THE VIROLOGY AND DYNAMICS OF THE EPIDEMIC

David O. Olaleye,* Tekena O. Harry,† and Georgina N. Odaibo*

UNIVERSITY OF IBADAN LIBRARY

Viruses are submicroscopic, obligate intracellular parasites that can neither grow nor reproduce outside a living cell. Their survival therefore depends completely on the continued survival of their hosts. They are unique among all other living organisms, with the following main features:

- The genome of a complete virion—or an infective virus particle—is either RNA or DNA;
- Virus particles are produced from the assembly of preformed components, whereas other agents "grow" from an increase in the integrated sum of their components and reproduce by division;
- Matured virus particles themselves do not "grow" in size or undergo division, but multiply by a process called replication (of their genetic material);
- Viruses lack the genetic information that encodes the apparatus (mitochondria) necessary for the generation of metabolic energy or for protein synthesis (ribosomes); they therefore depend on the host machinery for these functions; and
- They are composed mainly of nucleic acid and proteins (that is, products of the various genes).

*Department of Virology, College of Medicine, University of Ibadan, University College Hospital, Ibadan, Nigeria
†Department of Immunology and Microbiology, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria

Table 3-1. Host Range and Associated Diseases of Lentiviruses

Virus	Natural Host Species	Clinical Presentation
Equine infectious anemia virus	Horse	Cyclical infection in the first year, autoimmune hemolytic anemia, occasional encephalopathy, arthritis
Visna maedi virus	Sheep	Encephalopathy, pneumonitis, arthritis, wasting
Caprine arthritis-encephalitis virus	Goat	Lymphadenopathy, lymphocytosis, anemia, wasting, arthritis, encephalitis
Bovine immune deficiency virus	Cow	Lymphadenopathy, lymphocytosis, possible central nervous system disease
Feline immunodeficiency virus	Cat	Immune deficiency, wasting, encephalitis
Simian immunodeficiency virus	Primates	Immune deficiency and encephalopathy
Human immunodeficiency virus	Human	Immune deficiency and encephalopathy
	Chimpanzee	None

Source: Adapted from the International Committee on Taxonomy of Viruses database (http://phene.cpmc.columbia.edu/index.htm)

The morphology of viruses may be circular, oval, cylindrical, spiral, filamentous, hexagonal, or helical. The shape of the viral capsid usually determines its morphology. The smallest viruses are approximately 20 nm in diameter and the largest around 250 nm. The nucleic acid polymer may either be double-stranded or single-stranded DNA or RNA. This viral nucleic acid polymer may contain as few as 4 to 7 genes for the tiniest viruses to as many as 150 to 200 genes for the largest viruses. In some viruses, the nucleic acid may occur as more than one molecule or as intermediate forms during the process of replication.

Furthermore, some viruses contain a few enzymes while others contain none. All viruses are covered with a protein coat called the capsid. If a virus has only a protein capsid covering it, it is termed a naked capsid virus or a naked virus. Some viruses acquire a lipid membrane from the host cell during the process of release. This lipid membrane surrounds the virus capsid and is called the virus envelope; such viruses are termed enveloped viruses. In addition, virus particles may contain small quantities of carbohydrate moieties on the envelope, called glycoproteins.

Virus infection of higher organisms is the cumulative result of infection, replication, and expression of the viral genome. Virus infections range in complexity and duration from a brief, superficial interaction between the virus and its host, to chronic or persistent infection, which may encompass the entire life of the host organism. A common misconception is that virus infection invariably results in disease; instead, only a small proportion of viral infections give rise to any disease symptoms. The course of virus infections may be abortive, acute, chronic, latent, persistent, or steady state.

CHARACTERISTICS OF RETROVIRUSES

The virus family Retroviridae includes three sub-families: Oncovirinae, Lentivirinae, and Spumavirinae. Each member contains an enzyme called reverse transcriptase, which generates provi-

UNIVERSITY OF IBADAN LIBRARY

ral DNA from the infecting viral RNA genome. The provirus (also called the complementary or cDNA) integrates itself into the chromosome of the host cell with the aid of additional enzymes encoded by the viral *pol* gene. The integration of the viral cDNA into the host cell genome permits the continuous viral replication that characterizes retroviruses (1) as well as the unconventional method of "reverse" transcription used by this group of viruses (2).

Table 3-2. Summary of Properties of Lentiviruses

Family	Retroviridae
Major human viruses	HIV-1, HIV-2
Size of virion	80-130 nm
Capsid symmetry	Icosahedral
Envelope	Yes
Genome	Diploid linear + sense single-stranded RNA; 10kb
Genome replication site	Nucleus
Virus assembly	Cytoplasm
Characteristic feature	Slow disease
Associated diseases	AIDS; neurologic; arthritis; pneumonia

Source: Adapted from the International Committee on Taxonomy of Viruses database (http://phene.cpmc.columbia.edu/index.html)

The subfamily Lentivirinae, to which HIV belongs, includes multiple viruses that infect a diverse group of animals (Table 3-1). Interestingly, one of the first viruses identified in nature was a lentivirus, the equine infectious anemia virus, discovered in 1904 (3,4). Lentiviruses typically cause a slowly progressive disease with prolonged subclinical infection (1). Additional characteristics include a long incubation period, suppression of the immune system, tropism for cells of the hematopoietic system, involvement of the nervous system, malignancies, wasting disease, association with autoimmunity and arthritis, and sustained viremia in the absence of any obvious clinical disease. Lentiviruses are host specific and non-oncogenic. They induce syncytia, a phenomenon that occurs when viral fusion proteins that are normally used by the virus to enter host cells are transported to the cell surface, cause the host cell membrane to fuse with neighboring uninfected cells, and cause noncytopathic infection of macrophages. Formation of syncytia, which is caused by the fusion of HIV-infected cells to form large cells with many nuclei, has also been suggested as a possible cause of CD4+ cell depletion in HIV-infected people (5).

The viral cDNA, typically 9 to 10 kb in length, integrates into the host genome. In addition to the structural genes (*gag* and *env*) and enzyme genes (*pol*) that are found in other retroviruses, lentiviruses also encode regulatory proteins such as Tat and Rev, which have been shown to regulate viral transcription and viral RNA transport, respectively (6). Unlike oncoretroviruses, lentiviruses can efficiently infect terminally differentiated, non-dividing cells, such as macrophages and microglia. Table 3-2 shows additional properties of lentiviruses.

Lentiviruses were initially thought to have a latent stage due to their prolonged subclinical infection. It has been shown, however, that these viruses continue to replicate throughout the infection, regardless of the observation or diagnosis of clinical disease (3,4). The first cases of lentivirus-associated disease were reported in Iceland where a virus caused slow demyelinating brain disease (visna) and a progressive lung disease (maedi) in sheep (1). To date, at least six additional lentiviruses have been identified.

UNIVERSITY OF IBADAN LIBRARY

Human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) and related viruses in non-human primates, such as simian immunodeficiency virus (SIV), cause a severe immunodeficiency disorder in their primary hosts (7). HIV-1 is closely related to a naturally occurring virus of certain sub-species of chimpanzees called SIVcpz, while HIV-2 has been shown to be closely related to SIVsm, a virus that naturally infects sooty mangabey monkeys (8). Other members of the genus lentivirus include the feline immunodeficiency virus, the equine infectious anemia virus (3,4,6,7), the caprine arthritis-encephalitis virus, and the bovine immune deficiency virus.

HUMAN IMMUNODEFICIENCY VIRUSES

Retroviruses were initially believed to cause disease only in animals, as no retroviruses were isolated from humans for some time (9). In 1978, however, Poiesz et al. reported the isolation of a retrovirus called human T-cell lymphotropic virus (HTLV) from a patient with mycosis fungoids (10). Since the initial discovery of HTLV, many other retroviruses, including HIV-1 and HIV-2, have been isolated from humans.

Human immunodeficiency viruses, like other retroviruses, have a positive-sense, single-stranded RNA genome (11). In the mature virus, the genome is diploid, with a 60-70s complex of the two identical RNA copies. Electron-microscopy studies have showed that HIV has a dense, cylindrical core encoded by the Gag protein that surrounds the RNA genome. The central core is enclosed by a highly glycosylated protein envelope that is partially acquired from the surface of the host cell as mature virions are released (Figure 3-1A) (12).

HIV Genes and Proteins

The HIV proviral genome is approximately 10 kb in length with an open reading frame that codes for several viral proteins (3). The genome is flanked at both ends by long terminal repeat sequences that contain regulatory elements required for HIV replication (11). The genome includes the *gag*, *pol*, and *env* structural genes that code for the capsid proteins, the viral enzymes, and the internal and external envelope proteins, respectively. In addition to these major genes, the HIV genome has at least five other regulatory or accessory genes: *tat*, *rev*, *nef*, *vif*, and *vpu/vpx*. *Vpu* is present in HIV-1, while *vpx* is present in HIV-2 (3) (Figure 3-1B). At least four of these minor genes are involved in regulating HIV expression (11). Although much remains to be elucidated about the pathways for controlling HIV expression in infected cells, these genes have been characterized and their functions are now fairly well understood.

The *env* gene encodes the envelope precursor, gp160, which is split into two smaller glycoproteins, gp120 and gp41, via cellular enzymes in the Golgi apparatus (4). These glycoproteins have molecular weights of 120,000 and 41,000 (gp120 and gp41, respectively) for HIV-1 and 105,000 and 36,000 (gp105 and gp36, respectively) for HIV-2 (12). The gp120 forms the external surface envelope protein and contains the binding site for cellular entry receptors, as well as major immunodominant domains, while gp41 forms the transmembrane protein (Figure 3-1A; Table 3-3) (4). The structures of these small pro-

UNIVERSITY OF IBADAN LIBRARY

Figure 3-1A. Schematic Morphology of HIV-1

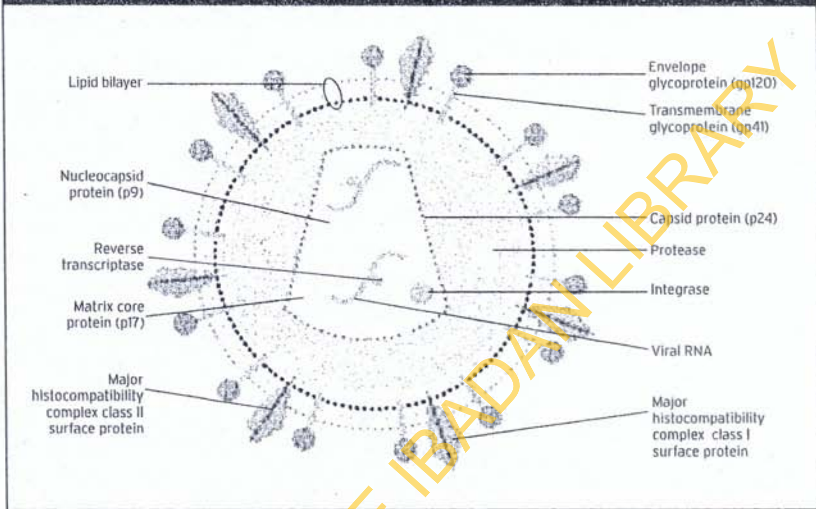
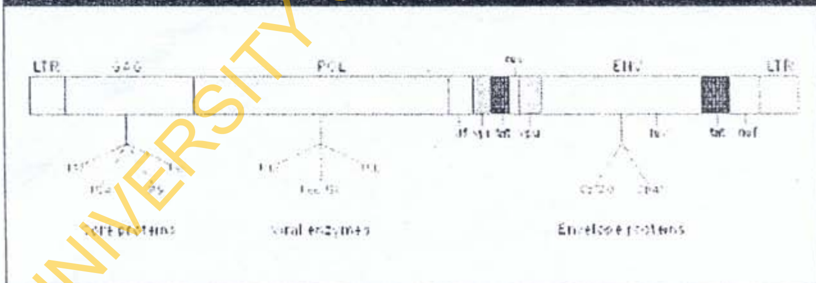


Figure 3-1B. Genomic Structure of a Typical HIV-1



UNIVERSITY OF IBADAN LIBRARY

Table 3-3. Structural Genes of Human Immunodeficiency Viruses			
Gene	**HIV-1**	**HIV-2**	**Protein/Function**
Gag	P24 P17 P9 P6	P26	Capsid (CA) structural protein Matrix (MA) protein-myristoylated RNA binding protein RNA binding protein
Pol	P66 P10 P32	P51 P14	Reverse transcriptase (RT) Post-translational processing of viral proteins (PR) Integration of viral DNA (IN)
Env	Gp120 Gp41	Gp105 Gp36	Envelope surface protein Envelope transmembrane protein
Tat	P14		Transactivation
Rev	P9		Regulation of viral mRNA expression
Nef	P27		Pleitropic, including virus suppression; myristoylated
Vif	P23		Increases virus infectivity and cell-cell transmission; helps in proviral DNA synthesis and/or in virion assembly
Vpr	P15		Necessary in virus replication; transactivation
Vpu	P16		Helps in virus release; disrupts gp160-CD4+ complexes
Vpx		P15	Associated with infectivity

jections appear to be one of the major differences between HIV-1 and HIV-2 (12); antibodies to these two sets of proteins do not usually cross-react and thus differentiate the serologic response to the two distinct human immunodeficiency viruses (12–14).

The envelope surrounds the core proteins that enclose the viral RNA genome and enzymes. The core proteins are encoded by the *gag* gene, whose precursor, p55, gives rise to four smaller proteins (p24, p17, p9, and p6) by proteolytic cleavage (4). The core itself is made up of two proteins of approximately 18,000 and 24,000 daltons in size (p18 and p24). The Pol precursor protein is cleaved into products consisting of the reverse transcriptase (RT), the protease (PR), and the integrase (IN) proteins by protease enzyme. The RT, IN, and PR enzymes have molecular weights of 66,000/51,000 (p66/p51), 32,000 (p32) and 12,000 (p12), respectively (11).

Cellular Receptors

The primary receptor for HIV is the CD4+ molecule located on T-helper cells; a number of other cell types—including macrophages, microglial cells, dendritic cells, and Langerhans cells—also bear these CD4+ receptors (15). Studies on the genetics of infectious diseases have shown that human genetic variation might influence susceptibility to pathogenic organisms, including HIV (16,17). Variation in the number of CD4+ molecules on the T-cell surface may influence the ability of HIV to bind and eventually penetrate the target cell (18). In addition, attachment to and fusion with target cells are determined not only by binding with CD4+ molecules, but also with secondary chemokine co-receptors (19–21).

UNIVERSITY OF IBADAN LIBRARY

The chemokine receptor family members have seven transmembrane helices and interact with G proteins. This family includes receptors for IL-8, MIP-1, and RANTES. The chemokine receptors CCR5 and CXCR4 are commonly used by HIV to preferentially enter either macrophages or T cells, respectively (16). CCR5 is the major co-receptor used for entry of macrophage (M-tropic or R5) isolates of HIV-1, while CXCR4 facilitates entry of T-tropic (or X4) HIV-1 strains. Cells of the myeloid lineage may be infected predominantly with R5 strains, although infection with dual-tropic isolates of HIV-1 or some strains of X4 isolates is possible. Several studies have found that individuals who are homozygous for a deletion in the CCR5 gene are less frequently infected with HIV, while those who are heterozygous for the same mutation still become infected but can be better protected against rapid progression than individuals who are homozygous for the wild-type CCR5 receptor gene (16,17,22).

Replication

Replication of the virus particle begins with attachment of gp120 to the CD4+ on the surface of a target cell. Following the gp120-CD4+ binding, a structural change allows for the interaction of the V3 loop region in the gp120 with a chemokine receptor, including CCR5 and CXCR4. The reaction with the co-receptor results in another conformational change in the viral surface glycoprotein, which exposes a fusion domain contained within the envelope transmembrane glycoprotein. Exposure of the fusion domain results in the insertion of the gp41 into the cellular membrane. Subsequent to the fusion event, the viral core is released into the cytoplasm of the host cell (1,7).

Once in the cytoplasm, the viral RNA genome is uncoated and reverse transcribed by the virally encoded RT enzyme to generate a double-stranded viral DNA preintegration complex. The double-stranded DNA is then transported into the host cell nucleus and, via catalysis by IN, becomes integrated into the host cell chromosome, where it resides as provirus. Once the viral genome has been integrated into the host cell genome, it can remain in a latent state for many years or can begin the production of new viral RNA. If the host cell is activated, the host cell enzyme RNA polymerase II will transcribe the proviral DNA into messenger RNA (mRNA). The mRNA is then translated into viral proteins that undergo extensive post-translational modifications. The viral RNA becomes the genetic material for the next generation of viruses. Viral RNA and viral proteins assemble at the cell membrane. After proper assembly and processing, new infectious virus particles are released by budding from the cell membrane.

GENETIC VARIABILITY OF HIV ISOLATES

An interesting feature of HIV is the marked genetic diversity among its different isolates. The viral reverse transcriptase is error-prone, with a mutation rate of approximately $7x10^6$ to $1.4x10^4$ base pair substitutions; thus, RT can quickly and efficiently give rise to mutations throughout the HIV genome (23). The average rate of base substitution would suggest that HIV-1, with a genome size of about 10 kb, mutates approximately one nucleotide per genome per replication cycle (24). Therefore, within an indi-

UNIVERSITY OF IBADAN LIBRARY

vidual or a population, HIV-1 will contain few, if any, identical genomes. HIV within any individual actually exists as a population of related, yet distinct, viral variants termed the viral *quasispecies*. This variation may have profound effects on the development of immunologic escape, drug resistance, and vaccine-induced immunity. The molecular epidemiology of HIV and the possible implications of this genetic heterogeneity will be discussed in greater detail in Chapter 4, *this volume*.

THE PATHOGENESIS OF HIV INFECTION

Cellular Targets of HIV

The name "human immunodeficiency virus" suggests that this virus ultimately brings about deficiencies in the immune system of infected individuals. To exert such effects, the virus attacks and destroys the very cells that form the immune defense mechanisms of humans. The most important of these cells and their functions are T-lymphocytes, B-lymphocytes, plasma cells, macrophages, and natural killer cells.

T-Lymphocytes

These cells, consisting of several subpopulations, play a vital role in cell-mediated immune responses and help B lymphocytes in humoral antibody production. The most important T-lymphocyte subpopulations are CD4+ T cells (or T4 cells) and CD8+ T cells (or T8 cells). CD4+ T cells help or induce other cells of the immune system to carry out their various functions; hence, they are called helper/inducer cells. CD4+ cells carry out these functions either by direct contact with these or other cells of the immune system or by secreting soluble substances, called cytokines, that serve as chemical messengers and/or chemoattractants that direct cells to the appropriate sites of infection throughout the body. The activities of T4 cells also result in the maturation, activation, and proliferation of other cells of the immune system. T8 cells either suppress the activities of other cells (T4 and B cells), or function as cytotoxic cells, killing virus-infected and tumor cells. They directly bind to cells carrying a foreign antigen and lyse such cells, thus eliminating them from the body. T8 cells have been proposed to play a key role in the immunologic defense against HIV by controlling viral replication through at least two mechanisms: direct antigen-specific cytolysis, which appears to be required for optimal suppression, and release of soluble antiviral factors (24,25). The antiviral activity of T8 cells was first described when investigators observed a depletion in CD8+ cells from the peripheral blood mononuclear cells of HIV-infected people and a corresponding increase in viral replication in the remaining CD4+ cells (26,27). On the other hand, replacement of the CD8+ cells caused a dose-dependent suppression of viral replication.

B-Lymphocytes and Plasma Cells

B-lymphocytes, on maturation, lead to the production of plasma cells, which, in turn, produce antibodies that clear infecting organisms. For the efficient performance of their functions, B-lymphocytes require the assistance of T4 lymphocytes. Plasma cells are small cells produced from the maturation of B cells and in turn produce antibodies against a variety of foreign agents.

UNIVERSITY OF IBADAN LIBRARY

Macrophages

Macrophages are large cells that engulf and kill pathogens. These pathogens are digested by macrophages into multiple antigens that are then presented on the surface of macrophages for recognition and processing by other cells of the immune system. Monocytes constitutively migrate out of the bloodstream into the tissues to replenish the tissue macrophage pool and scout for infection. This physiologic tissue migration of macrophages also provides a means by which HIV-1 can infect the brain, lungs, and many other organs.

Natural Killer Cells

Natural killer cells, otherwise known as NK cells, kill malignant, altered, or deformed cells. Morphologically, NK cells resemble T-lymphocytes. Like the B- and T-lymphocytes, they exist in resting form in normal individuals who have not previously encountered the particular infectious agent or cancerous cells. Unlike T and B cells, NK cells are not specific for the cells they attack.

Effects of HIV on the Immune System

As the principal cellular target of HIV infection is the CD4+ T helper lymphocyte, the depletion of T lymphocytes is a central factor in the progression of HIV/AIDS. Because these cells play a vital role in regulating and amplifying the immune response, any decline in their number results in deficits in both humoral and cell-mediated immunity, resulting in the immune dysfunction that is a hallmark of HIV infection (28). The mechanisms by which HIV induce CD4+ T-cell death are not yet fully understood, however. At least three major mechanisms have been proposed (29):

- the killing of productively infected CD4+ T-cells is caused either by direct viral cytopathic effects or by antiviral cytotoxic CD8+ T lymphocytes;
- viral-mediated killing of bystander uninfected CD4+ T-cells is induced by viral proteins that are released from infected cells; or
- excessive, ongoing immune activation caused by a high, persistent viral antigen load leads to the activation-induced death of various uninfected immune cells, including CD4+ T-cells.

The normal function of CD4+ cells in maintaining an efficient immune response to infection is thus progressively reduced by the cytopathic effects of HIV. Macrophages and CD8+ cells become less efficient at clearing virally infected cells, because CD4+ helper cells are no longer functioning properly. Subsequently, production of specific antibodies is also reduced. Collectively, this severe immunosuppression allows opportunistic pathogens to replicate unchecked within an individual. Thus, as HIV infection progresses, the victim develops immune abnormalities as the virus interferes with the normal interactions and regulation of cells in the immune system.

Viral Load and CD4+ Natural History

Before the widespread use of viral load assays, the CD4+ cell count has been used extensively as a surrogate marker for HIV disease progression. In Nigeria, CD4+ cell counts in healthy individuals have been found

UNIVERSITY OF IBADAN LIBRARY

to range from 636/mm^3 to 977/mm^3 of blood (unpublished data). In Western countries, the mean value has been reported to be 1,000/mm^3 to 1,100/mm^3 (30,31). Thus, constant exposure to a number of other pathogens in sub-Saharan Africa may result in an overall less healthy immune system with which to fight HIV infection.

The course of HIV infection varies within a population. Nonetheless, a typical infection can be divided into three stages: primary infection, asymptomatic infection, and symptomatic infection, or AIDS. Following primary HIV infection, the CD4+ cell count decreases, while HIV RNA rises to high levels. With sufficient exposure to viral antigens, cytotoxic T-lymphocyte responses are generated and the HIV viral load typically declines to an equilibrium known as a virologic "setpoint," which occurs within 6 to 12 months of initial infection. Once this viral setpoint is reached, the CD4+ cell count may rebound again marginally, although it does not often return to baseline values (32). Concurrent with these events are clinical manifestations of acute HIV infection in 30% to 60% of people (Figure 3-2). About half of newly infected people experience flu-like symptoms; the remainder are asymptomatic.

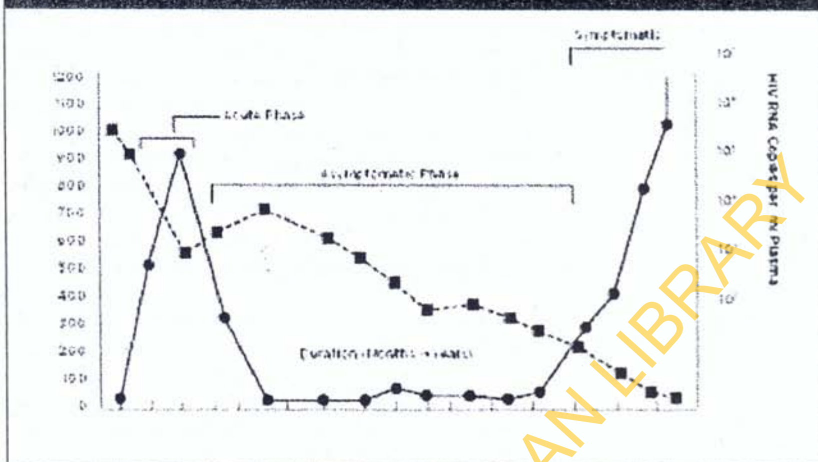
Once infected, humans experience an asymptomatic clinical latency that lasts 2 to 10 years, during which HIV is produced and removed by the immune system, and CD4+ T cells are killed and replaced. During this asymptomatic period, the number of infected circulating CD4+ cells and free virions is relatively low. Moreover, the hematopoietic system is able to replace most T cells that are destroyed, thus keeping the CD4+ cell counts in the normal range (800 to 1200/mm^3 of blood). Later in infection, replicating virus disrupts the follicular dendritic cells' architecture, and more infected T cells appear in the circulation. Viruses are no longer retained in the lymph nodes; thus, the circulating levels of free virus increase. Eventually, the circulating CD4+ T cell levels fall to less than 500/mm^3 and opportunistic infections may occasionally occur. During the later stage of infection, the CD4+ cell count declines below 200/mm^3, a level at which the infected individual is said to have developed AIDS. A number of opportunistic infections—including oral candidiasis and recurrent tuberculosis—are common during the early symptomatic phase of AIDS. As the CD4+ cell count declines to an even lower level, additional life-threatening opportunistic infections—such as herpes zoster, amoebiasis, and dermatomycoses—may occur with increasing frequency.

In the later stages of symptomatic HIV infection, the viral load levels rise again. Reports involving accurate quantification of virus in infected patients have revealed that much more virus is present than originally thought (33). Quantitative PCR methods, the so-called viral load assays, have shown that:

- Continuous replication of HIV occurs in nearly all infected individuals, although the rates of virus production vary by as much as 70-fold in different individuals;
- The average half-life of an HIV particle/infected cell in vivo is 2.1 days. Recent reports have suggested an even faster turnover of plasma virus of 28 to 110 per minute;
- Up to 10^9–10^{10} HIV particles are produced each day; and
- An average of 2×10^9 CD4+ cells are produced each day.

UNIVERSITY OF IBADAN LIBRARY

Figure 3-2. Dynamics of Virus and CD4+ Levels Over the Course of an Untreated HIV Infection



Thus, contrary to what scientists initially believed, there is a very dynamic situation in HIV-infected people involving continuous viral replication and destruction and replacement of CD4+ cells.

While the CD4+ cell count is a less expensive and less technical measure of HIV disease progression, quantifying the viral load is currently the most direct measurement of the HIV disease process. It has also been used to assess the risk of disease progression and the response to antiretroviral therapy (ART) (33). As the disease progresses, CD4+ cell count declines but may rebound if therapy is efficacious; however, this parameter alone is an incomplete marker for clinical assessment of a patient. Nevertheless, in resource-poor settings (which include a large proportion of the most affected countries), the CD4+ cell count is a more affordable and hence more practical yardstick for monitoring disease progression and ART efficacy. The following CD4+ cell counts are useful in staging a person's disease progression:

- Acute seroconversion syndrome: > 1000/mm^3
- Early disease: > 500/mm^3
- Middle-stage disease: 200–500/mm^3
- Late disease: 50–200/mm^3
- Advanced disease: < 50/mm^3

Clinical Latency (Asymptomatic Infection)

Studies in industrialized countries have shown that the median time from initial HIV-1 infection to the development of AIDS ranges from 9 to 11 years (32). In Nigeria, such figures are not available. The course

UNIVERSITY OF IBADAN LIBRARY

of infection with HIV-1 varies dramatically, even when the primary infections arose from the same source. In some individuals with a long-term non-progressive HIV-1 infection—that is, a lack of decline in CD4+ cell counts, or chronic infection for at least seven years without the development of AIDS—defective viruses have been identified (34–37). Thus, infection with a defective virus, or one with a poor capacity to replicate, may prolong the clinical course of HIV-1 infection.

As a result of immune activation and other factors, CD4+ cells in the lymph nodes produce virus more rapidly than latently infected cells. As these infected cells are ruptured, they release progeny virions that infect other susceptible cells. After acute HIV infection, a long clinical latency period occurs prior to the onset of symptomatic disease, or AIDS. Although the period is often called the latency period, the virus is not actually dormant. It continues to replicate, mostly in lymph nodes, leading to swollen lymph nodes or lymphadenopathy.

AIDS

Acquired immune deficiency syndrome, or AIDS, is a clinical diagnosis that represents the late stages of HIV disease progression. People classified as having progressed to AIDS either have CD4+ cell counts lower than 200/mm^3 or one or more AIDS-defining conditions. The most common AIDS-defining conditions in Nigeria are severe weight loss; pulmonary tuberculosis; candidiasis (of the esophagus, trachea, bronchi, and lungs); herpes zoster infection; recurrent bacterial pneumonia; and Kaposi's sarcoma (38,39). In industrialized countries, the most prominent AIDS-defining diagnosis is *Pneumocystis carinii* pneumonia, which has been rare among AIDS patients seen in Nigeria (4,7,38,39).

Upon diagnosis of AIDS or symptomatic disease, the median survival time ranges from 12 to 18 months. Nearly all patients who die of HIV-related complications are in this CD4+ cell count category. With the recent introduction of highly active antiretroviral therapy, or HAART, as well as better education and management of people living with HIV/AIDS in Nigeria, people infected with HIV live longer and are healthier even with CD4+ cell counts of less than 200/mm^3 (40–43).

TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS

After the first AIDS cases were reported in the United States, several risk groups were identified—homosexual men, hemophiliacs, and injection drug users—which suggested several possible modes of HIV transmission even before the isolation of the causative agent. Today, we know that both HIV-1 and HIV-2 are transmitted through three principal routes: sexual transmission; transfusion of blood and blood products; and mother-to-child transmission. These routes include the four bodily fluids responsible for all HIV transmission: semen, vaginal fluids, blood, and breast milk.

Sexual Transmission

HIV can be transmitted by vaginal, anal, or oral sexual intercourse. Like other sexually transmitted infections (STIs), the likelihood of infection with HIV is related to the number of sexual partners; the

UNIVERSITY OF IBADAN LIBRARY

infectiousness of infected individuals (for example, people with higher viral loads may be more likely to transmit HIV than people with lower viral loads); and the duration of infection. Globally, about 90% of HIV infection is acquired sexually (44).

In North America and Western Europe, homosexual anal intercourse was initially the major mode of HIV transmission (45). However, heterosexual transmission has since become the major mode of transmission in nearly all countries (46). In addition, heterosexual transmission of HIV-1 is more frequent than for HIV-2 (47). In Africa, the male-to-female ratio of HIV infection remains approximately 1:1 (48–51), suggesting that the main mode of transmission is heterosexual. It has been postulated, however, that the efficiency of male-to-female transmission is higher than female-to-male transmission (52). It also has been observed that some people became infected after a single exposure, while others escape infection even after prolonged, unprotected intercourse with HIV-infected people (53,54).

Moreover, the existence of highly exposed but uninfected individuals suggests that additional factors can influence the sexual transmission of the virus (53,54). Certain factors — such as duration of infection, genetic susceptibility of the host, infecting virus load, virulence of the particular HIV strain, and the status of the immune system — may also affect the rate of heterosexual transmission (52,55). Among the key factors are host infectiousness, viral infectivity and virulence, and host susceptibility.

Host Infectiousness

Some of the factors that influence the host infectiousness include:

- State of infection. Holmberg et al. suggested that HIV-infected people may be more infectious in later stages of infection (18). A study by Hirsch and Curran, however, found no association between stages of HIV infection and amount of virus in the semen (7).
- Severity of the disease and the presence of p24 antigenemia in the infecting person (56).
- CD4+ cell count. It has been shown that transmitting partners have significantly few T-helper cells than non-transmitters (57,58).
- Mode by which a person acquired infection. It has been shown that partners of infected injection drug users are much more likely to be HIV seropositive than partners of HIV-infected transfusion recipients (54).
- Vaginal sex during menstruation. Menstruation might increase the likelihood of HIV transmission to male sex partners (59).

Viral Infectivity and Virulence

Viral infectivity and virulence tend to relate directly to transmissibility. HIV manifests a high degree of genetic variability in vitro, and genetically distinct isolates can often be recovered from the same patient at different times. Nelson and Perelson observed fast replicating strains during early and late infection, and, in the same individual, slow replicating strains during the intermediate latent stage (60). Increased

UNIVERSITY OF IBADAN LIBRARY

virulence might lead to an increase in transmission rates by augmenting the number of viruses present in host secretions (61). Therefore, people infected with HIV variants of higher virulence could be expected to infect a higher proportion of their susceptible contacts than people with less virulent variants.

Host Susceptibility

It has been shown that a break in integrity of the vaginal or rectal mucosa also facilitates HIV infection (7). Herpes simplex virus infection and syphilis, which cause ulcerative diseases, are known risk factors for HIV transmission (4). Sources of abrasion for the rectum or vagina—such as rectal douching, perianal bleeding, and tampon use—may also facilitate HIV transmission (62–64).

Estrogen-containing contraceptives and sexual intercourse during menstruation have been associated with higher risk of HIV infection in women (18,59). Although estrogen thickens the cervical mucosa and might be expected to reduce HIV infection, it is associated with a change in the cervical ectopion and predisposes it to chlamydial infection, cervicitis, and cervical changes that may facilitate HIV infection. In men, a lack of circumcision has been associated with a higher risk of HIV infection (65).

Transfusion of Blood and Blood Products

Whole blood, cellular components, plasma, and clotting factors have all been implicated in HIV transmission. Most people infected with HIV through blood transfusions had an exposure to a single yet large inoculum and often had underlying medical conditions that influenced the rapid development of HIV-related illnesses (66).

Heat treatment of concentrated clotting factor, coupled with donor screening, has reduced the transmission risk associated with transfusion of blood and blood products in developed countries to fewer than one HIV infection per 500,000 transfusions. In developing countries, though, where HIV screening facilities for blood transfusion safety are inadequate, this mode of transmission remains a significant source of new HIV infections.

Parenteral exposure to HIV also results in a small but definite occupational risk of HIV infection for health care workers (67). For this transmission mode, the size of the inoculum is the most important factor determining the risk of HIV infection. Among adults and children in Africa, especially in rural areas, there is a relationship between AIDS and needle injection. These injections, administered primarily for medical purposes, are often by untrained practitioners who usually use a single needle for more than one person (68). In Africa, some practices such as facial scarification and group male and female circumcision may also promote transmission of HIV (48).

Injection drug use plays an important role in transmission of HIV in the developed world. In addition to sharing contaminated needles, injection drug users may also engage in high-risk sexual intercourse (69). It has been shown that female injection drug users and partners of male injection drug users represent the largest number of HIV-infected women of childbearing age in developed countries (70); thus, the association between perinatal transmission of HIV and injection drug use is strong in the developed world.

UNIVERSITY OF IBADAN LIBRARY

Mother-to-Child Transmission

Nearly all cases of HIV infection in infants in both developed and developing countries occur as a result of mother-to-child transmission through three potential routes: across the placenta (in utero); during delivery, through exposure to infected genital tract secretions (intrapartum); and, postnatally, through breastfeeding.

Studies have shown that breastfeeding accounts for most mother-to-child transmission of HIV (71–74). Profound stomatitis in children and cracks on mothers' nipples are additional factors leading to HIV transmission. Additional maternal factors that may influence perinatal transmission of HIV include a low CD4+ cell count, viral load, rupture of membrane more than 48 hours before delivery, and a low level or lack of anti-V3 loop antibodies (75,76). Recent reports also show that the absence of ART in the mother and/or child is an additional factor in transmission.

LABORATORY DIAGNOSIS AND MONITORING

The effectiveness of HIV control measures and the success of treatment of infected people in any country largely depend on the establishment and provision of accurate and reliable diagnoses. Testing techniques must be reliable for results to be unambiguous.

HIV testing programs tend to have the following objectives:

- To monitor the trends of HIV infection in a population or subgroup for planning interventions, such as surveillance or unlinked anonymous sentinel surveys;
- To ensure the safety of recipients by testing donated blood or donors of organs or tissues;
- To identify individuals with HIV infection for diagnosis of AIDS-related diseases or voluntary testing purposes in asymptomatic or AIDS cases; and,
- To enable research on various aspects of HIV infection and AIDS-related diseases.

HIV testing has generated interest not only in the scientific community, but also among the general public. Several factors—including HIV variability, type of laboratory facilities, and the competence of personnel handling the tests—are known to affect the accuracy and reliability of HIV testing in Africa (77). While some of these factors can be managed to a great extent in laboratory settings in some developing countries, the problem of genetic variability is a serious one in most parts of Africa, where multiple HIV-1 subtypes and/or HIV-2 may circulate.

The various technical, ethical, and legal issues that invariably accompany HIV testing have led many countries to develop their own HIV testing policies and guidelines. The development of tests to detect infection with HIV has made it possible to determine the prevalence of HIV and to monitor trends within various populations. While this information is of great value in designing, implementing, and monitoring public health programs for prevention and control, testing of any population for HIV requires careful consideration of a number of issues relating to logistics, laboratory operations, legal ramifications, and ethics.

UNIVERSITY OF IBADAN LIBRARY

Serologic Techniques

Detection of HIV-specific antibodies in the blood or other body fluids is the main method of testing for HIV and the standard procedure for diagnosis of HIV infection. The most commonly used serologic assay for diagnosing HIV infection is the enzyme-linked immunosorbent assay (ELISA) (7,78–80). In general, the assays used to detect specific HIV antibodies can be classified into two categories: screening tests, including ELISA/EIA, rapid, and simple; and supplemental or confirmatory tests, including Western blot, culture, antigen detection, and immunofluorescence assays.

Screening Tests

Screening assays are performed to test blood samples or blood products and for surveillance. These include different forms of ELISAs, which typically take two to three hours to complete. Rapid screening tests can provide results within a few minutes and, in most cases, include visual assays like dot-blot tests; particle (gelatin, latex, microbeads) agglutination; HIV spot and comb tests; and fluorometric microparticle technologies. Most of the simple screening tests are based on ELISA principles, but take about half an hour to conclude.

The indirect ELISA screening technique uses inactivated virus, synthetic peptides, or recombinant proteins as antigens to detect the presence or absence of HIV-specific antibodies in serum or plasma. While there may be some degree of cross-reactivity between HIV-1 and HIV-2, ELISAs that can specifically detect HIV-2 have also been developed. Thus, discrimination between HIV-specific antibodies against the two types of HIV can be ensured in the assay protocol.

A well-developed ELISA kit should have a specificity and sensitivity that exceed 98% to 99% (7). A sample reactive in an ELISA is usually retested using an ELISA with another antigen source. If the ELISA is found to be reactive a second time, it is considered to be repeatedly reactive and then a confirmatory test (Western blot) is performed. Cases of false positivity and false negativity have been reported, however (7). False positivity may reflect the presence of other retroviruses (48) or immunologic abnormalities. False negative reactions, on the other hand, may occur at the early stage of infection before HIV-specific antibodies have fully developed at sufficient quantities for detection (that is, during the "window period" of primary infection) (48). Several techniques—including PCR, p24 antigen detection assay, or viral culture—can detect HIV infection during the window period.

The first generation of ELISAs were sensitive yet not specific because whole viral lysates were used as antigens. These lysates usually contained small amounts of host cell components, which gave rise to false positive reactions. The ELISA technologies improved, and second- and third-generation kits were developed using recombinant and synthetic peptides as antigens. Thus, ELISA assays available in the market may be:

- First generation: Use antigens derived from detergent disruption of viruses grown in human lymphocytes.
- Second generation: Use artificially derived recombinant antigens expressed from bacteria.

UNIVERSITY OF IBADAN LIBRARY

- **Third generation:** Use chemically synthesized oligopeptides of 15 to 40 amino acids.
- **Fourth generation:** Detect both antibody and antigen in the same well of an ELISA plate.

First-generation ELISAs are no longer used for HIV testing, however, because of the high level of false positivity.

Rapid assays. A number of rapid assays based on the principles of agglutination and ELISA have been developed for ease of performance and quick results. These assays generally require less than 30 minutes to perform and do not require special equipment.

Agglutination assays. Agglutination assays incorporate various antigen-coated carriers, such as red cells, latex particles, gelatin particles, and microbeads. These particles are used to support or carry HIV antigens by non-specific attachment.

Agglutination assays have good sensitivity, do not require sophisticated equipment, are easy to perform and cost-effective, and require no wash procedures. Specificity is somewhat compromised, however, and a prozone reaction may occur. To overcome the prozone reaction, diluted specimens should be tested. During the agglutination reaction, HIV antibodies interact with HIV antigens on the carrier particles. Since all antibodies are multivalent, a lattice network between antibodies and antigens is formed that can be visualized macroscopically or microscopically.

Dot-blot assays/comb tests. These assays are rapid and easy to perform, can usually discriminate between HIV-1 and HIV-2, and do not require sophisticated equipment. The results are read by development of color. Sensitivity and specificity of most of these assays compare with ELISAs (81–84).

The assays use recombinant or synthetic peptides spotted onto nitrocellulose paper or micro particles. The antigen-containing matrix is housed in a plastic device containing absorbent pads to collect reactants or made as a comb and the antigens are spotted onto the tooth of the comb card. Each assay contains an immunoglobulin capture control to validate the result. These assays are good for single-test applications, such as in an emergency, during an autopsy, or in labor rooms or peripheral blood banks.

HIV Antibodies Detected in Other Fluids

As stated earlier, the standard specimens for detection of HIV antibodies are serum, plasma, or whole blood. Detection of HIV antibodies in other fluids is also possible, however. HIV antibodies can be detected in oral fluids, such as saliva and oral mucosal transudates. However, the level of HIV antibodies in these fluids is usually less than 1% of the level in serum (85–87). These tests may find better use if issues of confidentiality, counseling, and follow-up can be resolved. HIV antibodies can also be detected in urine using appropriate ELISA kits (88,89). Guidelines for using these tests need to be developed, however, as do adequate strategies for follow-up.

UNIVERSITY OF IBADAN LIBRARY

Choice of HIV Screening Assay

The following factors should be considered when choosing a particular protocol, kit, or strategy for HIV detection:

- The objectives of testing for HIV infection;
- The sensitivity and specificity of the test kit in a particular locality or country;
- The prevalence of HIV in the population;
- The cost-effectiveness of the choice;
- The appropriateness to the strategy and national guidelines of testing; and
- The infrastructure, facilities, and trained personnel available.

Sensitivity is the accuracy with which a test can establish the presence of an infection—that is, HIV antibodies in an appropriate specimen—and is determined as follows:

$$\text{Sensitivity} = \frac{\text{True positive (TP)}}{\text{TP + False negative (FN)}} \times 100$$

Specificity is the accuracy with which a test can confirm the absence of an infection—that is, truly negative specimens test negative. Tests with high specificity show few false positives; therefore, they are preferred for diagnosing HIV infection and/or are used as the second assay in screening. Specificity is defined as follows:

$$\text{Specificity} = \frac{\text{True negative (TN)}}{\text{TN + False positive (FP)}} \times 100$$

Confirmatory Tests

Studies have shown that the probability that a test will accurately determine the true infection status of a person being tested varies with the prevalence of HIV infection in the population (90–92); the higher the HIV prevalence, the greater the probability that a person testing positive is truly infected. This is referred to as the positive predictive value (PPV) of the test.

In contrast, the likelihood that a person showing a negative result is truly uninfected is termed the negative predictive value (NPV). NPV decreases as the prevalence of HIV among the general population increases (92).

Supplemental tests are performed on blood samples that are previously reactive in a screening test. When a blood specimen is reactive by any one of the screening tests, it is tested again by a different assay system to confirm the diagnosis. If a specimen is reactive in two different screening systems, it is tested again using a supplemental test, such as the Western blot or immunofluorescence assay.

HIV infection is confirmed by the detection of antibodies to specific HIV proteins using the Western blot—or immunoblotting—technique (93,94). This assay has certain drawbacks that limit its use, especially in developing countries. It is costly and technically demanding. Furthermore, indeterminate Western blot reactions are common, especially with African blood samples (95). Thus, alternatives to

UNIVERSITY OF IBADAN LIBRARY

Western blotting for developing countries have been proposed (96) and are currently being used in some laboratories.

These supplemental tests are used to resolve discordant results of ELISAs in voluntary counseling and testing or to establish diagnosis of HIV infection for the purpose of therapy. It is important to ensure that any commercial kit selected for this purpose is capable of detecting both HIV-1– and HIV-2–specific antibodies, as well as their respective subtypes.

Molecular Methods

Polymerase Chain Reaction

PCR is a molecular technique that rapidly amplifies specific nucleotide sequences (96). The usefulness of PCR for the direct detection of HIV genetic material was recognized immediately. Today, PCR has been widely applied to the detection and study of HIV infection in both clinical and basic research settings for detecting HIV during early or acute infection; subtyping HIV variants and identifying HIV strains; sequencing of the *pol* gene relevant to monitoring drug resistance and therapeutic efficacy; and detecting HIV in newborn babies of infected mothers.

Virus Isolation

HIV infection can also be detected by isolation of the virus from peripheral blood mononuclear cells, genital secretion, plasma, brain, bone marrow, or a variety of other tissues (97–99). Co-cultivation of the test specimen with uninfected mitogen-stimulated PBMCs is the technique most commonly used (7). After several days, the supernatant of the co-culture is evaluated for reverse transcriptase activity, the presence of HIV p24 antigen, or HIV RNA. In general, virus is present in PBMCs in high concentrations very early and very late in the course of HIV infection; thus, this approach is particularly important for the accurate detection of HIV infection during the window period before HIV-specific antibodies have fully developed. However, because virus isolation is quite slow and generates a large number of infectious particles, it must be conducted under conditions of strict biocontainment. Thus, it is not routinely attempted in most developing countries; in the developed world, it is primarily a research method.

Diagnosis of HIV Infection in Newborns

Transplacental transmission of HIV can occur from an infected pregnant mother to her fetus as early as eight weeks of gestation (100,101). Diagnosis of HIV infection in infants born to seropositive mothers is difficult because maternal antibody (IgG) to HIV-1 crosses the placenta and can persist for up to 15 months, making the distinction between maternal and neonatal IgG difficult. The various tests available to diagnose HIV infection in neonates under the age of 15 months include detection of IgA and/or IgM anti-HIV antibodies, detection of p24 antigen, PCR amplification of viral DNA or RNA, and virus isolation.

UNIVERSITY OF IBADAN LIBRARY

Monitoring Progression of HIV Infection and Therapy

Infection with HIV progresses to AIDS at different rates in different individuals, with a wide spectrum varying from rapid progression to long-term non-progression. This variability makes it essential to have tests that can accurately assess the stage of infection in an individual, as well as monitor the progression of disease. The response of individuals to ART is similarly monitored. The increased rate of HIV replication is reflected in an increase in plasma viral RNA load, which is considered the most representative and sensitive laboratory test for monitoring progression of HIV infection. Over time, increased HIV replication leads to a depletion of CD4+ cells. The CD4+ cell count, a useful surrogate marker for viral replication, was the first method described for monitoring HIV disease progression and is still used for staging infection and monitoring progression in many parts of the world.

The laboratory tests used for monitoring the progression of HIV disease can be classified into viral markers and surrogate markers.

Viral Markers

Plasma HIV RNA load. Plasma viral load (HIV RNA) quantification is considered the best method for monitoring progression and response to ART. Active replication of virus occurs during all clinical stages of infection; thus, it is possible to detect and quantify virus throughout the course of infection. The techniques available for quantifying viral RNA are: quantitative RNA-PCR; branched DNA assay (bDNA); and nucleic acid sequence based amplification (NASBA) (14).

P24 antigenemia. The *gag*-gene–encoded core protein antigen, p24, is one of the earliest viral antigens detectable in the blood after infection. It is useful for diagnosis during the "window period" of early infection and in the newborn. An increase in free p24 levels is an important predictor of increased virus replication (102–104). Until the development of HIV RNA load assays, the p24 assay was the major assay used to measure viral replication directly. The p24 antigen is poorly quantifiable, however, and may not be detectable in many individuals. In addition, p24 concentrations in the blood do not correlate well with ART efficacy.

Surrogate Markers

Virus-specific markers. A viral characteristic associated with progression of HIV infection is the conversion of the virus from a non-syncytium-inducing (NSI) phenotype to a syncytium-inducing (SI) phenotype (105–108).

Non-HIV–specific markers. A number of non HIV-specific cellular markers have been used for monitoring progression of HIV infection and assessing response to therapy. One of the most useful and commonly used cellular markers is the absolute count (or percentage) of CD4+ lymphocytes in the blood. Other lymphocyte phenotypic markers associated with progression include an increase in indicators of immune activation on T lymphocytes like CD38 (especially on CD8+ lymphocytes), HLA-DR, IL-2R, CD45RO, and markers of apoptosis, such as Fas. Flow cytometry is the most accurate method for determining these markers and CD4+ cell counts, though it is technically demanding and comparatively expensive both in terms of equipment and reagents.

UNIVERSITY OF IBADAN LIBRARY

CD4+ cell counts. The most commonly used cellular marker is the CD4+ lymphocyte count. Its decline is the hallmark of HIV infection, and the rate of CD4+ cell loss in each person is unique. CD4+ cell number changes during HIV infection in the following stages:

- A rapid decline for 6 to 18 months at the time of seroconversion;
- A plateau or gradual decline that can last several years during the asymptomatic period;
- A steeper decline for several months before AIDS develops; and
- A continued CD4+ cell decline and pronounced immunosuppression until the death of the infected individual.

The CD4+ cell count is extremely important in the staging of HIV infection. A revised classification of the U.S. Centers for Disease Control and Prevention stratifies HIV positive people into three CD4+ count categories: $> 500/mm^3$; $200–499/mm^3$; and $< 200/mm^3$ (along with three parallel clinical stages: A, B, and C). It is known that a low CD4+ count (less than 10% or $< 100/mm^3$) and a low CD4+/CD8+ ratio (< 0.2) are highly predictive of death from AIDS-related complications (109–114). In developed countries, a combination of the CD4+ cell count and HIV viral load assays are used as markers of progression. A persistently high viral load is predictive of a poor prognosis, especially when accompanied by a very low CD4+ cell count. Viral load is considered a superior prognostic marker compared with CD4+ cell counts when monitoring patients on ART.

Soluble markers: A large number of soluble markers of immune activation have been evaluated as prognostic indicators in HIV infection. These include serum/plasma levels of neopterin, β2-microglobulin, tumor necrosis factor alpha (TNF-α), soluble CD8+, and soluble cytokine receptors (sIL-2R for IL-2 and sTNF-α RII for TNF-α) (115–117).

HIV INFECTION IN NIGERIA

Initial concern about HIV infection in Nigeria was first raised between 1984 and 1986 by a number of medical scientists, including C. K. O. Williams and A. F. Fagbami, then of the Departments of Hematology and Virology, respectively, at the University College Hospital in Ibadan; Abdulsalami Nasidi and Tekena Harry at the National Institute for Medical Research in Yaba, Lagos; and Idris Mohammed of the University of Maiduguri Teaching Hospital. Their persistent call for action resulted in the establishment of the National Experts Advisory Committee on AIDS in 1987 by the minister of health at the time, the late Olikoye Ransome-Kuti. Also in 1986, the Nigerian government officially publicly recognized the first HIV/AIDS infection in the country (69). Subsequent work by individual researchers or groups led to the identification of more cases of HIV/AIDS in different parts of the country. It should be noted, however, that investigations at that period were limited to institutions or individual scientists who had access to the appropriate diagnostic assays through collaborations with scientists in institutions in the United States, France, the United Kingdom, and Germany.

UNIVERSITY OF IBADAN LIBRARY

In late 1987, the Federal Ministry of Health, with technical assistance from the Overseas Development Agency of the British Council and the World Health Organization, established HIV screening facilities in 13 teaching hospitals in the country with the sole objective of ensuring transfusion safety. Hence, the facilities were usually located in the departments of hematology or the blood banks of these centers. The number of such HIV screening centers was later increased to cover more hospitals in the country. Results of the first 2,000 blood units tested at the various centers were negative for HIV antibodies. In addition, none of 586 archival sera from blood samples collected from 1983, when the first evidence of HIV infection in Africa was reported, to 1986 was positive for HIV (59). Unfortunately, the announcement of these initial negative results led to complacency on the part of health authorities in Nigeria and consequently the general population.

Seroepidemiology

Data from screening of blood donors and limited seroepidemiologic studies of the general population indicated that the rate of HIV infection was less than 1% in Nigeria until the late 1980s (118–121). However, a study using 3,854 serum samples collected from 1985 to 1990 showed a 10-fold rise in the rate of HIV infection in the country within the five-year period (49). This observation was corroborated by other workers from different parts of the country, indicating increased HIV infections in Nigeria during the early 1990s (119,122). Thus, contrary to initial beliefs, the virus may have been introduced into the Nigerian population in the late 1970s, like many other African countries, but it remained at low levels within the population until its explosion in the late 1980s. In retrospect, blood samples from a woman who presented with severe loss of weight in the early 1980s, then called "slim disease," was found to be positive for HIV antibodies (unpublished data). The highly celebrated story of the "strange disease" was published on several occasions by a local newspaper, *Sketch*, in the early 1980s.

The first national surveillance of HIV infection among pregnant women attending antenatal clinics (ANC), as well as patients attending sexually transmitted infection clinics, was conducted in 1991 (123–125). In addition, groups of long-distance truck drivers and sex workers were tested. Surveys among the ANC attendees and people with high-risk behaviors have been repeated at various intervals since then (123–126). These studies have shown that HIV has spread extensively in urban and rural areas of Nigeria. The rate of infection also varies significantly from one location to another. The median rate of infection among the general population represented by ANC attendees in different parts of the country increased from 1.8% in 1991 to 4.5% in 1995, 5.4% in 1999, and 5.8% in 2001; a small decline to 5.0% was noted in 2003 (123–126). Similarly, a 2000 survey found high HIV infection rates of 11.0% (ranging from 5.2% to 23.0%) and 17.0% (ranging from 4.2% to 33.0%) among patients presenting with other sexually transmitted infections and pulmonary tuberculosis, respectively (123).

As HIV continues to spread in Nigeria, it is apparent that the epidemic manifests different patterns among various subpopulations and in different parts of the country. Initially, the age of highest infection was among people in their late twenties. More recent surveys, however, have shown high rates of HIV infection among people aged 15 to 24 years. The rate of HIV infection among pregnant women 15 to 24

UNIVERSITY OF IBADAN LIBRARY

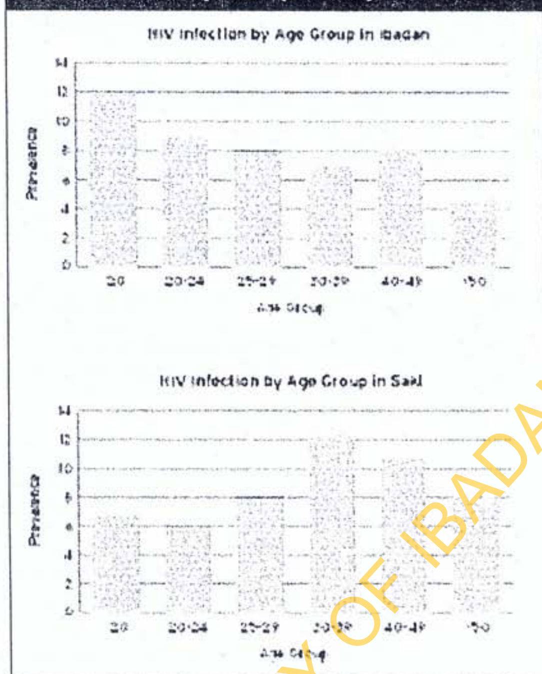


Figure 3-3. HIV Infection by Age Group in Two Communities in Oyo State (2001–2003)

years was 5.7% in 1999, 5.8% in 2001, and 5.4% in 2003 (125).

Apart from the periodic national surveys, testing of people with high-risk sexual behaviors, such as sex workers and long-distance truck drivers, also began in the late 1980s. The data showed a gradual increase in HIV infections among sex workers from about 2% in 1988–1989, to 15% in 1993, 24% in 1994, and greater than 50% in 1995 (126). By 2004, the rate of HIV infection among sex workers ranged from 35% to more than 80% in most parts of the country (126). Similar studies among long-distance truck drivers also demonstrated increased HIV infection rates from about 4% in the early 1990s to more than 20% in 2000 (126).

In a situation analysis report on HIV and other STIs in Nigeria in 2000, a ministerial committee of the Federal Ministry of Health identified several weaknesses in the national HIV data. With the rate based on the HIV seroprevalence among pregnant women attending government health facilities, little is known about HIV infections among pediatric populations or among men (124). The review also indicated a lack of information on incidence rates of HIV infection among various at-risk populations, few voluntary counseling and testing centers, and insufficient linkage of HIV with other STI prevention and control programs (124).

While several of these issues remain unresolved, surveillance has been the subject of the evidence-based prevention approach of the AIDS Prevention Initiative in Nigeria (APIN) of the Harvard School of Public Health. The goal of HIV surveillance is to determine the rate and incidence of the virus transmission among diverse subpopulations at the community level. Available baseline data from APIN-supported HIV surveillance projects in two occupational groups communities — Ibadan and Saki in Oyo State — showed that the actual HIV infection rate may be higher than previously suggested by the periodic

UNIVERSITY OF IBADAN LIBRARY

national testing of ANC attendees. For instance, while the 2003 national HIV survey estimated a 4% prevalence in Oyo State, the results of a population-based study by APIN and the Department of Virology at the University College Hospital in Ibadan involving approximately 10,000 apparently healthy volunteers found HIV infection rates of 7% and 8% in Ibadan and Saki, respectively (Figure 3.3). The rates varied widely between occupational groups from 5.4% to 13.6% in Ibadan and 3.2% to 16.9% in Saki. The rate of HIV infection was significantly higher among males than females in the same geographic area.

In Nigeria, HIV transmission remains predominantly due to heterosexual sex. Males have been shown to be the main bridging route between people who engage in high-risk sexual behavior, such as female sex workers, and the general population (Olaleye et al., *unpublished data*). In addition, use of unscreened or improperly screened blood units for transfusion constitutes a major source of transmission. It is also pertinent to note that fewer than 1% of pregnant women in Nigeria have access to free HIV testing and prevention-of-mother-to-child-transmission counseling. Hence, mother-to-child transmission of HIV remains a significant problem in Nigeria.

Reported AIDS Cases

As part of the global strategy to monitor the HIV pandemic, all countries are expected to report new AIDS cases to UNAIDS. Although the value of AIDS case reporting is limited, as it only provides information on transmission patterns during the previous five to ten years, the data remain useful for advocacy and estimating the burden of HIV-related morbidity, as well as planning of AIDS-related health care services. The data also provide information on the demographic and geographic characteristics of the infected and affected population groups, including their risk factors for HIV infection.

In Nigeria, several attempts have been made to organize a proper AIDS case reporting system. Like many other public sector agencies seeking to collect data, the Federal Ministry of Health has had difficulty obtaining accurate data on AIDS cases from the local and state levels. In 2000 the ministry reactivated the AIDS reporting program in collaboration with several international developmental partners. The process of active data collection at the hospital level started in 2002–2003.

Although the government of Nigeria has been reporting AIDS cases to the World Health Organization since 1998, the data do not correspond with the reality of the problem in the country. As an alternative, the Federal Ministry of Health has adopted the UNAIDS projection method of estimating AIDS cases from HIV infection rates emanating from sentinel survey data. Based on the 2001 and 2003 HIV sentinel surveys, it has been estimated that at least four million adults between 15 and 49 years of age are infected with HIV in Nigeria, with another 350,000 already diagnosed or living with AIDS (125).

CONCLUSION

Our understanding of the biology, diversity, and pathogenesis of HIV has increased tremendously in the two decades since its discovery. These advances have enabled newer methods for epidemiologic surveillance, more rapid diagnoses, and more effective drugs to treat infection. Nigeria, already home to a large

UNIVERSITY OF IBADAN LIBRARY

diversity of HIV viruses, may indicate that subtype-specific vaccine development will be difficult. The use of antiretrovirals in populations with limited resources may also suggest that drug-resistant virus is prevalent, creating obstacles for future ART programs if those strains begin to circulate widely. We remain hopeful, however, that continued research will advance the development of an effective vaccine that will prove a critical component in our efforts to stem the HIV/AIDS epidemic.

ACKNOWLEDGMENTS

We are very grateful for the kind opportunity given to us by the APIN director, Professor Phyllis Kanki, to contribute to this laudable initiative of the first book on AIDS in Nigeria. We would also like to thank APIN's senior program manager, Dr. Wole Odutolu, and project manager, Dr. Prosper Okonkwo, for their words of encouragement. The extensive and meticulous review work, advice, and constructive criticism by Dr. Jason Blackard is sincerely acknowledged, as is the deft editorial hand of Dr. Seema Thakore Meloni. The result of our collaboration with Dr. Jean-Louis Sankalé on the APIN surveillance project in Oyo State is evident in the data used on the molecular epidemiology of HIV in Nigeria. We are most grateful for this support. The tireless effort of Jalal Hosseini to get work done is appreciated. Many thanks are also due to M. O. Adewumi, A. S. Bakarey, and E. Donbraye, our graduate students in the Department of Virology at the University of Ibadan for their assistance. The versatility of Funke Adeniyi for the careful and faithful work of the illustrations and the typing of the manuscripts reaffirms her as a great asset to the group.

REFERENCES

1. Coffin M. Retroviridae and their replication. In: Fields BN, Knipe DM, Chanock RM, Hirsch MS, Melnick JL, Monath TP, Roizman B, eds. *Virology*. 2nd ed. Raven Press, 1990:1437–1488.
2. Gallo R, Salahuddin S, Popovic M. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS. *Science*, 1984; 224:497–500.
3. Essex M, Mboup S. The etiology of AIDS. In: Essex M, Mboup S, Kanki PJ, Marlink RG, Tlou SD, eds. *AIDS in Africa*. 2nd ed. New York: Kluwer Academic/Plenum Publishers, 2002:1–10.
4. Levy J. *HIV and the Pathogenesis of AIDS*. Washington, DC: ASM Press, 1994.
5. Zijenah L, Katzenstein D. Immunopathogenesis of AIDS. In: Essex M, Mboup S, Kanki PJ, Marlink RG, Tlou SD, eds. *AIDS in Africa*. 2nd ed. New York: Kluwer Academic/Plenum Publishers, 2002:34–52.
6. Yu X, Lichterfeld M, Addo M, et al. Regulatory and accessory HIV-1 proteins: potential targets for HIV-1 vaccines? *Curr Med Chem*, 2005;12:741–747.
7. Hirsch M, Curran J. Human immunodeficiency viruses: biology and medical aspect. In: Fields BN, Knipe DM, Chanock RM, Hirsch MS, Melnick JL, Monath TP, Roizman B, eds. *Virology*. 2nd ed. New York: Raven Press, 1990:1545–1570.
8. Diop O, Gueye A, Ayouba A, et al. Simian immunodeficiency viruses and the origin of HIVs. In: Essex M, Mboup S, Kanki PJ, Marlink RG, Tlou SD, eds. *AIDS in Africa*. 2nd ed. New York: Kluwer Academic/Plenum Publishers, 2002:104–120.
9. Gallo R, Mann D, Broder S, et al. Human T-cell leukemia lymphoma virus (HTLV) is in T but not B lymphocytes from a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA*, 1982;79:5680.

UNIVERSITY OF IBADAN LIBRARY

10. Poiesz B, Ruscetti F, Gazdar A, et al. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T cell lymphoma. *Proc Natl Acad Sci USA*, 1980; 77:7415–7418.

11. Wong-Staal F. Human immunodeficiency viruses and their replication. In: Fields BN, Knipe DM, Chanock RM, et al., eds. *Virology*. 2nd ed. New York: Raven Press, 1990:1529–1544.

12. Piot P, Plummer F, Mhalu F, et al. AIDS: An International Perspective. *Science*, 1998;239:573–579.

13. Clavel F, Guetard D, Brun-Vezinet F. Isolation of a new human retrovirus from West African patients with AIDS. *Science*, 1986;237:343–346.

14. Kanki P, Mani I. Monitoring viral load. In: Essex M, Mboup S, Kanki PJ, Marlink RG, Tlou SD, eds. *AIDS in Africa*. 2nd ed. New York: Kluwer Academic/Plenum Publishers, 2002:173–185.

15. Alimonti J, Ball T, Fowke K. Mechanisms of CD4+ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. *J Gen Virol*, 2003; 84:1649–1661.

16. Connor R, Sheridan K, Ceradini D, et al. Change in coreceptor use correlates with disease progression in HIV-1 infected individuals. *J Exp Med*, 1997;185: 621–628.

17. McDermott D, Zimmerman P, Guignard F, et al. CCR5 promoter polymorphism and HIV-1 disease progression. Multicenter AIDS Cohort Study (MACS). *Lancet*, 1998;352:866–870.

18. Holmberg SD, Horsburgh CR Jr, Ward JW, et al. Biologic factors in the sexual transmission of human immunodeficiency virus. *J Infect Dis*, 1989;3: 361–366.

19. Dean M, Carrington M, Winkler C. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. *Science*, 1996;273:1856–1862.

20. Wells T, Proudfoot A, Power C, et al. Chemokine receptors: the new frontier for AIDS research. *Chemica Biologica*, 1996;3:603–609.

21. Zhang L, Huang Y, He T, et al. HIV-1 subtype and second receptor use. *Nature*, 1996;383:768.

22. McDermott D, Beecroft M, Kleeberger C, et al. Chemokine RANTES promoter polymorphism affects risk of both HIV infection and disease progression in the Multicenter AIDS Cohort Study. *AIDS*, 2000;14:2671–2678.

23. Pathak V, Temin H. Broad spectrum of in-vivo forward mutations, hypermutations and mutational hot spots in a retroviral shuttle vector after a single replication cycle: substitutions, frame shifts, and hypermutations. *Proc Natl Acad Sci USA*, 1990;87: 6019–6023.

24. Oxenius A, Price D, Trkola A, et al. Loss of viral control in early HIV-1 infection is temporarily associated with sequential escape from CD8+ T cell responses and decrease in HIV-1-specific CD4+ and CD8+ T cell frequencies. *J Infect Dis*, 2004;190: 713–721.

25. Yang O, Walker B. CD8+ cells in human immunodeficiency virus type 1 pathogenesis: cytolytic and noncytolytic inhibition of viral replication. *Adv Immunol*, 1997;66:273.

26. Flamand L, Crowley R, Lusso P, et al. Activation of CD8+ T lymphocytes through the T cell receptor turns on CD4 gene expression: implications of HIV pathogenesis. *Proc Natl Acad Sci USA*, 1998;96:3111.

27. Livingstone W, Moore M, Innes D, et al. Frequent infection of peripheral blood CD8-positive T-lymphocytes with HIV-1. *Lancet*, 1996;348:649.

28. Douek D, Brenchley J, Betts M, et al. HIV preferentially infects HIV-specific CD4+ T cells. *Nature*, 2002;417:95–98.

29. Lelievre J, Mammona F, Arnoult D, et al. A novel mechanism for HIV-1-mediated bystander CD4+ T-cell death: neighboring dying cells drive the capacity of HIV-1 to kill noncycling primary CD4+ T cells. *Nature*, 2004;11:1017–1027.

30. Wood E, Hogg R, Yip B, et al. Using baseline CD4 cell count and plasma HIV RNA to guide the initiation of highly active antiretroviral therapy. *Rev Invest Clin*, 2004;56:232–236.

31. Yaman A, Cetiner S, Kibar F, et al. Reference ranges of lymphocyte subsets of healthy adults in Turkey. *Med Princ Pract*, 2005;14:189–193.

32. Fauci A. The human immunodeficiency virus infectivity and mechanism of pathogenesis. *Science*, 1988; 275:617–622.

33. Plantier J, Gueudin M, Damond F, et al. Plasma RNA quantification and HIV-1 divergent strains. *J Acquir Immune Defic Syndr*, 2003;33:1–7.

34. Barbour J, Grant R. The role of viral fitness in HIV pathogenesis. *Curr HIV/AIDS Rep*, 2005;2:29–34.

35. Bernier R, Tremblay M. Homologous interference resulting from the presence of defective particles of human immunodeficiency virus type 1. *J Virol*, 1995; 69:291–300.

UNIVERSITY OF IBADAN LIBRARY

36. Sanchez G, Xu X, Chermann J, et al. Accumulation of defective viral genomes in peripheral blood mononuclear cells of human immunodeficiency virus type 1-infected individuals. *J Virol*, 1997;71: 2233–2240.

37. Zaunders J, Geczy A, Dyer W, et al. Effect of long-term infection with *nef*-defective attenuated HIV type 1 on CD4+ and CD8+ T lymphocytes: increased CD45RO+CD4+ T lymphocytes and limited activation of CD8+ T lymphocytes. *AIDS Res Hum Retroviruses*, 1999;15:1519–1527.

38. Akinsete I, Akanmu A, Okany C. Spectrum of clinical diseases in HIV-infected adults at the Lagos University Teaching Hospital: a five-year experience (1992–1996). *Afr J Med Med Sci*, 1998;27:147–151.

39. Anteyi E, Idoko J, Ukoli C, et al. Clinical pattern of human immunodeficiency virus infection (HIV) in pulmonary tuberculosis patients in Jos, Nigeria. *Afr J Med Med Sci*, 1996;25:317–321.

40. Laurent C, Ngom Gueye N, Ndour C, et al. Long-term benefits of highly active antiretroviral therapy in Senegalese HIV-1–infected adults. *J Acquir Immune Defic Syndr*, 2005;38:14–17.

41. Moore R, Keruly J, Gebo K, et al. An improvement in virologic response to highly active antiretroviral therapy in clinical practice from 1996 through 2002. *J Acquir Immune Defic Syndr*, 2005;39:195–198.

42. Resino S, Bellon J, Ramos J, et al. Impact of highly active antiretroviral therapy on CD4+ T cells and viral load of children with AIDS: a population-based study. *AIDS Res Hum Retroviruses*, 2004;20: 927–931.

43. Sungkanuparph S, Kiertiburanakul S, Manosuthi W, et al. Initiation of highly active antiretroviral therapy in advanced AIDS with CD4 < 50 cells/mm^3 in a resource-limited setting: efficacy and tolerability. *Int J STD AIDS*, 2005;16:243–246.

44. World Health Organization. State from consultation on HIV and routine childhood immunization. *Wkly Epidemiol Rec*, 1987;62:297.

45. Padian N, Shiboski SC, Jewell NP. Female-to-male transmission of human immunodeficiency virus. *JAMA*, 1991;266:1664–1667.

46. World Health Organization. Global AIDS surveillance Part II. *Wkly Epidemiol Rec*, 1998;73:373–380.

47. Gilbert P, McKeague I, Eisen G, et al. Comparison of HIV-1 and HIV-2 infectivity from a prospective cohort study in Senegal. *Stat Med*, 2003;22:573–593.

48. Williams AO. *AIDS: An African Perspective*. Ann Arbor, Michigan: CRC Press, 1991.

49. Olaleye D, Bernstein L, Ekwezor C, et al. Prevalence of human immunodeficiency virus type 1 and 2 infections in Nigeria. *J Infect Dis*, 1993;167: 710–714.

50. WHO Global AIDS News: Newsletter of the World Health Organization. *Global Programme on AIDS*, 1994;1020:1–5.

51. Subbarao S, Schochetman G. Genetic variability of HIV-1. *AIDS*, 1996;10(Suppl A):S513–S523.

52. O'Brien W, Namazi A, Kalhor H, Mao SH, Zach JA, Chen IS. Kinetics of human immunodeficiency virus type-1 reverse transcriptase in blood mononuclear phagocytes are slowed by limitations of nucleotide precursors. *J Virol*, 1994;68:1258–1263.

53. Georgi C, Chou J, Gudeman C, et al. Cell-mediated immune response to human immunodeficiency virus (HIV) type-1 in seronegative homosexual men with recent sexual exposure to HIV-1. *J Infect Dis*, 1992;165:1012–1019.

54. Peterman T, Stonenburner R, Allen J, et al. Risk of human immunodeficiency virus transmission from heterosexual adults with transfusion associated infection. *JAMA*, 1988;259:55–58.

55. Aaby P, Airyoshi K, Bucker M, et al. Age of wife as a major determinant of male-female transmission of HIV-2 infection: a community study from rural West Africa. *AIDS*, 1996;10:1585–1590.

56. Fischl M, Dickinson G, Scott G, et al. Evaluation of heterosexual partners, children and household contacts of adults with AIDS. *JAMA*, 1987;257.

57. Rate of vertical transmission of HIV high in black South Africans. *AIDS Wkly Plus*, Aug 12, 1996:17–18.

58. Gray C, Williamson C, Bredell H, et al. Viral dynamics and CD4+ T cell counts in subtype C human immunodeficiency virus type 1-infected individuals from southern Africa. *AIDS Res Hum Retroviruses*, 2005;21:285–291.

59. Hill JA, Anderson DJ. Human vaginal leukocytes and the effects of vaginal fluid on lymphocyte and macrophage defense functions. *Am J Obstet Gynecol*, 1992;166:720-6.

60. Nelson G, Perelson A. A mechanism of immune escape by slow replicating HIV strains. *AIDS*, 1992; 5:82–93.

61. Sheppard H, Lang W, Ascher M, et al. The characterization of non-progressors: long-term HIV infection with stable CD4+ T-cell level. *AIDS*, 1993; 7:1159–1166.

UNIVERSITY OF IBADAN LIBRARY

62. Coates R, Read S, Fanning M, et al. Transmission of HIV in the male sexual contacts of men with ARC or AIDS. *IV International Conference on AIDS*, Stockholm, Sweden, 1988.

63. Goedert J, Eyster M, Biggarm R, et al. Heterosexual transmission of human immunodeficiency virus: association with severe depletion of T-helper lymphocytes in men with hemophilia. *AIDS Res Hum Retroviruses*, 1988;3:355–361.

64. Guinan JJ, Kronenberg C, Gold J, Morlet A, Cooper DA. Sexual behavioural change in partners of homosexual men infected with human immunodeficiency virus. *Med J Aust*, 1988,149:162.

65. Simonsen J, Cameron W, Gakinya M, et al. Human immunodeficiency virus among men with sexually transmitted diseases. Experience from a center in Africa. *New Engl J Med*, 1988;319:274–278.

66. Ward J, Bush J, Perkins M, et al. The natural history of transfusion associated infection with human immunodeficiency virus. *New Engl J Med*, 1989;321: 947–952.

67. Friedland G, Klein R. Transmission of the human immunodeficiency virus. *New Engl J Med*, 1987;317: 1125–1130.

68. Guay L, Horn D, Kabengera S, et al. HIV-1 ICD p24 antigen detection in Ugandan infants: use in early diagnosis of infection and as a marker of disease progression. *J Med Virol*, 2000;62:426–434.

69. Focus on AIDS. *Nig Bull Epidemiol*, 1992;2:1–23.

70. U.S. Centers for Disease Control and Prevention. *Drug-Associated HIV Transmission Continues in the United States*. Accessed at www.cdc.gov/hiv/pubs/facts/idu.htm on March 1, 2006.

71. Coutsoudis A. Influence of infant feeding patterns on early mother-to-child transmission of HIV-1 in Durban, South Africa. *Ann NY Acad Sci*, 2000;918: 136–144.

72. Ogundele M, Coulter J. HIV transmission through breastfeeding: problems and prevention. *Ann Trop Paediatr*, 2003;23:91–106.

73. Richardson B, John-Stewart G, Hughes J, et al. Breast-milk infectivity in human immunodeficiency virus type 1-infected mothers. *J Infect Dis*, 2003; 187:736–740.

74. Rouet F, Elenga N, Msellati P, et al. Primary HIV-1 infection in African children infected through breastfeeding. *AIDS*, 2002;16:2303–2309.

75. McCarthy M. No-needle, HIV test approved by FDA. *Lancet*, 1996;347:1683.

76. Ryder RW, Manzila T, Baende E, et al. Evidence from Zaire that breast-feeding by HIV-1-seropositive mothers is not a major route for perinatal HIV-1 transmission but does decrease morbidity. *AIDS*, 1991;5(6):709–714.

77. Odaibo G, Ibeh M, Olaleye D. Reliability of HIV testing in Africa. *2nd IAS Conference on HIV Pathogenesis and Treatment*, Paris, France, 2003.

78. Beelaert G, Vercauteren G, Fransen K, et al. Comparative evaluation of eight commercial enzyme linked immunosorbent assays and 14 simple assays for detection of antibodies to HIV. *J Virol Methods*, 2002;105:197–206.

79. Menard D, Mavolomade E, Mandeng M, et al. Advantages of an alternative strategy based on consecutive HIV serological tests for detection of HIV antibodies in Central African Republic. *J Virol Methods*, 2003;111:129–134.

80. Rouet F, Ekouevi D, Inwoley A, et al. Field evaluation of a rapid human immunodeficiency virus (HIV) serial serologic testing algorithm for diagnosis and differentiation of HIV type 1 (HIV-1), HIV-2, and dual HIV-1-HIV-2 infections in West African pregnant women. *J Clin Microbiol*, 2004;42:4147–4153.

81. Foglia G, Royster G, Wasunna K, et al. Use of rapid and conventional testing technologies for human immunodeficiency virus type 1 serologic screening in a rural Kenyan reference laboratory. *J Clin Microbiol*, 2004;42:3850–3852.

82. Meless H, Tegbaru B, Messele T, et al. Evaluation of rapid assays for screening and confirming HIV-1 infection in Ethiopia. *Ethiop Med J*, 2002;40(Suppl 1): S27–S36.

83. Menard D, Mairo A, Mandeng M, et al. Evaluation of rapid HIV testing strategies in under equipped laboratories in the Central African Republic. *J Virol Methods*, 2005;126:75–80.

84. Wright R, Stringer J. Rapid testing strategies for HIV-1 serodiagnosis in high-prevalence African settings. *Am J Prev Med*, 2004;27:42–48.

85. Abrao Ferreira P, Gabriel R, Furlan T, et al. Anti-HIV-1/2 antibody detection by Dot-ELISA in oral fluid of HIV positive/AIDS patients and voluntary blood donors. *Braz J Infect Dis*, 1999;3:134–138.

UNIVERSITY OF IBADAN LIBRARY

86. Margodo de Moura Machedo J, Kayita J, Bakaki P, et al. IgA antibodies to human immunodeficiency virus in serum, saliva and urine for early diagnosis of immunodeficiency virus infection in Uganda infants. *Pediatr Infect Dis J*, 2003;22:193–195.

87. Tiensiwakul P. Urinary HIV-1 antibody patterns by western blot assay. *Clin Lab Sci*, 1998;11:336–338.

88. Hashida S, Hashinaka K, Ishikawa S, et al. More reliable diagnosis of infection with human immunodeficiency virus type 1 (HIV-1) by detection of antibody IgGs to pol and gag proteins of HIV-1 and p24 antigen of HIV-1 in urine, saliva, and/or serum with highly sensitive and specific enzyme immunoassay (immune complex transfer enzyme immunoassay): a review. *J Clin Lab Anal*, 1997;11: 267–286.

89. Martinez P, Torres A, Ortiz de Lejarazu R, et al. Human immunodeficiency virus antibody testing by enzyme-linked fluorescent and western blot assays using serum, gingival-crevicular transudate, and urine samples. *J Clin Microbiol*, 1999;37:1100–1106.

90. de Freitas Oliveira C, Ueda M, Yamashiro R, et al. Rate and incidence estimates of recent human immunodeficiency virus type 1 infections among pregnant women in San Paulo, Brazil, from 1991 to 2002. *J Clin Microbiol*, 2005;43:1439–1442.

91. Gupta P, Kingsley L, Sheppard H, et al. High incidence and prevalence of HIV-1 infection in high risk population in Calcutta, India. *Int J STD AIDS*, 2003;14:463–468.

92. Tu X, Litvak E, Pagano M. Issues in human immunodeficiency virus (HIV) screening programs. *Am J Epidemiol*, 1992;136:244–255.

93. Delaporte E, Dupont A, Merlin M, et al. Seroepidemiology of HIV-1 and HIV-2 antibodies in Gabon. *AIDS*, 1988;2:136-7.

94. Ramirez E, Uribe P, Escanila D, et al. Reactivity patterns and infectious status of serum samples with indeterminate western immunoblot tests for antibody to HIV-1. *J Clin Microbiol*, 1992;30:801–805.

95. Janssens W, Buve A, Nkengasong J. The puzzle of HIV-1 subtypes in Africa. *AIDS*, 1997;11:705–712.

96. Simmond P. Polymerase chain reaction. In: Desselberger U, ed. *Medical Virology: A Practical Approach*. Oxford: Oxford University Press, 1995:107–145.

97. Alter H, Laurian Y, Paul D. Transmission of HTLV-III infection from human plasma to chimpanzee, an animal model for AIDS. *Science*, 1984;226:549–552.

98. Ho D, Rota T, Schooley R. Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. *New Engl J Med*, 1985;313:1493–1497.

99. Ou C, Kwok S, Mitchell S. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. *Science*, 1988;239:295–297.

100. Scarlatti G. Mother-to-child transmission of HIV-1: advances and controversies of the twentieth centuries. *AIDS Rev*, 2004;6:67–78.

101. Zijenah L, Moulton L, Iliff P, et al. Timing of mother-to-child transmission of HIV-1 and infant mortality in the first 6 months of life in Harare, Zimbabwe. *AIDS*, 2004;18:273–280.

102. Campbell T, Schneider K, Wrin T, et al. Relationship between in vitro human immunodeficiency virus type 1 replication rate and virus load in plasma. *J Virol*, 2003;77:12105–12112.

103. Kim A, Lauer G, Ouchi K, et al. The magnitude and breadth of hepatitis C virus-specific CD8+ T cells depend on absolute CD4+ T-cell count in individuals coinfected with HIV-1. *Blood*, 2005;105: 1170–1178.

104. Schupabach J. Measurement of HIV-1 p24 antigen by signal-amplification-boosted ELISA of heat-denatured plasma is a simple and inexpensive alternative to tests for viral RNA. *AIDS Rev*, 2002;4: 83–92.

105. Aquino-De Jesus M, Anders C, Miller G, et al. Genetically and epidemiologically related "non-syncytium-inducing" isolates of HIV-1 display heterogeneous growth patterns in macrophages. *J Med Virol*, 2000;61:171–180.

106. Callaway D, Ribeiro R, Nowak M. Virus phenotype switching and disease progression in HIV-1 infection. *Proc Biol Sci*, 1999;266:2523–2530.

107. Fitzgibbon J, Gaur S, Gavai M, et al. Effect of the HIV-1 syncytium-inducing phenotype on disease stage in vertically-infected children. *J Med Virol*, 1998;55:56–63.

108. Nicastri E, Ercoli L, Sarmati L, et al. Five human immunodeficiency virus type 1 phenotypic variants with different MT-2 cell tropisms correlate with prognostic markers of disease. *J Hum Virol*, 1998;1: 90–95.

UNIVERSITY OF IBADAN LIBRARY

109. Anastos K, Barron Y, Cohen M, et al. The prognostic importance of changes in CD4+ cell count and HIV-1 RNA level in women after initiating highly active antiretroviral therapy. *Ann Intern Med*, 2004; 140:256–264.

110. Anastos K, Shi Q, French A, et al. Total lymphocyte count, hemoglobin, and delayed-type hypersensitivity as predictors of death and AIDS illness in HIV-1 infected women receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr*, 2004; 35:383–392.

111. Kalish L, McIntosh K, Read J, et al. Evaluation of human immunodeficiency virus (HIV) type 1 load, CD4 T cell level and clinical class as time-fixed and time-varying markers of disease progression in HIV-1-infected children. *J Infect Dis*, 1999;180: 1514–1520.

112. Kozinetz C, Matusa R, Ruta S, et al. Alternatives to HIV-RNA and CD4 count to monitor HIV disease progression: a prospective cohort study in Romania. *J Med Virol*, 2005;77:159–163.

113. Lathey J, Hughes M, Fiscus S, et al. Variability and prognostic values of virologic and CD4 cell measures in human immunodeficiency virus type 1-infected patients with 200-500 CD4 cells/mm^3 (ACTG 175). AIDS Clinical Trials Group Protocol 175 Team. *J Infect Dis*, 1998;177:617–624.

114. Zhou J, Kumarasamy N. TREAT Asia HIV Observation Database. Predicting short-term disease progression among HIV-infected patients in Asia and the Pacific Region: preliminary results from the TREAT Asia HIV Observational Database (TAHOD). *HIV Med*, 2005;6:216–223.

115. Kamga I, Kahi S, Develioglu L, et al. Type I interferon production is profoundly and transiently impaired in primary HIV-1 infection. *J Infect Dis*, 2005;192:303–310.

116. Mildvan D, Spritzler J, Grossberg SE, et al. Serum neopterin, an immune activation marker, independently predicts disease progression in advanced HIV-1 infection. *Clin Infect Dis*, 2005;40(6): 853–858.

117. Shimizu Y, Miyazaki Y, Ibuki K, et al. Induction of immune response in macaque monkeys infected with simian-human immunodeficiency virus having the TNF-alpha gene at an early stage of infection. *Virology*, 2005;343(2):151–161.

118. Chikwem J, Mohammed I, Oyebode-Ola T. Prevalence of human immunodeficiency virus (HIV) infection in Borno State of Nigeria. *East African Med J*, 1988;65:342–346.

119. Harry T, Ekenna O, Chikwen J, et al. Sero-epidemiology of human immunodeficiency virus infection in Borno State of Nigeria by sentinel surveillance. *J Acquir Immune Defic Syndr*, 1993;6:99–103.

120. Harry T, Kyari O, Mohammed I. Prevalence of human immunodeficiency virus-infection among pregnant women attending ante-natal clinic in Maiduguri, north-eastern Nigeria. *Trop Geogr Med*, 1992;44:238–241.

121. Olusanya O, Lawoko A, Blomberg J. Seroepidemiology of human retroviruses in Ogun State of Nigeria. *J Infect Dis*, 1990;22:155–160.

122. Olaleye D, Ekweozor C, Sheng Z, et al. Evidence of serological cross-reactivities with human immunodeficiency virus types 1 and 2 and human T-lymphotropic virus. *Int J Epidemiol*, 1995;24:198–203.

123. Federal Ministry of Health. *Technical Report on 2000 HIV/Syphilis Sero-prevalence and STD Syndromes Sentinel Survey among PTB and STD Patients in Nigeria*. Abuja: Federal Ministry of Health, 2001.

124. Federal Ministry of Health. *The 2001 National HIV/Syphilis Sentinel Survey among Pregnant Women Attending Antenatals Clinic in Nigeria*. Abuja: Federal Ministry of Health, 2001.

125. Federal Ministry of Health. The 2003 National HIV Sero-prevalence Sentinel Survey. Federal Ministry of Health, 2003.

126. UNAIDS. WHO Epidemiological Fact Sheets on HIV/AIDS and Sexually Transmitted Infections. Geneva: UNAIDS, 2004.

UNIVERSITY OF IBADAN LIBRARY