhang, J. Ratajczak, and M. Z. Ratajczak.** 2004. CXCR4-SDF-1 signalling, locomotion, chemotaxis and adhesion. *J Mol Histol* **35**:233-45.

90. **Kwa, D., J. Vingerhoed, B. Boeser, and H. Schuitemaker.** 2003. Increased in vitro cytopathicity of CC chemokine receptor 5-restricted human immunodeficiency virus type 1 primary isolates correlates with a progressive clinical course of infection. *J Infect Dis* **187**:1397-403.
91. **Laing, K. J., and C. J. Secombes.** 2004. Chemokines. *Dev Comp Immunol* **28**:443-60.
92. **Larder, B. A., G. Darby, and D. D. Richman.** 1989. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **243**:1731-4.
93. **Lawrence, D. M., and E. O. Major.** 2002. HIV-1 and the brain: connections between HIV-1-associated dementia, neuropathology and neuroimmunology. *Microbes Infect* **4**:301-8.
94. **Lederman, M. M., A. Penn-Nicholson, M. Cho, and D. Mosier.** 2006. Biology of CCR5 and its role in HIV infection and treatment. *Jama* **296**:815-26.
95. **Levy, J. A.** 1993. The transmission of HIV and factors influencing progression to AIDS. *Am J Med* **95**:86-100.
96. **Levy, J. A., I. Scott, and C. Mackewicz.** 2003. Protection from HIV/AIDS: the importance of innate immunity. *Clin Immunol* **108**:167-74.
97. **Li, Q., L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carlis, C. J. Miller, and A. T. Haase.** 2005. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. *Nature* **434**:1148-52.
98. **Liu, R., W. A. Paxton, S. Choe, D. Ceradini, S. R. Martin, R. Horuk, M. E. MacDonald, H. Stuhlmann, R. A. Koup, and N. R. Landau.** 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **86**:367-77.
99. **Loetscher, M., T. Geiser, T. O'Reilly, R. Zwahlen, M. Baggiolini, and B. Moser.** 1994. Cloning of a human seven-transmembrane domain receptor, LESTR, that is highly expressed in leukocytes. *J Biol Chem* **269**:232-7.
100. **Ma, Q., D. Jones, P. R. Borghesani, R. A. Segal, T. Nagasawa, T. Kishimoto, R. T. Bronson, and T. A. Springer.** 1998. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. *Proc Natl Acad Sci U S A* **95**:9448-53.
101. **Maudsley, S., B. Martin, and L. M. Luttrell.** 2005. The origins of diversity and specificity in G protein-coupled receptor signaling. *J Pharmacol Exp Ther* **314**:485-94.

102. **Mehandru, S., M. A. Poles, K. Tenner-Racz, A. Horowitz, A. Hurley, C. Hogan, D. Boden, P. Racz, and M. Markowitz.** 2004. Primary HIV-1 infection is associated with preferential depletion of CD4<sup>+</sup> T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med* **200**:761-70.
103. **Mellors, J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley.** 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* **272**:1167-70.
104. **Mitsuya, H., K. J. Weinhold, P. A. Furman, M. H. St Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder.** 1985. 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. *Proc Natl Acad Sci U S A* **82**:7096-100.
105. **Moore, J. P., S. G. Kitchen, P. Pugach, and J. A. Zack.** 2004. The CCR5 and CXCR4 coreceptors--central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses* **20**:111-26.
106. **Murdoch, C.** 2000. CXCR4: chemokine receptor extraordinaire. *Immunol Rev* **177**:175-84.
107. **Nagasawa, T., S. Hirota, K. Tachibana, N. Takakura, S. Nishikawa, Y. Kitamura, N. Yoshida, H. Kikutani, and T. Kishimoto.** 1996. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* **382**:635-8.
108. **Naif, H. M., A. L. Cunningham, M. Alali, S. Li, N. Nasr, M. M. Buhler, D. Schols, E. de Clercq, and G. Stewart.** 2002. A human immunodeficiency virus type 1 isolate from an infected person homozygous for CCR5Delta32 exhibits dual tropism by infecting macrophages and MT2 cells via CXCR4. *J Virol* **76**:3114-24.
109. **Navenot, J. M., Z. X. Wang, J. O. Trent, J. L. Murray, Q. X. Hu, L. DeLeeuw, P. S. Moore, Y. Chang, and S. C. Peiper.** 2001. Molecular anatomy of CCR5 engagement by physiologic and viral chemokines and HIV-1 envelope glycoproteins: differences in primary structural requirements for RANTES, MIP-1 alpha, and vMIP-II Binding. *J Mol Biol* **313**:1181-93.
110. **Neer, E. J.** 1995. Heterotrimeric G proteins: organizers of transmembrane signals. *Cell* **80**:249-57.

111. Nishimura, Y., T. Igarashi, A. Buckler-White, C. Buckler, H. Imamichi, R. M. Goeken, W. R. Lee, B. A. Lafont, R. Byrum, H. C. Lane, V. M. Hirsch, and M. A. Martin. 2007. Loss of naive cells accompanies memory CD4<sup>+</sup> T-cell depletion during long-term progression to AIDS in Simian immunodeficiency virus-infected macaques. *J Virol* **81**:893-902.
112. Ohagen, A., A. Devitt, K. J. Kunstman, P. R. Gorry, P. P. Rose, B. Korber, J. Taylor, R. Levy, R. L. Murphy, S. M. Wolinsky, and D. Gabuzda. 2003. Genetic and functional analysis of full-length human immunodeficiency virus type 1 env genes derived from brain and blood of patients with AIDS. *J Virol* **77**:12336-45.
113. Olivieri, K., R. M. Scoggins, Y. C. Bor, A. Matthews, D. Mark, J. R. Taylor, Jr., D. Chernauskas, M. L. Hammarskjold, D. Rekosh, and D. Camerini. 2007. The envelope gene is a cytopathic determinant of CCR5 tropic HIV-1. *Virology* **358**:23-38.
114. Olson, T. S., and K. Ley. 2002. Chemokines and chemokine receptors in leukocyte trafficking. *Am J Physiol Regul Integr Comp Physiol* **283**:R7-28.
115. Ono, S. J., T. Nakamura, D. Miyazaki, M. Ohbayashi, M. Dawson, and M. Toda. 2003. Chemokines: roles in leukocyte development, trafficking, and effector function. *J Allergy Clin Immunol* **111**:1185-99; quiz 1200.
116. Oppenheim, J. J., C. O. Zachariae, N. Mukaida, and K. Matsushima. 1991. Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu Rev Immunol* **9**:617-48.
117. Oppermann, M. 2004. Chemokine receptor CCR5: insights into structure, function, and regulation. *Cell Signal* **16**:1201-10.
118. Owman, C., A. Garzino-Demo, F. Cocchi, M. Popovic, A. Sabirsh, and R. C. Gallo. 1998. The leukotriene B<sub>4</sub> receptor functions as a novel type of coreceptor mediating entry of primary HIV-1 isolates into CD4-positive cells. *Proc Natl Acad Sci U S A* **95**:9530-4.
119. Pantaleo, G., C. Graziosi, and A. S. Fauci. 1993. New concepts in the immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med* **328**:327-35.
120. Pantaleo, G., S. Menzo, M. Vaccarezza, C. Graziosi, O. J. Cohen, J. F. Demarest, D. Montefiori, J. M. Orenstein, C. Fox, L. K. Schrager, and et al. 1995. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. *N Engl J Med* **332**:209-16.

121. **Paredes, R., and B. Clotet.** 2003. New antiretroviral drugs and approaches to HIV treatment. *Aids* **17 Suppl 4**:S85-96.
122. **Pease, J. E.** 2006. Asthma, allergy and chemokines. *Curr Drug Targets* **7**:3-12.
123. **Perelson, A. S., A. U. Neumann, M. Markowitz, J. M. Leonard, and D. D. Ho.** 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science* **271**:1582-6.
124. **Persidsky, Y., and L. Poluektova.** 2006. Immune privilege and HIV-1 persistence in the CNS. *Immunol Rev* **213**:180-94.
125. **Picker, L. J.** 2006. Immunopathogenesis of acute AIDS virus infection. *Curr Opin Immunol* **18**:399-405.
126. **Platt, E. J., S. E. Kuhmann, P. P. Rose, and D. Kabat.** 2001. Adaptive mutations in the V3 loop of gp120 enhance fusogenicity of human immunodeficiency virus type 1 and enable use of a CCR5 coreceptor that lacks the amino-terminal sulfated region. *J Virol* **75**:12266-78.
127. **Platt, E. J., D. M. Shea, P. P. Rose, and D. Kabat.** 2005. Variants of human immunodeficiency virus type 1 that efficiently use CCR5 lacking the tyrosine-sulfated amino terminal have adaptive mutations in gp120, including loss of a functional N-glycan. *J Virol* **79**:4357-68.
128. **Price, R. W., B. Brew, J. Sidtis, M. Rosenblum, A. C. Scheck, and P. Cleary.** 1988. The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* **239**:586-92.
129. **Rambaut, A., D. Posada, K. A. Crandall, and E. C. Holmes.** 2004. The causes and consequences of HIV evolution. *Nat Rev Genet* **5**:52-61.
130. **Regoes, R. R., and S. Bonhoeffer.** 2005. The HIV coreceptor switch: a population dynamical perspective. *Trends Microbiol* **13**:269-77.
131. **Repits, J., M. Oberg, J. Esbjornsson, P. Medstrand, A. Karlsson, J. Albert, E. M. Fenyo, and M. Jansson.** 2005. Selection of human immunodeficiency virus type 1 R5 variants with augmented replicative capacity and reduced sensitivity to entry inhibitors during severe immunodeficiency. *J Gen Virol* **86**:2859-69.

132. **Samson, M., A. L. Edinger, P. Stordeur, J. Rucker, V. Verhasselt, M. Sharron, C. Govaerts, C. Mollereau, G. Vassart, R. W. Doms, and M. Parmentier.** 1998. ChemR23, a putative chemoattractant receptor, is expressed in monocyte-derived dendritic cells and macrophages and is a coreceptor for SIV and some primary HIV-1 strains. *Eur J Immunol* **28**:1689-700.
133. **Samson, M., O. Labbe, C. Mollereau, G. Vassart, and M. Parmentier.** 1996. Molecular cloning and functional expression of a new human CC-chemokine receptor gene. *Biochemistry* **35**:3362-7.
134. **Samson, M., F. Libert, B. J. Doranz, J. Rucker, C. Liesnard, C. M. Farber, S. Saragosti, C. Lapoumeroulie, J. Cognaux, C. Forceille, G. Muyldermans, C. Verhofstede, G. Burtonboy, M. Georges, T. Imai, S. Rana, Y. Yi, R. J. Smyth, R. G. Collman, R. W. Doms, G. Vassart, and M. Parmentier.** 1996. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* **382**:722-5.
135. **Schall, T. J., and K. B. Bacon.** 1994. Chemokines, leukocyte trafficking, and inflammation. *Curr Opin Immunol* **6**:865-73.
136. **Schmitz, J. E., M. J. Kuroda, S. Santra, V. G. Sasseville, M. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallan, J. Ghayeb, M. A. Forman, D. C. Montefiori, E. P. Rieber, N. L. Letvin, and K. A. Reimann.** 1999. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* **283**:857-60.
137. **Schoneberg, T., G. Schultz, and T. Gudermann.** 1999. Structural basis of G protein-coupled receptor function. *Mol Cell Endocrinol* **151**:181-93.
138. **Scoggins, R. M., J. R. Taylor, Jr., J. Patrie, A. B. van't Wout, H. Schuitemaker, and D. Camerini.** 2000. Pathogenesis of primary R5 human immunodeficiency virus type 1 clones in SCID-hu mice. *J Virol* **74**:3205-16.
139. **Sewell, A. K., D. A. Price, A. Oxenius, A. D. Kelleher, and R. E. Phillips.** 2000. Cytotoxic T lymphocyte responses to human immunodeficiency virus: control and escape. *Stem Cells* **18**:230-44.
140. **Shafer, R. W., and D. A. Vuitton.** 1999. Highly active antiretroviral therapy (HAART) for the treatment of infection with human immunodeficiency virus type 1. *Biomed Pharmacother* **53**:73-86.
141. **Shieh, J. T., A. V. Albright, M. Sharron, S. Gartner, J. Strizki, R. W. Doms, and F. Gonzalez-Scarano.** 1998. Chemokine receptor utilization by human immunodeficiency virus type 1 isolates that replicate in microglia. *J Virol* **72**:4243-9.

142. **Shimizu, N., Y. Haraguchi, Y. Takeuchi, Y. Soda, K. Kanbe, and H. Hoshino.** 1999. Changes in and discrepancies between cell tropisms and coreceptor uses of human immunodeficiency virus type 1 induced by single point mutations at the V3 tip of the env protein. *Virology* **259**:324-33.
143. **Shimizu, N., Y. Soda, K. Kanbe, H. Y. Liu, R. Mukai, T. Kitamura, and H. Hoshino.** 2000. A putative G protein-coupled receptor, RDC1, is a novel coreceptor for human and simian immunodeficiency viruses. *J Virol* **74**:619-26.
144. **Shioda, T., J. A. Levy, and C. Cheng-Mayer.** 1992. Small amino acid changes in the V3 hypervariable region of gp120 can affect the T-cell-line and macrophage tropism of human immunodeficiency virus type 1. *Proc Natl Acad Sci U S A* **89**:9434-8.
145. **Sierra, S., B. Kupfer, and R. Kaiser.** 2005. Basics of the virology of HIV-1 and its replication. *J Clin Virol* **34**:233-44.
146. **Simon, V., D. D. Ho, and Q. Abdool Karim.** 2006. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. *Lancet* **368**:489-504.
147. **Smit, T. K., B. Wang, T. Ng, R. Osborne, B. Brew, and N. K. Saksena.** 2001. Varied tropism of HIV-1 isolates derived from different regions of adult brain cortex discriminate between patients with and without AIDS dementia complex (ADC): evidence for neurotropic HIV variants. *Virology* **279**:509-26.
148. **Srivastava, I. K., J. B. Ulmer, and S. W. Barnett.** 2005. Role of neutralizing antibodies in protective immunity against HIV. *Hum Vaccin* **1**:45-60.
149. **Stevenson, M.** 2003. HIV-1 pathogenesis. *Nat Med* **9**:853-60.
150. **Tersmette, M., R. E. de Goede, B. J. Al, I. N. Winkel, R. A. Gruters, H. T. Cuypers, H. G. Huisman, and F. Miedema.** 1988. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *J Virol* **62**:2026-32.
151. **Tersmette, M., R. A. Gruters, F. de Wolf, R. E. de Goede, J. M. Lange, P. T. Schellekens, J. Goudsmit, H. G. Huisman, and F. Miedema.** 1989. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. *J Virol* **63**:2118-25.

152. **Thomas, E. R., R. L. Dunfee, J. Stanton, D. Bogdan, J. Taylor, K. Kunstman, J. E. Bell, S. M. Wolinsky, and D. Gabuzda.** 2007. Macrophage entry mediated by HIV Envs from brain and lymphoid tissues is determined by the capacity to use low CD4 levels and overall efficiency of fusion. *Virology* **360**:105-19.
153. **Tilakaratne, N., and P. M. Sexton.** 2005. G-Protein-coupled receptor-protein interactions: basis for new concepts on receptor structure and function. *Clin Exp Pharmacol Physiol* **32**:979-87.
154. **Trkola, A., S. E. Kuhmann, J. M. Strizki, E. Maxwell, T. Ketas, T. Morgan, P. Pugach, S. Xu, L. Wojcik, J. Tagat, A. Palani, S. Shapiro, J. W. Clader, S. McCombie, G. R. Reyes, B. M. Baroudy, and J. P. Moore.** 2002. HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use. *Proc Natl Acad Sci U S A* **99**:395-400.
155. **Tsibris, A. M., and D. R. Kuritzkes.** 2007. Chemokine antagonists as therapeutics: focus on HIV-1. *Annu Rev Med* **58**:445-59.
156. **Turner, B. G., and M. F. Summers.** 1999. Structural biology of HIV. *J Mol Biol* **285**:1-32.
157. **Ullum, H., A. Cozzi Lepri, J. Victor, H. Aladdin, A. N. Phillips, J. Gerstoft, P. Skinhoj, and B. K. Pedersen.** 1998. Production of beta-chemokines in human immunodeficiency virus (HIV) infection: evidence that high levels of macrophage inflammatory protein-1beta are associated with a decreased risk of HIV disease progression. *J Infect Dis* **177**:331-6.
158. **Walter, B. L., K. Wehrly, R. Swanstrom, E. Platt, D. Kabat, and B. Chesebro.** 2005. Role of low CD4 levels in the influence of human immunodeficiency virus type 1 envelope V1 and V2 regions on entry and spread in macrophages. *J Virol* **79**:4828-37.
159. **van't Wout, A. B., N. A. Kootstra, G. A. Mulder-Kampinga, N. Albrecht-van Lent, H. J. Scherpbier, J. Veenstra, K. Boer, R. A. Coutinho, F. Miedema, and H. Schuitemaker.** 1994. Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical transmission. *J Clin Invest* **94**:2060-7.
160. **Wang, J., G. J. Babcock, H. Choe, M. Farzan, J. Sodroski, and D. Gabuzda.** 2004. N-linked glycosylation in the CXCR4 N-terminal inhibits binding to HIV-1 envelope glycoproteins. *Virology* **324**:140-50.
161. **Verrier, F., A. M. Borman, D. Brand, and M. Girard.** 1999. Role of the HIV type 1 glycoprotein 120 V3 loop in determining coreceptor usage. *AIDS Res Hum Retroviruses* **15**:731-43.

162. **Williams, K. C., and W. F. Hickey.** 2002. Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. *Annu Rev Neurosci* **25**:537-62.
163. **Wu, L., and V. N. KewalRamani.** 2006. Dendritic-cell interactions with HIV: infection and viral dissemination. *Nat Rev Immunol* **6**:859-68.
164. **Wyatt, R., P. D. Kwong, E. Desjardins, R. W. Sweet, J. Robinson, W. A. Hendrickson, and J. G. Sodroski.** 1998. The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature* **393**:705-11.
165. **Yang, D., Q. Chen, D. M. Hoover, P. Staley, K. D. Tucker, J. Lubkowski, and J. J. Oppenheim.** 2003. Many chemokines including CCL20/MIP-3alpha display antimicrobial activity. *J Leukoc Biol* **74**:448-55.
166. **Zaitseva, M., K. Peden, and H. Golding.** 2003. HIV coreceptors: role of structure, posttranslational modifications, and internalization in viral-cell fusion and as targets for entry inhibitors. *Biochim Biophys Acta* **1614**:51-61.
167. **Zhang, L., T. He, Y. Huang, Z. Chen, Y. Guo, S. Wu, K. J. Kunstman, R. C. Brown, J. P. Phair, A. U. Neumann, D. D. Ho, and S. M. Wolinsky.** 1998. Chemokine coreceptor usage by diverse primary isolates of human immunodeficiency virus type 1. *J Virol* **72**:9307-12.
168. **Zhang, Y. J., T. Dragic, Y. Cao, L. Kostrikis, D. S. Kwon, D. R. Littman, V. N. KewalRamani, and J. P. Moore.** 1998. Use of coreceptors other than CCR5 by non-syncytium-inducing adult and pediatric isolates of human immunodeficiency virus type 1 is rare in vitro. *J Virol* **72**:9337-44.
169. **Zhang, Y. J., and J. P. Moore.** 1999. Will multiple coreceptors need to be targeted by inhibitors of human immunodeficiency virus type 1 entry? *J Virol* **73**:3443-8.
170. **Zheng, J., A. Ghorpade, D. Niemann, R. L. Cotter, M. R. Thylin, L. Epstein, J. M. Swartz, R. B. Shepard, X. Liu, A. Nukuna, and H. E. Gendelman.** 1999. Lymphotropic virions affect chemokine receptor-mediated neural signaling and apoptosis: implications for human immunodeficiency virus type 1-associated dementia. *J Virol* **73**:8256-67.
171. **Zhou, N., Z. Luo, J. W. Hall, J. Luo, X. Han, and Z. Huang.** 2000. Molecular modeling and site-directed mutagenesis of CCR5 reveal residues critical for chemokine binding and signal transduction. *Eur J Immunol* **30**:164-73.
172. **Zhu, T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup, and D. D. Ho.** 1993. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. *Science* **261**:1179-81.

173. **Zhu, T., D. Muthui, S. Holte, D. Nickle, F. Feng, S. Brodie, Y. Hwangbo, J. I. Mullins, and L. Corey.** 2002. Evidence for human immunodeficiency virus type 1 replication in vivo in CD14(+) monocytes and its potential role as a source of virus in patients on highly active antiretroviral therapy. *J Virol* **76**:707-16.