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IMMUNOBIOLOGY OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION

P Tripathi, *S Agrawal

Abstract

After the discovery of human immunodeficiency virus (HIV) and its role in the causation of most devastating epidemic acquired immune deficiency syndrome (AIDS), there has been an increasing trend to decipher the mechanism of infection and to understand why it cannot be controlled by our immune system. By evolution, our immune system has been empowered and enough trained to recognize, elicit immune response and remove antigens and pathogens from the body. Simultaneously, HIV has also gained enough mechanism to escape the natural immune response. On one hand, it downregulates HLA class I antigens, which may present viral antigens to specific CD8+ T cells; on the other hand, the viral genome get mutated very readily under the selection pressure of specific cytotoxic T lymphocytes. The high mutation rate and convertibility of its genotype makes it a moving target and poses a prime hurdle in vaccine development. This review explains how HIV enters into the cell, how it resists the host immune response and how HIV manages to escape from it and establish in the human body.

Key words: Acquired immune deficiency syndrome, human immunodeficiency virus, macrophages, NK cells, T cell

The most disastrous disease of its time is acquired immunodeficiency syndrome (AIDS). Along with the numerous efforts of scientific community and intervention of WHO to control the disease, the gross death due to human immunodeficiency virus (HIV) infection has increased up to 25 million.1 In the developed Western countries, the preventive measures are somewhat satisfactory; however, in developing countries like Asia and Africa, the rise in HIV infection still continues. The highest number of affected individuals is in Sub-Saharan Africa, followed by Asia, mainly India and China.1

In spite of continuous efforts, there has been no satisfactory success in providing a drug or vaccine to prevent the disease progression. HIV has evolved various immune evasion mechanisms to allow entry, integration and continuous viral replication inside the host. In this review, we have focused on how HIV invades immune system and reaches to nucleus, get incorporated in the genome and keep fending off further immune response to sustain in the body, further deteriorating the condition.

Molecular Organization of HIV

Structurally, HIV is very peculiar. It is enveloped by a lipid bilayer of host origin in which viral glycoproteins are embedded as knob-like structures.2 Glycoproteins play a very important role in the attachment and fusion of virus with host cells and then lead to their cellular entry.

As HIV is a retrovirus, its genome consists of RNA, which has various overlapping open-reading frame coding for several viral proteins3 (Table 1 and Fig. 1). The primary transcript of HIV is a full-length viral mRNA, which is translated into various proteins including Gag, Pol and Env. The Gag precursor is of 55 kDa (p55) and is subjected to proteolytic cleavage giving rise to the smaller proteins like capsid of 24 kDa (p24), matrix of 17 kDa (p17), nucleocapsid of 7 kDa (p7 and p6). The Pol precursor protein is also cleaved into smaller products including reverse transcriptase (RT; p66/51), protease (PR; p10) and integrase (IN; p32) proteins. RT is initially of 66 kDa but is further processed to remove 14 kDa from its ‘C’ terminus, leaving behind an ‘N’ terminus 51 kDa polypeptide, which then forms a dimer with original non-processed polypeptide of 66 kDa.4 RT is involved in the formation of cDNA from RNA molecules; PR is involved in the proteolytic cleavage of Gag and Pol polyproteins; and IN is involved in viral integration. The HIV Env gene codes for a polyprotein gp160, which then is cleaved into smaller proteins constituting viral envelope.

Similar to other retroviruses, HIV consists of various other proteins that include Rev, Tat, Nef, Vif, Vpr and Vpu. Rev is the regulator of viral gene expression, which is involved in the maintenance of relative amounts of unspliced to spliced mRNAs. It inhibits viral RNA. RNA splicing promotes nuclear export of incompletely spliced viral RNA. This interacts with a cis-acting RNA loop (Rev responsive element) present in the viral envelope mRNA, which further assists unspliced mRNA to enter the cytoplasm from nucleus and produces full-length viral proteins needed for the production of progeny virions.5,6

Similarly, the gene product of various mRNAs contributes to other viral regulatory and accessory proteins.
that can affect HIV replication and thus infectivity. One such regulatory protein is Tat, which is also known as transcriptional activator and can bind to RNA loop present in the 3′ viral LTR called Tat responsive region (TAR), which then phosphorylates COOH-terminal domain (CTD) of RNA polymerase II using a cellular protein kinase complex called TAK (Tat-associated kinase). In the presence of host cyclin T1 and CDK9, Tat enhances RNA pol II elongation and thus is involved in upregulating HIV replication. Further protein, Nef (negative effectors), is involved in various functions, including downregulation of host CD4 and MHC I expression to fend off immune response against infected cells. It is also involved in apoptosis and enhanced virion infectivity. In addition to aforesaid proteins, the accessory HIV viral gene products, Vif, Vpr and Vpu, are also involved to influence events such as assembly and budding of viruses. Viral infectivity factor (Vif) is involved in more stable RT complexes and thus increasing infectivity of viral particles, whereas viral protein R (Vpr) is involved in the degradation of CD4. Vpr can also interact with nuclear importins and also they can check cell cycle at G2 phase of cell cycle. Thus, functional evidences about these viral proteins are suggestive of the interplay of these proteins and would determine the extent of production of HIV or its infectivity.

**HIV’s voyage to nuclear destinations**

The genetic material of HIV is a single-stranded RNA. Using the enzyme RT, the RNA is copied into DNA, which is then used to incorporate into the host genome. This provirus is usually integrated into the host DNA by the action of viral IN. The better understating of viral entry and genome incorporation is of great interest, as it can provide footprints for developments of drugs that can affect their entry and proliferation. For a successful infection of a target cell, HIV must transfer its genetic material into the cytoplasm of the cell. Entry of the viral genome inside the cell involves fusion of the viral envelope with the host cell membrane and before that the specific interaction of envelope glycoproteins with specific host cell surface receptors. These glycoproteins are encoded by HIV-1 envelope Env gene that translates a polyprotein gp160, which then is cleaved into surface glycoprotein (gp120) and a transmembrane glycoprotein (gp41). gp41 consists of two helical heptad repeats, HR1 and HR2 and a fusion peptide. Before the fusion of virus with the host membrane, periodic, hydrophobic regions of gp41, along with fusion peptide is packed in a very high energy configuration in the gp120-gp41 trimer. A recent study has revealed the details of Env organization and the interaction between gp41 and gp120 in trimeric state. They have proposed that the V3 (hypervariable-3) loop of gp120 gp41 glycoproteins present on the cell surface are covered by gp120, which then interact with CD4 and chemokine receptors (CCR5 or CXCR4) of the host membrane. This initial interaction between virus and host cell surface molecules causes a conformational change that clears the steric hindrances by gp120 and releases gp41 from a high energy configuration. Further, HR1 of gp41 interacts with hydrophobic grooves of HR2 regions and constitutes stable six-helix configuration making gp41 to interact with host cellular membrane for fusion and entry. A recent study has revealed the details of Env organization and the interaction between gp41 and gp120 in trimeric state. They have proposed that the V3 (hypervariable-3) loop of gp120

![Table 1: Human immunodeficiency virus genes, their products and functions](image)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Product</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gag (Gag)</td>
<td>p24</td>
<td>Capsid (CA) structural protein</td>
</tr>
<tr>
<td></td>
<td>p17</td>
<td>Matrix (MA) protein, myristoylated</td>
</tr>
<tr>
<td></td>
<td>P7</td>
<td>RNA-binding protein</td>
</tr>
<tr>
<td></td>
<td>p6</td>
<td>RNA-binding protein; helps in virus budding</td>
</tr>
<tr>
<td>Polymerase (Pol)</td>
<td>p66/51</td>
<td>RT, RNase H (inside core)</td>
</tr>
<tr>
<td></td>
<td>p10</td>
<td>Protease (PR): Posttranslational processing of viral proteins</td>
</tr>
<tr>
<td></td>
<td>p32</td>
<td>Integrase (IN): Viral cDNA integration</td>
</tr>
<tr>
<td>Envelope (Env)</td>
<td>gp120</td>
<td>Envelope surface (SU) protein</td>
</tr>
<tr>
<td></td>
<td>gp41</td>
<td>Envelope transmembrane (TM) protein</td>
</tr>
<tr>
<td>Tat</td>
<td>p4</td>
<td>Transactivator of transcription</td>
</tr>
<tr>
<td>Rev</td>
<td>p19</td>
<td>Regulates viral mRNA expression</td>
</tr>
<tr>
<td>Vif</td>
<td>p23</td>
<td>Increases virus infectivity</td>
</tr>
<tr>
<td>Vpr</td>
<td>p15</td>
<td>Helps in virus release</td>
</tr>
<tr>
<td>Vpu</td>
<td>p18</td>
<td>Helps in virus replication; transactivation</td>
</tr>
<tr>
<td>Nef</td>
<td>p27</td>
<td>Pleiotropic effects</td>
</tr>
</tbody>
</table>

![Figure 1: Genome organisation of HIV](image)
is substantially masked by packing into the trimer axis. V3 exposure is increased due to a conformational change in Env initiated after CD4-gp120 engagement. This is evident with the increased accessibility of the V3 loop in the Env trimer to antibody binding and enzymatic proteolysis subsequent to CD4 engagement.

Though CD 4 is the main receptor for the viral fusion, but for viral entry into the host cell, some additional receptors (coreceptors) are further required. In the in vitro conditions, it is seen that 12 chemokine receptors can function as HIV coreceptors, but in vivo only two coreceptors play a crucial role. The anticipation of their role is based on evidences that certain chemokines affect the replication of viral strains, the mechanism that later on explained the difference in tropism. One of these coreceptor discovered is ‘Fusin’, expressed on variety of T cell lines, which functions as a coreceptor for the entry of T-cell tropic syncytium-inducing (X4) viruses. Later on it was found to be a receptor of CXC chemokine (SDF-1) and this receptor-chemokine interaction specifically blocks infection by T-tropic HIV-1 strains and hence later on was named as CXCR-4. Another important coreceptor is CC chemokine receptor 5 (CCR5), a β-chemokine receptor, which was found as a coreceptor for macrophage-tropic non-syncytium-inducing (R5) viruses. Where R5 types are found in most of the cases responsible for sexually transmitted HIV infection, X4 type is a dominant type in later stages of the disease and are implicated in rapid progression and death due to AIDS.

For successful infection and further replications of viruses, only entry inside the cell is not sufficient, as it faces cellular antiviral defense mechanism before reaching to nucleus and its incorporation in the host genome. Escaping through these hurdles is really essential for making an infection successful. After fusion of HIV-1 particle, viral core is subjected to uncoating. Though exact mechanism of this phenomenon is not clear, but studies have suggested involvement of phosphorylation of viral matrix proteins by a mitogen-activated protein (MAP) kinase, additional actions of cyclophilin A and the viral proteins Nef and Vif. Role of viral Nef is implicated as it is associated with a universal proton pump, V-ATPase, which could induce local changes in pH and thus may promote uncoating. With the end of uncoating viral core protein that results into the subviral particle, reverse transcription complex (RTC), composed of RNA and associated proteins including RT, which is released from cell membrane (Fig. 2).

For integration of the viral genome into host cell, RTC has to travel across the cytoplasm, through which RTC utilizes active cytoskeleton and later on microtubular network at subsequent stages. In this transportation, viral Nef protein plays a crucial role, probably by modulating cytoskeleton organization. In the later stages, Gag protein also contributes to intracellular trafficking by interacting with microtubules. Interaction of Gag protein with cytoplasmic light chain 8 (LC 8) of dynein protein of microtubule is evident of its role in intracellular trafficking towards nucleus. While invasion of viral components, they are resisted by cellular defense system having a recently identified protein termed CEM15/APOBEC3G. Vif protein overcomes their effect and stabilizes the reverse transcription complex in the cell. Another host protein that functions as cellular restriction factor is TRIM5α. Wu et al., has demonstrated in primates that TRIM5α resists virus invasion by blocking the early replication of HIV by preventing the accumulation of reverse transcription products by targeting them to proteasome-mediated degradation. At cellular level, interaction of tubulin with dynein supports the possibility that RTC complex utilizes cellular cytoskeleton at early stages, while at later stages it is transferred to microtubular network for further movements towards nucleus.

While transportation across the cytoplasm, reverse transcriptions is completed converting RTC into pre-integration complex (PIC), where majority of proteins are lost, but still retain viral cDNA, PRs, RT, IN and Vpr. The entry of PIC in nucleus is quite interesting as it has approximately 28 nm radius, which is approximately twice the radius of nuclear pore. Transportation of PIC in the nucleus is suggestive of its ample compaction and some fine molecular mechanism. It is further recognized that active transport of these complexes takes place with interaction of nuclear localization signal (NLS) present on viral proteins and shuttling receptors importins. Matrix protein of PIC consists of two NLSs, one at N-terminal region and other...
at C-terminal region. Another protein constituent of PIC is the IN that may be implicated in HIV-1 nuclear import as it consists of NLS. The HIV Vpr protein consists of at least three nuclear targeting signals. Vpr may bypass the importin system by mediating the direct docking of the PIC with the components of nuclear pore complex. Further, Vpr is expected to play a role here, as it is able to disrupt nuclear envelope. While Vpr is not required for infection of non-dividing resting T cells, it enhances viral infection in non-dividing macrophages.

Though it was initially expected that viral integration in the human genome is random, but now it is learnt that it occurs preferentially in genes highly transcribed by the RNA pol II and are flanked by matrix scaffold attachment region (MAR). After reaching to the nucleus, the viral double-stranded DNA may be inserted into the host genome, thus, establishing a functional provirus. Integration of double-stranded viral DNA into the host chromosome is catalysed by IN, which binds the ends of the viral DNA. IN, in fact, removes terminal nucleotides from the viral DNA and then leads to the actual insertion into the host DNA. The host proteins, HMG1(Y) and barrier to autointegration (BAF), are also required for proper integration (Fig. 2). Apart from these proteins, a recently identified host factor, named lens epithelium-derived growth factor (LEDGF/p75), has also been implicated in the HIV-1 integration. LEDGF/p75 interacts with both chromosomal DNA and HIV IN and thus leading their integration. IN also catalyses the subsequent joining reaction that establishes the HIV provirus within the chromosome. The cross-linked tetramers of HIV-1 IN are implicated for concerted integration, during which HIV IN constitutes highly stable complexes with viral DNA. These complexes progress from initial synthesis of a pair of viral DNA ends to the integration intermediate in which viral and host DNA are covalently joined, which leads to the formation of provirus.

HIV and Immune Response

Another front of HIV research is the immune response and how HIV circumvents it to have a successful and chronic infection. The studies not only provided the basic understanding of ‘how HIV invades’, but also provided clues for strategies and vaccines to understanding of ‘how HIV invades’, but also provided clues for strategies and vaccines to understanding of ‘how HIV invades’. The studies not only provided the basic understanding of ‘how HIV invades’, but also provided clues for strategies and vaccines to understanding of ‘how HIV invades’. The studies not only provided the basic understanding of ‘how HIV invades’, but also provided clues for strategies and vaccines to understanding of ‘how HIV invades’. The studies not only provided the basic understanding of ‘how HIV invades’, but also provided clues for strategies and vaccines to understanding of ‘how HIV invades’. The studies not only provided the basic understanding of ‘how HIV invades’, but also provided clues for strategies and vaccines to understanding of ‘how HIV invades’. The studies not only provided the basic understanding of ‘how HIV invades’, but also provided clues for strategies and vaccines to understanding of ‘how HIV invades’. The studies not only provided the basic understanding of ‘how HIV invades’, but also provided clues for strategies and vaccines to understanding of ‘how HIV invades’.
chemotaxis of T cells by releasing different chemokines i.e., macrophage inflammatory protein-1 (MIP-1) α and β.73 CD4+ T cells now coming closer to infected macrophages encounter more rapid infection and subsequent virus-mediated depletion. On the other hand, uninfected T cells may undergo CD4 cross-linking with macrophages, which may induce upregulated expression Fas on CD4+ T cells and FasL on macrophages. Further, the interaction of Fas and FasL may lead to activation apoptotic pathway in CD4+ T cells.74 Similarly, macrophages have also been implicated in apoptosis of CD8+ T cells by upregulating tumour necrosis factor-α (TNF-α) and TNF receptor (TNFR) on macrophages and CD8+ T cells, respectively;77,78 and this may account for increased CD8+ T cell turnover during HIV infection.

It is seen that survival of productively infected CD4+ T cells is prolonged, conditioned with intercellular interaction between macrophage and T cells infected with Nef positive HIV.79 Probably, interaction of Nef protein may block proapoptotic pathways, when infected T cells are in contact to macrophages. Thus, potentially infected macrophages create a double jeopardy during HIV infection, on one hand they induce apoptosis of uninfected immune cells and lead to depletion of functional effector cells and simultaneously they inhibit cell death of already infected T cells, to increase the pool of live viral reservoir, which can disseminate viral particles for a longer time. Recently, it is demonstrated that the mannose receptor (MR) of macrophages may function as a receptor for HIV-1 by interacting with carbohydrates of gp120. Entry of HIV-1 into the macrophages via MR resulted in the lack of observed viral replication.80 MR mediated uptake of HIV-1 finally lead their delivery into endosome, where HIV-1 is degraded and viral antigens are presented by CD1b to initiate cell-mediated immunity.80 However, the infectious pathway of HIV-1 uptake is mediated of HIV-1 gp120 proteins with host cellular protein receptors along with coreceptors. This information provides opportunity to suitably modify gp120 that may circumvent infectious pathway uptake of HIV-1.81

Role of NK cells in HIV infection

Another potent immune component, which can be anticipated to play a crucial role in HIV infection, is NK cells. As most of them do not possess CD receptor for HIV entry, hence, they may remain uninfected by HIV82 and may play an important role in control of HIV infection.

NK cells have potential to kill the HIV by direct lysis or by antibody-dependent cell-mediated cytotoxicity (ADCC) or by activating immune system. But practically, it is seen that in spite of all these properties of NK cell, HIV can infect and spread in human beings. To conquer this battle with NK cells, HIV can also modulate immune components to inhibit or competitively decrease functional NK cell-mediated lysis. Most of these strategies involve killer inhibitory receptors of NK cells, which can regulate their cytotoxicity. HIV infection on one hand decreases expression of HLA class I classical antigens, i.e., HLA-A and HLA-B and thus making them to susceptible to NK cell lysis, but simultaneously upregulate HLA-E, which can inhibit NK cell lysis by interacting to their inhibitory receptors.83,84

NK cell can secrete chemokines CCL3, CCL4 and CCL5, which are ligands for CCR5, the main receptor for HIV entry. These ligands can competitively bind to CCR5 and thus can prevent the entry of R5 viruses to target cells.85,86 It is further seen that in certain individuals who have profound exposure to HIV but remain uninfected have higher NK cell cytolytic and non-cytolytic (chemokine secretion) activity.87

Initially, it was seen that most of the NK cells do not possess CD4 receptor but they express CCR5 and CXCR4 coreceptors. But later on in 2002, Valentin et al, revealed a subset of NK cells that consists of CD4 as well as coreceptors, constituting a pool of NK cells that can be successfully infected and may function as reservoir of HIV particles.88 It is also evident that in the presence of HIV infection, there is an alteration in the pattern of NK cell surface receptors. Infection is progressively witnessed by the decreased CD16αi and CD56αβi cytolytic NK cells along with the increase of low cytokine secretor CD18αi and CD56- NK cell subsets. Low secretion of cytokines and defective lysis by the highly abundant NK cell subset may contribute to successful infection and proliferation of HIV.89,90 A recent study in animal model has shown that the NK cell subsets, i.e., CD3 and CD8+ NK cells, may regulate HIV-1 infection by increased production of IFN-γ, which may also be enhanced with IL-15 production.91 It is recently demonstrated that HIV envelopes may upregulate genes involved in apoptosis of NK cells and downregulate their genes involved in cell proliferation and survival. The study has confirmed reduced cytotoxicity, IFN-γ production, proliferative responses and increased apoptosis upon exposure to HIV envelopes.92

Along with aforementioned immunological immune escape mechanism that often involves activation of immune response, HIV also consists of various strategies that can make the infected cell go undetected by immune system and hence can avoid immunological confrontations. The detection of any cell depends on their cell surface markers and these strategies adopt to alter the organization and expression of these markers.

Viral-infected cells are recognized and induce the immune response by presenting viral-specific peptides on MHC class I molecules, this leads to activation of specific CTL and leads to killing of target cells. HIV-infected cells can redirect the HLA antigens to intracellular pathways and thus decrease cell surface molecules and recognition by specific CTLs. This recycling is carried out with the involvement of viral Nef protein,69 which reaches to cell membrane through amino-terminal myristoylation. It is further seen that Nef protein can interact with phosphofurin acidic cluster sorting protein 1 (PACS 1) and then can
activate phosphatidylinositol-3 kinase (PI3K), guanine exchange factor ARNO and finally ADP ribosylation factor-6 (ARF-6). This pathway leads to internalization of MHC class I molecules to ‘ARF compartments’ that finally reach to trans-Golgi Network (TGN). Another strategy of hijacking cellular machinery to go undetected by immune system and to maintain full pathogenicity of newly packaged virion is internalization of CD4 from cell surface. Here, Nef protein catalyses internalization of CD4 receptors in clathrin-coated vesicles, which then interacts with another coat constituent COP1 coatomers through Nef and is targeted to lysosomal degradation.94

**Role of dendritic cells in HIV infection**

Another component of immune system that plays a very important role in HIV infection is DCs, which are bone marrow-derived cells that also function as antigen-presenting cells (APCs). These cells are located in various tissues and include the Langerhans cells in the skin and mucous membranes, dermal DCs, interdigitating DCs in the thymic medulla and DCs in the blood and lymph. The main functions of DCs are to capture and process antigen in the periphery and, then to migrate to T cell-rich areas of lymphoid organs to activate T cells. DCs are proposed to be among the first cells that encounter HIV during sexual transmission. They carry the HIV to CD4+ T cells in lymphoid tissues in vivo and later constitute the main source of HIV replication and dissemination.

Unlike other immune cells, such as T cell, B cells and natural killer cells, DCs are a relatively rare population in the blood and tissues. However, the DC population is very complex and has many subsets.95 DC populations can be divided into several subsets based on their anatomical distribution, immunological function and expression of cell-surface markers. DCs develop from either myeloid or lymphoid precursor. Myeloid precursors give rise to the Langerhans cells, dermal DCs and interstitial DCs, which are all found in peripheral tissues, whereas lymphoid precursors generate DCs of primary and secondary lymphoid tissues.96

In the infection by HIV, the most important role is played by receptors of DCs. However, all the subsets of DCs have lower expression of the HIV-specific receptor CD4 and the coreceptors as CC-chemokine receptor 5 (CCR5) and CXC-chemokine receptor 4 (CXCR4).97,98 This decreased expression of HIV receptor and coreceptor has been implicated in less productive HIV replication in DCs as compared with CD4+ T cells.99 Apart from these HIV receptor and coreceptor, DCs also possess C-type lectin receptors (CLRs). One such CLR is DC-SIGN, which is able to interact with HIV in vivo and are thus suggested to play a key role in HIV dissemination by DC in vivo.100 DC-SIGN is a calcium-dependent C-type lectin that interacts with mannose oligosaccharide groups on the HIV Env glycoprotein.100 However, the recent investigations have revealed some contradicting results about their role in HIV infection. Hu et al., investigated the role of these DC receptors in the capture of HIV.101 In their experiment, blocking either mannose or DC-SIGN with antibodies has not affected the levels of infection suggesting that the interactions of HIV with DC-SIGN did not affect significantly to direct infection of local T cells and macrophages. Another group of DCs implicated in HIV pathogenesis is follicular DCs (FDCs). FDCs though are not typical DC subsets, but are involved in capture and retention of HIV.102 Unlike typical subsets of DCs, FDCs neither originate from the bone marrow nor involved in MHC-mediated antigen presentation.95 These cells are normally present in the B-cell follicles and germinal centres of peripheral lymphoid tissues where they can retain large quantities of HIV functioning as a reservoir of infectious virus surrounded by highly susceptible CD4+ T cells.103 Although FDCs are not productively infected, they can facilitate HIV infection of T cells and thus contribute to HIV pathogenesis.104

Further, HIV infection to T cells via DC could be through infectious synapses or DC exosome. Tsunetsugu-Yokota et al., have shown that the efficient virus transmission from DCs to CD4+ T cells depends on cell-to-cell interaction through adhesion molecules.105 This infectious synapse is similar to the immunological synapse between APCs and corresponding T cell. It has been demonstrated that during HIV infection, DCs through C-type lectins, e.g. DC-specific intercellular adhesion molecule, 3-grabbing non-integrin (DC-SIGN) capture and transmit the virus at the DC-T cell interface, the infectious synapse.100 However, recent studies have suggested that virus transmission to CD4+ T cells can occur in the absence of the classical immunological synapse. Wiley et al., have provided another possibility of HIV trans-infection apart from infectious synapse i.e., DC-derived exosome-mediated HIV infection by DCs.106 They have demonstrated that HIV particles captured by DCs can be transmitted to T cells by exocytosis without de novo infection. HIV-1 particles captured by DCs are rapidly endocytosed into endosomal multivesicular bodies (MVBs), which are endocytic bodies enriched with tetraspanin proteins. A fraction of the endocytosed HIV particles are constitutively secreted from the cell in endocytic vesicles, exosomes that could fuse with target cells and thus leads to the productive infections of CD4+ T cells.106

**CTL Immune Escape by HIV**

CD8+ cytotoxic T lymphocytes (CTLs) are anticipated to be a major player in HIV regulation, as they do not possess CD4 molecules, the main receptor for viral entry and infection. Practically, it is seen that they can use multiple effector mechanisms to regulate viral replication.107,108 Along with lytic mechanism, they can also release the chemokines, thus, leading to CC chemokine-mediated blockade of viral entry and thus infection.109,110 Further, existence of HIV-specific CTLs and their successful involvement in protection against disease transmission confirms their importance in disease regulation.111,112
HIV infection is counteracted by CTL-mediated immune response. However, this cellular immune response in most cases could not control HIV-1 infection, but their participation is evident in regulating viral load during infection. During acute infection, depletion of higher viral load occurs with the appearance of HIV-specific CTLs  making an inverse relationship between them. The initial CTL response may be directed against a few epitopes, which subsequently broaden during prolonged antigen stimulation.

The role of CTL could also be anticipated during chronic HIV-1 infection, where HIV-1-specific T cells remain at high frequency. The high concentration of these T cells may be due to continued antigenic stimulation. This observation is supported by the reduction of viraemia by HAART causing a steady decline in CD8+ T cells. However, in chronic infection without treatment, the high number of HIV-1-specific CD8+ T cells is seen. Although CTL response occurs in early as well as in later stages of infection, but epitopes targeted during acute infection often differs from those recognized during chronic infection.

As these CTLs can pose a strong regulatory force against HIV, it provides a selection advantage to resistant HIV that escapes CTL response. High turnover of CTL during viral infection and mutational rate, i.e., 1:10^5 bases, can produce various mutants but only those mutants could be selected, which do not cost to viral fitness. These mutational escapes lead to failure of vaccine development as well as of immune regulation, as escape variants do not generate specific CTLs, but keep on eliciting the proliferation of CTLs specific for wild type. These escape mutations can result into variation including, alteration of epitope on HLA for TCR, lack of antigen processing, absence of improper interaction with HLA and finally lack of recognition by TCR. All the aforementioned strategies are aimed to let the infected cell go undetected by immune cells. It has been seen while infection that under the selective pressure posed by CTLs, various escape mutations are generated and these variants may constitute majority of total viral pool. It is shown that ratio of non-synonymous substitutions to synonymous substitution was higher in CTL epitope. This further confirms the role of CTL selection pressure for occurrence and then for maintenance of these mutations. Later on, evidences of escape mutations in HLA-B8 restricted epitope in Nef, HLA-B44-restricted epitope in Env and HLA-B27-restricted Gag epitope KK10 have supported the CTL-mediated selection of these mutations.

For the recognition of infected cells, these viral epitopes have to be presented on MHC antigens to CTLs. It is further seen that homozygosity of HLA class I alleles have more rapid progression to disease than heterozygotes. This is suggestive of the important role played by variability of CTL-specific epitope in the regulation of HIV. HLA heterozygosity that implies more HLA diversity and hence possibility of more HIV-specific epitopes to be presented to CTLs and to escape from CTL recognition, more mutations will have to occur to avoid immune recognition as compared to HLA homozygosity.

It is also evident that the selection of escape mutation is further conditioned with lack of fitness cost to the virus. It is also supported by evidences that mutations those cost to viral fitness, revert back in the absence of selection pressure in new host. Individuals with HLA-B57, which is associated with successful regulation of HIV, in acute infection, have CTL specificity against p24 Gag epitope TW10. In most of HLA-B57 patients, escape occurs through T242N mutation at position 3 of epitope. But later on, transmission of this escape mutant to HLA-B57 negative individuals resulted in the reversal of this mutation to wild type. This reversal is suggestive of the important role of this epitope in HIV regulation, as it is further seen that lack of this mutation is required for suppression of viraemia. Furthermore, this mutation may abrogate the replication of viral particles. Though the escape mutations may only be selected when they do not cost to viral fitness, it is seen that they may do so if compensatory mutations are there to compensate the fitness cost to the escape mutations. HLA-B27-specific KK10 epitope in the Gag consists of an escape mutation R264K, substituting arginine with lysine at position 2, which is followed by a compensatory mutation L268M in the same epitope at position 6. It is further learnt that R264K mutation may impede structural organization compensated by compensatory mutation L268M.

These escape mutations often hinder the success of vaccine design and fail the immune response against them. In addition, transmission of these variants and then selection may shape the evolution of HIV, which will be marked with the signature of mutations persisted even after transmission to a new host.

Conclusions

Since the discovery of HIV and its implication in AIDS, attempts have been carried out to provide therapy as well as preventive medication. Better understanding of immune response and how retrovirus evades it to carry out successful infection is of immense interest as it can provide some clues for vaccine development. More information about the mechanisms adopted by HIV to render immune response ineffective and how genetic predisposition could affect susceptibility will assist in the development of HIV therapies.

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