HUMAN IMMUNODEFICIENCY VIRUSES

1. Exposure Data

1.1 Structure, taxonomy and biology

The human immunodeficiency virus type 1 (HIV-1) was discovered in 1983 (Barré-Sinoussi *et al.*, 1983) and firmly associated with the acquired immunodeficiency syndrome (AIDS) in 1984 (Gallo *et al.*, 1984). Later, a second virus was discovered in West Africa (HIV-2) that was sufficiently different from HIV-1 in its serological and molecular characteristics to be considered a separate, but related, virus (Clavel *et al.*, 1986). Initially the virus was referred to as lymphadenopathy-associated virus (LAV) or human T-cell lymphotropic virus type III (HTLV-III); the name human immunodeficiency virus was established in 1986. Between 1985 and 1989, several non-human primates were shown to harbour related retroviruses. All of these retroviruses belong to the lentivirus subfamily, have an RNA genome and replicate via a DNA intermediate (a 'provirus') by means of a viral RNA-directed DNA polymerase, more commonly called reverse transcriptase (RT). It is this 'backward' transfer of genetic information from RNA to DNA which classifies these viruses as retroviruses. HIV-1 and HIV-2 are the only known human lentiviruses.

1.1.1 *Structure*

All retroviruses share a similar overall morphology, but there is variation in detail (Table 1). Lentiviruses contain a diploid, single-stranded RNA genome within a protein core. Each HIV-1 virion measures approximately 120 nm in diameter and has a condensed cylindrical core surrounded by a lipid membrane. The inter-relationship of the genomic RNA, core proteins and surrounding viral envelope is schematically represented in Figure 1. The viral core is a complex made up of RT (p55/66), endonuclease or integrase (IN; p32), protease (PR; p10, p12 or p15[1]), and nucleocapsid proteins (NC; p6 and p7) and two copies of positive strand viral RNA, all of which is surrounded by an icosahedral capsid protein (CA; p24). The myristoylated matrix protein (MA; p17) lies just below the lipid bilayer which surrounds the virion. Embedded within the lipid bilayer are the viral envelope glycoproteins: the external surface glycoprotein (SU; gp120) and the transmembrane glycoprotein (TM; gp41), which are non-covalently associated on the virion surface (Gelderblom, 1991; Barker *et al.*, 1995).

[1]According to different researchers

Table 1. Morphological features of retroviruses

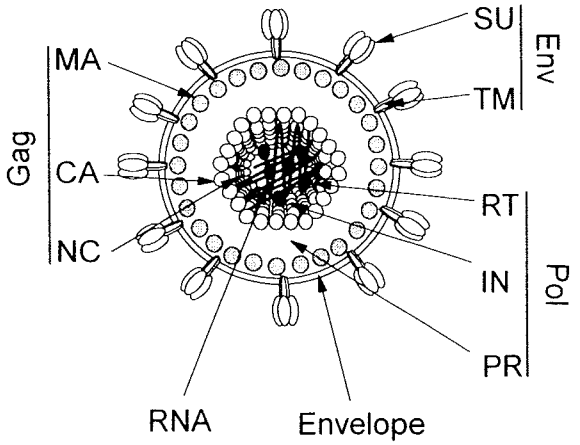
Classification	Morphological features	Examples
Oncoviruses		
A-type	Non-infectious, electron-dense, double shell, electron-lucent centre Intracytoplasmic particles: assembled core particles in B- or D-type infections Intracisternal particles: unknown function	Precursor of MMTV
B-type	Immature doughnut-shaped cores form prior to budding. Mature cores are located eccentrically within virus particles bearing prominent envelope spikes.	MMTV
C-type	No intracytoplasmic structures, immature cores; electron-lucent centres form simultaneously with budding. A centrally located electron-dense spherical core forms after maturation. Envelope spikes not always visible	MLV, ALV, FeLV, HTLVs, STLVs, BLV, GALV, SSAV, SNV
D-type	Ring-shaped immature cores; electron-lucent centres form prior to budding. Electron-dense, eccentrically located cores form on maturation. Less prominent spikes than MMTV	MPMV (SRV-2) Other SRVs
Lentiviruses	Immature cores form simultaneously with budding. Upon maturation, conical shaped cores are formed.	MVV, HIV-1, HIV-2, SIV, FIV
Spumaviruses	Electron-lucent cores form in the cytoplasm, which bud into extracellular medium or intracytoplasmic vacuoles. Very prominent envelope spikes	HFV, SFVs

MMTV, mouse mammary tumour virus; MLV, murine leukaemia virus; ALV, avian leukosis/sarcoma virus; FeLV, feline leukaemia virus; HTLV, human T-cell lymphotropic virus; STLV, simian T-cell lymphotropic virus; BLV, bovine leukaemia virus; GALV, gibbon ape leukaemia virus; SSAV, simian sarcoma-associated virus; SNV, spleen necrosis virus; MPMV, Mason–Pfizer monkey virus; SRV, simian retrovirus; MVV, maedi-visna virus; HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; FIV, feline immunodeficiency virus; HFV, human foamy virus; SFV, simian foamy virus
Adapted from Weiss *et al.* (1985); Coffin (1996)

1.1.2 *Taxonomy*

Traditionally, retroviruses (family *Retroviridae*) have been classified according to a combination of criteria including disease association, morphology and cytopathic effects *in vitro* (Table 1; Weiss *et al.*, 1985). On this basis three subfamilies were defined. The oncoviruses (Greek, *onkos* = mass, swelling) consist of four morphological subtypes which are associated with tumours in naturally or experimentally infected animals, and non-oncogenic related viruses. The second group, the lentiviruses (Latin, *lentus* = slow), cause a variety of diseases including immunodeficiency and wasting syndromes, usually after a long period of clinical latency. The third subfamily, the spumaviruses (Latin, *spuma* = foam), so called because of the characteristic 'foamy' appearance induced in infected cells *in vitro*, have not been conclusively linked to any disease (Schweizer *et al.*, 1994; Ali *et al.*, 1996).

Figure 1. Schematic representation of a mature retrovirus particle



Genomic RNA is contained within a core consisting of NC and CA proteins, along with RT and IN enzymes which are required for the formation of an integrated provirus following infection of a new target cell. MA is thought to be associated with the inner face of the lipid envelope by virtue of N-terminal myristoylation and basic amino acids, although a proportion may be associated with the viral core in some cases (see text). The lipid envelope is traversed by TM oligomers to which are bound SU proteins containing receptor recognition motifs. TM may also contact MA on the inner face of the envelope. The particle is also assumed to contain PR, since Gag and Pol proteins are incorporated into particles as polyprotein precursors, and mature morphology is achieved only after proteolytic processing.

NC, nucleocapsid; CA, capsid; RT, reverse transcriptase; IN, integrase (endonuclease); MA, matrix; TM, transmembrane; PR, protease; SU, surface

More recently, the International Committee on the Taxonomy of Viruses has divided the *Retroviridae* family into seven genera on the basis of genetic structure. The lentiviruses and spumaviruses each constitute a genus; the oncoviruses have been subdivided into five genera.

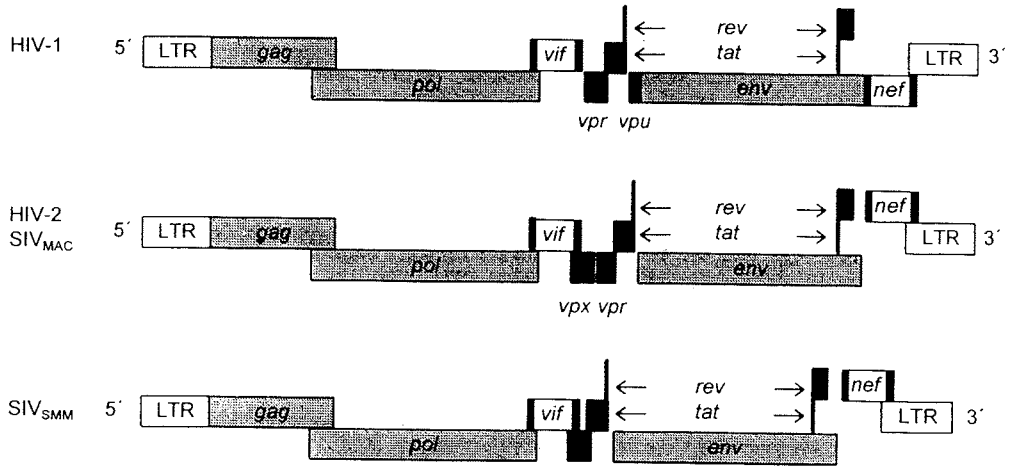
In addition to their morphological classification, retroviruses have been described as 'simple' or 'complex' according to their genome organization (Cullen, 1993; Figure 2). The defining feature of complex retroviruses is that in addition to *gag*, *pol* and *env* structural genes, they encode genes which regulate expression of structural genes (see Section 1.1.7). Most non-human and human primate lentivirus, oncovirus and spumavirus isolates so far analysed are complex retroviruses (Wilkenson *et al.*, 1994).

1.1.3 *Phylogeny*

(*a*) *Phylogenetic relationship of HIV-1 and HIV-2 to other retroviruses*

Several lentiviruses have been identified in various species of non-human primates as well as in other mammalian species. Genetically distinct simian immunodeficiency

Figure 2. Genomic organization of human and primate lentiviruses



Each genome is between 9 and 10 kb in length and has a similar overall organization of structural genes: *gag*, *pol*, *env* (grey), regulatory genes (*tat*, *rev*) and accessory genes (*nef*, *vif*, *vpr*, *vpx*, *vpu*) (black). The *vpu* gene is found exclusively in HIV-1, while HIV-2 and the closely related SIVs (SIV_{MAC}, SIV_{SMM}) have an additional gene, *vpx*. The genome is flanked by identical long terminal repeat (LTR) sequences (white).

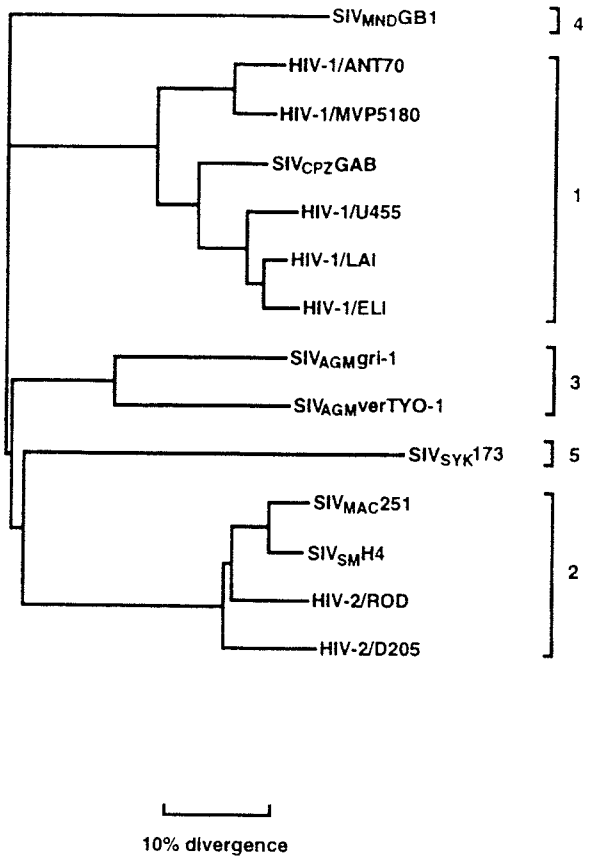
viruses (SIV) have been isolated from African green monkeys (*Cercopithecus aethiops*; SIV_{AGM}) (Kraus *et al.*, 1989), sooty mangabeys (*Cercocebus atys*; SIV_{SMM}) (Chen *et al.*, 1995; 1996), mandrills (*Mandrillus sphynx*; SIV_{MND}) (Tsujimoto *et al.*, 1988), Sykes' monkeys (*Cercopithecus mitis*; SIV_{SYK}) (Emau *et al.*, 1991) and chimpanzees (*Pan troglodytes*; SIV_{CPZ}) (Peeters *et al.*, 1989). The first primate lentivirus to be identified was SIV_{MAC} at the New England Regional Primate Research Center following an outbreak of lymphoma in rhesus (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*) (Daniel *et al.*, 1985). SIV_{MAC} is not naturally found in Asian macaques (*Macaca mulatta*) (Lowenstine *et al.*, 1986; Wu *et al.*, 1991), but its close relationship to SIV_{SMM} can be explained by the introduction of SIV_{SMM}-infected mangabeys into primate centres in the United States during the late 1960s and subsequent transfer of SIV into macaques. Each SIV appears to be endemic to the respective monkey species and none has yet been associated with disease in the natural host (Gardner *et al.*, 1994).

Both the human and non-human primate immunodeficiency viruses exist as quasi-species (Wain-Hobson, 1993), i.e., as a population of closely related, yet genetically distinct, viruses which co-exist simultaneously in each infected host. This is a consequence of the sequence diversity generated from the high rates of nucleotide evolution (Coffin, 1986; Hahn *et al.*, 1986). The latter results from a combination of the high error rate associated with RT activity during viral RNA transcription (Ricchetti & Buc, 1990), the extremely high turnover and the ability of retroviruses to undergo recombination (Zhang & Temin, 1994).

Comparison of structural gene sequence data for human and simian lentiviruses has allowed analysis of the evolutionary relationships of these viruses. Basing a phylogenetic analysis on *pol* gene sequences, the primate lentiviruses form five distinct and approximately equidistant lineages: (1) HIV-1 and SIV_{CPZ}, (2) HIV-2, SIV_{SMM} and

SIV_{MAC}, (3) SIV_{AGM}, (4) SIV_{MND} and (5) SIV_{SYK} (Figure 3). Extensive genetic diversity exists within the lineages 1–3. For example, HIV-1 falls into two distinct groups and diverse isolates of HIV-2 constitute another independent group. Diversity within HIV-1 is discussed below. Interestingly, the two HIVs are more closely related to the nearest primate viruses than they are to one another: HIV-1 to SIV_{CPZ} and HIV-2 to SIV_{SMM} (Hirsch *et al.*, 1989; Huet *et al.*, 1990).

Figure 3. Phylogenetic relationships of representative primate lentiviruses, derived from *pol* protein sequences



Numbered brackets at the right indicate the five major lineages. Horizontal branch lengths are drawn to scale: the *bar* indicates 0.10 amino acid replacements per site. The approximate position of the root of the tree (at the left) was determined from analyses using nonprimate lentiviruses as outgroups. The precise order of branching of the five major lineages (near the root) is unclear, but bootstrap values for all other nodes (with the exception of the branching order of $HIV\text{-}2_{D205}$ and $HIV\text{-}2_{ROD}$) are in the range 99–100%.

From Robertson *et al.* (1995)

An SIV_{SMM} evolutionary provenance for HIV-2 is supported by their gene sequence relatedness (Gao *et al.*, 1992) and by ecological and social considerations: sooty mangabeys, of which 30% are SIV-infected, are indigenous to West Africa, where HIV-2 is

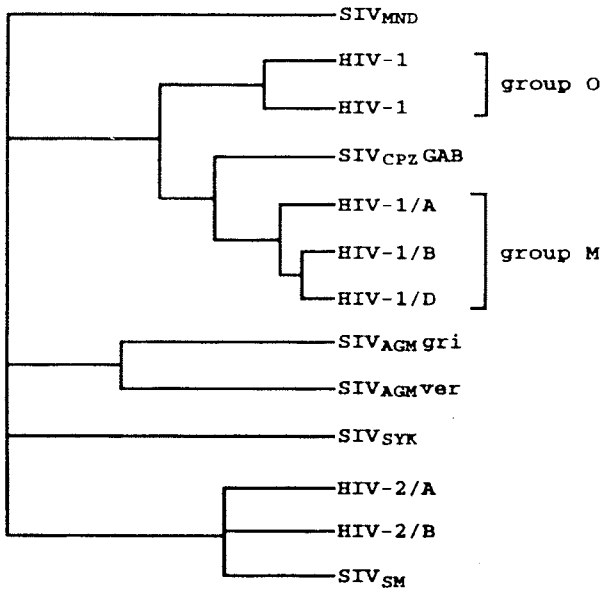
endemic. The human population is frequently exposed to SIV-infected monkey blood, since sooty mangabeys are hunted for food and kept as pets. Genetic characterization of diverse SIV_{SMM} isolates collected from a feral sooty mangabey troop suggests that each HIV-2 subtype found in West Africa originated from widely divergent strains of the simian virus, transmitted by multiple cross-species events in the same geographical region (Chen *et al.*, 1996). Since most chimpanzees in the wild appear to be seronegative for SIV_{CPZ}, there is less evidence for a similar transfer of HIV-1 from chimpanzees.

(*b*) *Relationship of HIV-1 and HIV-2 isolates to one another*

(i) *Genotypes*

Sequence analysis of the *env*, *gag* and *tat* genes from diverse geographical isolates of HIV-1 has revealed that the sequences cluster into two major groups: M, into which all the earliest known isolates fall, and a genetically distant and more diverse group containing more than 35% nucleotide differences, termed 'O' for outlier (Gürtler *et al.*, 1994; van den Haesevelde *et al.*,1994). Phylogenetic analyses of Group M sequences have revealed eight subgroups, designated A through H, also called clades (Greek, *klados* = branch) (Myers *et al.*, 1991) or sequence subtypes (Myers, 1993). The term 'genotype' has also been used (Ou *et al.*, 1993) to describe a distinct cluster of genetically related variants within a subtype (McCutchan *et al.*, 1991, 1992; Bobkov *et al.*, 1996) (see Figure 4).

Figure 4. Phylogeny of primate lentiviruses



Within the HIV-1 group M there are at least eight different sequence subtypes (A–H), of which just three are shown; within the HIV-2 group there are five known subtypes (A–E) and within the SIV_{AGM} group there are four lineages.
From Sharp *et al.* (1995)

Clade B is widespread and dominant (almost exclusively) in homosexual men and intravenous drug users throughout North America (Jain *et al.*, 1994) and Europe. With

the exception of F, clades A-H have been identified in sub-Saharan Africa (Jain *et al.*, 1994). Clade F has been identified only in Brazil and Romania (Dumitrescu *et al.*, 1994). Clade E is currently being transmitted heterosexually in Thailand (Jain *et al.*, 1994); a clade B variant (B') is circulating in Brazil (Potts *et al.*, 1993) and, besides southern Africa, clade C is found in India (Grez *et al.*, 1994). Moreover, more than one HIV-1 clade is found in some countries: in Uganda, clades A to D predominate over clade G (Kaleebu *et al.*, 1995); in Brazil, clades B, B', C and F have been identified, while in Thailand, clade A circulates among heterosexuals and clade B in intravenous drug users (Ou *et al.*, 1992).

Five clades (A–E) of HIV-2 have been identified (Gao *et al.*, 1994), but currently only clades A and B comprise more than one isolate.

(ii) *Antigenic diversity*

Although there is extensive literature on the genetic diversity of HIV-1 strains, less is known about antigenic diversity. It is clear that sequence data do not translate directly into antigenic information. A principal antigenic determinant of the virus envelope protein which elicits the greatest neutralizing antibody response is an epitope in the third variable domain of gp120, commonly called the V3 loop (Moore & Nara, 1991). A large number of HIV-1 V3 sequences have been reported, but it is still unclear how many distinct antigenic subtypes (also known as serotypes) exist.

HIV-1 neutralization assays were initially carried out using laboratory-adapted viral strains and immortalized T-cell lines (Weiss *et al.*, 1986). Primary isolates may have neutralizing phenotypes which are qualitatively and quantitatively different from T-cell line-adapted viruses. Viral diversity defined in terms of neutralization of field isolates propagated in peripheral blood mononuclear cells (PBMCs) remains to be determined, and may well be an important consideration in the development of a universally effective vaccine.

1.1.4 *Host range*

In addition to humans, HIV-1 and HIV-2 can infect some non-human primates (see Section 3.1).

1.1.5 *Cell tropism*

A distinguishing feature of HIV-1 and HIV-2 is their ability to infect $CD4^+$ T-lymphocytes and macrophages. Indeed, it was this early observation that led to the identification of the cell differentiation antigen CD4 as the receptor for HIV-1 entry into cells (Dalgleish *et al.*, 1984; Klatzmann *et al.*, 1984). All strains of HIV-1 and HIV-2 can infect peripheral blood $CD4^+$ lymphocytes (T-helper cells), but the extent to which immortalized or leukaemic T-cell lines are infected varies from strain to strain (Evans *et al.*, 1987).

Most primary HIV-1 strains (not adapted to propagate in T-cell lines) infect macrophages, although the limited extent of replication of some strains may necessitate co-cultivation of the macrophages with PBMCs to allow detection of the virus (Schrier

et al., 1990). Antigen presenting cells such as dendritic and Langerhans' cells may be important in mucosal and sexual transmission of HIV-1 (Pope *et al.*, 1994).

Since the identification of CD4 as the receptor for HIV-1 and HIV-2, it has become apparent that the virus is also capable of limited infection of certain $CD4^+$ cells, including fibroblasts, glial cells and rhabdomyosarcoma cells (Clapham *et al.*, 1991). The cellular tropism of HIV-1 appears to be determined primarily by its envelope, although other regions of the virus genome, e.g., *vpr*, may also have an influence. The identification of members of the seven-transmembrane G protein-coupled receptors which act as co-receptors helps to explain the cellular tropisms of HIV-1 (Alkhatib *et al.*, 1996; Deng *et al.*, 1996; Drajic *et al.*, 1996; Feng *et al.*, 1996).

1.1.6 *Target tissues*

(*a*) *Lymphoid tissue*

HIV-1 localizes in lymphoid tissue early in the course of infection (Biberfeld *et al.*, 1985; Tenner-Rácz *et al.*, 1985; Pantaleo *et al.*, 1993a). The presence of HIV-1 in lymphoid tissues throughout infection has been confirmed by in-situ methods (Embretson *et al.*, 1993). It remains uncertain whether HIV-1 infects other than lymphoid cells (Pantaleo *et al.*, 1993b).

(*b*) *Central nervous system*

HIV-1 frequently affects the brain. The microglial cells are the main location for viral replication in the central nervous system, although astroglial cells may be abortively infected (Shaw *et al.*, 1985; Epstein *et al.*, 1991; Donaldson *et al.*, 1994). However, there is controversy as to whether the productively infected cells of the brain are the resident microglia or are derived from invading macrophages.

(*c*) *Gastrointestinal tract*

HIV-1 isolated from the gastrointestinal tract of infected subjects has been reported to be biologically and molecularly different from viruses isolated from the peripheral blood of the same patient (Barnett *et al.*, 1991). In addition to lymphocytes and macrophages of the lamina propria (Smith, 1994), Nelson *et al.* (1988) reported HIV-1 to infect columnar epithelial cells and entero-chromaffin cells. Other investigators have failed to confirm these findings (DuPont & Marshall, 1995).

1.1.7 *The HIV-1 and HIV-2 genome and gene products*

The three major genes of HIV-1 and HIV-2 are the *gag*, *env* and *pol* genes, which initially give rise to polyproteins (respectively $Pr55^{gag}$, $Pr160^{gag\text{-}pol}$ and gp160) that are further processed to yield the structural proteins of the virus and enzymes (see Section 1.1.1 and Figure 1).

The *gag* gene products MA, CA and NC, the *pol* gene products PR, RT and IN and the *env* gene products SU and TM are always present in the same 5'–3' order. In addition, there are regulatory genes (*tat*, *rev*) and four accessory genes (*nef*, *vif*, *vpr*, *vpu*). In the proviral state, open reading frames are flanked by long terminal repeat (LTR) sequences

(Figure 2). These contain promoters of gene expression and specific enhancer elements which control viral gene expression and which are themselves influenced by cellular transcriptional proteins.

(i) *Structural proteins (Gag, Pol, Env)*

The primary product of the *gag* gene is a precursor polypeptide, p55, which undergoes systematic cleavage from its NH_2-terminus to yield the myristoylated MA, p17, and two antigens of the virus core: the CA, p24 and the PR, p15 or p14 (Levy, 1993). The latter is further processed into p7 and p6 (Barker *et al.*, 1995).

Enzymes which catalyse steps in the virus lifecycle are cleaved from the Gag-Pol polyprotein, $Pr160^{gag\text{-}pol}$ during virion morphogenesis. These are (i) the mature form of PR, composed of 99 amino acids with a molecular weight of 10 kDa (Katz & Skalka, 1994) and belonging to the category of aspartic proteinases, on the basis of the conserved Asp-Thr/Ser-Gly motif at the active site (Loeb *et al.*, 1989; Luciw, 1996); (ii) RT, which transcribes the viral RNA to DNA, and which has associated RNAse activity to degrade RNA/DNA hybrid molecules (Baltimore, 1970; Temin, 1976); (iii) IN, which results from the COOH-terminal of $Pr160^{gag\text{-}pol}$ to yield a 32 kDa protein with DNA cleavage and strand transfer activity, catalysing the covalent linkage of double-stranded DNA into the host genomic DNA (Luciw, 1996).

The initial envelope precursor protein gp160 is cleaved by a cellular protease to produce a mature glycosylated NH_2-terminal protein gp120 and the external spike glycoprotein gp41, which remain non-covalently linked (Figure 1) (reviewed by Moore *et al.*, 1993). The extracellular part of gp120 contains the binding site for the CD4 receptor, as well as the hypervariable region of about 36 amino acids referred to as the V3 loop (Freed *et al.*, 1991) (see Section 1.1.3). The gp 41 TM protein anchors gp120 in the viral lipid membrane and contains a hydrophobic peptide at its amino-terminus that is involved in membrane fusion.

(ii) *Regulatory proteins (Tat, Rev)*

The HIV-1 and HIV-2 genome encodes the major regulatory proteins Tat and Rev (reviewed by Peterlin, 1995; Luciw, 1996). Both are expressed from multiply spliced viral transcripts produced early after infection. Neither are packaged into virions and both are essential for virus replication.

The *tat* gene is bipartite, in that it has two coding exons, one located in the central region of the genome between *vpr* and *env* (Figure 2), the other overlapping the translation frames of *rev* and gp41. The 14 kDa Tat protein is localized in the nucleus by means of an arginine-rich nuclear localization signal within its basic domain. In the nucleus, Tat interacts with a stem-loop RNA structure in the LTR, designated the trans-activation response (TAR) element. Tat is essential for viral replication and acts to increase the steady-state levels of viral transcripts (for both structural and regulatory viral proteins) initiated in the LTR.

Viral structural protein expression is additionally regulated by the product of the *rev* gene. Rev is an essential 19 kDa protein which facilitates the appearance of partially spliced and unspliced transcripts in the cytoplasm. In the absence of Rev, only multiply

spliced transcripts are translated, so that no structural proteins, enzymes or genomic RNA can be packaged into the virus particle.

Rev, in keeping with its involvement with the splicing machinery, is located in the nucleolus. By binding to viral RNA at the Rev response element (RRE), Rev effectively shifts the balance from multiply spliced transcripts (encoding Tat, Rev, Nef and Vpr in the early stages of the virus replication cycle) to both unspliced and singly spliced transcripts which encode the viral structural proteins at a later stage in infection (Cullen, 1991).

(iii) *Accessory proteins (Nef, Vif, Vpr, Vpu)*

The role of the accessory proteins has been reviewed (Cullen, 1994; Hahn, 1994; Subbramanian & Cohen, 1994; Trono, 1995).

Nef, the first viral protein to be expressed, is a 25–30 kDa protein which is predominantly localized in the cytoplasm and inner surface of the membrane in infected cells (Yu & Felsted, 1992). Nef appears to be multi-functional: it down-regulates expression of the CD4 receptor in infected T-cells (Garcia & Miller, 1991; Aiken *et al.*, 1994), as indeed do Vpu and gp120, although the mechanism is unclear. Since the rate of CD4 endocytosis increases in the presence of Nef, it may be that Nef acts directly or indirectly via a cellular factor, to trigger removal of CD4 by endocytosis (Benichou *et al.*, 1994), thus preventing subsequent re-infection of cells already harbouring virus (Karn, 1991). The effects of Nef *in vivo* and *in vitro* are in sharp contrast. Deletion of *nef* appears to have little effect on infection by HIV-1 in T-cell lines (Cullen, 1994). However, macaques infected with SIV isolates expressing truncated Nef proteins maintain low-level viraemia and remain healthy, but if full-length Nef operates (due to a premature stop codon in SIV_{MAC239}), high-level viraemia and disease develop (Kestler *et al.*, 1991).

The *vpr* gene product is a 15 kDa oligomeric protein expressed from a singly spliced mRNA (Cohen *et al.*, 1990a,b; Zhao *et al.*, 1994). HIV-2 and most SIV strains carry an additional gene, *vpx*, which shares sequence homology with *vpr*, such that it has been suggested that *vpx* arose from *vpr* by gene duplication (Tristem *et al.*, 1992). Both Vpr and Vpx are packaged within the virions and by electron microscopy appear to be located outside the core structure (Wang *et al.*, 1994). Vpr induces differentiation and growth arrest in some tumour cell lines, even in the absence of other viral proteins (Rogel *et al.*, 1995). In terms of its effect on HIV replication, Vpr appears to enhance virus production in primary macrophages and, to a lesser extent in some T-cell lines (Hattori *et al.*, 1990; Connor *et al.*, 1995). Mutation in the nuclear localization signal of both the matrix protein p17 and Vpr of a macrophage tropic clone of HIV-1 led to a lower viral replication rate in macrophages and weakened the localization of uncoated viral complexes in the nucleus. Thus, p17 and Vpr appear to be able to mediate efficient nuclear importation of the pre-integration complex into non-dividing cells (Bukrinsky *et al.*, 1993; Heinzinger *et al.*, 1994). Vpr can also block the proliferation of human rhabdomyosarcoma cells and induce differentiation to muscle cells (Levy *et al.*, 1993a).

Vif, a 23 kDa cytoplasmic protein, is also essential for viral replication (Michaels *et al.*, 1993). In the absence of Vif, HIV-1 virions have abnormal morphology (Borman

et al., 1995) and have much reduced capacity to synthesize proviral DNA following infection of new target cells (Sova & Volsky, 1993).

HIV-1 and the related SIV_{CPZ} contain a *vpu* gene (Myers *et al.*, 1994), the 16 kDa phosphorylated product of which is localized in the perinuclear region of infected cells and is thus associated with the endoplasmic reticulum/Golgi system. Vpu-deficient HIV-1 mutants continue to replicate in $CD4^+$ T-cell lines, primary T-lymphocytes and macrophages, but at a reduced titre due to accumulation of virions in intracytoplasmic vesicles (Klimkait *et al.*, 1990). HIV-2 and other SIVs than SIV_{CPZ} lack a *vpu* gene, but its function is probably encoded elsewhere in the genome.

1.1.8 *Replication*

Infection by HIV is initiated when virus binds to the CD4 receptor on a target cell by means of the viral envelope glycoprotein, gp120 (Dalgleish *et al.*, 1984; Klatzmann *et al.*, 1984; Klasse *et al.*, 1993). This binding triggers a conformational change in the Env glycoprotein to expose the TM protein, gp41, resulting in fusion, possibly mediated by the co-receptor, between the virus and the host cell membrane (Weiss, 1993a). HIV-1 and HIV-2 enter the cell via a pH-independent mechanism (McClure *et al.*, 1990). After fusion, the viral core is released into the cell and single-stranded RNA, still associated with capsid protein, is converted to double-stranded proviral DNA through the polymerase and ribonuclease H activities of the viral reverse transcriptase.

The newly formed pre-integration complex enters the nucleus and the viral DNA integrates randomly in the host cellular DNA. This proviral DNA acts as a template for the production of viral RNA progeny. Transcription of the viral genome is driven by a promoter in the 5' LTR of the integrated provirus, resulting in the production of RNA molecules. These in turn serve both as messenger for synthesis of new viral proteins and as genomic RNA. Tat augments levels of viral RNA by increasing transcriptional initiation and/or elongation, and Rev regulates splicing and transport of viral RNA from the nucleus to the cytoplasm (reviewed by Cullen, 1993). Genomic RNA is subsequently packaged into virions which then bud at the surface from the cell membrane. As the virion matures, Gag and Gag–Pol polyproteins are cleaved by the viral protease into subunit proteins, resulting in the mature virion which is directed to the cell surface by the amino-terminal myristoylation of Gag (Smith *et al.*, 1993a). The virion is then released from the cell surface and this completes the life cycle (Figure 5).

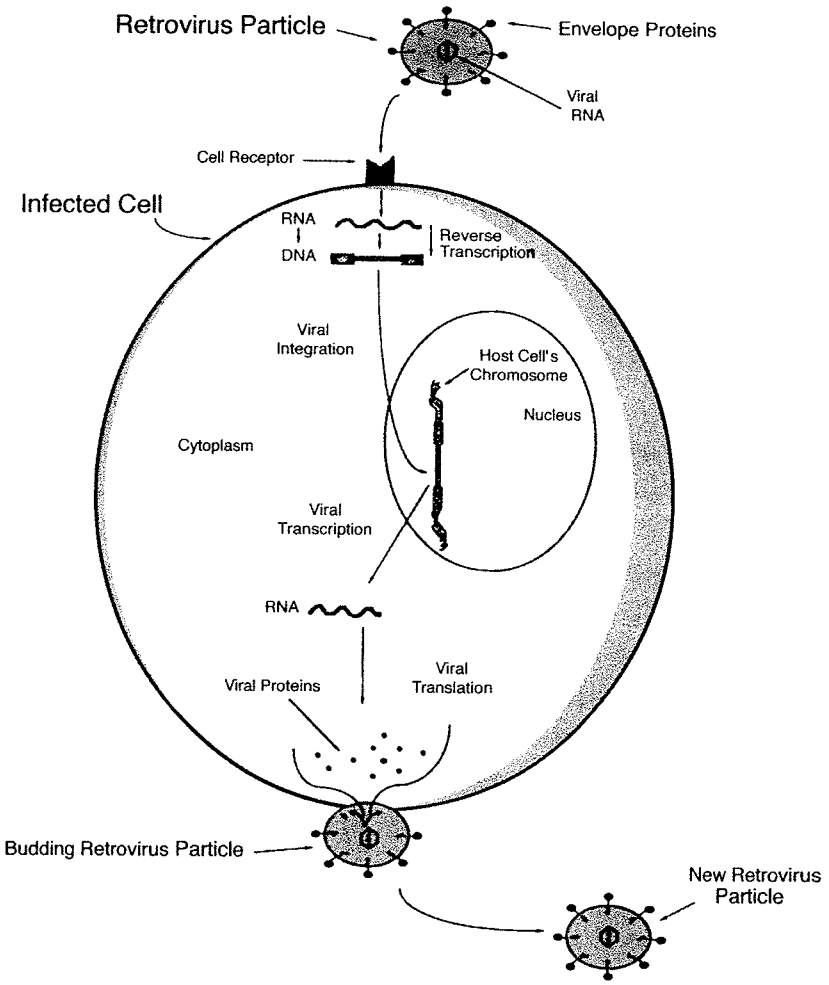
1.2 Methods of detection

In this section, HIV refers to both HIV-1 and HIV-2, unless otherwise specified.

1.2.1 *Antibody tests*

The confirmed presence of HIV antibodies is considered to represent current infection because as with other human retroviruses, once acquired, infection is lifelong. An antibody test for HIV-1 was first licensed in 1985, about two years after the virus was

Figure 5. Retrovirus life cycle



first isolated and identified as the causal agent for AIDS. The most widely used antibody tests for diagnosing HIV infection are enzyme-linked immunosorbent assays (ELISAs), with confirmation by western blot analysis.

(*a*) *ELISA*

Disrupted virions, purified from HIV-1-infected T-cells, were used as the antigen source in first-generation ELISAs. These partially purified antigens reacted with antibody to proteins from envelope (gp120 and gp41), core (p24) and reverse transcriptase (p55) regions of the virus. Early antigen preparations were often contaminated with non-viral antigens such as those originating from the major histocompatibility complex (MHC) expressed by the infected T-cells.

Sensitivity and specificity were substantially improved in second-generation ELISAs with the introduction of recombinant viral proteins or synthetic peptides. HIV-1 and HIV-2 are simultaneously detected in more sensitive third-generation ELISAs, also based on synthetic peptides of HIV or recombinant proteins (Simon *et al.*, 1992; Barbé *et al.*, 1994).

(*b*) *Western blot analysis*

In the western blot assay, enzyme-conjugated anti-human antibody is then used to detect membrane-bound HIV-specific antibody, observed as bands on the membrane corresponding to an antibody response to HIV proteins. The Centers for Disease Control (CDC; Atlanta, GA; United States of America) recommend that at least two bands corresponding to Gag and Env proteins must be reactive before a specimen can be classified as HIV-1 or HIV-2 antibody-positive (Centers for Disease Control, 1989a).

(*c*) *Indeterminate HIV antibody results*

Sera that do not meet the above criteria but exhibit reactivity to one or more bands are classified as 'indeterminate'. The proportion of serum samples that are repeatedly reactive on ELISA testing but interpreted as indeterminate by western blot analysis varies according to geographical region (Centers for Disease Control, 1989a).

HIV-1 indeterminate western blots can be seen in the early stages of HIV-1 infection (Gaines *et al.*, 1987; Ranki *et al.*, 1987; Sloand *et al.*, 1991) and throughout HIV-2 infection (Centers for Disease Control, 1989b). Indeterminate western blot patterns have rarely been found in healthy people with no identifiable risk for HIV infection (Dock *et al.*, 1991; Celum *et al.*, 1991), such as leprosy patients and pregnant women (Kashala *et al.*, 1994).

Further virological and immunological investigations such as HIV culture, quantification of p24 antigen and polymerase chain reaction (PCR) investigations can be used to diagnose HIV infection in individuals with indeterminate western blot results and a relevant exposure history (see Section 1.2.2).

(*d*) *Undetectable HIV antibody*

As with other infections, there is a delay between exposure and the development of antibodies (seroconversion), described as a 'window period'. Although antibodies to HIV-1 detectable by current ELISA may develop within weeks after infection, the usual public health practice is to retest 3–6 months after presumed exposure (Petersen *et al.*, 1994). The duration of the window period is variable and may be influenced by the mode of transmission, infectious dose and the host immune response. Improvement of ELISA has greatly reduced the window period.

(*e*) *Diagnosis of HIV infection in infants*

The serological diagnosis of HIV infection in children born to mothers with HIV infection is complicated by the passive transfer of maternal anti-HIV IgG antibodies to the baby. These antibodies decline steadily but can be detected for up to 15 months, so that standard serological assays cannot confirm or exclude HIV infection in the infant until then. The detection of IgA antibodies, which can only originate from the child (Livingston *et al.*, 1995), and serial testing to detect a rise in antibody titre after the initial fall during the first six months of life (Palasanthiran *et al.*, 1994), have been used to make an earlier diagnosis. However, where facilities exist, HIV infection is diagnosed in such infants by direct detection of HIV by culture and/or PCR on two occasions (McClure *et al.*, 1996; McMichael *et al.*, 1996).

Tests for HIV-specific antibodies remain useful for large-scale perinatal testing and in developing countries without facilities for viral culture or PCR analysis. To confirm that an infant is infected with HIV, antibody levels should be monitored to see if they persist beyond the first 15 months of life.

(*f*) *Detection of antibodies in saliva*

Testing for HIV antibody in saliva specimens has been shown to be a reliable technique for surveillance studies in populations with high prevalence of infection (Behets *et al.*, 1991; van den Akker *et al.*, 1992). The methods of collection of saliva specimens influence the detection of HIV antibody; therefore, these methods have not been recommended for individual diagnostic purposes (WHO, 1993).

1.2.2 *Direct detection of HIV*

Many of the problems encountered in antibody-based diagnosis of HIV infection, such as long seroconversion periods, the presence of cross-reactive antibody to non-viral proteins and diagnosis of HIV infection in neonates with maternal antibody to HIV, can be overcome by using techniques that detect virus or viral products directly.

HIV diagnosis is influenced by the amount of HIV present in the biological specimen tested. Table 2 shows how HIV load in various body fluids can vary dramatically.

Viral load varies greatly according to the stage of infection. In people recently infected with HIV and in those who have progressed to AIDS, viral load is high. Comparatively low levels of virus are found in asymptomatic individuals.

(*a*) *Viral culture*

Isolation of HIV by viral culture involves the co-culture of PBMCs with phytohaemagglutinin (PHA)-stimulated lymphocytes from an uninfected donor or a susceptible uninfected laboratory cell line (Feorino *et al.*, 1987). The presence of virus is then detected by measuring RT activity or p24 antigen.

Viral culture can take between two and four weeks to complete, requires experienced laboratory personnel to handle infectious material and is expensive.

(*b*) *p24 Antigen*

A quantitative p24 antigen capture assay has been developed, using a modified ELISA in which specific anti-p24 antibody is fixed to the wells of a microtitre plate so that free p24 antigen in serum is 'captured'. Enzyme-conjugated antibody specific to p24 is then added and the presence of immune complexes is visualized by a standard colour reaction.

The p24 antigen assay can detect HIV infection in some but not all recently exposed people before seroconversion. As antibodies to HIV develop, immune complexes form and p24 levels become low or undetectable. Late in the course of HIV disease, p24 antigen again becomes detectable.

Table 2. Representative data on isolation of HIV-1 from body fluids

Source	No. of specimens with virus isolated/ total specimens	Estimated quantity of HIV[a]
Free virus in fluid		
Plasma	33/33	1–5000[b]
Tears	2/5	< 1
Ear secretions	1/8	5–10
Saliva	3/55	< 1
Sweat	0/2	–[c]
Faeces	0/2	–[c]
Urine	1/5	< 1
Vaginal and cervical fluid	5/16	< 1
Semen	5/15	10–50
Milk	1/5	< 1
Cerebrospinal fluid	21/40	10–10 000
Infected cells in fluid		
Peripheral blood mononuclear cells	89/92	0.001–1%
Saliva	4/11	< 0.01%
Bronchial fluid	3/24	ND[d]
Vaginal and cervical fluid	7/16	ND[d]
Semen	11/28	0.01–5%

From Levy (1993)

[a] For cell-free fluid, quantities are given as infectious particles per millilitre; for infected cells, quantities are the percentage of total cells infected.

[b] High levels associated with symptoms and advanced disease

[c] –, no virus detected

[d] ND, not done

Acid dissociation of immune complexes in serum specimens increases the sensitivity of the p24 assay (Bollinger *et al.*, 1992).

(*c*) *Detection of viral genomes*

PCR and other nucleic acid amplification methods offer an alternative technique to cell culture for the detection and quantification of HIV in plasma or PBMCs. It is useful for diagnosing HIV infection in people at high risk for infection who remain antibody-negative, in people at low risk with an indeterminate western blot and in infants in whom maternal antibody is still present. Quantitative PCR is increasingly used to guide therapy; PCR is also used to detect mutations, including those which confer drug resistance.

(*d*) *HIV quantification*

The viral load can be quantified by viral culture and by nucleic acid detection methods (PCR, branched PCR, RT-PCR and nucleic acid sequence–base amplification).

The latter have the advantage of speed (2–3 h) and sensitivity (≤ 50 copies of HIV RNA can be detected per microlitre of plasma) (Holodniy *et al.*, 1991; Piatak *et al.*, 1993). In developed countries, viral load measurements are being introduced into routine patient management (see Section 1.4.2).

1.3 Epidemiology of HIV infection

In this section, HIV refers to HIV-1 unless otherwise specified.

1.3.1 *HIV transmission*

The three primary routes of HIV transmission — sexual intercourse, blood contact and from mother to infant — were proposed on the basis of AIDS case reports, even before the identification of this virus as the causative agent for AIDS. The appearance of AIDS first in homosexual men (Gottlieb *et al.*, 1981) suggested the possibility of sexual transmission, and its occurrence in recipients of blood and blood products (Anon., 1992a) and intravenous drug users (Small *et al.*, 1983) pointed strongly to transmissibility by blood contact. Once tests for detecting HIV antibodies became available in 1984, routes of transmission were established through identification of pairs of individuals with HIV antibody who were linked by a specific form of contact, such as blood donor–recipient, mother–child and members of the same sexual partnership.

(*a*) *Sexual contact*

There is extensive documentation of HIV transmission from man to woman and woman to man through vaginal and anal intercourse that is unprotected (i.e., without condom), and from man to man through unprotected anal intercourse. The risk of transmission associated with a single episode of unprotected intercourse appears to be highly variable and dependent on a number of factors (Mastro & de Vincenzi, 1996). Probably most important such factors are the disease stage of the infected partner (de Vincenzi, 1994; Nicolosi *et al.*, 1994a,b), which determines the amount of virus present in body fluids (Anderson *et al.*, 1992), and the presence of genital infection (Plummer *et al.*, 1991; Laga *et al*, 1993; Telzak *et al.*, 1993), particularly genital ulcerative disease (Cameron *et al.*, 1989). Other factors which have been less conclusively associated with an increased risk of transmission are lack of male circumcision (Cameron *et al.*, 1989; Hunter *et al.*, 1994), cervical ectopy (Moss *et al.*, 1991), intercourse during menstruation and older age for exposed women (European Study Group on Heterosexual Transmission of HIV, 1992). There may be an association between susceptibility to infection and specific HLA subtypes (Rowland-Jones *et al.*, 1995). The likelihood of HIV transmission per episode of sexual contact appears to be somewhat higher from man to woman than from woman to man, and anal intercourse presents a higher risk than vaginal intercourse for the receptive partner (de Vincenzi, 1994).

In the largest prospective study carried out to date (de Vincenzi, 1994), the cumulative risk of sexual transmission over the 20-month follow-up period of the study for couples practising unprotected intercourse was 13% from man to woman and 11% from woman to man. The transmission risks per episode were around 1/1000. A striking

feature of this study was that no transmission occurred among the 124 couples who consistently used condoms during sexual intercourse. Transmission risks per unprotected episode have been higher in studies of heterosexual partners from developing countries and in studies of homosexual men (Mastro & de Vincenzi, 1996). [The Working Group noted that some studies using a range of methodologies have found several-fold higher transmission risks than this study.]

A few cases of HIV transmission through penile-oral intercourse to the receptive partner have been reported (Mayer & DeGruttola, 1987; Rozenbaum *et al.*, 1988) but such transmission is thought to occur much less frequently than transmission by vaginal or anal intercourse.

HIV infection can occur through artificial insemination (Stewart *et al.*, 1985).

(*b*) *Blood contact*

The most efficient mode of HIV transmission is through direct blood-to-blood contact. In retrospective studies of people transfused with HIV-infected blood, transmission rates were essentially 100% (Donegan *et al.*, 1990). In a number of countries, the prevalence of HIV infection among haemophiliacs reached high levels due to the use of contaminated blood products before the introduction of systematic screening and heat treatment of donations. Transmission in the health care setting has also been documented following minor skin injury with needles and from splash exposure to mucous membranes. Overall, the risk of transmission following percutaneous or mucous membrane exposure to an HIV-infected source via occupational injury has been estimated to be around 0.3% per episode (Henderson *et al.*, 1990). However, the rate of transmission to health care workers who suffer a deep injury from a hollow-bore needle containing HIV-infected blood is much higher (Anon., 1995). HIV infection is also efficiently transmitted by organ transplantation.

Iatrogenic transmission of HIV infection has been minimized in developed countries and many developing countries through the use of procedures to defer (exclude) blood donors at risk of HIV infection and universal screening of blood and tissue donations for HIV antibody (Franceschi *et al.*, 1995a). However, a small number of cases of transmission still occur when a newly infected donor has not yet developed a detectable level of HIV antibody (Ward *et al.*, 1988). In a number of developing countries, the blood supply is not yet universally screened. In South Africa, 80% of HIV-positive donations came from first-time donors, and one approach has been to use only heat-treated blood products from first-time donors (Sitas *et al.*, 1994).

The other major pathway of blood-borne transmission is through the re-use of injecting equipment and related material by intravenous drug users (Friedman & Des Jarlais, 1991). The immediate re-use of a needle and syringe after they have been used by an HIV-infected person is an efficient means of transmitting the virus. Less clear is the extent to which the risk of transmission reduces with the time elapsed between use and re-use of the injecting equipment and by various methods of cleaning the equipment.

(*c*) *Mother-to-child transmission*

Between 15% and 35% of babies born to HIV-infected women acquire the infection, the risk depending on a range of factors which vary across population groups (Peckham & Gibb, 1995). As with sexual transmission, a key predictor is the HIV disease stage in the mother (European Collaborative Study, 1992), which is associated with viral load (Roques *et al.*, 1993). Breast-feeding is a strong independent risk factor, as shown by studies of women who became infected post-partum, either by blood transfusion (Ziegler *et al.*, 1985) or sexually (Van de Perre *et al.*, 1991) and of children of women already infected at the time of delivery. The majority of studies have found that delivery by Caesarian section reduces the risk of mother-to-child transmission (reviewed by the European Collaborative Study, 1994), suggesting that most transmission occurs during passage through the birth canal. This is supported by studies of twins in which the first-born twin has the higher risk of HIV infection (Goedert *et al.*, 1991).

(*d*) *Other modes of transmission*

There is no evidence that HIV transmission can occur through routes other than those described above (Friedland *et al.*, 1990; Gershon *et al.*, 1990; Anon., 1994). Although it is impossible to prove that a specific form of contact carries a zero likelihood of transmission, studies of the household and casual contacts of people with HIV infection have not revealed any risk of HIV transmission. Similarly, there is no evidence that mosquitoes, bed bugs or other arthropods act as vectors of HIV between humans.

Several well documented pairs or groups of cases of HIV infection are linked both epidemiologically and through molecular typing, but the specific mode of transmission has not been ascertained (Ciesiclski *et al.*, 1992; Chant *et al.*, 1993; Fitzgibbon *et al.*, 1993). It is believed that these cases represent unknowing or unacknowledged blood contact rather than evidence for new modes of transmission.

1.3.2 *Geographical distribution*

Assessment of the epidemiological pattern of HIV infection was initially based on AIDS case reporting (Buehler *et al.*, 1989). Since 1985, when HIV antibody testing became widely available, case reporting of HIV diagnoses (McDonald *et al.*, 1994) and serological surveys for HIV antibody in population subgroups (Dondero *et al.*, 1988) have complemented AIDS case reporting as mechanisms for monitoring the occurrence of HIV infection. Across geographical and administrative areas, there has been a wide variation in the specific approaches used for epidemiological surveillance of HIV infection, depending on a range of economic, political, cultural and ethical considerations. It is therefore difficult to compile an accurate and current picture of the HIV epidemic as it has spread around the world. Some countries, particularly those of the developed world, have produced national consensus reports on past and predicted patterns of HIV infection, while for other countries, there has been a reliance on estimates made by international bodies, such as WHO.

No single approach to epidemiological monitoring of HIV infection is fully satisfactory. Compilation and analysis of AIDS case reports only provide an indication of

past HIV infection patterns, because of the long and variable interval between the acquisition of infection and development of AIDS. AIDS case counts are also prone to substantial under-enumeration, because of reliance on individual medical practitioners to diagnose and report cases centrally. On the other hand, the occurrence of AIDS is generally a severe and life-threatening condition which almost always results in contact with the health system, thereby providing unbiased data in relative, if not absolute, terms within a population and over time. Surveillance based on HIV diagnosis suffers from its dependence on the extent of HIV testing and may be biased by variation in the level of testing across population subgroups. It can nevertheless provide an indication of transmission patterns earlier than would be available from AIDS case reports. Both AIDS and HIV reporting are difficult to implement on a routine basis in countries with limited resources.

Serological surveys for HIV antibody have been carried out in some countries on a routine basis (Gill *et al.*, 1989; Dondero & Gill, 1991; Ministry of Public Health, 1994), while in other countries they are implemented occasionally. Provided sampling frames are carefully chosen, such surveys can provide good estimates of HIV prevalence (and, with more difficulty, incidence) in selected population subgroups. Groups included in serological surveys have generally been either people considered to be at elevated risk of HIV infection, such as homosexual men, sexually transmitted disease clinic attendees, sex workers (prostitutes), intravenous drug users or prisoners. More representative of the general population may be people who are easily accessible within the health system or some other institutional setting, such as pregnant women, hospital in-patients, blood donors (who are now universally tested for HIV antibody in many countries) and military recruits and serving personnel.

(*a*) *Global estimates and projections*

At the end of 1995, WHO released a comprehensive set of estimates of HIV prevalence in adults by country (WHO, 1995), along with the Organization's routinely published counts of reported AIDS cases. The prevalence estimates (see Table 3) were provided by national bodies or expert groups in each country or were calculated by WHO if current national estimates were not available. The picture that emerges is one dominated by sub-Saharan Africa, where the HIV epidemic is believed to have started. The proportion of adults estimated to have HIV infection is above 14% in Malawi and Uganda and 17% in Zambia and Zimbabwe. Among the developed countries, the United States and Spain have the highest prevalence rates of HIV infection among adults, above 0.5%, while rates in other developed countries range down to below 0.05%. Apart from Cambodia, Myanmar and Thailand, with prevalence rates of 1.5–2.0%, HIV prevalence remains low in Asia, but India is now estimated to be the single country with the greatest number of people living with HIV infection.

Mathematical models have been used to carry out projections of the future course of the HIV epidemic globally, on the basis of available data and assumptions about future trends in transmission rates. These models predict that in the years up to 2000, there will be a declining annual incidence of AIDS in North America and Europe, a stable or slightly declining incidence in Africa and a sharply rising incidence in Asia (Chin, 1995).

By 2000, it is predicted that Asia will have over 1.3 million new infections per year, compared with 800 000 in Africa and 100 000 in North America and western Europe.

Table 3. Estimated prevalence of HIV infection among adults, in selected countries, at the end of 1994

Country	Number	%	Country	Number	%
North America			Greece	5 000	0.098
Canada	30 000	0.19	Hungary	3 000	0.058
United States	700 000	0.52	Ireland	1 700	0.094
Caribbean			Italy	90 000	0.31
Cuba	1 300	0.021	Netherlands	3 000	0.036
Dominican Republic	40 000	1.0	Norway	1 250	0.057
Haiti	150 000	4.4	Poland	10 000	0.05
Jamaica	12 000	0.91	Portugal	8 000	0.16
Latin America			Romania	500	0.004
Argentina	60 000	0.36	Russian Federation	3 000	0.004
Brazil	550 000	0.65	Spain	120 000	0.58
Chile	10 000	0.13	Sweden	3 000	0.072
Colombia	40 000	0.21	Switzerland	12 000	0.32
Mexico	200 000	0.42	Turkey	500	0.002
Peru	30 000	0.25	United Kingdom	25 000	0.087
Venezuela	35 000	0.32	Ukraine	1 500	0.006
Africa			**Asia**		
Egypt	7 500	0.025	Bangladesh	15 000	0.026
Ethiopia	588 000	2.5	Cambodia	90 000	1.9
Ghana	172 000	2.2	China	10 000	0.002
Kenya	1 000 000	8.3	India	1 750 000	0.38
Malawi	650 000	14	Indonesia	50 000	0.049
Morocco	5 000	0.036	Japan	6 200	0.01
Mozambique	400 000	5.7	Korea, Democratic	100	0.001
Nigeria	1 050 000	2.2	People's Republic of		
Rwanda	250 000	7.1	Korea, Republic of	2 000	0.008
Senegal	50 000	1.3	Malaysia	30 000	0.3
South Africa	650 000	3.2	Myanmar	350 000	1.5
Tanzania, United	840 000	6.4	Pakistan	40 000	0.063
Republic of			Philippines	18 000	0.054
Uganda	1 300 000	14	Thailand	700 000	2.1
Zaire	680 000	3.7	Vietnam	25 000	0.069
Zambia	700 000	17	**Oceania**		
Zimbabwe	900 000	17	Australia	11 000	0.12
Europe			New Zealand	1 200	0.065
Denmark	4 000	0.15	Papua/New Guinea	4 000	0.19
Finland	500	0.019	**Middle East**		
France	90 000	0.31	Israel	2 000	0.073
Germany	43 000	0.11	Saudi Arabia	1 000	0.012

From WHO (1995)

More detailed analyses of HIV prevalence and transmission patterns are available for most developed countries and a number of developing countries through national reports or papers published in the scientific literature.

(*b*) *United States and Canada*

As the country where AIDS was first recognized (Gottlieb *et al.*, 1981) and the developed country with the highest number of cases of HIV infection in absolute terms (WHO, 1995), the United States has carried out a large number of investigations into HIV infection. It is now apparent that two distinct HIV epidemics have occurred, beginning in the late 1970s and early 1980s. One was focused on the major communities of homosexual men, particularly in San Francisco, Los Angeles and New York. Retrospective tests of stored serum samples from homosexual men taken in the course of longitudinal studies of hepatitis B vaccination revealed a sharp rise in the incidence of HIV infection from the late 1970s (Hessol *et al.*, 1989; van Griensven *et al.*, 1993). These studies, as well as subsequent cohort studies (Winkelstein *et al.*, 1987; Kingsley *et al.*, 1991), showed that the incidence of new infection peaked at around 10% of homosexual men per year in the early 1980s. This finding was confirmed by back-projection (Rosenberg *et al.*, 1992; Rosenberg, 1995), a mathematical method that estimates past incidence of HIV infection based on AIDS case reports combined with knowledge about the rate of progression from HIV infection to the development of AIDS.

The other major epidemic in the United States was among inner-city, largely 'African-American' or 'Hispanic' residents of the major eastern cities, such as New York, Chicago, Philadelphia, Miami, Baltimore and Newark (Centers for Disease Control and Prevention, 1994a). Transmission was associated mainly with the use of illicit drugs, either directly through injection (Schoenbaum *et al.*, 1989) or indirectly through sexual contacts by people seeking money to buy drugs or partners of intravenous drug users (Diaz *et al.*, 1994; Ellerbrock *et al.*, 1995). To the end of 1994, 53% of AIDS cases reported in the United States were men who became infected through homosexual contact, but the proportion of such cases for 1994 alone had fallen to 44%, with corresponding increases in the proportion of AIDS cases attributed to intravenous drug use and heterosexual contact (Centers for Disease Control and Prevention, 1994b; Rosenberg, 1995).

In Canada, the patterns of HIV infection have generally been similar to those in the United States, but the overall rates of infection have been lower, and a higher proportion of cases have been transmitted through homosexual contacts between men (Remis & Sutherland, 1993).

(*c*) *Caribbean*

Early case reports of AIDS in the United States documented an association with Haitian origin (Anon., 1992b), and subsequent serological surveys confirmed high rates of HIV infection in Haiti and some other Caribbean countries (WHO, 1995; Cáceres & Hearst, 1996). The predominant mode of transmission in the Caribbean is heterosexual contact (Cáceres & Hearst, 1996). An apparent exception to the pattern of high HIV

infection rates in the Caribbean is Cuba, where the adult prevalence has been estimated to be 0.02% (WHO, 1995).

(*d*) *Latin America*

In the early 1980s, the pattern of HIV transmission in Latin American countries closely resembled those in the United States and Europe, being largely through sexual contact between men and among intravenous drug users (Cáceres & Hearst, 1996). More recently, some Latin American countries have experienced substantial increases in the extent of heterosexual transmission. In Brazil, the most populous country of the region, 23% of AIDS cases reported in 1992 were attributed to heterosexual transmission of HIV infection, compared with 7% in 1987 (Ministério da Saúde, 1993). There remains considerable variation between countries in the extent to which HIV transmission has extended beyond the population subgroups initially affected (Cáceres & Hearst, 1996).

(*e*) *Sub-Saharan Africa*

From retrospective testing of stored sera and tissue, HIV infection is known to have existed in Africa since before 1963 (Quinn *et al.*, 1986). Numerous serological surveys have documented the rapid spread of HIV infection through sub-Saharan Africa over the past decade. The most affected countries have been in central and southern Africa, including Kenya, Malawi, Rwanda, Tanzania, Uganda, Zambia and Zimbabwe (Nkowane, 1991; WHO, 1995). Within these countries, HIV prevalence has generally been substantially higher in cities than in rural communities (Berkley *et al.*, 1989) and epidemic spread has been associated with major transport routes (Grosskurth *et al.*, 1995), but is not strongly associated with social class, as measured by characteristics such as educational level attained (Malamba *et al.*, 1994). Transmission to adults has been mainly through heterosexual contact, with roughly equal numbers of men and women infected (Rwandan HIV Seroprevalence Study Group, 1989). Medical procedures such as injections and blood transfusion have also played a role.

Studies of women engaged in commercial sex work (prostitution) had already found HIV prevalence as high as 80% by the late 1980s in several African countries (Padian, 1988). The prevalence of infection in pregnant women has reached 30% in some urban surveys, resulting in high numbers of babies being born with HIV infection (Allen *et al.*, 1991).

In west Africa, HIV-2 was the predominant form in the mid-1980s, but in some urban areas, HIV-1 is now becoming more prevalent (Kanki *et al.*, 1994).

(*f*) *Europe*

In most European countries, HIV infection and AIDS were first reported among homosexual men in the early to mid-1980s (Downs *et al.*, 1987), but three distinct epidemiological patterns have emerged subsequently. In Germany, the Netherlands, the Nordic countries and the United Kingdom, sexual transmission between men has remained by far the most important route of transmission. In these countries, the cumulative proportions of AIDS cases attributed to male homosexual contact exceeded 60% in 1994 (European Centre for the Epidemiological Monitoring of AIDS, 1995a) and the

prevalence of HIV infection in pregnant women has generally been below 0.1% (European Centre for the Epidemiological Monitoring of AIDS, 1994). There are exceptions, such as parts of inner London, where large sections of the population are ethnic minority groups, in which the prevalence in pregnant women has been estimated at 0.4% (PHLS (Public Health Laboratory Service) Communicable Diseases Surveillance Centre, 1993).

In other European countries, the pattern of HIV infection became dominated by transmission related to intravenous drug use during the 1980s. Particularly affected were Italy, Spain and Switzerland, where HIV prevalence among people who inject drugs exceeded 50% in several cities (Friedman & Des Jarlais, 1991; European Centre for the Epidemiological Monitoring of AIDS, 1995a). As a consequence, these countries have experienced increasing rates of HIV infection and AIDS among women, acquired either through the sharing of injecting equipment or by sexual contact with male intravenous drug users, and of mother-to-child transmission of HIV infection (Franceschi *et al.*, 1994; European Centre for the Epidemiological Monitoring of AIDS, 1995b).

In a third group of European countries, primarily those of eastern Europe, HIV transmission appears to have been very limited so far (European Centre for the Epidemiological Monitoring of AIDS, 1995b). There are notable exceptions, such as a major outbreak of nosocomially-acquired HIV infection among children in Romania in the mid-1980s (Patrascu & Dumitrescu, 1993). In Poland, nearly half of the reported AIDS cases have been among intravenous drug users (European Centre for the Epidemiological Monitoring of AIDS, 1995a).

(*g*) *Asia*

There has been considerable variation between Asian countries in the extent to which rates of HIV infection have been monitored. However, there appears to be substantial heterogeneity, both within and across countries, in the patterns of HIV transmission (Kaldor *et al.*, 1994). As in Europe, the first Asian cases of HIV infection and AIDS were reported in homosexual men (Weniger *et al.*, 1991), but other routes of transmission later became predominant in a number of countries. In Myanmar (Htoon *et al.*, 1994) and Thailand (Brown *et al.*, 1994a), the prevalence of HIV infection among intravenous drug users increased rapidly during the mid- to late 1980s, reaching levels of 40–50% within a few years. High prevalences were reported among intravenous drug users in Yunnan Province, China (Xinhua *et al.*, 1994), the north-east Indian state of Manipur (Sarkar *et al.*, 1993) and, more recently (and to a lesser extent so far), in Malaysia (Singh *et al.*, 1994) and Vietnam (Kaldor *et al.*, 1994).

A separate HIV epidemic in Thailand initially arose through transmission between sex workers and their clients. Some surveys of prostitutes have found up to 70% having HIV infection, with a strong inverse association between prevalence of infection and the price charged per client, presumably through association with frequency of contact and prevalence in client groups (Brown *et al.*, 1994a).

A high prevalence of HIV infection has also been found among female prostitutes in a number of Indian cities (Jain *et al.*, 1994).

In several Asian countries, monitoring of population subgroups more representative of the general population, such as pregnant women and military recruits, has revealed a steady increase in HIV prevalence, presumably as a consequence of heterosexual transmission. By 1993, the prevalence of HIV infection in pregnant women had reached 2% in Thailand overall and 8% in the northern province of ChiangMai. The prevalence among military recruits in northern Thailand (men aged around 20) was of the order of 10% (Brown *et al.*, 1994a). In several other countries, including Cambodia and India, the reported HIV prevalence among volunteer blood donors has already exceeded 1% (Jain *et al.*, 1994; Kaldor *et al.*, 1994).

Nevertheless, a large part of the Asian population so far appears to be relatively untouched by the global spread of the HIV epidemic. The small numbers of cases reported from China (mostly from Yunnan province), Pakistan, Bangladesh and Indonesia (WHO, 1995) may to some extent be attributable to limited surveillance systems, but probably also reflect very low rates of HIV transmission in these countries.

(h) Oceania

In Australia and New Zealand, HIV transmission has overwhelmingly been through sexual contact between men (Crofts *et al.*, 1994). Transmission via this route occurred at high levels in the early 1980s but declined sharply in the second half of the decade.

In Papua New Guinea, heterosexual contact has emerged as the most important route of transmission (Malau *et al.*, 1994).

(i) Middle East

Few cases of HIV infection or AIDS have been reported from Middle Eastern countries (WHO, 1995), and distinct transmission patterns have not been discerned.

1.4 Clinical description of non-neoplastic disorders

1.4.1 *Seroconversion syndrome*

The 'seroconversion syndrome', also known as 'primary HIV infection' or 'acute retroviral syndrome', refers to a complex of symptoms that occur in the first one to six weeks after HIV-1 infection in many adult patients (Tindall *et al.*, 1988a,b) during the 'window period' before HIV antibody is detectable (see Section 1.2.1). Early observations on a few patients (Cooper *et al.*, 1985; Ho *et al.*, 1985a) indicated that these included truncal maculopapular rash, fever, arthralgia, myalgia, sore throat, lymphadenopathy, abdominal cramps, diarrhoea and headache (Ho *et al.*, 1985a). Subsequent studies of series of patients in the United States (Fox *et al.*, 1987), Australia (Tindall *et al.*, 1988a,b), Italy (Sinicco *et al.*, 1990) and Switzerland (Kinloch-de Loës *et al.*, 1993) have confirmed this constellation of signs and symptoms (see Table 4), although the frequency varies somewhat depending on the definitions used, the means of determination (e.g., self-reported versus observed) and the severity or persistence of symptoms. Additional signs and symptoms in persons with primary HIV infection include lethargy and malaise, anorexia and weight loss, retro-orbital pain and, more rarely, rhinorrhoea, dark urine and irritability (Cooper *et al.*, 1985; Tindall *et al.*, 1988a,b).

Table 4. Selected common symptoms in series of patients with seroconversion syndrome

Reference	No.[a]	Percentage with						
		Fever	Skin rash	Sore throat	Myalgia/ arthralgia	Headache	Diarrhoea	Enlarged[b] nodes
Kinloch-de Loës *et al.* (1993)	31	87	68	48	42	39	32	57
Sinicco *et al.* (1990)	12	100	58	75	75	NR	17	92
Tindall *et al.* (1988a)	39	77	23	56	56	49	28	43
Fox *et al.* (1987)	22	23	14	23	14	23	14	36

[a] Number of patients in series
[b] Enlarged nodes, polyadenomegaly; enlarged lymph nodes/lymphadenopathy
NR, not reported

In the first weeks of HIV-1 infection, there are very high levels of circulating virus (Clark *et al.*, 1991; Daar *et al.*, 1991) and 'antigen excess' as determined by p24 antigen assays (Kessler *et al.*, 1987; Henrard *et al.*, 1995). Numbers of peripheral $CD4^+$ T-lymphocytes decrease markedly and $CD8^+$ T-lymphocytes increase (Roos *et al.*, 1992; Weiss *et al.*, 1992; Zaunders *et al.*, 1995). Leukopenia and thrombocytopenia may be seen (Cooper *et al.*, 1985; Ho *et al.*, 1985a; Scully *et al.*, 1989; Kinloch-de Loës *et al.*, 1993) (Figure 6).

The occurrence of the seroconversion syndrome and its clinical severity may be prognostic of a rapid rate of progression to AIDS (Sinicco *et al.*, 1993; Henrard *et al.*, 1995).

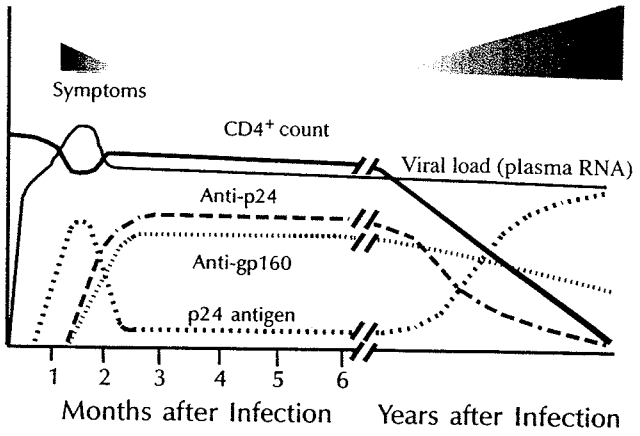
1.4.2 *Immunological decline*

Following infection, there is a variable period during which most patients are asymptomatic but undergo progressive immunological decline. This may be measured by various parameters such as $CD4^+$ T-cell counts and percentages of total lymphocytes, the ratio of $CD4^+$ to $CD8^+$ T-cells and serum levels of β_2-microglobulin and neopterin (Fahey *et al.*, 1990; Gruters *et al.*, 1991). Immunological decline is not smooth or consistent over the prolonged course of infection. As a general rule, some parameters, such as $CD4^+$ T-cell count, percentage and $CD4^+$ to $CD8^+$ ratio, decline with duration of HIV infection and appearance of symptomatic disease, whereas markers of lymphocyte activation, such as serum levels of β_2-microglobulin and neopterin, increase (see Figure 6).

Peripheral blood measurements, particularly the absolute $CD4^+$ T-cell count (or $CD4^+$ T-cell percentage), are used clinically to indicate the stage of HIV disease. $CD4^+$ T-cell decline and rate of decline have proven to be useful, if imperfect, markers of the development of the disease (Fahey *et al.*, 1990; Phillips *et al.*, 1991). During primary HIV infection, $CD4^+$ T-cells and their percentage typically fall rapidly, rise again with

the appearance of HIV antibody, then gradually decline during a long 'latent' (asymptomatic) period of several years (Margolick *et al.*, 1993, 1994; Holmberg *et al.*, 1995a). Subsequently, a more rapid drop in $CD4^+$ T-cell count or percentage presages the onset of AIDS-defining conditions and opportunistic infections (Krämer *et al.*, 1992; Galai *et al.*, 1993; Phillips *et al.*, 1994a). The prognostic value of rapidly declining or low $CD4^+$ T-cell counts as predictors of AIDS onset has been amply demonstrated in populations at risk for HIV infection, including homosexual and bisexual men (Schechter *et al.*, 1989; Veugelers *et al.*, 1993), intravenous drug users (Zangerle *et al.*, 1991; Margolick *et al.*, 1992; Muñoz *et al.*, 1992; Alcabes *et al.*, 1993a), heterosexual women (Flanigan *et al.*, 1992) and haemophilic men (Eyster *et al.*, 1987; Phillips *et al.*, 1989).

Figure 6. Schematic model of the natural history of HIV-1 infection



Markers of immunological decline other than $CD4^+$ T-cells have been investigated for prognostic purposes. In particular, serum levels of β_2-microglobulin and neopterin, non-specific markers of inflammation, correlate with declining immunity and the onset of AIDS-related conditions (Krämer *et al.*, 1992; Lifson *et al.*, 1992; Muñoz *et al.*, 1992; Galai *et al.*, 1993). Some investigators have found that addition of serum β_2-microglobulin or neopterin determinations to $CD4^+$ T-cell counts improves prognostic ability, but in general, the clinical role of these markers is diminishing (Melmed *et al.*, 1989; Fahey *et al.*, 1990; Krämer *et al.*, 1992; Muñoz *et al.*, 1992; Galai *et al.*, 1993).

The various immunological markers do not reflect accurately the total body burden of HIV (Pantaleo *et al.*, 1993a). HIV is actively replicating throughout the long asymptomatic period of infection. Although the decline in $CD4^+$ T-cells is gradual (Figure 6), up to 30% of the PBMCs may be infected by HIV and lost each day. The total viral load varies, but 10^{10} or more new virions may be generated per day and viral load measurements have been shown to have prognostic value beyond the $CD4^+$ count (Ho *et al.*, 1995; Wei *et al.*, 1995; Mellors *et al.*, 1996; O'Brien *et al.*, 1996).

During HIV-1 and HIV-2 infection, cellular immunity is compromised more than humoral immunity (Fauci *et al.*, 1991; Pantaleo & Fauci, 1995). Not only the number but also the function of $CD4^+$ and $CD8^+$ cytotoxic T-lymphocytes decrease, particularly in the

initial stages of HIV infection (Gruters *et al.*, 1991; Mackewicz *et al.*, 1991; Margolick *et al.*, 1993; Torpey *et al.*, 1993; Koup *et al.*, 1994). Anergy to delayed-type hypersensitivity skin tests is also more likely to occur as the disease progresses (Blatt *et al.*, 1993; Gordin *et al.*, 1994).

1.4.3 *Non-AIDS-defining manifestations of HIV infection*

(*a*) *Classification of HIV disease*

The use of the term 'AIDS' has been complicated by changes in its definition and the need to apply somewhat different definitions depending upon local situations. The initial definition of AIDS was developed in 1982 by the CDC and subsequently accepted by WHO in 1985. There were major revisions of the classification system in 1987 (WHO, 1988); cervical cancer, recurrent pneumonia, pulmonary tuberculosis and, for persons in the United States, a $CD4^+$ T-cell count of less than 200 cells/mm^3 (or percentage less than 14%) in HIV-positive individuals were added to the definition at the beginning of 1993 (Centers for Disease Control and Prevention, 1992a). Each of these revisions resulted in a large increase in reported numbers of AIDS cases in subsequent years, as AIDS was diagnosed earlier by including a broader range of conditions and, particularly in the United States, by including $CD4^+$ T-cell counts in patients who had not developed an AIDS-defining opportunistic infection or malignancy.

Because of the different spectrum of AIDS-related diseases in developing countries, and the shortage of sophisticated diagnostic equipment there, a WHO workshop in 1985 adopted a provisional clinical case definition of AIDS for use in such regions of the world (WHO, 1986).

Some non-malignant, non-AIDS-defining conditions have been described in the past as 'persistent generalized lymphadenopathy' and 'AIDS-related complex'. The former term was used to describe the lymphadenopathies often seen in HIV-infected persons before AIDS was recognized as an entity (Centers for Disease Control, 1982). In 1983, the Extramural AIDS Working Group of the US National Cancer Institute and National Institutes of Allergy and Infectious Diseases first defined the term 'AIDS-related complex' to cover the status of persons whose clinical condition did not meet the AIDS surveillance definition but who exhibited clinical and laboratory abnormalities that appeared to be related to AIDS (Abrams, 1988). This definition was never widely adopted. AIDS-related complex originally referred to persistent lymphadenopathy (Kaplan *et al.*, 1988), fever, weight loss, diarrhoea, fatigue and night sweats and, in standard laboratory tests, leukopenia, thrombocytopenia (Abrams, 1988; Sloand *et al.*, 1992) and anaemia. Later, other non-fatal conditions such as oral candidiasis, oral hairy leukoplakia and herpes zoster (Buchbinder *et al.*, 1992; Holmberg *et al.*, 1995b) were included, as well as some major manifestations that later became part of the most recent CDC definition of AIDS (Centers for Disease Control and Prevention, 1992a; see Table 5).

Table 5. Conditions included in the 1993 AIDS surveillance case definition[a]

Candidiasis of bronchi, trachea or lungs
Candidiasis, oesophageal
Cervical cancer, invasive[b]
Coccidiomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (> 1 month's duration)
Cytomegalovirus disease (other than liver, spleen or nodes)
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV-related
Herpes simplex; chronic ulcer(s) (> 1 month's duration); or bronchitis, pneumonitis or oesophagitis
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (> 1 month's duration)
Kaposi's sarcoma
Lymphoma, Burkitt's (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium complex or *M. kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis, any site (pulmonary[b] or extrapulmonary)
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis carinii pneumonia
Pneumonia, recurrent[b]
Progressive multifocal leukencephalopathy
Salmonella septicaemia, recurrent
Toxoplasmosis of brain
Wasting syndrome due to HIV
Immunodeficiency as measured by a $CD4^+$ T-cell count less than 200 cells/mm^3 or $CD4^+$ T-cell percentage less than 14%[b,c]

[a] From Centers for Disease Control and Prevention (1992a) [Appendix B]

[b] Added in the 1993 expansion of the AIDS surveillance case definition

[c] United States only

(*b*) *Non-AIDS illness*

To summarize a large body of research and clinical observations, it is clear that there are many pre-AIDS conditions, signs and symptoms of HIV infection. In persons with immunological impairment, many of these conditions reflect opportunistic or reactivated infection. Generally, these include 'constitutional' symptoms, namely persistent weight loss, diarrhoea, sweating and headaches (independent of intracranial causes) (Greenberg *et al.*, 1992; Hoover *et al.*, 1993; Holmberg *et al.*, 1995b); oral and sinus problems, including oral candidiasis, oral hairy leukoplakia and sinusitis (Farizo *et al.*, 1992;

Holmberg *et al.*, 1995b); skin manifestations, such as herpes zoster, seborrhoeic dermatitis and eczema; and anogenital problems, such as ulcers, fissures, warts and vaginal candidiasis (Renzullo *et al.*, 1991; Holmberg *et al.*, 1995b). Finally, several early neurological manifestations can be added to the spectrum of morbidity suffered by persons before they develop AIDS (Janssen *et al.*, 1989; Holmberg *et al.*, 1995b).

(*c*) *Time to AIDS*

The incubation time between HIV infection and the appearance of clinical AIDS conditions is of obvious importance to clinicians caring for HIV-infected patients, to epidemiologists and statisticians trying to model the size and direction of the HIV epidemic, to health care planners and administrators attempting to anticipate future health care needs of the HIV-infected population and last but not least to the patients themselves. This incubation period has been examined in populations in which dates of HIV-1 infection could be ascertained or interpolated, including homosexual and bisexual men (Lui *et al.*, 1988; Bacchetti & Moss, 1989; Biggar *et al.*, 1990; Giesecke *et al.*, 1990; Rutherford *et al.*, 1990; Kuo *et al.*, 1991) and transfusion recipients (Ward *et al.*, 1989). Almost all studies indicate that the median incubation period is 7–11 years (Alcabes *et al.*, 1993b). Many studies have attempted to discern host factors that may shorten or lengthen the incubation period of HIV infection, but only one 'cofactor', age, has been found consistently. In adults, the older the HIV-infected patient is, the shorter is the incubation period (Biggar & International Registry of Seroconverters, 1990; Mariotto *et al.*, 1992; Darby *et al.*, 1996). Antiretroviral therapies against HIV and prophylactic therapies against diseases associated with it, such as *Pneumocystis carinii* infection, have been shown to delay the onset of AIDS (Collier *et al.*, 1996).

1.4.4 *AIDS manifestations*

Table 5 lists the 26 AIDS-defining conditions recognized by CDC. Apart from the recognized HIV-associated malignancies, almost all are opportunistic infections. However, there is geographical variation, probably related to the varying prevalence of relevant pathogens. In Thailand, *Penicillium marneffei*, not included in CDC's definition of AIDS, is a very common fungal pathogen in AIDS patients (Sirisanthana & Sirisanthana, 1995).

The most frequently reported opportunistic infection of HIV-infected adults and children in the United States and most other developed countries is *Pneumocystis carinii* pneumonia (PCP) (Hughes, 1995). However, as treatment recommendations and guidelines have been published and promulgated (Centers for Disease Control and Prevention, 1992b), the incidence of cases of AIDS-defining PCP has declined (Muñoz *et al.*, 1993; Katz *et al.*, 1994; Centers for Disease Control and Prevention, 1995a; see Section 1.5.3).

Tuberculosis and non-tuberculous mycobacterial infections, particularly *Mycobacterium avium* complex (*M. avium* and *M. intracellulare*) (Horsburgh, 1991) are common. These have received much attention, because many multi-drug-resistant strains of *M. tuberculosis* have become epidemic in HIV-1-infected persons, especially in New York City in recent years (Frieden *et al.*, 1993). The continuing high rates of tuberculosis in

HIV-infected persons in developing countries present great problems for prevention, diagnosis and treatment (Pitchenik, 1990).

Candidiasis of the oesophagus, bronchi, trachea and lungs are all AIDS-defining conditions in HIV-infected persons.

Other fungal infections, such as cryptococcosis, coccidioidomycosis and histoplasmosis, are AIDS-defining opportunistic infections (Galgiani & Ampel, 1990; Currie & Casadevall, 1994; Stevens, 1995; Rinaldi, 1996) and have been included in several comprehensive clinical guidelines and preventive efforts for persons with HIV infection (Centers for Disease Control and Prevention, 1995b).

Parasitic infections of the central nervous system, notably with *Toxoplasma gondii,* are life-threatening complications in the HIV-immunocompromised host and require early diagnosis to optimize treatment (Wang *et al.*, 1995). The protozoans *Cryptosporidium* and *Isospora* have long been recognized as important causes of chronic diarrhoea in AIDS patients (DeHovitz *et al.*, 1986; Lopez & Gorbach, 1988).

Cytomegalovirus infections of the retina and intestines are often seen late in the course of HIV infection. Cytomegalovirus retinitis and colitis are much more difficult to prevent or treat than PCP and some other parasitic and bacterial infections. Therefore, as a proportion of AIDS diagnoses, their frequency has increased in developed countries, while that of PCP has decreased (Katz *et al.*, 1994).

Bacterial infections are frequent in HIV-infected persons, especially community-acquired pneumonia (Caiaffa *et al.*, 1993; Holmberg *et al.*, 1995b) and septicaemia (Whimbey *et al.*, 1986). Recurrent salmonellosis, an AIDS-defining condition, is an important, if less frequent, enteric infection (Lopez & Gorbach, 1988).

Progressive multifocal leukoencephalopathy is caused by the JC virus (Fong *et al.*, 1995). Focal neurological manifestations can be caused by opportunistic infections, such as toxoplasmosis, or by lymphoma. HIV can also directly cause peripheral nervous system abnormalities, such as sensory neuropathy, and AIDS-related dementia in late HIV infection (Simpson & Tagliati, 1994).

Wasting syndrome (DuPont & Marshall, 1995; Grunfeld, 1995), originally referred to as 'Slim disease' in Africa (Serwadda *et al.*, 1985), has long been recognized as a major cause of HIV-related morbidity and mortality. Reduced calorific intake is the prime determinant of this weight loss (Macallan *et al.*, 1995).

Paediatric AIDS has a somewhat different clinical profile, with an increased incidence of lymphocyte intestinal pneumonia in HIV-infected children (Horowitz & Pizzo, 1990; Chintu *et al.*, 1993).

1.4.5 *Long-term non-progressors*

'Long-term non-progressors', 'healthy long-term survivors' and other such terms describe persons known to be infected for several years but who have no or minor symptoms of HIV infection and who have $CD4^+$ T-cell counts that are normal or near normal (e.g., more than 500 $CD4^+$ T-cells/mm^3). About 5–10% of HIV-infected persons remain asymptomatic and maintain $CD4^+$ T-lymphocyte counts above 500 cells/mm^3 for 10 or more years (Buchbinder *et al.*, 1994). With time after infection, the percentage of

long-term non-progressors declines (Baltimore, 1995). While few in number, these persons have become the focus of much current research from two broad points of view: the host and the virus.

Most research into host factors has focused on factors associated with preserved immune function, and indicates that non-progressors, compared with other HIV-infected persons, have higher $CD8^+$ T-lymphocyte counts and lower antigenaemia and viral load (Lifson *et al.*, 1991; Buchbinder *et al.*, 1994; Cao *et al.*, 1995; Hogervorst *et al.*, 1995; Pantaleo *et al.*, 1995). $CD8^+$ T-cell function appears to be important in the control of viral replication (Lifson *et al.*, 1991; Landay *et al.*, 1993), while the role of neutralizing antibodies is unclear (Hogervorst *et al.*, 1995).

Viral variants may have different pathogenicity. Evidence of at least one less virulent strain of HIV-1 with a variant form of *nef* gene has come from a cluster of long-term healthy survivors infected from a single blood donor (Deacon *et al.*, 1995).

1.4.6 *Human immunodeficiency virus type 2 (HIV-2)*

HIV-2 has been recovered mainly from patients in west Africa. A seroconversion syndrome has also been described in relation to HIV-2 infection (Besnier *et al.*, 1990). Symptomatic patients usually have been described as having chronic diarrhoea, weight loss, lymphadenopathy and tuberculosis. However, HIV-2-infected persons can have the same immunological and clinical spectrum of disease as HIV-1 (Clavel *et al.*, 1987; Marlink *et al.*, 1988; Nauclér *et al.*, 1989; Odehouri *et al.*, 1989). Sexual and mother–child transmission seem to be less efficient (Matheron *et al.*, 1990; Markowitz, 1993; Kanki *et al.*, 1994). There is evidence that HIV-2 is less pathogenic than HIV-1. HIV-2-infected patients may have longer incubation periods between infection and AIDS-defining conditions than do HIV-1-infected patients (Burin Des Roziers *et al.*, 1987; Pepin *et al.*, 1991; Markowitz, 1993; Whittle *et al.*, 1994).

1.5 Control and prevention

1.5.1 *Behavioural prevention*

In the absence of a vaccine, behavioural change remains necessary to stem the worldwide HIV epidemic. To prevent sexual transmission, two general categories of preventive activity are usually urged: reducing the number of sexual partners and modifying the types of sexual contact; and the use of condoms.

Protection of sex partners from exposure to semen, blood and vaginal fluid during intercourse can be accomplished by the consistent and correct use of condoms, and this recommendation has been promulgated worldwide (Choi & Coates, 1994; Johnson, 1994; Stryker *et al.*, 1995). Other strategies to minimize risk of infection may be useful, such as penile withdrawal prior to ejaculation (de Vicenzi *et al.*, 1994) and the use of the vaginal pouch (or 'female condom') (Farr *et al.*, 1994).

Various programmes to change behaviour — such as increasing the use of condoms — have been effective to varying extents (Choi & Coates, 1994; Kelly *et al.*, 1994; Moore *et al.*, 1994; Stryker *et al.*, 1995). The greatest change has occurred among older

European and American homosexual men, who dramatically decreased their sexual exposures and HIV infection rates as early as the mid-1980s (Winkelstein *et al.*, 1987; Centers for Disease Control and Prevention, 1992c). The change in sexual behaviour and use of condoms among heterosexual men and women has been more modest (Catania *et al.*, 1992; Diaz *et al.*, 1994).

Empirical evidence indicates that behaviourally based HIV prevention programmes have had a favourable impact in specific populations, especially when delivered with sufficient resources, intensity and cultural sensitivity (Holtgrave *et al.*, 1995; Office of Technology Assessment, 1995). However, outcomes of prevention programmes, such as partner notification (Potterat *et al.*, 1989), have not been well evaluated. Some programmes or measures have been evaluated, and found to be ineffective, for example, programmes for counselling and testing (Higgins *et al.*, 1991a) and mandatory premarital testing for HIV (Turnock & Kelly, 1989).

Behavioural interventions are thought to have reduced the spread of HIV among intravenous drug users who share needles, syringes and other blood-tainted effects (Booth & Watters, 1994; Chitwood, 1994; Watters, 1994). Firstly, treatment for drug dependence can reduce the number of intravenous drug users in a community and so, presumably, decrease HIV transmission (Sisk *et al.*, 1990). Secondly, previously used needles may be disinfected, usually with bleach, but the contact times with bleach that are necessary to reduce or eliminate HIV in injection equipment are considerably longer than those generally applied by intravenous drug users (Centers for Disease Control and Prevention, 1994b; Garza *et al.*, 1994; Gleghorn *et al.*, 1994). Thus, it is not clear that bleach disinfection has reduced the risk of HIV infection among intravenous drug users (Booth & Watters, 1994; Titus *et al.*, 1994).

Recent attention has focused on the effectiveness of needle and syringe exchange and distribution programmes. There is accumulating evidence that providing sterile needles reduces the transmission of HIV among intravenous drug users (Donoghoe *et al.*, 1989; Hart *et al.*, 1989; Hartgers *et al.*, 1989; Stimson, 1989; van Ameijden *et al.*, 1994; Heimer *et al.*, 1994; Watters *et al.*, 1994; Centers for Disease Control and Prevention, 1995c; Hagan *et al.*, 1995). A recent international comparison of cities with and without needle exchange programmes supports the effectiveness of such measures (Feachem *et al.*, 1995). To provide sterile needles for injection, the deregulation of the sale and possession of needles and syringes has been advocated (Des Jarlais *et al.*, 1994; Vlahov, 1995). However, some countries in which disposable syringes are commercially available and cheap, such as Italy, have nevertheless experienced a high prevalence of HIV among intravenous drug users.

1.5.2 *Screening*

Antibody-test screening of all blood or plasma donors has been universal in developed countries since the mid-1980s and has resulted in a marked reduction in HIV transmission by blood transfusion or use of clotting factor concentrates. For example, it has been estimated that among 12 million blood donations collected in the United States, only 18–27 are now infectious (Lackritz *et al.*, 1995) because the donors were in the

'window period'. Blood transfusion has remained a major mode of HIV transmission in some developing countries, where screening of blood donors is not universal (N'tita *et al.*, 1991; Vos *et al.*, 1994).

Several countries recommend the counselling and voluntary screening of pregnant women for HIV infection (Centers for Disease Control and Prevention, 1995d) to allow them to take informed decisions about continuation of pregnancy, and enable suitable medical care and interventions to reduce the risk of vertical transmission to be applied. The rationale for screening mothers antenatally has received additional impetus from the finding that zidovudine (also called azidothymidine, AZT) taken by infected pregnant women and their newborns substantially reduces the probability of mother-to-child transmission (Connor *et al.*, 1994; Centers for Disease Control and Prevention, 1995e). Studies of simplified treatment protocols, particularly for use in developing countries, are being conducted (Dabis *et al.*, 1995).

1.5.3 *Treatment*

Zidovudine may reduce the levels of HIV in the semen of HIV-infected men (Anderson *et al.*, 1992) and hence its infectiousness; similarly, women taking zidovudine may be less likely to transmit HIV to their HIV-uninfected regular male partners (Nicolosi *et al.*, 1994b). However, the evidence that use of zidovudine prevents the sexual transmission of HIV should be considered as tentative and zidovudine-resistant strains of HIV are now being identified in newly acquired infections.

The literature on the efficacy of zidovudine and other reverse transcriptase inhibitors (e.g., didanosine (also called dideoxyinosine, ddI); dideoxycytidine (also called zalcitabine, ddC); stavudine) in prolonging survival of patients with HIV infection and AIDS is extensive. Briefly, improvements in survival time after AIDS diagnosis have been observed in America and Europe (Fischl *et al.*, 1987; Lafferty *et al.*, 1991; Jacobson *et al.*, 1993; Whitmore-Overton *et al.*, 1993; Blum *et al.*, 1994; Lundgren *et al.*, 1994). However, most recent reports indicate that zidovudine monotherapy is of modest benefit in the prolongation of this incubation time (Holmberg & Byers, 1993; Concorde Coordinating Committee, 1994; Volberding *et al.*, 1994, 1995). It has been suggested that improved incubation and survival times may be more attributable to improved prophylaxis and treatment of *Pneumocystis carinii* pneumonia than to use of zidovudine and other antiretroviral drugs (Lundgren *et al.*, 1994).

Antiretroviral therapy is in constant evolution. Chemotherapeutic agents have been evaluated on the basis of their ability to reduce viral load, as measured by the level of HIV-1 RNA in plasma (O'Brien *et al.*, 1996). A number of promising new agents may retard the development of HIV disease and prolong survival (Hirsch & D'Aquila, 1993; Saag *et al.*, 1993; Sande *et al.*, 1993). At present, interest has centred on the so-called 'protease inhibitors' (Danner *et al.*, 1995; Kitchen *et al.*, 1995), on combination therapy with two or more antiretroviral drugs used together or in rotation (Fauci, 1992; Kahn *et al.*, 1992; Abrams *et al.*, 1994; Yarchoan *et al.*, 1994; Collier *et al.*, 1996) and on the use of ILs (Schnittman *et al.*, 1994).

1.5.4 *Prospects for vaccines*

The development of a safe, effective and cheap preventive vaccine for HIV-1 or HIV-2 faces many obstacles: the considerable antigenic variability of the virus; the integration of proviral DNA in the host gene; the viability of the virus both inside and outside cells; the mucosal (sexual) and blood-borne modes of transmission; and the persistent nature of the infection even in the presence of host immunity (Girard, 1995; Graham & Wright, 1995; Hilleman, 1995). Nevertheless, more than 20 candidate vaccines have undergone preclinical evaluation for safety and immunogenicity in about 2000 volunteers. Several have entered phase I clinical testing in uninfected volunteers, and a few vaccines are now being evaluated in phase II studies in larger numbers of persons at risk for HIV infection. Candidate vaccines have been of various types, including whole killed virus and recombinant live vectors (e.g., canary pox) expressing antigens. Most of those still under consideration rely on immunization with recombinant or synthetic HIV peptides or envelope proteins such as gp120 or gp160 (see Section 1.1.7). These may induce neutralizing antibodies or lymphoproliferative responses (e.g., cytotoxic T-cell activity), but only variably and, even then, only to laboratory-adapted HIV-1 strains (not primary or wild-type isolates) (Johnston *et al.*, 1993; Dolin, 1995). Furthermore, several 'breakthrough' HIV infections have been documented in volunteers who received partial or complete series of vaccinations (Kahn *et al.*, 1995). In addition to immunization with antigenic peptides or proteins, another direction of research has been the use of live, attenuated mutant virus, which has provided immunological protection in some simian models. However, serious concerns about the use of live, attenuated virus vaccines in humans remain because viruses with deleted *nef* gene have been shown to cause disease in neonatal macaques (Baba *et al.*, 1995).

1.5.5 *Other approaches*

There is considerable interest in the safety and efficacy of agents such as Nonoxyl 9 (Elias & Meise, 1993) and dextrin sulfate (Stafford *et al.*, 1995) as vaginal virucides to protect against heterosexual transmission of HIV-1 and HIV-2. A perceived advantage of such agents over condoms is that they may be used unobtrusively by women in situations where condom usage is not acceptable to either or both partners.

Recent data from Tanzania show that HIV transmission can be reduced by effective, syndromic treatment of other sexually transmitted diseases (Grosskurth *et al.*, 1995; Hayes *et al.*, 1995; Dik *et al.*, 1995; Foulkes *et al.*, 1995; O'Reilly *et al.*, 1995; Rygnestad *et al.*, 1995; Whitaker & Renton, 1995).