T Follicular Helper Cells and B Cell Dysfunction in Aging and HIV-1 Infection

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T follicular helper (Tfh) cells are a subset of CD4 T cells that provide critical signals to antigen-primed B cells in germinal centers to undergo proliferation, isotype switching, and somatic hypermutation to generate long-lived plasma cells and memory B cells during an immune response. The quantity and quality of Tfh cells therefore must be tightly controlled to prevent immune dysfunction in the form of autoimmunity and, on the other hand, immune deficiency. Both Tfh and B cell perturbations appear during HIV infection resulting in impaired antibody responses to vaccines such as seasonal trivalent influenza vaccine, also seen in biologic aging. Although many of the HIV-associated defects improve with antiretroviral therapy (ART), excess immune activation and antigen-specific B and T cell responses including Tfh function are still impaired in virologically controlled HIV-infected persons on ART. Interestingly, HIV infected individuals experience increased risk of age-associated pathologies. This review will discuss Tfh and B cell dysfunction in HIV infection and highlight the impact of chronic HIV infection and aging on Tfh–B cell interactions.

Keywords: T follicular helper cells and HIV, T follicular helper cells and immunity, HIV and aging, T follicular helper cells and influenza vaccine, T follicular helper cells in aging and HIV

INTRODUCTION

Chronic infectious diseases, such as HIV infection, and the biological process of aging are known to impact humoral immune responses to vaccination and infection (1–5). The issue of aging during HIV infection has gained importance due to the success of antiretroviral therapy (ART) that can lead to near normal life expectancy and is resulting in increasing the numbers of aging HIV-infected people (3, 6, 7). Older HIV-uninfected individuals in the general population, especially those >80 years develop immune senescence, a term signifying immune defects affecting multiple cell types, characterized by quantitative reduction in hematopoietic stem cells, thymic involution with reduced naive cells and accumulation of effector and memory cell subsets with narrow TCR repertoires with low clonality, and reduced CD4:CD8 T cell ratio (8–11). Memory T cells tend to lose expression of CD28 and their antigen-specific responses are impaired (12). In addition, profound B cell alterations occur in biologic aging characterized by a reduction of the naive B cell pool and qualitative impairment of their function along with reduced vaccine induced immune responses (13–22). Concurrently, increased inflammation coined by the term inflamm-aging (21, 23) occurs with increased C-reactive protein (CRP), D-dimer, IL-6, and TNFα that correlate with occurrence of age-associated diseases.

Immunologic changes similar to biologic aging have been described in HIV infection, including accelerated immune senescence and inflammation, with increased IL-6, CRP, and D-dimer (24–26)
Tfh Cells in Lymph Node (LN) and Periphery

T follicular helper cells are a specialized subset of CD4 T cells in lymphoid organs that express the transcription factor B cell CLL/lymphoma 6 (Bcl-6), with high surface expression of programed death receptor 1 (PD-1) and CX3C chemokine receptor 5 (CXCR5) [reviewed in Refs. (33–37)]. During an immune response Tfh cells provide critical signals to antigen-experienced B cells in germinal centers (GCs) to undergo proliferation, isotype switching, and somatic hypermutation (SHM) in order to generate long-lived plasma cells and memory B cells through cellular interaction and cross-signaling for antibody production [reviewed in Refs. (37–39)]. Tfh cell differentiation requires dendritic cell (DC) priming of naive antigen-specific CD4 T cells followed by the interaction with B cells resulting in upregulation of costimulatory molecules such as inducible costimulator (ICOS) and CD40 ligand (CD40L) and secretion of cytokines IL-21 and IL-4 that play a critical role for the ensuing B cell response [reviewed in Refs. (33, 34, 39)].

Because of the difficulties in studying lymphoid tissue in humans, the field has increasingly relied on a circulating subset of memory CD4 T cells that partially resemble LN Tfh cells and have been designated as peripheral Tfh (pTfh) (40–47). The pTfh cells display a memory phenotype and are characterized by expression of CXCR5, the B cell follicle homing molecule, and by secretion of IL-21 during interactions with B cells (42, 48). Unlike LN Tfh cells, pTfh cells express only moderate levels of PD-1 and Bcl-6 but are similar in their ability to upregulate costimulatory molecules such as ICOS and CD40L upon antigen stimulation (42, 49–52). More recently, based on the surface expression of CXCR3, CCR6 and CXCR4 Tfh cells have been further characterized as Th1 (CXCR3 + CCR4 − CCR6−), Th2 (CXCR3 − CCR4 + CCR6+), and Th17 (CXCR3 − CCR4 − CCR6+4) memory CD4 T helper subtypes (42, 53, 54), indicative of reveals the heterogeneous nature of pTfh cells with respect to phenotypic, functional and transcription factor profiles (42, 54). It is now widely considered that a balance of pTfh subsets is important for maintaining healthy immune function.

Tfh, B cells, and HIV infection

T follicular helper cells are highly permissive to HIV becoming readily infected by follicular DC that transport infectious virions into lymphoid organs. Tfh cells are now considered as major reservoirs of transcriptionally silent integrated HIV genomes (55–58). In non-human primates, chronic infection with simian immunodeficiency virus (SIV) is associated with an expansion of Tfh cells within GC (59, 60), along with increase in numbers of B cells in LN, spleen, and gut tissues of rhesus macaques (60–63). Early initiation of ART can rapidly control the virus replication but not the early lymphoid activation, thereby increasing the risk of infection of Tfh and magnitude of viral reservoir (64). Contrary to the expansion of GC Tfh cells seen in chronic HIV/SIV infection (59, 60), we and others have reported a significant loss of circulating pTfh cells in chronic viremic HIV-infected subjects compared to HIV-uninfected persons (65, 66); 12 months of ART incorporating Raltegravir resulted in increased frequencies of pTfh cells (66). However, pTfh cells from HIV+ virologically suppressed patients on ART exhibit functional impairment in their ability to provide adequate B cell help in a number of systems (41, 67–69).

In chronic HIV infection, B cells exhibit immune dysfunction and altered B cell subset distribution, with a shift in resting memory (RM) B cells to an activated state with expression of activation markers such as CD71, CD80, and CD86 (70, 71). There is also an increase in inflammatory B cell subsets referred to as double negative (DN: CD27 − IgD − B cells) and tissue-like memory B cells (15, 72–75). ART-mediated viral suppression restores many of the B cell defects, especially when initiated during the acute phase of infection (76). However, reduced frequencies of RM B cells, elevated DN B cells, as well as chronic immune activation persist (31, 71, 77–79).

Vaccine-Induced Antibody Responses During HIV Infection

In healthy states, antibody responses to T-dependent antigens are generated in GCs within lymphoid tissue when antigen-primed B and T cells engage in interactions to promote B cell differentiation, SHM, and class switch recombination to develop into memory B cells and plasma cells (80–83). Studies in humans and animal models indicate that HIV infection affects the GC reaction, increases immune activation/exhaustion of lymphocytes, and results qualitative deficiency of Tfh and B cell function (57, 59–61, 69). These defects altogether lead to increased susceptibility to vaccine-preventable diseases (84, 85). Studies focusing on pTfh cells have been informative for understanding the phenotypic complexity within the Tfh subset and for determining the relationship between Tfh and B cells in immunological outcomes [reviewed in Ref. (86)].

Influenza vaccine studies have provided a valuable model system to analyze the immune system in vaccine induced antibody
responses (87). We initiated such studies in virally suppressed HIV+ adults on ART during the 2009/H1N1 pandemic influenza outbreak (43, 88, 89). Following monovalent H1N1 vaccination, vaccinees were classified as vaccine responders (VRs) if postvaccination hemagglutination inhibition (HAI) serum H1N1 Ab titer was 1:40 or more and exhibited a 4-fold increase, from baseline titer, and those who did not meet these criteria were classified as vaccine non-responders (VNRs). In study participants, administration of the vaccine resulted in VR status only in 50% HIV+, compared to all age matched healthy controls. In the HIV + VR and VNR, prevaccination CD4 and CD8 T cell counts, B cell frequencies, and plasma HIV RNA were similar, but phenotypic and qualitative immunological differences were identified. In VR, there was upregulation of IL-21R in B cells that correlated with plasmablasts and memory B cell responses post-vaccination (89), together with an expansion of pTfh cells with secretion of IL-21 and CXCL-13 in H1N1-stimulated PBMC culture supernatants. In coculture experiments, pTfh supported H1N1-stimulated IgG production by autologous B cells (43). More recent findings point to the ability to perform qualitative assessment of pTfh/CD4 T cells and B cells prior to immunization in previously vaccinated HIV+ children and young adults (90, 91). Examples of such assessments include (i) ex vivo stimulation with H1N1 resulting in induction of CXCR5 mRNA and protein in CD4 T cells and (ii) induction of IL21 gene in pTfh cells. These antigen-specific prevaccination measures strongly associated with H1N1-specific B cell responses by ELISPOT at postvaccination (91). Interestingly, CD4 T cells from VNR exhibit increased expression of IL2 and STAT5 genes, which are known to antagonize pTfh function (92). Our main findings of pTfh and B cells in relation to vaccine responses are summarized in Table 1. Other vaccine studies have shown associations between pTfh expansion and phenotype with vaccine response. Expansion of HIV-specific PD-1 + ICOS + pTfh correlated with vaccine-specific serum IgG after booster immunization in three different human HIV vaccine trials (93). Expression of ICOS, PD-1, CD38, and IL-21 in pTfh subsets have been useful for evaluating the influenza vaccine response in HIV-infected and -uninfected adults in other studies as well (50, 87, 93–95). Studies with Ebola vaccine (rVSV-ZEBOV) demonstrated that CXCR5 + PD-1 + pTfh correlated with expansion of plasmablasts (96). Taken together, these studies support the concept that both quality and quantity of pTfh cells are important determinants for the outcome of vaccine response in HIV infection.

**Tfh Cells and B Cells in HIV and Aging**

Our group has been interested in the question of immune function of aging HIV+ individuals who are well controlled on ART, the extent to which it resembles biologic aging of HIV+ individuals, and implications of aging with HIV infection. Earlier pilot studies in virologically suppressed postmenopausal women as representative of an aging population established the persistence of inflammation and gut microbial translocation and detrimental role of underlying immune activation on influenza vaccine responses that were associated with quantitative and qualitative deficiencies of pTfh cells (45, 97, 98). Our studies showed lower H1N1 influenza antibody titers in HIV-infected women compared to HIV-uninfected women at prevaccination. Following vaccination, magnitude of antibody responses and frequency of study participants achieving seroprotective titers were lower in HIV+ than in HIV− women. Frequencies of pTfh cells at postvaccination correlated with memory B cell function and H1N1 antibody titers. Antibody responses postvaccination were inversely correlated with inflammatory cytokine TNFα in plasma and with markers of cellular immune activation (CD38 and HLA-DR) on CD4 T cells, including pTfh subset, indicating an adverse influence of baseline immune activation and inflammation on vaccine induced antibody response in older age.

To examine the role of age and HIV infection further, we are engaged in a large ongoing study (99, 100) in virologically suppressed HIV+ and HIV− adults grouped by age as young (<40 years), middle aged (40–59 years), and old (≥60 years). Following seasonal trivalent influenza vaccine (TIV), magnitude of Ab titers against each vaccine strain were found to be lower in old age compared to others, regardless of HIV status. Baseline titers in seroprotective range were higher in HIV+ than in HIV− women. Frequencies of pTfh cells at postvaccination correlated with memory B cell function and H1N1 antibody titers. Antibody responses postvaccination were inversely correlated with inflammatory cytokine TNFα in plasma and with markers of cellular immune activation (CD38 and HLA-DR) on CD4 T cells, including pTfh subset, indicating an adverse influence of baseline immune activation and inflammation on vaccine induced antibody response in older age.

In statistical analysis somewhat surprisingly effect of age rather than HIV dominated the impaired immune response observed in old persons (age > 60 years), whereas HIV clearly had a strong effect on immunity at younger ages (99, 100).

We examined phenotypic characteristics of T and B cells in this group of participants prior to vaccination. T cell phenotypic analysis revealed a core signature of aging comprised of decreasing naïve T cells and a loss of CD38 expression on CD4 and CD8 T cells. Frequencies of activated CD4 T cells (and not CD8 T cells) identified by coexpression of HLA-DR and CD38,

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**TABLE 1** | Signature immunological changes in pTfh and B cells in vaccine responders (VRs) following influenza vaccine at TO (baseline), T1 (7 days), and T2 (4 weeks).

| Changes in pTfh cell compartment in vaccine responders |
|-------------------------------------------------------|
| Antigen induced IL-21 gene expression at TO |
| Expansion of pTfh at T1, T2 |
| Ag-stimulated intracellular IL-21 production in pTfh at T2 |
| “Help” to autologous B cells for H1N1-specific IgG production and B cell differentiation in pTfh plus B cell cocultures at T2 |

| B cell changes in vaccine responders |
|-------------------------------------|
| Increase in frequencies of plasmablasts at T1 |
| Increase in spontaneous H1N1-specific ASC at T1 |
| Increase in memory B cells and switch memory at T2 |
| Upregulation of IL-21R on total B and memory B cells at T2 |
| Increase in TACI expression on total B and memory B cells at T2 |
| Downregulation of BAFT-R expression on total B and memory B cells at T2 |

| PBMC culture sups/plasma findings in vaccine responders |
|---------------------------------------------------------|
| Production of IL-21 and CXCL13 in H1N1-stimulated culture sups with increases in plasma IL-21 |
| Increase in plasma BAFF and APRIL levels |

*pTfh, peripheral T follicular helper; PBMCs, peripheral blood mononuclear cells; Ab, antibody; BAFF-R, B cell activating factor receptor; APRIL, a proliferation inducing ligand; CXCL13, C-X-C motif chemokine ligand 13; ASCs, antibody secreting cells.*
as well as expression of PD-1, ICOS, and Ki-67 were higher in HIV+ participants compared to HIV− participants. Increases in activation markers previously associated with aging such as ICOS (87) were already evident in young HIV+ compared to young HIV−, indicative of HIV causing a state of premature immune senescence. Predictive modeling to determine the key T cell variables most closely associated with vaccine response revealed pTfh as an important biomarker. In HIV+, baseline pTfh frequency was positively associated with vaccine response, while in HIV− expression of multiple activation markers on pTfh (including PD-1) was negatively associated with vaccine response (99).

Prevaccination status of B cells also revealed perturbations as evidenced by alteration in markers of activation, exhaustion and immune regulation and were more prevalent in young HIV+ than in young HIV− (100). HIV infection in younger adults exhibited similarities with biological aging resulting in alterations in B cell phenotypic and functional characteristics similar to those observed in older HIV− individuals but underlying mechanisms appear to be distinct from that associated with biological aging (100). For example, the interaction between T and B cells through the PD-1:PD-L1 signaling pathway is involved only in HIV induced impairment of B cell function (101). These results provide the basis for immune correlates of premature aging in HIV+, even with prolonged ART-induced virological suppression (Figure 1). Additional mechanistic studies to understand the cellular basis of immunological impairments in pTfh and B cells in aging and HIV infection are currently ongoing in our laboratory.

Other factors that could influence the influenza vaccine response in aging also need consideration. Data from literature suggest that vaccine-induced immune responses are considerably influenced by demographic variables such as age, sex, ethnicity, and race (102–105). Many studies indicate that aged females consistently have higher antibody responses and increased vaccine efficacy to influenza vaccines than males [reviewed in Refs. (106, 107)].

Sex differences in HAI antibody titers to either the standard-dose or high-dose influenza vaccine are apparent, in which antibody responses are higher in older females than in males (108, 109). A role played by male hormone testosterone in lowering the immune response has been proposed (109, 110). There is growing interest in how latent cytomegalovirus (CMV) infections impact the outcome of vaccination [reviewed in Ref. (111)]. In young adults, CMV infection is associated with elevated antibody responses to influenza vaccines. In aged individuals, CMV seropositivity is associated with chronic inflammation and lower antibody responses to influenza vaccines (112, 113). However, lack of association between CMV status and influenza response in elderly population has also been reported (114). Thus the overall impact of CMV infection on influenza vaccine responsiveness remains controversial. A direct link between CMV seropositivity with increased risk of influenza illness in vaccinated older adults has not been reported in either HIV-infected or healthy individuals. Moreover, the influence of gender and CMV infection status on the cellular basis of immune impairment involving pTfh and B cell compartments are not been studied in aging and HIV infection. In aged mice, CD4+ and CD8+ T cells express several inhibitory receptor molecules, including PD-1, LAG-3, CTLA-4, and KLRG1 (115, 116) that could interfere with the immune response to vaccination. Prolonged expression of inhibitory molecules is a well-known feature of T cell exhaustion in chronic viral infections and exhausted T cells have also been identified in different viral infections, such as HIV and hepatitis A and B virus in humans [reviewed in Refs. (117–120)]. However, further studies are warranted to elucidate the significance of T cell exhaustion in HIV infection in the context of aging and its influence on vaccine induced immune response through regulation of pTfh and B cell function.

CONCLUSIONS AND FUTURE PERSPECTIVES

Development of a protective antibody response to vaccine or infection is important for the control or eradication of many pathogenic infections. Efficient Tfh–B cell interactions are required for regulating B cell differentiation toward the development of high affinity antibodies. Immune mechanisms underlying the regulation of Tfh–B cell interactions at the inductive sites of the immune response are an active area of immunology research. Several studies have highlighted the qualitative and quantitative impairment of Tfh compartment and their subsequent impact on humoral arm of immune response in treated HIV infection (43, 45, 67, 87, 94, 98). Since HIV-infected people are aging, research on the cumulative impact of premature and physiological immune senescence on immune function in HIV infection is of great importance. Our work underscores the adverse effect of inflammation, a cardinal feature associated with biologic aging and chronic HIV infection, on immune response to vaccination and functional impairment of Tfh and B cells as a consequence of persistent immune activation.

Recent advances in the field of immune checkpoint inhibitor-based immunotherapeutic approaches in cancer immunology
have highlighted the importance of cell to cell interactions on immune function. Many aspects of checkpoint molecule-based regulation of humoral immune response on Tfh and B cell interactions at the GC are not known. Trials employing checkpoint inhibitors in HIV infection will need to ensure that improved Tfh–B cell interactions not associated with autoimmunity. Immune checkpoints are negative regulators of T cell activation, T cell proliferation and effector functions and inhibiting immune checkpoints could influence and disrupt the resting status of latently infected cells and reverse latency with increase in HIV replication within GC (121). Future studies are needed to explore combination approaches targeting immune checkpoint molecules and costimulatory signaling pathways during an immune response to understand the coregulation of immunity by these molecules in the GC reaction. The ultimate goal should be to establish strategies to improve the immune function at inductive sites. Interventions aimed at reducing chronic inflammation and immune activation along with immunomodulatory approaches may improve response to vaccines in aging HIV+ individuals.

**AUTHOR CONTRIBUTIONS**

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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**Conflict of Interest Statement**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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