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Role of IL-21 and IL-21 Receptor on B Cells in HIV Infection

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Abstract

Interleukin (IL)-21 is a member of a family of cytokines which includes IL-2, IL-4, IL-7, IL-9 and IL-15 all of which utilize a common γ chain in their individual receptor complexes for delivering intracellular signals in their target cells. IL-21 is produced by CD4⁺ T cells, in particular T follicular helper cells, and is critically important in the regulation and maintenance of T cells and B cells in innate and adaptive immunity. Effects of IL-21 are pleiotropic, owing to the broad cellular distribution of the IL-21 receptor and it plays a critical role in T cell-dependent and independent human B cell differentiation for generating humoral immune responses. This chapter reviews current knowledge about the importance of IL-21 and IL-21 receptor interaction in human B cell responses, immune defects of B cells and IL-21 in HIV infection and the potential applicability of IL-21 in vaccines/immunotherapeutic approaches to augment relevant immune responses.

Keywords

IL-21 and IL-21 receptor; IL-21 and B cells; B cells and HIV; vaccines and IL-21

Introduction

Cytokines play a pivotal role in modulating immune responses. Agonist as well as antagonist therapies directed at cytokines, cytokine receptors or signaling pathways are being intensely investigated with some entering the clinical arena. Interleukin (IL)-21 is a member of the family of cytokines IL-2, IL-4, IL-7, IL-9, IL-15 that all utilize CD132, the common γ chain (γ_c), in their receptor complexes for delivering intracellular signals in their target cells.^{1,2} Among these, IL-21 has the most pleiotropic activities because the IL-21 receptor (IL-21R) is expressed on a broad range of cells.^{1–3} Production of IL-21 however is restricted predominantly to CD4 T cells, mainly to T follicular helper cells (TFH) and to some extent Th17 and other cells.^{1,4–7} Advances in the knowledge about the biology of IL-21 and its immunomodulatory activity have stimulated interest in this cytokine in different clinical and experimental settings.

There is now evidence for a critical role of IL-21 in protection against several virus infections.^{8–14} For example, in chronic lymphocytic choriomeningitis virus infection of mice, higher levels of IL-21 produced by virus-specific CD4 T cells were demonstrated to be essential for sustaining antiviral effector functions of CD8 T cells and preventing them from immune exhaustion.^{8,9,13} In chronic hepatitis B virus infected patients, increase in

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serum concentrations of IL-21 was associated with activation of the antiviral immune responses that could control hepatitis B virus replication.¹⁰ During acute hepatitis B virus infection, IL-21 mRNA levels were increased in patients who cleared the virus compared with patients who did not.¹² In hepatitis C virus infection as well, a recent study has demonstrated a possible role of IL-21 in distinguishing patients with hepatitis C virus clearance from those with hepatitis C virus persistence.¹¹ In patients infected with the human immunodeficiency virus (HIV), existing data suggest that IL-21 could play an important role in modulating T and B cell function, thereby influencing the immunodeficiency associated with it.^{15–21} IL-21 has been investigated as a therapeutic agent in humans for various malignancies [reviewed in^{3,22–24}]. In rhesus macaques infected with simian immune deficiency virus (SIV),¹⁸ IL-21 was shown to have a good safety record and to be biologically active, resulting in improved immune responses. The true potential of IL-21 as a therapeutic or vaccine adjuvant for HIV-infected individuals has not yet been established.

The hallmark of HIV infection is progressive decline of CD4 T cells^{25–27} with the virus infecting CD4 T cells, macrophages and dendritic cells. HIV-infected patients have widespread immune dysfunction that includes immunosuppression in concert with increased inflammation and immune activation;²⁸ this is because the virus has both direct and indirect effects that impact infected- as well as uninfected cells. Advances in antiretroviral therapy (ART) have vastly improved the lifespan of HIV-infected individuals, but it is intriguing that even with suppression of virus to below detectable levels in plasma, immune deficits can persist to varying degrees. These effects are attributed to ongoing low level virus replication in tissues as well as to gut associated microbial translocation, resulting in immune activation, inflammation and B cell related immune deficits. Thus, even though B cells are not infected, patients with chronic HIV infection manifest abnormal distribution of B cell subsets, loss of memory B cells and impaired humoral immune responses,²⁹ and [reviewed in^{30,31}]. Institution of ART early in the course of infection can to a large extent preserve the T and B cell immune system and reduce the persistent immune dysfunction that is noted when treatment is started later.²⁹

Emerging data suggests that IL-21 is associated with preservation of virus control and immunity in different stages of HIV infection.^{15,20,21} Although correlates of immune protection in HIV infection are not well understood, cytotoxic T cells^{32–34} and more recently, natural killer (NK) cells have been shown to play important roles in virologic control.^{35,36} Treatment of HIV-infected patient cells ex vivo with IL-21 and administration of IL-21 to SIV-infected rhesus macaques can augment the expression of the cytotoxic molecules perforin and granzyme B in CD8 T cells^{18,19} and NK cells^{18,37} without significant cellular proliferation or activation. Recent data suggests that IL-21 also plays an important role in preserving B cell immunity in HIV-infected patients.^{16,17} Many aspects in the life of B cells are regulated by IL-21, such as proliferation, differentiation, class switching or death, depending upon the type of antigenic stimulation and accessory signals.^{38–42} IL-21-elicited signaling has been identified as the most important among all the γ_c cytokines for long-lived humoral immunity.⁴² In this review we summarize the biology of IL-21 and its receptor (IL-21R), their role in modulating human B cell responses and their relevance to HIV infection.

I. Interleukin-21 and IL-21R expressing cells

The IL-21 polypeptide is 131 amino acids in length and has 57% amino acid sequence homology to its murine counterpart.^{1,2} In humans, the *IL21* gene is encoded on chromosome 4. This cytokine was first identified by Parrish-Novak *et al.* from the culture supernatants of activated human CD3 T cells.¹ Subsequently, it was established that IL-21 was secreted

predominantly by T cells, in particular by the CD4 TFH and Th17 subsets, as well as by NK T cells [reviewed in.^{3,43}]. The human *IL21R* gene is located adjacent to *IL4RA* gene on chromosome 16. It encodes a 538 amino acid protein and its amino acid sequence is most closely related to IL-2R β . Human IL-21R shares 62% amino acid sequence similarity with its murine counterpart, encoded on chromosome 7.^{1,44} The distinct IL-21R α chain couples with the γ_c to form the cytokine receptor complex.^{1,44} IL-21R is expressed on a broad range of cells [reviewed by,^{3,45}], predominantly in cells of lymphoid tissues including spleen, thymus and lymph nodes. It is constitutively expressed on B cells, CD4 T cells, NK cells, macrophages, monocytes, dendritic cells as well as hematopoietic and non-hematopoietic cells such as fibroblasts, keratinocytes and intestinal epithelial cells, and less often in cells from lung and small intestine.^{3,44} This broad range of expression of IL-21R explains the pleiotropic effect of IL-21.

Interestingly, the level of expression of IL-21R is highest on B cells.^{44,46} In human B cell subsets, IL-21R is constitutively expressed on naïve B cells and germinal center (GC) B cells, with memory B cells expressing it at low levels; recent reports show that plasma cells (PCs) also express IL-21R.^{46,47} The surface expression of IL-21R increases on human memory B cells following their activation, eg with anti-CD40 mAb.⁴⁶ IL-21 upregulates its own receptor expression on B cells by itself, or in combination with anti-CD40 stimulation.⁴⁸ Thus, the development and activation-dependent regulation of IL-21R expression on the surface of B cells suggests that IL-21 has important functions in B cell development and consequently on the immune response.

II. IL-21 signaling on B cells and its relevance to human B cell development

Like other γ_c -dependent cytokines, the binding of IL-21 to its receptor activates the Janus family tyrosine kinases (JAK), JAK1 and JAK3 which, in turn, activate signal transducer and activator of transcription (STAT)3, and to a weaker and more transient degree, STAT1, STAT4 and STAT5.^{44,49,50} Current views on IL-21/IL-21R signaling in B cells are depicted in Figure 1. Results from in vitro studies using human B cell lines indicate that binding of IL-21 to IL-21R leads to strong STAT3 activation^{50,51} as early as 5 minutes after binding and lasts for up to 6 days.⁵⁰ In B cells, IL-21 upregulates the master plasma cell transcription factor B lymphocyte induced maturation protein 1 (BLIMP-1), a transcriptional repressor that is important for plasma cell differentiation.^{6,39,42,52,53} BLIMP-1 is encoded by positive regulatory domain zinc finger protein 1 gene and its expression is regulated in a STAT3-dependent manner. In combination with CD40 ligation, IL-21 promotes class switch recombination, plasma cell differentiation, and immunoglobulin (Ig) production from B cells through the induction of activation-induced cytidine deaminase (AID), an essential factor for somatic hypermutation and class switch recombination. In primary human B cells as well, IL-21 induces activation of STAT3 which in turn triggers expression of BLIMP-1 and promotes Ig production.⁵⁴ In addition to JAK/STAT, IL-21 also activates extracellular signal-regulated protein kinases 1/2 that are mitogen-activated protein kinases and PI3K pathways in other cell types like neoplastic cells, epithelial cells, and monocytes.^{55,56} These signal transduction pathways eventually modulate the transcription program within the activated cell, thus regulating its phenotype, function and fate.

The significant role of IL-21/IL-21R induced B cell signaling in human B cell development and memory generation is exemplified in specific primary immunodeficiency disorders. In patients with severe combined immunodeficiency due to IL2RG/JAK3 deficiency, the B cells exhibit defective γ_c -dependent cytokine signaling that results in impaired B-cell responses, humoral dysfunction and significantly reduced memory B cells. This defect is not corrected if there is poor donor B-cell engraftment after hematopoietic cell transplantation, and the ex-vivo stimulation with CD40L/IL-21 induces B-cell proliferation, plasmablast

differentiation, and antibody secretion only in patients with donor B cell engraftment.⁴² These data indicate the importance of IL-21-mediated signaling in developing long-lived humoral immunity and antibody responses.

The critical role of STAT3 downstream of IL-21 is demonstrated in patients with STAT3 mutations who exhibit dramatically reduced numbers of functional, antigen specific memory B cells with inability of IL-21 to induce naïve B cells to differentiate into plasma cells. In contrast to STAT3, STAT1 deficiency has no effect on memory B cell formation *in vivo* or on IL-21-induced immunoglobulin secretion *in vitro* indicating that STAT3 plays a critical role in generating effector B cells from naïve precursors in humans.⁵² Specifically, levels of circulating anti-tetanus IgG were found to be reduced in STAT3 mutant patients and *in vitro* recall experiments showed that both the overall quantity and quality of antigen specific B cells was lost in these individuals. In addition, mutations in STAT3 largely impaired IL-21-mediated B cell expansion and plasma cell differentiation with failure to upregulate BLIMP-1 in response to IL-21 and CD40L while upregulation of AID was unaffected.⁵² In a more recent study STAT3-deficient CD4 T cells were shown to have defective expression of IL-21 with diminished B-cell helper activity *in vitro*. These results indicate the important role of STAT3 in IL-21-mediated differentiation of both, T and B cells.⁵⁷ Overall, these studies suggest that IL-21-mediated induction of AID and class switching is STAT3-independent, while STAT3 is critical for IL-21-induced B cell expansion, differentiation, and/or survival and support the concept that IL-21 is indispensable for the generation and maintenance of human B cell memory.

III. Role of IL-21 in human B cell development and plasma cell differentiation

IL-21 plays a critical role in B cell fate decisions and differentiation and influences the expression of many functionally important B-cell surface molecules. Direct stimulation of B cells with IL-21 alone induces upregulation of CD19, CD21, CD10, CD20, CD22, CD95, CD9, CD86, and the IL-21R, but not CD23 or CD27⁵⁸ with induction of AID without initiating somatic hypermutation.³⁹ In contrast, stimulation of B cells with anti-CD40 alone markedly increases expression of both CD23 and CD27⁵⁸ with minimal proliferation.^{1,6,39} The most potent and unique B cell stimulatory effects are seen with IL-21 and CD40 costimulation, unlike other B cell stimulatory cytokines like IL-2 or IL-10. The IL-21/CD40 costimulation induces expansion of both naïve and memory B cells isolated from either cord blood or peripheral blood, with inhibition of B cell death in cord blood together with augmentation of proliferation, class switch recombination, plasma cell differentiation, and Ig production. IL-21 induces IgG3 class switch in cord blood B cells and both IgG1 and IgG3 class switch in peripheral blood or splenic naïve B cells.^{39,53} In addition to IgG production, IL-21 is also capable of inducing IgA production from naïve cord blood or peripheral blood B cells^{38,39} but not IgE.^{39,53} CD40 and IL-21 costimulation also induces extensive proliferation of B cell subsets isolated from various anatomical sites such as human spleen or tonsil.^{46,59} These findings imply that IL-21 has a profound impact in direct T cell-driven stimulation of B cells.

Further support for the unique role of IL-21 in T cell-driven B cell responses was derived from experiments showing that blocking endogenously produced IL-21 following CD4 T cell activation was sufficient to significantly inhibit B cell expansion, plasma cell differentiation, and antibody production,^{6,60} whereas, blockade of other cytokines, specifically, IL-2, IL-4, or IL-10, did not have this effect. In addition to IgG, IgM production was also significantly reduced when IL-21 was blocked in this experimental system.⁶⁰ Blockade of IL-21 production inhibited IgM production from naïve B cells and IgG production from both naïve and memory B cells, whereas IgM production by pre-switched B

cells was less affected by IL-21 blockade.⁶⁰ These studies indicate that IL-21 is able to costimulate plasma cell differentiation more effectively in post-switched B cells, at later stages of B cell differentiation.^{6,60} It was shown that purified TFH cells from tonsils induced a marked increase of Ig production by tonsillar PC, and this effect was impaired when endogenous IL-21 production was blocked, indicating the efficiency of IL-21 in inducing immunoglobulin production in differentiated PC.⁴⁷ These studies strongly support the importance of IL-21 in effective plasma cell differentiation of naïve B cells and post-switched memory B cells which is a prerequisite for an effective humoral immune response.

IV. IL-21, TFH cells and Germinal Center (GC) reaction in antigen induced Ab formation

The most critical site for the generation of T dependent antibody responses is the GC within lymphoid follicles in secondary lymphoid organs (reviewed in⁶¹) wherein interaction of antigen-primed B cells with TFH drives the B cells to undergo proliferation and differentiation, with Ig isotype switching, somatic hypermutation and secretion of high affinity antibodies.^{43,62} The GC TFH cells are a specialized effector CD4 T helper cell subset [^{63,64} and reviewed⁶⁵], characterized by presence of the transcriptional regulator Bcl-6, surface expression of CXCR5, ICOS and programmed cell death 1 (PD-1) molecules and secretion of IL-21.^{42,66–68} Following pathogen invasion or vaccination, the antigen-activated specialized CD4 T cells and B cells are recruited into the lymphoid organs and into the GC where the GC reaction is activated. Experiments conducted ex-vivo demonstrated that IL-21 is capable of driving plasma cell differentiation and IgM, IgG, and IgA antibody production from human splenic GC B cells.⁶ Experimental evidence indicates that initial GC formation occurs within 6 days of primary immunization, when rapidly proliferating B cells begin to appear as foci in the B cell follicles of lymph nodes and spleen. These foci later increase rapidly in size and differentiate into the mature GC reaction.^{61,69} Within the GC, B cells move constantly and engage in short, dynamic interactions with both TFH and antigen that enhance B cell affinity and eventually the quality of immune response.⁶¹ TFH-derived IL-21 orchestrates GC development and maintenance and many aspects of B cell differentiation and function, including development of memory B cells and long-lived plasma cells.^{39,43,46,53,62} Thus TFH cells have emerged as key facilitators of Ab responses and are now being studied for their role in induction of Ab against HIV and SIV in systems aimed to inform vaccine design.

Although the role of TFH-B cell interaction for B cell differentiation and function was initially described for TFH cells residing within GC, the importance of TFH cells at other sites is becoming evident.^{43,70,71} While some GC TFH undergo apoptosis, recent data suggests that they are also capable of migrating out from the GC^{72,73} to interact with B cells at the border of the T cell-B cell zone in the lymph nodes to generate a heightened memory cell response or to migrate into the peripheral circulation.⁷¹ Human CXCR5⁺ memory CD4 T cells in peripheral circulation share functional properties with the GC TFH cells,^{63,64,74–78} such as the ability to secrete IL-21 and to induce autologous naïve and memory B cell subsets to produce immunoglobulins.^{16,79} These peripheral CXCR5⁺ CD4 T cells are absent in circulation of patients with ICOS deficiency⁸⁰ who also lack GC. Thus, these cells, termed peripheral TFH (pTFH), are easily accessible in peripheral blood and may serve as important biomarkers for assessing TFH function. Our findings related to pTFH following influenza vaccination in HIV-infected patients and healthy donors support this concept, and are discussed later in this review.

V. HIV infection and consequences on GC, TFH, and IL-21 responses

It has been shown that CD57⁺ CD4 GC T cells are one of the sites of HIV infection and replication that may play a pivotal role in the pathogenesis of HIV infection.⁸¹ In rhesus macaques, direct SIV infection of TFH has been demonstrated.⁸² In a recent study,⁸³ SIV-infected rhesus macaques were followed for changes in TFH and B cells *in situ* as the animals progressed from uninfected, to the acute and chronic stages of infection.⁸³ Progression of SIV infection was accompanied by an increased numbers of follicles containing GCs, as well as by increased frequency of PD-1-expressing TFH cells that correlated with accumulation of activated Ki-67⁺ B cells within GCs. If preferential infection of TFH is occurring in lymph nodes, these data suggest that the accumulation of TFH could initially stimulate GC formation and B cell proliferation. The absence of CD8 T cells in the GC further suggests that GC TFH could serve as reservoirs for HIV. We contend that HIV-infected TFH may not function efficiently and thus their helper function for B cells and Ab production could be impaired. These possibilities need to be evaluated.

Additional mechanisms contributing to poor Ab responses may relate to impaired B-T cell interaction. Chronic progressive HIV infection deeply alters lymph node architecture, with increased collagen deposition and interstitial fibrosis, resulting in perturbed cellular distribution and abnormal cytokine milieu [reviewed⁸⁴]. Consequently, alterations in the B-CD4 T cell interactions are to be expected. Under physiological conditions, the formation of a GC is mediated by changes in the expression of the chemokine receptors CXCR4, CXCR5 and CCR7 in B cells, together with changes in the local concentration of the chemokines that bind to these receptors, such as CXCL12, CXCL13, and CCL19/21.^{85–87} HIV infection is associated with alteration of the levels of these soluble mediators and their receptors, including increased levels of circulating CXCL12 and CXCL13, and decreased CXCR5 expression on B cells.⁸⁸ HIV-associated changes in the chemokines and their respective receptors that coordinate the GC formation and B-T cell crosstalk could impact the generation of Ab responses. Thus, together with CD4 T cell depletion and impaired TFH function, altered lymph node architecture could contribute to alterations in GC formation and contribute to impaired B cell derived Ab responses in HIV-infected individuals.

Recent studies have addressed the role of IL-21 and peripheral T cells in HIV infection and disease progression.^{20,21,89,90} In general these studies indicate that IL-21 plays a beneficial role in arming CD8 T cells. In a study of primary infection it was shown that IL-21-mediated T helper function is established with subsequent acquisition of CD8 T cell multifunctional traits, but this quality is rapidly lost in chronic, progressive infection.²⁰ In elite controllers in this study, not only CD4 T cells but also CD8 T cells secreted IL-21 in response to HIV-specific stimulation and both were more polyfunctional in elite controllers compared to viremic controllers and rapid progressors. Others have also found that elevated frequencies of HIV-specific IL-21-producing CD4 T cells correlate with relative viral control, probably by promoting CD8 T cell maintenance and function.²¹ In general, current data suggests that loss of ability to produce IL-21 accompanies disease progression. In cross-sectional and longitudinal studies in different groups of HIV-infected patients, plasma levels of IL-21 were lower in HIV-infected individuals compared to uninfected healthy control subjects. Moreover, the levels of IL-21 correlated with CD4 T cell counts, suggesting a predominant role for CD4 T cells as a source of IL-21 or a role of IL-21 in promoting CD4 T cell survival.^{89,90} It has also been suggested that HIV-infected individuals have greater circulating IL-21-producing CD4 T cells compared to uninfected individuals.²¹ One reason for differences in results is that antigen-specific IL-21 secreting cells are generally in extremely low frequency in circulation and data derived from PMA activated cells may not reflect deficiencies of antigen specific cells. The question of IL-21 secreting

peripheral T cells in HIV infection has not been conclusively established, and further studies are awaited.

Study of the role of TFH cells in HIV infection in the context of B cell function and HIV antibody responses as well as Ab responses to non-HIV vaccines has been limited. HIV-specific IL-21-producing CD4 T cells detected in blood during untreated acute and chronic HIV infection have been reported to express CXCR5 and to have an effector memory phenotype.²¹ A study by our group indicates an important role for peripheral TFH cells in influenza vaccine-induced antibody responses of HIV-infected patients.¹⁶ As IL-21 is produced mainly from CD4 cells, in particular from TFH,⁶ reconstitution of this subset of CD4 T cells following ART in HIV-infected patients may be critically important for restoring B cell function. Due to the fact that TFH-derived IL-21 orchestrates many aspects of B cell differentiation and function,^{39,43,46,53,62} it will be of interest to see how the overall depletion of CD4 T cells after HIV infection affects the TFH compartment of cells and how these changes affect the IL-21 levels and B cell mediated immune response during HIV infection. We have explored these questions in HIV-infected and uninfected volunteers following influenza H1N1/09 vaccination discussed below.^{16,17,91}

VI. B cells in HIV infection

It is well known that in addition to the defects in the cell-mediated arm of immune responses, HIV infection impairs the humoral arm of the immune system. The failure of the immune system to control the virus in infected individuals is a great challenge to the treatment of HIV infection and points to the need for the development of a successful HIV vaccine. Although CD4 T cells are the primary target for HIV, the B cell compartment is also profoundly affected by HIV infection.⁹² B cell abnormalities, that are both phenotypic and functional, can be ascribed to three mechanisms: a direct virus-induced B cell activation, bystander B cell polyclonal activation triggered by the abnormal milieu of cytokines, HIV proteins, other soluble mediators, and impaired cross-talk between B and CD4 T helper cells. These impairments lead to apparent loss of humoral immune control over HIV and also to other pathogens.

HIV does not productively infect B cells, but replication-competent HIV virions can be isolated from the surface of B cells obtained from HIV-infected individuals.⁹³ These virions have been shown to adhere to B cells because the complement and antibodies that opsonize such virions can be bound by CD21, that is expressed on B cell membrane.⁹⁴ As a consequence of this interaction, it has been suggested that triggering of CD21 by bound virions could alter physiological B cell responses,^{95,96} thus contributing to B cell dysfunction during the course of HIV infection. Several HIV proteins have also been reported to directly interact with B cells, resulting in their hyperactivation, altered functionality and increased propensity to cell death. Among them, Tat, released by infected CD4 T cells, has been shown to induce surface expression of Fas (CD95) in B cells,⁹⁷ that is associated with increased B cell apoptosis upon engagement with Fas ligand.⁹⁸ Paradoxically, while HIV protein Nef impairs immunoglobulin class switch by repressing AID expression,⁹⁹ Env promotes AID expression. This latter reaction leads to chronic polyclonal B cell activation and accumulation of nonprotective IgG and IgA.¹⁰⁰

Several members of γ_c -sharing cytokines^{101,102} which are important in B cell homeostasis, differentiation and function undergo alterations in HIV-infected patients. For example, reduction of physiological levels of IL-2 and IL-4, that provide important proliferation and survival signals to B cells^{103–106} may negatively impact B cell homeostasis and survival. Higher levels of IL-7,^{107,108} a cytokine that promotes the proliferation of B cell precursors¹⁰⁹ may be responsible for elevated levels of circulating B cell precursors in HIV-

infected individuals.¹¹⁰ As discussed earlier, ability of CD4 T cells to produce IL-21, is also affected during the course of HIV infection,^{15,20,21,89} with consequences on B cells. Other than γ_c -sharing cytokines, many other soluble mediators are altered during the course of HIV infection that may affect the B cell compartment [reviewed in^{30,31}]. Microbial translocation, that is the presence of bacteria-derived products such as lipopolysaccharide in the bloodstream due to compromised integrity of the gastrointestinal mucosa, has been suggested as a leading cause of immune activation that drives HIV disease progression.^{111,112} B cells express toll-like receptor 4 (TLR4)¹¹³ and CD180,¹¹⁴ two receptors that can sense lipopolysaccharide. Thus, increased microbial translocation could contribute to B cell hyperactivation both directly, stimulating TLR4 and CD180 on B cells, and indirectly, possibly by inducing the production of pro-inflammatory cytokines TNF and IFN α .¹¹¹

Overall, HIV infection affects the B cell compartment profoundly, leading to B cell functional defects in the face of intense polyclonal B-cell activation manifested as increased circulating levels of immunoglobulins and autoantibodies,¹¹⁵ increased incidence of B cell malignancies,¹¹⁶ expansion of B-cell areas within lymphoid tissues [reviewed in¹¹⁷], and accumulation of terminally differentiated B cells with high expression of activation markers and inhibitory surface molecules.^{92,118–120} Profound changes in B cell subpopulations occur in HIV-infected persons, with accumulation of terminally-differentiated B cells, that are characterized by loss of CD20 and CD21, that are markers of resting B cells, and an increased expression of the plasmacytoid-associated markers CD27 and CD38. Consequently, HIV-infected individuals have higher levels of circulating CD20[−]CD21^{lo}CD27⁺⁺CD38⁺⁺⁺ plasma cells¹²¹ as compared to uninfected controls. Concurrently, a progressive depletion of peripheral CD27⁺ memory B cells, that already occurs during the acute phase of HIV infection,^{122,123} is also observed in chronic HIV-infected subjects.¹²⁴ Immature/transitional B cells (CD20⁺CD10⁺CD27[−]) are also perturbed in HIV disease, being about three times more represented in HIV-infected subjects than in healthy controls,¹¹⁰ probably as a consequence of the elevated circulating levels of IL-7. Reduction of viremia with ART does not revert the loss of memory B cells,^{17,119,122,125–127} possibly due to residual disruption in IL-2 signaling that is required for memory B cell survival,¹⁰⁶ while other B cell subsets tend to normalize.^{128,129} Several inhibitory receptors that negatively regulate B cell function are over-expressed in B cells of HIV-infected individuals. These inhibitory receptors have been suggested to play a role in the exhaustion and dysfunction of B cells, in particular the tissue-like memory B cells.¹²⁰ These inhibitory receptors include: Fc receptor-like-4 (FcRL4); Fc γ RIIB (CD32b), a low-affinity IgG receptor; CD22 (Siglec-2), a sialic acid-binding Ig-like lectin; CD85j and CD85d, members of the leukocyte Ig-like receptor family; CD22; CD72; leukocyte-associated Ig-like receptor 1 (LAIR-1); PD-1; and cytotoxic T-lymphocyte antigen 4 (CTLA4).¹²¹ B cells from HIV-infected subjects are also characterized by increased expression of activation markers that are specific for the B cell compartment (including CD80 and CD86),¹³⁰ or that are also seen in CD4 and CD8 T cells (such as the proliferation marker Ki-67 and the surface molecule CD38).³⁰ Even though B cell activation markers are reduced by ART, a chronic state of B cell hyperactivation may persist for several years.¹³¹ More recent investigations suggest that early initiation of ART within the first year of HIV infection is associated with better preservation of humoral function.²⁹

The changes in B cell phenotype and differentiation subsets observed in HIV-infected individuals are unmistakably associated with altered effector function.⁹² The observation that HIV-infected subjects accumulate immature/transitional and terminally differentiated B cells can be used to explain the overall poor B cell responses that have been reported both *in vivo* and *ex vivo*, including hyperimmunoglobulinemia, loss of responsiveness to stimuli and increased susceptibility to apoptosis.^{119,121,132} Similarly, the loss of memory B cells in

HIV infection is associated with reduced serological memory against both T cell-dependent (such as influenza virus, measles virus and tetanus toxoid) and T cell-independent antigens (such as polysaccharide pneumococcal vaccine) and are not normalized by ART administration.^{127,133–135} The decreased B cell responses to vaccine immunogens have important consequences especially for HIV-infected pediatric patients,¹³⁶ and strategies such as re-vaccination after ART-induced virus suppression have been adopted to overcome persistent memory B cell deficits.

VII. Anti-HIV Ab in HIV infection

Studies of natural history of HIV-infected individuals showed approximately 10–30% of HIV-infected individuals develop broadly neutralizing antibodies (bNAbs), though it usually takes three or more years post infection for such antibodies to develop. But the antibodies with outstanding breadth and potency develop only in 1% of infected individuals and they are called “elite neutralizers”.¹³⁷ Monoclonal bNAbs isolated from these individuals provide more precise clues to the vulnerable regions on HIV Env that can then be targeted for vaccine design. Many bNAbs to HIV-1 gp140 have been isolated including those that bind to gp120 (b12, 2G12, PG9/PG16, HJ16 and VRC01) and others that are specific for gp41 (2F5, 4E10 and Z13),^{138–140} reviewed in.^{141–143}

One of the major challenges in the HIV-1 vaccine research is the limited development of bNAbs against Env antigen. This is mainly due to the conserved nature of viral Env epitopes that are poorly immunogenic because of their complex chemical nature. Moreover, Env glycans are antigenically similar to host carbohydrates and homology of Env proteins with self proteins might induce polyreactive antibodies and lead to alterations in tolerance mechanisms. In addition, selection of virus escape mutations will lead to transient neutralizing effect of the Abs to the virus [reviewed in^{144–146}]. Thus vaccine trials in non-human primates and humans with HIV-1 Env antigens have been unsuccessful. For the induction of protective antibodies, the focus is now directed to define ways to induce bNAbs systemically and mucosally and to understand the reason for the short lived nature of HIV-1 Env antibodies, based on a thorough understanding the B cell regulatory and tolerance pathways during humoral immune responses against HIV. Future studies need to identify traits of relevant bNAbs for their functions and *in vivo* regulation, understanding the ontogeny of bNAbs with specific focus towards defining the precursor B cell subsets from which bNAbs originate including identifying the checkpoints of bNAbs, and understanding how somatic hypermutation and affinity maturation impact acquisition of bNAbs functional properties. Interestingly, nearly all bNAbs that target gp120 are heavily hypermutated, and this property appears to be important for the neutralizing activity which is lost with reversion to the germline.

Design of optimal immunogens targeted at naive and memory B cell populations that can respond to HIV-1 Envelope and in this context the role of modulation of B cell function to enhance the specificity of Ab response may be an interesting approach. Recently, Mouquet et al. reported a novel and promising approach using a clade B YU-2 gp140 trimeric antigen and single-cell antibody cloning methods to obtain 189 new anti-gp140 Abs representing 51 independent B cell clones from the IgG memory B cells of 3 patients infected with HIV-1 clade A or B viruses and exhibiting broad neutralizing serologic activity.¹⁴⁷ Development of highly functional antigen-specific memory B cell is an essential requirement for these types of approaches. It is possible that during HIV infection defective B and TFH cell function could be a significant contributing factor to prevent the development of antigen-specific memory B cells and prevent desired immune responses.

While the gold standard for HIV Ab in vaccine research has been the elicitation of bNAbs, data from the RV144 trial (reviewed in^{143,148}) points to the role of non-neutralizing Ab with antibody dependent cellular cytotoxicity capability in affording protection from HIV acquisition. Thus efforts are being directed at not only eliciting bNAb but also to explore all other potential mechanisms of Ab mediated and cellular protection from HIV.

We have recently demonstrated cumulative defects in B cell intrinsic and extrinsic factors, IL-21/IL-21R and peripheral blood TFH cell dysfunction in the development of a H1N1/09 vaccine induced immune responses in a group of ART treated, virologically controlled HIV-infected patients.^{16,17,91} These studies underscore the fact that an important path to induction of long-lived systemic and mucosal antibody responses in HIV-infected individuals lies in the understanding of, and optimizing, the B-TFH cell interactions during a vaccine-induced antibody response. The role of IL-21 in elucidation of HIV Ab needs to be investigated further as a component in HIV vaccine design, owing to its potent effects on B cells; pre-clinical studies in non-human primates are showing promise as discussed below.

VIII. IL-21 and B cells in HIV infection

As discussed earlier, B cell subsets and function are disrupted in HIV infection, resulting from excessive B cell activation and impaired survival.^{31,106} The altered distribution includes accumulation of immature transitional B cells, activated mature cells, tissue-like exhausted and short lived plasmablasts with reduced naïve and resting memory B cells. Following effective ART, the B cell subsets do not completely revert to normal, and the CD27⁺ resting memory B cells remain significantly decreased in comparison to healthy uninfected donors, despite virologic control and CD4 T cell recovery.^{126,133} Studies of IL-21 and IL-21R in the context of B cells in HIV infection have been limited. Increased IL-21R expression has been described in B cells from HIV-1-infected patients compared to control subjects, with the highest levels in non-treated patients and an inverse correlation between IL-21R expression and percentages of circulating resting memory B cells.¹⁴⁹ Levels of plasma soluble CD14, a marker of monocyte/macrophage activation that is increased in association with microbial translocation were correlated with high IL-21R expression. The authors of this study attributed B cell defects to microbial translocation, as TLR activation of B cells in vitro resulted in IL-21R up-regulation, and IL-21R⁺ memory B cells were more susceptible to spontaneous apoptosis. Lower survival of memory B cells from HIV-infected subjects has also been attributed to the disrupted IL-2 signaling leading to increased transcriptional activity of Foxo3a and increased expression of its pro-apoptotic target TRAIL. In a study by Van Grevenynghe et al,¹⁰⁶ IL-21 could prevent the apoptosis by increasing phosphorylation of Foxo3a, which in turn downregulated the transcription of its pro-apoptotic target BimEL. These results support the concept that IL-21 could be beneficial in preserving the memory B cell compartment in HIV-infected people and thereby benefit the overall humoral immune responses.^{89,90}

In a study of 2009/H1N1 influenza vaccine recipients our data was supportive of the role of IL-21 in promoting vaccine-induced B cell responses in a cohort of HIV-infected subjects.^{16,17,91} At 4 weeks post vaccination, only 50% patients developed vaccine-specific Ab titers of >1:40 and 4 fold increase in Ab titers and were designated as H1N1 vaccine responders, while the rest of the patients with H1N1 antibody titers of 1:20 units were designated as H1N1 vaccine non-responders. Healthy controls who were also given the same vaccine and were all found to be responders. Pre-vaccination frequencies of B cells, CD4 and CD8 T cell counts, and mean age were statistically equivalent between the patient groups. Several interesting observations were made in this study (summarized in Table 1). Vaccine responders manifested expansion of plasmablasts and spontaneous Ab secreting cells and at 1 week post vaccination, with development of memory B cells that could secrete

Ab following ex-vivo antigen stimulation at 4 wks post vaccination. Plasma IL-21 and IL-21 secretion by PBMC following antigen stimulation increased in responders at 4 wks together with induction of IL-21R on total- and memory B cells. The upregulation of IL-21/IL-21R 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. These observations are consistent with a recent report showing the importance of IL-21 in developing humoral immune responses in humans with severe combined immunodeficiency mentioned earlier.⁴² In addition to the IL-21/IL-21R pathway, certain innate immune factors which influence the B cell development and differentiation were also defective in the H1N1/09 vaccine non-responder patient group.⁹¹

Based on the data discussed here and prior evidence for the role of IL-21 in promoting T-dependent B cell proliferation, isotype switching, differentiation of B cells into plasma cells and development of memory B cells,^{38,39,52,150} IL-21 merits continued attention in the future for non-HIV and HIV vaccine responses. Since IL-21 production is mainly stimulated by activation of TFH cells as discussed above, it is important to determine if the B cell defects in HIV-infected people are secondary to a deficiency in the CD4 TFH cell compartment and can be reversed by IL-21. In the H1N1/09 vaccine responders we found expansion of pTFH with secretion of IL-21. Moreover we could demonstrate that the antigen-stimulated purified pTFH cells could selectively help autologous antigen-stimulated B cells to secrete H1N1 Ab in vaccine responders whereas non-pTFH (CXCR5⁻ CD4 T cells) had poor helper function. Thus the novel H1N1 influenza vaccine was able to induce development of efficient IL-21 secreting pTFH, memory B cells and plasmablasts in about half of the patients, although all including vaccine non-responders had quantitative CD4 T cell reconstitution and virus control with ART. These findings point out that we still need to understand the basis of immune deficits that persist despite otherwise successful ART which leads to increases in CD4 cells and virologic control. Though not investigated, it is possible that the function of pTFH in vaccine non-responders is compromised because of higher HIV proviral burden and residual low-level virus replication.

Whether IL-21 can directly improve the survival and function of immune cells including B cells was investigated in chronically SIV-infected rhesus macaques by administering rMamu-IL-21 to them. We found a small but significant increase in the frequencies of CD27⁺ memory B cells with upregulation of IL-21R on both CD27⁺ memory and CD27⁻ naïve B cells, together with an increase in anti-SIV antibodies in the serum of the IL-21-treated animals,¹⁸ providing proof of a biologic activity of IL-21 on B cell populations. In addition to peripheral blood, peripheral lymph nodes also showed an increase in CD27⁺ memory B cells with increased expression of IL-21R, illustrating the potential of IL-21 to increase B cell memory at different anatomical sites. It is interesting to note that administration of IL-21 was able to increase the memory B cells and anti-SIV Abs despite an advanced chronic phase of SIV infection, highlighting its immunotherapeutic properties in SIV infection.¹⁸ At this time IL-21 has not been evaluated as an adjuvant for its B cell stimulating properties in HIV/SIV vaccine strategies to prevent infection. The importance of induction of mucosal and systemic HIV/SIV Ab with vaccines is being increasingly emphasized as discussed earlier based on encouraging results in humans and rhesus macaques with vaccines utilizing boosting with envelope protein.¹⁵¹

IX. IL-21/IL-21R involvement in human inflammatory diseases and cancer

Because IL-21 exerts regulatory effects on lymphoid and myeloid cells, the role of IL-21 has been investigated in certain human immune-mediated diseases such as those involving the gastrointestinal tract, skin, rheumatoid arthritis and systemic lupus erythematosus and

malignancies (Reviewed^{3, 48}). IL-21 is over-expressed in the gut of patients with Crohn's disease and ulcerative colitis, the two major forms of inflammatory bowel disease. High IL-21 production is also seen in other Th1-cell-associated gastrointestinal diseases, such as *Helicobacter pylori*-related gastritis and celiac disease. IL-21 and IL-21R exhibit increased expression in skin lesions of patients with atopic dermatitis, and IL-21R expression is increased in epidermal keratinocytes of patients with systemic sclerosis. The synovial macrophages and fibroblasts express high IL-21 and IL-21R in patients with rheumatoid arthritis. Interestingly, reduced IL-21R expression in peripheral blood B cells of patients with lupus has been associated with nephritis and high titers of autoantibody (Reviewed^{3, 48}).

Recent studies have highlighted the potential of IL-21 as an immunotherapeutic agent for hematological malignancies based on its ability to induce apoptosis of leukemic cells. Importantly, the ability of IL-21 to enhance CD8⁺ T and NK cell cytolytic activity have led to clinical trials in patients with renal cell carcinoma and metastatic melanoma, with evidence for improved anti-tumor CD8 T cell responses. IL-21 was well-tolerated, with mild adverse side effects and showed some efficacy in tumor control or regression.^{152–155} There is a need for a better understanding of how and when IL-21 exerts its effects optimally in vivo. This knowledge could lead to future studies moving IL-21 into the therapeutic setting

Conclusions

IL-21 is an important cytokine that regulates both humoral and cellular immune responses in health and disease states. In HIV infection a beneficial role has been ascribed to IL-21 based on studies in animal models and humans with viral infections, including HIV/SIV infected states and with the influenza H1N1/09 vaccine in HIV-infected and uninfected individuals. In chronically SIV-infected rhesus macaques rMamuIL-21 alone has shown promising effects on humoral immune responses. Importantly, IL-21 also favorably influenced cellular immunity in these animals without inducing immune activation that adversely influences disease progression, thus pointing to its potential applicability in preventative or therapeutic strategies for HIV. Strategies incorporating IL-21 are favored on the basis of IL-21's B, T and NK stimulating activities. Besides its direct effects on these subsets, another mechanism by which IL-21 could augment immune responses is through inhibition of regulatory T cell activity, by inhibiting the transforming growth factor beta-mediated differentiation of naive CD4 T cells into regulatory T cells, or as recently demonstrated, through inhibiting IL-2, which is necessary for Treg growth and survival.¹⁵⁶ Studies of cancer, autoimmune and infectious disease models suggest that administration or blocking of IL-21 may be an attractive therapeutic strategy in infectious and non-infectious disease settings.

Defects in the humoral arm of the immune system during HIV infection are prominent as antibody titers against antigens previously encountered through vaccination or natural infection are low in some patients even among those who are stable on ART. Contributing factors could involve intrinsic B cell defects including altered expression of molecules involved in the B-cell homing process, abnormal T cell activation, impaired TFH function and dysfunctional interaction between T and B cells in the GC of lymphoid tissues. The significant association of IL-21 and IL-21R with H1N1/09 vaccine-mediated immune response in HIV-infected individuals (Table 1) indicates that alterations in IL-21/IL-21R function can contribute to defects in the overall immune response to vaccine antigens. Defects in IL-21 signaling within T and B cells would also impair the immune response against T dependent and independent vaccine responses. Incorporation of more than one costimulatory molecules involved in T-B interaction may be the most effective approach for optimal vaccine induced humoral immune responses. The pleiotropic actions of IL-21 on multiple lineages of cells indicate that modulation of IL-21 and IL-21R signaling could be

harnessed for therapeutic effects. Continued research is needed to understand the IL-21/IL-21R driven optimal B cell responses and factors that interfere with them.

Current knowledge of the biologic activity of IL-21, promising data in viral infections in mice, interesting findings in cells of HIV-infected patients, and in-vivo trials in humans and non-human primates all point to a favorable profile for IL-21, but as yet a definitive role of IL-21 in the prevention or treatment of HIV infection has not been established. Knowledge gained from studies on the effect of IL-21 in HIV infection, autoimmunity and immunity to tumors will advance the field for the development of IL-21-based adjuvants and therapeutics for prevention or treatment that could potentially be used in the fight against HIV.

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Abbreviations used

IL-21R	IL-21 receptor
TFH	T follicular helper cells
NK	natural killer
HIV	human immunodeficiency virus
SIV	simian immunodeficiency virus
Ab	antibody
ART	antiretroviral therapy
GC	germinal center
PCs	plasma cells
JAK	Janus family tyrosine kinases
STAT	signal transducer and activator of transcription
AID	activation-induced cytidine deaminase
pTFH	peripheral TFH
BLIMP-1	B lymphocyte induced maturation protein 1
bNAbs	broadly neutralizing antibodies

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Figure 1. Role of IL-21 in B cell differentiation and function

CD4 T cells provide a fundamental help to B cell differentiation and function through a complex network of signaling pathways. The conjunct activation of CD40 and IL-21R by interaction with CD40 ligand and IL-21 secreted by CD4 T cells activates the transcription factors NF-κB and STAT3, that orchestrate the transcription of target genes responsible for B cell proliferation and differentiation into antibody-secreting plasma cells or memory B cells. These genes include: *PRDM1*, encoding for the transcription repressor Blimp-1, and *AID*. Concurrently, they promote class switch recombination and somatic hypermutation. IL-21 also induces the expression of its own receptor in activated B cells.

Table 1

Immunological changes in B and peripheral TFH cells associated with successful humoral response to seasonal influenza vaccine¹

Cells	Changes in vaccine responders
B cells	<i>In vivo</i> up-regulation of IL-21R upon vaccination
	<i>In vivo</i> development of spontaneous Ab secreting plasmablasts 7 days post-vaccination
	<i>in vivo</i> generation of memory B cells 28 days post-vaccination
pTFH cells	<i>In vivo</i> expansion after vaccination
	<i>In vivo</i> development of Ag-specific memory pTFH cells post-vaccination
	<i>Ex vivo</i> IL-21 production upon antigen stimulation
	Induction of Ag-specific IgG Ab production in co-cultures with autologous B cells
	CXCL13 secretion upon antigen stimulation <i>ex vivo</i>

¹ Mechanisms underlying a successful influenza vaccine-induced Ab response were evaluated in a cohort of HIV-infected persons on combination antiretroviral therapy who received a single dose of nonadjuvanted H1N1/09 influenza vaccine during the 2009 H1N1 epidemic. Peripheral TFH (pTFH) cells from individuals who responded to the influenza vaccination expanded. IL-21 and CXCL13 secretion was increased, together with the ability of pTFH cells to promote antigen-specific IgG secretion when co-cultured with autologous B cells. Moreover, IL-21R expression on B cells was increased upon vaccination, indicating the importance of the IL-21/IL-21R pathway in both the pTFH and the B cell compartment. Ab production and generation of memory B cells was observed in response to the vaccine. These changes in B and pTFH compartments were not observed in HIV-infected individuals who did not respond to the H1N1/09 vaccine. The table reports the changes in both B and peripheral TFH cell compartments that were associated with responsiveness to the vaccine.