Molecular Virology of HIV-1 and Current Antiviral Strategies

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Over 40 million people are currently infected with the human immunodeficiency virus (HIV), and over 25 million deaths have been attributed to the virus since the beginning of the epidemic. It has been theorized that the virus originated from monkeys during the development of an oral polio vaccine in Africa in the 1950's.[1] The HIV virus is part of the lentivirus family, and is a sexually transmitted pathogenic retrovirus which can be divided into two types. HIV-1 is currently widespread among humans, and begins showing symptoms within 5 years of infection. HIV-2 is localized in Africa, and it takes longer for symptoms to appear. HIV-1 is classified into three sub-groups based on the sequences of *gag* and *env* genes: Group O (outliers), Group M (majority), and Group N (non-M/O). Within Group M lies 10 clades, classified A through J. Clade B is the most common in North America and Western Europe, B and F are prevalent in Brazil, E is prevalent in South Eastern Asia, and A, C, D and E are localized to the developing world.

The HIV virus targets the host immune system, making it a very difficult pathogen for the human body to fight. In addition to making the host highly susceptible to secondary infections, rapid mutation rates within the viral genome make vaccine and drug development difficult. During the 20 years since the discovery of the HIV virus, many significant breakthroughs have been made concerning the molecular biology and pathogenesis of the virus. This review article outlines current strategies for combating the virus, which have arisen thanks to these advances.

HIV infection is characterized by a decline in T-cell count and function, leading to a weakened immune system. HIV also induces B-cell polyconal activation and a lack of antibody specificity. The host receptors important in viral recognition are CD4 and one of two chemokine receptors: CCR5 or CXCR4.

Molecular Virology of HIV

Viral Structure

The HIV virion's diploid genome consists of 2 single-stranded RNA molecules within a host-derived lipid bilayer. Trimers of surface glycoproteins gp41 and gp120 protrude from the virion. The latter's outermost part contains a variable region known as a V3 loop, and is responsible for inducing a strong immune response.[2] A p9 nucleocapsid protein interacts non-covalently with the viral genome, a p17 matrix protein anchors the internal face of the viral envelope, and a p24 capsid protein encloses the genome. Within the capsid of the virus particle are enzymes essential to reverse transcription and integration within the host.

Viral Entry

HIV has an affinity for CD4+ T-cells and monocytes. The viral glycoproteins that mediate entry are transmembrane protein gp41 and gp120.[3] gp120 is non-covalently linked to gp41, and recognizes the CD4 ligand on host cells. Upon binding, a conformational change within gp120 is induced which exposes co-receptor binding sites in gp120. The co-receptors bind a host chemokine receptor - either CXCR4 or CCR5 depending on the type of HIV particle. M-tropic particles recognize CCR5, infect macrophages and primary T-cells, and have a low specificity for CD4+ T-cell lines. T-tropic particles recognize CXCR4, which is highly expressed in CD4+ T-cells, and induce syncitia.[4] (Syncitia is the fusion of cells to create one large cell with many nuclei. It is a precursor of cell death.) Upon interaction between gp120 and the host chemokine receptor, gp41 binds to a host herparan sulfate, triggering fusion of the host

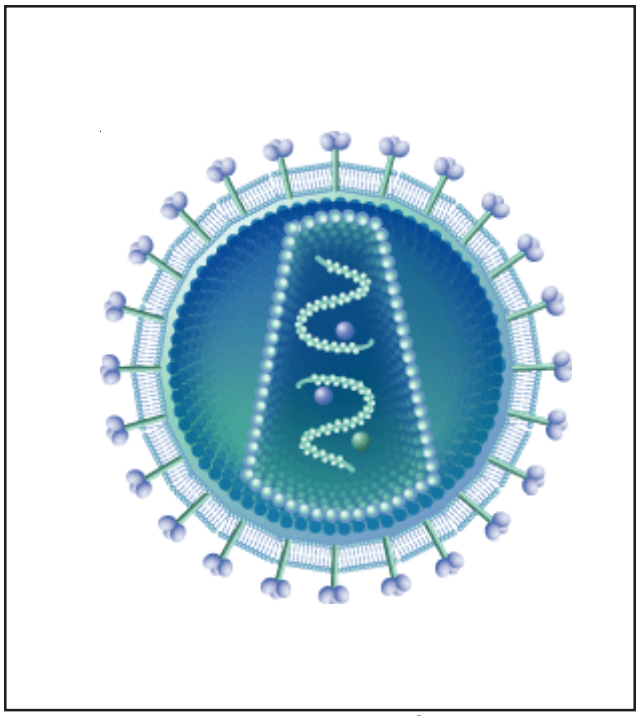

Figure 1. The general structure of the HIV virus

and viral membranes, and the entry of the capsid into the cytoplasm. [5]

Life Cycle

The viral lipid envelope is left behind in the host's lipid bilayer, and the viral capsid is released into the cell. Immediately after entry, viral reverse transcriptase (RTase) transcribes the viral genome into cDNA, using a cellular lysine tRNA molecule as a primer[4]. RTase has a very high error rate (as high as one mismatched base pair per 1.5 x 10^3 bases). In spite of having a 5' cap and a poly A tail, the viral RNA does not serve as mRNA. The RTase's nuclease activity degrades the viral RNA template, and the cDNA travels to the nucleus. Nuclear entry is mediated by *Vpr* and *Vif* accessory proteins, as well as by nuclear localization signals within the *Vpr* and p17 matrix sequences. Viral integrase proteins insert the viral genome into the host's chromosomal DNA, and it has been proposed that distortion characteristics in the DNA (for example bound proteins) influence where the viral cDNA is inserted.[7]

The HIV RNA genome contains 9 open reading frames and is about 9 kilobases in length. After integration, cellular factors mediate transcription of viral transcription factors located on the *pol* or *env* genes. A protein called *Tat* increases transcription rate[5]. Upon transcription of the *env* gene, *Rev* (a regulatory protein) binds a specific region on both spliced RNA (used for structural components) and un-spliced RNA (used for genome packaging) and mediates *env*'s export from the nucleus.

Viral structural components are located on the *gag* gene, and are initially transcribed and translated (in the cytoplasm) into a pr55 pre-protein, which is cleaved upon maturation of the virus. A *gag-pol* derived pre-protein, also translated in the cytoplasm, yields the viral protease, integrase, and RTase. Both the *gag* and *gag-pol* genes contain 2 different reading frames which are differentially expressed, favoring transcription of structural products over enzymatic ones.[9] The mRNA of the *env* gene, which codes for viral surface glycoproteins, is translated in the ER to yield a gp160 pre-protein. This precursor travels to the cell membrane where it remains in a non-covalent trimer. During virion formation, the gp160 polyprotein is cleaved into gp120 and gp41, and also yields 3 regulatory proteins. *Vpr* aids in the transport of cDNA into the nucleus, *Vpu* aids in virion assembly, and *Vif* is important in maintaining infection efficiency.[10]

Immunological Response

Infection by HIV is characterized by several effects on the host immune system. B cells decline in number and function[12], and, because of the toxicity of HIV antigens, cytokine regulation is distorted causing a decrease in CD4+ T-cells. [13]

There is a distinct interplay between HIV and the immune defenses. Typical non-progressors (those who have been infected with HIV but do not show symptoms) display several responses that are different than those of progressors. Non-progressors show more T_H1-type cytokines like IL-2 and IFN-ã, and an elevated response by CD4+ T-cells and cytotoxic CD8+ T-cells towards HIV is observed. Additionally, there is an increased synthesis of â-chemokines. The HIV virus counters these defenses by varying antigenic sites[14], (preventing an effective immune response and overwhelming the immune system) and by reducing MHC on the surface of cells, and reducing the number of CD8+ T-cells. [15]

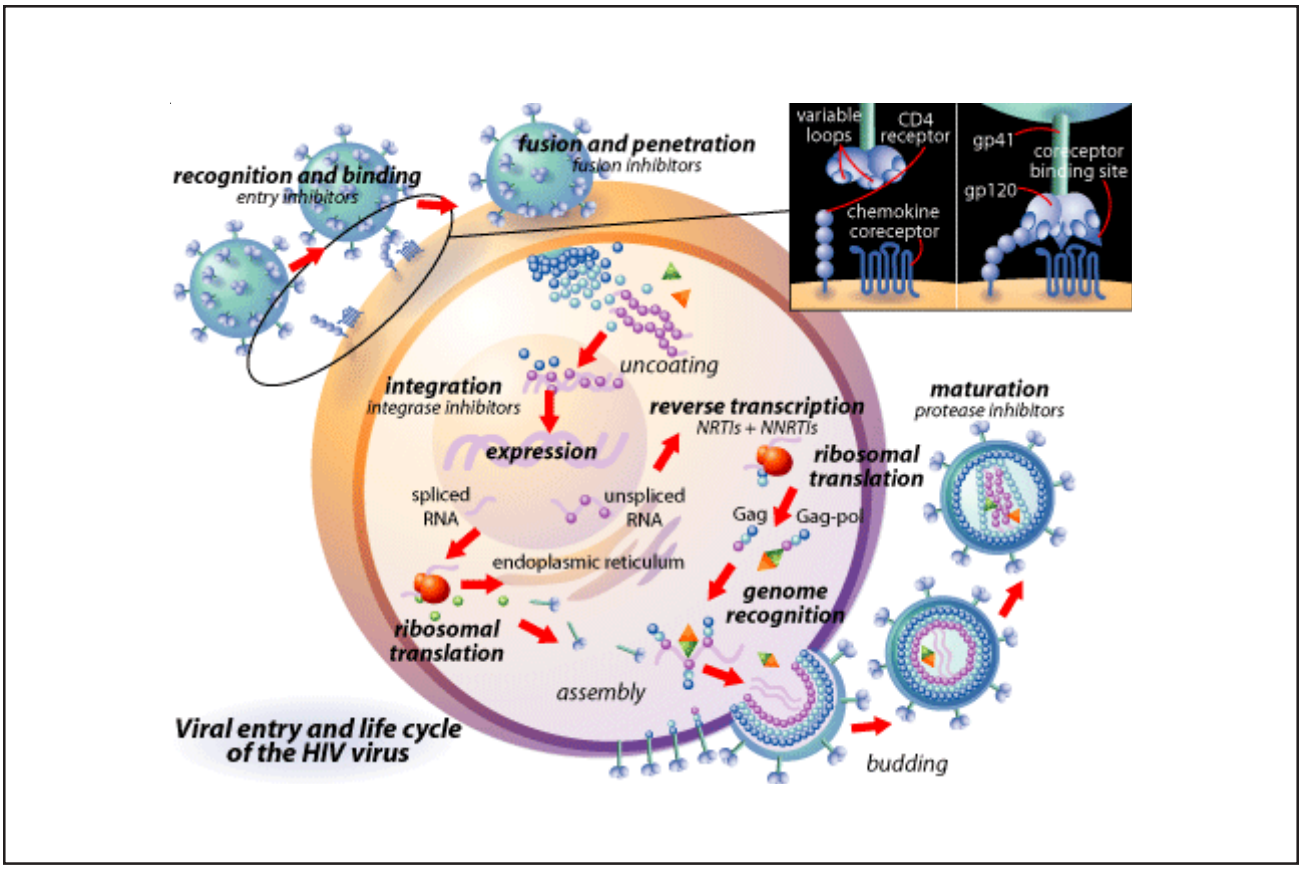

Figure 2. The life cycle of HIV and its mechanism of infection.

Current Antiviral Strategies

Many methods of combating the HIV virus have been investigated over the last two decades. Most of these strategies involve inhibiting normal viral functions. Ideally, drugs that are developed will provide effective resistance for as long as possible, be safe with minimal side effects, and be chemically stable and inexpensive. The following techniques are examples of newer methods for combating HIV, and are currently entering or undergoing clinical trials.

Antiretroviral Drugs

Inhibiting viral replication gives the host immune system a chance to recover from infection. This is the idea behind antiretroviral drugs. Although they inhibit a crucial step in viral replication, these drugs do not address latent viral reservoirs and are therefore only a temporary solution. The efficacy of these drugs increases when used in tandem with similar drugs, the dosage required of each drug is lowered, and the side effects are minimized. Drug resistance is also minimalized because only a small amount of each drug is used.

There are two main categories of antiretroviral drugs currently under investigation. The first type, nucleoside inhibitors, inhibits RTase by binding the enzyme's active site and adding to the growing DNA chain. As a result, normal 5' to 3' synthesis halts.

The second category of antiretroviral drugs are the non-nucleoside inhibitors. These drugs bind RTase at a site that is distal from the active site, inducing a detrimental conformational change within the enzyme. These drugs show high antiviral activity and low toxicity *in vitro*, but are too highly specific. Single mutations have been shown to reduce or eliminate efficiency. For this reason, drugs of this type are usually administered alongside other drugs.[16]

In addition to RTase inhibitors, other enzymes such as protease have been identified as potential drug targets. Drugs which block the protease substrate site

DRUG	TOXICITY
nucleoside reverse transcriptase inhibitors	
Class associated	Lactic acidosis
	Hepatic steatosis
	Lipodystrophy (fat wasting)
Drug specific:	
Zidovudine	bone marrow suppression, nausea, myopathy
Stavudine	peripheral neuropathy, hepatits
Didanosine	pancreatitis, dry mouth
Zalcitabine	peripheral neurophathy, mouth ulcers
Lamivudine	Few side effects
Abacavir	Hypersensitivity reaction
non-nucleoside reverse transcriptase inhibitors	
Nevirapine	rash, hepatitis, Steven-Johnson syndrome
Efavirenz	rash, dysphoria, mood changes, vivid dreams
protease inhibitors	
Class specific	Lipodystrophy, hyperlipidaemia, Didbetes Mellitus
Drug specific:	
Nelfinavir	Diarrhoea, rash
Saquinavir	Few side effects
Indinavir	hyperbilirubinaemia, nail changes, nephrolithiasis, dry nails
Ritonavir	perioral dysathesia, flushing, hepatitis, diarrhoea, nausea
Amprenavir	rash, nausea, diarrhoea
Lopinavir	diarrhoea

Table 1. Side effects of various HIV drugs.

prevent the cleavage of gp160 at the cell surface, preventing virion maturation.

Currently, patients infected with HIV-1 undergo a treatment called Highly Active AntiRetroviral Therapy (HAART). Patients are administered cocktails containing combinations of drugs that inhibit RTase (such as Nevirmpine and Efavirez) or viral protease (Sequinavir, Ritoavir, Indinavir, Nelfinavir, Amprenavir, and Lopinavir). HAART has reduced HIV-related illness and mortality between 1994 and 1998. [17]

Inhibition of Fusion

Preventing HIV from accessing its host has been a major focus in drug development. Although, in theory, any of the proteins mediating viral fusion could be potential drug targets, viral glycoprotein gp41 has recently proven a worthy target *in-vivo*. A synthetic peptide named T-20 targets two consensus motifs in gp41 which occur commonly in hydrophobic alpha-helices. The sequences targeted by T-20 have been shown to be flexible and important in mediating movement of gp41 upon binding of gp120 to the host chemokine receptor. As a result, fusion between the viral and host membranes cannot occur, preventing viral entry in the cell. *In-vivo* studies in humans saw a reduced viral-load in all participants, even those that had undergone HAART. Side effects were minimal, and because the sequence targeted by T-20 is highly conserved, mutations are not likely to negate the drug's effects.

Other efforts to inhibit fusion have included soluble chemokine receptor ligands, which cause receptor internalization, thereby preventing gp120 from binding.[18]

Integrase Inhibition

Drugs aimed at viral integrase can either act on viral DNA by preventing its association with the enzyme, or they can act directly on the enzyme itself. A naturally occurring product of *Actinoplanes sp.*, termed Integramycin, has been shown to prevent the transfer of viral cDNA into the host genome.[19] Another small, recently synthesized 12-mer called EBR28 has been found to interact with the catalytic core of integrase, preventing 3' processing and strand transfer into the host genome *in-vitro*. EBR28 targets a highly conserved domain, making drug-resistance unlikely. The lack of integrase homologues in humans suggests that integrase inhibitors are non-toxic to humans.[20]

Accessory Proteins

Viral accessory proteins play numerous critical roles in the HIV life cycle. Strategies to combat the virus

from this standpoint are currently under development. One method of targeting accessory proteins involves the use of lentivirus vectors, which can be packed with plasmids. One such use of transducing vectors includes the murine retrovirus vector, which can carry defective transdominant-negative *Rev* (*TdRev*) genes. *TdRev* inhibits nuclear export of *Rev* by forming useless multimers. *Rev* serves to export un-spliced mRNA from the nucleus. Blocking this function causes the accumulation of transcription products within the nucleus, preventing viral assembly.[21]

In addition to using vectors, synthetic analogues of a 99 amino acid N-terminal stretch of *Vif* have been shown to bind HIV-1 protease *in-vitro* and prevent viral replication. *Vif* normally functions to prevent processing of *gag* and *gag-pol* precursors until viral assembly, so it stands to reason that too much of this protein would prevent protease from ever carrying out its normal function.[22]

Vaccine Development

HIV vaccines aim to reduce the spread of HIV and eliminate viral existence in the host. The development of immunostimulatory vaccines has been a priority in the fight against HIV, primarily because of their low cost, simplicity of administration, and low storage requirements. Few HIV vaccines have proven themselves in phase I clinical trials. Many have been proven unsafe and unable to elicit strong CD4+ and CD8+ responses. Only about a third of all vaccines have been shown to elicit strong cytotoxic T-cell responses, albeit with weak neutralizing antibody responses. Many antigens have been tried in vaccine development (Table II), including *env*, gp120, gp160, 120 multimers, V3 peptides, and recombinant envelope proteins. Many vectors have also been investigated for their abilities to deliver the antigens, such as alpha viruses, polioviruses, adenoviruses, herpes viruses, and Venezuelan equine encephalitis viruses to name a few.

Several vaccines are currently going through phase I clinical trials. They take different approaches towards accomplishing the same goal. One method involves a plasmid that encodes *env* and *Rev* genes, and has been shown to elicit strong T-cell response and chemokine secretion in non-HIV volunteers.[25] In addition to antigen-encoding plasmids, immunostimulatory DNA sequences and cytokine-encoding plasmids can be co-administered.[26]

Innate Immunity

In the mid-1980's, it was discovered that individuals with a certain CD8 T lymphocyte phenotype secreted a protein called CAF and were highly resistant to the effects of HIV-1 infection. This year, using gene chip technology and antibody tagging techniques, a group discovered that expression of 3 genes, alpha-Defensin-1,-2, and -3 was stimulated during HIV-1 infection. Synthetic preparations of these proteins were shown to inhibit HIV-1 replication *in-vitro*.[27]

Sex-workers known to be resistant to the progression of HIV do not produce antibodies specific to HIV. Instead, they have elevated CD8+ T-cell counts, leading researchers to investigate vaccines which stimulate their production. Studies on macaques have shown such strategies to protect against the simian immunodeficiency virus.[28]

Conclusion

Much of the work over the last two decades has elucidated the molecular workings of the HIV virus and the human immunological response to it. In spite of the many antigenic sites targets available for antiviral drugs or vaccines, only a small handful lacks the variability which inevitably leads to drug resistance. Of these, the EBR28 integrase inhibitor and the T-20 fusion inhibitor show the most promise. More information needs to be gathered on issues like genetic factors determining resistance, the role of chemokines, and the role of latent viral reservoirs before a successful vaccine can be developed.

The evidence reported in this review suggests that there is hope that humanity will eventually conquer HIV.

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