



LUND UNIVERSITY

Virus tropism and neutralization response in SIV infection

Laurén, Anna

2006

[Link to publication](#)

Citation for published version (APA):

Laurén, A. (2006). *Virus tropism and neutralization response in SIV infection*. [Doctoral Thesis (compilation), Division of Medical Microbiology]. Department of Laboratory Medicine, Lund University.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Virus tropism and neutralization response in SIV infection

Anna Laurén

Institutionen for Laboriemedicin
Lunds universitet

Akademisk avhandling

Som med vederbörligt tillstånd av Medicinska fakulteten vid Lunds universitet för
avläggande av doktorsexamen i medicinsk vetenskap kommer att offentlig
försvaras i Rune Grubb-salen, Biomedicinskt centrum, Sölvegatan 19, Lund
lördagen den 13 maj 2006, kl 9.30

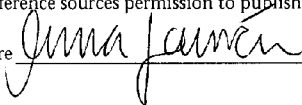
Fakultetsopponent: docent Gunilla B. Karlsson Hedestam,
Mikrobiologiskt och tumörbiologiskt centrum, Karolinska Institutet, Stockholm

Organization LUND UNIVERSITY		Document name DOCTORAL DISSERTATION	
Department of Laboratory Medicine Division of Medical Microbiology Unit of Virology Sölvegatan 23, 223 62 Lund, Sweden		Date of issue May 13, 2006	
		Sponsoring organization	
Author(s) Anna Laurén			
Title and subtitle Virus tropism and neutralization response in SIV infection.			
<p>Abstract</p> <p>Simian immunodeficiency virus (SIV) infections in macaques are commonly used as models to study the pathogenesis of human immunodeficiency virus (HIV). Both SIV and HIV normally use the CD4 receptor and an additional coreceptor for cell entry. The most common coreceptors used by HIV are CCR5 and CXCR4. SIV use CCR5 and rarely CXCR4 but may use other coreceptors as alternatives.</p> <p>We have studied virus tropism and evolution of the humoral immune response in relation to pathogenesis in cynomolgus macaques experimentally infected with SIVsm (of sooty mangabey origin) by intravenous (IV) or intrarectal (IR) route. Route of transmission did not influence disease progression or virus tropism. CCR5 was the major coreceptor used and isolates were in general multitropic, using CCR5, CXCR6 and/or gpr15. Furthermore, both macrophages and peripheral blood mononuclear cells (PBMC) could be readily infected with all the SIVsm reisolates. CXCR4-using viruses could be isolated, but only when human cells were used for virus isolation. Human cells may also select for virus variants with increased CD4-dependence. Comparisons of SIV and HIV-1 showed differences in mode of CCR5 use which in turn may explain the CD4-independence of SIVsm. Virus production after CD4-independent infection occurred both intracellularly and extracellularly. Neutralizing antibodies appeared earlier in IV-infected animals than in IR-infected animals. In progressors, this early humoral immune response was accompanied by early appearance of neutralization resistant variants.</p> <p>The majority of monkeys with progressive disease showed broadening of coreceptor use, stable coreceptor use or fluctuation in the use of different coreceptors. Viruses maintained effective replication in macrophages and PBMC and evolved to escape neutralizing antibodies already at three months after infection. In contrast, viruses from long-term non-progressor (LTNP) monkeys became less fit as shown by decreased frequency of successful virus isolations, narrowing of coreceptor use or stable CCR5 use and evolution to a more limited macrophage-tropism. Appearance of neutralization escape variants was delayed as compared to the early neutralization resistance in progressors. Neutralization resistance, whenever evident correlated with increased CD4-dependence in LTNP monkeys.</p>			
Key words: SIV, HIV, pathogenesis, evolution, neutralization, coreceptor use, macrophage tropism, CD4-independence, mode of CCR5 use			
Classification system and/or index terms (if any):			
Supplementary bibliographical information:		Language English	
ISSN and key title: 1652-8220		ISBN 91-85481-83-1	
Recipient's notes		Number of pages 174	
		Price	
		Security classification	

Distribution by (name and address)

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature



Date March 22, 2006

Virus tropism and neutralization response in SIV infection

Anna Laurén

Lund 2006

From the Department of Laboratory Medicine,
Division of Medical Microbiology, Unit of Virology
Faculty of Medicine, Lund University, Sweden

Anna Laurén
Virus tropism and neutralization response in SIV infection
Lund University, Lund, 2006

All published papers were reproduced with the permission from the publisher

This work was supported by the Medical Faculty, Lund University; the Swedish Research Council; the Swedish International Development Cooperation Agency/Department for Research Cooperation (SIDA/SAREC); and the Crafoord Foundation.

© Anna Laurén 2006
ISSN 1652-8220
ISBN 91-85481-83-1
Lund University, Faculty of Medicine Doctoral Dissertation Series 2006:58

Printed by Media-Tryck, Lund, 2006

To my family

CONTENTS

LIST OF PAPERS.....	3
ABBREVIATIONS.....	4
AIMS OF THIS THESIS	6
SUMMARY	7
INTRODUCTION.....	8
Discovery of HIV and the epidemic.....	8
Origin of HIV	9
HIV pathogenesis	11
Primate animal models of HIV.....	12
Virus structure and genome organization.....	13
<i>Structure</i>	13
<i>Genes and proteins</i>	14
Replication cycle.....	15
<i>Entry</i>	15
<i>Transcription, integration and translation</i>	16
<i>Assembly</i>	18
Antigen variation.....	18
Receptor usage	19
Cell tropism	22
<i>T cells</i>	22
<i>Macrophages</i>	23
<i>Dendritic cells</i>	24
The immune response to HIV	24
<i>Innate immune response</i>	25
<i>Humoral immune response</i>	25
<i>Cellular immune response</i>	27
Host factors in virus infection	28
MATERIALS AND METHODS	30
Cynomolgus macaques.....	30
Virus isolates	32
Serum	32
Characterization of coreceptor use	32

Infection of macrophages and PBMC	33
Virus titrations and neutralization assay on GHOST(3) cells	33
CD4-independent infections.....	34
RESULTS AND DISCUSSION	35
Coreceptor use of SIVsm	35
Replication capacity in macrophages and PBMC	36
Evolution of virus tropism in disease progression	37
Kinetics of the neutralizing antibody response in the infected host.....	40
Evolution of neutralization resistant virus variants.....	42
Can heterologous sera neutralize virus?.....	42
CD4-independence of SIVsm reisolates	43
Does mode of CCR5-use explain CD4-independence of SIVsm isolates?.....	45
Is there a correlation between macrophage tropism, neutralization sensitivity and CD4- independence?	47
CONCLUSIONS	49
SAMMANFATTNING PÅ SVENSKA	51
ACKNOWLEDGEMENTS	54
REFERENCES.....	56
APPENDICES: PAPER I-IV	71

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their roman numerals (I-IV):

- I **Anna Laurén, Dalma Vödrös, Rigmor Thorstensson and Eva Maria Fenyő.** Comparative studies on mucosal and intravenous transmission of SIVsm: evolution of coreceptor use varies with pathogenic outcome. *J Gen Virol.* 2006;87:581-594.

- II **Anna Laurén, Rigmor Thorstensson and Eva Maria Fenyő.** Comparative studies on mucosal and intravenous transmission of SIVsm: the kinetics of evolution to neutralisation resistance is related to progression rate of disease. *J Gen Virol.* 2006;87: 595-606.

- III **Anna Laurén, Rigmor Thorstensson, and Eva Maria Fenyő.** Replication capacity of sequential SIVsm isolates in monocyte-derived macrophages - relationship to pathogenesis. *Submitted.*

- IV **Anna Laurén, Elzbieta Vincic, Rigmor Thorstensson, Hiroo Hoshino and Eva Maria Fenyő.** CD4-independent use of the CCR5 receptor by sequential primary SIVsm isolates. *Manuscript*

ABBREVIATIONS

ADCC	antibody dependent cellular cytotoxicity
AIDS	aquired immunodeficiency syndrome
APJ	apelin receptor
C1 to C5	constant region one to five
CAF	CD8+ cell antiviral factor
CCR	CC chemokine receptor
CD	cluster of differentiation
CTL	cytotoxic T lymphocyte
CX3CR	CXC3C chemokine receptor
CXCL	CXC chemokine ligand
CXCR	CXC chemokine receptor
DC	dendritic cell
DNA	deoxyribonucleic acid
Env	envelope protein
env	envelope gene
gag	group antigen gene
GFP	green fluorescent protein
gp	glycoprotein
gpr	G protein-coupled receptor
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hMDM	monocyte derived macrophages of human origin
hPBMC	peripheral blood mononuclear cells of human origin
HTLV	human T-lymphotropic virus
ICAM	Intercellular adhesion molecule
IDU	injecting drug users
IFN	interferon
IL	interleukin
LAV	lymphadenopathy-associated virus
LFA	leukocyte factor antigen
LTNP	lon-term non-progressor
LTR	lon terminal repeat
mamu	macaque MHC protein
MCP	monocyte chemoattractant protein
MDM	monocyte derived macrophages
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
mMDM	monocyte derived macrophages of macaque origin
mPBMC	peripheral blood mononuclear cells of macaque origin
mRNA	messenger RNA
nef	negative regulatory factor gene
NK cell	natural killer cell
NSI	non-syncytium inducing
ORF	open reading frame
P	progressor
PBMC	peripheral blood mononuclear cells
PHA	phytohemagglutinin
pol	polymerase gene

R5 phenotype	virus using CCR5
RANTES	regulates on activation norma T cell expressed and secreted
rev	regulator of virion protein gene
RNA	ribonucleic acid
RRE	Rev responsive element
sAIDS	simian aquired immunodeficiency syndrome
SDF	stromal cell derived factor
SHIV	hybrid virus between HIV and SIV
SI	syncytium inducing
SIV	simian immunodeficiency virus
SP	slow progressor
SU	surface envelope protein
TAR	target sequence for viral transactivation
tat	transactivator gene
TCR	T cell receptor
Th cell	T helper cell
TM	transmembrane protein
tRNA	transport RNA
V1 to V5	variable region one to five
vif	viral infectivity factor gene
vpr	viral protein R
vpr	viral protein R
vpu	viral protein U
vpx	viral protein X
X4 phenotype	virus using CXCR4

AIMS OF THIS THESIS

The general aim of the thesis was to follow evolution of virus tropism and sensitivity to neutralization during Simian immunodeficiency virus (SIV) pathogenesis in cynomolgus macaques infected intrarectally or intravenously with SIV_{sm} (sooty mangabey origin). In addition, our question was if there is a relationship between virus tropism, neutralization sensitivity and mode of CCR5 use. The long study period of the animals (up to five years) and the collection of sequential virus isolates and sera made the material unique.

Paper I

To follow the evolution of coreceptor use in sequential isolates in relation to disease progression and route of transmission.

Paper II

To further dissect the effect of transmission, we examined the kinetics of appearance of autologous neutralizing antibodies and evolution of virus neutralization resistance.

Paper III

To study whether disease progression was related to changes in virus tropism, as reflected by replication capacity in monocyte-derived macrophage (MDM) and PBMC cultures.

Paper IV

To analyze if primary SIV_{sm} isolates could infect CCR5 expressing cells independent of CD4 and to study the mode of CCR5 use.

SUMMARY

Simian immunodeficiency virus (SIV) infections in macaques are commonly used as models to study the pathogenesis of human immunodeficiency virus (HIV). Both SIV and HIV normally use the CD4 receptor and an additional coreceptor for cell entry. The most common coreceptors used by HIV are CCR5 and CXCR4. SIV use CCR5 and rarely CXCR4 but may use other coreceptors as alternatives.

We have studied virus tropism and evolution of the humoral immune response in relation to pathogenesis in cynomolgus macaques experimentally infected with SIV_{sm} (of sooty mangabey origin) by intravenous (IV) or intrarectal (IR) route. Route of transmission did not influence disease progression or virus tropism. CCR5 was the major coreceptor used and isolates were in general multitropic, using CCR5, CXCR6 and/or gpr15. Furthermore, both macrophages and peripheral blood mononuclear cells (PBMC) could be readily infected with all the SIV_{sm} reisolates. CXCR4-using viruses could be isolated, but only when human cells were used for virus isolation. Human cells may also select for virus variants with increased CD4-dependence. Comparisons of SIV and HIV-1 showed differences in mode of CCR5 use which in turn may explain the CD4-independence of SIV_{sm}. Virus production after CD4-independent infection occurred both intracellularly and extracellularly. Neutralizing antibodies appeared earlier in IV-infected animals than in IR-infected animals. In progressors, this early humoral immune response was accompanied by early appearance of neutralization resistant variants.

The majority of monkeys with progressive disease showed broadening of coreceptor use, stable coreceptor use or fluctuation in the use of different coreceptors. Viruses maintained effective replication in macrophages and PBMC and evolved to escape neutralizing antibodies already at three months after infection. In contrast, viruses from long-term non-progressor (LTNP) monkeys became less fit as shown by decreased frequency of successful virus isolations, narrowing of coreceptor use or stable CCR5 use and evolution to a more limited macrophage-tropism. Appearance of neutralization escape variants was delayed as compared to the early neutralization resistance in progressors. Neutralization resistance, whenever evident correlated with increased CD4-dependence in LTNP monkeys.

INTRODUCTION

Human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) are retroviruses and belongs to the primate lentiviruses in humans and monkeys, respectively (64). The viruses in the retrovirus family, *Retroviridae*, are found in all vertebrates. Based on properties of the viruses they are classified as simple or complex retroviruses. Other retroviruses that are infecting humans are human T-lymphotropic virus (HTLV) I and II. The lentiviruses comprise a separate genus of the family and are complex viruses. These viruses are associated with long incubation periods, a characteristic that has earned them the name slow (lenti) viruses. Lentiviruses that infect non-primates are feline immunodeficiency virus in cats, Visna/maedi virus in sheep, bovine immunodeficiency virus in cattle, caprine arthritis-encephalitis virus in goats and the equine anemia virus in horses.

Discovery of HIV and the epidemic

In 1981, rare cases of pneumonia infection caused by a fungus, was reported in young homosexual men in Los Angeles affected by severe immune depletion of T cells (81). In the same year, Kaposi sarcoma, a cancer form usually affecting elderly, was reported among homosexuals in New York (98). The first reports were followed by several others describing acquired immunodeficiency syndrome (AIDS). Clinical and epidemiological investigations provided persuasive evidence that the disease was caused by an infectious agent, probably a virus, transmitted by sexual routes and in blood derivatives (10). All initial attempts to establish a link between the epidemiological and clinical features of this disease and a known virus failed. A French group first discovered a new virus in a lymph node biopsy from an individual suffering from generalized lymphadenopathy (11). The virus was suspected as the causative agent of AIDS, described as a T-lymphotropic retrovirus and was named lymphadenopathy-associated virus (LAV). Shortly thereafter, American groups confirmed that retrovirus could be isolated from patients with AIDS and designated the virus as HTLV-III. These two viruses were later shown to be the same and were renamed to human immunodeficiency virus (HIV), later known as HIV-1 (38). In 1986, another HIV virus (HIV-2) was isolated from West African patients (36).

HIV has until 2005 killed more than 25 million people since it was first recognized in 1981, making it one of the most destructive epidemics in recorded history. Despite recent, improved access to antiretroviral treatment and care in many regions of the world, the AIDS epidemic

claimed 3.1 million lives in 2005; more than half a million were children (244). The total number of people living with HIV in 2005 reached the highest level so far to an estimated 40.3 million people. Close to five million people were newly infected. Two thirds of all people living with HIV are in sub-Saharan Africa, as are 77% of all women with HIV. Growing epidemics are underway in Eastern Europe, Central Asia, and in East Asia. The reduction in life expectancy has significant economic consequences and the number of orphans has increased dramatically in regions most affected by HIV.

Origin of HIV

SIV in the natural host, African monkeys in the wild and captivity, is non-pathogenic. More than 20 different primates are known to harbor SIV variants (85). There are at least six lineages of primate lentiviruses: SIVsm from sooty mangabeys; SIVagm from African green monkeys; SIVsyk from sykes monkeys; SIVcpz from chimpanzees; SIVlhoest and SIVsun from L'Hoest and suntailed monkeys and SIVcol from colobus monkeys (89) (Figure 1). Genetically, HIV-1 is most closely related to SIVcpz from chimpanzees (*Pan troglodytes*) and HIV-2 is more closely related to SIVsm from sooty mangabeys (*Cercocebus atys*). The two groups share only about a 50% homology in the Gag and Pol genes whereas within each group, they share more than 80% homology of amino-acid sequence (48). The viruses are thought to have been transmitted to humans by exposure to infected animal blood as a result of hunting, butchering and consumption of uncooked contaminated primate meat (85). Transmission has occurred at several occasions, as indicated by the fact that HIV-1 can be genetically divided into three distinct virus groups termed M, N and O and HIV-2 can be divided into seven lineages, subtype A through G. The predominant HIV-1 group M can be further subtyped into A1, A2, B, C, D, F1, F2, G, H, J and K (141). The transmission of HIV-1 group M from SIVcpz infected chimpanzees has been dated to around 1930 ± 15 years (119, 204). However, AIDS do not qualify as a zoonosis since transmission per se was not the major requirement for the generation of the AIDS epidemic (146). Human exposure of SIV is thousands of years old, but the HIV epidemic emerged only in the 20th century. If AIDS were a true zoonosis the virus would have spread from Africa earlier, for example during slave trade. A combination of various factors has been proposed for the spread of HIV in the 20th century; social disruption, urbanization, and prostitution, but the real cause of the epidemic spread of HIV has not yet been fully understood (85). HIV-1 group M has subsequently spread globally, generating the pandemic observed today. The date of the transmission of

SIVsm to establish HIV-2 subtype A and B strains in the human population was estimated to 1940-1945 (130). HIV-2 is mainly spread in West Africa with epicenters in Senegal, Guinea-Bissau and Côte-d'Ivoire (85). The epidemic initiation of HIV-2 in Guinea-Bissau coincided with the independence war (1963–1974), suggesting that war-related changes in sociocultural patterns has had major impacts on the HIV-2 epidemic (130).

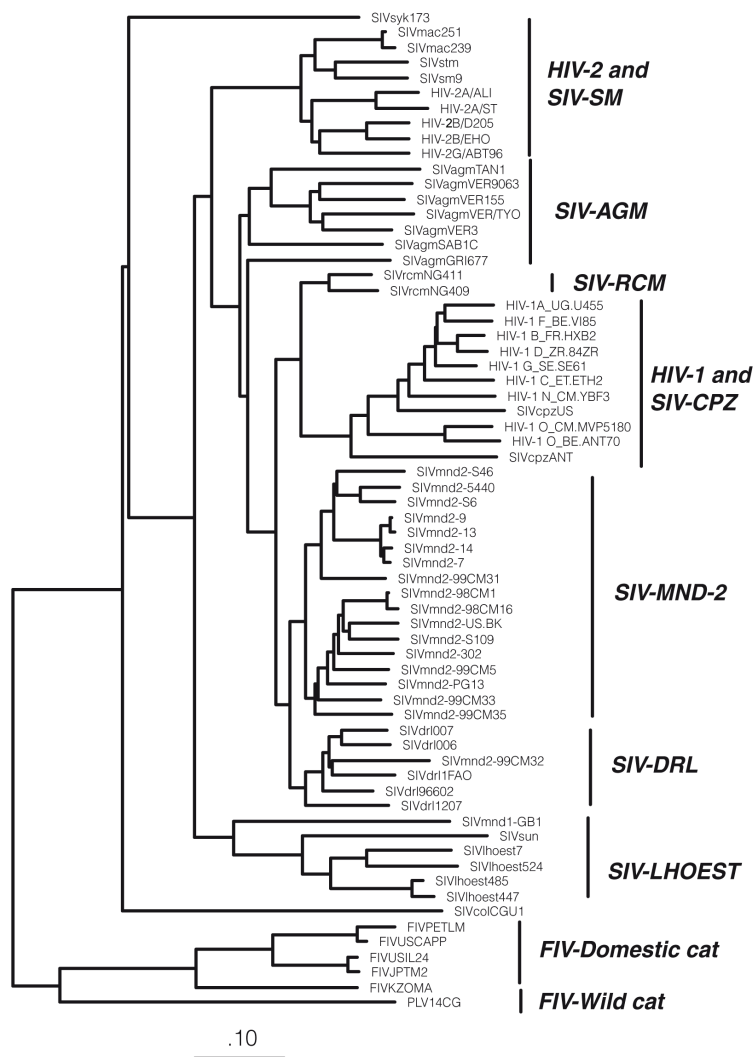


Figure 1. The phylogenetic relationship of a conserved fragment of the pol gene between the various SIV and HIV strains. Adapted from (89).

HIV pathogenesis

HIV transmission occurs through sexual intercourse, from infected needles, from infected pregnant or breastfeeding mother to her child or from transfusion of blood and blood products. The primary infection may be subclinical or accompanied by few symptoms like fever, fatigue, skin rash, headache and swollen lymph nodes. Symptoms are usually associated with production of virus-specific antibodies by the host, also known as seroconversion (181). Infections at the mucosal barrier, the predominant route of heterosexual transmission, have been the most studied in animal models. HIV/SIV cross the mucosa by infection of intraepithelial lymphocytes or dendritic cells (DC) or are merely captured by dendritic cells and transferred across the mucosa. After this first infection or transfer of virus the viruses will encounter and infect macrophages, DCs, and resting or activated T cells in the submucosa. Newly produced virus and virus infected cells establish infection in local lymph nodes which further spreads the infection to multiple organs such as the brain, spleen and peripheral lymphoid tissues. This acute stage of infection lasts for a few days up to a couple of weeks and is accompanied by a rapid decrease of CD4⁺ T lymphocytes (47) and a rise in plasma viremia with a production of up to 10¹⁰ viral particles per day (92, 176). By use of the SIV model it became clear that both activated and surprisingly high number of resting CD4⁺ T cells are productively infected shortly after transmission (47, 91, 135, 149, 232, 251, 261). According to these studies the gastrointestinal tract appeared to be the major site of CD4⁺ T cell depletion within a few days after transmission. After a few weeks viral replication is normally more or less suppressed by the host immune system, but a chronic infection remains (47). The virus infects and eliminates activated T cells, which in combination with the general loss of T cells compromises the virus specific and the general immune-response. After the initial virus replication there is a set point of the viral load in the infected individual which is predictive of prognosis (154, 236). This set point is usually followed by the chronic and asymptomatic phase of the infection. The chronic phase may continue for several years in an HIV infected individual. However, there is a continuous virus production and loss of CD4⁺ T cells and in the end the immune system is fatigued and AIDS follows. The time to AIDS is individual and the median time to AIDS is longer in HIV-2 than in HIV-1 infections. The final stage is associated with various symptoms such as chronic fever, diarrhea, weight loss, opportunistic infections and lymphomas (24). The exhausted immune system will in the end lead to immunosuppression and opportunistic infections and in the end cause death. The most frequent opportunistic infections are *Candida albicans* infection (causing oral/pharyngeal

candidiasis), *Pneumocystis jiroveci* (previously called *P. carinii*) pneumonia, lung disease caused by *Mycobacterium tuberculosis* and *Karposi's sarcoma* (an unusual skin tumor).

Primate animal models of HIV

Shortly after the discovery of HIV, chimpanzees were experimentally infected with both cell-free and cell-associated HIV-1 (4, 68, 71, 72). More experiments with chimpanzees followed and the infections with HIV-1 contributed important steps in understanding transmission, primary infection and the immune response (240). Infections of chimpanzees with HIV-1 were in some cases followed by mild symptoms but in most cases there were no apparent clinical disease stage. The long incubation period, the expenses that followed handling of these animals and most of all the fact that the chimpanzee is an endangered species led to an end of the chimpanzee experiments.

SIV is non-pathogenic in its natural host, but when macaques are infected with the virus they develop symptoms of immunodeficiency, similar to humans. This was discovered in 1985 when a lentivirus, SIVmac, was isolated from captive rhesus macaques (*Macaca mulatta*) with lymphomas and immunodeficiency-associated disorders (42, 131). SIVmac and other SIV isolated from macaques, i.e. SIVstm from stumptailed macaques (*M. arctoides*) and SIVmne from pigtailed macaques (*M. nemestrina*) are all different SIVsm lineages and are most likely the result of cross-species transmission from sooty mangabeys to macaques in captivity (89). Experimental infections of macaques, such as rhesus macaques, pigtailed macaques and cynomolgus macaques (*M. fascicularis*) with viruses from the SIVsm lineage was shown to be adequate animal models for AIDS (12, 69, 122, 132, 150, 185, 187). In these models, disease is followed by loss of CD4⁺ T cells and immunodeficiency is acquired within months up to a few years after infection. Since then experimental infections of macaques with SIVsm lineages have been used to study pathogenesis and immunity (86). Hybrids between SIV and HIV-1 (SHIV) have been developed to evaluate the behavior of HIV-1 genes in the SIV animal model. The first SHIVs used to infect macaques were proven to be avirulent (134, 203, 219). Improved SHIVs were later shown to be pathogenic causing acute T cell loss and AIDS (87, 106, 193, 220).

Virus structure and genome organization

Structure

The lentiviruses are roughly spherical, enveloped RNA viruses, approximately 100 nm in diameter and with two copies of RNA genome (Figure 2). Virus encoded envelope glycoproteins are incorporated in the cell-derived lipid membrane that also contains cellular membrane proteins from the host cell (i.e. integrins and tetraspanins). The outer surface envelope protein (SU) gp120 forms trimers of knob-like structures which are non-covalently anchored into the membrane by triplets of the transmembrane protein (TM) gp41. An inner matrix protein lines the envelope and helps maintain the structure of the virion. The capsid-protein (p24 in HIV-1 or p26 in HIV-2 and SIV) forms a cone-shaped shell that surrounds the viral genome and virus encoded enzymes. The two copies of single-stranded RNA genome of positive polarity are coated by nucleocapsid proteins. A t-RNA, functioning as primer for transcription, is bound to each genome. The virus particle carries three types of virus encoded enzymes: protease, reverse transcriptase and integrase. In addition to these enzymes the virus particle also brings some accessory proteins; Vif, Vpr and Vpx in HIV-2 and SIV or Vpu in HIV-1.

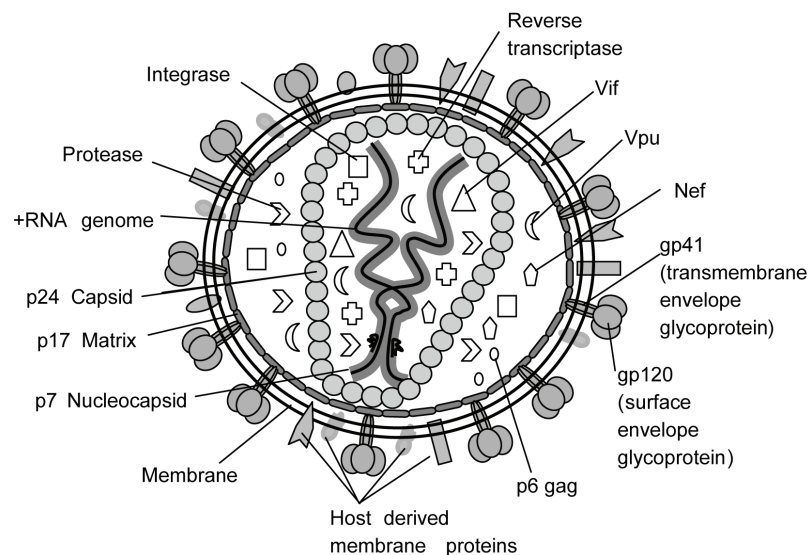


Figure 2. Structure of the HIV-1 virion. Adapted from www.images.md

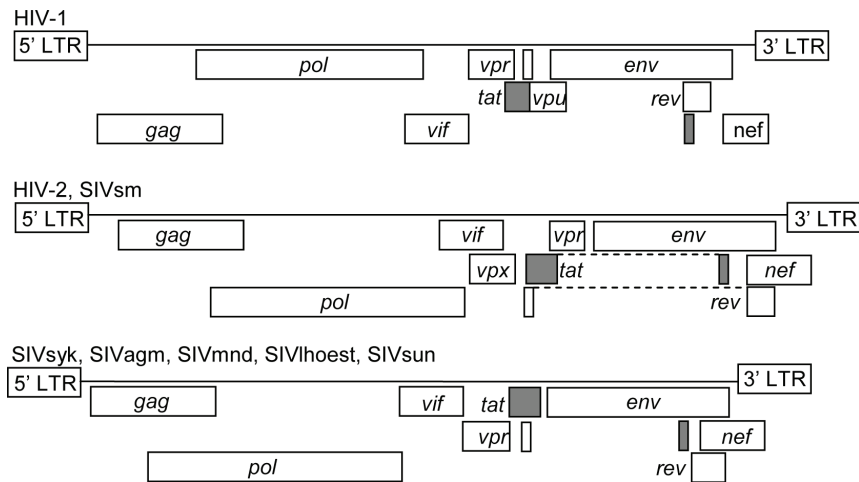


Figure 3. Genomic organisation of HIV and SIV. Adapted from (141).

Genes and proteins

The 10^4 base-pair long genome is flanked by long terminal repeats (LTR) and contains open reading frames (ORFs) for a dozen of viral genes (141). (Figure 3) The major ORFs carry genes encoding structural and enzymatic proteins (*gag*, *pol*, and *env*) and the additional ORFs (*tat*, *rev*, and *nef*) encode non-structural, RNA-binding regulatory proteins as well as genes for the accessory proteins *vif*, *vpr*, *vpu* or *vpx*. LTR contains important regulatory regions, especially those for transcription initiation and polyadenylation. The genome organization of HIV-1, HIV-2 and SIVs are similar and the major difference is that HIV-1 has both the *vpr* and *vpu* gene, HIV-2 and SIVsm lineages have the *vpr* and the *vpx* gene, while other SIVs only carry the *vpr* gene (Figure 2).

The *gag* genomic region encodes the p55 polyprotein precursor, which is cleaved by the viral protease to capsid (p24 or p26), matrix (p17), nucleocapsid (p7) and Vpr binding (p6) proteins. The *pol* region codes for three viral enzymes: protease, reverse transcriptase and integrase. These enzymes are produced as a Gag-Pol precursor, which is produced by site-specific frame shifting during translation. These precursors are processed by the viral protease. The membrane-associated proteins are produced as a precursor (gp160) from the *env* gene, which is processed to the surface glycoprotein gp120 and the transmembrane glycoprotein gp41. Control of HIV gene expression is regulated by cellular and viral factors. Tat is one of the two viral gene products that are essential for viral replication. The other

necessary gene product is Rev. These proteins are regulatory factors for HIV gene expression and are mainly found in the nucleus of infected cells. Two forms of Tat are known, Tat-1 exon (minor form) and Tat-2 exon (major form). Tat acts by binding to the target sequence for viral transactivation (TAR) in the LTR and activating transcription initiation and elongation from the LTR promoter. The LTR contains an enhancer and promoter that are recognized by cellular proteins. These regulatory sequences support proviral transcription to a lesser extent after integration but transcription is enhanced after Tat binding. Rev is a phosphoprotein and acts by binding to the Rev responsive element (RRE), encoded within the *env* region. This binding promotes the nuclear export, stabilization and utilization of the viral mRNAs containing RRE. By binding of Rev to RRE the balance is shifted from early expression of multiple-spliced transcripts of regulatory proteins to late expression of single spliced mRNAs, which encode the viral structural proteins. The *nef* genes of HIV and SIV are dispensable *in vitro*, but are essential for efficient viral spread and disease progression *in vivo*. Nef is important for virion infectivity, downregulates CD4, the primary viral receptor and major histocompatibility complex (MHC) class I and has the capacity to alter the activation state of cells. Vif is the viral infectivity factor that promotes the infectivity but not the production of viral particles. In the absence of Vif the produced viral particles are defective, vif inhibits the cellular enzymes APOBEC3G and APOBEC3F by introducing numerous G→A mutations into the viral genome. APOBEC3G and APOBEC3F are cytidine deaminases and inhibit replication of Δ vif HIV-1 by deaminating the minus-strand of the viral reverse transcripts. Two biological functions that have been tied to Vpu (viral protein U) are degradation of CD4 in the endoplasmic reticulum and enhancement of virion release from the plasma membrane of HIV-1-infected cells. Vpr (viral protein R) is homologous to the Vpx (viral protein X). In the infected cell, Vpr is localized to the nucleus and proposed functions include targeting of preintegration complexes for nuclear import of, cell growth arrest, transactivation of cellular genes, and induction of cellular differentiation.

Replication cycle

Entry

HIV replication begins by interaction between the viral envelope glycoprotein and two cellular receptors (Figure 4). Shortly after the discovery of HIV, CD4 was demonstrated as its receptor (41, 117). The entry process of HIV begins when the surface viral envelope protein,

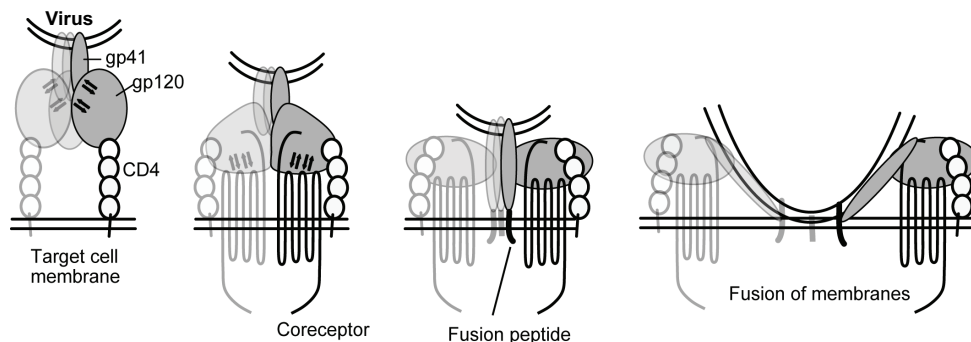


Figure 4. HIV binding to receptors on the target cell. All proteins in the envelope trimers may be involved in binding to clusters of receptors on the target cell membrane. Adapted from (158).

gp120, binds to CD4. Later it has been shown that CD4 alone cannot promote fusion with gp120. The first identified cofactors for HIV-1 entry into target cells were the chemokine receptors CXCR4 and CCR5 (45, 51, 62). The glycoprotein gp120 consists of five constant regions, designated C1 to C5, and five variable regions or loops designated V1 to V5. Binding to CD4 leads to major conformational changes within gp120, forming and moving the variable loops away from a conserved chemokine receptor-binding site (Figure 4). Crystallization of gp120 unligated or CD4-bound, showed that after binding to CD4, the envelope retains the secondary structure of gp120, but reshuffles the location and relative orientation of different domains with conformational changes up to 40 Å (26, 125). The exposed region in gp120 binds to the chemokine receptor and this leads to further conformational changes that expose a hydrophobic fusion peptide (gp41) (78). The fusion peptide reaches the cell surface and this result in membrane fusion and the viral core and particles are released into the cytoplasm. It is likely that several envelope trimers need to undergo conformational change in order to form a fusion pore.

Transcription, integration and translation

The single-stranded RNA virus genome is reversely transcribed to double-stranded DNA by the viral reverse transcriptase, within the decomposing capsid (Figure 5) (64). The reverse transcriptase lacks proof-reading mechanisms of mismatches in nucleotide bases when the viral genome is transcribed. This leads to a high mutation frequency of the viral genome. The viral DNA associates with matrix and Vpr protein as well as with the viral integrase to form a preintegration complex. This complex has nuclear localization signals which direct the complex into the nucleus. The viral DNA is integrated into the host cell genome by the viral

integrase. Transcription of proviral DNA by host cell RNA polymerase II is regulated by viral and cellular factors. mRNAs are transcribed and will be used for templates of viral enzymes and proteins. RNA copies of the full-length provirus will be transported to the site of assembly and incorporated as viral genome in newly formed virus particles. mRNA for Gag and Gag-Pol polyprotein precursors and other full length RNAs are transported to the cytoplasm after encapsidation and will serve as new viral genomes. RNA is also spliced within the nucleus to form Env mRNA.

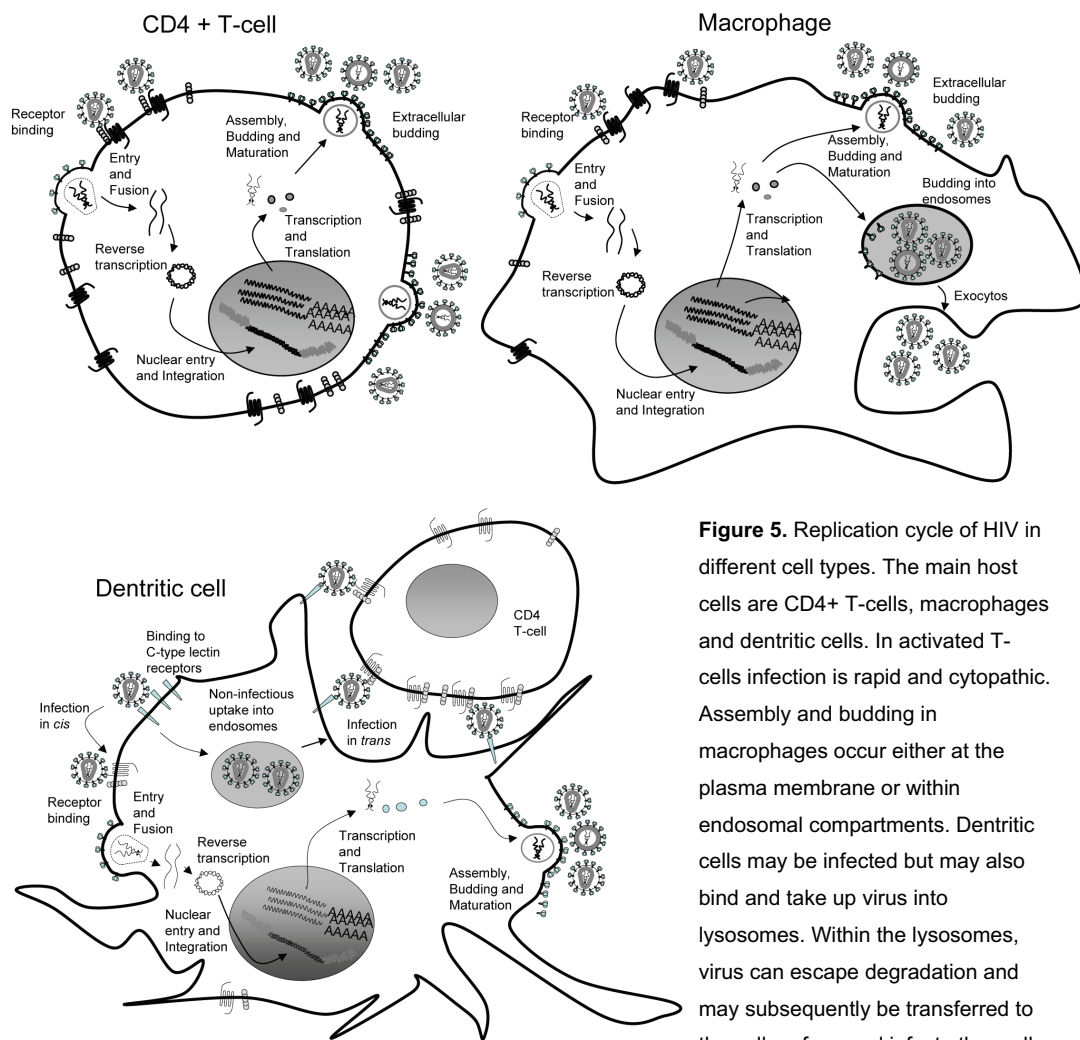


Figure 5. Replication cycle of HIV in different cell types. The main host cells are CD4+ T-cells, macrophages and dendritic cells. In activated T-cells infection is rapid and cytopathic. Assembly and budding in macrophages occur either at the plasma membrane or within endosomal compartments. Dendritic cells may be infected but may also bind and take up virus into lysosomes. Within the lysosomes, virus can escape degradation and may subsequently be transferred to the cell surface and infect other cells. Adapted from (235).

Assembly

The Gag p55 precursor plays a central role in virus assembly (19). Gag is important for recruitment of viral proteins and genome as well as cellular proteins during assembly. Gag is synthesized in the cytoplasm and is modified after translation with the myristic fatty acid at the N-terminus of the matrix protein (195). After modification, large complexes of Gag form (Gag multimerization) together with Gag-Pol precursors. The myristylation together with a cluster of basic residues promotes localization of Gag complexes to membranes. The envelope gene is translated by ribosomes in the endoplasmic reticulum to the precursor protein gp160, followed by extensive glycosylation and maturation into functional gp120 and gp41. By separate routes, Env and Gag traffics to lipid rafts in multivesicular bodies (MVB) which contain cellular proteins machineries that are necessary for particle budding. In macrophages, where exocytosis is regulated, budding occurs into the lumen of MVB (175, 190) (Figure 5). In other cell-types, Gag and Env rapidly transit to the plasma membrane where viral particles buds from the cell. Cleavage of Gag and Gag-Pol precursors is essential for virus maturation and infectivity (19). The viral protease is active during or soon after budding, and Gag and Gag-Pol precursors are cleaved within the virion. The cleavage and maturation induces condensation of the nucleocapsid and the RNA genome into a cone-shaped core with the capsid as a shell. Besides from budding of new virus particles the virus can also spread to new cells by cell to cell transmission. The most recognizable mode of cell to cell transmission is explained by expression of mature Env on the plasma membrane of infected cells that can induce fusion with CD4+ cells, which leads to formation of gigantic multinucleated cells (syncytia).

Antigen variation

Antigen variation is a fundamental feature of many pathogens and is a way for bacteria and viruses to escape the immune response (162). The most effective antigenic variation usually occurs in the surface proteins of the pathogen, and in the case of HIV and SIV in the gp120 envelope protein. Since the reverse transcriptase has no proof reading and is extremely error prone there will be many mistakes made during transcription that can lead to new virus variants. The mutation frequency of reverse transcriptase is about $1/10^4$ bp (142) which is extremely high compared to cellular DNA polymerases with a mutation frequency of $1/10^9$. As many as 10^{10} new viral particles are produced every day (92, 176) and many of these may result in variants with successful mutations. The viral genome has many conserved regions,

important for protein function, where mistakes in reverse transcription usually result in nonfunctional viruses. The envelope gp120 protein has five conserved regions, C1 to C5, and five variable regions, V1 to V5. In the tertiary protein structure, the variable regions or loops hide the conserved regions that are important for binding to the receptors. These variable loops may change in length, glycosylation sites and in the charge of the amino-acids (66, 225). Another way of mutation is by recombination between the two strands of the RNA genome (96). This may occur if the same cell is infected by more than one virus and two different genomes are assembled and packed within one virus particle. Indeed, in splenocytes taken from HIV-1-infected patients contain a median proviral copy number of three copies per cell, ranging from one to eight proviral copies in each cell (83, 108). After infection of a new cell the reverse transcriptase jumps between the two copies of RNA when transcribing the genome and recombination(s) follows.

Recombinations and mutations have given rise to the extraordinary scale of HIV variation resulting in the various subtypes that are spread around the world (118). The sequence variation of the quasispecies of viruses in a single HIV-1-infected individual a few years after infection is greater than the global variation of epidemic influenza strain during the flu season in one year. The effect of mutations of HIV and SIV can be resistance to anti-viral inhibitors, escape from cellular immune response, changes in receptor preferences, as well as escape from neutralizing antibodies.

Receptor usage

CD4 is regarded as the main receptor for primate lentiviruses, in addition gp120 binds to a coreceptor, to induce the fusion of membranes. However, it has been shown that HIV-2 and SIV strains may enter cells independently of CD4 (35, 58, 60, 139, 191). This was followed by reports on laboratory adapted HIV-1 variants that were able of CD4-independent infections (52, 93, 95, 127). Primary HIV-1 isolates that can infect cells independently of CD4 are rare and have not been isolated until recently (257). Laboratory adapted CD4-independent HIV-1 was found to have a stable exposure of the coreceptor binding site (93). However, similar conformational changes in the envelope have not yet been shown for SIV or HIV-2 and it may be that HIV-1 is more dependent on conformational envelope changes for efficient infection than HIV-2 and SIV.

The binding to a coreceptor is important for fusion for both HIV and SIV. Coreceptors are usually chemokine receptors belonging to the seven-transmembrane G-protein-coupled receptor family (198). The receptor forms a barrel-like structure, crossing the plasma membrane seven times, where the extracellular (N-terminal) part of the receptor binds the natural ligand, the chemokine, and the intracellular (C-terminal) region is coupled to a signal transducing G-protein. There are three extracellular and three intracellular loops. Chemokine receptors are classified into C, CC, CXC and CX3C based on the molecular structure of the ligands bound. Chemokines are 92-125 amino acids in length and their normal functions are to act as chemoattractant cytokines for lymphocytes and monocytes as well as activators and attractants for eosinophils and basophils. Ligands for chemokine receptors important in HIV and SIV are listed in Table 1.

Table 1. Main receptors used by HIV and SIV, their ligands and expression.

Receptor	Ligand*	Expressed by
CD4	Coreceptor for MHC II, binds Lck	T helper cells, monocytes, macrophages, dendritic cells (102)
CCR3	Eotaxin 1, 2, and 3, MIP-1 α , MIP-1 β , RANTES, MCP-2, 3, and 4	Eosinophils, neutrophils, basophils, monocytes, macrophages, dendritic cells, bone marrow cells, T cells, platelets, keratinocytes (102)
CCR5	MIP-1 α , MIP-1 β , RANTES	Monocytes, macrophages, NK cells, basophils, dendritic cells, bone marrow cells, T cells especially activated/memory subset (16, 102)
CXCR4	SDF-1 α/β	T cells especially naive, unstimulated T lymphocytes, bone marrow stromal cells, progenitor lymphocytes, dendritic cells, B cells, plasma cells, and (16, 94, 102)
CXCR6	CXCL16	T cells, dendritic cells, colorectal epithelial cells, bone marrow stromal cells (94, 101, 216, 246)
gpr15	unknown	CD4 ⁺ T cells, macrophages, and cells in the epithelium of small intestine (2, 46, 61, 144)

Virus isolates from HIV-1 infected patients may use different coreceptors depending on stage of disease. HIV-1 isolates that use CCR5 (R5 phenotype) predominate early in asymptomatic HIV-1 infection, while CXCR4-using (X4 phenotype or multi-tropic) viruses can be isolated in approximately half of the patients that progress to AIDS (15, 109, 213, 237, 238). CXCR4-using HIV-1 isolates are regarded as more pathogenic (7, 30, 39, 63, 109, 237, 238). It must

be remembered that individuals that maintain the R5 phenotype still progress to AIDS (15, 43, 109) and that isolates from these patients evolve to more cytopathic over time (123). The R5 phenotype was previously classified as slow/low or non-syncytium inducing (NSI), whereas CXCR4-using viruses were called rapid/high or syncytium inducing (SI) (13). CCR5 and CXCR4 are the major and most common coreceptors for HIV-1 (Table 1). Evolution of the coreceptor use in a HIV-1 infected individual is a continuous process where ability of viral gp120 to bind coreceptors change. By using receptor chimeras between CCR5 and CXCR4, Karlsson et al. showed that the R5 phenotype of HIV-1 undergoes evolution over time and changes the mode of CCR5 use (110, 111). From first being dependent on the N-terminal, the R5 phenotypes evolved to be able to use other parts of the CCR5 receptor for infection of cells. Changes in the mode of CCR5-use correlated with disease progression and resistance to inhibition by RANTES. In patients that maintained the R5 phenotype despite progression to AIDS, R5 variants that replicated more efficiently seemed to evolve and had a reduced sensitivity to RANTES and other entry inhibitors like T-20 and TAK-779 (194). In addition to CCR5 and CXCR4 use, several other coreceptors have been identified for HIV-1 but the importance of these minor coreceptors is yet not known. Minor coreceptors include: CCR2b (50), CCR3 (32, 50), CCR8 (105, 200), CXCR6 (46, 137), gpr1 (222), gpr15 (46), RDC1 (223) and APJ (31, 57). CXCR4-using HIV-1 are often characterized by dual tropism (R5X4) or multi-tropism and may use CCR5, CCR3, CCR2b in combination with CXCR4. Coreceptor use of HIV-2 is similar to that of HIV-1, but shows promiscuity in the coreceptor usage, such as use of one or more of the CCR1, CCR2b, CCR3 and gpr15 coreceptors in addition to CCR5 or CXCR4 (17, 152, 163)

Change in coreceptor use of sequential SIV isolates has not been evident although late SIV isolates are often more cytopathic than early isolates (114, 115, 201). CCR5 is, as for HIV-1, the major coreceptor for SIV (28, 55, 143), while CXCR4 use is rare and was shown with virus isolated and grown on human peripheral blood mononuclear cells (hpBMC) (170, 210, 255). Instead SIV isolates often use CXCR6, and the orphan receptor gpr15 (Table 1) (2, 46, 61). CXCR6- and gpr15-use by HIV-1 are much less frequent. Furthermore, differences in the ability of various SIV strains to interact with CCR5 have been observed. SIVmac with an efficient capacity to replicate in macrophages has been shown to depend on both the amino-terminal and the second extracellular loop of CCR5 for cell entry, while SIVmac with a preference to replicate in T cells require only CCR5 extracellular loop-2 (55). Comparable to HIV-1 and 2, SIV strains have been shown to use a wide set of alternative coreceptors,

including CCR1 (116), CCR2b (147), CCR3 (116), CCR8 (200), CX3CR1 (192), gpr1 (57, 61), APJ (58), ChemR23 (205) and RDC1 (223) but the relevance of these receptors is still unknown.

Cell tropism

The distribution of the viral receptors on different cell types renders many cells, normally involved in the immune system, permissive to infection by HIV and SIV (Table 1). The major cell types infected by HIV and SIV are T cells, macrophages and dendritic cells. The ability of HIV-2 and SIV to infect cells independently of CD4 increases the number of putative target cells.

T cells

Primate lentiviruses are normally thought to infect CD4⁺ T cells. Indeed, virus replication of HIV is rapid and efficient in activated CD4⁺ T cells. Several studies suggest that HIV isolates obtained late in infection from patients with a progressive disease, regardless of coreceptor use, have a higher replication capacity and cytopathicity both in cell lines and peripheral blood mononuclear cells (PBMC) (7, 30, 39, 63, 109, 123, 194, 237, 238). In vitro infection of PBMC appear to require activated cells (33, 88, 197, 209, 231). Analysis of preferential integration sites *in vitro* in CD4⁺ T cells suggests that HIV-1 preferentially integrates into active genes (211). However, Nef may create conditions that allow the virus to enter quiescent non-activated cells (155, 230). In addition, cytokine signals may be sufficient for HIV-1 infection of resting T cells (245) and *ex vivo* infection of lymphoid tissue with HIV-1 support infection of non-activated CD4⁺ T cells (53, 79). HIV-1 isolates from long-term non-progressors have been shown to have lower replication. In addition, HIV-1 isolates from long-term non-progressors had a reduced replicative fitness in PBMC compared to isolates from patients who progressed to AIDS (189, 241).

By use of the SIV model it has been shown that both activated and a surprisingly high number of resting CD4⁺ T cells were productively infected shortly after infection (47, 91, 135, 149, 232, 251, 261). At later stages most of the HIV-1 infected CD4⁺ T cells were activated (261). Major CD4⁺ T cell depletion occurs within a few days after transmission (47, 91, 135, 149, 232, 251, 261). The viral envelope has been implicated as the determinant of cytopathicity *in*

vitro (126, 138, 229). However, uninfected CD4⁺ and CD8⁺ cells from infected individuals undergo spontaneous apoptosis indicating other mechanism of bystander apoptosis (235).

Macrophages

Macrophages are considered important viral reservoirs throughout the chronic infection in individuals infected by HIV (235). The amount of infected CD4⁺ T cells outnumber infected macrophages in an infected individual by 10-100 fold (59). However, the contribution of virus production from macrophages exceeds the frequency at which macrophages are represented in the infected cell reservoir (54). Nevertheless, the importance of the capacity of HIV and SIV to replicate in macrophages has been debated. Early reports showed that shortly after seroconversion, HIV-1 variants able to readily infect macrophages, termed macrophage-tropic (M-tropic) variants, can usually be detected (212). With progression to AIDS, HIV-1 isolates were reported to lose the capacity to infect macrophages. More recent studies showed that macrophage tropism of HIV-1 isolates from patients with advanced immunodeficiency was enhanced over-time (136, 243). Enhanced M-tropism of R5 HIV-1 may result from adaptive viral evolution, resulting in HIV-1 variants that have increased ability to utilize relatively low levels of CD4 and CCR5 expressed on macrophages (9, 80). Nevertheless, it must be remembered that a strict correlation between CCR5 use and macrophage tropism cannot always be found (29) and primary X4 HIV-1 isolates are also capable of replication in macrophages (226, 233, 247, 248, 254).

Varying results of the importance of macrophage-tropism have been reported in the SIV model in macaques over the years. Infection of rhesus monkeys with highly pathogenic SHIV results in a depletion of the majority of CD4⁺ T cells and plasma viremia is maintained by infected tissue macrophages (99). Infection of rhesus monkeys with the non-M-tropic molecular clone SIVmac239 resulted in either evolution of M-tropic variants or lack of appearance of M-tropic virus regardless of whether the animals developed AIDS (49, 214). Stephens et al. suggested that all SIV reisolates are both M-tropic and T-tropic but viruses may vary in the efficiency of infection in macrophage lineage cells (234). An experimental pathogenesis study using the molecular clone SIVMneCL8 showed that late variants reisolated from the monkeys were more cytopathic in human T cell lines, and less efficient in infecting macrophages compared to the early reisolates and the inoculum virus (201).

Dendritic cells

Dendritic cells (DC) may be infected by HIV and SIV and are important for the transport of the virus from the site of infection to local lymph nodes where the viruses are transmitted to CD4+ T cells. However, the viruses can also exploit a route not leading to infection of DCs but rather a mean for transmission to T cells (Figure 5). By the use of C-type lectins, DC-SIGN being the most studied, viruses are captured and internalized by DC, subsequently transmitted to T cells *in trans* (76). Internalized virus can retain infectivity for more than 4 days (76), or may be rapidly degraded (within hours), likely in the lysosomal compartments (160, 242). Moreover, the compartment where viruses are captured by DC after DC-SIGN uptake appears to be a non-lysosomal compartment (74, 124) and transfer of HIV from DC to T cells has been shown to occur through an infectious synapse that locally concentrates virus and receptors between infected and uninfected cells (73, 151). Viruses transferred by the infectious synapse are poorly accessible to neutralizing antibodies (73). Interestingly, the infectious or virological synapse has similarities with the immunological synapse that is formed between antigen presenting cells and T cells at peptide presentation through MHC and the T cell receptor (177). For example, adhesion molecules like ICAM-1 and LFA-1 as well as CD4 are recruited to the infectious synapse.

Other C-type lectins that have been shown to be important in HIV infections include the mannose-receptor, heparans, and LFA-1/ICAM-1 (40, 128, 156, 173). None of the C-type lectins have been shown to induce the conformational changes needed for entry. However, cells can be infected after virus binding to C-type lectins, known as transmission and infection *in cis* (129, 167). In this case attachment of virus to C-type lectins may concentrate virus on the plasma membrane for sequential binding to CD4 and coreceptors on the same cell.

The immune response to HIV

In the host, the invading virus must overcome the host defenses against infections. The first shields to cross are physical and chemical barriers: the skin, mucous secretions, tears, low pH and surface cleansing mechanisms (102). When viruses have overcome the first hindrance the immune responses are encountered, consisting of the innate and the adaptive defense. The adaptive immune response has two arms, the humoral response and the cellular response. All three, the innate, humoral and cellular immune responses interact between helper and effector

cells. In HIV and SIV the situation is complex since the actual target cells also are cells involved in the immune defense.

It is not known why the immune system cannot control HIV infection, when SIV is apathogenic in its natural host, the African monkeys. It has been suggested, that SIV infection in sooty mangabeys or African green monkeys lacks intense immune activation and apoptosis, and maintain T lymphocyte populations, the opposite to what is characteristic of pathogenic HIV and SIV infections (25, 224). Unfortunately, models suggesting immune tolerance to SIV in natural infections are too simplistic to explain the lack of disease (89). It is clear that sooty mangabeys and African green monkeys mount both cellular and humoral immune responses to the virus.

Innate immune response

The innate immune system is governed by cytokines, complement, and effector cells like phagocytic cells (i.e macrophages and neutrophils) and natural killer (NK) cells (102). This early defense is activated rapidly, within hours after an infection. The innate immune response also instructs the adaptive immune system to respond to different microbes. The most powerful antiviral cytokines are the interferons (IFN- α and IFN- β) that are released by virus infected cells and induce an anti-viral state of the infected and neighboring cells. The anti-viral state of a cell seems to involve many different mechanisms and may lead to upregulation of MHC genes and apoptosis. IFN production in infected individuals has physiological consequences and gives fever, chills, nausea and malaise. The complement system has various functions that involve cytolysis of lipid membranes by the membrane attack complex, activation of inflammation and opsonization. NK cells can kill infected cells directly or by antibody dependent cellular cytotoxicity (ADCC).

Humoral immune response

Antibodies to HIV can be detected shortly after acute infection, sometimes already after a few days, but generally within one to three months. B cells produce antibodies after a first encounter of the antigen and further activation by cytokines and via interactions of MHC class II on B cells and T cell receptors (TCR) on CD4⁺ T helper (Th) cells (102). The most important antibodies in virus infections are antibodies that upon binding to the virus can inhibit infection of cells. These antibodies are called neutralizing antibodies and are used as

markers of protective immunity in many virus infections. The importance of the humoral immune response in primate lentivirus infection has been investigated by depleting the B cells from macaques before exposure to SIV. Depletion of B cells results in less immunological control and much higher viral loads, suggesting an importance of neutralizing antibodies at the early infection and in the post-acute phases of infection (107, 208). Passive immunization of macaques with high titer neutralizing antibodies before virus challenge protects animals from disease or from virus infection (8, 148, 166, 186, 252).

Various studies suggest an association between neutralizing antibody responses and disease progression in HIV-1 and infections (21, 157, 172, 178, 258). Serum from asymptomatic long-term non-progressor patients contain antibodies capable of neutralizing both heterologous and autologous virus-isolates compared to serum from fast progressor with weakly neutralizing properties. Autologous HIV-1 neutralizing antibodies may evolve already after two to four weeks after infection but not surprisingly this restricted immune response leads to the selection of neutralization resistant variants (1, 6, 196, 253). Escape from neutralization has not been demonstrated in HIV-2 infected individuals (14, 218) and HIV-2 reisolates obtained from HIV-2 infected macaques remained neutralization sensitive during the entire course of the non-pathogenic infection (260).

In SIV, the ability to produce autologous neutralizing antibodies in experimental SIVsm infections correlated with the progression of disease (20, 259). Viruses resistant to neutralization by autologous sera emerged during the entire course of infection. Neutralizing antibodies was showed against the challenge virus SIVsmE660 already at seven weeks after infection (202). The timing of neutralizing antibodies in these macaques correlated with an increase in the number of envelope gene variants within the host, suggesting an escape from the antibody response.

Laboratory adapted HIV-1 variants have been shown to be more neutralization sensitive than primary isolates. These laboratory adapted viruses have been shown to have a stable exposure of the coreceptor binding site (93) and are capable of CD4-independent infections (52, 93, 95, 127). Neutralization sensitivity of SIVmac envelopes have been correlated to CD4-independent entry into target cells as well as to enhanced macrophage tropism (153, 184).

A few different epitopes on the HIV envelope are considered to induce neutralizing antibodies. These epitopes have been mapped with different human monoclonal antibodies and are both in variable and conserved regions of the envelope (262). Antibodies that can neutralize isolates of different strains and subtypes are known as broadly neutralizing antibodies. V2 and V3 loops are highly immunogenic, but antibodies are virus strain specific. The CD4 binding domain of gp120 can induce neutralizing antibodies. However, only one monoclonal antibody has been found that elicit broad neutralizing activity. Antibody fragments may bind to the CD4-induced domain of gp120, unfortunately this epitope is not accessed by intact IgG molecules. The transmembrane protein gp41 has an immuno-dominant region which induces high levels of antibodies, most are not neutralizing, but might mediate other important functions after binding. The region of gp41 that is proximal to the membrane is mostly covered and is exposed during a limited time, nevertheless antibodies to this region are broadly neutralizing. The envelope proteins are heavily glycosylated and carbohydrates are normally not recognized by antibodies, interestingly one neutralizing monoclonal antibody is known to target a cluster of carbohydrates in gp120.

Cellular immune response

The cell-mediated immune response includes actions of cytotoxic T cells and Th cells (102). Th cell activities include stimulation and activation of both B cells and cytotoxic T lymphocytes (CTL). The majority of CTL are CD8+, and their role in viral infections is recognition of MHC class I presented peptides on infected cells followed by lysis of the presenting cell. CD4+ Th cells recognize MHC class II molecules presenting peptides on specialized antigen presenting cells like dendritic cells and macrophages. After binding to MHC II, the Th cells produce cytokines that either activates maturation of CTLs (Th1 response: IL-2, IL-12 and IFN- γ) or stimulate B cells development (Th2 response: IL-4, IL-5, IL-6 and IL-10). The human MHC molecules are also known as HLA (human leukocyte antigen).

Many groups emphasise the importance of the cellular adaptive immune response in primate lentivirus infections. The appearance of CTL in HIV and SIV infections correlates with the decline and control of viral load from peak levels (104, 121, 207). Strong CTL responses have been reported in a cohort of prostitutes in Nairobi who have been exposed to HIV but remain uninfected (67, 112, 199). However the CTL immune responses were not combined with a

memory since reduction in sex work resulted in loss of HIV-specific CD8⁺ responses and was followed by HIV infection (113). The protective role of CTL in infection have been shown in macaque studies (104, 207). Depletion of CD8⁺ cells by monoclonal antibodies resulted in dramatic increases in viral load. Viremia was suppressed coincident with the reappearance of SIV-specific CD8⁺ T cells. Nevertheless, like in the humoral immune response where viruses resistant to neutralization by autologous sera emerge during the entire course of infection, HIV- and SIV-specific CTL responses select for viral escape variants during chronic infection (3, 82, 169, 182, 250).

Beside from the normal role of cell lysis by CTL in infection, CD8⁺ cell have a non-cytotoxic innate antiviral response. This response have been shown to be mediated by one or more secreted soluble factors, known as the CD8⁺ cell antiviral factor (CAF) (133). A large number of interleukins, interferons, chemokines, granzymes, growth factors and other cytokines with anti-HIV activity have been evaluated as possible CAF candidates. However, none of these candidates fulfilled the property of CAF. The natural ligands to CCR5, MIP-1 α , MIP-1 β , RANTES are produced by transformed CD8⁺ T cells and have been shown to inhibit HIV activity in cell culture (37) and spontaneous and antigen-induced production of these β -chemokines are associated with a more favorable outcome of HIV-1 infection (75).

Certain HLA class I alleles (HLA-B27 and B-57) have been associated with slow disease progression in HIV disease (23). In contrast, the presence of another HLA allele (HLA-B35) was more frequent in rapid progressing patients. Genetic associations between HIV disease and HLA class II have not been as strong as for those observed for class I. The repertoires of MHC class I and II have been studied rather thoroughly in rhesus monkeys (22). MHC class I allele, Mamu-A26 have been shown to protect from infection and the Mamu-A*01 molecule is considered to control slow progression.

Host factors in virus infection

Differences in disease progression may be related to route of infection. Comparisons of disease progression in HIV-1 infection between injecting drug users (IDU) and homosexual men, have shown that homosexual men had a significantly accelerated progression rate, when adjusting for non-AIDS mortality in the IDU group (174). On the other hand others found little evidence for different disease progression between IDU and homosexual men (183).

HIV is transmitted to women primarily via heterosexual contact, and the virus must therefore penetrate the mucosal barrier to establish a systemic infection. Physiologically, women are more susceptible to HIV infection than are men (188). Increased susceptibility among women has been linked to specific cofactors, including the use of hormonal contraceptives and the increased presence of sexually transmitted diseases. Several studies have shown that women have lower viral loads compared with men, however, the risk to develop AIDS was similar among men and women (77). Comparisons of viral loads between male and female monkeys have not been performed, and one retrospective analysis showed that gender and age at experimental infection with SIV could not be correlated to survival time (65).

A 32 base-pair deletion within the exon of the CCR5 gene (CCR5- Δ 32) results in almost complete protection against HIV-1 infection and a slower progression to AIDS in individuals homozygous for the allele (44, 97, 140, 206). The phenotype of another chemokine receptor (CCR2-64I) is also associated with slower progression to AIDS (120, 227). The beneficial effect on HIV-1 infection by CCR2 V64I has been proposed to be mediated by down-regulation of CCR5 (164). However, there are also genes that may accelerate AIDS progression. For example, point mutations within the promoter sequence of the CCR5 gene increase the expression of CCR5 and cause an accelerated AIDS progression (145). The roles of similar defects in infection of non-human primate species have not been documented, although mutations influencing the expression of functional CCR5 have been found in mangabey monkeys (27, 171). SIV isolated from one red-capped mangabey monkey with homozygous 24-bp deletion in the CCR5 gene was not R5-tropic, but used CCR2b as its major coreceptor (27).

MATERIALS AND METHODS

Cynomolgus macaques

Thirty cynomolgus macaques were intravenously (IV) or intrarectally (IR) inoculated with 10 MID₅₀ of cell free virus stocks of SIV_{sm} (strain SMM-3) isolated from a naturally infected sooty mangabey (70). The monkeys were monitored for general clinical status, virus isolation, viral load and CD4⁺ T-cell count and kept until development of AIDS, or if asymptomatic, until the end of the study period, when they were euthanized. When observing the rate of change of the CD4⁺ lymphocytes, values obtained before infection were set to 100% for each animal and following values were calculated in relation to these set-point values. The rate of change as percentage of the CD4⁺ lymphocyte population was fitted by linear regression analysis.

Monkeys were divided into three groups based on the rate of disease progression, CD4 decline, time of death and, whenever available, viral load (Table 2 and paper I). The three groups were named progressor (P), slow progressor (SP) and long-term non-progressor (LTNP). As expected, CD4 decline was more pronounced in the first three months of infection, while the decline of CD4⁺ T-cells was less thereafter. There was no difference in either the CD4 decline or viral load when comparing monkeys inoculated by different routes of infection. For the majority of animals the patterns of viral load was consistent with the observations by Ten Haaft et al. in that a threshold plasma virus load which was greater than 10⁵ RNA equivalents/ml of plasma 6 to 12 weeks after inoculation could predict a faster disease progression (236). The progressor monkeys showed the most evident decline in CD4⁺ T-cell count and all animals developed simian AIDS or AIDS-related symptoms. All progressor animals had to be euthanized within 27 months after infection due to disease symptoms and the median time to AIDS in this group was 18 months. Four out of five monkeys in the slow progressor group did not show disease symptoms during the study period (median 43 months), while one monkey developed lymphoma 47 months post infection. The other monkeys, although without signs of disease, showed a higher rate of CD4 cell decline (Table 2) and had a higher virus isolation frequency (median 75%) than the monkeys in the LTNP group. With one exception, the monkeys in the LTNP group did not show any symptoms of disease over the observation period of 34 to 60 months. Overall, relative number of CD4⁺ cells in this group remained for a long time close to the CD4 values before infection. Virus isolation from LTNPs was unsuccessful at many time points and virus isolation frequencies varied between 10 and 72 % for individual monkeys.

Monkey	Group ^a	Route of inoculation ^b	End of study		CD4+ T-cell decline ^d	Viral load 8-12 weeks post-infection ^e
			Disease ^c	Time (months)		
D24	P	IR	SaIDS	12	-8.1	1.1×10^6
C87	P	IR	SaIDS	12	-7.4	9.9×10^5
D23	P	IR	SaIDS	12	-7.0	9.5×10^4
B174	P	IV	SaIDS	15	-5.3	ND
C83	P	IR	SaIDS	15	-4.5	ND
D27	P	IR	SaIDS	18	-4.5	ND
C45	P	IR	SaIDS	24	-3.9	4.4×10^5 *
C2	P	IR	SaIDS	15	-3.6	2.1×10^4 *
C73	P	IR	SaIDS	18	-3.3	ND
C38	P	IR	lymphoma, SaIDS	24	-3.3	2.1×10^5
C37	P	IR	SaIDS	15	-3.3	7.5×10^5 *
C86	P	IR	SaIDS	24	-3.1	7.8×10^4 *
D26	P	IR	SaIDS	18	-2.9	ND
C27	P	IV	weight loss, diarrhea	18	-2.8	5.5×10^2 *
C26	P	IV	SaIDS	21	-2.7	1.2×10^5
C39	P	IR	lymphoma, SaIDS	18	-2.6	7.0×10^4 (1.4×10^5 , $<1.0 \times 10^2$)
56-3	SP	IV	none	38	-1.9	1.1×10^3 *
C20	P	IV	SaIDS	18	-1.8	$<1.0 \times 10^2$ *
C24	SP	IV	none	38	-1.0	ND
C68	SP	IR	none	53	-1.0	ND
C54	SP	IR	lymphoma, SaIDS	47	-0.9	1.7×10^3 *
59-3	SP	IV	none	36	-0.9	ND
B173	LTNP	IV	none	39	-0.8	ND
C44	P	IR	SaIDS	27	-0.7	4.6×10^6 (9.1×10^6 ; 3.4×10^3)
C82	LTNP	IR	none	35	-0.5	ND
C93	LTNP	IR	none	50	-0.4	3.5×10^3 *
D25	LTNP	IR	none	39	-0.3	$<1.0 \times 10^2$ *
C1	LTNP	IR	lymphopenia, weight loss	60	-0.2	ND
C35	LTNP	IR	none	34	-0.04	ND
D28	LTNP	IR	none	39	0.5	1.6×10^3 *

Foot notes to Table 2:

^a P, progressors; SP, slow progressors; LTNP, long-term nonprogressors.

^b IR, intrarectal; IV, intravenous.

^c Symptoms of disease at the end of study; sAIDS, simian AIDS.

^d CD4+ cell decline is presented as regression coefficient of linear regression analysis, taking into account 10-27 determinations per monkey.

^e Viral load was measured at 8 and 12 weeks post infection for the majority of monkeys, values are expressed as means. Monkeys indicated with * denote measurements only at one occasion. For monkeys C39 and C44 values at 8 and 12 weeks were at high variance and therefore both values are indicated in parenthesis. For the majority of cases the pattern of viral load was consistent with the previous observation that a threshold plasma virus load greater than 10^5 RNA equivalents/ml of plasma 6 to 12 weeks after inoculation could predict a faster disease progression (236). ND, not determined.

Virus isolates

Two to four isolates were available from each monkey, depending on virus isolation frequency and the length of the monkey's survival time. Virus isolation was performed by cocultivation of peripheral cynomolgus macaque PBMC (mPBMC) with mPBMC or with human PBMC (hPBMC) stimulated by phytohaemagglutinin (PHA-P) (165). The first isolate was usually obtained as early as two weeks post infection, the second isolate analyzed was obtained by three or four months, the third isolate was chosen at a time in between the second and the last isolate and the last (fourth) isolate was collected by the time of euthanization.

Serum

Sera from 16 animals, collected prior to infection, two weeks, one, two, and three months after infection, was available to study the kinetics of mounting a neutralizing antibody response. A late serum obtained shortly before the end of the study was also used from each monkey. The positive control serum (H55:16) was obtained from an infected monkey that remained asymptomatic and had high neutralizing titers towards SIVsm. Monkey H55 was one of four monkeys that remained healthy in an earlier study on disease progression of 33 cynomolgus macaques infected with SIVsm (185). All sera were heat inactivated (30 min at 56°C) to remove complement activity before tested in the neutralization assay.

Characterization of coreceptor use

Coreceptor use was determined using the human osteosarcoma cell line GHOST(3) and the human glioma cell line U87.CD4. The cell lines have been engineered to stably express the

CD4 receptor alone or together with one of the chemokine receptors CCR3, CCR5, CXCR4, CXCR6 or the orphan receptor gpr15 (46, 161). Chimera between CCR5 and CXCR4 previously described and expressed in U87.CD4 cells were used to characterize the mode of CCR5 use (5). GHOST(3) cell lines are known to express a low but detectable level of endogenous CXCR4, therefore the CXCR4-antagonist AMD3100 was included in a set of experiments. Infection of GHOST cells was analyzed by FACS analysis since the GHOST(3) cells have been modified to express green fluorescent protein upon infection by HIV and SIV. Microscopic read-out of syncytia formation was used to analyze infection of U87.CD4 cells. Infection in both assays was confirmed by ELISA for p26 antigen production (239).

Infection of macrophages and PBMC

Infection of PBMC from seronegative human or cynomolgus macaque blood donors, stimulated for four days with PHA-P, was analyzed seven days after infection. Monocyte derived macrophages (MDM) were obtained by plastic adherence of PBMC for five or six days in RPMI medium with 10% human serum and 20% fetal bovine serum (FBS) as previously described (247). The cells were then rinsed extensively to remove non-adherent cells and infected one or two days later. Virus production was analyzed at day 1, 3, 7, 11 and 15 after infection. Cavid HS-kit Lenti RT (Cavidi Tech, Sweden) was used to evaluate productive infection by the amount of active reverse transcriptase (RT) in cell culture supernatants of both PBMC and MDM.

Virus titrations and neutralization assay on GHOST(3) cells

We adopted a neutralization assay based on plaque reduction in the GHOST(3) cell line from the previously published U87.CD4 plaque reduction assay (217). The GHOST(3) cell line-based plaque assay is a single cycle infectivity assay for HIV and SIV, where GFP expression is used for read-out (168) and paper II). The assay was adapted to 96-well microtiter plates and the readout was optimally performed by fluorescence microscopy. Infected single cells or syncytia appearing as distinct fluorescent plaques were counted and infectious virus titers were expressed as plaque-forming units (PFU). The neutralizing property of a serum was determined by its ability to reduce the number of plaques as compared to infection without serum. The intra-assay variation with microscopic reading showed good reproducibility, with a standard deviation of 9.9%. The GHOST(3) plaque assay was also adopted for reading by

flow cytometry (168), however, in this case the virus dose had to be at least five times higher and therefore we routinely used microscopic evaluations.

CD4-independent infections

NP-2 cells expressing CD4 and CCR5 or CCR5 alone were used to determine if the isolates could establish infection independently of CD4. NP-2 is human glioma cell line and has been engineered to stably express human CD4 and/or human CCR5 (139, 221, 228). Infection of NP-2 cells was measured by amount of reverse transcriptase in cell culture supernatants. In addition, infection of NP-2 cells expressing only CCR5 was analyzed by four other means. The first method was coculture of infected NP-2/CCR5 cells with PBMC, starting one week after infection. Additional methods to evaluate infections were to infect PBMC with cell culture supernatants or cell lysates from infected NP-2/CCR5 cells. Infection was also confirmed with PCR for proviral *gag* (249).

RESULTS AND DISCUSSION

Coreceptor use of SIVsm

Coreceptor use was studied in SIVsm reisolates from eighteen progressor monkeys, five slow progressor animals and seven LTNP animals (paper I). The inoculum virus SIVsm strain SMM-3 used CCR5, CXCR6 and gpr15 for entry. Human PBMC (hPBMC) was used in isolations of 105 of the virus isolates and in eleven cases we also isolated viruses using macaque PBMC (mPBMC). CCR5 was the most common coreceptor, regardless of disease progression, used by 99 of the 105 reisolates obtained by virus isolation on hPBMC (Table 3). CXCR6 and gpr15 were commonly used in parallel with CCR5 by 63 and 59 hPBMC virus isolates, respectively. Interestingly, as many as 20 isolates derived by cocultivation with hPBMC could infect cells by the CXCR4 coreceptor. In earlier studies, CXCR4 use of SIV has rarely been encountered and was in at least two cases shown with virus isolated and/or passaged on hPBMC (170, 210, 255). Also, SIV adaptation to human cells has been described and was associated with a shorter form of gp41 (gp31) (90). Therefore it was important to compare viruses isolated on hPBMC and mPBMC, respectively. A comparison of exact matches of isolates derived on hPBMC and mPBMC showed that CCR5 and CXCR6 use was similar and CCR3 use was more frequently present than among hPBMC isolates, while gpr15 use was rare (paper I). The fact that, we could isolate CXCR4-using viruses on hPBMC but not on mPBMC, suggested that hPBMC may select for variants with CXCR4 use. Comparisons of IV-infected versus IR-infected monkeys could not link coreceptor use to route of transmission.

CCR5 has been shown to be the major coreceptor for SIV and is essential for cell entry in combination with CD4 (28, 55, 143). In addition, CXCR6 and gpr15 have been shown to be common coreceptors for SIV (2, 46, 61). Our results are in agreement with previous studies on sequential SIV isolates where CCR5 has been shown to be an important coreceptor for both early and late SIVsm and SIVmne variants (114, 255). *In vivo* studies have shown that gpr15 plays a minor role in pathogenicity (180) and this is further supported by the rare isolation of gpr15-using viruses on mPBMC in our material.

Table 3. Summary of coreceptor use of viruses isolated on hPBMC from 30 animals.

Group	Total number	Coreceptor use on cells expressing CD4						
		Weak ^a	Unknown ^b	CCR3	CXCR4	CCR5	CXCR6	gpr15
P – 18 animals								
early isolates ^c	36	0	3	5	6	36	24	23
late isolates ^c	30	1	0	1	5	28	15	17
SP – 5 animals								
early isolates	10	1	2	4	4	9	8	8
late isolates	10	0	2	2	2	10	7	5
LTNP – 7 animals								
early isolates	13	2	1	3	3	11	8	5
late isolates	6	1	0	0	0	5	1	1
Summary	105	5	8	15	20	99	63	59

^a Isolates gave indeterminate results on GHOST(3)-CCR5 cells.

^b Infection of the parental cell line and GHOST(3)-CXCR4 could not be inhibited by AMD3100, suggesting use of an unknown receptor.

^c Early isolates were obtained two weeks and three or four months post infection and late isolates were usually obtained at twelve months or later (by the time of euthanization).

Isolates from both mPBMC and hPBMC could infect GHOST(3)-parental and GHOST(3)-CXCR4 cells in the presence of the CXCR4-antagonist AMD3100 (paper I). Interestingly, this unknown way of infection of GHOST(3) cells was observed frequently with isolates obtained by cocultivation with mPBMC (six isolates out of eleven) but only with a minority of hPBMC isolates (eight isolates out of 105). Incomplete inhibition by AMD3100 of SIVsm infection of CXCR4-expressing GHOST(3) cells have previously been observed and was suggested to be the result of an additional unknown coreceptor also present on the GHOST(3) cells (255). We hypothesize that this unknown coreceptor may be similar to CXCR4, and that use of this receptor can promote evolution of SIV to CXCR4 use when replicating in human cells. Another possibility is that the interaction of the SIV envelope with CXCR4 is different from the interaction of the HIV-1 envelope and CXCR4 (170). Accordingly, when SIV replicates in hPBMC, there may be a selection towards interactions more similar to the HIV-1/CXCR4 interactions.

Replication capacity in macrophages and PBMC

Replication capacity in macrophages and PBMC was monitored with sequential isolates from all 30 animals. PBMC and MDM could be readily infected with reisolates from all animals (paper III). We infected macrophage and PBMC cultures of both human and macaque origin.

Only a few reports have evaluated the capacity of SIV isolates to infect human macrophages (18, 84, 179). Grimm et al. showed that 12 out of 16 SIV isolates from five different species could infect human macrophages (84). However, the capacity of the isolates to infect macrophages of primate origin was not tested. Our results indicate that viruses isolated on mPBMC or hPBMC have the same replication capacity in macrophages from macaque (mMDM) and in PBMC of both human and macaque origin. Performing the same experiment on macrophages from human blood donors (hMDM) showed that isolates obtained from mPBMC tended to replicate to lower levels relative to isolates obtained by cocultivation with hPBMC. However, the relative differences in replication capacity between isolates were the same. Based on these results and the limited availability of blood from macaques, we decided to work with cells of human origin.

The experiments involved macrophages from thirteen different blood donors and every isolate was tested on 2 to 4 donors in independent experiments (paper III). The M-tropic SIVmac251 isolate was included in all experiments as a positive control and productive infection of macrophage cultures with SIVmac251 varied up to 22-fold among different blood donors at peak level (day 15 after infection). SIVsm strain SMM-3, the inoculum virus, was tested on four hMDM donors and replication was found to be similar to the SIVmac251 control. To account for the variation in virus replication between macrophages from different blood donors, RT production of infected macrophage cultures of interest was divided by the RT production of SIVmac251 infected macrophages at day 15. Peak replication for reisolates was the same as for SIVmac251.

Evolution of virus tropism in disease progression

Our results indicate that SIV evolves to a less pathogenic virus in LTNP monkeys using decreasing numbers of receptors and replicates less efficiently over time (paper I). Virus could usually be isolated at early time-points from LTNP monkeys, while isolation was unsuccessful later in infection in many of the LTNP animals, resulting in few late isolates from LTNP monkeys to be analyzed. Coreceptor use of isolates from LTNP monkeys was less promiscuous than isolates from progressors and slow progressors. In six out of seven cases the pattern of coreceptor use either narrowed and included use of lower number of coreceptors or was stable CCR5 use (Table 4). The same pattern was seen in three out of five slow

progressor animals, while a minority of progressor monkeys showed the narrowing pattern. On the contrary, progressors showed broadening of coreceptor use, stable use of several coreceptors or fluctuation between these different coreceptor usage patterns.

Table 4. Summary of the different patterns of evolution of coreceptor use in SIVsm infected macaques.

	Evolution of coreceptor use		
	Narrow ^a	Fluctuating ^b	Broad ^c
Progressors			
No CXCR4 use	6	3	1
Early CXCR4 use	1	2	0
Late CXCR4 use ^d	0	3	2
<i>Summary</i>	7	8	3
Slow progressors			
No CXCR4 use	2	0	1
Early CXCR4 use	1	0	0
Late CXCR4 use	0	0	1
<i>Summary</i>	3	0	2
LTNP			
No CXCR4 use	3	1	0
Early CXCR4 use	3	0	0
Late CXCR4 use	0	0	0
<i>Summary</i>	6	1	0

^a narrowed to include use of lower number of receptors or was stable CCR5 use

^b fluctuation between the different coreceptor usage patterns, changing from the capacity to use many coreceptors to use of less coreceptors and late isolates using several coreceptors again.

^c broadening of coreceptor use to include capacity to use several coreceptors or stable coreceptor use of more than two coreceptors.

^d Includes monkeys with early and late CXCR4 use

In spite of the rare detection of CXCR4-using SIV isolates reported, we could recover CXCR4-using viruses from 13 monkeys, when isolated on hPBMC (paper I). Two patterns of CXCR4 use were demonstrated. CXCR4 use either appeared early during the acute phase of infection and disappeared later or, CXCR4 use only appeared late in infection during immunodeficiency. While the first pattern has been encountered in an earlier work (255, 256), late appearance of CXCR4 use has not previously been demonstrated in SIV infection. In our SIVsm material altogether five out of 18 monkeys in the progressor group yielded CXCR4-using viruses. One animal (D24) evolved to a unique phenotype, X4X6, using CXCR4 as the major coreceptor and not at all CCR5. Viruses from three monkeys (C38, C86 and C87) showed evolution to include CXCR4 use, although use of CCR5 and CXCR6 was more efficient than CXCR4 use and one progressor monkey (C73) showed fluctuation in the evolution of CXCR4 use in that CXCR4-using virus was isolated early and late in infection, but not in between. When large populations of HIV-1 subtype B infected individuals are

considered, appearance of syncytium inducing virus (SI = CXCR4-using) has been estimated to involve 50% of AIDS cases (109, 213, 237, 238). Large variations between groups and also according to subtype have been reported. For example, in HIV-1 subtype C infections, the X4 phenotype occurred less frequently (17% of the AIDS cases) (34). In our study of evolution of SIV coreceptor use, CXCR4-using virus variants appeared in later stages of disease in 28% of the animals. Our results indicate that SIVsm evolves to acquire a broader receptor use in progressive disease to include CXCR4 use or use of an unknown CXCR4-like coreceptor. This suggests that evolution of coreceptor use in SIV infections may be more similar to HIV infections than previously anticipated.

A broad use of coreceptors could be linked to a more efficient replication in macrophages (Paper III). Faster disease progression in SIVsm infected monkeys was associated with maintained or fluctuating macrophage tropism of sequential isolates (Table 5). The capacity of isolates from progressor monkeys to replicate well in macrophages was in agreement with the replicative capacity in hPBMC (Table 6). In contrast, isolates from LTNP monkeys became less fit with decreasing replicative capacity in macrophages and to some extent in hPBMC. It must be remembered that the limited fitness of isolates from LTNP monkeys was also reflected by unsuccessful virus isolations. There was a significant difference in the evolution of the replicative capacity in macrophages between LTNP monkeys and monkeys with a more progressive disease (P+SP versus LTNP, $p=0.0253$, Fischer's exact test). In hPBMC there was no apparent correlation between progressors and LTNP monkeys (P+SP versus LTNP, $p=0.1432$, Fischer's exact test), since the isolates from LTNP monkeys replicated better in PBMC than in macrophages.

Table 5. Evolution of replication capacity in hMDM in experimentally SIVsm infected macaques^a.

	Pattern of evolution ^b		
	Decreased	Fluctuating	Increased or no change
Progressors (n=18)	7	4	7
Slow progressors (n=5)	1	1	3
Long-term non-progressors (n=7)	6	1	0

^a Fisher's exact test was used to analyze disease progression in relation to macrophage tropism, by comparing the number of monkeys in the two categories (P+SP versus LTNP) for decrease versus increase + fluctuation in macrophage tropism over time ($p=0.0253$).

^b Pattern of evolution: **Increase or no change**, productive infection was higher or equal to that of SIVmac251 or no change of productive infection over time of sequentially collected isolates; **Decrease**, productive infection decreased over-time relative to SIVmac251; **Fluctuating**, increase or decrease between two virus isolate time-points.

Table 6. Evolution of replication capacity in hPBMC in experimentally SIVsm infected macaques^a.

	Pattern of evolution ^b		
	Decreased	Fluctuating	Increased or no change
Progressors (n=18)	4	4	10
Slow progressors (n=5)	2	1	2
Long-term non-progressors (n=7)	4	0	3

^a Fisher's exact test on disease progression in relation to replication in PBMC ($p=0.1432$), see also table 4,

^b Pattern of evolution, see table 4.

Over the last years macrophages have been recognized as important viral reservoirs for HIV-1 throughout the chronic infection (235). Recent studies showed that macrophage tropism of HIV-1 isolates was enhanced if derived from patients with advanced immunodeficiency (136, 243). Our results from a large cohort of infected macaques further support the notion that the capacity to replicate in macrophages is important to establish a progressive disease in the host. For many years it was assumed that shortly after seroconversion, HIV-1 variants able to readily infect macrophages dominate, while with progression to AIDS HIV-1 loses the capacity to infect macrophages (212). Similar results were obtained in the macaque model with the molecular clone SIVMneCL8 (201). Late variants reisolated from the monkeys were more cytopathic in human T cell lines, and less efficient in infecting macrophages compared to the early reisolates and the inoculum virus. However, as early as 1992, Mori et al. showed that even if a non-macrophage tropic molecular clone, SIVmac239, was inoculated into macaques, pathogenesis was associated with emergence of macrophage tropic virus (159). Similarly, in rhesus monkeys inoculated with a highly pathogenic SIV/HIV-1 chimera (SHIV), rapid depletion of CD4⁺ T cells was followed by the emergence of macrophage tropic viruses (99, 100). This implies that tissue macrophages are important reservoir of virus *in vivo*. SHIV infection is followed by increase in number of infected macrophages during the pathogenic process providing a high viral burden. Consequently, early suppression of viruses could lead to long-term non-progression. Indeed, our results demonstrate that suppression of the replicative capacity of SIVsm in macaques is related to a delayed disease progression and to failure of virus isolation.

Kinetics of the neutralizing antibody response in the infected host

Evolution of the neutralization response was evaluated in 16 monkeys: ten progressors; two slow progressors; and four LTNPs. All monkeys developed a neutralization response to the inoculum virus (paper II). There was no pathogenesis-related difference in the efficacy of

antibody response to the inoculum virus. One and two months after infection, a difference in the capacity to neutralize the inoculum virus was observed between IV-infected monkeys (six animals) and IR-infected monkeys (ten animals) (Figure 6). The IV-infected monkeys had a significantly earlier neutralization response than the IR-infected monkeys ($p=0.002$ and $p=0.022$, respectively, Mann-Whitney test). Later at three months after infection the IR-infected animals had reached the same levels of neutralization to the inoculum virus. Likewise, others have shown that neutralizing antibodies could be detected at seven weeks after infection in IV-inoculated macaques when using SIVsmE660 as challenge virus, while, at this time, the IR-inoculated macaques did not have detectable neutralizing antibodies (202). In HIV-1 infection, antibody responses to HIV-1 antigens are higher in HIV-1 positive injecting drug users than in HIV-1 positive homosexual men (103). In addition, comparisons of disease progression in HIV-1 infection between injecting drug users and homosexual men have shown that homosexual men had a significantly accelerated progression rate, when adjusting for non-AIDS mortality in the drug user group (174). It is tempting to speculate that these results reflect differences in antigen presentation. The intravenous route may lead to a sooner transport to lymph nodes, followed by an early presentation to B cells, than what occurs after infection by the intrarectal route. Alternatively, the capacity or response of B cells or T-helper cells may differ according to the compartment of virus entry.

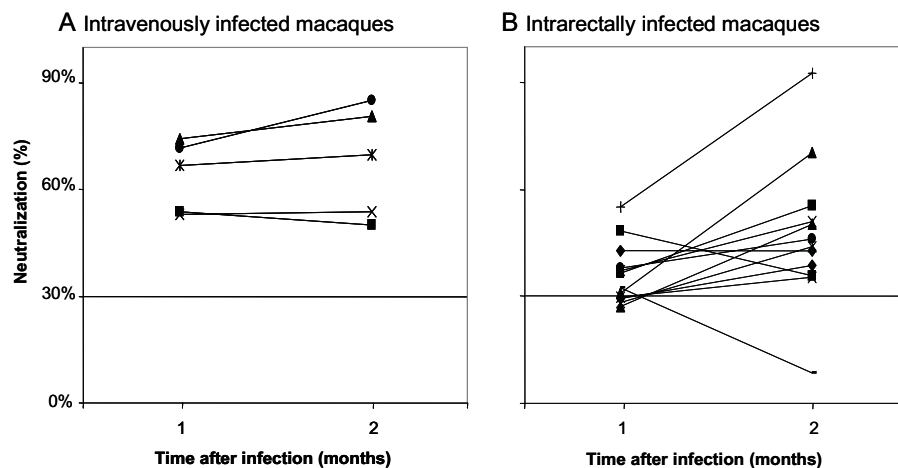


Figure 6. The early kinetics of the neutralization response to the inoculum SIVsm strain SMM-3 with serum from IV-infected macaques (A) or IR-infected macaques (B). Each line represents individual macaques. Values are means of two independent neutralization assays.

Evolution of neutralization resistant virus variants

Neutralization resistant isolates could be recovered from both IV- and IR-inoculated monkeys already at three or four months after infection (paper II). Notably, six out of ten progressor monkeys escaped neutralization by autologous sera, while neutralization resistant variants could not be observed at this early time-point for the SP and LTNP group of animals. Isolates obtained late in infection were all more or less resistant to neutralization with early sera.

Our results suggest a role for neutralizing antibodies in controlling viremia. When the capacity of serum to neutralize autologous virus isolated three months post-infection was analyzed in relation to viral load, two progressor monkeys (C39 and C44) had low viral loads at early time points and had no neutralization escape variants at three months after infection. Progressor animal C27, also with a latency to develop neutralization resistant virus had low viral load throughout the study, similar to the LTNP animals. However, in most cases control was transient and was overridden by the emergence of neutralization resistant variants.

Can heterologous sera neutralize virus?

Our results indicate that the viruses resistant to autologous neutralizing activity were also resistant to neutralization in the heterologous reaction. All viruses were tested with a high-titer neutralizing serum from a LTNP monkey (H55:16, paper II). This serum neutralized the inoculum virus and virus obtained two weeks after infection to over 80%. However, the 3- or 4-month isolates from the IV-infected progressor monkeys (four animals) were completely or partially resistant to neutralization with this high-titer neutralizing serum, while the IR-infected progressor monkeys (six animals) did not show the same pattern. The differences between neutralization of 2-week and 3- or 4-month isolates was significantly larger for IV infected than IR-infected progressors ($p=0.001$, Mann-Whitney test). All late isolates, except one obtained from a LTNP monkey, were highly resistant to neutralization. The results indicate that a strong neutralization response, as the response from IV-infected monkeys with an earlier neutralization response to the inoculum virus, may select for neutralization resistant variants. Moreover, an early and effective humoral immune response may not serve as the sole explanation for the differences in progression rate between injecting drug users and homosexual men in HIV-1 infection, as discussed above(174).

Heterologous neutralization was also tested in a checkerboard fashion with sera and virus isolates from the four progressor monkeys (B174, D23, C39 and C44). The virus isolates were chosen to represent two monkeys with neutralization escape variants at three months after infection and two monkeys with no detectable escape variants at three months. Similar to the autologous reaction, virus isolates from monkeys C39 and C44 were sensitive to neutralization with the heterologous sera. The 3-month viruses from monkeys B174 and D23 were neutralization resistant, however these two monkeys elicited broadly neutralizing antibodies that could neutralize 3- or 4-months virus isolates from monkeys C39 and C44. These results indicate that monkeys B174 and D23 do have an efficient neutralization response but that the viruses evolve to escape neutralization.

To further analyze the heterologous reaction, virus isolates from the same four progressor monkeys (B174, D23, C39 and C44) were tested for neutralization with a pool of late sera from LTNP progressor monkeys (B173, C82, C93 and D28). Interestingly the pooled sera could control the three or four months isolates from all four monkeys with neutralization close to or more than 90%. The results indicate that a serum pool, presumably containing a spectrum of neutralizing antibodies, is more potently neutralizing than one serum from a single animal. Neutralization resistance can thus be controlled by a reagent with broad neutralizing capacity.

CD4-independence of SIVsm reisolates

Sequential reisolates from 13 monkeys with different disease progression were monitored for CD4-independent use of CCR5. The majority of CCR5-using isolates were capable of CD4-independent infection in NP-2/CCR5 cells, however CD4-independent infection was generally not apparent as measured by reverse transcriptase in supernatants or syncytia formation (paper IV and Table 7). Infection of NP-2 cells expressing CCR5 but not CD4 with viruses obtained by cocultivation with mPBMC was in most cases more efficient than virus isolated on hPBMC. The mPBMC isolates induced both syncytia and virus production in seven cases out of eleven, while only a few isolates obtained on hPBMC were able to induce syncytia or were positive for virus production after infection of NP-2/CCR5 cells. The more apparent infection of NP-2/CCR5 cells with isolates obtained on mPBMC indicates that SIV evolves to be more dependent on CD4 in human cells. However, cocultivation of infected NP-2/CCR5 cells with hPBMC revealed successful CD4-independent infection with 93 % of the

Table 7. Summary of infection of NP-2 cells with CCR5-using isolates.

	% of isolates positive for infection of NP-2/CCR5 cells			% of isolates positive after cocultivation of NP-2/CCR5 cells with hPBMC ^b
	Syncytia	RT ^a	both RT and syncytia	
P hPBMC isolates (n=24)	17	35	17	96
SP hPBMC isolates (n=8)	13	38	13	88
LTNP hPBMC isolates (n=12)	25	58	25	92
All hPBMC isolates (n=44)	18	43	19	93
mPBMC isolates (n=11)	64 ^c	64 ^c	64 ^c	100

^a Virus production in supernatants as measured reverse transcriptase activity.

^b Measured by production of reverse transcriptase activity in supernatants of cocultures.

^c A portion of the isolates were infected with low virus doses that may have influenced the outcome of syncytia formation and productive infection

Table 8. Summary of infection of hPBMC with lysates and supernatants of NP-2/CCR5 cultures.

	% of isolates positive for infection of NP-2/CCR5 cells			% of isolates positive after infection of hPBMC with ^b	
	Syncytia	RT ^a	DNA-PCR ^c	NP-2/CCR5 lysates	NP-2/CCR5 supernatants
hPBMC isolates (n=19)	16	32	89	95	100
mPMC isolates (n=6)	50	50	100	83	67

^a and ^b see Table 6.

^c Nested PCR was performed on DNA from infected NP-2/CCR5 cells with *gag* specific primers.

hPBMC isolates and all of the mPBMC isolates. In addition, a nested PCR performed on DNA from infected NP-2/CCR5 cells with *gag*-specific primers confirmed presence of provirus (Table 8). Infection of hPBMC with cell lysates or cell culture supernatants from infected NP-2/CCR5 cells revealed both intracellular and extracellular virus production.

Intracellular virus production has previously been observed in infected macrophages (175, 190). Virus particles were observed within intra-cytoplasmic vesicles with characteristic multivesicular bodies known as late endosomes or major histocompatibility complex class II compartments. Sharova et al. demonstrated that virus may assemble and bud intracellularly into endosomal compartments in primary macrophages and that the viruses retain infectivity for at least 6 weeks (215). Macrophages may be infected in a CD4-independent manner since these cells express low levels of CD4 (9). It is possible that transfer of virus after intracellular budding to uninfected cells occurs through an infectious synapse, similar to what has been observed between dendritic cells and T cells (73, 151). Viruses transferred by the infectious

synapse are poorly accessible to neutralizing antibodies (73). Accordingly, this may be a means for the virus to transfer to uninfected cells and infect cells without encounter of the immune system. It is also important to note that CD4-independent infection may greatly enhance the number of cells that are permissive to infection.

Does mode of CCR5-use explain CD4-independence of SIVsm isolates?

Mode of CCR5 use was evaluated in U87.CD4 cell lines expressing chimeric receptors constructed of CCR5 and CXCR4 (5). In the present experiments we used three chimeras (FC-1, FC-2 and FC-4b), where CCR5 had been exchanged gradually, beginning with the N-terminal, for corresponding parts of the CXCR4 molecule. Our results showed that the FC-1 receptor was frequently used by SIVsm (93% of the hPBMC reisolates) and FC-2 and FC-4b were also used by a high number of isolates (Paper IV). However use of FC-2 and FC-4b was rarely as effective as FC-1 use. The majority of the hPBMC reisolates (29 out of 45) and most of the mPBMC reisolates (8 out of 11) could use all three chimeric receptors. It seems that the ability to use different variants, especially FC-1, of the CCR5 receptor may predispose the SIVsm isolates (tested in paper IV) for CD4-independence. This is in contrast to a previous report where it was shown that the N-terminal of CCR5 is the critical domain for CD4-independent entry of SIVsm envelope clones (56). The N-terminal of CCR5 was also sufficient for entry of the M-tropic strain SIVmac316 and, by contrast, the strains SIVmac239 and SIVmac251 required the presence of the second extracellular loop of CCR5 (55). In our hands, the N-terminal of CCR5 was usually not needed for infection. However, we could not estimate the importance of the CCR5 second extracellular loop since this loop was expressed by all of the chimeric receptors tested in our present study.

HIV-1 primary isolates with R5 phenotype use FC-2 more often than FC-1. In fact FC-1 appeared to be the most restrictively used chimeric receptor by HIV-1 isolates (110, 111). The results indicate that SIVsm uses CCR5 in a different mode than HIV-1. We decided to explore whether potential effects of the mode of CCR5 use and CD4-independence could be found in HIV-1 and HIV-2 isolates. For this purpose we chose 15 primary HIV-1 isolates previously tested for coreceptor use and mode of CCR5 use (110, 111) and seven primary HIV-2 isolates previously tested for coreceptor use (163). Ability to infect FC-1 was observed in six out of 15 of the HIV-1 isolates and in five out of seven HIV-2 isolates (Table 8). Interestingly, one HIV-1 primary isolate (1023:6761) could infect cells independent of CD4 as revealed by

cocultivation of NP-2/CCR5 with hPBMC. This isolate also induced the highest number of syncytia in FC-1 among all HIV-1 isolates tested. CD4-independence of CCR5-using HIV-2 isolates was less prominent still, in four cases out of five these isolates efficiently used the FC-1 chimera.

Table 8. HIV-1 and HIV-2 infection of U87.CD4 expressing wild type or chimeric receptors compared to CD4-independent infection of NP-2/CCR5 cells.

Isolate	U87.CD4					NP-2/CCR5	
	Syncytia or RT ^a					Syncytia or RT ^a	Cocultivation with hPBMC ^b (RT pg/ml)
	CCR5	CXCR4	FC-1	FC-2	FC-4b		
HIV-1 1023:6761	+++	++	++	++	++	-	>1000
HIV-1 1276:962	+++	-	-	-	-	-	0
HIV-1 1276:3514	+++	-	-	++	-	-	0
HIV-1 1276:8112	+++	-	-	++	-	-	0
HIV-1 2242:477	+++	-	-	-	-	-	0
HIV-1 2242:1886	++++	-	+	++	-	-	0
HIV-1 2242:3700	++++	-	-	+++	-	-	0
HIV-1 2242:4874	++++	++++	+	+	++++	-	0
HIV-1 2112:171	++++	-	-	-	-	-	0
HIV-1 2112:2574	++++	-	-	+++	+/-	-	0
HIV-1 2112:3502	++++	-	+/-	+++	+	-	0
HIV-1 2112:5309	++++	++	+/-	++	+	-	0
HIV-1 958:5229	+++	-	-	-	-	-	0
HIV-1 958:6789	++++	-	-	-	+/-	-	0
HIV-1 958:8567	+++++	++++	+	++	++++	-	0
HIV-2 1010	+	+++	+	++	++++	-	0
HIV-2 6669	-	+++	-	+	+++	-	0
HIV-2 1808	+++	-	++	+	+/-	-	194
HIV-2 1812	+++	-	++	+++	+	-	86
HIV-2 1816	++++	-	++	+/-	+/-	-	93
HIV-2 1654	+++	-	-	+	-	-	94
HIV-2 1682	+++	+/-	++	+	+/-	+/-	>1000

^a Induction of syncytia was observed in light microscope. Supernatant culture fluids were collected at day 7 and analyzed for production of RT. -, no syncytia; +/-, <10 syncytia per well or RT positive; +, 10-20 syncytia per well; ++, syncytia covering 20-50% of the wells; +++, syncytia covering 50-90% of the wells; +++++, syncytia covering >90% of the wells.

^b Virus production was by measured reverse transcriptase activity six days after start of cocultures with hPBMC. Supernatants were tested undiluted in the RT assay and therefore values above 1000 pg RT/ml cannot be separated.

HIV-2 isolate 6669 does not use CCR5 and as expected could not infect NP-2/CCR5 cells. CXCR4 use of HIV-2 isolate 1010 was more efficient than CCR5 use and this might explain why we did not observe infection NP-2/CCR5 cells. The results allow us the suggestion that mode of CCR5 use may influence CD4-independence. FC-1 and FC-2 differ in the first transmembrane region where FC-1 is CCR5, while FC-2 is CXCR4. The capacity to replicate in FC-1 and FC-2 indicates two different interactions with the CCR5 receptor, one that is used by isolates with an envelope conformation that allows CD4-independence and another conformation that is strictly CD4-dependent. In addition, the rare observations of CD4-independent HIV-1 isolates and the inefficient CD4-independent HIV-2 isolates indicate that HIV has evolved to be dependent on CD4 in humans. This is also supported by our notion that a majority of SIV isolates is CD4-independent and that isolation of SIV on human cells selects for more CD4-dependent SIV variants (Table 7 and 8).

Is there a correlation between macrophage tropism, neutralization sensitivity and CD4-independence?

Neutralization sensitivity of SIVmac envelopes has been correlated to CD4-independent entry into target cells as well as to enhanced macrophage tropism (153, 184). In addition, neutralization sensitive laboratory adapted HIV-1 viruses have been shown to have a stable exposure of the coreceptor binding site (93) and are capable of CD4-independent infections (52, 93, 95, 127). We chose to separate the P+SP and the LTNP groups when comparing CD4-independence and neutralization sensitivity. Neutralization sensitivity in the LTNP group correlated with a higher ability of CD4-independent use of CCR5, estimated by RT production in cocultures of NP-2/CCR5 with hPBMC (paper IV). No such correlation was found for isolates from progressor and slow progressor monkeys. It is tempting to speculate that in LTNP monkeys the overall immunity is more potently controlling the virus. However, since we did not find any correlations between CD4-independence and neutralization sensitivity in the P and SP groups it is less likely that our CD4-independent SIVsm isolates have an open envelope structure that may be exposed to neutralizing antibodies.

In our material, neutralization sensitivity did not correlate to replication capacity in MDM or PBMC (paper III). We further compared CD4-independent use of CCR5 and macrophage tropism separately in the two groups (P + SP and LTNP). There was no correlation between macrophage tropism and CD4-independence in the progressor and slow progressor monkeys

(paper IV). Virus from LTNP monkeys showed a trend towards a correlation between CD4-independent use of CCR5 (as estimated from cocultures of NP-2/CCR5 cells with hPBMC) and relative replication capacity in macrophages. Nevertheless, even if we did not find any clear correlations between macrophage tropism and CD4-independence it must be remembered that hMDMs could be readily infected with reisolates from all animals and the majority of these isolates were CD4-independent. Therefore our results are in line with those suggesting that less dependence on CD4 of SIV and HIV leads to an increased ability to utilize relatively low levels of CD4 and CCR5 expressed on macrophages (9, 80, 153, 184). However, it appears that there is no clear relationship between disease progression and efficacy of CD4-independent infections, indicating that CD4-independence is a means to broaden the tissue tropism, but do not necessarily make the virus more pathogenic.

CONCLUSIONS

CCR5, CXCR6 and gpr15 are common receptors used by primary SIVsm isolates. Isolates, especially viruses obtained by cocultivation on macaque PBMC, may use an unknown receptor expressed on the GHOST(3) cells. Alternatively they may use CXCR4, endogenously expressed and present on GHOST(3) cells, in a way that cannot be inhibited by the CXCR4-antagonist AMD3100. Furthermore, CXCR4-using SIVsm, sensitive to AMD3100, may be isolated on human PBMC, but not when isolating virus on macaque PBMC suggesting that human and monkey cells select for different virus variants. In addition, human cells may also select for more CD4-dependent virus variants, indicated by the fact that viruses isolated on mPBMC were in most cases more efficient in infecting NP-2 cells expressing CCR5 but not CD4 than viruses isolated on hPBMC. Comparisons of SIV and HIV-1, showed that the differences in mode of CCR5 use may be explained by the ability of SIVsm to use CCR5 independently of CD4 for infection. CD4-independent infections seem to result in both intracellular and extracellular virus production. Intracellular virus production may serve as an additional way for the virus to evade the immune system.

All animals mounted a neutralizing antibody response to the inoculum virus. However, neutralizing antibodies appeared earlier in IV-infected animals than in IR-infected animals. This early humoral immune response might have induced faster neutralization resistance in the IV-infected progressor animals than in IR-infected animals, as evidenced by resistance to a heterologous high titre serum

Evolution of virus occurred in the infected macaques. The majority of sequential isolates from progressor monkeys gradually used more coreceptors, or showed stable use of multiple coreceptors or fluctuation between the different coreceptor usage patterns. In addition, in five animals out of 18, late isolates included CXCR4 use. Late isolates from progressor monkeys also maintained an effective replication capacity in macrophages and PBMC and evolved to escape neutralizing antibodies already at three months after infection. The majority of virus isolates from progressors used CCR5 independently of CD4.

The LTNP animals seemed to control the virus evolution. Virus became less fit as shown by decreased frequency of successful virus isolations, coreceptor use generally narrowed to stable CCR5 use and late virus from LTNP monkeys was also less capable for replication in

macrophages. In addition, virus evolution to neutralization escape was delayed. Furthermore, there was a significant correlation between a higher neutralization sensitivity and more efficient CD4-independent use of CCR5, estimated by RT production in cocultures of NP-2/CCR5 with hPBMC. Pooled sera from four LTNP monkeys showed a broad neutralizing capacity, including neutralization of escape variants. The results suggest an important role for neutralizing antibodies in controlling viremia. Although this control is transient in the infected host, neutralization resistance is relative and variant viruses may be neutralized by a broadly cross-neutralizing serum pool.

SAMMANFATTNING PÅ SVENSKA

Sedan 1981, då de första fallen av AIDS¹ beskrevs, har 25 miljoner människor dött på grund av att de har blivit smittade av immunbristviruset HIV². Det finns idag mer än 40 miljoner människor som lever med HIV eller AIDS. SIV³ är apslåktets variant på HIV och orsakar normalt ingen sjukdom hos sina värdjur som är olika afrikanska apor, men då asiatiska apor, t. ex. makaker, blir smittade av SIV utvecklar de en immunbristsjukdom som liknar AIDS hos människor. HIV tros ha uppstått genom att människor har jagat och ätit kött från afrikanska apor och att SIV har förändrats så att det kan smitta från människa till människa. SIV-infektion hos makaker används som en modell för att förstå HIV så man kan utveckla nya mediciner och förhoppningsvis vaccin. Det finns idag inga botemedel för sjukdomen, däremot finns det ett antal bromsmediciner som förlänger livslängden och minskar symptomen för HIV-smittade.

Virus består av en bit arvs massa (DNA eller RNA) som omges av ett proteinhölje och alla virus kräver levande celler och cellernas förmåga att kopiera arvs massa för att bli fler. När HIV har infekterat en cell kommer virusets arvs massa att infogas i cellens DNA och cellen kan aldrig bli fri från virus smittan igen. För att ta sig in i och infektera en cell använder virus speciella proteinstrukturer på cellens utsida, så kallade receptorer. Receptorer används normalt för kommunikation mellan celler och är viktiga för normala kropps funktioner. HIV angriper immunförsvarets celler som bär på speciella receptorer. För att ta sig in i en cell kräver HIV två receptorer. Man har sedan länge känt till att den primära receptorn är CD4, medan den andra receptorn, coreceptorn, kan variera mellan ett tiotal olika receptorer. Av dessa coreceptorer använder HIV oftast två, CCR5 eller CXCR4, medan SIV oftast använder CCR5 och mycket sällan CXCR4. Ett problem med virus, speciellt HIV, är att när de kopieras och blir fler sker ofta små misstag, så kallade mutationer. Dessa mutationer ger upphov till att det finns en mängd olika varianter av HIV som kan infektera människor. Mutationer gör att virus kan variera användandet av coreceptorer samt att virus kan undvika immunförsvaret eftersom de nya varianterna inte känns igen. Mutationerna orsakar även resistens mot bromsmediciner.

¹ AIDS = "förvärvat immunbristsyndrom" eller "acquired immunodeficiency syndrome"

² HIV = "humant immunbristvirus" eller "human immunodeficiency virus"

³ SIV = "apimmunbrist virus" eller "simian immunodeficiency virus"

I denna avhandling jämförde vi virus (så kallade virus-isolat) från 30 krabbnakaker (*Macaca fascicularis*) som hade infekterats experimentellt med SIV. Åtta av aporna var smittade intravenöst via blodet och 22 av aporna var infekterade intrarektalt via slemhinnor. Aporna utvecklade sjukdom olika snabbt, 18 apor insjuknande snabbt, fem apor långsammare medan sju apor förblev friska länge. Virus hade isolerats från dessa apor olika dagar efter infektionen och vi ville jämföra om infektionsvägen och sjukdomsförloppet spelade någon roll för vilka receptorer och celltyper som virusen utnyttjade. Vi ville även studera hur immunförsvarets antikroppar utvecklades för att hindra att viruset kunde infektera celler (det neutraliserande antikroppssvaret) och när, och till vilken grad, virus muteras för att undvika neutralisation. Våra studier kommer att ge ökade kunskaper om hur SIV och HIV fungerar och främja möjligheterna för utveckling av effektiv behandling och vaccin.

För att testa vilka celler och receptorer som var viktiga använde vi olika celltyper och mätte om cellerna kunde infekteras med de olika virus-isolaten. Vi kom fram till att det inte fanns någon skillnad mellan infektion via slemhinnor eller blod för infektion av olika celltyper eller på användandet av receptorer. Däremot fann vi att intravenöst smittade apor utvecklade neutraliserande antikroppar snabbare än intrarektalt smittade apor. Tyvärr gav detta inte något skydd mot sjukdom utan att det verkade snarare som om detta selekterade fram muterade virus som inte kunde kontrolleras av immunförsvaret. CCR5, CXCR6 och gpr15 var de mest använda coreceptorerna. Vi var förvånade över att kunna isolera virus som använde CXCR4. Det är möjligt att när vi odlade virus på humana celler så fick vi fram virus som använde CXCR4 och även virus som var mer beroende av CD4 receptorn. Allmänt var inte SIV-isolaten särskilt beroende av CD4-receptorn utan de kunde även infektera celler som bara hade CCR5 på sin cellyta. Vi fann ett troligt samband mellan hur SIV eller HIV kunde interagera med CCR5 receptorn och hur beroende dessa virus var av CD4-receptorn. HIV-1 är mycket beroende av CD4-receptorn och kanske har behovet att binda till CD4 har uppkommit hos människan. Att virus kan infektera celler som inte bär på CD4 gör att antalet målceller i en individ blir fler, och dessutom så verkar det som om nyproducerade viruspartiklar kan gömma sig från immunförsvaret inuti celler.

Vid jämförelser mellan olika sjukdomsförlopp fann vi intressanta skillnader. Virus från apor som blev snabbt sjuka bibehöll en effektiv replikationskapacitet i makrofager och i T-celler. Dessa virus kunde även efter hand som sjukdomen utvecklades infektera celler med hjälp av flera olika coreceptorer och dessutom så förändrades virusen redan efter tre månader till att

vara resistent mot neutraliserande antikroppar. Apor som förblev friska länge kunde däremot kontrollera virusen bättre. Virus från dessa apor var ofta svårt att isolera eller så var det svårt att få det att växa i olika cellförsök. Speciellt sena virus från dessa apor replikerade dåligt i makrofager och T-celler och använde oftast bara CCR5 som coreceptor. Utöver detta, så muterade inte virus för att fly undan neutraliserande antikroppar lika snabbt hos friska apor som hos sjuka apor. Detta visar att utvecklandet av antikroppar kan vara viktigt för att kontrollera sjukdomsförloppet, men att antikroppar också kan selektera för nya virus varianter.

ACKNOWLEDGEMENTS

I would like to take the opportunity to thank all of you who in one way or another have contributed to completing this work. Especially I would like to thank:

Eva Maria Fenyö, my supervisor, for your enthusiasm and never-ending support and who have taught me to think of obstacles as opportunities. I will always remember comments like: “Of course there is a pattern here” or “Negative results are also results”. Thank you, for your scientific guidance and the opportunity to be a member of your group.

Rigmor Thorstensson, my co-author. Even if never a co-supervisor on the paper, you have always been there to encourage me and to share your great knowledge. Thank you for sharing the unique material this thesis was based on.

Other co-authors in this thesis, thank you for important contributions to finalize this thesis.

My colleagues at Virology in Lund:

Mattias Mild, Johanna Repits, Marie Nilsson, Marianne Jansson, Birgitta Holmgren, Ingrid Karlsson, Salma Nowroozalizadeh, Joakim Esbjörnsson, Dalma Vödrös and Yu Shi, for a fun time together and lot of help in the lab.

The expert technicians in the lab: Monica Öberg and Elzbiata Vincic, without you this work would not have been possible.

Students who have participated in my projects over the years.

People at SMI and MTC, Stockholm:

Hélène Fredlund, Reinhold Benthin, Eva Olausson-Hansson, Andreas Mörner, Lena Vetterli and Kerstin Andreasson. Thank you for helping me when needed; with material, for searching for in those deep freezers, and taking care of me when I have been visiting Stockholm.

Gabriella Scarlatti and her students for nice collaborations.

The VIAV group for the opportunity to join a very innovative vaccine project.

My mentor, Einar Everitt, for introducing me to Virology and for fruitful discussions.
Liselotte Antonsson and Ulf Karlsson, and other colleagues in the house and at journal club,
and especially “onsdags-kak-gänget” for bringing joy to those Wednesdays!

All my friends for never giving up on me and for always being there. Frida and Janka for help with proofreading this thesis and nice discussions over a bottle of wine. Anki, Sofia, Kristina, and Magdalena: You go jungle-girls! Jessica, Louise and Elin: my great friends way back in time. The other NL girls and all the TL boys for all fun get-togethers. Alexandra and Flash and all my friends in the stable. And all others, you are too many to mention here, but no-one is forgotten!

The Laurén family: Bosse, Lena, Joakim, Carina, Anna, Josefin, Mårten, Thea, Ella and Malte for all the nice and fun times together.

My family, my parents Monica and Jan Nordqvist and my sister Marie for your great support and for always believing in me.

Ulrik, for your love and for sharing your life with me. For your unlimited patience and encouragement. I could never have done this without you!

REFERENCES

1. **Albert, J., B. Abrahamsson, K. Nagy, E. Aurelius, H. Gaines, G. Nystrom, and E. M. Fenyo.** 1990. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS* **4**:107-112.
2. **Alkhatib, G., F. Liao, E. A. Berger, J. M. Farber, and K. W. Peden.** 1997. A new SIV co-receptor, STRL33. *Nature* **388**:238.
3. **Allen, T. M., P. Jing, B. Calore, H. Horton, D. H. O'Connor, T. Hanke, M. Piekarczyk, R. Ruddersdorf, B. R. Mothe, C. Emerson, N. Wilson, J. D. Lifson, I. M. Belyakov, J. A. Berzofsky, C. Wang, D. B. Allison, D. C. Montefiori, R. C. Desrosiers, S. Wolinsky, K. J. Kunstman, J. D. Altman, A. Sette, A. J. McMichael, and D. I. Watkins.** 2002. Effects of cytotoxic T lymphocytes (CTL) directed against a single simian immunodeficiency virus (SIV) Gag CTL epitope on the course of SIVmac239 infection. *J Virol* **76**:10507-10511.
4. **Alter, H. J., J. W. Eichberg, H. Masur, W. C. Saxinger, R. Gallo, A. M. Macher, H. C. Lane, and A. S. Fauci.** 1984. Transmission of HTLV-III infection from human plasma to chimpanzees: an animal model for AIDS. *Science* **226**:549-552.
5. **Antonsson, L., A. Boketoff, 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**:2571-2579.
6. **Arendrup, M., C. Nielsen, J. E. Hansen, C. Pedersen, L. Mathiesen, and J. O. Nielsen.** 1992. Autologous HIV-1 neutralizing antibodies: emergence of neutralization-resistant escape virus and subsequent development of escape virus neutralizing antibodies. *J Acquir Immune Defic Syndr* **5**:303-307.
7. **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**:660-662.
8. **Baba, T. W., V. Liska, R. Hofmann-Lehmann, J. Vlasak, W. Xu, S. Aychunie, L. A. Cavacini, M. R. Posner, H. Katinger, G. Stiegler, B. J. Bernacky, T. A. Rizvi, R. Schmidt, L. R. Hill, M. E. Keeling, Y. Lu, J. E. Wright, T. C. Chou, and R. M. Ruprecht.** 2000. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. *Nat Med* **6**:200-206.
9. **Bannert, N., D. Schenten, S. Craig, and J. Sodroski.** 2000. The level of CD4 expression limits infection of primary rhesus monkey macrophages by a T-tropic simian immunodeficiency virus and macrophagetropic human immunodeficiency viruses. *J Virol* **74**:10984-10993.
10. **Barre-Sinoussi, F.** 2003. The early years of HIV research: integrating clinical and basic research. *9*:844-846.
11. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, 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**:868-871.
12. **Benveniste, R. E., W. R. Morton, E. A. Clark, C. C. Tsai, H. D. Ochs, J. M. Ward, L. Kuller, W. B. Knott, R. W. Hill, M. J. Gale, and et al.** 1988. Inoculation of baboons and macaques with simian immunodeficiency virus/Mne, a primate lentivirus closely related to human immunodeficiency virus type 2. *J Virol* **62**:2091-2101.
13. **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.
14. **Bjorling, E., G. Scarlatti, A. von Gegerfeldt, J. Albert, G. Biberfeld, F. Chiodi, E. Norrby, and E. M. Fenyo.** 1993. Autologous neutralizing antibodies prevail in HIV-2 but not in HIV-1 infection. *Virology* **193**:528-530.
15. **Bjorndal, A., H. Deng, M. Jansson, J. R. Fiore, C. Colognesi, A. Karlsson, J. Albert, G. Scarlatti, D. R. Littman, and E. M. Fenyo.** 1997. Coreceptor usage of primary human immunodeficiency virus type 1 isolates varies according to biological phenotype. *J Virol* **71**:7478-7487.
16. **Bleul, C. C., L. Wu, J. A. Hoxie, T. A. Springer, and C. R. Mackay.** 1997. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc Natl Acad Sci U S A* **94**:1925-1930.
17. **Bron, R., P. J. Klasse, D. Wilkinson, P. R. Clapham, A. Pelchen-Matthews, C. Power, T. N. Wells, J. Kim, S. C. Peiper, J. A. Hoxie, and M. Marsh.** 1997. Promiscuous use of CC and CXC chemokine

- receptors in cell-to-cell fusion mediated by a human immunodeficiency virus type 2 envelope protein. *J Virol* **71**:8405-8415.
18. **Broussard, S. R., S. I. Staprans, R. White, E. M. Whitehead, M. B. Feinberg, and J. S. Allan.** 2001. Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease. *J Virol* **75**:2262-2275.
 19. **Bukrinskaya, A. G.** 2004. HIV-1 assembly and maturation. *Arch Virol* **149**:1067-1082.
 20. **Burns, D. P., C. Collignon, and R. C. Desrosiers.** 1993. Simian immunodeficiency virus mutants resistant to serum neutralization arise during persistent infection of rhesus monkeys. *J Virol* **67**:4104-4113.
 21. **Cao, Y., L. Qin, L. Zhang, J. Safrin, and D. D. Ho.** 1995. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. *N Engl J Med* **332**:201-208.
 22. **Carrington, M., and R. E. Bontrop.** 2002. Effects of MHC class I on HIV/SIV disease in primates. *Aids* **16 Suppl 4**:S105-114.
 23. **Carrington, M., and S. J. O'Brien.** 2003. The influence of HLA genotype on AIDS. *Annu Rev Med* **54**:535-551.
 24. **Centers for Disease Control and Prevention.** 1992. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *Morbidity Mortal Wkly Rep* **41**:1-18.
 25. **Chakrabarti, L. A., S. R. Lewin, L. Zhang, A. Gettie, A. Luckay, L. N. Martin, E. Skulsky, D. D. Ho, C. Cheng-Mayer, and P. A. Marx.** 2000. Normal T-cell turnover in sooty mangabeys harboring active simian immunodeficiency virus infection. *J Virol* **74**:1209-1223.
 26. **Chen, B., E. M. Vogan, H. Gong, J. J. Skehel, D. C. Wiley, and S. C. Harrison.** 2005. Structure of an unliganded simian immunodeficiency virus gp120 core. *Nature* **433**:834-841.
 27. **Chen, Z., D. Kwon, Z. Jin, S. Monard, P. Telfer, M. S. Jones, C. Y. Lu, R. F. Aguilar, D. D. Ho, and P. A. Marx.** 1998. Natural infection of a homozygous delta24 CCR5 red-capped mangabey with an R2b-tropic simian immunodeficiency virus. *J Exp Med* **188**:2057-2065.
 28. **Chen, Z., P. Zhou, D. D. Ho, N. R. Landau, and P. A. Marx.** 1997. Genetically divergent strains of simian immunodeficiency virus use CCR5 as a coreceptor for entry. *J Virol* **71**:2705-2714.
 29. **Cheng-Mayer, C., R. Liu, N. R. Landau, and L. Stamatatos.** 1997. Macrophage tropism of human immunodeficiency virus type 1 and utilization of the CC-CKR5 coreceptor. *J Virol* **71**:1657-1661.
 30. **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**:80-82.
 31. **Choe, H., M. Farzan, M. Konkel, K. Martin, Y. Sun, L. Marcon, M. Cayabyab, M. Berman, M. E. Dorf, N. Gerard, C. Gerard, and J. Sodroski.** 1998. The orphan seven-transmembrane receptor apj supports the entry of primary T-cell-line-tropic and dualtropic human immunodeficiency virus type 1. *J Virol* **72**:6113-6118.
 32. **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**:1135-1148.
 33. **Chou, C. S., O. Ramilo, and E. S. Vitetta.** 1997. Highly purified CD25- resting T cells cannot be infected de novo with HIV-1. *Proc Natl Acad Sci U S A* **94**:1361-1365.
 34. **Cilliers, T., J. Nhlapo, M. Coetzer, D. Orlovic, T. Ketas, W. C. Olson, J. P. Moore, A. Trkola, and L. Morris.** 2003. The CCR5 and CXCR4 coreceptors are both used by human immunodeficiency virus type 1 primary isolates from subtype C. *J Virol* **77**:4449-4456.
 35. **Clapham, P. R., A. McKnight, and R. A. Weiss.** 1992. Human immunodeficiency virus type 2 infection and fusion of CD4-negative human cell lines: induction and enhancement by soluble CD4. *J Virol* **66**:3531-3537.
 36. **Clavel, F., D. Guetard, F. Brun-Vezinet, S. Chamaret, M. A. Rey, M. O. Santos-Ferreira, A. G. Laurent, C. Dauguet, C. Katlama, C. Rouzioux, and et al.** 1986. Isolation of a new human retrovirus from West African patients with AIDS. *Science* **233**:343-346.
 37. **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**:1811-1815.
 38. **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.
 39. **Connor, R. I., K. E. Sheridan, D. Ceradini, S. Choe, and N. R. Landau.** 1997. Change in coreceptor use correlates with disease progression in HIV-1--infected individuals. *J Exp Med* **185**:621-628.

40. **Curtis, B. M., S. Scharnowske, and A. J. Watson.** 1992. Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp120. *Proc Natl Acad Sci U S A* **89**:8356-8360.
41. **Dagleish, A. G., P. C. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312**:763-767.
42. **Daniel, M. D., N. L. Letvin, N. W. King, M. Kannagi, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers.** 1985. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* **228**:1201-1204.
43. **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**:1106-1115.
44. **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 *CCR5* structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* **273**:1856-1862.
45. **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**:661-666.
46. **Deng, H. K., D. Unutmaz, V. N. KewalRamani, and D. R. Littman.** 1997. Expression cloning of new receptors used by simian and human immunodeficiency viruses. *Nature* **388**:296-300.
47. **Derdeyn, C. A., and G. Silvestri.** 2005. Viral and host factors in the pathogenesis of HIV infection. *Curr Opin Immunol* **17**:366-373.
48. **Desrosiers, R. C.** 1990. HIV-1 origins. A finger on the missing link. *Nature* **345**:288-289.
49. **Desrosiers, R. C., A. Hansen-Moosa, K. Mori, D. P. Bouvier, N. W. King, M. D. Daniel, and D. J. Ringler.** 1991. Macrophage-tropic variants of SIV are associated with specific AIDS-related lesions but are not essential for the development of AIDS. *Am J Pathol* **139**:29-35.
50. **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 *CCR-5*, *CCR-3*, and *CCR-2b* as fusion cofactors. *Cell* **85**:1149-1158.
51. **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-CCR-5*. *Nature* **381**:667-673.
52. **Dumonceaux, J., S. Nisole, C. Chanel, L. Quivet, A. Amara, F. Baleux, P. Briand, and U. Hazan.** 1998. Spontaneous mutations in the *env* gene of the human immunodeficiency virus type 1 NDK isolate are associated with a CD4-independent entry phenotype. *J Virol* **72**:512-519.
53. **Eckstein, D. A., M. L. Penn, Y. D. Korin, D. D. Scripture-Adams, J. A. Zack, J. F. Kreisberg, M. Roederer, M. P. Sherman, P. S. Chin, and M. A. Goldsmith.** 2001. HIV-1 actively replicates in naive CD4(+) T cells residing within human lymphoid tissues. *Immunity* **15**:671-682.
54. **Eckstein, D. A., M. P. Sherman, M. L. Penn, P. S. Chin, C. M. De Noronha, W. C. Greene, and M. A. Goldsmith.** 2001. HIV-1 Vpr enhances viral burden by facilitating infection of tissue macrophages but not nondividing CD4+ T cells. *J Exp Med* **194**:1407-1419.
55. **Edinger, A. L., A. Amedee, K. Miller, B. J. Doranz, M. Endres, M. Sharron, M. Samson, Z. H. Lu, J. E. Clements, M. Murphey-Corb, S. C. Peiper, M. Parmentier, C. C. Broder, and R. W. Doms.** 1997. Differential utilization of *CCR5* by macrophage and T cell tropic simian immunodeficiency virus strains. *Proc Natl Acad Sci U S A* **94**:4005-4010.
56. **Edinger, A. L., C. Blanpain, K. J. Kunstman, S. M. Wolinsky, M. Parmentier, and R. W. Doms.** 1999. Functional dissection of *CCR5* coreceptor function through the use of CD4-independent simian immunodeficiency virus strains. *J Virol* **73**:4062-4073.
57. **Edinger, A. L., T. L. Hoffman, M. Sharron, B. Lee, Y. Yi, W. Choe, D. L. Kolson, B. Mitrovic, Y. Zhou, D. Faulds, R. G. Collman, J. Hesselgesser, R. Horuk, and R. W. Doms.** 1998. An orphan seven-transmembrane domain receptor expressed widely in the brain functions as a coreceptor for human immunodeficiency virus type 1 and simian immunodeficiency virus. *J Virol* **72**:7934-7940.
58. **Edinger, A. L., J. L. Mankowski, B. J. Doranz, B. J. Margulies, B. Lee, J. Rucker, M. Sharron, T. L. Hoffman, J. F. Berson, M. C. Zink, V. M. Hirsch, J. E. Clements, and R. W. Doms.** 1997. CD4-independent, *CCR5*-dependent infection of brain capillary endothelial cells by a neurovirulent simian immunodeficiency virus strain. *Proc Natl Acad Sci U S A* **94**:14742-14747.

59. **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**:359-362.
60. **Endres, M. J., P. R. Clapham, M. Marsh, M. Ahuja, J. D. Turner, A. McKnight, J. F. Thomas, B. Stoebenau-Haggarty, S. Choe, P. J. Vance, T. N. Wells, C. A. Power, S. S. Sutterwala, R. W. Doms, N. R. Landau, and J. A. Hoxie.** 1996. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. *Cell* **87**:745-756.
61. **Farzan, M., H. Choe, K. Martin, L. Marcon, W. Hofmann, G. Karlsson, Y. Sun, P. Barrett, N. Marchand, N. Sullivan, N. Gerard, C. Gerard, and J. Sodroski.** 1997. Two orphan seven-transmembrane segment receptors which are expressed in CD4-positive cells support simian immunodeficiency virus infection. *J Exp Med* **186**:405-411.
62. **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**:872-877.
63. **Fenyo, E. M., L. Morfeldt-Manson, F. Chiodi, B. Lind, A. von Gegerfelt, J. Albert, E. Olausson, and B. Asjo.** 1988. Distinct replicative and cytopathic characteristics of human immunodeficiency virus isolates. *J Virol* **62**:4414-4419.
64. **Fields, B. N., and D. V. Knipe.** 2001. *Fields Virology*, 4 ed, vol. 2. Lippincott Williams & Wilkins, Philadelphia.
65. **Fortgang, I. S., S. K. Srivastav, G. B. Baskin, P. M. Schumacher, and L. S. Levy.** 2004. Retrospective analysis of clinical and laboratory factors associated with lymphoma in simian AIDS. *Leuk Lymphoma* **45**:161-169.
66. **Fouchier, R. A., M. Groenink, N. A. Kootstra, M. Tersmette, H. G. Huisman, F. Miedema, and H. Schuitemaker.** 1992. Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. *J Virol* **66**:3183-3187.
67. **Fowke, K. R., R. Kaul, K. L. Rosenthal, J. Oyugi, J. Kimani, W. J. Rutherford, N. J. Nagelkerke, T. B. Ball, J. J. Bwayo, J. N. Simonsen, G. M. Shearer, and F. A. Plummer.** 2000. HIV-1-specific cellular immune responses among HIV-1-resistant sex workers. *Immunol Cell Biol* **78**:586-595.
68. **Francis, D. P., P. M. Feorino, J. R. Broderon, H. M. McClure, J. P. Getchell, C. R. McGrath, B. Swenson, J. S. McDougal, E. L. Palmer, A. K. Harrison, and et al.** 1984. Infection of chimpanzees with lymphadenopathy-associated virus. *Lancet* **2**:1276-1277.
69. **Fultz, P. N., D. C. Anderson, H. M. McClure, S. Dewhurst, and J. I. Mullins.** 1990. SIVsmm infection of macaque and mangabey monkeys: correlation between in vivo and in vitro properties of different isolates. *Dev Biol Stand* **72**:253-258.
70. **Fultz, P. N., H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, and A. Srinivasan.** 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercopithecus atys*). *Proc Natl Acad Sci U S A* **83**:5286-5290.
71. **Fultz, P. N., H. M. McClure, H. Daugharty, A. Brodie, C. R. McGrath, B. Swenson, and D. P. Francis.** 1986. Vaginal transmission of human immunodeficiency virus (HIV) to a chimpanzee. *J Infect Dis* **154**:896-900.
72. **Gajdusek, D. C., H. L. Amyx, C. J. Gibbs, Jr., D. M. Asher, P. Rodgers-Johnson, L. G. Epstein, P. S. Sarin, R. C. Gallo, A. Maluish, L. O. Arthur, and et al.** 1985. Infection of chimpanzees by human T-lymphotropic retroviruses in brain and other tissues from AIDS patients. *Lancet* **1**:55-56.
73. **Ganesh, L., K. Leung, K. Lore, R. Levin, A. Panet, O. Schwartz, R. A. Koup, and G. J. Nabel.** 2004. Infection of specific dendritic cells by CCR5-tropic human immunodeficiency virus type 1 promotes cell-mediated transmission of virus resistant to broadly neutralizing antibodies. *J Virol* **78**:11980-11987.
74. **Garcia, E., M. Pion, A. Pelchen-Matthews, L. Collinson, J. F. Arrighi, G. Blot, F. Leuba, J. M. Escola, N. Demaurex, M. Marsh, and V. Piguet.** 2005. HIV-1 trafficking to the dendritic cell-T-cell infectious synapse uses a pathway of tetraspanin sorting to the immunological synapse. *Traffic* **6**:488-501.
75. **Garzino-Demo, A., R. B. Moss, J. B. Margolick, F. Cleghorn, A. Sill, W. A. Blattner, F. Cocchi, D. J. Carlo, A. L. DeVico, and R. C. Gallo.** 1999. Spontaneous and antigen-induced production of HIV-inhibitory beta-chemokines are associated with AIDS-free status. *Proc Natl Acad Sci U S A* **96**:11986-11991.
76. **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**:587-597.

77. **Gilad, J., A. Walfisch, A. Borer, and F. Schlaeffer.** 2003. Gender differences and sex-specific manifestations associated with human immunodeficiency virus infection in women. *European Journal of Obstetrics & Gynecology and Reproductive Biology* **109**:199-205.
78. **Gomez, C., and T. J. Hope.** 2005. The ins and outs of HIV replication. *Cell Microbiol* **7**:621-626.
79. **Gondois-Rey, F., J. C. Grivel, A. Biancotto, M. Pion, R. Vigne, L. B. Margolis, and I. Hirsch.** 2002. Segregation of R5 and X4 HIV-1 variants to memory T cell subsets differentially expressing CD62L in ex vivo infected human lymphoid tissue. *Aids* **16**:1245-1249.
80. **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**:6277-6292.
81. **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**:1425-1431.
82. **Goulder, P. J., R. E. Phillips, R. A. Colbert, S. McAdam, G. Ogg, M. A. Nowak, P. Giangrande, G. Luzzi, B. Morgan, A. Edwards, A. J. McMichael, and S. Rowland-Jones.** 1997. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat Med* **3**:212-217.
83. **Gratton, S., R. Cheynier, M. J. Dumaurier, E. Oksenhendler, and S. Wain-Hobson.** 2000. Highly restricted spread of HIV-1 and multiply infected cells within splenic germinal centers. *Proc Natl Acad Sci U S A* **97**:14566-14571.
84. **Grimm, T. A., B. E. Beer, V. M. Hirsch, and K. A. Clouse.** 2003. Simian immunodeficiency viruses from multiple lineages infect human macrophages: implications for cross-species transmission. *J Acquir Immune Defic Syndr* **32**:362-369.
85. **Hahn, B. H., G. M. Shaw, K. M. De Cock, and P. M. Sharp.** 2000. AIDS as a zoonosis: scientific and public health implications. *Science* **287**:607-614.
86. **Haigwood, N. L.** 2004. Predictive value of primate models for AIDS. *AIDS Rev* **6**:187-198.
87. **Harouse, J. M., R. C. Tan, A. Gettie, P. Dailey, P. A. Marx, P. A. Luciw, and C. Cheng-Mayer.** 1998. Mucosal transmission of pathogenic CXCR4-utilizing SHIVSF33A variants in rhesus macaques. *Virology* **248**:95-107.
88. **Helbert, M. R., J. Walter, J. L'Age, and P. C. Beverley.** 1997. HIV infection of CD45RA+ and CD45RO+ CD4+ T cells. *Clin Exp Immunol* **107**:300-305.
89. **Hirsch, V. M.** 2004. What can natural infection of African monkeys with simian immunodeficiency virus tell us about the pathogenesis of AIDS? *AIDS Rev* **6**:40-53.
90. **Hirsch, V. M., P. Edmondson, M. Murphey-Corb, B. Arbeille, P. R. Johnson, and J. I. Mullins.** 1989. SIV adaptation to human cells. *Nature* **341**:573-574.
91. **Hirsch, V. M., M. E. Sharkey, C. R. Brown, B. Brichacek, S. Goldstein, J. Wakefield, R. Byrum, W. R. Elkins, B. H. Hahn, J. D. Lifson, and M. Stevenson.** 1998. Vpx is required for dissemination and pathogenesis of SIVSM PBj: Evidence of macrophage-dependent viral amplification. *4*:1401-1408.
92. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* **373**:123-126.
93. **Hoffman, T. L., C. C. LaBranche, W. Zhang, G. Canziani, J. Robinson, I. Chaiken, J. A. Hoxie, and R. W. Doms.** 1999. Stable exposure of the coreceptor-binding site in a CD4-independent HIV-1 envelope protein. *Proc Natl Acad Sci U S A* **96**:6359-6364.
94. **Honzarenko, M., Y. Le, M. Swierkowski, I. Ghiran, A. Glodek, and L. E. Silberstein.** 2005. Human Bone Marrow Stromal Cells Express a Distinct Set of Biologically Functional Chemokine Receptors. *Stem Cells*.
95. **Hoxie, J. A., C. C. LaBranche, M. J. Endres, J. D. Turner, J. F. Berson, R. W. Doms, and T. J. Matthews.** 1998. CD4-independent utilization of the CXCR4 chemokine receptor by HIV-1 and HIV-2. *J Reprod Immunol* **41**:197-211.
96. **Hu, W., and H. Temin.** 1990. Genetic Consequences of Packaging Two RNA Genomes in One Retroviral Particle: Pseudodiploidy and High Rate of Genetic Recombination. *PNAS* **87**:1556-1560.
97. **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**:1240-1243.
98. **Hymes, K. B., T. Cheung, J. B. Greene, N. S. Prose, A. Marcus, H. Ballard, D. C. William, and L. J. Laubenstein.** 1981. Kaposi's sarcoma in homosexual men—a report of eight cases. *Lancet* **2**:598-600.
99. **Igarashi, T., C. R. Brown, Y. Endo, A. Buckler-White, R. Plishka, N. Bischofberger, V. Hirsch, and M. A. Martin.** 2001. Macrophage are the principal reservoir and sustain high virus loads in rhesus

- macaques after the depletion of CD4⁺ T cells by a highly pathogenic simian immunodeficiency virus/HIV type 1 chimera (SHIV): Implications for HIV-1 infections of humans. *Proc Natl Acad Sci U S A* **98**:658-663.
100. **Igarashi, T., H. Imamichi, C. R. Brown, V. M. Hirsch, and M. A. Martin.** 2003. The emergence and characterization of macrophage-tropic SIV/HIV chimeric viruses (SHIVs) present in CD4⁺ T cell-depleted rhesus monkeys. *J Leukoc Biol* **74**:772-780.
 101. **Ignatius, R., Y. Wei, S. Beaulieu, A. Gettie, R. M. Steinman, M. Pope, and S. Mojssov.** 2000. The immunodeficiency virus coreceptor, Bonzo/STRL33/TYMSTR, is expressed by macaque and human skin- and blood-derived dendritic cells. *AIDS Res Hum Retroviruses* **16**:1055-1059.
 102. **Janeway, C. A., P. Travers, M. Walport, and M. J. Shlomchik.** 2004. *Immunobiology - the immune system in health and disease*, 6 ed. Garland Science Publishing, New York.
 103. **Jiang, J. D., and G. J. Bekesi.** 2001. Antibody responses to HIV-1 antigens are higher in HIV-1(+) intravenous drug users than in HIV-1(+) homosexuals. *Biomed Pharmacother* **55**:313-315.
 104. **Jin, X., D. E. Bauer, S. E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C. E. Irwin, J. T. Safrit, J. Mittler, L. Weinberger, L. G. Kostrikis, L. Zhang, A. S. Perelson, and D. D. Ho.** 1999. Dramatic rise in plasma viremia after CD8⁺ T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* **189**:991-998.
 105. **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.
 106. **Joag, S. V., Z. Li, L. Foresman, E. B. Stephens, L. J. Zhao, I. Adany, D. M. Pinson, H. M. McClure, and O. Narayan.** 1996. Chimeric simian/human immunodeficiency virus that causes progressive loss of CD4⁺ T cells and AIDS in pig-tailed macaques. *J Virol* **70**:3189-3197.
 107. **Johnson, W. E., J. D. Lifson, S. M. Lang, R. P. Johnson, and R. C. Desrosiers.** 2003. Importance of B-cell responses for immunological control of variant strains of simian immunodeficiency virus. *J Virol* **77**:375-381.
 108. **Jung, A., R. Maier, J. P. Vartanian, G. Bocharov, V. Jung, U. Fischer, E. Meese, S. Wain-Hobson, and A. Meyerhans.** 2002. Multiply infected spleen cells in HIV patients. *Nature* **418**:144.
 109. **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**:364-370.
 110. **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**:2561-2569.
 111. **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**:11807-11815.
 112. **Kaul, R., F. A. Plummer, J. Kimani, T. Dong, P. Kiama, T. Rostron, E. Njagi, K. S. MacDonald, J. J. Bwayo, A. J. McMichael, and S. L. Rowland-Jones.** 2000. HIV-1-specific mucosal CD8⁺ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. *J Immunol* **164**:1602-1611.
 113. **Kaul, R., S. L. Rowland-Jones, J. Kimani, T. Dong, H. B. Yang, P. Kiama, T. Rostron, E. Njagi, J. J. Bwayo, K. S. MacDonald, A. J. McMichael, and F. A. Plummer.** 2001. Late seroconversion in HIV-resistant Nairobi prostitutes despite pre-existing HIV-specific CD8⁺ responses. *J Clin Invest* **107**:341-349.
 114. **Kimata, J. T., J. J. Gosink, V. N. KewalRamani, L. M. Rudensey, D. R. Littman, and J. Overbaugh.** 1999. Coreceptor specificity of temporal variants of simian immunodeficiency virus Mne. *J Virol* **73**:1655-1660.
 115. **Kimata, J. T., L. Kuller, D. B. Anderson, P. Dailey, and J. Overbaugh.** 1999. Emerging cytopathic and antigenic simian immunodeficiency virus variants influence AIDS progression. *Nat Med* **5**:535-541.
 116. **Kirchhoff, F., S. Pohlmann, M. Hamacher, R. E. Means, T. Kraus, K. Uberla, and P. Di Marzio.** 1997. Simian immunodeficiency virus variants with differential T-cell and macrophage tropism use CCR5 and an unidentified cofactor expressed in CEMx174 cells for efficient entry. *J Virol* **71**:6509-6516.
 117. **Klatzmann, D.** 1984. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. *Science* **225**:59-63.
 118. **Korber, B., B. Gaschen, K. Yusim, R. Thakallapally, C. Kesmir, and V. Detours.** 2001. Evolutionary and immunological implications of contemporary HIV-1 variation. *Br Med Bull* **58**:19-42.

119. **Korber, B., M. Muldoon, J. Theiler, F. Gao, R. Gupta, A. Lapedes, B. H. Hahn, S. Wolinsky, and T. Bhattacharya.** 2000. Timing the Ancestor of the HIV-1 Pandemic Strains. *Science* **288**:1789-1796.
120. **Kostrikis, L. G., Y. Huang, J. P. Moore, S. M. Wolinsky, L. Zhang, Y. Guo, L. Deutsch, J. Phair, A. U. Neumann, and D. D. Ho.** 1998. A chemokine receptor CCR2 allele delays HIV-1 disease progression and is associated with a CCR5 promoter mutation. *Nat Med* **4**:350-353.
121. **Koup, R. A., J. T. Safrit, Y. Cao, C. A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D. D. Ho.** 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol* **68**:4650-4655.
122. **Kuller, L., W. R. Morton, R. E. Benveniste, C. C. Tsai, E. A. Clark, M. J. Gale, S. L. Hu, M. E. Thouless, and M. G. Katze.** 1990. Inoculation of *Macaca fascicularis* with simian immunodeficiency virus, SIV_{mac} immunologic, serologic, and pathologic changes. *J Med Primatol* **19**:367-380.
123. **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**:1397-1403.
124. **Kwon, D. S., G. Gregorio, N. Bitton, W. A. Hendrickson, and D. R. Littman.** 2002. DC-SIGN-mediated internalization of HIV is required for trans-enhancement of T cell infection. *Immunity* **16**:135-144.
125. **Kwong, P. D., R. Wyatt, J. Robinson, R. W. Sweet, J. Sodroski, and W. A. Hendrickson.** 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* **393**:648-659.
126. **LaBonte, J. A., T. Patel, W. Hofmann, and J. Sodroski.** 2000. Importance of membrane fusion mediated by human immunodeficiency virus envelope glycoproteins for lysis of primary CD4-positive T cells. *J Virol* **74**:10690-10698.
127. **LaBranche, C. C., T. L. Hoffman, J. Romano, B. S. Haggarty, T. G. Edwards, T. J. Matthews, R. W. Doms, and J. A. Hoxie.** 1999. Determinants of CD4 independence for a human immunodeficiency virus type 1 variant map outside regions required for coreceptor specificity. *J Virol* **73**:10310-10319.
128. **Larkin, M., R. A. Childs, T. J. Matthews, S. Thiel, T. Mizuochi, A. M. Lawson, J. S. Savill, C. Haslett, R. Diaz, and T. Feizi.** 1989. Oligosaccharide-mediated interactions of the envelope glycoprotein gp120 of HIV-1 that are independent of CD4 recognition. *Aids* **3**:793-798.
129. **Lee, B., G. Leslie, E. Soilleux, U. O'Doherty, S. Baik, E. Levroney, K. Flummerfelt, W. Swiggard, N. Coleman, M. Malim, and R. W. Doms.** 2001. cis Expression of DC-SIGN allows for more efficient entry of human and simian immunodeficiency viruses via CD4 and a coreceptor. *J Virol* **75**:12028-12038.
130. **Lemey, P., O. G. Pybus, B. Wang, N. K. Saksena, M. Salemi, and A.-M. Vandamme.** 2003. Tracing the origin and history of the HIV-2 epidemic. *PNAS* **100**:6588-6592.
131. **Letvin, N. L., M. D. Daniel, P. K. Sehgal, R. C. Desrosiers, R. D. Hunt, L. M. Waldron, J. J. MacKey, D. K. Schmidt, L. V. Chalifoux, and N. W. King.** 1985. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. *Science* **230**:71-73.
132. **Letvin, N. L., and N. W. King.** 1990. Immunologic and pathologic manifestations of the infection of rhesus monkeys with simian immunodeficiency virus of macaques. *J Acquir Immune Defic Syndr* **3**:1023-1040.
133. **Levy, J. A.** 2003. The search for the CD8+ cell anti-HIV factor (CAF). *Trends Immunol* **24**:628-632.
134. **Li, J., C. I. Lord, W. Haseltine, N. L. Letvin, and J. Sodroski.** 1992. Infection of cynomolgus monkeys with a chimeric HIV-1/SIV_{mac} virus that expresses the HIV-1 envelope glycoproteins. *J Acquir Immune Defic Syndr* **5**:639-646.
135. **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**:1148-1152.
136. **Li, S., J. Juarez, M. Alali, D. Dwyer, R. Collman, A. Cunningham, and H. M. Naif.** 1999. Persistent CCR5 utilization and enhanced macrophage tropism by primary blood human immunodeficiency virus type 1 isolates from advanced stages of disease and comparison to tissue-derived isolates. *J Virol* **73**:9741-9755.
137. **Liao, F., G. Alkhatib, K. W. Peden, G. Sharma, E. A. Berger, and J. M. Farber.** 1997. STRL33, A novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J Exp Med* **185**:2015-2023.
138. **Lifson, J. D., M. B. Feinberg, G. R. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moss, F. Wong-Staal, K. S. Steimer, and E. G. Engleman.** 1986. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. *Nature* **323**:725-728.

139. **Liu, H.-y., Y. Soda, N. Shimizu, Y. Haraguchi, A. Jinno, Y. Takeuchi, and H. Hoshino.** 2000. CD4-Dependent and CD4-Independent Utilization of Coreceptors by Human Immunodeficiency Viruses Type 2 and Simian Immunodeficiency Viruses. *Virology* **278**:276-288.
140. **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**:367-377.
141. **Los Alamos National Laboratory.** HIV sequence database. <http://hiv-web.lanl.gov/>
142. **Mansky, L. M., and H. M. Temin.** 1995. Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. *J Virol* **69**:5087-5094.
143. **Marcon, L., H. Choe, K. A. Martin, M. Farzan, P. D. Ponath, L. Wu, W. Newman, N. Gerard, C. Gerard, and J. Sodroski.** 1997. Utilization of C-C chemokine receptor 5 by the envelope glycoproteins of a pathogenic simian immunodeficiency virus, SIVmac239. *J Virol* **71**:2522-2527.
144. **Maresca, M., R. Mahfoud, N. Garmy, D. P. Kotler, J. Fantini, and F. Clayton.** 2003. The virotoxin model of HIV-1 enteropathy: involvement of GPR15/Bob and galactosylceramide in the cytopathic effects induced by HIV-1 gp120 in the HT-29-D4 intestinal cell line. *J Biomed Sci* **10**:156-166.
145. **Martin, M. P., M. Dean, M. W. Smith, C. Winkler, B. Gerrard, N. L. Michael, B. Lee, R. W. Doms, J. Margolick, S. Buchbinder, J. J. Goedert, T. R. O'Brien, M. W. Hilgartner, D. Vlahov, S. J. O'Brien, and M. Carrington.** 1998. Genetic acceleration of AIDS progression by a promoter variant of CCR5. *Science* **282**:1907-1911.
146. **Marx, P. A., C. Apetrei, and E. Drucker.** 2004. AIDS as a zoonosis? Confusion over the origin of the virus and the origin of the epidemics. *J Med Primatol* **33**:220-226.
147. **Marx, P. A., and Z. Chen.** 1998. The function of simian chemokine receptors in the replication of SIV. *Semin Immunol* **10**:215-223.
148. **Mascola, J. R., M. G. Lewis, G. Stiegler, D. Harris, T. C. VanCott, D. Hayes, M. K. Louder, C. R. Brown, C. V. Sapan, S. S. Frankel, Y. Lu, M. L. Robb, H. Katinger, and D. L. Birx.** 1999. Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. *J Virol* **73**:4009-4018.
149. **Mattapallil, J. J., D. C. Douek, B. Hill, Y. Nishimura, M. Martin, and M. Roederer.** 2005. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature* **434**:1093-1097.
150. **McClure, H. M., D. C. Anderson, P. N. Fultz, A. A. Ansari, E. Lockwood, and A. Brodie.** 1989. Spectrum of disease in macaque monkeys chronically infected with SIV/SMM. *Vet Immunol Immunopathol* **21**:13-24.
151. **McDonald, D., L. Wu, S. M. Bohks, V. N. KewalRamani, D. Unutmaz, and T. J. Hope.** 2003. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science* **300**:1295-1297.
152. **McKnight, A., M. T. Dittmar, J. Moniz-Periera, K. Ariyoshi, J. D. Reeves, S. Hibbitts, D. Whitby, E. Aarons, A. E. Proudfoot, H. Whittle, and P. R. Clapham.** 1998. A broad range of chemokine receptors are used by primary isolates of human immunodeficiency virus type 2 as coreceptors with CD4. *J Virol* **72**:4065-4071.
153. **Means, R. E., T. Matthews, J. A. Hoxie, M. H. Malim, T. Kodama, and R. C. Desrosiers.** 2001. Ability of the V3 loop of simian immunodeficiency virus to serve as a target for antibody-mediated neutralization: correlation of neutralization sensitivity, growth in macrophages, and decreased dependence on CD4. *J Virol* **75**:3903-3915.
154. **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**:1167-1170.
155. **Miller, M. D., M. T. Warmerdam, I. Gaston, W. C. Greene, and M. B. Feinberg.** 1994. The human immunodeficiency virus-1 nef gene product: a positive factor for viral infection and replication in primary lymphocytes and macrophages. *J Exp Med* **179**:101-113.
156. **Mondor, I., S. Ugolini, and Q. J. Sattentau.** 1998. Human immunodeficiency virus type 1 attachment to HeLa CD4 cells is CD4 independent and gp120 dependent and requires cell surface heparans. *J Virol* **72**:3623-3634.
157. **Montefiori, D. C., G. Pantaleo, L. M. Fink, J. T. Zhou, J. Y. Zhou, M. Bilska, G. D. Miralles, and A. S. Fauci.** 1996. Neutralizing and infection-enhancing antibody responses to human immunodeficiency virus type 1 in long-term nonprogressors. *J Infect Dis* **173**:60-67.
158. **Moore, J. P., and R. W. Doms.** 2003. The entry of entry inhibitors: a fusion of science and medicine. *Proc Natl Acad Sci U S A* **100**:10598-10602.
159. **Mori, K., D. J. Ringler, T. Kodama, and R. C. Desrosiers.** 1992. Complex determinants of macrophage tropism in env of simian immunodeficiency virus. *J Virol* **66**:2067-2075.
160. **Moris, A., C. Nobile, F. Buseyne, F. Porrot, J. P. Abastado, and O. Schwartz.** 2004. DC-SIGN promotes exogenous MHC-I-restricted HIV-1 antigen presentation. *Blood* **103**:2648-2654.

161. **Morner, A., A. Björndal, J. Albert, V. N. Kewalramani, D. R. Littman, R. Inoue, R. Thorstensson, E. M. Fenyö, and E. Björling.** 1999. Primary human immunodeficiency virus type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in coreceptor usage. *J Virol* **73**:2343-2349.
162. **Murray, P. R., K. S. Rosenthal, G. S. Kobayashi, and M. A. Pfaller.** 2002. *Medical Microbiology*, 4 ed. Mosby, Inc., St. Louis.
163. **Mörner, A., Å. Björndal, J. Albert, V. KewalRamani, D. R. Littman, R. Inoue, R. Thorstensson, E. M. Fenyö, and E. Björling.** 1999. Primary human immunodeficiency virus type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in coreceptor usage. *J Virol* **73**:2343-2349.
164. **Nakayama, E. E., Y. Tanaka, Y. Nagai, A. Iwamoto, and T. Shioda.** 2004. A CCR2-V64I polymorphism affects stability of CCR2A isoform. *Aids* **18**:729-738.
165. **Nilsson, C., R. Thorstensson, G. Gilljam, S. Sjölander, K. Hild, K. Broliden, L. Akerblom, B. Morein, G. Biberfeld, and P. Putkonen.** 1995. Protection against monkey-cell grown cell-free HIV-2 challenge in macaques immunized with native HIV-2 envelope glycoprotein gp125. *Vaccine Res* **4**:165-175.
166. **Nishimura, Y., T. Igarashi, N. Haigwood, R. Sadjadpour, R. J. Plishka, A. Buckler-White, R. Shibata, and M. A. Martin.** 2002. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. *J Virol* **76**:2123-2130.
167. **Nobile, C., A. Moris, F. Porrot, N. Sol-Foulon, and O. Schwartz.** 2003. Inhibition of human immunodeficiency virus type 1 Env-mediated fusion by DC-SIGN. *J Virol* **77**:5313-5323.
168. **Nordqvist, A., and E. M. Fenyö.** 2005. Plaque-reduction assays for human and simian immunodeficiency virus neutralization. *Methods Mol Biol* **304**:273-285.
169. **O'Connor, D. H., T. M. Allen, T. U. Vogel, P. Jing, I. P. DeSouza, E. Dodds, E. J. Dunphy, C. Melsaether, B. Mothe, H. Yamamoto, H. Horton, N. Wilson, A. L. Hughes, and D. I. Watkins.** 2002. Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. *Nat Med* **8**:493-499.
170. **Owen, S. M., S. Masciotra, F. Novembre, J. Yee, W. M. Switzer, M. Ostyula, and R. B. Lal.** 2000. Simian immunodeficiency viruses of diverse origin can use CXCR4 as a coreceptor for entry into human cells. *J Virol* **74**:5702-5708.
171. **Palacios, E., L. Digilio, H. M. McClure, Z. Chen, P. A. Marx, M. A. Goldsmith, and R. M. Grant.** 1998. Parallel evolution of CCR5-null phenotypes in humans and in a natural host of simian immunodeficiency virus. *Curr Biol* **8**:943-946.
172. **Pantaleo, G., S. Menzo, M. Vaccarezza, C. Graziosi, O. J. Cohen, J. F. Demarest, D. Montefiori, J. M. Orenstein, C. Fox, L. K. Schragar, J. B. Margolick, S. Buchbinder, J. V. Giorgi, and A. S. Fauci.** 1995. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. *N Engl J Med* **332**:209-216.
173. **Paquette, J. S., J. F. Fortin, L. Blanchard, and M. J. Tremblay.** 1998. Level of ICAM-1 surface expression on virus producer cells influences both the amount of virion-bound host ICAM-1 and human immunodeficiency virus type 1 infectivity. *J Virol* **72**:9329-9336.
174. **Pehrson, P., S. Lindback, C. Lidman, H. Gaines, and J. Giesecke.** 1997. Longer survival after HIV infection for injecting drug users than for homosexual men: implications for immunology. *Aids* **11**:1007-1012.
175. **Pelchen-Matthews, A., B. Kramer, and M. Marsh.** 2003. Infectious HIV-1 assembles in late endosomes in primary macrophages. *J Cell Biol* **162**:443-455.
176. **Piatak, M., Jr., M. S. Saag, L. C. Yang, S. J. Clark, J. C. Kappes, K. C. Luk, B. H. Hahn, G. M. Shaw, and J. D. Lifson.** 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science* **259**:1749-1754.
177. **Piguet, V., and Q. Sattentau.** 2004. Dangerous liaisons at the virological synapse. *J Clin Invest* **114**:605-610.
178. **Pilgrim, A. K., G. Pantaleo, O. J. Cohen, L. M. Fink, J. Y. Zhou, J. T. Zhou, D. P. Bolognesi, A. S. Fauci, and D. C. Montefiori.** 1997. Neutralizing antibody responses to human immunodeficiency virus type 1 in primary infection and long-term-nonprogressive infection. *J Infect Dis* **176**:924-932.
179. **Poaty-Mavoungou, V., R. Onanga, I. Bedjabaga, and E. Mavoungou.** 2001. Simian immunodeficiency virus from mandrill (*Mandrillus sphinx*) SIVmnd experimentally infects human and nonhuman primate cells. *Microbes Infect* **3**:599-610.
180. **Pohlmann, S., N. Stolte, J. Munch, P. Ten Haaf, J. L. Heeney, C. Stahl-Hennig, and F. Kirchhoff.** 1999. Co-receptor usage of BOB/GPR15 in addition to CCR5 has no significant effect on replication of simian immunodeficiency virus in vivo. *J Infect Dis* **180**:1494-1502.

181. **Pope, M., and A. T. Haase.** 2003. Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. *Nat Med* **9**:847-852.
182. **Price, D. A., P. J. Goulder, P. Klenerman, A. K. Sewell, P. J. Easterbrook, M. Troop, C. R. Bangham, and R. E. Phillips.** 1997. Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. *Proc Natl Acad Sci U S A* **94**:1890-1895.
183. **Prins, M., and P. J. Veugelers.** 1997. Comparison of progression and non-progression in injecting drug users and homosexual men with documented dates of HIV-1 seroconversion. European Seroconverter Study and the Tricontinental Seroconverter Study. *Aids* **11**:621-631.
184. **Puffer, B. A., S. Pohlmann, A. L. Edinger, D. Carlin, M. D. Sanchez, J. Reitter, D. D. Watry, H. S. Fox, R. C. Desrosiers, and R. W. Doms.** 2002. CD4 independence of simian immunodeficiency virus Envs is associated with macrophage tropism, neutralization sensitivity, and attenuated pathogenicity. *J Virol* **76**:2595-2605.
185. **Putkonen, P., E. E. Kaaya, D. Bottiger, S. L. Li, C. Nilsson, P. Biberfeld, and G. Biberfeld.** 1992. Clinical features and predictive markers of disease progression in cynomolgus monkeys experimentally infected with simian immunodeficiency virus. *Aids* **6**:257-263.
186. **Putkonen, P., R. Thorstensson, L. Ghavamzadeh, J. Albert, K. Hild, G. Biberfeld, and E. Norrby.** 1991. Prevention of HIV-2 and SIVsm infection by passive immunization in cynomolgus monkeys. *Nature* **352**:436-438.
187. **Putkonen, P., K. Warstedt, R. Thorstensson, R. Benthin, J. Albert, B. Lundgren, B. Oberg, E. Norrby, and G. Biberfeld.** 1989. Experimental infection of cynomolgus monkeys (*Macaca fascicularis*) with simian immunodeficiency virus (SIVsm). *J Acquir Immune Defic Syndr* **2**:359-365.
188. **Quinn, T. C., and J. Overbaugh.** 2005. HIV/AIDS in Women: An Expanding Epidemic. *Science* **308**:1582-1583.
189. **Quinones-Mateu, M. E., S. C. Ball, A. J. Marozsan, V. S. Torre, J. L. Albright, G. Vanham, G. van Der Groen, R. L. Colebunders, and E. J. Arts.** 2000. A dual infection/competition assay shows a correlation between ex vivo human immunodeficiency virus type 1 fitness and disease progression. *J Virol* **74**:9222-9233.
190. **Raposo, G., M. Moore, D. Innes, R. Leijendekker, A. Leigh-Brown, P. Benaroch, and H. Geuze.** 2002. Human macrophages accumulate HIV-1 particles in MHC II compartments. *Traffic* **3**:718-729.
191. **Reeves, J. D., S. Hibbitts, G. Simmons, A. McKnight, J. M. Azevedo-Pereira, J. Moniz-Pereira, and P. R. Clapham.** 1999. Primary Human Immunodeficiency Virus Type 2 (HIV-2) Isolates Infect CD4-Negative Cells via CCR5 and CXCR4: Comparison with HIV-1 and Simian Immunodeficiency Virus and Relevance to Cell Tropism In Vivo. *J. Virol.* **73**:7795-7804.
192. **Reeves, J. D., A. McKnight, S. Potempa, G. Simmons, P. W. Gray, C. A. Power, T. Wells, R. A. Weiss, and S. J. Talbot.** 1997. CD4-independent infection by HIV-2 (ROD/B): use of the 7-transmembrane receptors CXCR-4, CCR-3, and V28 for entry. *Virology* **231**:130-134.
193. **Reimann, K. A., J. T. Li, R. Veazey, M. Halloran, I. W. Park, G. B. Karlsson, J. Sodroski, and N. L. Letvin.** 1996. A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. *J Virol* **70**:6922-6928.
194. **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**:2859-2869.
195. **Resh, M. D.** 2005. Intracellular trafficking of HIV-1 Gag: how Gag interacts with cell membranes and makes viral particles. *AIDS Rev* **7**:84-91.
196. **Richman, D. D., T. Wrin, S. J. Little, and C. J. Petropoulos.** 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc Natl Acad Sci U S A* **100**:4144-4149.
197. **Roederer, M., P. A. Raju, D. K. Mitra, and L. A. Herzenberg.** 1997. HIV does not replicate in naive CD4 T cells stimulated with CD3/CD28. *J Clin Invest* **99**:1555-1564.
198. **Rollins, B. J.** 1997. Chemokines. *Blood* **90**:909-928.
199. **Rowland-Jones, S. L., T. Dong, K. R. Fowke, J. Kimani, P. Krausa, H. Newell, T. Blanchard, K. Ariyoshi, J. Oyugi, E. Ngugi, J. Bwayo, K. S. MacDonald, A. J. McMichael, and F. A. Plummer.** 1998. Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. *J Clin Invest* **102**:1758-1765.
200. **Rucker, J., A. L. Edinger, M. Sharron, M. Samson, B. Lee, J. F. Berson, Y. Yi, B. Margulies, R. G. Collman, B. J. Doranz, M. Parmentier, and R. W. Doms.** 1997. Utilization of chemokine receptors, orphan receptors, and herpesvirus-encoded receptors by diverse human and simian immunodeficiency viruses. *J Virol* **71**:8999-9007.

201. **Rudensey, L. M., J. T. Kimata, R. E. Benveniste, and J. Overbaugh.** 1995. Progression to AIDS in macaques is associated with changes in the replication, tropism, and cytopathic properties of the simian immunodeficiency virus variant population. *Virology* **207**:528-542.
202. **Rybarczyk, B. J., D. Montefiori, P. R. Johnson, A. West, R. E. Johnston, and R. Swanstrom.** 2004. Correlation between env V1/V2 region diversification and neutralizing antibodies during primary infection by simian immunodeficiency virus sm in rhesus macaques. *J Virol* **78**:3561-3571.
203. **Sakuragi, S., R. Shibata, R. Mukai, T. Komatsu, M. Fukasawa, H. Sakai, J. Sakuragi, M. Kawamura, K. Ibuki, M. Hayami, and et al.** 1992. Infection of macaque monkeys with a chimeric human and simian immunodeficiency virus. *J Gen Virol* **73 (Pt 11)**:2983-2987.
204. **Salemi, M., K. Strimmer, W. Hall, M. Duffy, E. Delaporte, S. Mboup, M. Peeters, and A.-M. Vandamme.** 2001. Dating the common ancestor of SIVcpz and HIV-1 group M and the origin of HIV-1 subtypes using a new method to uncover clock-like molecular evolution. *FASEB J.* **15**:276-278.
205. **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**:1689-1700.
206. **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**:722-725.
207. **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**:857-860.
208. **Schmitz, J. E., M. J. Kuroda, S. Santra, M. A. Simon, M. A. Lifton, W. Lin, R. Khunhkhun, M. Piatak, J. D. Lifson, G. Grosschupff, R. S. Gelman, P. Racz, K. Tenner-Racz, K. A. Mansfield, N. L. Letvin, D. C. Montefiori, and K. A. Reimann.** 2003. Effect of humoral immune responses on controlling viremia during primary infection of rhesus monkeys with simian immunodeficiency virus. *J Virol* **77**:2165-2173.
209. **Schnittman, S. M., H. C. Lane, J. Greenhouse, J. S. Justement, M. Baseler, and A. S. Fauci.** 1990. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. *Proc Natl Acad Sci U S A* **87**:6058-6062.
210. **Schols, D., and E. De Clercq.** 1998. The simian immunodeficiency virus mnd(GB-1) strain uses CXCR4, not CCR5, as coreceptor for entry in human cells. *J Gen Virol* **79**:2203-2205.
211. **Schroder, A. R., P. Shinn, H. Chen, C. Berry, J. R. Ecker, and F. Bushman.** 2002. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* **110**:521-529.
212. **Schuitemaker, H., M. Koot, N. A. Kootstra, M. W. Dercksen, R. E. de Goede, R. P. van Steenwijk, J. M. Lange, J. K. Schattenkerk, F. Miedema, and M. Tersmette.** 1992. Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus population. *J Virol* **66**:1354-1360.
213. **Shankarappa, R., J. B. Margolick, S. J. Gange, A. G. Rodrigo, D. Upchurch, H. Farzadegan, P. Gupta, C. R. Rinaldo, G. H. Learn, X. He, X. L. Huang, and J. I. Mullins.** 1999. Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection. *J Virol* **73**:10489-10502.
214. **Sharma, D. P., M. C. Zink, M. Anderson, R. Adams, J. E. Clements, S. V. Joag, and O. Narayan.** 1992. Derivation of neurotropic simian immunodeficiency virus from exclusively lymphocytotropic parental virus: pathogenesis of infection in macaques. *J Virol* **66**:3550-3556.
215. **Sharova, N., C. Swingler, M. Sharkey, and M. Stevenson.** 2005. Macrophages archive HIV-1 virions for dissemination in trans. *Embo J* **24**:2481-2489.
216. **Sharron, M., S. Pohlmann, K. Price, E. Lolis, M. Tsang, F. Kirchhoff, R. W. Doms, and B. Lee.** 2000. Expression and coreceptor activity of STRL33/Bonzo on primary peripheral blood lymphocytes. *Blood* **96**:41-49.
217. **Shi, Y., J. Albert, G. Francis, H. Holmes, and E. M. Fenyo.** 2002. A new cell line-based neutralization assay for primary HIV type 1 isolates. *AIDS Res Hum Retroviruses* **18**:957-967.
218. **Shi, Y., E. Brandin, E. Vincic, M. Jansson, A. Blaxhult, K. Gyllensten, L. Moberg, C. Brostrom, E. M. Fenyo, and J. Albert.** 2005. Evolution of human immunodeficiency virus type 2 coreceptor usage, autologous neutralization, envelope sequence and glycosylation. *J Gen Virol* **86**:3385-3396.

219. **Shibata, R., and A. Adachi.** 1992. SIV/HIV recombinants and their use in studying biological properties. *AIDS Res Hum Retroviruses* **8**:403-409.
220. **Shibata, R., F. Maldarelli, C. Siemon, T. Matano, M. Parta, G. Miller, T. Fredrickson, and M. A. Martin.** 1997. Infection and pathogenicity of chimeric simian-human immunodeficiency viruses in macaques: determinants of high virus loads and CD4 cell killing. *J Infect Dis* **176**:362-373.
221. **Shimizu, N., M. Kobayashi, H.-Y. Liu, H. Kido, and H. Hoshino.** 1995. Detection of tryptase TL2 and CD26 antigen in brain-derived cells non-permissive to T-cell line-tropic human immunodeficiency virus type 1. *FEBS Letters* **358**:48-52.
222. **Shimizu, N., Y. Soda, K. Kanbe, H. Y. Liu, A. Jinno, T. Kitamura, and H. Hoshino.** 1999. An orphan G protein-coupled receptor, GPR1, acts as a coreceptor to allow replication of human immunodeficiency virus types 1 and 2 in brain-derived cells. *J Virol* **73**:5231-5239.
223. **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**:619-626.
224. **Silvestri, G., D. L. Sodora, R. A. Koup, M. Paiardini, S. P. O'Neil, H. M. McClure, S. I. Staprans, and M. B. Feinberg.** 2003. Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. *Immunity* **18**:441-452.
225. **Simmonds, P., P. Balfe, C. A. Ludlam, J. O. Bishop, and A. J. Brown.** 1990. Analysis of sequence diversity in hypervariable regions of the external glycoprotein of human immunodeficiency virus type 1. *J Virol* **64**:5840-5850.
226. **Simmons, G., J. D. Reeves, A. McKnight, N. Dejuq, S. Hibbitts, C. A. Power, E. Aarons, D. Schols, E. De Clercq, A. E. Proudfoot, and P. R. Clapham.** 1998. CXCR4 as a functional coreceptor for human immunodeficiency virus type 1 infection of primary macrophages. *J Virol* **72**:8453-8457.
227. **Smith, M. W., M. Dean, M. Carrington, C. Winkler, G. A. Huttley, D. A. Lomb, J. J. Goedert, T. R. O'Brien, L. P. Jacobson, R. Kaslow, S. Buchbinder, E. Vittinghoff, D. Vlahov, K. Hoots, M. W. Hilgartner, and S. J. O'Brien.** 1997. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. *Science* **277**:959-965.
228. **Soda, Y., N. Shimizu, A. Jinno, H.-Y. Liu, K. Kanbe, T. Kitamura, and H. Hoshino.** 1999. Establishment of a New System for Determination of Coreceptor Usages of HIV Based on the Human Glioma NP-2 Cell Line. *Biochemical and Biophysical Research Communications* **258**:313-321.
229. **Sodroski, J., W. C. Goh, C. Rosen, K. Campbell, and W. A. Haseltine.** 1986. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. *Nature* **322**:470-474.
230. **Spina, C. A., T. J. Kwok, M. Y. Chowers, J. C. Guatelli, and D. D. Richman.** 1994. The importance of nef in the induction of human immunodeficiency virus type 1 replication from primary quiescent CD4 lymphocytes. *J Exp Med* **179**:115-123.
231. **Spina, C. A., H. E. Prince, and D. D. Richman.** 1997. Preferential replication of HIV-1 in the CD45RO memory cell subset of primary CD4 lymphocytes in vitro. *J Clin Invest* **99**:1774-1785.
232. **Stahl-Hennig, C., R. M. Steinman, K. Tenner-Racz, M. Pope, N. Stolte, K. Mätzt-Rensing, G. Grobuschupff, B. Raschdorff, G. Hunsmann, and P. Racz.** 1999. Rapid Infection of Oral Mucosal-Associated Lymphoid Tissue with Simian Immunodeficiency Virus. *Science* **285**:1261-1265.
233. **Stent, G., G. B. Joo, P. Kierulf, and B. Asjo.** 1997. Macrophage tropism: fact or fiction? *J Leukoc Biol* **62**:4-11.
234. **Stephens, E. B., D. Galbreath, Z. Q. Liu, M. Sahni, Z. Li, R. Lamb-Wharton, L. Foresman, S. V. Joag, and O. Narayan.** 1997. Significance of macrophage tropism of SIV in the macaque model of HIV disease. *J Leukoc Biol* **62**:12-19.
235. **Stevenson, M.** 2003. HIV-1 pathogenesis. *Nat Med* **9**:853-860.
236. **Ten Haaf, P., B. Verstrepen, K. Uberla, B. Rosenwirth, and J. Heeney.** 1998. A pathogenic threshold of virus load defined in simian immunodeficiency virus- or simian-human immunodeficiency virus-infected macaques. *J Virol* **72**:10281-10285.
237. **Termette, 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**:2026-2032.
238. **Termette, 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**:2118-2125.

239. **Thorstensson, R., L. Walther, P. Putkonen, J. Albert, and G. Biberfeld.** 1991. A capture enzyme immunoassay for detection of HIV-2/SIV antigen. *J Acquir Immune Defic Syndr* **4**:374-379.
240. **Trimble, J. J., J. R. Salkowitz, and H. W. Kestler.** 2000. Animal models for AIDS pathogenesis. *Adv Pharmacol* **49**:479-514.
241. **Troyer, R. M., K. R. Collins, A. Abraha, E. Fraundorf, D. M. Moore, R. W. Krizan, Z. Toossi, R. L. Colebunders, M. A. Jensen, J. I. Mullins, G. Vanham, and E. J. Arts.** 2005. Changes in human immunodeficiency virus type 1 fitness and genetic diversity during disease progression. *J Virol* **79**:9006-9018.
242. **Turville, S. G., J. J. Santos, I. Frank, P. U. Cameron, J. Wilkinson, M. Miranda-Saksena, J. Dable, H. Stossel, N. Romani, M. Piatak, Jr., J. D. Lifson, M. Pope, and A. L. Cunningham.** 2004. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. *Blood* **103**:2170-2179.
243. **Tuttle, D. L., C. B. Anders, M. J. Aquino-De Jesus, P. P. Poole, S. L. Lamers, D. R. Briggs, S. M. Pomeroy, L. Alexander, K. W. Peden, W. A. Andiman, J. W. Sleasman, and M. M. Goodenow.** 2002. Increased replication of non-syncytium-inducing HIV type 1 isolates in monocyte-derived macrophages is linked to advanced disease in infected children. *AIDS Res Hum Retroviruses* **18**:353-362.
244. **UNAIDS, and WHO** December 2005, posting date. AIDS epidemic update. [Online.]
245. **Unutmaz, D., V. N. KewalRamani, S. Marmon, and D. R. Littman.** 1999. Cytokine signals are sufficient for HIV-1 infection of resting human T lymphocytes. *J Exp Med* **189**:1735-1746.
246. **Wagsater, D., and J. Dimberg.** 2004. Expression of chemokine receptor CXCR6 in human colorectal adenocarcinomas. *Anticancer Res* **24**:3711-3714.
247. **Valentin, A., J. Albert, E. M. Fenyo, and B. Asjo.** 1994. Dual tropism for macrophages and lymphocytes is a common feature of primary human immunodeficiency virus type 1 and 2 isolates. *J Virol* **68**:6684-6689.
248. **Valentin, A., J. Albert, E. M. Fenyo, and B. Asjo.** 1990. HIV-1 infection of normal human macrophage cultures: implication for silent infection. *Virology* **177**:790-794.
249. **Walther, L., O. Grankvist, B. Mirzai, Z. da Silva, H. Fredlund, G. Biberfeld, and R. Thorstensson.** 1998. Optimization of polymerase chain reaction for detection of HIV type 2 DNA. *AIDS Res Hum Retroviruses* **14**:1151-1156.
250. **Van Baalen, C. A., M. Schutten, R. C. Huisman, P. H. Boers, R. A. Gruters, and A. D. Osterhaus.** 1998. Kinetics of antiviral activity by human immunodeficiency virus type 1-specific cytotoxic T lymphocytes (CTL) and rapid selection of CTL escape virus in vitro. *J Virol* **72**:6851-6857.
251. **Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner.** 1998. Gastrointestinal Tract as a Major Site of CD4+ T Cell Depletion and Viral Replication in SIV Infection. *Science* **280**:427-431.
252. **Veazey, R. S., R. J. Shattock, M. Pope, J. C. Kirijan, J. Jones, Q. Hu, T. Ketas, P. A. Marx, P. J. Klasse, D. R. Burton, and J. P. Moore.** 2003. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. *Nat Med* **9**:343-346.
253. **Wei, X., J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar-Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong, and G. M. Shaw.** 2003. Antibody neutralization and escape by HIV-1. *Nature* **422**:307-312.
254. **Verani, A., E. Pesenti, S. Polo, E. Tresoldi, G. Scarlatti, P. Lusso, A. G. Siccardi, and D. Vercelli.** 1998. CXCR4 is a functional coreceptor for infection of human macrophages by CXCR4-dependent primary HIV-1 isolates. *J Immunol* **161**:2084-2088.
255. **Vodros, D., R. Thorstensson, G. Biberfeld, D. Schols, E. De Clercq, and E. M. Fenyo.** 2001. Coreceptor usage of sequential isolates from cynomolgus monkeys experimentally Infected with simian immunodeficiency virus (SIVsm). *Virology* **291**:12-21.
256. **Vodros, D., R. Thorstensson, R. W. Doms, E. M. Fenyo, and J. D. Reeves.** 2003. Evolution of coreceptor use and CD4-independence in envelope clones derived from SIVsm-infected macaques. *Virology* **316**:17-28.
257. **Zerhouni, B., J. A. Nelson, and K. Saha.** 2004. Isolation of CD4-independent primary human immunodeficiency virus type 1 isolates that are syncytium inducing and acutely cytopathic for CD8+ lymphocytes. *J Virol* **78**:1243-1255.
258. **Zhang, Y. J., C. Fracasso, J. R. Fiore, A. Bjorndal, G. Angarano, A. Gringeri, and E. M. Fenyo.** 1997. Augmented serum neutralizing activity against primary human immunodeficiency virus type 1 (HIV-1) isolates in two groups of HIV-1-infected long-term nonprogressors. *J Infect Dis* **176**:1180-1187.