

Basic HIV virology

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The acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). HIV-1 was initially identified by Luc Montanier at the Institute Pasteur, Paris, in 1983 and was then more fully characterised in 1984 by Robert Gallo in Washington and Jay Levy in San Francisco. A second virus, HIV-2, was isolated from West African patients in 1986. Viruses similar to HIV-1 and HIV-2 have been isolated from chimpanzees and wild African monkeys. It is most likely that HIV-1 and HIV-2 crossed species from primates to humans in Africa several times over the last hundred years.

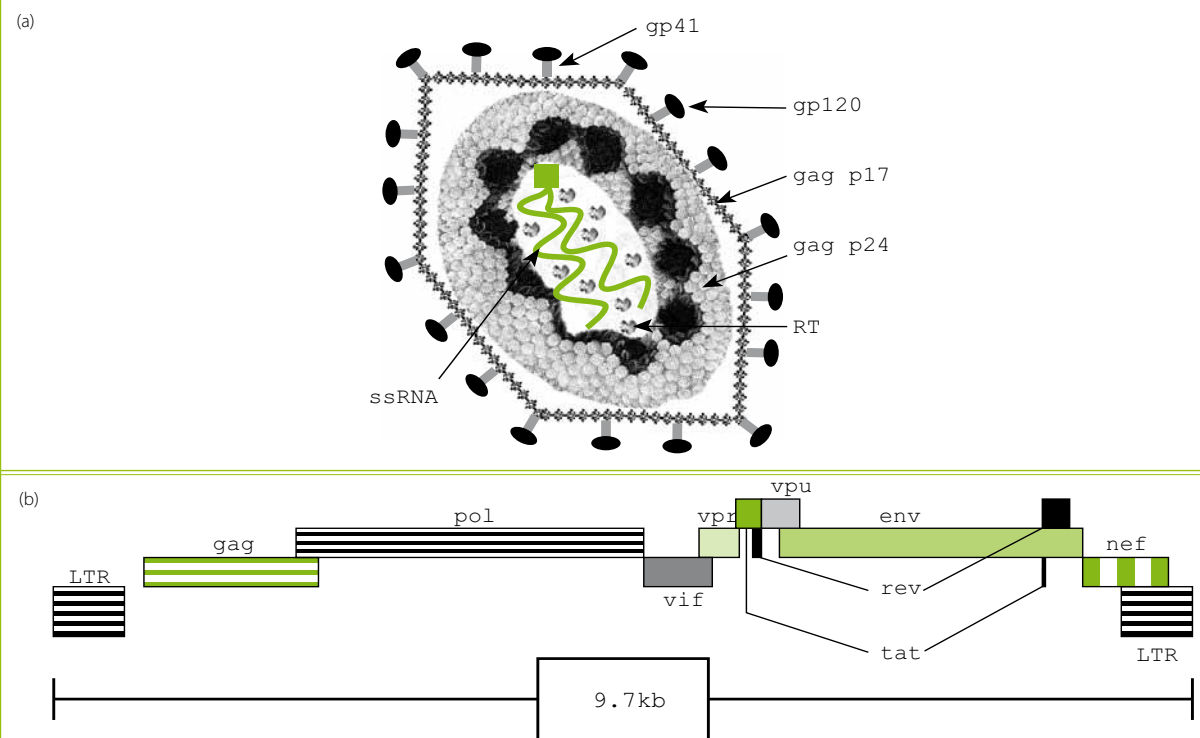
1.1 HIV structure and organisation

HIV is classified in the family *Retroviridae*, subfamily *Lentivirinae*, and genus *Lentivirus*.^{1,2,3} The structure of HIV follows the typical pattern of the retrovirus family, comprising a single-stranded, positive-sense ribonucleic acid (RNA) genome of about 9.7 kilobases. There are two strands of HIV RNA and each strand has a copy of the virus's nine genes (Figure 1.1a). The RNA is surrounded by a cone-shaped capsid which consists of approximately 2000 copies of the p24 viral protein. Surrounding the capsid is the viral envelope. The viral envelope is composed of a lipid bilayer membrane, formed from the cellular membrane

of the host cell during budding of the newly formed virus particle. Host-cell proteins, such as the major histocompatibility complex (MHC) antigens and actin, remain embedded within the viral envelope, along with the viral envelope protein. Each envelope subunit consists of two non-covalently linked membrane proteins: glycoprotein (gp) 120, the outer envelope protein, and gp41, the transmembrane protein that anchors the glycoprotein complex to the surface of the virion. The envelope protein is the most variable component of HIV, although gp120 itself is structurally divided into highly variable (V) and more constant (C) regions. The variability of V regions may be a product of envelope functionality, as has been especially well described in V3, where amino acid changes alter coreceptor use. The variability of the HIV envelope also confers a uniquely complex antigenic diversity.

The genomic organisation of HIV is extremely efficient. Use of all three reading frames (the triplet codes) of the genetic sequence permits overlapping of gene-coding regions. There are nine genes of HIV (Figure 1.1b). These encode proteins that may be broadly classified into structural, catalytic, regulatory, and accessory classes (Table 1.1).

Figure 1.1 HIV genomic structure



Notes: (a) HIV virion structure highlighting envelope (gp120, gp41) and structural (gag p17, gag p24) proteins. RT = reverse transcriptase; ssRNA = single-stranded RNA. (b) The single-stranded RNA genome of HIV efficiently encodes nine major structural and catalytic proteins by using overlapping parts of the genome. Additionally, the nucleic acid secondary and tertiary structure performs functions independent of translation. LTR = long-terminal repeat

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In addition to the functions performed by these proteins in the viral lifecycle, the nucleic acid sequence of the virus possesses intrinsic functions. For example, the Rev responsive element, within the coding region for gp41, interacts with the Rev protein to assist export of spliced RNA transcripts from the nucleus of the cell.⁴ The long-terminal repeat (LTR) region has a transcription-promoter function in the integrated deoxyribonucleic acid (DNA) provirus, and contains regions essential for reverse transcription, integration into the host-cell genome⁵ and genomic RNA dimerisation.⁶

1.2 HIV life cycle

The life cycle of a retrovirus is that of an obligatory intracellular parasite, and thus HIV can not replicate outside human cells (Figure 1.2).

Table 1.1 The nine major genes of HIV and their associated gene products

Protein class	Gene	Gene products
Structural	gag	p17 (matrix) p24 (capsid) p7 (nucleocapsid)
	env	gp120 gp41
Catalytic	pol	protease reverse transcriptase integrase
Regulatory	tat	Tat
	rev	Rev
Accessory	vpu	Vpu
	vif	Vif
	vpr	Vpr
	nef	Nef

1.2.1 HIV binding and entry

Infection of the host cell commences when HIV binds to specific receptors on the cell membrane. In general, the interaction requires the recognition of two host-cell surface-receptor proteins by the viral gp120 envelope protein. The presence or absence of these cellular proteins restricts the range of host-cell types that are susceptible to infection by a strain of HIV.

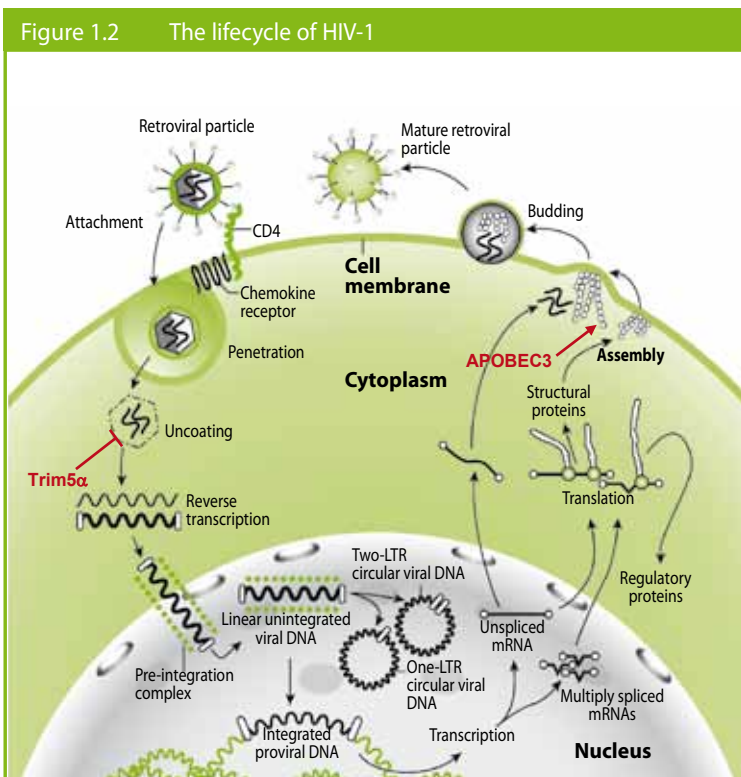
The first coreceptor described was the CD4 protein,⁷ which is present predominantly on cells of the T lymphocyte and macrophage lineages. The distribution of CD4 receptors has been thought to restrict HIV susceptibility to cells of the lymphocyte, monocyte/macrophage and other CD4-expressing lineages, although more recent studies have shown efficient viral entry into cells not expressing CD4.⁸⁻¹⁰

Subsequently, the requirement of a second coreceptor for viral entry was recognised.¹¹⁻¹³ This function may be performed by a range of proteins within the class of seven-transmembrane receptors, although the most important are CCR5 (CC chemokine receptor 5) and CXCR4 (CX chemokine receptor 4). The seven-transmembrane class of receptors is very large, containing over 100 related proteins. Several of these proteins have been shown to facilitate the binding of HIV *in vitro*.¹⁴⁻¹⁶ The *in vivo* significance of entry via these minor co-receptors, however, remains unclear.

After HIV gp120 binds to CD4 receptor and the co-receptor, a conformational change in gp41 causes insertion of the N-terminal hydrophobic fusion-peptide region into the target-cell membrane.¹⁷ This insertion results in membrane fusion and the entry of the viral particle contents into the cytoplasm, a process critically dependent upon interactions between the N- and C-terminal regions of the gp41 ectodomain. This intra-protein interaction led to the discovery of a novel class of antivirals called fusion inhibitors, e.g. T-20 (enfuvirtide) which is a short peptide that mimics the structure of the conserved C-terminal region of gp41.^{18,19,20}

Although all HIV strains will recognise and bind to CD4, affinity for either CCR5 or CXCR4 varies. These differences account for the observed distinct tropisms between HIV strains.²¹ Binding ability and tropism of the virus is dependent on the protein structure of gp120. Particular patterns of sequence of the V3 and V4 variable regions, and other regions of gp120, relate to CD4 binding and differential coreceptor affinity.²²⁻²⁵

In general, viral strains that bind to CCR5 (R5 strains) infect macrophages and T cells, and are characterised by less aggressive growth *in vitro*. Strains that recognise CXCR4 (X4 strains), by contrast, infect only T cells and T cell lines.²⁶ The growth of X4 strains *in vitro* is characterised by high viral titre and the presence of syncytial cells, which are formed by the fusion of multiple infected cells and can be observed by light microscopy. This classification, however, is by no means rigid.^{27,28} Viral replicative capacity depends on many variables apart from coreceptor use, and syncytium-forming ability may be related more to efficiency of viral growth than to tropism. At present, however, this working classification of viral strains remains broadly used.



Source: Furtado MR, Callaway DS, Phair JP, Kunstman KJ, Stanton JL, Macken CA, et al. Persistence of HIV-1 transcription in peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. *N Engl J Med* 1999;340:1614-22. Used with permission.

An important new strategy for antiretroviral therapy has been the development of agents that block HIV entry into the cell.²⁹ A number of small molecules that block binding of HIV to either CCR5 and CXCR4 have been described, several of which are now in preclinical and clinical trials.³⁰⁻³³

1.2.2 The pre-integration complex

The genetic information of HIV is contained within an RNA genome. Following infection of a new host cell, the RNA genome is first reverse transcribed into single-stranded DNA that is then further transcribed to double-stranded DNA. These two polymerase steps are performed by viral reverse transcriptase, which is copackaged in the viral particle. Self-priming of the single-stranded RNA and DNA and removal of the transcribed RNA strand occur by a complex series of steps dependent upon interactions between the viral LTR and host-cell enzymes. The double-stranded DNA genome forms a complex with host-cell and viral proteins (including matrix, integrase and Vpr) that is actively transported to the nucleus.^{34,35}

During the early steps of the HIV-1 replication cycle, the virus counteracts specific host proteins that have evolved to limit retroviral replication. One of these host cell factors is called human tripartite motif 5 alpha (TRIM5alpha). TRIM5alpha acts in the early steps of HIV-1 replication cycle, soon after the entry process and before reverse transcription.³⁶ TRIM5alpha restricts retroviral infection by specifically recognising HIV-1 capsid and promoting its rapid, premature disassembly.^{37,38} TRIM5alpha from rhesus macaques and African green monkeys inhibit HIV-1 replication, whereas the human homologue is inactive against simian immunodeficiency virus (SIV) and HIV-1, leading to the susceptibility of human cells to both viruses.^{31,39} TRIM5alpha also restricts other retroviruses such as the Moloney leukemia virus.^{37, 40-42} Variants of this factor have been detected in some patients with HIV-1 infection.⁴³ The presence of these isoforms of TRIM5alpha was associated with a relatively long asymptomatic phase, suggestive of some degree of suppression of HIV-1 replication by TRIM5alpha. Whether the polymorphisms of TRIM5alpha are associated with disease progression is debatable. A recent study has however reported that different genetic variants of TRIM5alpha may lead to different clinical outcomes.⁴⁴

1.2.3 Integration and transcription

The double-stranded HIV genome is then either randomly integrated into the host-cell genome by means of DNA splicing, performed by the viral integrase, or forms stable DNA circles.⁴⁵ The integrated form of HIV is known as the provirus and takes the form shown in Figure 1.1, with identical LTR copies flanking the coding regions. Proviral DNA is replicated as part of the normal cell genome and may persist in this form for long periods and through many rounds of mitotic cell division.

The 5' end of the LTR now functions as a promoter, regulating the production of RNA transcripts dependent on the presence of host-cell transcription factors (such as promoter-specific transcription factor, SP1, and nuclear factor - kappa beta) and the viral protein Tat.⁴⁶ The transcribed HIV RNA molecules may either be spliced in preparation for translation of viral proteins, or exported from the nucleus in an unspliced form for packaging into newly produced virions. Nuclear export of spliced RNA is assisted by the viral protein Rev.

Viral proteins perform a variety of roles to subvert normal cellular function and facilitate viral replication. Much about these processes remains poorly understood. Vpr acts to alter host-cell transcription⁴⁷ and arrest infected cells at the G2/M phase of cell division.⁴⁸ Nef induces downregulation of the CD4 receptor and MHC class I molecules.⁴⁹⁻⁵¹ Vpu promotes degradation of CD4 in the endoplasmic reticulum⁵² and Vif is necessary for subsequent efficient infectivity of the newly produced viral particles.^{53,54} Vif counteracts cytidine deaminases (enzymes present especially in macrophages and T cells) that are naturally occurring host defence mechanisms against retroviruses. These proteins include APOBEC3G and APOBEC3F and are degraded by HIV.⁵⁵

1.2.4 HIV assembly and release

Immature viral polypeptides are processed into their functional forms by the enzyme protease and assembled with full-length HIV RNA transcripts into nascent viral particles. The main structural immature viral polypeptide, gag, encodes the majority of such proteins. gag itself uses cellular proteins to make its way to the plasma membrane for assembly into the new progeny. It is now known that the p6 protein located at the C-terminus of the gag polypeptide interacts with tumour suppressor gene 101 (TSG101), an interaction which is critical for the release of the newly assembled particles.⁵⁶ Immature viral polypeptides together with the full-length HIV RNA transcripts are initially assembled into immature particles. During budding from the plasma membrane, viral proteins within these particles are processed into their functional forms by the enzyme protease and rearranged into mature particles. The Vpu protein facilitates virion release from the cell membrane in the late stage of the replication cycle.⁵⁷ Vpu interacts with a host cell factor named tetherin, an interferon-alpha-induced human protein.⁵⁸ Tetherin is an endogenous membrane associated protein that blocks release of viral particles. Without Vpu, HIV-1 particles become tethered to the cell membrane and cannot be released.

1.3 The taxonomy of HIV and the primate immunodeficiency viruses

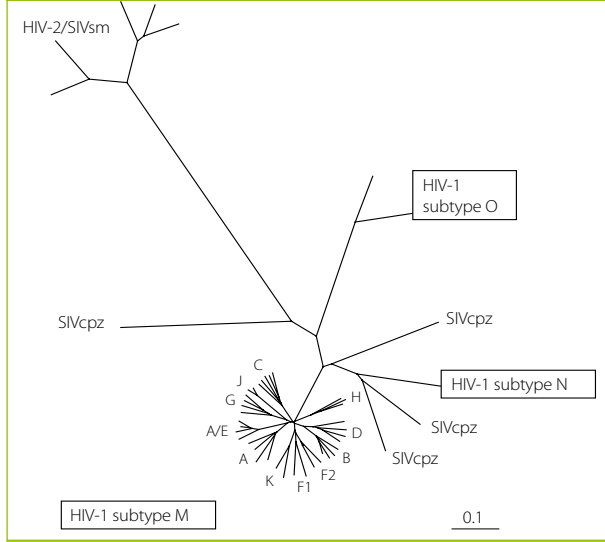
HIV is subdivided into two very broad types: HIV-1 and HIV-2 (Figure 1.3). HIV-1 is by far the most common and broadly distributed HIV type, accounting for most of HIV infections worldwide. Significant prevalence of HIV-2 is confined to western central Africa and southern and western India,^{59,60} although sporadic occurrences and transmission have been reported from many countries, including the United States of America (USA), Europe and Australia.⁶¹⁻⁶⁴

The differences between HIV types 1 and 2 reflect their distinct zoonotic origin.⁶⁵ HIV-1 is most similar to SIV strains isolated from chimpanzees and HIV-2 to those from the sooty mangabey. There is some evidence that HIV-2 results in a less virulent infection than HIV-1, with generally lower viral loads, lower rates of vertical transmission and slower progression of the disease in an individual with the infection.⁶⁶⁻⁶⁸

1.3.1 HIV-1 lineages - groups

HIV-1 is divided into three quite distinct lineages: the groups M, N and O. Again, the worldwide distribution of these groups is not equal: group M (for Main)

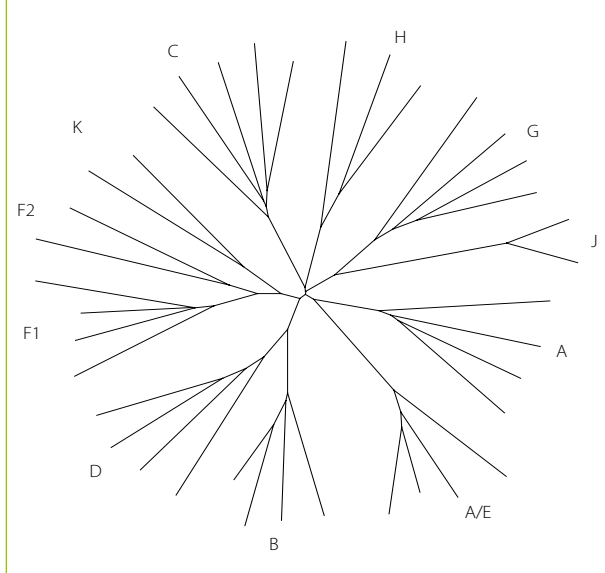
Figure 1.3 The molecular phylogeny of primate immunodeficiency viruses



Note: An unrooted phylogenetic tree estimated using an alignment of the envelope gene (gp160). HIV is classified into types 1 and 2, the latter co-segregating with simian immunodeficiency virus (SIV) strains from sooty mangabey and the former with those from chimpanzee indicating probable distinct zoonotic origins. HIV forms three groups: M, N and O. Group M contains the nine subtypes and further circulating recombinant forms that contribute to the overwhelming majority of HIV infections worldwide. (In this analysis of envelope, CRF01_AE is the only circulating recombinant strain included. The scale bar indicates 10% nucleotide sequence divergence.

strains are substantially more common in the global epidemic than the group O (Outlier) strains, which are largely confined to Africa, with sporadic cases reported elsewhere. The group N (non-M, non-O) strains have only been isolated in Cameroon.⁶⁹

Figure 1.4 Phylogeny of HIV subtype M envelope gene



Note: A tree based on an alignment of envelope gene sequences (gp120 portion) from reference-subtype strains currently recommended by the Los Alamos National Laboratory (USA) HIV Sequence Database. Group M strains are clearly segregated into subtypes A to K (without I and with the further subdivision of subtype F into sub-subtypes F1 and F2). The tree topology is star-shaped, indicating a shared common ancestor and roughly equal divergence times for these strains.

1.3.2 Subtypes and circulating recombinant forms

There are currently nine subtypes that are consistently identified as different subtypes regardless of the genomic region analysed (Figure 1.4). Additionally, several recombinant forms of the virus, the genomes of which made up of different regions from distinct subtypes, have been separately classified as circulating recombinant forms (CRFs).⁷⁰

The subtypes and CRFs show strong patterns of distribution in the global pandemic. Western countries, including Australia, continue to have an epidemic that is almost exclusively subtype B in all risk groups. Thailand originally showed a sharp segregation of subtypes between risk groups, with heterosexual transmission largely due to CRF01-AE, and injecting drug use (IDU) transmission due to subtype B; since then CRF01-AE has come to predominate in all risk groups.⁷¹⁻⁷³ India and South Africa are experiencing explosive epidemics of subtype C.^{74,75} All HIV subtypes described to date have also been detected in sub-Saharan Africa.

HIV subtype A/E was first described in Thailand as a new subtype E. Subsequent analysis of the entire genome of this form showed it to be a recombinant between subtypes A (that is most prevalent in Africa) and subtype E (that is unique). Isolates of this strain were then termed subtype A/E. As this strain is so prevalent in Asia, it was among the first to be renamed under the new nomenclature of CRFs. It is now referred to as HIV CRF01-AE.

1.3.3 Sub-subtype classification

A further refinement in the subtype classification has been made recently to distinguish strains that form distinct groups within subtypes, but are not sufficiently different to warrant classification as a novel subtype. A detailed description of the most recent HIV taxonomy has been published by the Los Alamos National Laboratory (USA) HIV Sequence Database and is continuously updated on the Laboratory's database website (<http://www.hiv.lanl.gov/content/index>).

1.3.4 Phenotypic differences between HIV subtypes

Although all HIV subtypes are similar there is some evidence that the genotypic subtype classification may have different phenotypes. Phenotypic differences between HIV subtypes are of clinical importance for the following reasons:

- transmission and viral fitness
- drug resistance
- vaccine design.

Transmission and viral fitness

Researchers and public health officials have viewed with increasing alarm the growing dominance of HIV subtype C in the global pandemic.^{76,77} Several viral characteristics suggest this particular subtype might have adapted better to transmission in human populations. The viral promoter in subtype C strains is of a different structure to that of other subtypes,⁷⁸ driving higher rates of viral replication and potentially higher levels of circulating virus in patients with the infection— leading to the potential for increased transmission. Recent studies from Thailand have shown differences between subtypes B and CRF01-AE both in level of viral replication during seroconversion,⁷⁹ and in transmissibility in a cohort of injecting drug users.⁸⁰

Drug resistance

The pattern of mutations associated with drug resistance in subtype B strains, although complex, is well defined and is used routinely in clinical practice. Several non-B subtypes commonly show genetic mutations that are associated with resistance to antiretroviral therapy in subtype B, despite no exposure to these agents.⁸¹⁻⁸³ Subtypes also differ in the rate or pattern of acquisition of resistance mutations.^{84,85}

Vaccine design

Vaccine epitopes have been identified that elicit immune responses common to multiple subtypes, but most of the candidate vaccines under development or trial have been designed to specifically protect against the HIV subtype predominantly circulating in the target population. Genetic characterisation of the prevalent subtype in each country, and each distinct sub-epidemic within some countries, is thus a fundamental first step in vaccine design.⁸⁶

1.4 The global pandemic of HIV

Despite the improved access to antiretroviral treatment, the implementation of prevention programs and development of low cost testing for early detection, the HIV epidemic is on the rise in many countries. More than 2.6 million more people were living with HIV in 2006 than in 2004. The total number of individuals with the infection in 2006 reached 39.5 million of which 17.7 million were women and 2.3 million were children under the age of 15 years. Sadly, the number of children with HIV infection accounted for 40% of the overall number of newly diagnosed people in 2006.⁸⁷ The 2007 statistics show that this number has remained almost steady. (Figures 1.5a and 1.5b).

1.4.1 North America and Western and Central Europe

In the USA, sexual activity among men who have sex with men (MSM) is still the main mode of HIV transmission (accounting for approximately 44% of new HIV infections in 2001-2004). IDU has become a more prominent route of HIV infection in

Figure 1.5 (a) Adults and children newly diagnosed with HIV infection in 2007



Figure 1.5 (b) Adults and children estimated to be living with HIV in 2007



Note: The figures above shows current statistical data provided by UNAIDS on newly diagnosed infections and the total number of people living with HIV in 2007. The Sub-Saharan region remains the most affected country in the world by the global epidemic. Of the 22.5 million people living with HIV in this region, 61% are women.

Source: Joint United nations programme on HIV/AIDS (UNAIDS). Used with permission.

the USA, where an estimated 17% of new reported HIV cases are related to this mode of transmission.⁸⁸ In Canada, the number of people with HIV infection increased by 16% with an increase in the percentage of women with the infection each year. The main mode of transmission remains MSM (46% of infections), while women represented 27% of new HIV infections in 2005, compared with 22% in 2002.⁸⁷

Throughout the world, ethnic minorities and migrant communities are unevenly affected by HIV and AIDS. In the USA, there is a higher incidence of HIV among African-Americans and Hispanics, accounting for 69% of all HIV cases.⁸⁸ It is well recognised that migrant populations are at heightened risk for HIV infection and that initiatives to prevent HIV transmission do not necessarily reach ethnic minority or migrant communities.^{89,90}

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Minority populations often miss out on mainstream prevention efforts in the countries in which they are living. They also have limited access to care and health services.⁹¹

According to the UNAIDS surveillance report for 2006, there are now 740 000 people with HIV infection living in Western and Central Europe, with more than half of these infections being acquired via heterosexual sex. Again, similar to North America, the data show an increased trend of new infections among migrant and immigrant communities. In most Central European countries, the number of people with HIV infection remains low compared to Western Europe and the number of new infections in the Baltic region (i.e. Latvia, Lithuania and Estonia) has been rising at a slower rate.

1.4.2 Australia and the South Pacific

At the end of 2006, in Australia there were 26 267 diagnoses of HIV, 10 125 diagnoses of AIDS and 6723 deaths following AIDS. The number of new HIV infections increased from 763 to 998 cases per year accounting for a 31% increase between 2000 and 2006. Within this group, the number of people born in countries with a high prevalence of HIV (i.e. sub-Saharan Africa) was five times higher than for people born in Australia.⁹²

The main route of HIV transmission in Australia remains MSM, followed by heterosexual sex and IDU. Concomitant with an increase in HIV cases among the MSM population, a significant increase in sexually transmissible disease has been observed. Acquisition by IDU is more common in Indigenous compared with non-Indigenous Australians.⁹² The predominance of subtype B in the Australian epidemic is the product of multiple introductions of the virus from the USA and Europe.⁹³

In New Zealand, the highest number of recorded HIV cases was 218 in 2005, with 51% of these cases acquired via MSM and 37% acquired via heterosexual sex. Almost 90% of these cases were acquired outside the country primarily in Asia and sub-Saharan Africa. MSM also form a major risk group for HIV acquisition in other countries of the region such as Kiribati, Vanuatu, Fiji, Samoa and the Solomon Islands, with an estimated 300 new cases reported in 2005.

Papua New Guinea is by far the most affected country by HIV in the Pacific region, with a prevalence of 1.8% or more than 57 000 adults living with HIV in 2005. Out of 7100 new cases reported within Australia, New Zealand and the Pacific Islands in 2005, three quarters were acquired in Papua New Guinea. National prevalence data are still extremely limited, because of the very small number of sentinel sites and low levels of testing outside Port Moresby. In this city, there has been a rapid increase in HIV prevalence over the last several years with only 24% of young men and 13% of women admitting to using condoms.⁸⁷ A behavioural survey that analysed samples from 1175 participants from seven different provinces found that of the 71 HIV positive cases, 33 (46%) had subtype CHIV-1 infection. Within the global pandemic, PNG subtype C isolates most closely resemble those from East Africa. The remaining 2 (6%) were subtype B and further phylogenetic analysis found no province-specific clustering among the samples.⁹⁴ Lack of awareness, poor condom use, early sexual relationship initiation, concurrent sexual relationships, transactional activity and increasing sexual violence against women, have all contributed to the growth of the epidemic in PNG according to the UNAIDS 2006 and the *Risk Behaviour and HIV Prevalence in Tanah Papua* (2006) reports, clearly suggesting an urgent need for expanded national surveillance and prevention programs.⁹⁵

1.4.3 Sub-Saharan Africa

The sub-Saharan African region accounts for 63% of people with HIV infection worldwide. The number of people with HIV infection in 2006 reached 24.7 million and the number of people who died from HIV/AIDS reached 2.1 million, accounting for 72% of HIV/AIDS related deaths worldwide.⁸⁵ According to UNAIDS surveillance data, one in five adults has HIV infection in Zimbabwe, a country where the life expectancy for women and men is 34 and 37 years, respectively, which is the lowest in the world.

One of the regions with the highest adult HIV incidence in the world is Swaziland with a prevalence of 33%, followed by Botswana, Lesotho and Namibia. In Botswana, almost 40% of pregnant women have HIV infection. In some African countries, the numbers of new infections have decreased or stabilised. For example, over the last few years in Angola the number of pregnant women diagnosed with HIV has decreased. In Malawi, in 2005, one million people were newly diagnosed with HIV. However since 2000, the overall number of new infections appears to have stabilised. This trend is reported to be the result of an increased uptake of safe sex practices and abstinence (22% of young women see abstinence as one of the best ways to prevent infection).⁸⁷ West and Central Africa have a much lower HIV prevalence compared to Southern Africa and Nigeria, which continue to report high HIV prevalence among adults.

References

- 1 Chiu IM, Yaniv A, Dahlberg JE, Gazit A, Skuntz SF, Tronick SR, et al. Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses. *Nature* 1985;317(6035):366-8.
- 2 Wain-Hobson S, Alizon M, Montagnier L. Relationship of AIDS to other retroviruses. *Nature* 1985;313(6005):743.
- 3 Vogt PK. Historical introduction to the general properties of retroviruses. In: Coffin JM, Hughes SH, Varmus HE, editors. *Retroviruses*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1997:1-27.
- 4 Le Rouzic E, Benichou S. The Vpr protein from HIV-1: distinct roles along the viral life cycle. *Retrovirology* 2005;2:11-25.
- 5 Granoff A, Webster RG, editors. *Encyclopedia of virology*. 2nd edition. San Diego: Academic. Press, 1999.
- 6 Paillart JC, Shehu-Xhilaga M, Marquet R, Mak J. Dimerization of retroviral RNA genomes: an inseparable pair. *Nat Rev Microbiol* 2004;2(6):461-72.
- 7 McDougal JS, Nicholson JK, Cross GD, Cort SP, Kennedy MS, Mawle AC. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. *J Immunol* 1986;137(9):2937-44.
- 8 Borsetti A, Parolin C, Ridolfi B, Sernicola L, Geraci A, Ensoli B, et al. CD4-independent infection of two CD4(-)/CCR5(-)/CXCR4(+) pre-T-cell lines by human and simian immunodeficiency viruses. *J Virol* 2000;74(14):6689-94.
- 9 Liu HY, Soda Y, Shimizu N, Haraguchi Y, Jinno A, Takeuchi Y, et al. CD4-dependent and CD4-independent utilization of coreceptors by human immunodeficiency viruses type 2 and simian immunodeficiency viruses. *Virology* 2000;278(1):276-88.
- 10 Saha K, Zhang J, Gupta A, Dave R, Yimen M, Zerhouni B. Isolation of primary HIV-1 that target CD8+ T lymphocytes using CD8 as a receptor. *Nat Med* 2001;7(1):65-72.
- 11 Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, et al. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 1996;85(7):1135-48.

- 12 Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, et al. Identification of a major coreceptor for primary isolates of HIV-1. *Nature* 1996;381(6584):661-6.
- 13 Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CCR-5. *Nature* 1996;381(6584):667-73.
- 14 Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, Peiper SC, et al. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* 1996;85(7):1149-58.
- 15 Hoffman TL, Stephens EB, Narayan O, Doms RW. HIV type 1 envelope determinants for use of the CCR2b, CCR3, STRL33, and APJ coreceptors. *Proc Natl Acad Sci USA* 1998 ;95(19):11360-5.
- 16 Zhang L, He T, Huang Y, Chen Z, Guo Y, Wu S, et al. Chemokine coreceptor usage by diverse primary isolates of human immunodeficiency virus type 1. *J Virol* 1998;72(11):9307-12.
- 17 Eckert DM, Kim PS. Mechanisms of viral membrane fusion and its inhibition. *Annu Rev Biochem* 2001;70:777-810.
- 18 Kilby JM, Hopkins S, Venetta TM, DiMassimo B, Cloud GA, Lee JY, et al. Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nat Med* 1998;4(11):1302-7.
- 19 Robertson D. US FDA approves new class of HIV therapeutics. *Nat Biotechnol* 2003;21(5):470-1.
- 20 Este JA, Telenti A. HIV entry inhibitors. *Lancet* 2007;370(9581):81-8.
- 21 Berger EA, Murphy PM, Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu Rev Immunol* 1999;17:657-700.
- 22 Rizzuto CD, Wyatt R, Hernandez-Ramos N, Sun Y, Kwong PD, Hendrickson WA, et al. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science* 1998 ;280(5371):1949-53.
- 23 Groenink M, Fouchier RA, Broersen S, Baker CH, Koot M, van't Wout AB, et al. Relation of phenotype evolution of HIV-1 to envelope V2 configuration. *Science* 1993;260(5113):1513-6.
- 24 De Jong JJ, De Ronde A, Keulen W, Tersmette M, Goudsmit J. Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the syncytium-inducing phenotype: analysis by single amino acid substitution. *J Virol* 1992;66(11):6777-80.
- 25 Hoffman NG, Seillier-Moiseiwitsch F, Ahn J, Walker JM, Swanstrom R. Variability in the human immunodeficiency virus type 1 gp120 Env protein linked to phenotype-associated changes in the V3 loop. *J Virol* 2002 ;76(8):3852-64.
- 26 Cho MW, Lee MK, Carney MC, Berson JF, Doms RW, Martin MA. Identification of determinants on a dualtropic human immunodeficiency virus type 1 envelope glycoprotein that confer usage of CXCR4. *J Virol* 1998;72(3):2509-15.
- 27 Cheng-Mayer C, Liu R, Landau NR, Stamatatos L. Macrophage tropism of human immunodeficiency virus type 1 and utilization of the CC-CCR5 coreceptor. *J Virol* 1997;71(2):1657-61.
- 28 Yu X, McLane MF, Ratner L, O'Brien W, Collman R, Essex M, et al. Killing of primary CD4+ T cells by non-syncytium-inducing macrophage-tropic human immunodeficiency virus type 1. *Proc Natl Acad Sci USA* 1994;91(21):10237-41.
- 29 Strizki J. Targeting HIV attachment and entry for therapy. *Adv Pharmacol* 2008;56:93-120.
- 30 Dorr P, Westby M, Dobbs S, Griffin P, Irvine B, Macartney M, et al. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob Agents Chemother* 2005;49(11):4721-32.
- 31 Fatkenheuer G, Pozniak AL, Johnson MA, Plettenberg A, Staszewski S, Hoepelman AI, et al. Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat Med* 2005;11(11):1170-2.
- 32 Seto M, Aikawa K, Miyamoto N, Aramaki Y, Kanzaki N, Takashima K, et al. Highly potent and orally active CCR5 antagonists as anti-HIV-1 agents: synthesis and biological activities of 1-benzazocine derivatives containing a sulfoxide moiety. *J Med Chem* 2006;49(6):2037-48.
- 33 Schurmann D, Fatkenheuer G, Reynes J, Michelet C, Raffi F, van Lier J, et al. Antiviral activity, pharmacokinetics and safety of vicriviroc, an oral CCR5 antagonist, during 14-day monotherapy in HIV-infected adults. *AIDS* 2007;21(10):1293-9.
- 34 Gallay P, Hope T, Chin D, Trono D. HIV-1 infection of nondividing cells through the recognition of integrase by the importin/karyopherin pathway. *Proc Natl Acad Sci USA* 1997;94(18):9825-30.
- 35 Fouchier RA, Malim MH. Nuclear import of human immunodeficiency virus type-1 preintegration complexes. *Adv Virus Res* 1999;52:275-99.
- 36 Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 2004;427(6977):848-53.
- 37 Stremlau M, Perron M, Lee M, Li Y, Song B, Javanbakht H, et al. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. *Proc Natl Acad Sci USA* 2006;103(14):5514-9.
- 38 Sakuma R, Noser JA, Ohmine S, Ikeda Y. Rhesus monkey TRIM5alpha restricts HIV-1 production through rapid degradation of viral Gag polyproteins. *Nat Med* 2007;13(5):631-5.
- 39 Zhang F, Perez-Caballero D, Hatzioannou T, Bieniasz PD. No effect of endogenous TRIM5alpha on HIV-1 production. *Nat Med* 2008;14(3):235-6.
- 40 Perron MJ, Stremlau M, Lee M, Javanbakht H, Song B, Sodroski J. The human TRIM5alpha restriction factor mediates accelerated uncoating of the N-tropic murine leukemia virus capsid. *J Virol* 2007;81(5):2138-48.
- 41 Hatzioannou T, Perez-Caballero D, Yang A, Cowan S, Bieniasz PD. Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5alpha. *Proc Natl Acad Sci USA* 2004;101(29):10774-9.
- 42 Keckesova Z, Ylinen LM, Towers GJ. The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities. *Proc Natl Acad Sci USA* 2004;101(29):10780-5.
- 43 Kootstra NA, Navis M, Beugeling C, van Dort KA, Schuitemaker H. The presence of the Trim5alpha escape mutation H87Q in the capsid of late stage HIV-1 variants is preceded by a prolonged asymptomatic infection phase. *AIDS* 2007;21(15):2015-23.
- 44 van Manen D, Rits MA, Beugeling C, van Dort K, Schuitemaker H, Kootstra NA. The Effect of Trim5 Polymorphisms on the Clinical Course of HIV-1 Infection. *PLoS Pathog* 2008;4(2):e18.
- 45 Bushman FD, Fujiwara T, Craigie R. Retroviral DNA integration directed by HIV integration protein *in vitro*. *Science* 1990;249(4976):1555-8.
- 46 Nekhai S, Jeang KT. Transcriptional and post-transcriptional regulation of HIV-1 gene expression: role of cellular factors for Tat and Rev. *Future Microbiol* 2006;1:417-26.
- 47 Cohen EA, Dehni G, Sodroski JG, Haseltine WA. Human immunodeficiency virus vpr product is a virion-associated regulatory protein. *J Virol* 1990;64(6):3097-9.
- 48 Poon B, Grovit-Ferbas K, Stewart SA, Chen IS. Cell cycle arrest by Vpr in HIV-1 virions and insensitivity to antiretroviral agents. *Science* 1998;281(5374):266-9.
- 49 Garcia JV, Miller AD. Serine phosphorylation-independent downregulation of cell-surface CD4 by nef. *Nature* 1991;350(6318):508-11.

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- 50 Greenway AL, McPhee DA, Grgacic E, Hewish D, Lucantoni A, Macreadie I, et al. Nef 27, but not the Nef 25 isoform of human immunodeficiency virus-type 1 pNL4.3 down-regulates surface CD4 and IL-2R expression in peripheral blood mononuclear cells and transformed T cells. *Virology* 1994;198(1):245-56.
- 51 Schwartz O, Marechal V, Le Gall S, Lemonnier F, Heard JM. Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. *Nat Med* 1996;2(3):338-42.
- 52 Willey RL, Maldarelli F, Martin MA, Strebel K. Human immunodeficiency virus type 1 Vpu protein induces rapid degradation of CD4. *J Virol* 1992;66(12):7193-200.
- 53 Strebel K, Daugherty D, Clouse K, Cohen D, Folks T, Martin MA. The HIV 'A' (sor) gene product is essential for virus infectivity. *Nature* 1987;328(6132):728-30.
- 54 Fisher AG, Ensoli B, Ivanoff L, Chamberlain M, Petteway S, Ratner L, et al. The sor gene of HIV-1 is required for efficient virus transmission *in vitro*. *Science* 1987;237(4817):888-93.
- 55 Huthoff H, Malim MH. Cytidine deamination and resistance to retroviral infection: towards a structural understanding of the APOBEC proteins. *Virology* 2005;334(2):147-53.
- 56 Bieniasz PD. Late budding domains and host proteins in enveloped virus release. *Virology* 2006;344(1):55-63.
- 57 Strebel K, Klimkait T, Martin MA. A novel gene of HIV-1, vpu, and its 16-kilodalton product. *Science* 1988;241(4870):1221-3.
- 58 Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 2008;451(7177):425-30.
- 59 Berry N, Ariyoshi K, Jaffar S, Sabally S, Corrah T, Tedder R, et al. Low peripheral blood viral HIV-2 RNA in individuals with high CD4 percentage differentiates HIV-2 from HIV-1 infection. *J Hum Virol* 1998;1(7):457-68.
- 60 Kumar S. India has the largest number of people infected with HIV. *Lancet* 1999;353:48.
- 61 Downie JC, Dwyer DE, Kazazi F, Chew CB, Dowton DN, Randle C, et al. Identification of infection of an Australian resident with the human immunodeficiency virus type 2 (HIV-2). *Med J Aust* 1992;157(6):415-7.
- 62 van der Ende ME, Schutten M, Ly TD, Gruters RA, Osterhaus AD. HIV-2 infection in 12 European residents: virus characteristics and disease progression. *AIDS* 1996;10(14):1649-55.
- 63 Sullivan MT, Guido EA, Metler RP, Schable CA, Williams AE, Stramer SL. Identification and characterization of an HIV-2 antibody-positive blood donor in the United States. *Transfusion* 1998;38(2):189-93.
- 64 Damond F, Apetrei C, Robertson DL, Souquiere S, Lepretre A, Matheron S, et al. Variability of human immunodeficiency virus type 2 (HIV-2) infecting patients living in France. *Virology* 2001;280(1):19-30.
- 65 Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, et al. Origin of HIV-1 in the chimpanzee *Pan troglodytes*. *Nature* 1999;397(6718):436-41.
- 66 Ariyoshi K, Jaffar S, Alabi AS, Berry N, Schim van der Loeff M, Sabally S, et al. Plasma RNA viral load predicts the rate of CD4 T cell decline and death in HIV-2-infected patients in West Africa. *AIDS* 2000;14(4):339-44.
- 67 O'Donovan D, Ariyoshi K, Milligan P, Ota M, Yamuah L, Sarge-Njie R, et al. Maternal plasma viral RNA levels determine marked differences in mother-to-child transmission rates of HIV-1 and HIV-2 in The Gambia. MRC/Gambia Government/University College London Medical School working group on mother-child transmission of HIV. *AIDS* 2000;14(4):441-8.
- 68 Berry N, Ariyoshi K, Balfe P, Tedder R, Whittle H. Sequence specificity of the human immunodeficiency virus type 2 (HIV-2) long terminal repeat u3 region *in vivo* allows subtyping of the principal HIV-2 viral subtypes a and b. *AIDS Res Hum Retroviruses* 2001;17(3):263-7.
- 69 Simon F, Mauclere P, Roques P, Loussert-Ajaka I, Muller-Trutwin MC, Saragosti S, et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. *Nat Med* 1998;4(9):1032-7.
- 70 Peeters M. Recombinant HIV sequences: Their Role in Global E Epidemic. In: Kuiken C, Foley B, Hahn B, et al. (eds). *HIV Sequence Compendium*. Los Alamos: Theoretical Biology and Biophysics Group, Los Alamos national laboratory. 2000:39-54.
- 71 Ou CY, Takebe Y, Weniger BG, Luo CC, Kalish ML, Auwanit W, et al. Independent introduction of two major HIV-1 genotypes into distinct high-risk populations in Thailand. *Lancet* 1993;341(8854):1171-4.
- 72 Mastro TD, Kunanusont C, Dondero TJ, Wasi C. Why do HIV-1 subtypes segregate among persons with different risk behaviors in South Africa and Thailand? *AIDS* 1997;11(1):113-6.
- 73 McCutchan FE, Viputtigul K, de Souza MS, Carr JK, Markowitz LE, Buapunth P, et al. Diversity of envelope glycoprotein from human immunodeficiency virus type 1 of recent seroconverters in Thailand. *AIDS Res Hum Retroviruses* 2000;16(8):801-5.
- 74 Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, et al. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *J Virol* 1999;73(1):152-60.
- 75 Van Harmelen JH, Van der Ryst E, Loubser AS, York D, Madurai S, Lyons S, et al. A predominantly HIV type 1 subtype C-restricted epidemic in South African urban populations. *AIDS Res Hum Retroviruses* 1999;15(4):395-8.
- 76 Novitsky VA, Montano MA, McLane MF, Renjifo B, Vannberg F, Foley BT, et al. Molecular cloning and phylogenetic analysis of human immunodeficiency virus type 1 subtype C: a set of 23 full-length clones from Botswana. *J Virol* 1999;73(5):4427-32.
- 77 Oelrichs RB, Shrestha IL, Anderson DA, Deacon NJ. The explosive human immunodeficiency virus type 1 epidemic among injecting drug users of Kathmandu, Nepal, is caused by a subtype C virus of restricted genetic diversity. *J Virol* 2000;74(3):1149-57.
- 78 Montano MA, Nixon CP, Ndung'u T, Bussmann H, Novitsky VA, Dickman D, et al. Elevated tumor necrosis factor-alpha activation of human immunodeficiency virus type 1 subtype C in Southern Africa is associated with an NF-kappaB enhancer gain-of-function. *J Infect Dis* 2000;181(1):76-81.
- 79 Hu DJ, Vanichseni S, Mastro TD, Raktham S, Young NL, Mock PA, et al. Viral load differences in early infection with two HIV-1 subtypes. *AIDS* 2001;15(6):683-91.
- 80 Hudgens MG, Longini IM, Jr., Vanichseni S, Hu DJ, Kitayaporn D, Mock PA, et al. Subtype-specific transmission probabilities for human immunodeficiency virus type 1 among injecting drug users in Bangkok, Thailand. *Am J Epidemiol* 2002;155(2):159-68.
- 81 Descamps D, Apetrei C, Collin G, Damond F, Simon F, Brun-Vezinet F. Naturally occurring decreased susceptibility of HIV-1 subtype G to protease inhibitors. *AIDS* 1998;12(9):1109-11.
- 82 Vergne L, Peeters M, Mpoudi-Ngole E, Bourgeois A, Liegeois F, Toure-Kane C, et al. Genetic diversity of protease and reverse transcriptase sequences in non-subtype-B human immunodeficiency virus type 1 strains: evidence of many minor drug resistance mutations in treatment-naive patients. *J Clin Microbiol* 2000;38(11):3919-25.
- 83 Fonjungo PN, Mpoudi EN, Torimiro JN, Alemnji GA, Eno LT, Lyonga EJ, et al. Human immunodeficiency virus type 1 group m protease in Cameroon: genetic diversity and protease inhibitor mutational features. *J Clin Microbiol* 2002;40(3):837-45.
- 84 Julg B, Goebel FD. HIV genetic diversity: any implications for drug resistance? *Infection* 2005;33(4):299-301.
- 85 Geretti AM. HIV-1 subtypes: epidemiology and significance for HIV management. *Curr Opin Infect Dis* 2006;19(1):1-7.

- 86 Hurwitz JL, Zhan X, Brown SA, Bonsignori M, Stambas J, Lockey TD, et al. HIV-1 vaccine development: tackling virus diversity with a multi-envelope cocktail. *Front Biosci* 2008;13:609-20.
- 87 Joint United nations programme on HIV/AIDS (UNAIDS). 2006 Report on the global AIDS epidemic Geneva. 2006.
- 88 Centre for Disease Control and Prevention. HIV/AIDS Surveillance Report, 2005. Vol 17. Rev ed. Atlanta: US Department of Health and Human Services, CDC: 2007. Available at: <http://www.cdc.gov/hiv/topics/surveillance/resources/reports/2005report/default.htm> (cited October 2008)
- 89 Quinn TC. Population migration and the spread of types 1 and 2 human immunodeficiency viruses. *Proc Natl Acad Sci USA* 1994;91(7):2407-14.
- 90 McMunn AM, Mwanje R, Paine K, Pozniak AL. Health service utilization in London's African migrant communities: implications for HIV prevention. *AIDS Care* 1998 ;10(4):453-62.
- 91 Haour-Knipe M. Migration and ethnicity issues. Vancouver Conference Review. *AIDS Care* 1997;9(1):115-9.
- 92 National Centre in HIV Epidemiology and Clinical Research. HIV/AIDS, viral hepatitis and sexually transmissible infections in Australia. Annual Surveillance Report 2006. Sydney: National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, 2008. Available at: [http://www.nchechr.unsw.edu.au/NCHECRweb.nsf/resources/SurvReports_4/\\$file/ASR2008-v2.pdf](http://www.nchechr.unsw.edu.au/NCHECRweb.nsf/resources/SurvReports_4/$file/ASR2008-v2.pdf) (cited February 2009).
- 93 Oelrichs RB, Lawson VA, Coates KM, Chatfield C, Deacon NJ, McPhee DA. Rapid full-length genomic sequencing of two cytopathically heterogeneous Australian primary HIV-1 isolates. *J Biomed Sci* 2000;7(2):128-35.
- 94 Ryan CE, Gare J, Crowe SM, Wilson K, Reeder JC, Oelrichs RB. The Heterosexual HIV Type 1 Epidemic in Papua New Guinea is dominated by Subtype C. *AIDS Res Hum Retroviruses* 2007;23:7: 941-4
- 95 Oelrichs RB, Crowe SM. The molecular epidemiology of HIV-1 in South and East Asia. *Curr HIV Res* 2003;1(2):239-48.

