Immunopathogenesis of HIV infection

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Progression of HIV infection is largely dependent on the interaction between the viral factors and host factors. HIV primarily infects the CD4 lymphocytes in the body. It brings about the destruction of CD4 cells through multiple mechanisms including apoptosis. The loss of CD4 cell population ultimately leads to the inability of infected person to deal with opportunistic organisms. Host genetic factors such as HLA polymorphism and HIV co-receptor polymorphism may influence either susceptibility to infection or disease progression. Innate immune mechanisms may play a role in disease progression. However, adaptive immune response is the most critical component of immune system for control of HIV infection. HIV-specific CD4 helper response and HIV-specific CTL responses have clearly emerged as the most important host factors that may decide the rate of disease progression. However, the role of neutralizing antibodies still remains to be understood in context with the disease progression. One of the gray areas is the role of mucosal immune response in HIV infection. However, it is clear that it is not a single component but orchestrated action of different immune mechanisms will decide the outcome of HIV infection. The studies in persons exposed to HIV infection but who are uninfected and the long term non-progressors will be critical for understanding the immunopathogenesis of HIV infection.

Key words Cytotoxic T lymphocytes - genetics factors - innate immunity - neutralizing antibodies - plasma viral set point - syncytium

The progression of the HIV infection may be measured through various outcome measures such as the plasma virus load set point, the rate of decline in CD4 count, increase in plasma virus load and the HIV associated opportunistic infections. Though a number of factors may influence the course of disease progression, a balance between the host factors and the virus factors are major determinants of the outcome of HIV infection. At one end of the spectrum are the persons who are able to mount immune response that is able to control the virus multiplication successfully and such individuals may remain clinically normal with minimal loss of CD4 cells and very low to undetectable plasma virus load. These are “long term non-progressors (LTNPs)”. At the other end of spectrum are individuals who are unable to control virus multiplication successfully and show rapid decline in CD4 cell counts and may develop AIDS within 2-3 yr of acquiring HIV infection. This review highlights the mechanisms responsible for CD4 cell depletion, and the role of genetic factors, innate immune response, and the adaptive immune response in HIV disease progression.

Depletion of CD4 positive cells: The principal impact of HIV infection on the immune system is destruction of the CD4+ T lymphocytes. The half-life of most HIV infected T cells in vivo is
Apoptosis: In HIV infection T cell apoptosis is one of the mechanisms that contribute to T cell destruction. Elevated levels of apoptosis are found to correlate directly with disease progression and inversely with T helper cell counts. HIV proteins Nef, Env and Tat may upregulate CD95 and FasL levels; thus enhancing the susceptibility to Fas-mediated killing. HIV proteins such as gp120, Tat, Nef and Vpu have been shown to induce cell death in uninfected cells. Cross linking of CD4 molecules is probable mechanism of gp120 induced apoptosis in HIV uninfected T cells. HIV Nef protein in the extracellular matrix can induce cell death in uninfected T cells. HIV Tat protein may induce apoptosis through both Fas-mediated and Fas independent mechanisms. Apoptosis, a mechanism normally used by the body to eliminate redundant cell populations and defective cells, is utilized thus by HIV to destroy both infected and uninfected cells.

Other mechanisms of CD4 T cell destruction: CXCR4 tropic HIV isolates generally found in the late stage of HIV infection, preferentially infect T cells and induce membrane fusion between adjacent cells to form a giant multinucleate cell called syncytium. Such syncytia are short lived and are more often seen in advanced stage of infection. Both HIV infected and uninfected cells participate in syncytium formation thus accelerating the cell destruction during advanced disease.

Continuous budding of the viruses from infected cells may cause membrane disruption and increased permeability resulting in ultimate death of the cell. Specific HIV proteins such as Vpu can induce membrane permeability leading to cell death. The build up of unintegrated linear viral DNA in the cell also increases cellular cytotoxicity. HIV-specific cytolytic T lymphocytes (CTL) recognize HIV infected cells through T cell receptor in HLA restricted manner.

Fig. 1. The rate decline in CD4 cell, plasma virus load set point, rate of increase in plasma virus load and immune response to HIV (CTL response and neutralizing antibody response) influence the progression of HIV disease. CTL, Cytotoxic T lymphocyte.
The cell-to-cell contact leads to the activation of mechanisms for the lysis of target cell i.e., HIV infected cell. However, this mechanism may not contribute significantly to the CD4 cell decline as the infected cell in the peripheral blood account for relatively small percentage of lymphocytes. HIV not only infects and destroys CD4 cells, but also affects other cells of immune system. It adopts multiple strategies to destroy the immune system of infected individuals.

The host factors such as genetic factors, innate immunity and most importantly HIV-specific immune responses impact greatly the progression of HIV infection. How effectively the host factors can counter these strategies decides the outcome of infection.

**Influence of genetic factors on HIV disease progression**

Three particular genetic factors that have been studied in the context of HIV disease progression include alleles at major histocompatibility complex (MHC) loci, mutations and polymorphism in HIV co-receptor genes and polymorphic immunoregulatory genes present in the MHC region and are in strong linkage equilibrium with them.

*Human leucocyte antigen (HLA) and HIV infection:* A number of studies have revealed association between certain HLA alleles with rapid or slow disease progression. The alleles B57, B14, C8 and B27 have been shown to be associated with long term non-progressor status. Homozygosity for HLA BW-4 was found to be associated with long term non-progression to AIDS. A study in the cohort of long term non-progressors revealed a strong association between LTNP status and expression of HLA B*5701 class I allele with 85 per cent of LTNP expressing this allele against only 9.5 per cent of non LTNP expressing this allele. On the contrary, HLA B3501 and Cw4 have been consistently associated with accelerated disease progression. Heterozygosity at all HLA loci is more likely to be associated with better control of viraemia and slower disease progression.

The only study on HIV and HLA alleles from India carried out among 38 HIV infected persons and 120 uninfected healthy controls revealed strong association between HLA alleles B*1801(OR 5.57), B*3520(OR 4.588) and C1507(OR 13.37) and HIV infection.

**Chemokine receptors:** Receptors for chemokines, chemoattractant substances secreted at the sites of infection or injury, were found to act as elusive second receptor for HIV. Haplotype structure of chemokine receptor has subsequently been studied in context with HIV infection and disease progression. One of the strongest association of genetic polymorphism and HIV susceptibility and disease progression has been reported in case of a mutation of 32 base pair deletion in the CCR5 receptor gene (CCR5Δ32). Individuals homozygous for this mutation have decreased susceptibility to HIV infection. Persons heterozygous for CCR5Δ32 are not less susceptible to HIV infection, but are more likely to become long term non-progressors/survivors. CCR5 gene promoter region has nine stable haplogroups. Certain haplogroups may influence the HIV disease progression. CCR5 haplogroup E speeds the decline in CD4 counts and is shown to be associated with accelerated disease progression in Thai population. HIV disease progression also has been reported to be influenced by mutation in co-receptor CCR2. Persons exhibiting this mutation may progress to AIDS more slowly. A mutation in stromal derived factor 1 (SDF-1), ligand for CXCR4, may delay progression to AIDS and also increase survival after

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<tr>
<th><strong>Table I. Soluble factors with HIV suppressive activity</strong></th>
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<td>Amino-terminal peptide fragment of Urokinase type plasminogen activator</td>
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<tr>
<td>Eosinophil derived neurotoxin</td>
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<td>Interferon-α</td>
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<td>Macrophage derived chemokine (MDC)</td>
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<td>Urokinase-type plasminogen activator receptor</td>
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<td>STAT- signal transducer and activator of transcription</td>
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<td>Secretory leucocyte protease inhibitor (SLPI)</td>
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<td>Salivary soluble proteins, Mucin, Histatins</td>
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**Source:** References 20, 21
diagnosis of AIDS. The mutated gene SDF-1 $3'\alpha$, involves mutation in an untranslated region of the gene and may upregulate SDF-1, thus competitively inhibiting T-tropic strains of HIV from binding.

Other genetic mechanisms: Central MHC region contains many immunoregulatory genes that are in strong linkage disequilibrium with MHC alleles. These include genes for cytokines, chaperon proteins, etc. These genes may influence the capacity to recognize and destroy the pathogens. The adaptive immune response may be influenced by polymorphism in killer inhibitory receptor (KIR) gene cluster.

The evidence in literature strongly suggests that host genetic factors may impact the disease progression. Considering the fact that various alleles may be present in different frequencies in different populations, it is impossible to pinpoint at a single genetic factor as an important influence. These genetic factors may not only influence disease progression but may be of importance in developing patient specific anti-retroviral strategies and development of HIV vaccines.

Innate immunity and HIV infection: Early processes that try to contain the pathogen during the interim period when adaptive immune response is not yet ready are innate immune responses. The innate responses may play a major role in initial containment of infection and hence may be crucial in acute primary HIV infection.

Anti-viral non-cytotoxic responses: Walker et al in 1986 described for the first time that the CD8+ T cells are able to block active HIV replication through non-cytolytic virus suppressive mechanisms. This activity was attributed to soluble factors that are capable of suppressing primary HIV isolates that are both CCR5 tropic and CXCR4 tropic. These soluble factors have been collectively coined as ‘CD8 antiviral factor’ or CAF. In 1995, Cocchi et al reported that ligands for chemokine receptors RANTES, macrophage inflammatory proteins $\alpha$ and $\beta$ (MIP-$\alpha$ and MIP-$\beta$) are involved in suppressor activity when released by activated CD8 cells. These chemokines can account for the suppression of R5 strains of HIV that use CCR5 as a co-receptor. However, the ability of CAF to suppress X4 strains that use CXCR4 for virus entry cannot be accounted for by chemokines. It is now clear that HIV suppression by non-cytolytic mechanisms use multiple mechanisms of suppression such as CD4 cross-linking, inhibition of pro-viral integration, down

Fig. 2. Potential mechanisms of CD4+ T cell destruction. HIV-1 infected and bystander uninfected CD4+ T cells are destroyed by multiple mechanisms; 1. action of cytotoxic T lymphocytes; 2. By mechanism of apoptosis; 3. Killing by natural killer (NK) cells; and 4. due to cytopathic effect such as syncytia formation.
regulation of receptors and inhibition of viral entry. The suppressor activity may vary based on various factors such as the cell types producing the factor, the stimulus for activation of the cells and time kinetics in culture system.

The role of soluble suppressor factors has not yet been fully understood in the pathogenesis of HIV infection. However, it may be most crucial in the acute primary infection. This may also play important role in the control of viraemia in structured treatment interruptions.

**Innate cellular responses:** Two cell populations, plasmacytoid dendritic cells (PDC) and natural killer (NK) cells play predominant role in innate cellular immune response. PDCs were first detected in lymph nodes in 1975 and described as T-like plasmacytoid cells, which initially had no known function\(^2\). Only recently has this cell type been recognized as the major producer of type 1 Interferons. Loss of these cells, reflected by decreased interferon-alpha (INF-\(\alpha\)) production, is associated with higher HIV RNA levels and progression to AIDS\(^2\). Normal or high PDCs were observed in HIV infected patients, who remain healthy without receiving any therapy, despite their low CD4+ cell count. PDCs not only participate in the innate immune response through Interferon production, they may become mature dendritic cells and participate in the adaptive immune response. The presence of PDCs in advanced disease may indicate that PDCs may operate innate immune mechanisms even when adaptive immune response is lost.

Mature dendritic cells are potent antigen presenters and play role in inducing adaptive immune response. DC also acts as a conduit for HIV transmission. The DC-specific lectin DC-SIGN (Dendritic Cell-Specific ICAM-3 non-integrin), which has been shown to bind HIV virions, is expressed by DCs located in mucosal sub-epithelia and may 'shuttle' viruses to susceptible T cells.

NK cells form a critical component of host innate immune response against a variety of viruses, fungi, parasites, and bacteria. They were originally identified on a functional basis because of their ability to lyse certain tumour cells without previous stimulation. NK cells are large granular lymphoid cells that lack surface markers characteristic of T- or B-cells.

NK cells have the natural capacity to kill virus infected cells in the absence of prior sensitization and without MHC restriction. NK cells can kill target cells either by direct contact in the absence of antibody or by antibody-dependent cellular cytotoxicity (ADCC). Although the importance of NK cells in some viral infections is well documented, their role in the protection and control of HIV-1 infection is currently unclear. NK cells display a decreased lytic activity in HIV-1 infected patients as compared to uninfected controls. This effect appears to be associated with the very low expression of natural cytotoxicity receptors (NCR) on HLA-DR+ NK cells and possibly, at least in part, with a differential content in perforin molecules\(^2\). The role of NK cells in defense against HIV infection was highlighted by studies in European intravenous drug users (IDUs)\(^2\). The finding of an enhanced NK-cell function (production of the cytokines IFN-\(\gamma\) and TNF-\(\alpha\) and the \(\beta\) chemokines CCL3, CCL4, and CCL5) in exposed but uninfected IDUs, especially in comparison to those who became HIV-1 infected strongly supports the protective role of NK cells\(^2\). NK cells may also cause suppression of viraemia through CC-chemokine production. Suppression of HIV replication by NK cell culture supernatant was predominantly mediated by CC-chemokine secretion and was considerably greater in patients without viraemia than in patients with viraemia\(^2\). Although considerable information is available on the role of

| Table II. HIV-1 neutralizing monoclonal antibodies and epitopes recognized by them |
|--------------------------------|----------------------------------|
| Human monoclonal antibodies showing broad HIV-1 neutralizing activity | Recognized Epitope |
| IgG1b12 | CD4 binding domain of gp120 |
| 2F5 | Transmembrane-proximal region of gp41 |
| 17b, 48D | CD4-induced epitope of gp120 |
| 2G12 | Mannose residues of gp120 |
PDCs and NK cells on the HIV infection, the exact role of these cellular components of innate immunity needs to be studied further.

Adaptive immune response

Almost all HIV infected individuals mount HIV-specific immune response. However, only few can control the viraemia successfully for prolonged period. The adaptive immune response plays major role in the control of viraemia. Adaptive immune response uses multiple effector mechanisms to control the virus multiplication. HIV-specific neutralizing antibodies, CTL responses and T helper response are the major players in HIV-specific adaptive immune response (Fig. 3).

Neutralizing antibodies: The role of neutralizing antibody response in HIV-1 infection is one of the most debated issues in HIV immunopathogenesis. The role of neutralizing antibodies in the early control of infection was studied in simian immunodeficiency virus (SIV)/macaque experimental model by artificially depleting the B cells from animals before their exposure to SIV. Although both studies emphasized role of neutralizing antibodies during chronic infection, one concluded that neutralizing antibodies play a limited role during acute infection whereas the other suggested that role of neutralizing antibodies during this period is under-appreciated.

Study on recently HIV-1 infected individuals show that initial HIV-1-specific immune response is characterized by appearance of non-neutralizing antibodies detected by ELISA 2 to 4 wk after infection. These antibodies serve as the basis for the diagnosis for HIV infection but may not have any
clinical relevance. Neutralizing antibodies may be detected 8 wk or more after infection\textsuperscript{32,33}. However, these antibodies appear after initial containment of burst of HIV replication in newly infected individual\textsuperscript{34}, making their role in acute phase of infection debatable. The neutralizing antibodies seem to place strong immune pressure on the virus as evidenced by appearance of escape variants along with an explosion of viral diversity\textsuperscript{35}. Several studies suggest that neutralizing antibodies are generated sequentially, at first against the autologous infecting HIV-1 variant and then against variants that appear subsequently as a result of immune pressure; thus resulting in broadening of neutralizing antibody response\textsuperscript{36}. Appearance of neutralizing antibody response results in complete replacement of neutralization sensitive virus by successive population of resistant virus. Escape virus may contain mutation in Env protein that alters conformation of gp120 protein, which diminish antibody binding\textsuperscript{37} or may modify glycosylation pattern of envelope protein\textsuperscript{32}.

Studies of neutralizing antibody response in individuals with established infection shows that LTNPs have shown broad and high titered neutralizing activity compared to individuals that show rapid disease progression\textsuperscript{34,38,39}. Neutralization of contemporaneous autologous virus is more likely to be seen in LTNPs compared to short-term non progressors and progressors\textsuperscript{34}. Another evidence of protective role of neutralizing antibodies comes from studies which indicate that antibodies transferred across placenta might protect against transmission of HIV-1 from infected pregnant women to their infants\textsuperscript{40,41}. Some investigators have reported that presence of neutralizing antibodies to heterologous HIV-1 isolate is not predictive of rate of HIV-1 disease progression\textsuperscript{42}. It is important to mention that the patients showing signs of immunological escape retain their ability to make neutralizing antibodies, although these antibodies are not directed against their predominant autologous strain.

HIV-1 neutralizing monoclonal antibodies have been generated from lymphocytes of infected individuals and their specificities have been determined\textsuperscript{43} (Table II).

Though protective role of neutralizing antibodies in an infected individual is debatable, many passive immunization experiments in various experimental models have established that broadly neutralizing monoclonal antibodies can block infection and can provide sterilizing immunity\textsuperscript{14-34,37}. However, these studies show that high titer neutralizing antibodies are required to block infection and such high titres may not be usually seen in natural infection or as a response to HIV vaccine.

First phase III HIV-1 vaccine trial conducted in USA and Thailand by Vaxgen Inc. measured efficacy of a vaccine that primarily induced neutralizing antibody response. The results were disappointing, once again emphasizing the fact that the neutralizing antibodies may not play important role in protection from HIV infection. This may be because vaccine induced antibodies were capable of neutralizing laboratory strains of HIV-1\textsuperscript{48} but not the primary isolates. Neutralization resistance of primary isolates of HIV-1 might be due to dense glycan shield that masks the neutralizing epitopes of envelope protein of HIV-1 and due to extremely high variability of envelope protein.

Variability of HIV-1 envelope protein, poor immunogenicity and steric restriction for antibodies to access neutralizing epitopes are major concerns for development of an immunogen that induces broadly HIV-1 neutralizing antibodies. Several new approaches are being explored for development of such immunogen that could bring us closer to the goal of successful AIDS vaccine\textsuperscript{49}.

The limitation in studying HIV-specific neutralizing antibody response is the nature of the assays available for studying neutralizing antibodies, use of different viruses and selection of neutralization threshold. Several different assays have been described for detection of antibody mediated HIV-1 neutralization. These assays use peripheral blood mononuclear cells (PBMCs) or cell line like MT2, MT4, U87 or indicator cell lines like HeLa-MAGI (Multinuclear activation of galactosidase indicator) or U373-MAGI, GHOST (human osteosarcoma cells that produce green fluorescence protein). In PBMC based assay there
is certain level of variability from donor to donor. The cell line need to be selected depending upon co-receptor usage of test virus.

Different investigators use different levels of virus suppression (such as 50, 80 or 90%) as a threshold of neutralization. In neutralization assay some investigators use natural infectious viruses, some employ clonal viruses. The results from various studies cannot be compared meaningfully as the results may depend on the viruses used in the assay, culture conditions, different threshold values for significant neutralization, the method used, etc. Use of recombinant viruses with or without embedded reporter gene and Env-pseudotyped viruses has also been reported. A standardized panel of viruses may help greatly in obtaining widely comparable results.

**Helper T cell response:** HIV infects CD4+ T lymphocytes leading to the death of not only infected CD4 cells but uninfected bystander cells also. The decrease in CD4 cell count affects the CD4 T cell responses; HIV-specific response, response to recall antigens as well as response to T cell mitogens. T cell help is required for generation of neutralizing antibody response as well as cytotoxic T cell responses. The CD4 T cells produce an array of cytokines that are very important in activating and maintaining effector cells. The impairment of lymphocyte proliferative response to HIV-1 antigens is a relatively early functional defect of cell mediated immunity found in HIV-1 infected individuals. The magnitude of CD4 T lymphocyte proliferation and quantity and pattern of cytokine production correlate with the clinical status. Patients who have controlled the viraemia will show a strong proliferative response to HIV antigens and production of Th1 type of cytokines and the responses inversely correlate with plasma virus load. Similar findings have been reported in SIV and simian human immunodeficiency virus (SHIV) infected monkeys. A strong CD4 T lymphocyte response in primary HIV infection was found to correlate with slower disease progression and better control of viraemia and those with poor CD4 response in primary HIV infection failed to control viraemia effectively. Loss of HIV-1-specific CD8+ T cell function that correlates with progressive infection was restored in chronic infection by augmentation of HIV-1-specific T helper cell function either by vaccine induction or infusion of autologous CD4 helper cells. CD4 cells apparently do not appear to have significant cytotoxic activity. However, their role in maintaining HIV-1-specific CD8 CTL activity is well recognized. It is yet not clear how CD8 CTL activity is maintained even when CD4 helper activity is not evident during chronic stage of infection.

CD4+ T cells produce various cytokines that activate the effector cells of the immune system. A fine balance between various cytokines is the key to immune response that would ultimately equip immune system to fight the infection. In several infectious diseases including HIV, the profile of the cytokines produced by the CD4 helper cells may decide the progression of the disease. Cytokines not only activate the cells of immune system, they may also influence the expression of various critical molecules on the cell surface and may upregulate or downregulate HIV multiplication in infected cells. Cytokines such as IL2, IFNγ, IL12 are proregulatory cytokines (Th1 type) while IL4, IL10, IL5, IL13 are proinflammatory cytokines (Th2 type). Since the cytokines are shown to have an immunomodulatory role and also have ability to modulate HIV expression the interplay between cytokines and HIV infection has been intensively investigated. Earlier studies in HIV-1 infection suggested that HIV disease is associated with overproduction of type 2 cytokines and decreased production of type 1 cytokines. However, there are contradictory observations regarding production of type 2 cytokines and there is general agreement that IL2 is decreased in HIV infection. Few studies have demonstrated decreased production of both type1/type2 cytokines rather than polarization of cytokine profile. In a recent study impaired production of cytokine was found to be predictor of mortality in HIV-1-infected patients and preserved capacity to produce cytokines was associated with prolonged survival. This indicates clinical significance of impaired cytokine production in HIV-1 infection. Another study has demonstrated that balanced type1/type2 response is associated with long-term non-progressive HIV-1 infection.
Considering potential therapeutic and immunomodulatory role, several cytokines (IL2, IL12, IL15, IFNα, IFNγ) have been tested or are under investigation in phase I/II human study in infected individuals. Some cytokines are also being used as adjuvant for candidate prophylactic vaccine.

**HIV-1-specific cytotoxic T lymphocyte (CTL) response:** Cytolytic T lymphocytes recognize HIV infected cells through HIV antigens expressed on the surface of the infected cells in association with HLA Class I peptides. This activity requires cell-to-cell contact. The cytotoxic T cells induce biochemical pathways in the target cell that ultimately lead to the destruction of cells. By destroying infected cells the CTLs puts halt to multiplication of HIV. Presence of HIV-1-specific cytolytic T lymphocyte responses were reported in HIV-1 seropositive individuals as early as in 1987.

Three kinds of evidence support the role of CTLs in the control of HIV infection. (i) Studies in animal models; (ii) Cytolytic T cell responses in early HIV infection and in LTNPs; and (iii) CTL responses in persons who are exposed to HIV infection but are not infected.

Rhesus monkeys were administered with anti-CD8 monoclonal antibodies to eliminate the CD8 populations in vivo. When CD8 depleted monkeys were challenged with SIV, uncontrolled viraemia and rapid progression of disease was observed. Depletion of CD8 cells in the monkeys with chronic SIV infection resulted in transient increase in viraemia which lasted for the period coinciding with CD8 depletion. A number of candidate HIV/SIV vaccines, either alone or in prime and boost strategy, have been able to induce potent cytotoxic T cell response that was able to provide at least a degree of protection against non-pathogenic as well as pathogenic viral challenges. In humans, HIV-1-specific CD8+ T-cell response is the earliest adaptive immune response generated and is temporally associated with the decline in viremia during acute HIV-1 infection. The appearance of CTLs in early HIV infection is found to influence disease progression and be responsible for favourable outcome. Strong virus-specific CTL responses have also been demonstrated in HIV-1-infected individuals with long-term non progressive disease. The non-progressors have also shown highly activated and broadly directed HIV-specific CTLs in setting of low viral loads. The HIV-specific CTL response has been detected in highly exposed but uninfected individuals (HEPS). In a study among uninfected sexual partners of HIV infected persons, 41 per cent showed HIV-specific MHC-Class I-restricted memory CTLs. The studies in Nairobi cohort of commercial sex workers have shown that the subjects considerably exposed to HIV infection but still not infected exhibit HIV-specific CTL response at vaginal mucosal level. It has also been shown that the epitopes recognized by these HEPS subjects were different than the epitopes recognized by HIV infected individuals. Thus, there is compelling evidence to believe that CTLs play a crucial role not only in controlling the HIV multiplication, but also protection from new HIV infections. However, CTLs are unlikely to be the only mechanism to control and prevent HIV infection. Although CTL responses are reported to be associated with long term non-progressor status and control of viraemia in early HIV infection, several studies have failed to show a clear association between plasma virus load and breadth or magnitude of CTL response in humans.

CTLs recognize 7-11 amino acid peptides, antigenic determinants (epitopes), expressed on antigen presenting cells in association with class I HLA molecules. The CTL response of an individual is thus genetically governed through the HLA haplotype of the individual and the alleles expressed. Studies performed on uninfected HIV vaccinees have shown association between expression of certain class I HLA alleles and CD8+CTL responses. In a recent study on 84 patients participating in the Swiss-Spanish Intermittent Therapy Trial showed that HLA types associated with rapid disease progression often failed to elicit a killer T-cell response, potentially because the HLA failed to bind and display the appropriate viral epitope. The investigators hypothesized that rare HLA types would be more likely to elicit an effective immune response than commonly occurring HLA types. They also suggested that rare HLA types were advantageous because HIV variants that have mutated to evade recognition by rare HLAs should also be rare.
The HIV-specific CTL response has been observed against all HIV antigens. HIV-1 Gag and Nef specific CTLs have been found to appear in early HIV infection. HIV-1 Gag-specific response appearing in early HIV infection has been found to inversely correlate with plasma viral load. Since cytotoxic T lymphocyte response is crucial for control of viraemia, most strategies for prophylactic HIV vaccines are aimed at developing potent anti-HIV CTL response. One of the critical issues in this approach is whether candidate vaccines based on one HIV subtype will be able to control infection with other subtypes. Several studies have demonstrated such cross-clade CTL response.

In a study in Pune, India, vigorous CTL response was reported against subtype B antigens among subtype C infected individuals studied within first year of infection. Frequencies of precursors of cytotoxic T lymphocytes were measured by limiting dilution analysis using subtype B specific antigens. Strong response was seen in most participants and response was seen against more than one antigen. In a follow up study, 12 patients were studied for HIV-1 subtype B- and subtype C-specific T cell response in an ELISPOT assay. The responses were studied on two occasions within one year of documented seroconversion. The results confirmed our earlier finding that HIV-1 subtype C infected patients demonstrated cross-clade response. However, it was also seen that subtype C-specific CTL response is significantly associated with lower viraemia. Such association was not seen with subtype B-specific response. HIV uninfected volunteers immunized with HIV-1 subtype B based ALVAC/gp160 vaccine showed both a broad pattern of cytolysis in which viruses from other clades including HIV-1 subtypes A and C were recognized. It has also been shown that the estimation of CTL response against autologous virus will provide better understanding of the extent of CTL responses. A study has shown that 29 per cent of the targeted peptides were detected only with the peptides representing autologous viral strains. However, the approach is not practical and hence not being used for estimation of HIV-specific CTL responses.

Considering the protective role of HIV-specific CTLs, the efforts for development of vaccine have been principally aimed at a vaccine that could generate strong, sustained cross subtype specific CTL response. Several HIV vaccine candidates containing HIV-1 proteins especially, Gag and Env are under phase I and II trials.

CTL responses are unable in most cases to prevent HIV-1 infection or thwart viral escape from immune recognition. The apparent inability of HIV-specific CTLs in controlling viral replication has been attributed to several reasons. First, depletion of essential helper CD4+ T cells by HIV may be an important cause for the progressive loss of CTL function. These cells are critical in sustaining optimum virus-specific CTL response. A direct correlation between HIV-specific CTL response and helper responses has been reported. Secondly, HIV-specific CTLs may suffer functional defects that make them unable to control HIV infection. Defects in perforin expression and IFN-γ have been reported. HIV-specific CTLs detected by tetramer staining, were detectable throughout the study but their ability to produce IFN-γ declined with disease progression. It has also been shown that the stage of maturity of CTLs is also important for effective response. Thirdly, CTLs are not able to maintain the control of HIV multiplication as a result of viral escape from recognition by CTLs.

Viral escape from CTL control may be influenced by various factors such as the immunological pressure in favour of escape mutants, purifying selection in the virus, fitness of the escape mutant for replication and HLA haplotype of the individual. The generation of escape mutants and their transmission may contribute to the evolution of the virus within the individual as well as population. This may have influence on the efficacy of vaccines. Accumulation of escape mutants in infected individuals and establishment of escape mutants in population may affect the response to the vaccine over time. A single amino acid change in the anchor residues within the epitopes can abrogate CTL recognition. The most compelling evidence for CTL selecting for mutations in HIV-1 comes from an analysis of the 473 HIV-1 infected subjects of the Western Australia HIV cohort study.
Viral sequence analyses demonstrated that the regions of HIV-1 hypervariability coincide with the regions encoding dominant CTL epitopes in humans with particular MHC Class I haplotype. Leslie et al. demonstrated that HLA-B57 and HLA-B5801-restricted CTL escape mutations in Gag epitope TW10 behave differently on transmission to HLA-B57/B5801 negative individuals. While one escape mutant underwent reversion to wild type, the other did not. Similar findings were reported by Friedrich et al. in SIVppm infection in Indian Rhesus macaques. This study also demonstrated that the escape mutants may show lesser fitness. These findings established the fact that CTLs may play important role in the evolution of HIV in population.

One of the limitations in studying CTL responses has been less than optimum technology available to the researchers. The traditional assays like classical chromium release assay analyze bulk populations of CTLs for cytotoxicity after HIV-specific stimulation. This is the functional assay that estimates the ability of CD8+ T cells to lyse target cells expressing specific HIV antigens. However, the methodology is labour-intensive and the results are usually qualitative. The single cell assay such as enzyme linked immunospot assay (ELISPOT), MHC-peptide tetramer staining assay and flow cytometry based intracellular cytokine (ICC) assays have come into use. While ELISPOT and ICC assay use antigen driven cytokine production as surrogate marker for cytotoxic activity, tetramer assay determines the expression of HIV-specific T cell receptor by fluorescent staining. Though antigen-specific production of Interferon-γ has been used as surrogate for cytotoxic activity, recent evidence demonstrates that HIV-1-specific cytotoxicity of CD8(+) T cells is preferentially mediated by a subset of CD8(+) T cells secreting both interferon-gamma and TNF-alpha.

Each of these assays can provide quantitative estimates since they enumerate antigen-specific cells without need for ex vivo expansion of reactive cell population. However, each of these assays differs in their sensitivity. In general, ELISPOT requires fewer cells and is less technically demanding than ICC. ICC, on the other hand, allows precise immuno phenotyping using lineage-specific markers to identify the responding cell populations.

**Mucosal antibody response**

The mucosal surfaces provide first contact for HIV and hence the defense mechanisms at mucosal levels may be important in preventing establishment of HIV infection. Recent studies on anti-HIV immune response at mucosal surfaces have suggested that HIV immunopathogenesis at mucosal surfaces probably involves both innate and acquired immunity.

The innate defence mechanisms at the mucosa primarily include the antimicrobial products derived from the epithelial cells and neutrophils such as lysozyme, lactoferrin and defensins, the bacterial flora and pH of the local mucosal microenvironment.

The studies on adaptive mucosal immunity suggested a key role for mucosal antigen presenting cells (APCs), and more specifically DCs in HIV infection. DCs residing within epithelial surfaces are the initial cells infected by HIV after mucosal exposure to virus. DC-SIGN, a novel C-type lectin expressed by DCs, has recently been shown to play a role in transport of HIV to the local lymph nodes. It has been observed that virus bound to DC-SIGN on DCs can remain infectious for several days, and virus-pulsed DCs efficiently transmit virus when they come into contact with CD4- and co-receptor-positive cell types.

HIV-1-specific IgA has been detected in mucosal compartment in exposed seronegative partners of HIV-seropositive persons and HIV-1 resistant sex workers. The CD8+ T-cell mediated immune responses have been detected from the vaginal epithelium of SIV-infected macaques. CD4 and CD8 CTLs have been recovered from cervix of HIV infected women. These HIV-1-specific MHC-restricted CD8+ and CD4+ CTL have been found to be capable of both destroying HIV-1 infected cells and releasing anti-viral cytokines. These cytokines might be locally active in women with vaginal or cervical infection. HIV-specific CD8+ CTLs have also been shown in the cervicovaginal secretions of exposed but uninfected commercial sex workers (CSWs) from Nairobi. The CD8+ CTL response in systemic compartment of HIV-1 resistant women...
was found to be directed against different subdominant or new CTL epitopes than the epitopes recognized by HIV infected women99. It has also been postulated that continuous antigen stimulation is needed for eliciting anti-HIV CTL responses in these women. However, still there is no conclusive evidence to suggest protective role of mucosal CTL activity against HIV transmission. Though mucosal immunity may hold key as the first barrier for establishing HIV infection, the data on mucosal immune response is still very limited.

In summary, HIV disease progression is largely dictated by the balance between the viral factors and host factors. Although virus affects almost all cells of the immune system directly or indirectly, the gradual loss of CD4 cells is the prime cause of the immune suppression. The host genetic factors may be associated with the rate of disease progression, although the evidence suggests that the genetic factors may not be critical for HIV control. HIV infection may be contained by innate immune response in the early phase. However, adaptive immune response is the most critical component in control of HIV infection. The role of neutralizing antibodies in control of HIV infection is yet a mute point. HIV-specific CD4 helper response and HIV-specific CTL responses clearly emerge as the most important host factors that may decide the rate of disease progression.

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