Published in final edited form as:

Immunol Res. 2013 December ; 57(1-3): 279–291. doi:10.1007/s12026-013-8457-0.

Interleukin-21 and T follicular helper cells in HIV infection: research focus and future perspectives

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Abstract

Interleukin (IL)-21 is a member of the γ chain-receptor cytokine family along with IL-2, IL-4, IL-7, IL-9, and IL-15. The effects of IL-21 are pleiotropic, owing to the broad cellular distribution of the IL-21 receptor. IL-21 is secreted by activated CD4 T cells and natural killer T cells. Within CD4 T cells, its secretion is restricted mainly to T follicular helper (Tfh) cells and Th17 cells to a lesser extent. Our research focus has been on the role of IL-21 and more recently of Tfh in immunopathogenesis of HIV infection. This review focuses on first the influence of IL-21 in regulation of T cell, B cell, and NK cell responses and its immunotherapeutic potential in viral infections and as a vaccine adjuvant. Second, we discuss the pivotal role of Tfh in generation of antibody responses in HIV-infected persons in studies using influenza vaccines as a probe. Lastly, we review data supporting ability of HIV to infect Tfh and the role of these cells as reservoirs for HIV and their contribution to viral persistence.

Keywords

IL-21 and T cells; Tfh cells; IL-21 and B cells; HIV persistence; HIV and IL-21; IL-21 and immunity

Introduction

Interleukin-21 (IL-21) belongs to the family common γ chain (γ_c) cytokines that include IL-2, IL-4, IL-7, IL-9, and IL-15, all of which utilize the γ_c (CD132) in their receptor complexes for regulating multiple innate and adaptive immune responses. IL-21 is produced by CD4 T cells, in particular T follicular helper (Tfh) cells, T helper 17 (Th17) cells, and also by natural killer T cells [1–4], and by CD8 T cells under certain conditions [5, 6]. The IL-21 receptor (IL-21R) is a heterodimer composed of the common γ_c and the unique IL-21R, and is expressed on a broad range of lymphoid tissues including spleen, thymus, and lymph nodes [2, 7, 8], and less often in cells from lung and small intestine. In lymphocytes, IL-21R is constitutively expressed on T, B, and natural killer (NK) cells with the highest expression on B cells. T cells express low levels of IL-21R that increase upon T cell receptor (TCR) stimulation. Constitutive expression of IL-21R has been reported on

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Conflict of interest The authors have no financial conflicts of interest.

dendritic cells (DC), macrophages, fibroblasts, and epithelial cells [9–12]. This wide range of expression of IL-21R explains the pleiotropic effect of IL-21 in the regulation of immune response. The binding of IL-21 to its receptor leads to the activation of the Janus kinase family proteins (JAK) 1 and 3. Downstream of JAK recruitment, IL-21 mainly activates signal transducer and activator of transcription (STAT) 3, and to a weaker and more transient degree, STAT1, STAT4, and STAT5 leading to the activation of Akt and MAPK pathways [8, 13].

Based on their immunomodulatory properties, several γc cytokines, notably IL-2, IL-7, IL-15, and IL-21 have been investigated in the context of acute and chronic phases of simian immunodeficiency virus (SIV) or human immunodeficiency virus (HIV) infection [14–24]. For example, administration of rIL-7 to uninfected or SIV-infected Rhesus macaques (RM) demonstrated alterations in T cell homeostasis [20–22] with no effect on SIV replication [22]. In a recent study, IL-7 administered during the acute phase of SIV infection was found to be effective in preventing decline of circulating naïve and memory CD4 T cells [25]. In humans, IL-7 therapy has shown promise for immune reconstitution [26–29]. Administration of rIL-15 to healthy RM was found to increase the frequency of long-lived effector memory CD4 and CD8 T cells [17]. In chronically SIV-infected RM, rIL-15 augmented effector memory CD8 T cells without reduction in viral replication in chronic infection [18, 19], whereas in acute SIV infection, rIL-15 administration resulted in increased peak viremia, which was attributed to increased CD4 T cell activation and proliferation [30]. We chose to study IL-21 in the context of HIV/SIV infection because of its wide range of target cells, its interesting effects in clinical trials in certain human cancers [31, 32], and because it was known to be produced by CD4 T cells, the main cellular target of HIV infection.

IL-21 and modulation of T cells and NK cell cytotoxic properties in HIV infection

Our group was the first to initiate research with IL-21 in the HIV/AIDS field [33]. Based on the T cell-potentiating properties of IL-21 that had been described in tumor models [31, 32], we hypothesized that IL-21 could augment the effector function of CD8 T cells in patients with HIV infection. We found that indeed ex vivo treatment of peripheral blood mononuclear cells (PBMC) with IL-21 resulted in augmented cytotoxic properties of CD8 T cells of HIV-infected patients with HIV RNA of < 50 copies/mL and CD4 counts > 200 cells/mm³ [33]. Cytotoxic molecules such as perforin and granzyme B were upregulated in memory and effector CD8 T cell subsets and in HIV gag antigen-specific CD8 T cells. Unlike other γc cytokines like IL-15 or IL-2, treatment with IL-21 resulted in selective increase in cytotoxic molecules in CD8 T cells without driving their proliferation or degranulation.

To further understand the mechanisms by which IL-21 increases the cytotoxic molecules on CD8 T cells in HIV infected patients and modulates their cytotoxic potential, we performed in vitro studies using CD8 T cells from healthy volunteers that were pre-activated via T cell receptor signals to mimic their heightened state of immune activation in HIV-infected patients [34]. We found that IL-21 upregulates the cytotoxic effector function and the

expression of the costimulatory molecule CD28 on CD8 T cells. The acquisition of cytotoxic molecules in IL-21-treated CD8 T cells was accompanied by sustained expression of surface markers characteristic of memory T cells (i.e., CD27, CD28, and CD127). By gene expression analysis, we further showed that IL-2 and IL-21 operate different transcriptional regulation of perforin and granzyme B induction in CD8 T cells; IL-2 orchestrated the expression of cytotoxic effector genes RUNX3 and EOMES, while IL-21 only induced EOMES [34]. Furthermore, although IL-21 and IL-2 induced mRNA expression of GZMB and PRF1 equally, IL-21 promoted higher accumulation of granzyme B and perforin at the protein level, suggestive of a post-transcriptional regulation mechanism, perhaps similar to that described for perforin expression by NK cells [35]. We also found that CD8 T cells treated with IL-21 *ex vivo* displayed augmented antiviral activity comparable to that induced by IL-2, but unlike IL-2 it did not induce HIV-1 replication *in vitro* in CD4 T cells [34].

HIV replication requires CD4 T cell activation. For a molecule to be considered for immunotherapy, it is important to demonstrate that it does not enhance viral replication. Modulation of cytotoxic molecules without induction of generalized cellular activation by IL-21 provided a rationale for investigating immunotherapeutic properties of IL-21. Our results have been confirmed and expanded by several other studies, showing the importance of IL-21 in induction, maintenance, survival, and cytotoxicity of CTL during HIV infection [6, 36–38]. Collectively, these studies favor the concept that IL-21 can modulate the phenotypic and enhance antiviral properties of CD8 T cells that may be impaired during HIV-1 infection. A cytokine that has been used as an adjuvant in many vaccine trials is IL-12 [39–43] and has been tested for its immunomodulatory benefits during chronic SIV infection [44, 45]. IL-12 is a potent inducer of IL-21 [46–48], and it is possible that some beneficial effects of IL-12 can be mediated in part indirectly by the induction of IL-21 [47].

In addition to CD8 T cells, we found that cytotoxic properties of human NK cells were also profoundly influenced by exposure to IL-21 *ex vivo*. NK cells are important components of the innate arm of the immune response and have been shown to mediate cytotoxic activity against infected CD4 T cells in very early stages of HIV infection [49, 50]. These cells express IL-21R and can be influenced by *in vitro* exposure to IL-21 [51]. IL-21R is equally expressed on all NK cell subsets, as defined by the differential expression of CD16 and CD56. We analyzed the effects of IL-21 on resting peripheral blood NK cells in chronically HIV-infected individuals. We studied the *in vitro* effects of IL-21 on perforin expression, proliferation, degranulation, IFN- γ production, cytotoxicity, and induction of STAT phosphorylation in NK cells [51]. Our data indicated that the CD56dim subset of NK cells, which is preferentially dependent upon IL-21, is reduced during HIV infection. *Ex vivo* treatment with IL-21 enhanced the responses of NK cells from HIV-infected subjects by stimulating perforin production in a STAT3-dependent manner. IL-21 could also enhance HIV-specific antibody-dependent cell-mediated cytotoxicity, secretory, and cytotoxic functions, as well as the viability of NK cells from HIV-infected persons [38]. IL-21-activated NK cells were found to inhibit viral replication when co-cultured with HIV-infected autologous CD4 T cells in a perforin-dependent manner [38]. IL-21 activates STAT3, MAPK, and Akt to enhance NK cell functions [38, 51]. Together, these studies of immunomodulatory properties of IL-21 resulting in augmentation of virus-specific CD8 T

cells and NK effector functions in chronically HIV-infected individuals point to the potential utility of IL-21 for immunotherapy or as a vaccine adjuvant.

IL-21 as an immunotherapeutic agent: administration of IL-21 in vivo

The therapeutic utility of IL-21 has been currently investigated in a number of malignant disorders and in viral infections (reviewed in [52–55]). In human clinical trials, therapeutic benefits of IL-21 have been reported in patients with metastatic renal cell carcinoma, metastatic melanoma, and relapsed/refractory indolent non-Hodgkin's lymphoma, with demonstrable antitumor activity (reviewed by Hashmi and Van Veldhuizen [56]). In phase I and phase IIa studies in patients with metastatic melanoma, administration of IL-21 was well tolerated and resulted in increases in CD8 T cells and NK cells expressing mRNA for IFN- γ , perforin, and granzyme B [57–60]. Role of IL-21 in regulating the immune responses has been reported in both acute [61–64] and chronic [65–68] viral infections in mice and also in chronic hepatitis B and C virus infections [69–77] in humans.

Our group investigated the effects of IL-21 administration to SIV-infected rhesus macaques. To evaluate safety, biological activity, and immunomodulatory effects of IL-21, we conducted a pilot study of recombinant mamuIL-21 (rMamuIL-21) administration in chronically SIV-infected RM [24]. IL-21 was well tolerated up to the highest dose of 100 μ g/kg body weight, and enhanced the expression of cytotoxic molecules perforin and granzyme B in T cells and NK cells within 48 h. After each dose of IL-21, increases were noted in frequency and median fluorescence intensity of granzyme B and perforin expression in memory and effector subsets of CD8 T cells in peripheral blood, in peripheral and mesenteric lymph node cells, and in peripheral blood memory and effector CD4 T cells. Consistent with our prior observation in IL-21 treated cells of HIV-infected individuals [33], administration of IL-21 to SIV-infected RM did not induce T cell activation, proliferation, or increase the levels of plasma virus load [24]. In addition to augmentation of cytotoxic molecules in CD8 T cells, we also noted an increase in memory B cells and SIV-specific Ab response in IL-21-treated animals. Thus, this first in vivo IL-21 study in chronically SIV-infected animals provided evidence that IL-21 could modulate the immune responses in chronic SIV infection without having any adverse effect on the markers of disease progression.

To analyze the effect of IL-21 during the early stages of SIV infection, we conducted another study in which IL-21 was administered during acute SIV infection [23]. We infected 12 RM with SIV-mac239, and at day 14 postinfection, six of the animals were treated with five weekly subcutaneous doses of 50 μ g/kg rhesus rIL-21-IgFc, which represents a dosage similar or lower to that tested in phase I and II clinical trials in humans. Thus, IL-21 treatment was safe and did not increase plasma viral load or result in systemic immune activation. Consistent with our observation in chronic SIV-infected animals [24], acutely infected RM showed higher expression of perforin and granzyme B in total and SIV-specific CD8 T cells with IL-21 treatment in comparison with untreated animals. IL-21 treatment increased the levels of intestinal Th17 cells along with reduced levels of intestinal T cell proliferation, microbial translocation, and systemic activation/inflammation in the chronic phase of SIV infection without T cell exhaustion or undesirable effects on viral load [23]. In

addition, IL-21 also increased the frequencies of memory B cells. Results of this study are significant for HIV immunopathogenesis research, as any immunotherapeutic modality that preserves Th17 cells in the gut mucosa can potentially minimize gut microbial translocation and ultimately prevent immune activation and disease progression. Overall, our data demonstrate that IL-21 treatment in SIV-infected RM can improve mucosal immune function through enhanced preservation of Th17 cells. However, further studies are needed to extend this observation to see whether IL-21 given prior to SIV infection can modulate the course of acute SIV infection and whether it can be used as a suitable adjuvant in vaccines to bolster cellular and humoral immunity.

T follicular helper (Tfh) cells and IL-21 in humoral immune responses

Several investigators have performed extensive studies to elucidate T-B cell interaction in lymph nodes that leads to antibody responses. Germinal centers (GC) are sites within lymphoid follicles in secondary lymphoid organs that are critical for the generation of T-dependent antibody responses mediated via cooperative interaction between resident B cells and Tfh cells (reviewed in [78, 79]). Tfh cells in GC are characterized by the high expression of the transcriptional regulator Bcl-6, surface expression of follicular homing receptor CXCR5, and co-stimulatory molecules like ICOS, CD40L, and PD-1, with secretion of their signature cytokine, IL-21 [80–83]. Upon receiving cognate T cell help, B cells might undergo three potential fates. They can differentiate into an extrafollicular focus of antibody-secreting plasmablasts, or form a GC within the B cell follicle, or differentiate into early recirculating memory B cells [84–86]. As shown in Fig. 1, early humoral immune responses occur in extrafollicular areas of lymphoid tissues where B cells differentiate into short-lived extrafollicular plasmablasts (ePB). Following interactions with extrafollicular helper T cells (eTh), ePB produce low-affinity antibodies that are predominantly short-lived. Similar to Tfh cells, the function of eTh is mediated by CD40L, ICOS, and IL-21 and they do not express high levels of CXCR5 [87, 88]; rather, they show higher CXCR4 expression [89]. eTh cells might also give rise to GC Tfh cells following interactions with cognate B cells [90]. However, the quality of the immune responses is different between these two pathways. With the help of extrafollicular Th cells, ePB produce low-affinity antibodies that are predominantly short-lived, whereas the end product of immune response after a GCs reaction is the production of high-affinity Abs along with the generation long-lived PCs and memory B cells with antigen affinity. During GC reaction, antigen-activated Tfh cells interact with antigen-primed B cells via unique surface molecules that regulate Tfh differentiation and function and through the production of cytokine IL-21 (Fig. 1). Although the importance of Tfh cells for B cell differentiation and function was initially described for Tfh cells residing within GC, the role of Tfh B cell interaction in the T cell zone–B cell zone border of the GC is also under scrutiny [91–93].

Association of IL-21 in the regulation of Tfh cell differentiation was first identified by microarray profiling of human Tfh cells [94]. Experiments using IL-21 reporter mice have shown that among Tfh cells, about one-third of the population express IL-21 in the context of a T cell-dependent immunization [95]. IL-21 can also induce c-Maf, thus providing a positive self-regulatory loop that maintains IL-21 expression in Tfh cells [96]. In addition, IL-21 can induce Bcl6 expression in Tfh cells [4, 97]. Expressions of Bcl6 and cMaf on Tfh

cells are important for the induction of migration genes of Tfh cells that control homing to the lymph nodes, namely CXCR4, CXCR5, CC-chemokine receptor (CCR)7, and genes that are involved in T–B interactions including CD40L, ICOS, CXC chemokine ligand (CXCL)13, the SLAM-associated protein (SAP), and programmed death (PD)-1 receptor [98].

Tfh-derived IL-21 orchestrates many aspects of B cell differentiation and function, GC development and maintenance, and development of memory B cells and plasma cells (PC) [81, 87, 93, 99]. Within GC, IL-21 promotes persistence of GC B cells through the upregulation of Bcl6 in GC B cells [81, 87]. In GC, IL-21 is important in developing and maintaining the GC reaction by regulating Bcl6 expression on both B cells and Tfh cells [81, 97, 100]. IL-21 plays an important role in vaccine-induced humoral immune responses by regulating B cell function. IL-21 is capable of inducing B cell proliferation, differentiation, class switching or death, depending upon the type of antigenic stimulation and accessory signals [7, 101], making the IL-21-elicited signaling the most important among all the γ_c cytokines for long-lived humoral immunity [83, 102]. Naïve human B cells are efficiently induced to secrete immunoglobulin via IL-21 following cognate T–B interaction [103, 104]. Recently, expression of the IL-21R on B cells was shown to be critical for the development of memory B cells [105]. IL-21-elicited STAT3 activation induces B cell maturation with the expression of PC-associated genes, phenotypic PC formation, and antibody secretion [87, 103, 106–109]. In experiments conducted ex vivo, IL-21 is capable of driving PC differentiation and IgM, IgG, and IgA antibody production from human splenic GC B cells, [108]. Consistent with these observations, the importance of IL-21 in developing humoral immune responses has been reported in humans with severe combined immunodeficiency [83].

HIV infection significantly alters several aspects of distribution of B cell subsets and B cell function, resulting from excessive B cell activation and impaired survival (reviewed in [110–113]). Following the initiation of effective antiretroviral therapy (ART), most of the B cell phenotypic alterations revert back to normal but the CD27⁺ resting memory B cells remain significantly decreased in comparison with healthy uninfected donors, despite virologic control and CD4 T cell recovery [114, 115]. In a study by Van Grevenynghe et al., lower memory B cell survival from HIV-infected subjects was attributed to disrupted IL-2 and IL-21 signaling leading to increased Foxo3a transcriptional activity and increased expression of its pro-apoptotic target TRAIL [111]; results from this study support the concept that IL-21 could rescue the memory B cell from apoptosis in HIV-infected people and thereby benefit the overall humoral immune responses by supporting the persistence and survival of memory B cells during HIV infection [116, 117]. The fact that IL-21 production is mainly stimulated by the activation of Tfh cells, it is important to determine whether the B cell defects in HIV-infected patients are secondary to a deficiency in the CD4 Tfh cell compartment and if it can be reversed by IL-21.

Peripheral Tfh cells (pTfh)

The fate of the GC Tfh after their interaction with B cells is not known. Although widely believed that they undergo apoptosis in the GC, recent data suggest that they are capable of

exiting the GC to generate a heightened memory cell response [118, 119]. Persistence of Tfh cells within the lymph nodes several weeks after immunization has been reported [118]. However, as antigen persisted in this model, it was not clear whether those cells were true memory cells. Later studies conclusively demonstrated the generation of memory Tfh cells after GC reaction [120, 121].

It was recently demonstrated that human peripheral CXCR5⁺ memory CD4 T cells share functional properties with the GC Tfh cells [122–127], such as the ability to secrete IL-21 and to induce autologous naïve and memory B cell subsets to produce immunoglobulins [104, 128]. We observed that the pTfh cells displayed a memory phenotype with the characteristics of central memory cells [128]. Marshall et al. also observed that a subset of memory cells with similar phenotypic features as Tfh cells existed within the memory cell pool, and these CXCR5⁺ memory cells enhance the kinetics of B cell expansion and class switching, and suggested that such CXCR5 expression on memory cells promotes migration to areas where encounter with cognate B cells can occur following reintroduction of antigen [129, 130]. Notably, these peripheral CXCR5⁺ CD4 T cells are absent in circulation of patients with ICOS deficiency [131] who also lack GC.

In HIV infection, a recent study has identified a resting memory subpopulation of circulating memory PD-1⁺CXCR5⁺CD4⁺T cells to be most closely related to bona fide GC Tfh cells by gene expression profile, cytokine profile, and functional properties [132], and also demonstrated that frequencies of PD1⁺CXCR5⁺CXCR3^{neg}pTfh cells directly correlated with broad neutralizing Ab development. We have noted that in HIV infected treatment naïve patients, 12 months of combination antiretroviral therapy (cART) incorporating raltegravir was highly effective in CD4 T cell immune reconstitution [133]. These patients manifested increase in central memory CD4 T cells and pTfh cells as well as IL-21 producing CD4 T cells after phorbol 12-myristate 13-acetate (PMA) + Ionomycin stimulation implying improved pTfh function. In well-controlled HIV infection, pTfh frequency was found to be equivalent in patients and healthy controls (HCs) [128]. We have investigated pTfh and their relationship to serologic responses to influenza vaccination in HIV-1-infected patients [128].

HIV infection and B cell responses: role of IL-21 and pTfh

We have utilized influenza vaccination as a probe to investigate the immune competence of pTfh cells and B cells in HIV infection. We started these studies at the time of the 2009/ H1N1 pandemic influenza outbreak [128, 134, 135] in virally suppressed HIV-infected adults on combination ART and healthy controls of similar age and with similar frequencies of B cells. Blood was drawn at pre-, 1, and 4 weeks post-vaccination. At 4 weeks post-vaccination, only 50 % patients were classified as vaccine responders based on Ab titers of > 1:40 and > 4-fold increase in Ab titers, whereas 100 % of healthy controls were responders. Pre-vaccination CD4 and CD8 T cell counts and plasma HIV RNA were not different between the responder and non-responder patients. Characteristic immunologic findings related to vaccine responses in this cohort [128, 134, 135] are summarized in Table 1. The upregulation of IL-21/IL-21R in B cells and CD4 T cells in the vaccine responders corresponded with development of plasmablasts and memory B cells suggesting that

responsiveness to IL-21 was important for the Ab response. Patients who did not respond to the H1N1/09 vaccine failed to develop these vaccine-induced characteristic B cells changes.

In vaccine responders, pTfh cells underwent expansion with secretion of IL-21 and CXCL13 in H1N1-stimulated PBMC culture supernatants at week 4 (T2) post-vaccination. These changes were not seen in vaccine non-responders. In purified pTfh and B cell co-culture experiments, pTfh cells supported H1N1Ag-stimulated IgG production by autologous B cells only in vaccine responders. At T2, frequencies of pTfh were correlated with memory B cells, serum H1N1 Ab titers, and Ag-induced IL-21 secretion. Our results showed for the first time a role of pTfh cells in inducing vaccine-induced immune response and indicate that the expansion of pTfh could be considered as a biomarker for ensuing immune response following vaccination. Consistent with our findings, a later study by Bentebibel and colleagues found that a small population of activated ICOS⁺CXCR3⁺CXCR5⁺ cells transiently appear in human blood after influenza vaccination and that these cells correlate with influenza antibody titers [136]. Importantly, as mentioned earlier, a recent study indicates that the frequency of pTfh correlated with the development of bnAbs against HIV in a large cohort of HIV infected individuals [132]. Taken together, these studies support the concept that Tfh cells exist as memory cells in the periphery. As pTfh cells are easily accessible from peripheral blood their utility as surrogate Tfh biomarkers needs to be investigated. Our data support the concept that pTfh cells could be used as a tool for studying the relationship between Tfh and B cells in generation of immune responses. Studies using lymph node Tfh and peripheral blood Tfh cells pre- and post-immunization are needed to conclusively establish the relationship of pTfh with lymph node Tfh with respect to an ongoing immune response. The molecular signatures of these cell subsets from the two sites during an immune response also need to be investigated in order to understand their precise functional relationship.

IL-21 and pTfh cells in aging

The major reason underlying the susceptibility to infection and for poor vaccine responses in both the HIV uninfected elderly and HIV infected of all ages is the decline of or impairment in functioning of the immune system, often termed as immunosenescence [137, 138]. Advances in antiretroviral therapy have dramatically increased the life expectancy of HIV infected persons up to 70 years [139], and incidence of new HIV infections at older ages has also increased. It is estimated that by 2015, 50 % of HIV infected population will be 50 years of age [140]. Given the independent detrimental effects of aging and of HIV infection on the immune system, research into cumulative immune decline with HIV and aging has taken on increasing significance.

We have expanded our research focus to study the effect of aging on immune responses in HIV infected individuals. To address the relationship between aging and HIV infection, we first studied the distribution of factors contributing to the HIV disease progression in young and older HIV-infected individuals [141]. In this study, we investigated immune activation and microbial translocation in HIV infected aging women in postmenopausal ages. Twenty-seven postmenopausal women with HIV infection receiving cART with documented viral suppression and 15 HIV-negative age-matched controls were included in this study. Levels

of immune activation markers (T cell immune phenotype, sCD25, sCD14, sCD163), microbial translocation (lipopolysaccharide), and biomarkers of cardiovascular disease and impaired cognitive function (sVCAM-1, sICAM-1, and CXCL10) were evaluated. Our results indicate that T cell activation and exhaustion, monocyte/macrophage activation, and microbial translocation were significantly higher in HIV-infected women when compared to uninfected controls [141]. Microbial translocation correlated with T cell and monocyte/macrophage activation. Biomarkers of cardiovascular disease and impaired cognition were elevated in women with HIV infection and correlated with immune activation. Our data indicate that HIV-infected antiretroviral-treated aging women who achieved viral suppression are in a generalized state of immune activation and therefore are at an increased risk of age-associated end-organ diseases compared to uninfected age-matched controls.

We investigated whether the age-associated changes in combination with the factors affecting HIV disease progression might also negatively impact the immune function in HIV infected older patients. To address this issue, preliminary studies were conducted in which immune response after influenza vaccination was measured in these HIV-infected postmenopausal women and compared with healthy HIV-negative postmenopausal volunteers [142]. Antibody titers to trivalent influenza vaccination given during the 2011–2012 season were determined before and 4 weeks after vaccination. Our results showed reduced seroprotective influenza antibody titers ($<1:40$) in HIV infected women compared to HIV uninfected women (31 vs. 58 %, respectively) pre-vaccination. Following vaccination, magnitude of antibody responses and frequency of seroprotection were lower in HIV infected (75 %) than in HIV uninfected (91 %) women [142]. Seroconversion was associated with an increase in plasma IL-21, the signature cytokine of Tfh cells. A novel observation in this study was that post-vaccine antibody responses were inversely correlated with pre-vaccination plasma TNF α levels and with markers of immune activation (CD38 and HLA-DR) on CD4 T cells, including pTfh subset, indicating detrimental consequences of immune activation and inflammation on vaccine-induced antibody response in older age. Plasma TNF α levels were found to correlate with activated pTfh cells, and inversely with the post-vaccination levels of plasma IL-21. Higher frequencies of activated and exhausted CD8 T and B cells were noted in HIV infected women [142]. Our results indicate that in aging, activated CD4 and pTfh cells may compromise influenza vaccine-induced antibody response, for which a mechanism of TNF α -mediated impairment of pTfh-induced IL-21 secretion is postulated. It is hypothesized that interventions aimed at reducing chronic inflammation and immune activation may improve response to vaccines [142]. We are now conducting a larger study of influenza vaccination in different age groups to investigate the molecular and immunologic mechanisms that could explain the defect in aging-associated immune response and to understand the effect of HIV on aging. Studies in SIV-infected aged Rhesus macaques are needed to understand the immune responses in inductive sites such as GC following vaccination. The question whether IL-21 supplementation or cellular therapy using in vitro expanded Tfh cells could improve the immune responses after vaccination needs to be investigated.

pTfh cells and HIV reservoirs

In addition to its role in regulating the immune responses, recent studies have ascribed an important role of Tfh cells in the LN in contributing to HIV persistence and reservoirs. In SIV infection models of Rhesus macaques and pig tail macaques, studies have clearly demonstrated that Tfh cells within LNs are highly susceptible to SIV infection [77, 143, 144] and suggest that Tfh cells have relevance for assessing the size of the viral reservoir [143]. Within weeks of SIV inoculation, these cells are often maintained or expanded in frequency and spatial localization within B cell follicles [77, 143]. Early infection of Tfh cells represents an unexpected focus of viral infection. However, infection of Tfh cells does not interrupt antibody production but may be a factor that limits the quality of antibody responses.

Consistent with the findings in macaques, susceptibility of LN Tfh cells and HIV infection in human has been reported recently [145]. This study showed that both Tfh (CXCR5⁺PD1⁺) and CXCR5^{neg}PD1⁺ cell populations are enriched in HIV-specific CD4 T cells, and these populations are significantly expanded in viremic HIV-infected subjects and the percentage of Tfh cells correlated with the levels of plasma viremia. Tfh cell population contained the highest percentage of CD4 T cells harboring HIV DNA and was the most efficient in supporting productive infection in vitro [145]. These studies indicate that Tfh cells could be an important target of HIV, and it was suggested that compared with T cell zones, GCs seemed to exclude CD8 T cells while harboring increasing numbers of virus-specific CD4 T cells, suggesting an environment particularly beneficial for virus replication and reservoirs [146]. Moreover, it has been suggested that reduced tissue penetration of ART in lymph nodes also would favor the persistence of the virus at sites within LNs that could favor Tfh cells as an important cellular subset for HIV persistence and viral reservoirs [147]. In humans, prior studies have identified central memory CD4 T cells (T_{CM}) as a major reservoir for HIV in virally suppressed individuals along with transitional memory (T_{TM}), which is consistent with their capacity to survive for long periods in vivo that makes it ideal for HIV to persist within these cells [148]. A close association of PD1⁺CXCR5⁺ Tfh cells with GC Tfh has been reported recently with respect to their gene signatures and helper function of B cells [132]. Moreover, a recent study has shown that PD1⁺CXCR5⁺ Tfh cells from lymph nodes of patients with HIV infection contained the highest percentage of HIV infected cells and were the most efficient in supporting virus replication and production, indicating a close association of PD1 with HIV reservoirs in lymph nodes [145]. We are currently investigating the role of pTfh cells in viral persistence and its association with HIV reservoirs. Consistent with the reported data in lymph node Tfh cells, we noted a higher permissiveness of pTfh cells to HIV-1 infection compared to non-pTfh cells and PD1⁺ pTfh cells showed higher frequencies of virally infected cells (Pallikkuth et al., unpublished). This research is in progress, and we are now identifying the factors that contribute to the higher permissiveness of pTfh cells to HIV and also how ART-induced decrease in virus levels affects the HIV burden on pTfh cells. Overall, our preliminary investigations revealed that in addition to their role in immune responses, pTfh cells could also serve as the major CD4 T cell compartment for HIV infection, replication, and production. Our preliminary results indicate that pTfh cells could be considered as one of the important memory cell subsets that

support HIV infection and persistence. Further investigation is needed to clarify the potential utility of pTfh cells as tools for measuring the burden of HIV for HIV persistence and in cure research.

Conclusions and future perspectives

In this review, we have highlighted the favorable properties of IL-21 in modulation of adaptive immune responses in the context of HIV infection in humans and SIV infection in Rhesus macaques. Three major properties of IL-21 are as follows: 1. augmentation of cytotoxic molecules perforin and granzyme B in CTL and NK cells; 2. induction of antibody responses and autocrine regulation; and 3. lack of induction of T cell activation that leads to HIV replication. Our studies in chronic and acute SIV-infected rhesus macaques using IL-21 showed enhancement of cellular immunity without associated immune activation and increase in virus replication [23, 24], indicating a immunomodulatory effect of IL-21 at different stages of SIV infection. In humans, in vitro studies [33, 51] as well as investigations of HIV-infected patients with different disease states including elite controllers have ascribed an important role to IL-21 in preservation of virus control [6, 36, 37, 116, 117]. There is ample support for the beneficial role of IL-21 in other acute and chronic virus infections. Mice models of acute and chronic LCMV infection highlighted the importance of IL-21 in promoting and sustaining immune response during chronic and acute viral infections [65–67]. Immunomodulatory properties of IL-21 have also been described in human hepatitis B and C infections. *IL-21* mRNA levels were increased in PBMC of patients with acute infection who cleared the virus compared with patients who did not clear the virus [149]. A study by Ma et al. suggests that serum IL-21 levels may be a biomarker for HBeAg seroconversion during therapy in chronic hepatitis B virus-infected patients, a good prognosis marker of HBV. HBeAg seroconversion coincides with the development of the HBV-specific CD8T cell repertoire that is believed to control viral replication. This study suggests that IL-21 may also have a role in immunotherapy for chronic hepatitis B infection [150].

The recent advances in the field of immunity by understanding the role of GC in regulating the adaptive immune responses further strengthen the cooperative role of IL-21 and Tfh cells in regulating antigen-specific antibody production. In HIV infection, a role for Tfh in HIV Ab responses [132] and importantly in flu vaccine-induced Ab responses [128, 136] has been demonstrated. Together, there is compelling rationale to investigate IL-21 as a vaccine adjuvant to augment T and B cell immune responses and also in immunotherapeutic approaches to enhance CTL for eliminating virally infected target cells in “cure” strategies aimed at achieving permanent virologic control. The lack of an effect of IL-21 on immune activation after in vivo administration in chronic and acute SIV infection indicates that IL-21 has promise in immunotherapeutic approaches in SIV/HIV infection as it does not appear to affect factors that favor disease progression.

Thus far, definitive studies have not been performed that demonstrate the utility of IL-21 either in vaccine design or as a therapeutic moiety in HIV/SIV infection. For example, it is not known whether IL-21 by itself or as a vaccine adjuvant in healthy RM can elicit cellular or humoral immune responses that impact infection status in any way after a virus challenge.

IL-21 could potentially promote the generation of high-affinity antibodies during an immune response by modulating signals required for antigen-specific expansion, and differentiation of B cells and Tfh cells. Current data on interaction between Tfh cells, B cells, and IL-21 during a humoral immune response support the notion that IL-21 supplementation could enhance the efficacy of vaccines or immunity against infection in immunocompromised individuals. Current knowledge supports the rationale for continued exploration of the properties of IL-21 in experimental models of HIV/SIV infection in prevention and treatment during early and chronic stages in conjunction with ART and other immunotherapeutic strategies

The studies of pTfh cells point to the potential utility of pTfh as biomarkers of ongoing immune response. Further studies are needed to establish the relationship of pTfh with lymph node Tfh and to determine whether pTfh cells can provide insight into the immune responses at the inductive sites such as GC. Knowledge in this field will open avenues for translational research using IL-21 and Tfh cells and may help in the development of IL-21-based adjuvant or pTfh-based cellular therapies that could potentially be used in understanding HIV immunopathogenesis, in strategies to improve the immune response in situations where it is deeply compromised, and in approaches aimed at “curing” HIV. The role of pTfh as reservoirs for HIV is particularly relevant and in need for further investigation as strategies to eliminate virus from cellular sanctuaries are developed.

Acknowledgments

This work was supported by NIH Grant A1077501, AI 108472 to SP and a CFAR developmental award to S Palli. We acknowledge support from the Miami Center for AIDS Research (CFAR) at the University Of Miami Miller School Of Medicine, which is funded by a Grant (P30AI073961) from the National Institutes of Health (NIH). The CFAR program at the NIH includes the following co-funding and participating Institutes and Centers: NIAID, NCI, NICHD, NHLBI, NIDA, NIMH, NIA, FIC, and OAR.

Biography

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Fig. 1.

Tfh cells and IL-21 secreted by Tfh cells coordinate the follicular and extrafollicular immune responses: DC-primed T cells get activated, upregulate Bcl6, and CXCR5, and migrate toward the B cell follicle. Activated T cells then interact with antigen-activated B cell and further undergo differentiation and maturation, and secrete IL-21. IL-21 then initiates an autocrine loop that promotes Tfh cell development. Early events in the development of humoral immune responses occur in extrafollicular areas of lymphoid tissues where B cells differentiate into short-lived extrafollicular plasmablasts (ePB). Following interactions with extrafollicular helper T cells (eTh) cells, ePB produces low-affinity antibodies that are predominantly short-lived. Similar to Tfh cells, the function of eTh is mediated by CD40L, ICOS, and IL-21 and they do not express high levels of CXCR5; rather, they show higher CXCR4 expression. eTh cells might also give rise to GC Tfh cells following interactions with cognate B cells. Alternatively, both T and B cells migrate to the GC where Tfh cells reach their fully differentiate and produce high levels of IL-21. In the GC, Tfh cells interact with GC B cells through an array of molecular pairings, including pairings of T cell antigen receptor (TCR) and major histocompatibility complex (MHC) class II; CD28 and B7 family members; the costimulatory molecule CD40 and its ligand CD40L; ICOS and its ligand ICOSL; SLAM family members on both cell types; and PD-1 and PD-L1. These interactions further enhance the production of IL-21 and the IL-21 produced by Tfh cells helps in the maintenance and persistence of Tfh cell phenotype,

function, and is a major B cell helper cytokine to induce B cell class switching and for antibody diversification mechanisms. After the completion of the GC reaction, long-lived plasma cells (PC) and memory B cells and antigen-specific memory Tfh cells exit the follicular region and enter the circulation. Memory Tfh cells could reenter to the GC and induce GC reaction during a secondary immune response [90]

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Table 1

Signature immunologic changes in pTfh and B cells in vaccine responders (VR) following influenza vaccine at T1 (7 days), T2 (4 week) [128, 134, 135, 142]

pTfh cell changes in VR (not seen in non-pTfh or VNR)
Expansion of pTfh at T1 and T2
Ag-stimulated IL-21 production in pTfh (ICC)
“Help” to autologous B cells for Ag-specific IgG production and B cell differentiation in purified pTh plus B cell co-cultures at T2
B cell changes in VR (deficient in VNR)
Increase in plasmablasts at T1
Spontaneous H1N1 influenza Ab-secreting cells (ASC) at T1
Increase in total memory B cells and switch memory at T2
Upregulation of IL-21R on total B and memory B cells at T2
Increase in plasma cells at T2
Increase in TACI expression on total B memory B cells at T2
Downregulation of BAFF-R expression on total B and memory B cells at T2
PBMC/plasma findings in VR (deficient in VNR)
Production of IL-21 and CXCL13 in Ag-stimulated culture supernatants with increases in plasma IL-21
Increase in plasma BAFF and APRIL levels

VR vaccine responders, *VNR* vaccine non-responders, *pTfh* peripheral T follicular helper cells, *ICC* intracellular cytokine staining, *PBMC* peripheral blood mononuclear cells, *Ab* antibody