MOLECULAR BIOLOGY OF HUMAN IMMUNODEFICIENCY VIRUSES: HIV-1 AND HIV-2

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OVERVIEW

Since the emergence of the acquired immunodeficiency syndrome (AIDS) and discovery of its etiological agents, human immunodeficiency viruses type 1 and type 2 (HIV-1 and HIV-2), much new information on the epidemiology, pathology and basic biology of these viruses has been discovered. This review will focus on some of the highlights in the field of HIV from the perspective of basic molecular virology to some of the recent issues in clinical studies in the past and present, and by no means reflects the voluminous information and research data gathered worldwide on HIV/AIDS. Current complexities in HIV pathogenesis are still responsible for the lack of an effective cure or vaccine to control the spread of the virus. Most notably, the genetic heterogeneity and mechanisms of molecular pathogenesis of the virus have been the counteracting force behind the unceasing efforts to obtain an effective therapy or vaccine.

INTRODUCTION

HIV-1 and HIV-2 comprise a spectrum of retroviruses with varied potential to cause latent infection and pathogenic effects known as AIDS.1 According to the World Health Organization (WHO), a country-by-country review of HIV/AIDS data indicate that since 1995, a total of 6 million new cases, including adults and children, is present worldwide and currently 20.1 million adults are estimated to be infected with HIV.2 Most cases are concentrated in eastern, central and southern Africa. The epidemic is rapidly spreading to West and South Africa, India and Southeast Asia with proportional shifts to heterosexual infections in North America, western Europe and Latin America. Moreover, WHO estimates a cumulative total of about 40 million HIV infections in men, women and children by the year 2000.2

In Asia, the spread of AIDS has recently increased more than ten-fold; nearly 40% of these are women.3 Only one out of every five infected mothers passes HIV on to her children; however, more than 80% of the AIDS cases among children occur because of transmission from mother to child during pregnancy or at birth.3 According to a WHO projection, the annual rate of HIV infections in Asia will exceed that in Africa in the not-so-distant future.

Since the discovery of HIV over a decade ago, two novel epidemiological findings have emerged in recent years. First, the progression of AIDS involves certain developing countries; for example, in Japan, HIV infection is actually on the rise4, and in one city of Thailand, 20% of young men and 8% of young women are infected with HIV-1. Second, individuals with HIV disease live longer with a median life expectancy of 15 to 20 years.5 It is of concern that a prolonged infection period can shift the disease epidemics into emergence of new resistant strains, resulting in a poor prognosis of the AIDS epidemic. Moreover, recent findings of some new strains of HIV-1, such as subtype "O" (Outgroup), originally found in Cameroon and later among
Western Europeans, were missed by the available antibody screening diagnostic tests, i.e. enzyme-linked immunosorbent assays (ELISA), which further complicates the AIDS epidemic.6,11

Epidemiological data over the years have shown a number of significant points, some of which are: a) the nature of this pandemic disease remains complex, b) health care authorities should give special attention to the proper recruitment of the available preventive measures to contain further spread of the disease, and c) policy officials for public health and infectious diseases should make serious decisions on many aspects of the preventive care for this disease and a tight control over blood and blood products that harbor HIV and other infectious agents.

HIV SUBTYPES

Several clades of HIV-1 and HIV-2 genotypes have been identified from recent studies of HIV isolates throughout the world. This classification is by-and-large based on env and gag sequences. Variation in envelope gp 120 is considered the most common. We know very little of how sequence variation affects the conformation of gp 120 or its antigenicity. It has been suggested that there may be related but not identical, antigenic subtypes, to the genetic subtypes.7 The following classifications are based on the predicted amino acid sequence variations. These include two classes of HIV-1, designated as “M” (Major) and “O” (Outgroup). The former is subdivided into clades A through H that are distributed in many regions of the world.6 Clade B is the most predominant strain in North America and Europe, clades B and F in South America, clades B and E in Asia, and clades A to H and “O” in Central Africa. The clade “O” shows the highest prevalences in the endemic populations, i.e., Cameroon, equatorial Guinea and Gabon, from the start of the AIDS outbreak and its spread simply had not been detected effectively by available ELISA assays because of its unusual antigenicity.5,11

In HIV-2, at least five different clades, A through E, have been detected, including the existence of “mosaic” HIV-2 genomes indicative of genetic recombination between at least two diverse clades. Moreover, because of the sequence homology among HIV-2 and Sifian immunodeficiency virus (SIV) isolates, recent data support the notion that the genetically diverse sooty mangabey viruses have been introduced into human populations.12 Unlike HIV-1, infection by all five HIV-2 clades can be detected by commercially available serological Western blot assays. One HIV-2 strain, referred to as EHO from an Ivory Coast patient, has shown 26-30% divergence in the envelope glycoprotein which migrates as gp 100 as compared to gp 125 of the prototype HIV-2 ROD. This difference in molecular size is seen in gel electrophoresis because the EHO envelope is less glycosylated than that of ROD.13,14

HIV in the patient is present as a mixture of strains, "quasi species", prone to differential selection in culture.15 The body of information on HIV variability was accumulated initially from DNA sequence analyses on laboratory isolates adapted to grow in cell lines. Most recent sequence analyses of the viral genome are done preferably on uncultured patient material (usually from plasma or lymphocytes), since minor species can overgrow in culture, leading to misrepresentation of the actual viral pool. Because of the lack of well-established animal models to study HIV, many studies evaluating anti-viral approaches face numerous obstacles. Nonetheless, a recent demonstration of an AIDS-like condition induced in baboons by HIV-2 may provide a valuable model for studying HIV pathogenesis.16 Moreover, the key to a better understanding of HIV immunopathogenesis might lie in the studies on long-term survivors whose immune response is unable to prevent establishment of infection, but apparently does delay the development of AIDS. These patients, called "long-term non-progressors" (LTNP)16,17 account for about 5% of infected individuals and are defined as: a) seropositive, yet with no apparent disease for more than 12 to 15 years, b) having low levels of HIV (viral-load) in blood, c) having strong CD4+ T-cell response to HIV, d) having high levels of neutralizing antibodies in the plasma, and e) having defective viruses, i.e., mutations in nef gene. However, isolating HIV from LTNP individuals is difficult and often such viruses are attenuated for replicative ability.

PATHOGENESIS

Cytopathology

The mechanism of cell killing in vivo is generally deduced from numerous in vitro findings on the cytopathic effects of the virus. Many studies recently showed that in addition to direct cell killing, HIV can cause indirect single cell killing as a consequence of apoptosis.18-20 Apoptosis is triggered by many physiologic, chemical and other viral agents and is distinct from the pathogenic cell lysis known as necrosis.21 The magnitude of in vivo complexity leads one to believe that the mechanism of cell depletion in the body may involve a variety of physiological and immunological responses.

Destruction of infected cells in HIV-infected monkeys has been linked to the viral nef gene expression.22 In contrast, Fauci and co-workers at the National Institutes of Health and many others believe that AIDS is a multifactorial and multi-disease phenomenon and thus it would be naive to attribute it to a single mechanism.23 Whatever the mechanism(s) responsible may be, HIV presence in the blood leads to AIDS and eventual death. However, there are exceptions, as documented by Chen and co-workers— one
Neurological Damage

The mechanism for high prevalence of the neurological disease associated with AIDS in HIV-infected people remains poorly understood. The presence of HIV-infected cells in perivascular regions suggests that the virus enters the central nervous system (CNS) by traversing the blood-brain barrier (BBB). In addition to HIV-1 infection of macrophages and microglial cells in the brain, it has been shown that human neuronal cells can be infected in vitro and the ensuing infection is enhanced by neuronal growth factors. Early studies showed that HIV RNA and proteins are present at autopsy in brain endothelial cells from patients with AIDS dementia. Recently, a neurovirulent strain of simian immunodeficiency virus (SIV) has been shown to productively infect endothelial cells of macaque CNS in vivo and in vitro, using double immunohistochemistry, in situ hybridization and electron microscopy. These studies propose that changes in endothelial cell function can potentially alter the endothelial cells and this may promote the influx of inflammatory cells to the CNS.

When cells die in the natural course of CNS development, macrophages can be observed to phagocytize these cells. The macrophage is therefore an immune cell with the potential to release neuroactive and numerous signal molecules which can freely enter/penetrate the blood-brain barrier. Monocytes/macrophages (microglia) display the CD4 antigen and are productively infected by HIV.

Nitric oxide (NO) appears to be emerging as an important molecule involved with normal physiological functions as well as one that has the potential to damage tissues due to its free radical nature. Interestingly, it appears to work as an neurotransmitter, as well as an immune cytotoxic substance produced by activated macrophages. Given the significance of NO and the role of macrophages in both harboring HIV as well as gaining entrance to the CNS, it is possible that the AIDS-associated neuronal damage is, in part, due to abnormal levels of NO. Indeed, gp 120 appears to stimulate the release of NO from macrophages and contribute to the neuropathogenic manifestations.

One possible scenario for HIV neuropathogenesis could be that both the cellular slowing and deregulation of chemotactic responses would upset the normal "protective" immune response. This may contribute to the overall inability of immune cells to deal effectively with HIV. Infected macrophages may be literally wandering through neural tissues unable to "sense" the site of antigenic challenge. By doing so they may release potentially harmful molecules, such as cytokines and NO, in otherwise healthy tissues, thus disrupting cellular chemotaxis and initiating tissue damage. This same scenario may be exacerbated when uninfected macrophages enter the CNS on a random basis, based on antigenic activation of gp 120 on existing infected cells.

Kaposi's Sarcoma

Kaposi's sarcoma (KS) seen in association with AIDS has a wide range of initial clinical presentations from small, innocuous-looking macular lesions in inconspicuous locations to symptom-producing visceral, oral cavity, or cutaneous lesions, which may be extremely prominent and troublesome. Data on AIDS-associated KS is primarily derived from studies on HIV-1 infected patients. The lesions typically begin as macular and papular lesions that progress to plaque-like or nodular tumors. Lesions vary in size and shape but are generally nonpruritic and painless. The color of the lesions ranges from brown to pink to deep purple, and they can be found anywhere on the body surface, but seem to appear predominantly in the upper body and head and neck areas. KS is often the presenting sign of HIV disease predominantly among male homosexuals, but lesions may appear at any time in the course of the illness. Moreover, lesions that appear after the occurrence of opportunistic infection may have a more aggressive course than do those presenting as the first manifestation of AIDS. Occasionally, lesions may disappear spontaneously, even as other lesions
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appear in neighboring sites.43

The histopathological nature of the KS lesion in vivo remains ambiguous because of its complex pattern of cell types. Much of the recent progress was obtained through in vitro studies by culturing KS-lesion-derived cells using conditioned media prepared from a HTLV-II infected T-cell line. The KS-derived cells were originally isolated from pleural effusions; however, other cloned cell lines with spindle cell morphology have been established from KS skin biopsy specimens, as well as from the peripheral blood of patients with AIDS-related KS.44 AIDS associated KS cells show a greater proliferative response to platelet-derived growth factor (PDGF) than normal endothelial cells, thus giving them a growth advantage in the presence of this cytokine.46 In addition, KS cells, but not normal endothelial or smooth muscle cells, grow in response to interleukin-6 (IL-6) and to oncostatin-M, a cytokine that is derived from activated lymphocytes and that can increase IL-6 expression in endothelial cells.46,47 Generally, the proliferative response of KS-derived cells to these cytokines distinguishes them from normal mesenchymal cells.

Some cytokines, such as basic fibroblast growth factor (b-FGF) are expressed at high levels in KS-derived cells indicating the role of b-FGF in an autocrine pathway. HIV-1 Tat can increase the proliferation of KS cells, but has no effect on normal smooth muscle or endothelial cells in vitro.48 A recent report indicates that HIV Tat can induce angiogenesis in experimental animals, further arguing for the contributory role of Tat in induction of KS in AIDS.49 It should be mentioned that HIV and other retroviral genes have not been found directly in KS tumors. A recent finding indicates that DNA sequences from KS-associated herpes virus (KSHV) were found in KS biopsies.50 However, KSHV, as the etiological culprit for this disease, remains controversial.51

GENETIC STRUCTURE-FUNCTION

In addition to gag, pol and env, which are present in all retroviruses, HIV-1 has six auxiliary genes called vif, vpr, tat, rev, vpu, and nef that are located in the middle and at the 3' end of the HIV-1 genome52,53 (Fig. 1). In order to provide a concise yet definitive review, a glossary of HIV proteins are summarized later in the text. Tat and rev have been the focus of extensive studies as these genes are essential for virus replication.54 Others (vif, vpr, vpx, vpu, nef) are dispensable in certain cell types. Many of these genes may play important role(s) in vivo. The "virion infectivity factor" (vif) is present in all lentiviruses and plays an important role in the infection of relevant target cells; both HIV-1 and HIV-2 vif mutant viruses are unable to replicate in peripheral blood mononuclear cells but replicate to wild type efficiency in certain cell lines such as SupT1 and Molt-4/8.55

![Genomic organization of HIV-1 and HIV-2](image)

Fig. 1. Genomic organization of HIV-1 and HIV-2. The diagram depicts the major open reading frames and the relative positions of the accessory genes of HIV-1 as compared to HIV-2. Boxes indicate the approximate sizes of the various viral genes aligned (not drawn to scale).

Also, HIV-1 genome complexity is further confounded by the observation that an anti-sense RNA, transcribed in the opposite direction from the env gene encodes a 19 kD protein which is recognized by antibodies from HIV-infected individuals.56,57 This RNA is apparently transcribed from a negative strand promoter within the 3' LTR, but its significance in vivo has not yet been determined. It has been speculated that sense/anti-sense RNA duplex formation may regulate the maturation and/or stability of the sense RNAs and vice-versa.

Studies from many laboratories have shown that HIV accessory genes possess multi-functional properties. For example, vpr has been demonstrated to influence nuclear transport of HIV-1 provirus in non-dividing cells such as macrophages, transactivation of cellular genes, and cellular differentiation of the human muscle cell line.58 Moreover, vpr plays a critical role in the productive infection of macrophages, but not activated or resting T-cells.59-61 In HIV-2 and SIV, vpr gene is called vpx (Fig. 1). These two genes share significant homology and are selectively incorporated into the virus particle.62-64 It is possible that the latter two proteins may have a function within the virion or influence viral replication early, prior to new viral protein synthesis.

Both vpu and nef have been shown to down-regulate surface expression of CD4,65 and this is in part due to accelerated CD4 internalization, and indirectly due to down-regulation of transcription factors NF-kB and AP-1.66 The effect of nef on viral gene expression and pathogenesis remains controversial. In SIV, both nef and vpx seem to be essential for rapid disease progression.67,68 Ruprecht and colleagues found that after mucosal exposure of newborn rhesus macaques to the nef-vpr-deleted HIV-1, there was a rapid increase in virus replication and progression to AIDS roughly one year after infection.69 It is believed that this discrepancy may be in part due to the route of administration and dose of infection, as well as differences in the infants' and adults' immune systems.
MECHANISM OF INFECTION AND LIFE CYCLE

HIV can productively infect CD4+ as well as CD4- cells of a variety of primate origins. The nature of viral tropism is related directly to the viral genetic structure and on the number of viral passages in specific laboratory cell lines. For example, a Zairian HIV-1 isolate, but not the prototype, has been demonstrated to successfully infect human intestinal cells, in addition to its highly cytopathic property in CD4+ lymphocytes.76 This route of infection could occur via CD4-independent entry, making use of other receptors such as galactosylceramide.71,72

T cell line-tropic HIV-1 isolates require additional cell surface cofactors. One such cofactor, formerly named "Fusin", is a heterotrimeric GTP-binding protein (O protein)-coupled receptor, or a family of receptors, named CC CKR5, utilized by chemokines such as RANTES, MIP-1 alpha, and MIP-1 beta. The latter receptor serves as the fusion cofactor for macrophage tropic HIV-1 strains and this may be required for infection of macrophages when a person first becomes infected.73 Furthermore, nonhuman murine (3T3) cells expressing CD4 are ordinarily not infectable with HIV-1 unless they are additionally engineered to express the CC CKR5 receptor.74 In a separate study,75 the chemokines MIP-1 alpha, MIP-1 beta and RANTES have been shown to inhibit infection of CD4+ T cells by primary, non-syncytium-inducing (NSI) HIV-1 strains at the virus entry stage, and also block env-mediated cell-cell membrane fusion. Interestingly, it has also been shown that a mutant allele of this receptor, now invariably called CCR5, is present at a high frequency in caucasian populations (allele frequency, 0.092), but is absent in black populations. A 32-base pair deletion within the coding region results in a frame shift, and generates a non-functional receptor that does not support membrane fusion or infection by macrophage- and dual-tropic HIV-1 strains.76 This finding implies that host genetic variations in infected individuals may determine the course of the disease.

In an infected cell, the HIV life cycle is divided into early and late stages of virus replication. In the early stage, HIV reverse transcriptase (RT) plays the most critical role by copying viral RNA to DNA which confers the ability to integrate into the host genome. This proviral DNA can subsequently be expressed or undergo latency. During the dormant state, the provirus persistently spreads into a large pool of productively infected individuals may determine the course of the disease.77 For example, an Atherian HIV-1 isolate, but not the prototype, has been demonstrated to successfully infect human intestinal cells, in addition to its highly cytopathic property in CD4+ lymphocytes.78

Among many facets of the virus lies its ability to translocate a full complement of molecules needed for initiation and persistence of viral replication. In addition to RT, other viral gene products, i.e., the accessory proteins Vpr and
Vpx, are believed to be transported by the virions into the host cells. More recently, it has also been shown that the tRNA primer required for RT function that initiates the synthesis of the complementary DNA strand also resides in viral particles. In transfected cells, about 8-12 molecules of tRNA are incorporated selectively into the virus particle. This incorporation is via specific interaction with RT protein which is independent of genomic RNA encapsidation.

Thus, the virally-encoated RT catalyzes the replication of single-stranded viral RNA to yield double-stranded viral DNA which is then integrated into the host genome. After integration, proviral DNA undergoes latency or immediate activation of its promoter which is the beginning of the late stage, which results in synthesis of the full complement of the viral products. Upon completion of the de novo protein synthesis, gag and gag-pol precursors cluster with envelope glycoproteins and budding occurs from the cell membrane. However, virus maturation occurs when the viral protease gene is activated, resulting in the cleavage of the gag-pol precursor and formation of infectious particles. The mechanism of this process is still unknown.

Using improved techniques, two independent groups of researchers reported an interesting observation that steady-state levels of viral RNA are present in patients' blood, suggesting that virus particles are continuously produced by newly infected cells and then rapidly cleared, presumably by the immune system, and it is argued that many new immune cells are infected constantly. These continuous rounds of de novo infection, replication, and turnover of both virus and infected cells, when sustained for a long time, form the basis for the pathology seen in AIDS.

Figure 2 shows a simplified schematic representation of how HIV enters an active cell and follows multiple steps for a complete round of replication. The figure also indicates more than a dozen steps involved in virus replication. Interrupting any one of these steps could potentially prevent the virus from reproducing.

Shown in Figure 3 is the thin section electron microscopy evaluation of HIV-1 indicating massive productive infection of cultured human lymphocytes 3-5 days after cultivation in vitro. The figure also depicts the morphological transition from budding to maturation of the virions. The immature and mature cell-released particles show distinguishable morphologies. The immature virions indicate diffuse and ill-defined patterns, whereas the fully developed particles show complete conical cores (p24 gag) connected at the smaller end to the envelope-associated matrix protein (p17 gag) which is encapsulated with the lipid bilayer containing the embedded viral envelope proteins (gp 120/41 env), seen usually as fine spike-like structures in the circumference of the complete particles. These structural components are depicted schematically in Figure 4.

**Fig. 3.** Electron-microscopy analysis of the cultured T cells, indicating newly formed virus particles (A) and steps in the budding (B) and maturation of the cell-released (C) particles. Magnification × 25,000 (A) and 200,000 (B,C) (Micrographs by Hans Gelderblom of Robert Koch Institute of Germany).

**VIRAL GENE EXPRESSION**

Tat/TAR and Rev/RRE axes are two pivotal HIV autoregulatory pathways. This section will mainly focus on Tat, and not on Rev protein. Rev and its viral RNA target sequence RRE (rev-responsive element), i.e., Rev/RRE axis, regulates posttranscriptional events in the viral life-cycle including splicing, polyadenylation, degradation, transport and translation. The Rev/RRE axis has drawn considerable attention and scrutiny in particular mechanisms involving cellular factors which participate in the Rev transport of viral RNAs encoding the HIV structural proteins. For further information on Rev, the reader is referred to reference 82.

HIV-1 Tat, a 14-16 kD nuclear protein, is the product of a doubly spliced transcript early in the viral life-cycle. It binds to the 5' bulge of an RNA-stem loop, TAR, a structure which is located at the 5' end of all nascent viral transcripts adjacent to the transcription initiation site spanning the nucleotide positions +1 to +59. Tat is a very potent transactivator that is required for high levels of viral
The function of Tat at the level of TAR element has been under considerable scrutiny, since Tat plays multiple roles in transcription of HIV and various other cellular genes including interleukin-2 (IL-2), tumor necrosis factor-beta (TNF-β), tumor growth factor-beta (TGF-β), glucose-6-phosphate dehydrogenase (G6PD) and interleukin-6 (IL-6). Tat also allows replication of TAR-negative mutant viruses in cells other than T-lymphocytes, i.e. in an astrocytic glial cell line, U87-MG.

In HIV-2, Tat (Tat2) is composed of 130 amino acids versus the prototype 86 aminoacid form of HIV-1 Tat (Tat1). Tat2 differs from Tat1 in that about 20% of Tat2 at the amino-terminus and 30% at the carboxy-terminus are essential for its transactivation function. There is little homology between the two Tat proteins. However, like Tat1, Tat2 contains conserved cysteine- and arginine-rich domains which are important for its function.

Tat1 has also been shown to have cytokine-like effects on various cell types. For example, transgenic mice harboring the HIV tat gene develop Kaposi sarcoma (KS)-like lesions where Tat appears to synergize with basic fibroblast growth factor (bFGF) in inducing angiogenic lesions. Transcriptionally, Tat stimulates both transcription initiation and elongation in vivo. Moreover, Tat facilitates the formation of the HIV preinitiation complex, and interacts directly with the TATA binding protein (TBP) subunit of transcription factor TFIIID. Two laboratories have independently shown that TBP/TFIID plays a key role in binding to Tat and up-regulating more RNA polymerase-II processive complexes. The ability of Tat to bind to TBP is further strengthened by the finding that viral clones defective in NF-κB and/or Sp1 binding sites create spontaneous new

**Fig. 4.** Schematic diagram of HIV-1. Electron microscopic findings on HIV-1 particles shown in this diagram indicates the major entities of the virus with the two letter codes of nomenclature for the structural components; SU: surface, TM: transmembrane, MA: matrix, LI: link, CA: capsid, NC: nuclear capsid, PR: protease, RT: reverse transcriptase, IN: integrase. CEL: core envelope link morphopoietic protein which is apparently essential to finish the budding process, and MHC: major histocompatibility complex.

**Fig. 5.** Diagram of HIV-1 promoter and a stepwise assembly of adjacent and basal transcriptional factors on DNA. The top line depicts the most well-studied transcription factor binding sites upstream of the TATA box. They include negative regulatory element (NRE) followed by interleukin-2 (IL-2) signal transduction binding site. Both of these sites could act as modulatory NF-κB elements that are affected by the state of the cell cycle (i.e. resting cells have signals occupying the NRE whereas cells entering G1 or the activated state would have proteins occupying the IL-2 site, subsequently leading to activation of promoter). The NF-κB sites are important for binding of p50 homodimer.

The NF-κB family of proteins are released as active homodimers or heterodimers into the nucleus upon stimulation by DNA viruses, lymphokines or immune activation pathways. Following binding of NF-κB, the promoter is signalled to be activated where a strong cooperation between NF-κB/Sp1/TFIID takes place. This cooperative activity makes a series of short transcripts where the polymerase is named "non-processive". However, upon synthesis of viral protein Tat, the non-processive complex becomes "processive" and through a series of recruitments of new proteins, i.e. transcription factor TFIIA and Tat-associated kinase (TAK), into the promoter, the polymerase is now capable of reading through the genome in a very efficient manner. It is indeed this step of viral transcription that acts as a master switch to turn on viral replication and subsequent progeny formation.

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revertant viruses that have a stronger TATA sequence, hence allowing better initiation complexes to be made on the viral promoter.34 Some of these mechanisms and various step-by-step processes of the HIV gene expression are illustrated in Figure 5. Taken together, Tat upregulates HIV transcription at the preinitiation complex and during elongation of nascent transcript.

One of the distinguishing features between Tat and Rev is that Tat is essential for the expression of all genes including Tat itself by its positive autostimulatory effect on the viral promoter. This effect of Tat is enhanced partially when Rev is absent, resulting in the further accumulation of the viral regulatory proteins. In addition, there is a lack of a full complement of viral structural proteins or virus production, implying that Rev is involved positively in the production of tat and the viral accessory genes. Thus, Rev in turn regulates the optimal level of viral gene expression.34 For these reasons, the two regulatory proteins, in particular Tat, have been the subject of numerous studies analyzing systematically their structure-function.98-101 In an attempt to inhibit Tat function, a number of various approaches have been reported. For instance, Tat transdominants,102 Tat peptides,103 anti-sense ribozymes with cleavage capacity,104 pharmacological drugs such as benzodiazepine derivative Ro 5-3335,105 and single chain antibodies for intracellular immunization.106,107 Not many of these approaches have proceeded to clinical trials. However, due to lack of other effective therapies, Tat remains to be an extraordinarily attractive HIV target for therapeutic intervention.

VACCINE

To date, no vaccines or effective therapies have been developed that stop the spread of AIDS.108-110 Many researchers believe that an effective vaccine against human immunodeficiency virus (HIV) will never exist. Their major concerns often revolve around the fact that the retroviral genome integrates into the DNA of the immune system itself, and the infection traverses from cell to cell, thus the virus escapes from immune surveillance. Some investigators are optimistic since an effective vaccine against a retrovirus such as feline leukemia virus (FeLV) exists,111 and such investigators argue that human trials of an AIDS vaccine should not be delayed unnecessarily and efficacy trials should be carried out, especially in certain developing countries, where there are high incidences of infection.

The types of HIV immunogens currently being tested or being considered for testing in human clinical trials include inactivated HIV, protein subunit immunogens (individual HIV proteins such as gp 120, gp 160, or various synthetic peptides of HIV proteins, multivalent HIV subunit immunogen mixtures, subunit immunogens in live vectors (e.g., Vaccinia, Salmonella, Calmette-Guerin bacillus, poliovirus, rhinovirus, or adenovirus), immunization with host proteins (CD4 or MHC molecules), anti-idiotypic antibody to CD4, gene therapy through ex vivo transduction of desired genes or intracellular immunization, or direct immunization with complementary DNAs of HIV proteins. The advantages, disadvantages and concerns of these approaches and the current status of almost 30 clinical trials in human have been extensively reviewed.112

Fig. 6. A PCR strategy to detect the presence of HIV-1 RNA or DNA in infected cells. The top panel depicts 5' or 3' nested primers against the most conserved region of all retroviruses, namely the pol gene. The primers are against HIV-1 reverse transcriptase from 2.3 Kb to 4.2 Kb. Both sense and anti-sense sequences are written in the box below the diagram. As a routine procedure, all PCR products after amplification must be sequenced or hybridized with an internal 40 to 50 base oligonucleotide. A confirmatory result on a sample is divided into two steps. The first step includes amplification of a known abundant RNA or DNA, namely globin. Only if the globin primers allow amplification should one then proceed with the HIV PCR. A lack of globin amplification usually is an indication of poor quality of samples prepared.

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Table 1. Pharmacologic comparison of nucleoside analog HIV drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Bioavailability(%)</th>
<th>Oral Serum: CSF</th>
<th>Serum Half-Life</th>
<th>Intracellular Half-Life</th>
<th>Indication</th>
<th>Dosage (Total Daily)</th>
<th>Principal Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zidovudine</td>
<td>63</td>
<td>60-70</td>
<td>1.1</td>
<td>3.4</td>
<td>HIV+, CD4 &lt; 500</td>
<td>500-600 mg</td>
<td>Anemia, Neutropenia, Nausea</td>
</tr>
<tr>
<td>Didanosine</td>
<td>33</td>
<td>30-25</td>
<td>1.4</td>
<td>12</td>
<td>HIV+, advanced disease, disease failed or intolerant to Zidovudine or following AZT</td>
<td>400 mg</td>
<td>Neuropathy, Pancreatitis</td>
</tr>
<tr>
<td>Zalcitabine</td>
<td>80</td>
<td>20</td>
<td>1.2</td>
<td>2.6</td>
<td>Advanced HIV disease after prior AZT</td>
<td>2.25 mg</td>
<td>Neuropathy, Oral ulcers, rash</td>
</tr>
<tr>
<td>Stavudine*</td>
<td>83</td>
<td></td>
<td></td>
<td></td>
<td>In trials</td>
<td>In trials</td>
<td>Neuropathy</td>
</tr>
</tbody>
</table>

*: 2', 3'-dihydro-3'-deoxythymididine. CSF, cerebrospinal fluid. Compiled from Volberding.[130]

ANTI-VIRAL DRUGS

The field of HIV therapy continues to evolve as new drugs are developed. The most effective drugs are those targeting AIDS-related diseases. These classes of drugs show only a reasonable success rate for the better management of the disease. On the other hand, direct antiviral drugs encountered thus far have a lack of long-term efficacy. Although new drugs are urgently needed, attention is also drawn to use the existing compounds as rationally as possible to achieve a positive clinical outcome. In almost all studies, new insights into the pathogenesis of HIV are gained as the results of clinical trials become available.

To date there are two classes of anti-HIV drugs that are currently approved for treatment of HIV infection. First, chemical derivatives of nucleosides which are known as zidovudine (AZT), didanosine (ddI) and zalcitabine (ddC), that inhibit HIV reverse transcriptase enzyme.[131] Four nucleoside analogs are currently available for clinical use (Table 1). The extensive use of these compounds, in particular AZT, has resulted in the emergence of drug-resistant virus strains in cell culture and in vivo. Not all mutant strains are equally resistant to all the compounds, which are phosphorylated by intracellular enzymes to triphosphate, representing the active forms of the drugs.[114][115] These compounds, also inhibit DNA chain synthesis and may have other intracellular activities which can contribute to their toxic side-effects.

Second, three peptide derivative inhibitors of HIV-1 protease have recently been used in clinics, i.e., saquinavir (Hoffman-La Roche), ritonavir (Abbott Laboratories) and indinavir (Merck, Inc.). HIV-1 protease is an aspartyl protease and is composed of two identical 99 aminoacid subunit peptides. In brief, the peptide mimetic compounds have demonstrated the following characteristics: a) all three protease inhibitors can extend survival only when administered in combination with standard therapy, i.e., using nucleoside analogs, b) protease inhibitors have synergistic effects, c) common pathways of resistance via multiple mutations (some specific) are induced within the HIV-1 protease gene, including the active site, and d) non-infectious HIV-1 virions still cause cell death in the presence of protease inhibitors in vitro by syncytia and apoptosis. Nonetheless, after taking a combination of ritonavir and saquinavir for six weeks for instance, 43 patients have demonstrated a median reduction of HIV-1 RNA by 99.6%.[116]

In one study, five patients enrolled had extensive prior nucleoside therapy, a low mean CD4 T-cell count, a high soluble tumor necrosis factor-alpha type II (sTNFII) receptor concentration and high viral load. The drug used was indinavir which was tolerated at a dose of 600 mg every 6 hours. Both CD4 count and the boost in immune response was partially restored.[117] In a separate study, the safety and activity of saquinavir was evaluated in combination with zalcitabine (ddC) and/or zidovudine (ZDV). This study demonstrated that in subjects with prior extensive experience with antiretroviral therapy, the triple combination (saquinavir, ddC and ZDV) was well-tolerated, safe and remained superior to other regimens as measured by CD4+ cell counts, quantitative HIV-1 microculture assay and plasma HIV-1 RNA levels.[118][119]
**HIV DIAGNOSTIC ASSAYS**

Conventional virus diagnosis has been based on the direct demonstration of virus in clinical samples, using viral antigen detection and virus culture, or on indirect detection of a specific antibody response. Prototypic ELISA tests often fail to detect antibodies to HIV-1 subtype 'O', and confirmatory immunoblot assays give false negative results.\(^{11,12}\)

Therefore, modified procedures should be considered for screening of blood and blood products for transfusion, or hematopoietic cells intended for use in transplantation. For example, a new procedure, named product-enhanced reverse transcriptase (PERT) assay, has been reported that detects HIV-1 in plasma with a similar sensitivity to a polymerase chain reaction (PCR)-based assay, i.e., with a corresponding sensitivity of detecting HIV from about 553 to 417,000 particles/mL.\(^{121}\) It remains to be determined if this assay would hold the claimed performance for large scale screenings.

Currently used HIV detection assays are rapidly shifting from ELISA, i.e., antibody or antigen screening, or more specific tests such as competitive ELISA, toward "nucleic-acid-based assays".\(^{120}\) The core component of such assays include a PCR procedure. These assays can allow not only the differentiation of HIV clades or subtypes, but are useful in detecting infection in newborn babies, forensic identification, therapeutic monitoring, identification of the seroconversion window period, and in the future, may be also used in distinguishing 'infection' from 'seroconversion' in HIV-vaccinated subjects. Most current nucleic acid-based assays designed for the major HIV-1 strains (subtype 'M') do not amplify sequences from HIV-1 subtype 'O' which show the highest variations in their envelope gene. It is also pointed out that other unidentified subtypes are likely to exist.\(^{11}\)

PCR is especially useful as an additional technique where serological results are repeatedly doubtful or in situations where serology is not interpretable. Furthermore, PCR offers several advantages over virus culture: it requires shorter hands-on time, provides faster results, uses a small sample volume and is more sensitive than virus culture in assessing asymptomatic HIV-infected individuals. Using appropriate PCR primers, it is possible to detect new emerging viruses from patients under clinical trials for various drugs.\(^{122}\)

Recently it has been shown that using cell in situ-PCR technique,\(^{123}\) the virus reservoir in the lymphoid tissues is much larger than previously thought.\(^{124}\) This study measured the dynamics of immune depletion during HIV infection. During latency, it was found that fewer than five copies of RNA per infected cell could be detected with this technique. The technique uses two pairs of PCR primers with a short two base pair overlap that was sensitive enough to detect a single copy of integrated DNA. Using a double staining method which stained CD4 cells first then PCR amplified HIV DNA, this technique allowed for the identification of CD4 cells infected in different lymphoid compartments. Data shows that almost 25% of CD4+ cells harbored HIV DNA, but less than 1% of them were HIV RNA+.\(^{125}\) This suggests that infected cells contain dormant proviral DNA which may slowly convert to transcriptionally active and replicative provirus.

A diagram of a possible laboratory approach to PCR detection of HIV is depicted in Figure 6. The key to success of this diagnostic technique is to use the conserved regions of the viral sequences for amplification and obtaining quantitative RNA or DNA PCR representative of the viral genome.\(^{126}\)

**PERSPECTIVE**

The authors wish to emphasize three independent but equally important points that will have impact on the future of AIDS and related diseases. These include: 1) safety, 2) clade variations and 3) opportunistic infections. Safety practices are among the most important aspects of preventive medicine today. Because the lives of uninfected people are constantly at risk due to HIV's nondiscriminatory host selection, continuous education must be made available to the general public and health-care workers. Moreover, good clinical practices (GCP) and current good manufacturing procedures (cGMP) must be employed routinely; particularly for screening of blood and blood products. Failure to enforce rigorous programs usually results in the spread of the virus through blood banks and other public domains as seen in East Asia today. It is estimated that by the year 2000, the AIDS epidemic will shift from the western world to the Far East and neighboring countries. Therefore, a growing need for world policies on safety issues from organizations such as the WHO, will undoubtedly be encouraged.

A challenging issue that faces all facets of the AIDS epidemic is the genetic variations known as clades. The emergence of new HIV clades has affected our view of virus biology and therapeutic interventions, i.e., new definition of open reading frames, effectiveness of diagnostic assays, vaccine development and drug treatment. Diagnostics have classically relied on the presence of antigen or antibody in serum/plasma. Antibody screening tests are the least to be used to monitor the virus spread, especially as the risk of acquiring HIV positive specimens, for most available assays in the market, remains around 1 in 500,000. However, these methods are rapidly being replaced by nucleic acid (DNA and/or RNA)-based detection assays, such as PCR. Although this technique has not yet dominated hospitals and clinical laboratories, it will undoubtedly be valuable in: a) validation of clinical findings, b) detection of virus during the early
seroconversion window period, c) better prognosis of the disease, d) patient monitoring in response to treatment, e) evaluation of drug-resistant strains and, f) perhaps more reliable and cost-effective.

As viruses undergo rearrangement and recombination, their open reading frames, like regulatory and accessory genes, may acquire new functions. This is evident in the ever-growing literature on viral genes tat, vpr and nef. It is clear from a number of published reports that small regions of tat for example perform multiple functions. Another clear example is nef which was initially thought to be non-essential. Now there is a direct correlation between mutations in nef gene and “non-progressor” status of the patient. Careful attention to details on genomic rearrangement of new viral clades, as well as understanding and defining new acquired functions of these genes, will be the central focus of molecular genetic studies.

Vaccine development for HIV is also one of the most challenging issues, the reason being that retroviruses have two methods of transmission, either through extracellular secretion or cell-to-cell contact. In fact, it is estimated that most of the viral particles released into the extracellular environment are not infectious in part because of the rapid shedding of the viral envelope or instability of the matrix proteins. Instead, the route that is most preferred by virus for transmission is cell-to-cell contact. This method of transmission along with the heterogeneity of envelope and matrix proteins, makes classical vaccine development obsolete. Although approaches such as whole inactivated virus have been successful on animal retroviruses like feline immunodeficiency virus, the same approach fails to work on HIV. One primary reason may be that feline immunodeficiency virus is a much simpler virus as compared to HIV or that there are fundamental differences in the host susceptibility and virus life-cycle. Future vaccine approaches will likely focus on replication-defective strains carrying mutations in more than one gene, e.g., nef(-), tat(-) and rev(-). These live attenuated-viruses will probably include conditional lethal genes inserted into the viruses, such as the herpes simplex virus thymidine kinase (TK) gene, in an attempt to safeguard the vaccine preparation, should the need arise to inactivate the mutant virus post-vaccination.

Drug treatment for AIDS patients may be the only plausible solution for rapid treatment. Numerous inhibitors have been tested and succeeded in in vitro studies, but never made it to the clinic. The primary reasons were lack of specificity or in vivo efficacy. Other issues such as toxicities associated with the anti-viral compounds have been one of the major concerns. Emphasis on combination therapy (CT) regimens, e.g., using RT and protease inhibitors, will continue to grow as new drugs become available. The obvious drawback to these regimens remains the emergence of new drug resistant strains. It is also important to note that unorthodox approaches exemplified in transdominant mutants, or use of peptide mimetics, will continue to be the focus of interest in the future.

The most manageable scenario becomes a formidable task when HIV infection predisposes the patient to neoplastic conditions such as non-Hodgkin’s lymphoma (NHL) and Kaposi’s sarcoma (KS). Opportunistic agents like human herpes virus type 8 (HHV-8), the possible etiological agent of Kaposi’s sarcoma, represent an example of homeostasis between HIV and the herpes virus members. Indeed, the symbiotic interactions between different classes of viruses have long been known. Moreover, cytokine signals involving IL-2, bFGF, glucocorticoid receptors and NF-xB-like family of proteins are all well documented to share common pathways between these two distinct viruses. Finally, understanding the dynamic interplay between opportunistic agents and HIV may be of great importance in boosting the immune system to fight off these viruses.

**GLOSSARY OF HIV ENCODED PROTEINS**

The following glossary is a summary of the HIV-encoded proteins obtained from the Los Alamos Reference Manual.1

a) Structural Proteins:

**Gag:** Polypeptides of group-specific capsid proteins; the precursor is the p55 myristoylated protein, which is processed to p17 matrix (MA), p24 capsid antigen (CA), p7 nucleocapsid (NC) which binds two zinc ions to form zinc fingers, and p6 (Vpr binding). Products of partial proteolysis, p1 and p2, by the viral protease are also found in the infectious virus.

**Pol:** This open reading frame generates the viral enzymes protease (p10), precursor reverse transcriptase (p66) which in turn is processed to (p51), and endonuclease/integrase (p31). Pol is primarily generated from the processing of a Gag-Pol precursor polyprotein (p170) by the viral protease. Pol is produced by ribosome frameshifting to -1 translation site, as seen in many retroviruses, from a single mRNA.

**Env:** Viral glycoproteins are produced as one precursor (gp160) and processed to the surface (gp120) and the transmembrane (gp41) glycoproteins. The mature proteins are held together by noncovalent interactions; as a result a substantial amount of gp120 is released extracellularly. The glycoprotein (gp120) contains the binding site for the CD4 receptor present on human lymphoid, monocytoid and some neuronal cells.

b) Regulatory Proteins:

**Tat:** transactivator of HIV gene expression on its long
terminal repeat (LTR). Two forms are known, tat-1 exon (minor form) of 72 amino acids, and tat-2 exon (major form) of 86 amino acids. The electrophoretic mobility of these two predominant forms in SDS gels is very similar and they are approximately 15 kD and 14 kD in weight. Low levels of both proteins are found in persistently infected H9 cells. Tat is localized primarily in the nucleolus/nucleus; it acts by binding to the TAR (transactivation responsive) RNA element and activating transcription from the LTR promoter. Post-transcriptional effects of tat have been postulated. Tat has also been shown to upregulate several endogenous promoters, namely IL-2, IL-4, and TNF, which may act as cytokines for upregulation of viral production. Tat has been proposed to be virion-associated.

Re: a necessary regulatory factor for HIV expression. A 19kD phosphoprotein localized primarily in the nucleolus/nucleus, which acts by binding to RRE (rev-responsive element) and promoting the nuclear export, stabilization and utilization of the viral mRNAs containing RRE. All HIV messages contain RRE which are transported to the cytoplasm for transactivation.

c) Accessory Proteins:

Tat: a tripartite 28 kD viral phosphoprotein produced very early in infection by some HIV-1 strains. Found primarily in the nucleolus/nucleus, Tat contains the first exon of tat, a small part of env and the second exon ofrev. It has both Tat and Rev functions in the same polypeptide and can functionally replace both the accessory proteins of HIV-1.

Vif: viral infectivity factor, typically 23 kD, not found in the virion; required for the efficient transmission of cell-free virus in tissue culture and possibly in vivo. In the absence of Vif, the produced viral particles are defective for cell-free infection, while the cell-to-cell transmission of virus is not affected significantly. It has been reported that the cellular localization is primarily in the Golgi.

Nef: an approximately 27 kD non-virion protein found in the cytoplasm of infected cells. It is myristoylated and associated with the inner plasma membrane. One of the first HIV proteins to be produced in infected cells, it is the most immunogenic of the accessory proteins and may be used in the future for diagnosis and staging of the disease. Nef is dispensable and possibly suffers counter-selection during ex vivo viral propagation. Recent evidence suggests that SIV nef is required for viral propagation in vivo.

Vpr: virion-associated protein of unknown function found in HIV-1, HIV-2, SIVmac, and SIVmne; typically 15 kD. May be functionally homologous to Vpx. Also called "rap" for rapid.

Vpu: protein that promotes extracellular release of viral particles. Found only in HIV-1. Integral membrane phosphoprotein of 16 kD; similar to M2 protein of influenza virus. It may be involved in Env maturation. It is not found in the virion.

Vpx: virion protein of 12 kD found only in HIV-2/SIV and SIVmac; not in HIV-1 or SIVmne. Function unknown. The vpu/vpx genes may be used to distinguish between HIV-1 and HIV-2 infection (vpx may have some homology with vpr).

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