Immunotherapy in HIV Infection

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Abstract

Substantial progress has been made in devising successful therapies against Human Immunodeficiency Virus (HIV) replication, and antiretroviral therapy (ART) can provide a sustained control of HIV replication. It is, however, associated with at best partial immune reconstitution, as well as lack of elimination of viral reservoirs. Both innate and adaptive immune cell compartments that suffer as a result of HIV replication, fail to recover completely under ART, hence, the need for lifelong therapy once infected. Novel therapeutic approaches are being tested at an encouraging rate and have the potential to improve the odds against the virus. Among them, immunotherapeutic approaches are one of the exciting areas and can be considered as adjunct to ART for improving immune competence. Cells of the innate immune system, including natural killer (NK) cells, gamma delta (γδ) T cells and natural killer T (NKT) cells have cytotoxic potential against viruses, and therapies including such effector cells will be useful. The fact that HIV infection results in a severe dysfunction in most of these effector subsets, warrants approaches that take this into consideration. One reason for the dysfunction of immune cells is the disruption of the common γ chain family of cytokines during HIV infection. These cytokines play vital role in regulating functional activities of such immune effector cells. Consequently, besides and along with cells, these cytokines including IL-2, IL-7, IL-15 and IL-21 have shown some promise against HIV infection. This review briefly summarizes the immune therapy options in HIV infection, with special focus on innate cells and cytokines.

Keywords: Immunotherapy; HIV; Antiretroviral therapy; T cells; Effector cells

Introduction

HIV is one of the most challenging pathogen as there is still no cure or successful vaccine available, despite the unprecedented research efforts. Immune control of HIV involves both cellular and humoral responses. Besides the classical MHC restricted cellular response orchestrated by CD8 and CD4 T cells, several other "killer" cells play an important role. NK cells, γδ T cells and NKT cells are the main cells in this latter category. Numerous research articles describe importance of NK cells in controlling HIV, as well as their dysfunction during infection [1-5]. Clearly, NK cells are potent cytotoxic cells, and strategies for improving their function in HIV infected patients will be valuable. Another cellular subset with potential is NKT cell. These natural killer T cells are a subset of T lymphocytes that express NK cell markers, bridge innate and adaptive immune responses, and have potent immunoregulatory properties. A subset of NKT cells, termed invariant NKT cells (iNKT), express a highly restricted T cell receptor (TCR), and respond to CD1d-restricted lipid ligands. Studies have shown a role for these iNKT cells in controlling immune activation during HIV and SIV infections [6]. Recent advances in NKT ligand discovery pave way for use of NKT cell-specific agonists as immunotherapeutic adjuvants to vaccine or therapies [7]. γδ T cells also play a critical role in linking innate and acquired immunity. Various studies report a dysfunction in gamma delta T cells during HIV infection [8-10], and *in vitro* studies show a cytotoxic effect of these cells on HIV infected cells [10]. Approved drugs for stimulating this subset of killer lymphocytes are already being tried for cancer therapy and have potential against viral infections, including HIV.

A challenge to utilizing these effector cells as therapeutic option in HIV infection is their severe depletion, and/or dysfunction caused by the virus. Antiretroviral therapy, if started very early, will likely limit the damage and also results in partial restoration in effector cell function. The common γ chain cytokines with a potent stimulating effect on these cytotoxic cells have been used to partly improve their function. The fact that HIV infection causes a dysregulation in these cytokines means that exogenous supplementation will result in restoration of the cellular subsets affected by them. Studies in HIV patients, as well

as rhesus macaque SIV model, point to a beneficial effect on effector or memory immune cell reconstitution using these cytokines [11,12]. A combination of ART and innate immune cell stimulation has the potential to be more effective against the virus.

Innate Immune Cells and HIV Infection

'Classical' T cells recognize antigenic peptides bound to major histocompatibility complex (MHC) molecules. Besides classical αβ T cell response, cytotoxic lymphoid cells of innate immune system are involved in HIV pathogenesis and control. These cytotoxic cells are adversely affected by virus infection and consequently, the host capacity for effective virus control goes down.

NK cells are the most studied subset among these innate cytotoxic effectors. NK cells recognize target cells lacking self-MHC, and their function is positively or negatively regulated through a range of HLA-specific and non-HLA-specific receptors. NK cells recognize HIV infected cells by both activating and inhibitory killer immunoglobulin-like receptors (KIRs) [4,13]. Recently, it was shown that NK cells mediate direct antiviral immune pressure on HIV [13]. Researchers found that particular variants of HIV viral proteins are recognized by specific NK cell KIR genes, suggesting that HIV mutates in response to such NK-cell-mediated immune pressure. This is the first time such immune pressure on HIV evolution is reported to be exerted by cells of innate immune system. Excellent reviews on role of NK cells in HIV infection are available [5,14,15]. Functional defects in NK cells during HIV infection are also well documented [3,16-18]. An expansion of the functionally defective CD56$^-$/CD16$^+$ population of NK cells in

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viremic patients was reported to be responsible for impaired function of NK cell population [3,19]. HIV infection is accompanied by an alteration in activating and inhibitory KIRs, and resulting defects in cyotoxic capacity of these cells [3]. Both NK cell loss and chronic NK cell activation occur as a result of HIV viremia [2], and ART has partial effect on NK cell restoration [14].

NKT cells express NK-like and T cell like recognition structures and respond to lipid antigens. A subset of them termed 'invariant' NKT, has a restricted T cell repertoire.

iNKT cell frequency is significantly reduced among HIV-1 positive individuals [20], with a specific depletion of the CD4$^+$ iNKT subset, compared to the CD4$^-$ subset. The iNKT subset displays functional impairment, even if the numbers are not severely depleted during HIV, where these cells exhibit reduced proliferation and IFNγ, TNFα, and IL-4 secretion in response to stimulation with iNKT ligand [6,21]. Antiretroviral therapy has a variable effect on restoration of iNKT numbers and function [22].

We reviewed recently the impact of HIV on γδ T cells and potential strategies for exploiting Vδ2+ gamma delta T cells in HIV disease [23]. These cells are present at relatively low frequency of 1-7% in normal human peripheral blood. These cells express the non-classical γδ T cell receptor (TCR), with majority of cells expressing variable Vδ2 segment associated with Vγ9 segment [24], and are therefore, referred to as Vδ2 T cells for purpose of brevity. These Vδ2+ cells recognize phosphorylated nonpeptidic microbial metabolites *like* isopentenyl pyrophosphate (IPP) and aminobiphosphonates [25,26]. These compounds directly trigger T cells expressing the Vγ9Vδ2 TCR, without the need for antigen processing and presentation, allowing for a very rapid response to microbial immune challenge. HIV infection leads to major dysfunction, in both numbers and functions of these cells [27,28]. In HIV infected humans, these cells are severely depleted in peripheral blood. Since these cells don't express the HIV receptor CD4 and hence, are not directly infected by the virus, their depletion mechanism has long been subject of research. Very recently, it was shown that HIV binds the CCR5 and α4β7 co-receptors present on these cells and signal through CCR5 to cause cell death [29], providing one explanation for their depletion during infection. ART has minimal effect on restoration of cell numbers or proliferative responsiveness of this subset [27].

Both NKT and γδ T cells share common features, such as expression of antigen receptors of limited diversity, lack of MHC restriction, expression of NK like receptors and rapid release of cytokines following stimulation. There are also shared cytokine signaling pathways among these innate cell types, e.g. all the innate cells described here have receptors for common γ chain cytokine family members, and respond to stimulation with these cytokines.

Cytokine Effector Cell Activation and Therapeutic Potential

Antiretroviral therapy has been very successful in controlling virus replication, resulting in near normal life spans for continuously treated patients. However, the deleterious impact of HIV infection on immune cells is profound and despite prolonged ART, virus-specific and innate immune cells are not fully restored. Additionally, ART does not tackle the latent virus reservoir. Other interventions to improve immune responsiveness of these effector cells are being explored as a result. Immunotherapy with common γ chain cytokines is a promising approach in this direction. These cytokines, *viz.* IL-2, IL-7, IL-15 and IL-21 are main regulators of T cell homeostasis, and hence, are good immunotherapeutic candidates. During HIV infection, disruption of signaling pathways of these cytokines results in a deleterious effect on immune cell function [30]. Down-regulation of common γ(c) subunit was shown to occur during SIV infection of macaques [31], and could be related to inhibited immune cell functions. Thus, using these cytokines as adjunct to ART is likely to promote immune reconstitution in HIV infected patients.

IL-2 therapy has been extensively tested in multiple clinical trials, but no clinical benefit of this cytokine on HIV disease was found. IL-7, IL-15 and IL-21 have showed some promise, and are under investigation. Some selected clinical trials testing these cytokines in HIV infection are presented in table 1. For most of the cytokines, clinical trials are not concluded/not done, however, data from laboratory research using *in vitro* systems or animal models has been relatively promising.

IL-2 therapy in HIV infected individuals resulted in peripheral expansion of naive and memory CD4 T cells [32]. Data from clinical trials show some immune restorative effect of IL-2 on CD4 T cells; however, no effect on CD8 T cells or any clinical benefits were observed [11,33]. IL-2 therapy, as an adjunct to vaccines, has been tested in animal models like SIV infection of rhesus macaques, and resulted in both increase in anti-viral immunity and slower disease progression [11,34]. Another study testing the effect of IL-2 adjunct therapy with antiretroviral treatment in HIV infection [35], found that both NK and NKT cells expanded during IL-2 treatment. The functional response of NKT cells was not boosted, whereas NK cell showed expansion of the CD56^{dim} effector subset and enhanced IFNγ production.

IL-7 has effects on these immune cells, varying from activation to cell survival and maintenance. It promotes survival of CD56^{bright} NK cells, and inhibits apoptosis by promoting BCl-2 production [36], and maintains NKT cells. Recently IL-7 was shown to induce IL-17 production in Vδ2 T cells [37]. Use of IL-7 in HIV infection is also tested. Whereas there was no effect observed on levels of viral replication in ART naive and ART treated SIV infected macaques [38], IL-7 therapy induced proliferation of CD4 and CD8 subsets [39,40].

Clinical trial identifier (name)	Cytokine	Remarks
NCT00004978(ESPRIT)	IL-2 in ART patients	Improved absolute CD4 cell counts; no clinical benefit over ART group; serious side effects
NCT00013611(SILCAAT)	IL-2 in ART patients	Improved CD4 T cell counts; no clinical benefit over ART group; serious side effects
NCT00477321(Inspire)	IL-7 in ART patients	rhIL-7 at 20 ug/kg is well tolerated and resulted in CD4 and CD8 T cell increase and broadening of TCR diversity
NCT01190111(Inspire 2)	IL-7	Ongoing
NCT01241643(Inspire 3)	IL-7 in immune non-responders	Ongoing
NCT00115960	HIVgag DNA with IL-15	IL-15 tested as adjuvant to HIV gag vaccine. Minimal response to DNA vaccine alone and no augmentation with cytokine observed
NCT00775424	Pennvax-B with IL-15	IL-15 tested as adjuvant to an HIV pol gag env DNA vaccine; no improvement in IFNg or IL-2 producing CD4 or CD8 T cells with IL-15

Table 1: Clinical trials using common γ chain cytokines in HIV infection.

Recombinant human IL-7 treatment in antiretroviral-treated HIV-infected persons led to a dose-dependent CD4 T cell increase, as well as broadening of T cell receptor diversity in a phase I/II trial [41].

IL-15 therapy has shown more promise for improving anti-viral immunity, as well as memory response. IL-15 has a contrasting effect on HIV replication during acute versus chronic stages. Administration of IL-15 during acute SIV infection in ART naive animals resulted in significant increase in viral replication [42], whereas there was no impact on viral replication, when IL-15 was administered during chronic stage. IL-15 treatment, however, improved SIV specific CD8 T cells, as well as NK cells in all stages [42]. Procedures using IL-15 to obtain large scale therapy grade NK cells are described [43]. Considering sometimes harmful or not potent beneficial effects of these therapies, it is suggested that cytokine therapy may be used as adjunct to vaccine candidates. The animal studies utilizing IL-15 co-administration with SIVgag vaccine shows variable results [38,44]; however, at least one study shows a clear benefit of IL-15 co-administration, resulting in more rapid reduction in SIV viral load and for improving vaccine induced immunity [44]. IL-15 can also potentially be used as immune modulator for improving CD8 T cell effector response in patients whose viral loads are effectively suppressed by ART.

IL-21 also has stimulating effect on immune effector cells. IL-21 was shown to regulate the differentiation of a human γδ T subset that supports antibody production by B cells [45,46]. NKT cells are known to be both producers of IL-21 and respond to IL-21 [47]. IL-21 enhances NK and NKT cell granzyme B expression, and some inhibitory NK receptors, including Ly49C/I and CD94, as well as NKT cell cytokine production in response to anti-CD3/CD28 *in vitro* [48]. IL-21 has shown some beneficial effects against HIV infection. IL-21 administration in chronically SIV infected macaques showed benefit for cytotoxic and B cells [49]. Although no effect on plasma viral loads was observed, IL-21 therapy resulted in increase in virus specific IFNγ+CD107a+CD8 T cells, CD27+ memory B cells and NK cells. In a study of HIV elite controllers (individuals who control virus replication in absence of therapy), IL-21 producing HIV specific CD8 T cells were higher in these rare individuals compared with other HIV infected groups [50], suggesting they play role in effective virus control. In fact, another study shows that IL-21+ HIV specific CD4 T cells modulate HIV-specific CD8 T cells function and contribute to effective virus control [51].

Use of these cytokines alone or in combinations for stimulation of innate cells to be used as adoptive transfer therapy can also be considered. While these cytokines stimulate NK, NKT and CD8 T cells, stimulating the γδ T cell subset will require additional antigens, to which these cells respond. Human Vδ2 subset of γδ T cell can be readily expanded using combinations of aminobisphosphonate drugs (FDA approved for treatment of osteoclastic bone resorption), and cytokines such as IL-2 or IL-15 [52]. Several preclinical and clinical studies utilizing such γδ T cell activation seek to activate direct cytotoxicity against tumor cells. In another approach, therapeutic tumor or viral antigen specific monoclonal antibodies can be used in combination with activated killer cells for killing through ADCC [53]. IL-21 therapy has been shown to improve Trastuzumab- and Cetuximab mediated ADCC by NK cells against squamous cell carcinoma [54]. For such strategies to succeed, however, the critical factors remain the frequency of γδ T cell in peripheral blood of HIV infected patients, and their responsiveness to stimulants. As discussed above, during HIV infection, there is significant impact on Vδ2 T cell phenotype and function, with significantly low frequency of this subtype present in infected individuals, along with poor responsiveness

to natural gamma delta ligands like IPP [27]. We showed recently that aminobisphosphonate (Zoledronate), but not IPP, can partly restore Vδ2 T cell expansion from HIV infected patients, and these cells have functional capacity [55]. Whether *in vivo* activation will be sufficient to raise the effector activity remains to be tested in clinical studies. It is likely that for clinical studies, selection of patients will be required on the basis of their initial γδ frequency and phenotype, to test patients most likely to have benefit from such therapies. One area where such cellular activation may be useful is for designing combination therapeutic vaccines, involving γδ T cells activation, in conjunction with traditional vaccine antigens. This may have superior therapeutic effects, in comparison with the peptide-based vaccines alone.

Conclusion

Innate immune cells like NK, γδ T cells and NKT cells are potent cytotoxic effectors, and can inhibit HIV replication through direct or indirect cytolytic and noncytolytic pathways. These antiviral innate effector cells are depleted or dysregulated in HIV-infected individuals with chronic disease, but may partly recover after successful antiretroviral therapy. The use of common γ chain cytokines to stimulate these cells has shown some promise against HIV replication and reconstitution of some effector cell components. While IL-2 therapy alone is not likely to garner more support due to negative results in major clinical trials, there is optimism for use of IL-7, IL-15 and IL-21. Such approaches most likely will benefit individuals whose viral load are relatively low, in the absence of therapy, or are suppressed by antiretroviral drugs. Also, there is potential for these therapies, as an adjuvant to peptide vaccine. In future, it can be possible to tackle the latent reservoir in such patients, using combinations of these approaches. Our research is aimed at development of therapeutic strategies, combining innate cell stimulating compounds and antiretroviral drugs, to optimally stimulate immunity.

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