Review Article

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The enduring tale of T cells in HIV immunopathogenesis

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HIV continues to be a major health problem worldwide even today. Owing to the intricate nature of its interactions with the immune system, HIV has remained an enigma that cleverly utilizes the host machinery to survive. Its ability to evade the host immune system, at both levels, innate and adaptive, allows the pathogen to replicate and transmit from one host to another. It has been shown that HIV has multipronged effects especially on the adaptive immunity, with CD4+ T cells being the worst affected T cell populations. Various analyses have revealed that the exposure to HIV results in clonal expansion and excessive activation of the immune system. Also, an abnormal process of differentiation has been observed suggestive of an alteration and blocks in the maturation of various T cell subsets. Additionally, HIV has shown to accelerate immunosenescence and exhaustion of the overtly activated T cells. Apart from causing phenotypic changes, HIV has adverse effects on the functional aspect of the immune system, with evidences implicating it in the loss of the capacity of T cells to secrete various antiviral cytokines and chemokines. However, there continues to be many aspects of the immunopathogenesis of HIV that are still unknown and thus require further research to convert the malaise of HIV into a manageable epidemic.

Key words Differentiation - HIV - immune activation - immune exhaustion - polyfunctionality - proliferation - T cells

Introduction

T cells are one of the most potent cell populations against invading pathogens and ironically, these are also the cells that make the body vulnerable to HIV. Dalgleish *et al*[1] were the first to arrive at this conclusion and provided the evidence that CD4+ T lymphocytes were potential target cells of HIV. Although initial studies by Chun *et al*[2] proposed the resting CD4+ T cells to be the stable latent reservoir for HIV, but recent latency models suggest other cells such as macrophages and monocytes to be involved in viral latency[3]. However, the subject remains debatable to date. In recent years, the most predominant theory about HIV immunopathogenesis is that of HIV associated chronic immune activation. This theory suggests hyper and sustained systemic immune activation to be the trigger for perturbations in T cell dynamics and function. Brenchley *et al*[4] proposed that the translocation of microbial products such as lipopolysaccharide (LPS), from leaky gut mucosa could be a cause for the persistent activated state of the immune system. While considerable evidence has been gathered, the most alarming questions of viral replication, viral reservoir, correlates of disease progression, CD4 depletion and vaccine development are still to be answered. One of the major leads gathered so far is the role of adaptive

immune response against HIV. In the quest for all these outstanding questions, researchers have found that adaptive immune response could be the connecting link which could provide considerable clues to solve the HIV conundrum. Being at the forefront of adaptive immune response, T cells and more specifically, their activation, proliferation, differentiation, polyfunctionality and exhaustion have been the focus of research efforts to delineate mechanisms leading to HIV/AIDS and effective vaccine strategies. The two recent HIV vaccine efficacy trials, namely the Merck STEP trial and the Thai RV144 trial[5] are testament to not only the varying degrees of success but also the setbacks faced in the field of HIV vaccine development. The STEP trial was conducted keeping in mind that the MRKAd5 HIV-1 Gag Pol Nef vaccine would elicit an effective immune response in HIV uninfected individuals, at the initial phase of the trial the vaccine was found to be highly immunogenic. But as the study progressed further to the test of concept trial, the vaccine failed to provide protection as compared to the placebo. The trial also pointed out the fact that interferon gamma (IFN-γ) alone cannot be assumed to be the definite correlate of immune protection[5].

In contrast, the Thai RV144 test of concept trial evaluated both the humoral as well as the cell mediated arm of the immune system. Although negligible cell mediated immune response was observed in the trial, still the vaccine was found to be 31.2 per cent efficacious in preventing HIV infection. While the vaccine showed moderate protection, the lack of effective cytotoxic T lymphocyte (CTL) response remained unexplained[5]. The trials brought out the differences in approach as well as underscored our inadequacy to conclusively identify correlates of protection.

The studies and trials so far have employed non-human primate models (NHP) to decipher the mechanisms of immunological imbalance and optimization of vaccination strategies. Furthermore, the comparative analysis of cellular immune responses generated by vaccine candidates in humans and macaques underscores the importance of NHP models. However, the recent vaccine trials based on initial NHP studies failed to achieve the desired protective threshold in humans, and thus a critical re-evaluation of the used NHP models and the generation of new suitable models that are able to precisely imitate HIV-1 infection in humans is the need of the hour[6].

Taking all this into consideration, this review attempts to recapitulate the past achievements and also sheds light on the questions that are road blocks on the way to an effective vaccine. In recent years immune activation has emerged as a linchpin in HIV immunopathogenesis; however, evidence implicating this phenomenon in T cell dysfunction and exhaustion remains elusive. Thus, to enlighten these aspects of HIV and the host, this enduring tale has been discussed in two phases: acute viral infections and chronic HIV infection. It draws up a character sketch of T cells, their strength and weaknesses, and their journey during the course of HIV infection.

T cells and their types

It is now a well established fact that the T cells are a key component of the immune system with numerous studies elucidating their role in thwarting infections and keeping the body disease free. Within the T cell compartment, there are two well characterized subpopulations namely, T helper (CD4) and T cytotoxic cells (CD8)[7]. Mosmann *et al*[8] further characterized CD4+ T cells into two distinct subsets based on their ability to produce signature cytokines, TH1 subset secreting IFN-γ and TH2 subset secreting interleukin IL-4 and IL-13. In addition to their secretory capacity, Th1 and Th2 cells promote the cell mediated and humoral branch of the immune system respectively[8]. Recent discovery of a few more subsets of T cells namely Th17 and Treg (regulatory T cells) has further complicated the whole picture. Th17 cells as their name suggests, are active producers of IL-17[9] and their role against extracellular bacteria and fungi was first reported by Milner *et al*[10] in individuals lacking IL-17 production who were susceptible to bacterial and fungal infections[13]. Apart from IL-17 production, studies have also reported secretion of other pro-inflammatory cytokines by TH17 cells such as tumour necrosis factor TNF-α, IL-1, IL-2, IL-21 and IL-22. Being localized in the gastrointestinal tract[11], these play a significant role in mucosal immunity and are involved in regeneration of the epithelial cells. In contrast to the above mentioned protective T helper populations, the Tregs (CD25+FoxP3+) are suppressive in nature and regulate T cell hyperactivation, proliferation and cytokine production[12]. Like Th17 cells, these have also been reported in autoimmunity and other disorders[12]. Initial description of Tregs was reported by Gershon and Kondo in 1970[13] and later on, these were fully characterized by Sakaguchi *et al*[14]. Recent reports suggest that although Th17 and Tregs have a common lineage, but their differentiation pathways are inversely modulated in a number of immune disorders.

The common developmental link between these two populations is transforming growth factor (TGF-β). In the Th17 developmental pathway, TGF-β upregulates the master transcription factor retinoic acid receptor-related orphan receptor-γt (*RORc* gene encoding ROR-γt,)[15]. But the same TGF-β also induces the Treg developmental pathway by activating the transcription factor Fox P3[15]. Bettelli *et al*[16] reported that IL-6 along with TGF-β can favour the development of Tregs over Th17 cells. These evidences indicate a "see-saw" equation between Th17 and Treg population.

Apart from this, several new models of T cell lineage have been proposed which include T_{FH} (Follicular helper T cell), Th3, Tr1, Th9[17]. The precise role of all these newly identified subset is still under investigation[9].

Immune response against acute infections

T cell development: The journey of T cells begins in the bone marrow where haematopoiesis results in the production of progenitor T cells which then migrate to thymus for maturation and differentiation to develop into functionally distinct populations. Post negative and positive selection, CD4+ and CD8+ T cells exit the thymus and enter the circulatory system as "Naïve" (CD45RA+CCR7+) resting T cells having little transcriptional activity and accumulate in the lymphoid tissues, which are also the sites of accumulation of infected cells and pathogens[7]. Along with functional differences, these subsets exhibit a modified characteristic phenotype. Initial studies using flowcytometry techniques allowed the identification of the specific phenotypes using specific markers such as CD45RA, unprimed T cell marker and CD45RO, a primed memory T cell marker[18]. Further characterization of naïve T cell subset was made possible using CD27 and CD28, co-stimulatory molecules.

On exposure to antigens, CD4+ and CD8+ T cells undergo changes at the cellular as well as the sub-cellular levels and get activated. During their activated state, T cells undergo a number of phenotypic and functional changes resulting in the upregulated expression of stimulatory and co-stimulatory molecules such as CD28. Also, one of the activation markers which has recently come into limelight is CD38, a glycoprotein that is highly expressed on early T cell precursors[19], is downregulated upon maturation and finally in the presence of antigenic stimulus, its expression gets upregulated again[19].

The activated CD4 T cells secrete IL-2, a growth factor that acts on the antigen activated lymphocytes and stimulates their proliferation resulting in clonal expansion[20]. At the phenotypic level receptors such as CC-chemokine receptor 7 (CCR7), the lymph-node homing receptor CD62 ligand (CD62L) and the co-stimulatory receptors CD28 and CD27 are initially expressed on naïve T cells but once primed, there expression is downregulated[20]. The model developed by Hamann *et al*[21] allowed for simultaneous use of CD45RA and CD27 to define 3 distinct subsets of T cells: naive (N; RA^+27^+), effector (E; RA^+27^-), and memory (M; RA^-27^+) $CD8^+$ subpopulations. These differentiated effector cells exhibit polyfunctional capacity, *i.e.* the ability to produce a plethora of cytokines such as IFN-γ, TNF-α, IL-2 *etc.* In addition to this, CD8 T cells upregulate the expression of granzyme and perforin, thus making them cytolytic in nature[21].

While both CD4 and CD8 T cells differentiate simultaneously, CD8+ T cells proliferate at a much faster rate thereby forming a larger pool of effector cells as compared to CD4+ T cells. Also, at the genotypic level, the daughter CD8+ effector population is programmed such that no repeated stimulation is required for further proliferation and differentiation making them independent of the antigenic stimulus[22]. Subsequently, irrespective of the antigen clearance, the expansion phase is followed by a contraction of the effector population where 90-95 per cent of the activated CD8+ effector cells undergo programmed cell death primarily through Fas-mediated apoptosis[20]. The transition from the death to memory phase is not specific to a predetermined population. A number of factors such as changes in gene expression, function and phenotype decide the fate of effector cells to become memory cells. Now, this newly formed memory compartment is characterized by low granzyme B production and high expression of Bcl-2 (B-cell lymphoma-2) *i.e.* upregulated by the IL-7/IL-7R pathway[23]. IL-7 is one of the cytokines involved in T cell homeostasis, maturation, differentiation and long term T cell survival. It is actively produced by the thymic epithelium, stroma and bone marrow. In addition to these changes, a peculiar feature of this memory compartment is its ability to sustain itself for much longer period of time[24]. This is achieved through slow division of memory CD8 T cells which ensures a steady homeostatic turnover[24]. Further investigation into this compartment revealed that memory cells

residing in lymph nodes were phenotypically and functionally different from those present in blood and spleen. The cells present in the non lymphoid locations were found to be $CD62L^{Lo}$ $CCR7^{Lo}$ and could produce IFN-γ and TNF-α efficiently whereas memory cells in lymphoid zones expressed $CD62L^{Hi}$ $CCR7^{Hi}$, and produced IFN-γ, TNF- α along with IL-2. Thus based on their homing and functional capacity, these were categorized as T effector memory (T_{EM}) and T central memory cells (T_{CM}), respectively[43]. Recent studies have observed a high degree of heterogeneity in the effector memory (EM; CD45RA-CCR7-) subset with differential expression of CD28 and CD27 molecules. Thus Romero *et al*[25] proposed the categorization of this section as well, the resultant being 4 phenotypically distinct subpopulations called EM1(CD27+CD28+), EM2(CD27+CD28-), EM3(CD27-CD28-) and EM4(CD27-CD28+). Of these, three subsets are functionally distinct, with EM1 being memory like, EM2 exhibiting partial replicative history and effector functions and EM3 being effector like. The findings so far have not only enabled researchers to understand the multidimensional process of T cell differentiation but can also be considered as an archetype of immune response in the presence of acute infections.

In this way sufficient numbers of CD4 and CD8 T cells are maintained throughout the life of an individual by slow and steady homeostatic process.

***Immune response in chronic infection*:** In the event of a chronic infection like HIV, where the virus persists in the body in both latent and replicative forms, T cell dynamics play a significant role in deciding the susceptibility to a pathogen. The initial weeks post HIV infection witness a sharp rise in the viral titre along with a depletion of CD4+ T cells most prominently observed in the gastrointestinal tract associated lymphoid tissue[26]. However, a partial retaliation by the immune system is observed when the decline in viraemia coincides with the appearance of HIV specific CD8+ T cells. It must be noted here, that this initial CD8 response is limited to a few viral epitopes[27]. Ordinarily, this decline in viraemia plateaus and the virus establishes latent viral reservoirs in the lymph nodes and eludes the cytotoxic response of the immune system and thus the chronic phase of HIV sets in. But investigators have studied a few exceptions to this trend. In the Nairobi cohort, commercial sex workers who were repeatedly exposed to HIV remained uninfected exhibited HIV specific CTL responses in the vaginal mucosa. They also found that the epitopes recognized by CTLs were different from those recognized in HIV infected individuals[28]. Additionally, studies on long term non progressors have reported stronger CTL responses and control of viral replication[29]. These studies contradictory to prevalent beliefs prove that CTLs could be a part of the solution.

Chronic phase

***The lull before the storm*:** One of the hallmarks of the chronic phase of HIV infection is the depletion of CD4 T cell pool. While the classic notion of HIV mediated CD4 depletion points to the direct killing of CD4 infected T cells, recent developments have challenged this theory and given rise to newer paradigms. Recent findings suggest that majority of the CD4+ T cells that undergo cell death are in fact not infected with HIV. This loss of uninfected bystander T cells indicates the existence of an alternate mechanism of CD4 T cell depletion independent of productive HIV infection[30].

One of the mechanisms that has gained popularity is that of chronic immune activation. Giorgi and colleagues[31] demonstrated that CD38 expression on CD8+ T cells correlates strongly with HIV disease progression in HIV infected than HIV uninfected individuals. Further support came from the studies in Simian immunodeficiency virus (SIV) infected primates. Rhesus macaques infected with SIV showed high levels of immune activation, suffered progressive CD4+ T cell depletion and exhibited high rates of viral replication. On the other hand, Sooty mangabey, the natural hosts of SIV, registered low immune activation levels and maintained normal reservoir of CD4 T cell population without developing any immunodeficiency despite high viral replication[32]. When translated into the pathogenesis of HIV/AIDS in humans, primate studies provide strong support to the hypothesis: Immune activation is the leading cause of bystander CD4 T cell death[33]. However, the major paradox of immune activation is that HIV causes immunodeficiency but at the same it is responsible for chronic immune activation. The answer lies in the complexity of the immune response against HIV. Although the exact cause of immune activation is still unknown, studies over the past few decades have gathered evidence to the theory that there is no single cause for chronic immune activation[33]. Rather it is a resultant of a multitude of factors which are still debatable.

(i) Chronic innate immune activation: Innate being the foremost line of defence encounters HIV first and is activated within hours. The first cells to come across HIV are the plasmacytoid dendritirc cells (pDCs)[34].

pDCs through their CD4 surface molecule interact with HIV and get activated. The persistent stimulation of pDCs by HIV beyond the acute early phase causes continued secretion of IFN-α and indoleamine 2, 3 dioxygenase (IDO)[35]. *In vitro* studies have shown IFN-α to be closely associated with increased expression of CD38 on CD8+T cells[36]. In contrast to this, IFN-α has also been reported to induce apoptosis in CD4+T cells in HIV infected and SIV infected macaques but not in non-human primates with non-pathogenic infection[35,36]. Thus uncontrolled innate immune activation may lead to dysregulated adaptive immune response. This finding suggests a link between players of activation in innate and adaptive immunity. Also IDO which is required for degradation of tryptophan to kynurenine[37] has suppressive effect on T cell proliferation. Two evidences supporting this were murine models where inhibition of HIV induced IDO enhanced the clearance of HIV-infected macrophages[38] and *in vitro* studies which demonstrated the improvement in CD4 T cell proliferation on blocking of HIV-induced IDO[39]. Thus, the ripples of chronic immune activation in the innate arm of immunity can be felt in the form of immune activation as well as deficiency in adaptive immunity.

(ii) Direct stimulation by HIV proteins: The most obvious mechanism is the direct stimulation of the immune system by the virus itself. Activation sets right in the acute phase of HIV where its presence triggers a cascade of signals. *In vitro* studies have reported that HIV gene encoded products can directly stimulate the immune system without direct infection[40]. HIV proteins such as gp-120 through their interaction with CD4 and co-receptors have been shown to activate lymphocytes and macrophages through production of pro-inflammatory cytokines like TNF-α which in turn boosts viral replication[41]. Two other important proteins that induce hyperactivation of monocytes and macrophages are Nef and Vpr. The Nef and Vpr proteins partially mimic the TNF receptor signalling in these cells and stimulate NFk-β leading to HIV LTR (long terminal repeat) activation and subsequent HIV replication[42]. However, at the same time, pro-inflammatory cytokines and chemokines production is blocked by Vpr protein[43] thereby favouring the recruitment of T cells, monocytes and macrophages[44].

In other words, these viral proteins by fooling the immune system ensure a continuous secretion of TNF-α thereby creating an environment of constant inflammation and viral replication. These events ensure a closed loop for immune activation as well as HIV-1 replication thereby creating a vicious cycle.

(iii) Microbial translocation: The human gut has vast surface area that allows it to absorb nutrients and water without infiltration of the gut flora and thereby restricts the translocation of microbial products. Contrary to this notion, the presences of histological abnormalities and lymphocytes in the gut associated lymphoid tissue (GALT) of chronically HIV infected individuals have been reported[7,45] highlighting a possible association between mucosal immunity and HIV disease progression. Since then, substantial data have been gathered and different theories have been proposed as the underlying mechanisms. Some researchers endorse the most apparent mechanism of virus mediated toxicity in enterocytes[46]. This could be due to the inhibitory role of Tat, an HIV protein, on uptake of glucose by enterocytes. Also, gp120 has shown to increase the concentration of calcium in enterocytes leading to ionic imbalance[47]. Thus, these changes affect the integrity of the mucosa lining of the gastrointestinal tract leading to mobilization of microbial products such as LPS, sCD14 and EndoCAb. To prove this hypothesis, Brenchley *et al*[48] showed that LPS, a common component of bacterial cell wall was increased in plasma of chronically infected HIV individuals and SIV-infected macaques and correlated with immune activation. Supporting evidence was provided by measurement of soluble CD14 (sCD14) in HIV infected individuals and significantly higher levels of plasma CD14 were found in the acute and chronic HIV infected individuals as compared to the HIV seronegative individuals[49]. Apart from this, they also reported that LPS levels strongly correlated with plasma IFN-α level which led to their hypothesis that certain factors in the intestinal flora could also induce IFN-α production. Thus, they concluded that the microbial products translocated across a leaky gut, may result in systemic hyperactivation of the innate immune system and may further impair the adaptive immune responses[4] (Figure). Other microbial products such as flagellin, bacterial 16sDNA and peptidoglycans have also been shown to translocate across the mucosal barrier. These microbial products through their interaction with Toll-like receptors (TLRs) activate the innate immune system. Boasso and Shearer[39] proved this hypothesis through their work on murine models and reported that in mice lacking the IFN-α receptor showed milder physiological and functional alterations in their lymphoid organs on treatment with CpG oligodeoxynucleotides (ODN) as compared to those

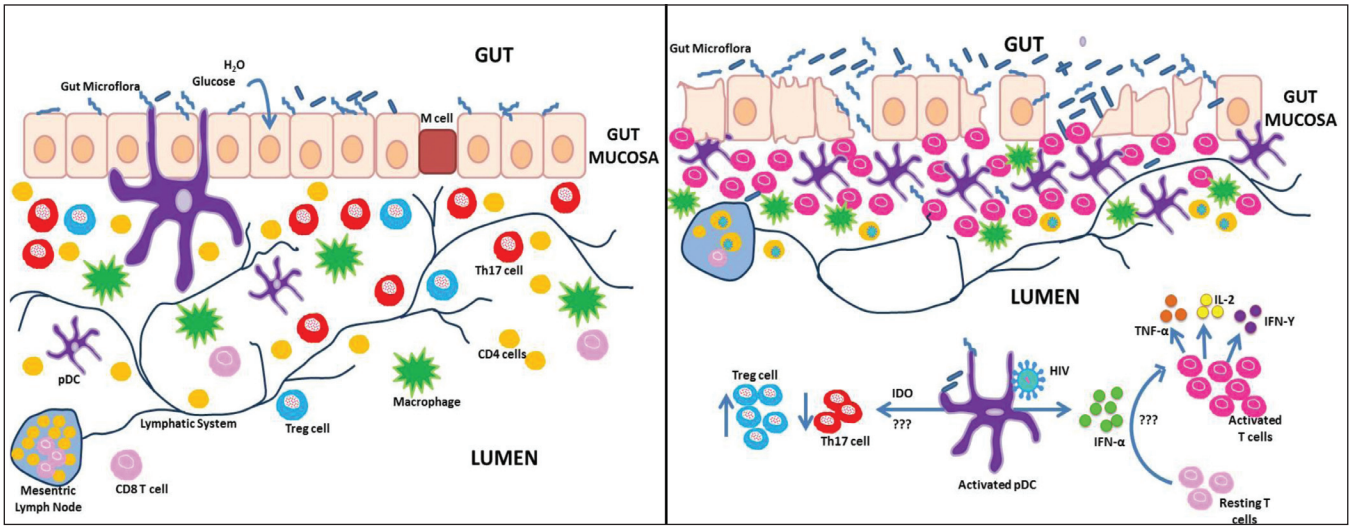

Fig. Hypothetical model of gastrointestinal tract **(A)** Normal Healthy state and **(B)** chronic HIV infection. (**A)** In a normal healthy state, the epithelial and M cells lining the mucosal barrier of the gastrointestinal tract forms an effective barrier against invading pathogens. The lamina propria contains loose clusters of T cells, macrophages, plasmacytoid dendritirc cells (pDCs) and follicles. **(B)** During chronic HIV infection, the mucosal barrier lining the gastrointestinal tract becomes leaky facilitating translocation of microbial products. This results in increased infiltration of inflammatory cells into the lamina propria leading to more proinflammatory cytokine production. Lipopolysaccharide has been reported to activate IFN-α and Indoleamine 2, 3 dioxygenase (IDO) production by (pDCs) promoting immune activation and a shift in the balance from Th17 to Treg population by unknown mechanisms.

with IFN-γ receptor. Therefore, they propounded the theory that the administration of CpG ODN activates pDC through TLR9 and results in alterations[50] which were similar to those seen in HIV infection[39].

The investigators have also identified a preferential loss of Th17 subsets from the GI tract[51]. Further studies comparing the pathogenic (pigtailed macaques) with the non pathogenic SIV models (African green monkey) revealed loss of Th17 cells with a concomitant increase in Treg[7,52] in the pigtail macaques. The loss of Th17/Treg balance was associated with sustained systemic immune activation[52]. Our group[53] has also observed a gradual loss of Th17 cells in the peripheral blood of HIV-1 infected Indian individuals which revealed that this loss of Th17 cells during late stage of HIV-1 infection could render them more prone to opportunistic infection. Furthermore, an enhancement in the proportion of Treg cells was noted during the HIV disease progression. This indicative of progressive HIV infection was associated with the loss of Th17/Treg immune balance. Therefore, our group speculated that the relative balance between Th17 and Treg cellular subsets rather than the function of either alone was critical for immunopathogenesis of HIV infection[53].

Consistent with our study, others have also reported a loss of Th17 cells in blood and rectosigmoid mucosal biopsies[51] and was found to be associated with sustained immune activation. However, the reason behind this selective loss of Th17 cells remains unclear. One possible mechanism involves the role of IDO metabolism. As IDO catabolises tryptophan to kynurenine and in the case of HIV, an excess of IDO production deprives the cell of the tryptophan pool resulting in the accumulation of catabolites[37]. These catabolites, on the one hand, prove toxic[54] for the cell and induce apoptosis and on the other hand, due to the ability to stimulate FoxP3 expression and downregulate RORc expression, these promote a shift in the balance from Th17 to Treg population[55].

Thus constant damage of the gastrointestinal track, decline in Th17 cell population and translocation of microbial products set up an enduring cycle of immune activation and viral replication leading to poor immune response.

***Co-infections*:** A number of opportunistic infections can also contribute to immune activation. Among bacterial infections *in vivo* studies have revealed tuberculosis (TB) to be a driving factor for HIV replication. Pro-inflammatory cytokines such as TNF-α produced against TB bind to the cell receptors leading to the secretion of active nuclear factor (NF)-kB in large quantities[56]. NF-kB activates transcription of a

number of host genes including HIV-1 LTR sequences subsequently enhancing viral replication[57] which in turn maintains the systemic immune activation. Evidence in support of this came from co-infected Ugandan patients whose pleural fluid samples recorded four times higher amount of HIV-1 load than in plasma samples. High levels of TNF-α, IL-6 and other soluble markers were found to be strongly correlated with HIV-1 viral load in the pleural space[58].

Looking at this scenario, it appears that it is the innate immune system which initiates the process of immune activation but it is the adaptive immunity that sustains it and gets affected in the process. HIV through immune activation is able to generate new targets for infection and propagation. While these events have been labelled as causes of immune activation, these along with other factors play an important contributory role in immune deficiency. Whether these causes are linked through an unknown network or are a series of events occurring simultaneously still remains to be determined. Also, other governing factors that are contributing to this phenomenon need to be explored.

After-effects of immune activation

I CD4 T cell depletion

(i) Direct virus mediated killing: Brenchley *et al*[59] reported that GI tract harbours the majority of CD4+ T cells followed by lymph nodes and other lymphatic tissues rather than peripheral blood. Most of CD4+ T cells are CCR5+ activated memory cells making them the susceptible targets for HIV. Also as compared to the periphery productive infection rate is higher in mucosal sites. Massive depletion of these cells occurs during the early phase of infection followed by a slow and stable decline of the remaining cells in the chronic phase. In addition to the mucosa, a marked loss in CD4 T cells was also noted in the periphery by many research groups including ours[60]. We observed significant negative correlations of higher magnitude between CD4+ T cell percentages and plasma HIV-1 RNA levels when adjusted for the effects of the immune activation markers. These data support the idea that viral replication leads to immune activation, which in turn drives CD4+ T cell depletion. However, a lucid mechanism explaining the loss of CD4 T cells in HIV infection has yet to be defined[60].

Several mechanisms have been proposed to explain the depletion of CD4 T cells. Studies have shown an increase in CTL responses during the acute phase of infection which coincided with the drop in plasma viraemia. From this it is evident that CTLs try and control the infection by directly killing infected CD4 T cells by cytolysis. Thus, this virus mediated immune response directly results in the preferential loss of CD4 T cells. However, as the error prone reverse transcriptase enzyme generates variants of viral epitopes and the virus evolves constantly, the existing CTLs no longer recognize the mutant epitopes and the immune system fails to generate new cytolytic response against the variant epitopes thereby allowing the virus to escape. In addition to this, HIV encoded protein Nef downregulates the expression of MHC I and prevents recognition by CTLs and thus avoid CTL mediated killing of infected cells[61]. Hence, the killing effects of CTLs get shadowed by continues evolution of HIV and its proteins. However, this clearly indicates the presence of other factors that are involved in depletion of CD4 T cells.

(ii) Activation induced cell death (AICD): Cell signalling studies have shown that immune activation shares molecular steps that are also involved in apoptosis giving rise to the notion that activation and apoptosis may be two sides of the same coin[61]. AICD is a mechanism through which the body keeps immune activation in check by directing the activated cell to undergo apoptosis[61]. During the chronic phase of infection, pro-inflammatory cytokines such as TNF and C-C chemokines are produced in significant amounts by the infected cells[62]. Studies have shown that TNF family of cytokines can induce apoptosis in a direct manner by inducing the appropriate (or inappropriate) apoptotic signals[63]. However, their precise role in inducing apoptosis is still under investigation. While, these mechanisms describe the cytopathic role of immune activation through cytokine production, these could not explain apoptosis in bystander cells.

It has been reported that apoptosis occurs predominantly in non-infected bystander cells as compared to the infected cells in HIV infected children and SIV infected macaques[64]. During the hyperactivated state of HIV infection which is also associated with high viral replication, the expression of host factors such as TNF superfamily ligands and receptors (Fas/Fas-L along with TRAIL-DR5) and viral factors such as Tat, Nef, Vpu, Vpr are upregulated[65]. These simultaneous events result in depletion of CD4 T cells leading to acquired immunodeficiency. Studies have shown higher expression of both CD95 (Fas) and FasL on peripheral blood mononuclear cells (PBMCs) of HIV infected individuals and have also reported that

both CD4 and CD8 T cell populations are vulnerable to Fas mediated apoptosis[66]. This mechanism was suggested to be the reason for bystander death by AICD. Moreover, Tat has also been reported to induce the production of reactive oxygen intermediates, activation of caspases and other transcription factors such as NF-kB. These signalling events ensure HIV production by NF-kb activation but at the same time, these prepare the cell for apoptosis by caspase activation. The Tat protein in plasma induces apoptosis in infected as well as uninfected cells through its activation of FOXO3a (Forkhead box transcription factor O class 3a) which controls the expression of several proapoptotic genes, including FasL, Bim and TRAIL[67], Env (another HIV protein), binds to CD4 molecule and sensitizes the cell to Fas-mediated killing[68] with an increase in caspase activity[69]. Both the secreted and the membrane bound form of Env are able to induce apoptosis in uninfected cells. When these cross-linked cells encounter antigen presenting cells (APCs), stimulatory signals through T cell receptor (TCR) leads to apoptosis in uninfected cells and these negative signals are more enhanced during chronic immune activation resulting in AICD[70]. This env mediated apoptosis is significantly higher in lymphoid tissues of HIV infected individuals[30].

Unlike Env and Tat, Vpr (Virion associated protein) is involved in cell cycle arrest[71]. By inhibiting the activation of p34cdc2-cyclin B which is required by the cell to enter mitotic phase, Vpr arrests both infected and uninfected cells in G2 phase[72]. Studies[73] have demonstrated that the carboxy-terminal truncation of Vpr induces apoptosis via G1 arrest of the cell cycle. On one hand, Vpr benefits HIV by enhancing viral replication and on the other hand induces cell death[73,74].

Like Tat, Nef, another potent HIV protein induces apoptosis in bystander cells through upregulation of Fas and FasL or directly engaging CXCR4 on infected cells[75]. In addition to this, free Nef in plasma can be present in the form of microvesicles expressing CD45 which are known to cause AICD in resting CD4 T cells[75,76].

However, HIV being a true opportunist delays cell death for its sustenance. It can modulate the activity of Nef protein, depending on the stage of infection, to inhibit apoptosis and thus promotes continuous production of progeny virions. In such a scenario, Nef can switch roles and be anti-apoptotic in nature[77].

II. Loss of homeostasis

The adverse effects of chronic immune activation in HIV are not restricted to CD4 T cells depletion alone as the expansion of CD4 and CD8 T cells is also seen as an equally detrimental effect. The indiscriminate and chronic immune activation results in the creation of a pool of rapidly proliferating T cells with effector functions which subsequently undergo apoptosis[33]. This may represent compensatory homeostasis in response to cells killed either directly or indirectly by the virus. In contrary to this, studies monitoring immunological changes with the initiation of HAART have shown that in comparison to the rapid decline in viral titres, the restoration of CD4 T cell counts takes more time with therapy[78]. Detailed analysis of T cell subsets and their response to HAART has revealed that the normalization of CD4 T cell counts is preceded by an initial drop in the proliferation rates of all the subsets of CD4 and CD8 T cells, *i.e.* naïve, effector and memory[79]. These findings clearly indicate that once the viraemia is contained, antigenic stimulus decreases and opportunity for immune activation reduces suggesting that immune activation plays a crucial role in heightened T cell proliferation and dysregulates a normal homeostatic balance. The unremitting rounds of activation and expansion exert a negative pressure on naïve T cell pool. This would not only result in the exhaustion of the T cell compartment but also weaken the immune response to newer infections[38,80].

Many studies have proposed two different pathways, thymic-dependent (thymopoiesis) and thymic-independent (homeostatic proliferation of existing cells) for the observed dysregulation in homeostasis[81].

(i) Thymic-independent mechanisms: Studies have reported elevated levels of IL-7 during HIV infection which may be produced in response to the depleted pool of T cells to restore homeostasis. Decreased levels of CD127 expression in HIV infected individuals on both the CD4+ and CD8+ T cell compartments have been shown[82]. Similar findings have also been reported by our group, wherein a decreased expression of CD127 was observed and this decrease was found to be more pronounced on CD8 T cell subsets than CD4 T cells. Additionally, on further investigations, the CD127 expression was observed to be less in naïve, effector, and memory T cell subsets and was more marked for the central and effector memory cell subsets[83].

As signaling through CD127-IL-7 receptor complex is fundamental to T cell survival, a depletion of CD127 from these memory cells signifies a functional aberration of these cells that can result in loss of these cells eventually in the course of infection. Reduced expression of CD127 disables the cell to respond to high levels of IL-7 which are mostly seen in HIV infected individuals[84]. Studies have also shown reduced expression of Bcl-2 in case of HIV infection, suggesting that the reduced expression of CD127 and Bcl-2 might predispose the cell to apoptosis raising the question of a possible association between correlates of disease progression and CD127[84,85]. In this context, studies were conducted to measure correlation between markers of immune activation and CD127 and have been found to be strongly correlated[82]. Investigations have also revealed strong correlation between central memory pool affected by immune activation and reduced CD127 expression[86]. This is further supported by evidences in elite controllers, non-pathogenic retroviral infection models, HIV-2 infection and controlled HIV infection models. Elite controllers are able to maintain stable CD4 counts with an undetectable viral load for long period of time in the absence of therapy. Reports have suggested that these HIV infected individuals show a conserved CD4 central memory cells expressing high levels of CD127. Also it was found that immune activation was restricted to CD4+ TEM compartment which had decreased expression of CD127[86]. In comparison to the pathogenic HIV infection, SIV infected sooty mangabeys with low levels of immune activation showed stable expression of CD127 on CD4+T cell population which correlated with preserved T cell count[87]. In another scenario of low immune activation, elevated levels of CD127 were observed in HIV-2 infected individuals[88]. In addition to this, patients who display recovery of T cell numbers on initiation of antiretroviral therapy have also showed upregulated expression of CD127 on the T cell compartment. However, individuals who despite therapy are not able to control the viral loads recorded poor restoration of CD127 expression[85,89].

These findings support the theory that the immune activation and not the viral load, is an independent regulator of CD127 expression.

(ii) Thymic dependent mechanisms: It is apparent that immune activation is one of the major mechanisms for CD4 depletion. However, thymic impairment has also been frequently attributed to the progressive loss of CD4+ T cells[90]. In support of this, several studies have highlighted the role and effect of HIV on the thymus and to its functions. First line of evidence comes from a study of aborted foetuses from HIV-infected mothers wherein a loss of HIV infected thymocytes has been observed. Furthermore, similar results were seen in thymus biopsies of HIV-infected neonates[91], in post mortem thymic tissue from HIV infected individuals[92], and in SIV-infected rhesus macaques[93]. However, till date the precise contribution of HIV in thymic impairment and its possible role in CD4 depletion remain ambiguous. In 1998, Douek *et al*[94] proposed the use of T cell receptor (TCR) excision circle (TREC) as a mean to quantify thymic output. TRECs are extra-chromosomal DNA fragments which are produced during TCR gene rearrangement. These are relatively stable, do not replicate, and have been proposed to be surrogate markers for thymic output[95].

To further understand the role of thymus better, studies involving the removal of the thymus were undertaken. A group HIV infected individuals thymectomized before the infection, were studied and it was found that the thymectomy did not exclude long term survival. Also, thymectomized persons on HAART recorded an increase in CD4 T cells which was similar to that observed in non-thymectomized HIV infected individuals[92]. To directly measure the impairment of thymic output in SIV infection, Arron *et al*[96] infected a group of thymectomized and group of sham operated juvenile rhesus macaques with the assumption that if SIV infection had a negative effect on the thymic output, then in thymectomized SIV infected macaque models, the condition should not deteriorate. On the contrary, they reported that the sham-operated, SIV infected macaques displayed a slower decline in CD4+ T cells and total TRECs in the CD4+ T cell population than the thymectomized, SIV-infected macaques. Also, when compared with the healthy uninfected thymectomized macaques, the SIV infected thymectomized macaques showed a major loss of CD4 T cells and TRECs. When translated into HIV infection, perhaps the decline in CD4 and TREC content could be due to the immune activation and increased proliferation resulting in dilution of TRECs[96].

These findings point to the fact that *(i)* the impairment of thymus due to SIV does not result in the decline of CD4 T cells and TRECs, rather the peripheral effects of SIV should be taken into account, and *(ii)* TREC numbers are strongly dependent on T

cell division rather than thymic dysfunction and thus these cannot be a marker for thymic function.

III. Maturational and differentiation defects

With growing evidence of HIV induced disturbance of the T cell differentiation and its impact on the immunopathogenesis, Clerici *et al*[97] questioned the possibility of immune abnormalities occurring in foetuses of HIV infected pregnant women. Abnormal maturation of the T cell naïve and memory compartments were observed in infected aborted foetuses as well as uninfected children of HIV infected mothers leading the group to emphasize on the persistent effects HIV had on the immune system even after termination of the antigen stimulus. It also raised various concerns of the impaired immune system making the children susceptible to autoimmune diseases[98]. While the consequences of HIV infection of the immune system were becoming less ambiguous, Appay *et al*[99] set out to understand the dynamics of T cell responses specifically in the acute and chronic phases of HIV infection. They pointed out that during the acute phase of the infection, the T cell compartment mostly comprises naïve T cells (CD27+CD28+) which underwent maturation by differentiating rapidly (within 2-4 wk) into intermediately differentiated cells (CD27-CD28+) and as the infection progresses to the chronic stage, the T population displayed a quantum shift towards the CD27-CD28- subsets. Although the study highlighted the shift in the phenotype, it cautioned against the oversimplified classifications of T cell subsets as a clear distinction between the CD28- and CD28+ population, suggesting the need for further scrutiny. However, the subsequent study by Tussey *et al*[100] challenged this conclusion by stating that well before seroconversion, the CD8 population may exhibit a mixed expression of CD28 indicating a period of priming where the expression of CD28 is downregulated. They further argue that there is a flux between the maturation subsets in response to the antigenic stimulus, *i.e.* when the antigen load is low, the cells mature to the memory stage but during recrudescence, the memory subsets are recruited back to their previous state[101].

To understand how the continuous antigenic stimulus disturbs the differentiation process better, Younes *et al*[102] examined the effect of HIV viraemia on the differentiation of T cells. They found that persistent viraemia resulted in the alteration of the differentiation pathway by blocking the formation of stable long term central memory T cells. Instead, the continuous stimulation caused further differentiation of the effector T cells (Tem1) to form a functionally limited effector T cell population (Tem2). Furthermore, their data supported the previous findings that chronic progressive HIV-1 infection is related to a maturation block from effector memory phenotype (EM, CD45RA/CCR7) to full effector phenotype (E, CD45RA+/CCR7)[103].

These conclusions also raised critical questions regarding the possible role of terminally differentiated HIV specific effector cells in mediating viral control. Our group also investigated quantitative alterations in the naive (CD45RA+CD62L+), memory/effector (CD45RO+) and activated (HLA-DR+CD38+) T lymphocyte subpopulations in treatment naive, HIV-1 infected Indian patients. Significant correlation obtained between different CD4+ and CD8+ T cell subsets reflected the quantitative alterations in the T- lymphocyte subpopulations and activation of the immune system during HIV-1 infection[104].

Our group also observed an overrepresentation of intermediate (CD27+CD28-) differentiated cells in the naïve, effector memory, central memory and TEMRA CD8 subsets[104]. Our study has pointed out that this skewed maturation is likely a pathogenic strategy utilized by the virus, whereby CD8 T cells are prevented from achieving full effector functions[104]. Such abnormal CD8 T cell maturation, in turn may lead to a dearth of effective immune responses to clear the virus, thus establishing disease chronicity. Another factor that has been under intense research is the effect of HAART on maturation of T cells. On examining a group of patients receiving HAART, Le´curoux *et al*[105] found a particular subset of CD8+T cells displaying CD27-CD45RO-/RA+ phenotype, originally which were sparsely present during primary HIV infection, increased in numbers after the early initiation of HAART. This newly identified subset was found to be stable, nonactivated, and resting, presenting memory characteristics, and can develop effector capacities after stimulation thus providing direction for future studies to explore their capacity to control HIV infection. While the ideal vaccine against HIV remains elusive to man, intense research over the decades has revealed and such encouraging findings may prove to be an impetus for future scientists to understand the deleterious effects of HIV on the adaptive immune system.

The dark side of HIV/AIDS

Polyfunctionality: Loss or gain?: In recent years, polyfunctionality of CD8+ T cells has gained popularity and many research groups have identified

HIV specific CD8+ T cell subsets capable of producing multiple cytokines simultaneously. The quantitation of these immune parameters has been possibly largely due to the advancements in flow-cytometry where multiple markers can be examined at a time. Using these techniques, investigators have characterized cells producing cytokines such as IFN-γ, TNF-α, and macrophage inflammatory protein (MIP)1-b exhibiting degranulation. It was also demonstrated that these cells produced multiple cytokines on a per cell basis as compared to the monofunctional CD8 T cells. Current notion regarding polyfunctional CD8+T cells suggest that "the more the number of cytokines produced, the better is the quality of the immune response". Such a response was observed in Elite Controllers (ECs) who are a rare subset of treatment naïve HIV infected individuals capable of maintaining stable CD4 T cell counts for a longer period of time with a viral load of <50 copies/ml[106] and long term non-progressors (LTNP) who greatly vary in their levels of viraemia but are able to sustain CD4 T cell counts over 7-10 years in the absence of therapy[107]. Thus, their natural resistance to HIV disease progression has made them ideal models to delineate the involved mechanisms. ECs and LTNPs have been shown to be able to maintain these responses as compared to non progressors. Additionally, Migueles *et al*[108] revealed that the HIV specific CD8 T cells of LTNPs apart from secreting multiple cytokines also demonstrate high proliferative and cytotoxicity capacity against autologous HIV infected CD4 T cells when compared to progressors. This capacity was attributed to efficient release of perforin and granzyme B production which may be helpful in suppressing HIV replication. However, these polyfunctional HIV-specific CD8 T cells are rarely found in the peripheral blood of individuals with progressive HIV infection[42,109]. Progressors after mounting a strong polyfunctional anti-HIV CD8 T cell response during the early phase of infection are unable to sustain it over a period of time unlike LTNPs[110]. Similar findings have also been reported from our laboratory wherein we observed that HIV specific CD8 T cells are significantly impaired with regard not only to IFN-γ but also in IL-2 and TNF-α production in chronically infected individuals[111]. Interestingly, the degranulation capacity was found to be largely conserved. It was also observed that HIV specific responses were mostly mono functional followed by dual responses and responses with three or more functions were almost absent. The finding also showed that the ability of CD8+ T-cells to respond to Gag and Nef stimulation with cytokine secretion and degranulation did not differ substantially and was irrespective of HIV antigen. Also, the loss of functionality was found to be restricted to only HIV-1-specific CD8+ T cells, whereas no significant changes were found in the functionality of CD8+ T-cell responses to polyclonal stimulation in the same participants concluding that it is a quality of CD8 T cells *i.e.* paramount for virus control[111,112].

Although, CD8+T cells have the potential to suppress viral replication, many LTNPs are completely devoid of these subsets thus, questioning the importance of CD8 T cells as an immune correlate of protection. This clearly indicates either a gap in our knowledge of CTL responses or the possible existence of another subset controlling viral replication.

(ii) Immune exhaustion: As the disease progresses in HIV infected individuals, both HIV specific CD4 and CD8 T cells lose their capacity to mount an effective immune response and gradually become exhausted. Immune exhaustion was initially described in mouse model of lymphocytic choriomeningitis (LCMV) infection[113]. The LCMV specific CD4 and CD8 T cells were found to be deficient in their cytotoxicity and were termed 'exhausted'. Subsequently this phenomenon was observed and reported in chronic HIV infection. T cell exhaustion is a slow and steady process, with partial loss of effector functions (proliferation, IL-2 secretion)) in the early phase followed by a hierarchical loss of other functions like TNF-α, IFN-γ secretion during the late phase of infection[114]. However, this phenomenon of immune exhaustion is not only restricted to the cell but can be seen at a systemic level. On a cellular level, numerous changes have been observed including altered gene expression patterns especially in the expression of inhibitory receptors, exhibition of metabolic and bioenergetic insufficiencies[114], dysregulation of TCR and cytokine signalling pathways along with maturational and differentiation defects. On the systemic level Tregs, homeostatic growth factors and regulatory cytokines such as IL-10 and TGF-β have been proposed to have a significant role in immune exhaustion. Although the phenomenon is not fully understood, a few underlying mechanisms have been proposed.

Inhibitory receptors

Many inhibitory receptors (like PD-1, LAG-3, Tim-3, CTLA-4, 2B4, and CD160) have garnered attention due to their upregulation during chronic phase of infection and their possible role in immune suppression.

PD-1: A member of the B7:CD28 family of molecules, PD-1 was first studied in T cell hybridoma undergoing apoptosis and hence was named as programmed death-1. However, further studies have highlighted its association with immune exhaustion. In 2008, Kaufmann and Walker[115] elucidated the crucial part PD-1 essays in balancing CD4 and CD8 T cell activation and tolerance. Additionally, this molecule is expressed on an array of cells including natural killer (NK) cells, B cells and monocytes and binds to its ligand, PD-L1, expressed on APCs[116]. Barber *et al*[117] were the first to report the involvement of PD-1 in chronic viral infections and showed marked upregulation of messenger RNA encoding PD-1 using LCMV mouse models. Expression of PD-1 in LCMV specific CD8 T cells was further confirmed by antibody staining thereby establishing it a marker of T cell impairment. The findings of this study triggered interest in understanding the role of PD-1 in HIV infection. Day *et al*[118] studied the expression of PD-1 in 71 antiretroviral therapy (ART) naïve HIV-infected subjects and found heightened levels of PD-1 expression on both CD4 and CD8 molecules which in turn correlated positively with viral load and inversely with CD4 counts. Furthermore, on blocking PD-1, they found a marked rise in the HIV-1 specific CD4 and CD8 T cells.

The molecular expression of PD-1 in HIV specific CD8 T cells was investigated by Quigley *et al*[119] and they reported that the silencing of Basic leucine transcription factor (BATF), a transcriptional factor downstream of PD-1 receptor, was associated with recovery of IFN-γ secretion along with proliferation of HIV specific CD8 T cells. They also observed an augmentation of IL-2 secretion by CD4 T cells and have concluded that the downstream PD-1/PD-L1 signalling pathway is operative in exhaustion of T cell immune response to HIV. However, these studies failed to explain the association of immune exhaustion with immune activation. Sauce *et al*[120] revealed that the upregulation of PD-1 was accompanied by a concomitant increase in the expression of activation markers: CD38 or HLA-DR. They also proposed that such an increase in inhibitory and activation markers was restricted to antigen experienced cells rather than the naive population. On the contrary, not all exhausted cells express PD-1 indicating the presence of other inhibitory players in T cell exhaustion.

T-cell immunoglobulin and mucin domain-containing molecule-3 (TIM 3)

PD-1 is not the only inhibitory receptor expressed on exhausted T cells, a variety of other molecules including TIM-3, LAG-3, CTLA-4, CD160, CD244 and GP49 are also expressed. TIM-3 is one such inhibitory receptor that has recently been highlighted to play an important role in chronic HIV infection. TIM-3 was initially identified to be expressed on Th1 cells and not on Th2 cells in murine models[116]. Interaction of TIM-3 with its ligand galactin-9 induces CD4 T cell death. The inhibitory role of TIM-3 was confirmed by blockade of TIM-3 with small interfering RNA (siRNA) or antibodies. In HIV infected individuals, high expression of TIM-3 has been shown to correlate with markers of disease progression along with immune activation[121]. They also observed that the heightened expression of TIM-3 was more prominent in individuals with progressive acute and chronic HIV infection than in individuals who are able to control viraemia or remain uninfected. In another study it was observed that both TIM-3 and PD-1 were expressed synergistically on LCMV specific CD8 T cells. Also, the severity of immune exhaustion was more when both the markers were co-expressed as compared to singular expression of PD-1. Further on blocking both receptors Jin *et al*[122] recorded restoration of antiviral functions indicating possible therapeutic avenues. However, the role of both the receptors and their co-expression remains to be determined in case of HIV infection.

CTLA-4 and lymphocyte activation gene-3 (LAG-3)

Another negative regulatory receptor belonging to the B7-CD28 family is CTLA-4 which has been found to play an important role in autoimmunity[7]. Studies in human cohorts have also illustrated its role in tumour reduction and cancer treatment[123]. Like PD-1 and TIM-3, CTLA 4 expression was found to be elevated in HIV specific CD4 T cells[124]. Kaufmann *et al*[115] also demonstrated that CTLA-4 expression correlated directly with viral load and indirectly with CD4 T cell counts.

Like TIM-3, LAG-3 is preferentially expressed on Th1 cells and belongs to the immunoglobulin superfamily[125]. Investigations carried out in LCMV mice models have revealed elevated expression of LAG-3 and like other inhibitory markers, inhibition of LAG-3 resulted in proliferation and production of cytokines by Th1 cells in HIV infection[121]. Further investigations are

required to understand and establish the role of all the inhibitory receptors in T cell exhaustion.

Although, these studies have highlighted the importance of inhibitory receptors in chronic HIV infection, yet many alarming questions are to be answered: *(i)* whether immune exhaustion is a by-product of high antigen load or is it the cause?, *(ii)* is the concomitant expression of negative regulatory receptors along with high immune activation a possible regulatory mechanism by the host to control hyper-activation or is it a virus survival tactic?, and *(iii)* will blockade of inhibitory receptors - with or without therapeutic intervention- abet HIV-specific immune responses or place the immune system in an uncontrolled hyper activated state?

Regulatory cytokines

Apart from inhibitory receptors, regulatory cytokines such as IL-10 and TGF-β have been implicated in T cell exhaustion. LCMV studies have shown enhanced virus specific responses in the absence of IL-10 secretion[126]. In case of HIV infection elevated levels of IL-10 were shown to have considerable effect on CD41 T cell function[127]. Recent studies have shown the existence of a novel IL-10 producing HIV specific CD8 T cell population[122]. Jin *et al*[122] showed phenotypic similarities between IL-10 producing CD8 T cells and severely exhausted CD8 T cells co-expressing PD-1 and TIM-3. Said *et al*[128] studied the possible link between immune exhaustion and microbial translocation and reported upregulation of PD-1 along with plasma IL-10. Also on blocking the IL-10 and PD-1 pathways, viral clearance and improved T cell function were observed in animal models of chronic viral infection. Their findings suggest that translocated microbial products such as LPS result in the upregulation of PD-1 expression on monocytes and positively correlated with high levels of plasma IL-10. The data from this study highlight the need to explore the missing links between microbial translocation and exhaustion.

Conclusion

The vaccine trials have been a turning point in the field of HIV immunopathogenesis and have given enough reasons to re-evaluate our strategies to tackle HIV. The data reviewed here provide new insights into the dynamics of T cells and HIV. Additionally, these findings revealed unique strategies that the virus utilizes in the host system to sustain itself. One of the main pathways that HIV explores to deteriorate the immunity is chronic activation of the immune system. Though the review highlights involvement of multiple mechanisms that are likely to cause aberrant activation of T cells, the role of each factor and their relationship with each other are still incompletely understood and are a matter of debate and speculation. Chronic immune activation has been portrayed as the major driver of CD4 T cell depletion especially in gut associated lymphoid tissues; however, its association with this massive depletion is still ambiguous. Recently the focus has been shifted to investigate the effects of HIV on the intestinal epithelial barrier and studies have shown a link between immune activation and microbial translocation. The details of how infection and loss of intestinal CD4 T cells lead to this leaky gut are unclear, but multiple avenues of investigation have begun to be explored, including the role of Th17 cells and Tregs. Unlocking the role of Th17 and Tregs can provide important clues in unravelling the mystery of HIV pathogenesis.

The last few years have witnessed the exploration of newer avenues of research to understand the impact of HIV on the immune system, with one of these being innate immune system. Emerging data suggest activation of this compartment at an early stage but to what extent this innate activation affects the adaptive immune response and helps in establishment of sustained chronic activation needs to be investigated. Apart from this, immune activation has also been shown to be involved in increased turn over, homeostatic imbalance, exhaustion and apoptosis which represent a massive task for the immune system. To address all these issues, additional studies are required which would help us broaden our view on the dynamics of T cells and HIV.

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