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REVIEW

Cytokines Focus

Regulation of the germinal center and humoral immunity by interleukin-21

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Cytokines play critical roles in regulating the development, survival, differentiation, and function of immune cells. Cytokines exert their function by binding specific receptor complexes on the surface of immune cells and activating intracellular signaling pathways, thereby resulting in induction of specific transcription factors and regulated expression of target genes. While the function of cytokines is often fundamental for the generation of robust and effective immunity following infection or vaccination, aberrant production or function of cytokines can underpin immunopathology. IL-21 is a pleiotropic cytokine produced predominantly by CD4$^+$ T cells. Gene-targeting studies in mice, in vitro analyses of human and murine lymphocytes, and the recent discoveries and analyses of humans with germline loss-of-function mutations in *IL21* or *IL21R* have revealed diverse roles of IL-21 in immune regulation and effector function. This review will focus on recent advances in IL-21 biology that have highlighted its critical role in T cell–dependent B cell activation, germinal center reactions, and humoral immunity and how impaired responses to, or production of, IL-21 can lead to immune dysregulation.

Introduction

Cytokines are a diverse group of small soluble proteins that have profound autocrine or paracrine effects on the development and function of hematopoietic and nonhematopoietic cells. To date, >60 cytokines, including ILs (IL-1 through IL-40), IFNs (type I [IFNα, β, ε, κ, ω], type II [IFNγ], and type III [IFNλ1/IL29, IFNλ2/IL28A, IFNλ3/IL28B]), CSFs (GM-CSF, G-CSF, M-CSF, and erythropoietin), transforming growth factors, and members of the TNF superfamily (e.g., TNFα and lymphotoxin) have been identified (Akdis et al., 2016; Catalan-Dibene et al., 2018; Crow et al., 2019; Locksley et al., 2001; Metcalf, 2008). Each of these cytokines play important roles in innate and adaptive immune responses, including leukocyte proliferation, differentiation, migration, and survival, as well as immune regulation, homeostasis, and tolerance. What is remarkable about cytokines is both their pleiotropy and redundancy. For example, IL-4 has potent effects on B cells, T cells, myeloid cells, and granulocytes, yet many of these functions on B cells and monocytes are shared by IL-13 (Akdis et al., 2016; Zurawski and de Vries, 1994). These shared functions of several cytokines, coupled with the broad effects of individual cytokines, ensure compensatory pathways underpin intact immunity if intrinsic (e.g., genetic) or extrinsic (e.g., toxins, drugs, and infections) factors compromise the function of particular cytokines or their signaling pathways. However, some cytokines have unique and nonredundant functions. For example, gene targeting of *Il7* or *Il7ra* in mice, or germline autosomal recessive (AR) biallelic mutations in *IL7RA* in humans, completely abolish T cell development, resulting in T cell lymphopenia and SCID, a fatal condition that can only be cured by allogeneic hematopoietic stem cell transplant (Giliani et al., 2005).

Detailed analyses of animal models in vivo, human cell culture in vitro, inborn errors of immunity, and pharmacological targeting of specific signaling pathways have revealed key functions of cytokines in health and disease (Akdis et al., 2016; Catalan-Dibene et al., 2018; Crow et al., 2019; Metcalf, 2008). In fact, as outlined below, studies using these approaches over the past 20 yr have delineated the nonredundant polyfunctionality of IL-21, which can affect the behavior of most immune cell types.

Germinal center (GC) reactions underpin the efficacy and longevity of humoral immune responses

GCs are specialized structures that transiently form in B cell follicles of secondary lymphoid tissues following infection or vaccination (Brink and Phan, 2018; Victora and Nussenzweig,

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2012). GCs result following cognate interactions between antigen (Ag)-specific B cells, CD4+ T cells, and accessory cells (dendritic cells [DCs], follicular DCs), which cooperate to induce extensive proliferation (i.e., clonal expansion) of responding B cells. This is accompanied by somatic hypermutation of Ig V-region genes expressed by, and subsequent selection of, high-affinity B cells, resulting in affinity maturation of the humoral response (Brink and Phan, 2018; Victora and Nussenzweig, 2012). In true Darwinian style, GC B cells that successfully compete for and integrate signals provided by CD4+ T cells, DCs, and follicular DCs, in the form of Ag, cell–cell interactions and soluble factors (i.e., cytokines), survive this environment to differentiate into long-lived memory B cells or plasma cells (PCs), the effector cells of serological memory (Brink and Phan, 2018; Victora and Nussenzweig, 2012). These dynamic processes ensure the development of long-lived pathogen-specific humoral immunity, often for the lifetime of the host, and represent a cornerstone of adaptive immune responses in higher vertebrates. Thus, understanding the fundamental drivers of GC responses will reveal mechanisms underpinning the generation of robust humoral immunity and potentially identify strategies to target these processes to modulate the GC in health and disease. In this review, we will discuss the dominant function of IL-21 in regulating the outputs of T cell–dependent (TD) B cell activation in the setting of GC reactions, long-lived humoral immunity, and immunological memory.

Discovery of IL-21/IL-21 receptor (IL-21R) identified a novel cytokine that regulates immune cell function

IL-21R was identified in 2000 by the groups of Parrish-Novak et al. at ZymoGenetics, and Warren Leonard from the National Institutes of Health (Ozaki et al., 2000; Parrish-Novak et al., 2000). IL-21R was discovered based on its structural similarity to class I cytokine receptors and was subsequently found to have significant homology with human IL-2Rβ and IL-4Rα chains. Functional studies established that a chimeric IL-21R induced proliferation of a growth factor–dependent cell line in vitro, thereby providing a platform to identify a putative ligand. Indeed, culture supernatants from activated human T cells contained a soluble factor capable of inducing proliferation of the IL-21R–expressing cell line, but not the parental line that lacked intrinsic IL-21R expression. Subsequent molecular cloning approaches led to the identification of a cDNA enriched in activated CD3+ T cells encoding a 162-amino acid polypeptide that was termed IL-21 (Parrish-Novak et al., 2000).

Consistent with the structure of its receptor, IL-21 was found to belong to the type I family of cytokines that also includes IL-2, IL-4, IL-7, IL-9, and IL-15 (Parrish-Novak et al., 2000). Receptors for these cytokines form a dimeric (IL-2 and IL-15) or trimeric (IL-4, IL-7, and IL-9) complex with IL-2Rγ, the γ-chain (γc) common to these receptors (Leonard, 2001). IL-21R was also found to associate with γc to form IL-21R, and signal via JAK1 and JAK3 kinases, STAT1, STAT3, and STAT5 (Asao et al., 2001; Habib et al., 2002). These findings immediately implicated disrupted IL-21 signaling in the pathogenesis of X-linked and AR SCID due to *IL2RG* and *JAK3* mutations, respectively.

IL-21R is expressed by nonhematopoietic cells (fibroblasts, keratinocytes, and intestinal epithelial cells), but also lymphocytes, macrophages, and DCs, with levels often increasing following activation (Good et al., 2006; Ozaki et al., 2000; Parrish-Novak et al., 2000). These initial studies also established that IL-21 is predominantly produced by activated CD4+ T cells and that IL-21 efficaciously induced B and T cell proliferation and generated highly lytic natural killer cells (Parrish-Novak et al., 2000). However, it took several more years for the importance of IL-21 in TD B cell differentiation to become apparent.

Early studies into the effect of IL-21 on murine and human B cell function

Murine B cells

Studies published from 2002 to 2005 started to provide the foundation for the realization that IL-21 has a fundamental role in regulating B cell differentiation. While germline targeting of *Il21r* in mice had minimal if any effect on lymphocyte development, responses to TD Ags were blunted, with dramatic reductions in total and Ag-specific IgG (Ozaki et al., 2002). A decreased IgG_1 response was due to reduced generation of Ag-specific IgG_1–producing PCs. This phenotype was exacerbated by combined deletion of *IL21r* and *Il4* (Ozaki et al., 2002), establishing the importance of IL-21/IL-21R signaling in not only inducing humoral immune responses but also cooperating with other cytokines previously characterized to be potent B cell growth and differentiation factors (Moens and Tangye, 2014). IL-21 was also found to enhance CD86 expression on murine B cells, enabling them to provide superior T cell costimulatory capacity (Attridge et al., 2014).

Next came the generation of mice transgenically expressing human IL-21 and analysis of in vivo and in vitro effects of IL-21 on murine B cells. Human IL-21 transgenic mice exhibit hypergammaglobulinemia and increased frequencies of class-switched B cells and PCs (Ozaki et al., 2004). Similarly, delivery of exogenous IL-21 to mice promoted B cell expansion and class switching in vivo (Ozaki et al., 2004) while reducing production of Ag-specific IgE (Suto et al., 2002). This latter finding is reminiscent of the hyper-IgE phenotype of IL-21R$^{-/-}$ or IL-21$^{-/-}$ mice (Ozaki et al., 2002; Shang et al., 2006). Consistent with these in vivo discoveries, IL-21 enhanced murine B cell proliferation, IgG class switching, and plasmablast differentiation (Ozaki et al., 2004) and inhibited IL-4–induced IgE production by these cells (Suto et al., 2002) in vitro.

Human B cells

While the function of cytokines is often preserved across different species, there are striking examples of where this bifurcates when comparing human and murine B cells. For instance, IL-7 is fundamentally required for the generation of B cells from hematopoietic progenitors in mice, but not humans (Akdis et al., 2016; Giliani et al., 2005). Similarly, B cell activating factor of the TNF family (BAFF) is necessary for development and survival of murine B cells in peripheral lymphoid tissues, while it plays a subtler role in humans (Moens and Tangye, 2014; Tangye et al., 2006). In contrast, IL-13 promotes class switching to IgE, and IL-10 induces proliferation, class

switching, and Ig secretion in human, but not murine, B cells (Akdis et al., 2016; Moore et al., 2001; Zurawski and de Vries, 1994). Thus, to further our understanding of IL-21 biology, it was important to establish its effect on human B cells.

While it was initially reported that IL-21 can promote proliferation of activated human B cells (Parrish-Novak et al., 2000), the first indication of a role in human B cell differentiation was reported by Hans Yssel and colleagues, who demonstrated IL-21 preferentially induced class switching to IgG1 and IgG3 by human naive B cells and increased secretion of these Ig isotypes by human memory B cells (Pène et al., 2004; Fig. 1 A). These findings were subsequently confirmed and extended by Ettinger et al. (Ettinger et al., 2005, 2007; Kuchen et al., 2007) and our own studies (Avery et al., 2008; Bryant et al., 2007; Good et al., 2006; Suryani et al., 2010) to establish that IL-21 was an incredibly potent growth and differentiation factor for all human B cells, irrespective of their site of isolation (cord blood, adult peripheral blood, spleen, or tonsils) or stage of differentiation (transitional, naive, memory, or GC B cells), inducing copious quantities of secreted IgM, IgG (mostly IgG1/G3), and IgA (mostly IgA_1) by these B cell populations (Fig. 1 A). This is further substantiated by constitutive or inducible expression of IL-21R on human B cell subsets (Good et al., 2006). Indeed, the effect of IL-21 surpassed that of previously established human B cell tropic cytokines (IL-2, IL-4, IL-10, and IL-13) by up to 100-fold (Avery et al., 2008; Bryant et al., 2007; Ettinger et al., 2005, 2007; Good et al., 2006; Moens and Tangye, 2014; Pène et al., 2004). IL-21 also induced naive B cells to express CD25, thereby enabling IL-21–primed naive B cells to respond to the stimulatory effects of IL-2, resulting in enhanced differentiation and Ig secretion (Berglund et al., 2013; Fig. 1, A and B). Furthermore, as part of the differentiation program, IL-21 up-regulated expression of IL-6 receptor components on plasmablasts induced in vitro (Ettinger et al., 2005), allowing these cells to respond to autocrine or paracrine IL-6, a well-established survival factor for human Ig-secreting cells (Moens and Tangye, 2014; Fig. 1, A and B). In addition to inducing plasmablasts from human transitional, naive, memory, and GC B cells, IL-21 can support the survival of and Ig secretion by in vivo–generated PCs isolated from secondary lymphoid organs (tonsils, lymph nodes, or spleen; Fig. 1), but not those in bone marrow (Rodríguez-Bayona et al., 2012). The distinct effect of IL-21 on lymphoid tissue versus bone marrow PCs correlated with differential expression of IL-21R on PCs from these diverse sites (Ettinger et al., 2005; Good et al., 2006; Rodríguez-Bayona et al., 2012; Fig. 1). Thus, IL-21 contributes to humoral immunity by mediating the generation and subsequent survival of Ig-secreting plasmablasts.

These studies clearly established that IL-21 could guide multiple fates of activated B cells in vitro and in vivo (class switching, PC differentiation/survival, Ig secretion, and GC/memory B cell formation). This was achieved by IL-21 regulating the molecular machinery required for these processes, inducing *AICDA* (encoding activation-induced cytidine deaminase [AID]), *PRDM1* (B-lymphocyte induced maturation protein 1 [BLIMP1]), *XBP1* (X-box–binding protein 1), *IRF4*, and *BCL6* and suppressing *PAX5* (Bryant et al., 2007; Diehl et al., 2008; Ettinger et al., 2005; Ozaki et al., 2004; Fig. 1 A). These factors cooperatively and antagonistically interact to regulate class switching (AID and IRF4), GC formation (BCL6), PC formation (Blimp-1, XBP-1, and IRF4), and B cell identity (PAX5; Nutt et al., 2015).

Mechanism of action of IL-21

Like most cytokines, IL-21 can activate several JAK kinases (JAK1 and JAK3) and STAT transcription factors (STAT1, STAT3, and STAT5), at least in vitro (Asao et al., 2001; Diehl et al., 2008; Habib et al., 2002; Leonard, 2001). However, these initial observations did not delineate the dominant or nonredundant functions of individual STAT molecules in mediating the effect of IL-21 on human lymphocytes. Constitutive activation of STAT3 in primary human B cells induced *PRDM1* (BLIMP1), *IRF4*, and *XBP1*, thereby initiating differentiation of B cells into Ig-secreting plasmablasts (Diehl et al., 2008). These molecular and cellular changes induced by constitutively activated STAT3 largely phenocopied the effects of IL-21 stimulation of primary human B cells (Diehl et al., 2008), inferring STAT3 is the predominant mediator of IL-21–induced human B cell differentiation. Our detailed analysis of in vitro responses of naive B cells from individuals with specific inborn errors of immunity confirmed these findings. Thus, IL-21–induced expression of *PRDM1* (*BLIMP1*), *XBP1*, and *BCL6*, as well as *IL2RA*, was abolished in naive B cells from individuals with heterozygous dominant-negative (DN) germline mutations in *STAT3* or AR mutations in *IL2IR*. In contrast, loss-of-function (LOF) mutations in *STAT1* or *STAT5* did not prevent these events (Avery et al., 2010; Berglund et al., 2013; Deenick et al., 2013; unpublished data). Interestingly, IL-21–induced expression of *AICDA* in naive B cells, as well as of *PRDM1*, *IRF4*, and *XBP1* in memory B cells, was unaffected by hypomorphic *STAT3* mutations, suggesting that class switching in naive B cells and plasmablast differentiation from memory B cells require less STAT3 function than the generation of plasmablasts from naive B cells (Avery et al., 2010; Deenick et al., 2013). Thus, by activating STAT3, IL-21 induces the suite of molecular changes necessary for human naive B cell differentiation and function.

T follicular helper (Tfh) cells

$CD4^+$ T cells can differentiate into distinct effector subsets that can be defined by expression of specific transcription factors, production of canonical cytokines, and specialized roles in immune responses. Thus, Th1 cells produce IFNγ and are important for host defense against intracellular pathogens; Th2 cells produce IL-4, IL-5, and IL-13 and mediate immunity following infection with parasites; while Th17 cells produce IL-17A, IL-17F, and IL-22 and underpin protection against fungal infections (Ma et al., 2012b; O'Shea and Paul, 2010). In contrast to these cells, Tfh cells play a general but critical role in immune responses against diverse pathogens inasmuch that they induce TD B cell activation and differentiation (Crotty, 2011, 2014; Ma et al., 2012b; Tangye et al., 2013). Tfh cells can be identified by expression of the chemokine receptor CXCR5, together with other surface molecules such as CD40L, ICOS, and PD-1 (Ma et al., 2009). Acquisition of CXCR5 positions Tfh cells to the border of the T and B cell zones of secondary lymphoid tissues; modulation of expression of additional trafficking receptors (CCR7,

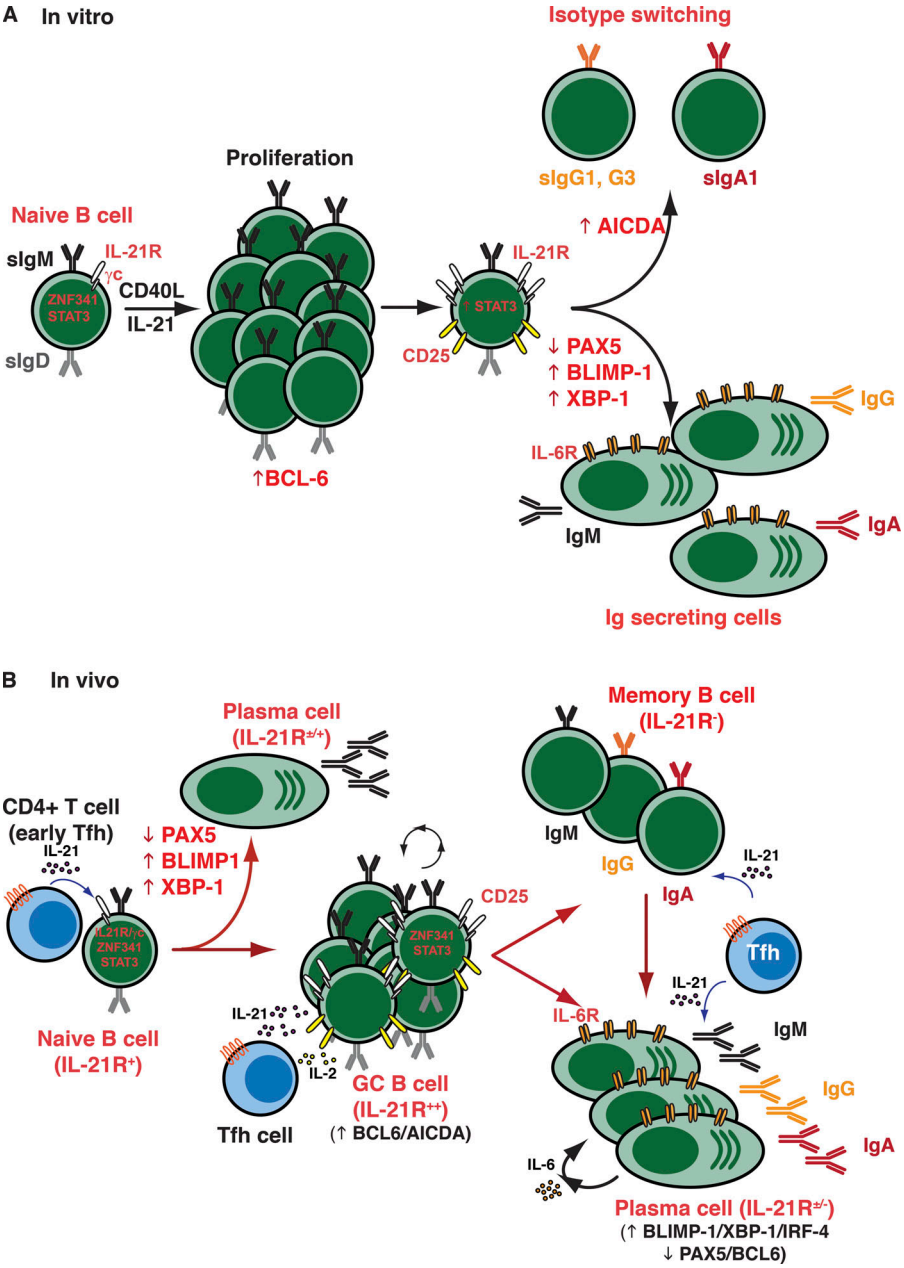

Figure 1. **IL-21 imprints multiple differentiation fates in activated B cells to yield GC cells, memory cells, and PCs for effective humoral immunity. (A)** Effects of IL-21 on human B cells in vitro. When human naive B cells are stimulated with CD40L and IL-21, they undergo intense proliferation, followed by induction of Ig class switching (predominantly to IgG_3, IgG_1, and IgA_1), as determined by acquisition of expression of switched isotypes or differentiation to plasma-like cells capable of secreting all major Ig isotypes (Avery et al., 2008; Bryant et al., 2007; Ettinger et al., 2005, 2007; Good et al., 2006; Pène et al., 2004). IL-21–activated B cells also upregulate expression of CD25 (Berglund et al., 2013), while IL-21–induced plasmablasts acquire expression of the IL-6R complex (Ettinger et al., 2005). These differentiation events coincide with induction of BCL-6, AID (encoded by *AICDA*, required for class switching), and BLIMP-1 and XBP-1 (required for PC formation; Avery et al., 2010; Bryant et al., 2007; Ettinger et al., 2005). The ability of IL-21 to induce naive B cells to differentiate into PCs in vitro was abolished by DN mutations in *STAT3*, as well as AR mutations in *ZNF341* or *IL21R* or hemizygous mutations in *IL2RG* (Avery et al., 2010; Berglund et al., 2013; Béziat et al., 2018; Deenick et al., 2013; Miggelbrink et al., 2018; Recher et al., 2011). **(B)** Model of effects of IL-21 on human B cells in vivo. Extrapolating from the in vivo data, we infer that IL-21 derived from $CD4^+$ T cells present in the microenvironment drive naive B cells to undergo activation and differentiate into either extrafollicular short-lived PCs (secreting predominantly IgM; Avery et al., 2008; Bryant et al., 2007; Kuchen et al., 2007), or seed a GC. Within GCs, B cells undergo AID-mediated somatic hypermutation; only those B cells with the highest affinity are selected and then differentiate into long-lived memory B cells or PCs under the influence of Tfh cells. Throughout this process, IL-21 induces expression of CD25, enabling the B cells to respond to IL-2, also derived from Tfh cells, which promotes the effect of IL-21 (Berglund et al., 2013). Similarly, IL-21 induces expression of IL-6R on PCs, which allows these cells to integrate survival signals by autocrine or paracrine IL-6 (Moens and Tangye, 2014). Tfh cell–derived IL-21 likely plays additional roles during humoral immunity by (1) promoting survival and Ig secretion by PCs (Rodríguez-Bayona et al., 2012) and (2) initiating the rapid differentiation of memory B cells into PCs following reencounter with the initiating Ag (Bryant et al., 2007; Deenick et al., 2013; Ettinger et al., 2005). IL-21–mediated differentiation of naive B cells to these distinct effector fates in vivo is controlled by the balanced expression and function of various transcription factors, including (but not exclusively) PAX5, BCL-6, BLIMP-1, XBP-1, and IRF4. The generation of memory B cells and PCs secreting high-affinity Ig is compromised by DN mutations in *STAT3* (Avery et al., 2010; Deenick et al., 2013) and also by AR mutations in *ZNF341* (Béziat et al., 2018; Frey-Jakobs et al., 2018) or *IL21R* (Deenick et al., 2013) or hemizygous mutations in *IL2RG* (Miggelbrink et al., 2018; Recher et al., 2011).

CXCR4, and EBI2) enables migration and localization of Tfh cells to distinct areas of GCs, thereby allowing them to be precisely and ideally positioned to provide survival and helper signals to B cells for their differentiation into memory cells and PCs (Crotty, 2011, 2014; Ma et al., 2012b; Tangye et al., 2013).

IL-21 is produced by Tfh cells

Investigation into the effects of IL-21 on murine and human B cells coincided with determining which cells were the predominant producers of this cytokine. While the initial discovery of IL-21 reported rapid induction of *IL21* mRNA in activated human $CD4^+$, but not $CD8^+$, T cells (Parrish-Novak et al., 2000), it was not until Chtanova et al. (2004) reported the transcriptional profile of human tonsil $CD4^+$ T cell subsets that it became apparent that Tfh cells were strongly enriched for cells expressing the highest levels of *IL21*. These findings were promptly confirmed for murine Tfh cells (Vinuesa et al., 2005), thereby implicating a key role for IL-21 in GC reactions. Indeed,

Table 1. **Inborn errors of immunity impacting IL-21 signaling and humoral immunity**

Gene	Clinical features	Cellular defects and mechanisms of disease
IL2RG (X-linked) *JAK3* (AR)	$B^+T^-NK^-$ SCID; recurrent, severe, and often fatal microbial infections; impaired B cell responses due to lack of T cell help	Persistent B cell defect (poor responses in vivo, ↓ total and class-switched memory B cells) after HSCT in nonconditioned patients due to intrinsic B cell requirement of IL-21R/γc/JAK3 cytokine signaling
STAT3 (AD DN) *ZNF341* (AR)	*S. aureus, Streptococcus pneumonia,* and *C. albicans* infections; eczema; vascular/musculoskeletal/dental/connective tissue defects; impaired humoral immunity; and hyper-IgE	↓ Memory B cells in vivo; ↓ naive B cell plasmablast differentiation in vitro to IL-21; ↓ IL-21–dependent upregulation of *IL2RA* expression, impairing responses to IL-2; ↓ Tfh memory cells in vivo; ↓ Tfh cell generation from naive $CD4^+$ T cells in vitro
IL21R/IL21 (AR)	Combined immunodeficiency; disseminated cryptosporidium infection, susceptibility to *Pneumocystis jirovecii* and fungal infections; impaired humoral immunity; hypogammaglobulinemia; increased serum IgE	↓ Total memory and class-switched memory B cells in vivo; ↓ naive B cell differentiation into plasmablasts in vitro to IL-21; ↓ Tfh memory $CD4^+$ T cells in vivo; ↓ Tfh cells from naive $CD4^+$ T cells in vitro

AD, autosomal dominant; NK, natural killer.

IL-21 derived from Tfh cells was responsible for $CD4^+$ TD B cell differentiation into Ig-secreting cells in vitro (Avery et al., 2008; Bryant et al., 2007; Kuchen et al., 2007; Ma et al., 2009). Notably, most Tfh cells in human tonsils that express IL-4 also expressed IL-21 (Ma et al., 2009), revealing Tfh cells as a source of cytokines well characterized for B cell activation (Moens and Tangye, 2014). Kinetic analysis of T cell responses revealed that acquisition of cytokine expression by murine Tfh cells was temporally regulated. Thus, IL-21 is expressed during the early stages of a GC response; Tfh cells coexpressing IL-4 and IL-21 subsequently accumulate, while $IL\text{-}4^+IL21^-$ Tfh cells predominate at later stages of the response (Gonzalez et al., 2018; Weinstein et al., 2016). Thus, studies in mice and humans revealed the existence of distinct subsets of Tfh cells which likely play specialized roles is regulating the dynamics, quality, quantity, class, and outcome of Tfh-mediated humoral immune responses (Gonzalez et al., 2018; Ma et al., 2015; Morita et al., 2011; Reinhardt et al., 2009; Weinstein et al., 2016).

Regulation of IL-21 expression in $CD4^+$ T cells

Induction of IL-21 in $CD4^+$ T cells requires cytokine-mediated STAT3 signaling. This was first demonstrated in vitro with the findings that culturing murine $CD4^+$ T cells with IL-6, IL-21 itself, IL-23, or IL-27 resulted in substantial production of IL-21 (Batten et al., 2010; Dienz et al., 2009; Eto et al., 2011; Karnowski et al., 2012; Nurieva et al., 2008; Suto et al., 2008). While similar findings were made for humans, the cytokine inducing greatest expression of IL-21 in human naive $CD4^+$ T cells was IL-12 (Batten et al., 2010; Diehl et al., 2012; Ma et al., 2009, 2016; Schmitt et al., 2009, 2013, 2014). Despite these species' differences, STAT3 signaling downstream of receptors for these cytokines was required for IL-21 induction in human and murine $CD4^+$ T cells (Batten et al., 2010; Ma et al., 2012a, 2016; Nurieva et al., 2008; Table 1).

The physiological significance of these findings investigating the cytokine-mediated induction of IL-21 in in vitro–cultured $CD4^+$ T cells was underscored by several key observations. First, induction of IL-21 by IL-6, IL-12, IL-23, or IL-21 was sufficient to mediate the differentiation of co-cultured B cells into Ig-secreting plasmablasts in vitro (Diehl et al., 2012; Dienz et al., 2009; Ma et al., 2009, 2012a, 2016; Schmitt et al., 2009, 2013, 2014). Second, in vivo analysis of murine Tfh cells revealed IL-21 expression was significantly reduced when IL-6 signaling was abrogated (Harker et al., 2015; Karnowski et al., 2012). Third, expression of IL-21 by human naive and memory $CD4^+$ T cells was reduced in patients with LOF mutations in *IL12RB1*, *IL21R*, or *STAT3* (Kotlarz et al., 2013; Ma et al., 2012a, 2015, 2016; Schmitt et al., 2013; Table 1). These findings of the importance of $CD4^+$ T cells acquiring expression of IL-21 in response to signals provided by cytokines to facilitate B cell differentiation were further substantiated by detailed analysis of the impact of IL-21/IL-21R deficiency on GCs, Tfh cells, and humoral immunity in mice and humans, as discussed next.

B cell–intrinsic IL-21R signaling has nonredundant roles in the efficiency of GC reactions

Insights from murine models

The weak humoral responses to TD Ags originally documented in IL-21R–deficient mice (Ozaki et al., 2002) were subsequently found to be accompanied by compromised, but not abolished, GC reactions, as evidenced by reduced magnitude and premature dissolution of GCs due to less B cell proliferation, fewer Ag-specific splenic and long-lived bone marrow PCs, lower levels of Ag-specific serum Ig, and minimal affinity maturation in both the memory and PC pools (Bessa et al., 2010; Eto et al., 2011; Gonzalez et al., 2018; King et al., 2010; Linterman et al., 2010; Nurieva et al., 2008; Rankin et al., 2011; Rasheed et al., 2013; Vogelzang et al., 2008; Zotos et al., 2010). In fact, most of the memory B cells detected in IL-21R– or IL-21–deficient mice continued to express unmutated Ig V-region genes, indicating greatly relaxed stringency for affinity-based transition of precursor B cells into the memory compartment (Zotos et al., 2010). Notably, even though memory B cells could be generated in IL-21R–deficient mice, these cells exhibited limited anamnestic responses, with reduced generation of IgG-secreting PCs following reexposure to the initiating Ag, resulting in ineffective recall responses (Rankin et al., 2011).

Low-affinity unmutated memory B cells have also been detected in mice with Bcl6-deficient B cells, which are unable to form GCs (Toyama et al., 2002). Interestingly, expression of

Bcl-6 (Gonzalez et al., 2018; Linterman et al., 2010; Zotos et al., 2010) and the c-Myc–induced transcription factor AP4 (Chou et al., 2016) was greatly reduced in GC B cells from immunized IL-21R–deficient mice. Given the fundamental requirement for Bcl-6 in GC formation (Brink and Phan, 2018; Nutt et al., 2015; Victora and Nussenzweig, 2012) and the recent finding that AP4 facilitates ongoing proliferation of GC B cells (Chou et al., 2016), impaired expression of these transcriptional regulators provides a plausible mechanism for inefficient formation, proliferation, maintenance, and output (i.e., affinity maturation and selection of high-affinity variants) from GC reactions in the absence of IL-21 signaling. These findings also raise the possibility that memory B cells generated in the absence of IL-21 arise independently of the GC (Toyama et al., 2002; Zotos et al., 2010). This scenario is supported by the ability of IL-21 to induce AID expression in activated B cells in vitro (Avery et al., 2010; Diehl et al., 2008; Ettinger et al., 2005; Ozaki et al., 2004), a process partially dependent on AP4 (Chou et al., 2016), and diminished somatic hypermutation, affinity maturation, and class switching in IL-21R–deficient B cells (Rasheed et al., 2013; Zotos et al., 2010). Many of these defects in humoral immunity were also observed when IL-21R was lacking only from B cells (Bessa et al., 2010; Chou et al., 2016; King et al., 2010; Rasheed et al., 2013; Zotos et al., 2010), establishing a requirement for B cell–intrinsic IL-21 signaling to generate long-lived humoral immune responses.

Defects in humoral responses in mice with IL-21R–deficient B cells were largely recapitulated in mice generated to selectively lack *Stat3* in the B cell lineage. Thus, while mice with *Stat3*-deficient B cells have normal levels of total serum IgM, IgG, and IgA, production of IgM and IgG (IgG1, 2b, and 3) following immunization with TD Ags was significantly reduced (Fornek et al., 2006; Kane et al., 2016). This resulted from reduced clonal expansion, impaired class switching, and enhanced apoptosis of Ag-specific B cells within GC reactions (Fornek et al., 2006; Kane et al., 2016). Selection of high-affinity Ag-specific GC B cells was also severely compromised by B cell–intrinsic Stat3 deficiency (Kane et al., 2016), which possibly contributed to increased apoptosis of these cells. Although not tested directly, one hypothesis would be that reduced Ig class switching and somatic hypermutation in Stat3-deficient B cells results from impaired induction of *Aicda* in response to STAT3-activating cytokine such as IL-21 (Avery et al., 2010; Ettinger et al., 2005; Ozaki et al., 2004).

Collectively, although GC formation can be initiated by *Il21r*- or *Stat3*-deficient murine B cells, these studies established that B cell–intrinsic IL-21/IL-21R/STAT3 signaling plays a critical and nonredundant function in sustaining GC reactions and establishing long-lived humoral immunity mediated by high-affinity, Ag-specific memory and PCs.

Insights from human inborn errors of human immunity

Primary immunodeficiencies (PIDs) result from monogenic mutations that predispose affected individuals to recurrent, severe, and often fatal infectious diseases (Picard et al., 2018). As the genetic lesion underlying many PIDs is known (Picard et al., 2018), these conditions can reveal the unique functions of specific genes and related signaling pathways in immune cells and the importance of these pathways in productive and protective immune responses. Thus, analysis of PIDs unravels the molecular requirements for lymphocyte development and function in health and disease.

Clinical manifestations of several PIDs result from defective responses to, or production of, IL-21. This includes patients with inactivating mutations in *STAT3*, *ZNF341*, *IL21*, *IL21R*, or *IL2RG* (Picard et al., 2018; Table 1 and Fig. 1 A). Patients with DN mutations in *STAT3* or AR mutations in *ZNF341* present with recurrent *Staphylococcus aureus* and *Candida albicans* infections, pneumonia, and elevated levels of serum IgE (Béziat et al., 2018; Frey-Jakobs et al., 2018; Holland et al., 2007; Minegishi et al., 2007). These patients also fail to generate Ag-specific antibody (Ab)-secreting B cells and high-affinity serum Abs following infection or routine vaccination (Avery et al., 2010; Béziat et al., 2018; Deenick et al., 2013; Dreskin et al., 1985; Frey-Jakobs et al., 2018; Leung et al., 1988; Sheerin and Buckley, 1991; Table 1). Consistent with impaired humoral immunity, and despite normal frequencies of total peripheral blood B cells, *STAT3* DN or *ZNF341* deficient patients have few circulating memory B cells, and their naive B cells are unable to differentiate into plasmablasts in vitro in response to IL-21 (Avery et al., 2010; Berglund et al., 2013; Béziat et al., 2018; Deenick et al., 2013; Frey-Jakobs et al., 2018; Ma et al., 2015; Table 1 and Fig. 1 A). *STAT3* DN naive B cells also failed to up-regulate CD25 expression in response to IL-21 stimulation, thereby limiting the ability of IL-21–primed B cells to respond to the effects of IL-2 (Berglund et al., 2013; Table 1). IL-21–induced up-regulation of IL-6R on plasmablasts would also be impaired by DN *STAT3* or AR *ZNF341* mutations, further compromising responses of these B cells to IL-6–mediated survival and differentiation. This would be consistent with reduced serum Ig levels in individuals with LOF mutations in *IL6R* (Spencer et al., 2019). The clinical, cellular, and molecular similarities between patients with mutations in *STAT3* or *ZNF341* resulted from the ability of ZNF341 to regulate expression and function of STAT3 downstream of STAT3-activating cytokine receptors, particularly IL-21 in the setting of B cell responses (Béziat et al., 2018). These findings revealed ZNF341 and STAT3 have nonredundant roles in generating Ag-specific memory and Ab-secreting cells in vivo and implicated IL-21 as the predominant cytokine acting upstream of ZNF341/STAT3 to maintain humoral immunity.

A likely role of IL-21R/STAT3 signaling in efficient Ab responses in humans was supported by the finding that B cells with mutations in *IL2RG*, a component of the IL-21R complex, or the downstream kinase *JAK3* phenocopy *STAT3* DN B cells with respect to impaired memory cell formation and unresponsiveness to IL-21 (Miggelbrink et al., 2018; Recher et al., 2011; Table 1). Importantly, investigation of SCID patients who had undergone hematopoietic stem cell transplant and engrafted with donor T cells but retained *IL2RG* mutant B cells established a clear requirement for expression of γc, and by extension IL-21R, by B cells for the successful generation of effective Ag-specific Ab responses (Miggelbrink et al., 2018; Recher et al., 2011; Table 1).

IL21R-deficient patients present with recurrent respiratory and gastrointestinal infections, including pneumonia, otitis media, and cryptosporidia, as well as reduced serum IgG levels

and poor Ab responses following vaccination with TD Ags (Erman et al., 2015; Kotlarz et al., 2013, 2014; Stepensky et al., 2015; Table 1). A single *IL21* patient who developed early-onset inflammatory bowel disease, recurrent respiratory infections, and hypogammaglobulinemia has also been reported (Salzer et al., 2014). IL-21/IL-21R–deficient patients have near-normal numbers of peripheral B cells but a paucity of memory B cells, essentially lacking isotype-switched (IgG^+, IgA^+) cells (Deenick et al., 2013; Erman et al., 2015; Kotlarz et al., 2013, 2014; Ma et al., 2015; Stepensky et al., 2015; Table 1). Predictably, IL-21R–deficient naive B cells failed to undergo IL-21–induced proliferation, class switching, and plasmablast differentiation in vitro due to an inability to acquire expression of *AICDA*, *PRDM1*, *XBP1*, and *IL2RA* (Berglund et al., 2013; Deenick et al., 2013; Table 1). The clinical features of IL-21/IL-21R–deficient patients, coupled with the documented functional and molecular defects of their B cells, unequivocally established the criticality of IL-21 in establishing and/or maintaining long-lived humoral immune responses and serological memory.

IL-21 restrains production of IgE

Intriguingly, deficiency of IL-21 or IL-21R in mice and humans causes elevated basal and/or Ag-specific levels of serum IgE (Erman et al., 2015; King et al., 2010; Kotlarz et al., 2013, 2014; Ozaki et al., 2002; Rankin et al., 2011; Salzer et al., 2014; Shang et al., 2006; Stepensky et al., 2015; Zotos et al., 2010). A defining clinical feature of DN *STAT3* or AR *ZNF341* mutations is extreme levels of serum IgE (Béziat et al., 2018; Frey-Jakobs et al., 2018; Holland et al., 2007; Minegishi et al., 2007). We recently found that *Stat3*-deficient murine B cells produce dramatically increased levels of Ag-specific IgE compared with Stat3-sufficent B cells, and this defect was B cell intrinsic (Kane et al., 2016). Thus, IL-21/ZNF341/STAT3 signaling also plays an important role in regulating IgE production, with impaired B cell responses to IL-21 likely contributing to the hyper-IgE phenotype of *STAT3* DN and *ZNF341* or *IL21/IL21R*–deficient individuals.

While Stat3 operates intrinsically in B cells to maintain IgE levels (Kane et al., 2016), exact mechanisms underlying IL-21/STAT3–mediated IgE regulation remain to be completely elucidated. Interestingly, elevated serum IgE in IL-21R–deficient mice was abolished in $Il4^{-/-}Il21r^{-/-}$ mice (Ozaki et al., 2002), indicating this dysregulated IgE production was IL-4 dependent (Ozaki et al., 2002). Interestingly, $CD4^+$ T cells from patients with hyper-IgE syndromes due to mutations not only in *STAT3* but also *DOCK8* or *ZNF341* have impaired production of IL-21 but increased production of Th2 cytokines (IL-4, IL-5, and IL-13; Béziat et al., 2018; Ma et al., 2015, 2016; Tangye et al., 2017). Remarkably, a population of $IL4^{hi}IL\text{-}5^{hi}IL\text{-}13^{hi}IL\text{-}21^{lo}$ $CD4^+$ T cells was recently identified in humans and mice and was associated with production of allergenic IgE (Gowthaman et al., 2019). Thus, threshold levels of IL-21 in $CD4^+$ T cells are required to directly suppress Th2-induced production of IgE by B cells to regulate IgE production and subsequent IgE-mediated immune pathologies.

IL-21 is dispensable for Tfh cell formation

In contrast to B cell output from GCs, many studies have reported that Tfh cell generation is independent of IL-21/IL-21R (Bessa et al., 2010; Eto et al., 2011; Karnowski et al., 2012; King et al., 2010; Linterman et al., 2010; Rasheed et al., 2013). However, combined blockade of IL-6 and IL-21 signaling significantly impeded Tfh cell generation during GC responses (Eto et al., 2011; Karnowski et al., 2012). Thus, IL-21 plays a complementary, rather than obligatory, role in regulating Tfh cell differentiation and fate, with inputs from different cytokines cooperating to establish the Tfh cell pool.

This is supported from findings obtained from murine models and human PIDs. First, *Stat3*-deficient murine $CD4^+$ T cells are inefficient at generating Tfh cells, acquiring IL-21 expression, and supporting GC formation and effective humoral responses following Ag challenge in vivo (McIlwain et al., 2015; Ray et al., 2014; Wu et al., 2015). Second, frequencies and numbers of circulating $CD4^+CXCR5^+$ Tfh-type (cTfh) cells were only modestly reduced in patients with *IL21* or *IL21R* mutations (Erman et al., 2015; Kotlarz et al., 2013; Ma et al., 2015; Salzer et al., 2014; Stepensky et al., 2015). However, cTfh cells were significantly reduced in individuals with *STAT3* DN mutations (Ma et al., 2012a, 2015; Mazerolles et al., 2013), indicating a requirement for signaling through multiple STAT3-activating cytokines (IL-6, IL-21, IL-23, and IL-27; Batten et al., 2010; Diehl et al., 2012; Ma et al., 2009, 2012a, 2015, 2016; Schmitt et al., 2009, 2014) for adequate cTfh cell formation. Notably, like *STAT3* DN cTfh cells, cTfh cells from *IL21R*-deficient patients were predominantly of a nonhelper Th1-type and thus likely to be poor B cell helpers despite these cells being present in normal numbers in the absence of IL-21 signaling (Ma et al., 2015, 2016). Third, some studies have reported numerical or functional deficiencies in Tfh cells in *Il21r*-deficient mice; however, these deficits were often mild, transient or could be restored by the use of different adjuvants or infections (Eto et al., 2011; Karnowski et al., 2012; Linterman et al., 2010; Nurieva et al., 2008; Vogelzang et al., 2008). Thus, while IL-21 may not be required for the generation/maintenance of Tfh cells, it can influence the quality and functionality of these cells, indicating the nuanced effects of specific cytokines on imprinting differentiation and effector function during lymphocyte differentiation.

IL-21, Tfh cells, and immune (dys)regulation

Murine models and human autoimmune diseases

Aberrant immune function can manifest clinically as autoimmunity, with many of these diseases being caused by the production of pathogenic autoreactive antibodies. The potent effect of IL-21 on human and murine B cell differentiation would invoke the scenario of IL-21 contributing to dysregulated B cell differentiation and function in the setting of autoimmunity. IL-21 was first implicated in disease etiology by the finding that it is highly expressed in splenocytes of BXSB-*Yaa* mice, which develop severe systemic lupus erythematosus–like pathology including lymphadenopathy, hypergammaglobulinemia, and immune complex-mediated glomerulonephritis (Ozaki et al., 2004). Critically, immune pathology in this and other mouse models of lupus was dramatically reduced by in vivo blockade of IL-21 function by soluble IL-21R-Fc (Herber et al., 2007) or neutralizing anti–IL-21R mAbs (Zhang et al., 2015) or gene

targeting of *Il21r* (Bubier et al., 2009; Rankin et al., 2012). These studies paralleled investigations into the contribution of IL-21 to the pathophysiology of other murine models of human autoimmune diseases. Specifically, IL-21 was found to be overexpressed in immune cells and/or inflamed tissues in models of type 1 diabetes (Kenefeck et al., 2015; Liu and King, 2013; McGuire et al., 2011; Spolski et al., 2008; Sutherland et al., 2009; Vogelzang et al., 2014), rheumatoid arthritis (Jang et al., 2009; Sakuraba et al., 2016; Young et al., 2007), and experimental uveitis (Wang et al., 2011). Furthermore, disease severity was ameliorated by impeding IL-21/IL-21R signaling in these conditions. Notably, selective deficiency of IL-21R from B cells was sufficient to protect mice from disease (McPhee et al., 2013; Sakuraba et al., 2016), revealing the indispensable role of B cell–intrinsic IL-21 signaling in the pathophysiology of these models. IL-21 blockade could also ameliorate disease progression when administered to mice with preexisting disease (McGuire et al., 2011; Zhang et al., 2015), suggesting IL-21 neutralization as a possible therapeutic for various autoimmune conditions. Interestingly, several studies also reported increased frequencies and/or function of Tfh cells in these models of human disease (Kenefeck et al., 2015; Rankin et al., 2012; Subramanian et al., 2006; Vinuesa et al., 2005; Zhang et al., 2015), inferring Tfh cell–mediated, IL-21–dependent dysregulation of B cell differentiation may contribute to humoral autoimmunity.

To determine the physiological significance of these findings in mice, and test the validity of targeting IL-21 as a therapy for human immune dyscrasias, expression and production of IL-21 has been examined in a vast array of human autoimmune conditions (reviewed in Gensous et al., 2018). Serum levels of IL-21, IL-21 production by $CD4^+$ T cells, and proportions of cTfh cells have been found to be increased in patients with systemic lupus erythematosus (Dolff et al., 2011; Feng et al., 2012; He et al., 2013; Le Coz et al., 2013; Nakou et al., 2013; Simpson et al., 2010; Terrier et al., 2012; Wang et al., 2014), rheumatoid arthritis (Liu et al., 2012; Rao et al., 2017; Wang et al., 2013), Sjogren's syndrome (Simpson et al., 2010; Szabó et al., 2016; Verstappen et al., 2017), type 1 diabetes (Kenefeck et al., 2015; Xu et al., 2013), and multiple sclerosis (Fan et al., 2015; Romme Christensen et al., 2013). Many of these conditions are characterized by the formation of ectopic lymphoid tissues that often contain GC-like structures (Gensous et al., 2018). Notably, increases in serum IL-21 and cTfh cells often positively correlated with disease activity, including levels of auto-Abs (Feng et al., 2012; He et al., 2013; Liu et al., 2012; Rao et al., 2017; Simpson et al., 2010; Szabó et al., 2016; Verstappen et al., 2017; Wang et al., 2013, 2014; Xu et al., 2013). As these parameters were attenuated by immunosuppressive treatments (Fan et al., 2015; Feng et al., 2012; Romme Christensen et al., 2013; Verstappen et al., 2017; Wang et al., 2013, 2014; Xu et al., 2013), excessive production of IL-21 by Tfh-like cells in ectopic lymphoid tissues in inflamed tissues is likely to directly contribute to disease severity. Collectively, these studies identify aberrant IL-21 production/Tfh cell differentiation as biomarkers of human autoimmunity and infer that targeting IL-21 is a valid approach to developing therapies to treat such conditions. Importantly, the clinical features of IL-21R–deficient individuals highlight potential complications relating to sustained therapeutic IL-21 blockade in the context of infectious susceptibility.

T follicular regulatory (Tfr) cells

Regulatory T cells (T reg cells) restrain the effector function of activated immune cells, thereby limiting collateral damage caused by a stimulated immune response following pathogen exposure (Josefowicz et al., 2012). Many studies over the past decade have established that B cell/Tfh cell–mediated humoral immune responses are controlled by Tfr cells, a specialized T reg cell subset that has co-opted the transcriptional machinery of Tfh cells to migrate into the B cell follicles and GCs to exert their regulatory effect (reviewed in Stebegg et al., 2018; Wing et al., 2018). Interestingly, IL-21 appears to play a key role in balancing the opposing influences of Tfh and Tfr cells on humoral immunity (Fig. 2).

Tfr cells inhibit proliferation and production of IL-21 and IL-4 by Tfh cells, thereby suppressing B cell expansion and differentiation (Miles et al., 2015; Sage et al., 2014; 2016; Fig. 2). The inhibitory effect of Tfr cells on Tfh cell–induced B cell differentiation was overcome by adding exogenous IL-21 to cocultures of Tfr, Tfh, and responding B cells (Ding et al., 2014; Sage et al., 2016). In this setting, IL-21 restored metabolic function in Tfr cell–suppressed B cells, enabling their differentiation into plasmablasts (Sage et al., 2016). These results established that Tfr cell–mediated repression of IL-21 production by Tfh cells was a predominant mechanism of humoral immune regulation (Fig. 2). This provides additional evidence for the critical requirement for Tfh cell–derived IL-21 in inducing and guiding B cell immune responses.

While Tfr cells repress IL-21 production by Tfh cells, IL-21 reciprocally restrains Tfr cell generation. Tfr cells are increased in *Il21*-gene targeted mice, resulting in a skewed Tfr/Tfh cell ratio in favor of Tfr cells (Ding et al., 2014; Jandl et al., 2017). The Tfr cells that expand in the absence of IL-21 were functional, as evidenced by suppression of autoantibody titers following transfer into autoimmune-prone mice. Furthermore, administration of recombinant IL-21 to *Il21* KO mice reduced the imbalance in the Tfr/Tfh cell ratio, substantiating this key effect of IL-21 on Tfr cell proliferation and maintenance (Ding et al., 2014), reminiscent of the antagonistic effect of IL-21 on the formation of conventional T reg cells (Attridge et al., 2012; Schmitz et al., 2013).

Thus, Tfh and Tfr cells represent the yin and the yang of humoral immunity, with IL-21 availability being the key determinant in orchestrating the balance of inputs and outputs from these cells to achieve robust and long-lived serological immune responses without invoking immune dysregulation and potential autoimmunity (Fig. 2).

Conclusions

Since its discovery in 2000, IL-21 has established itself as a cytokine critical for TD B cell/GC responses. This has been evidenced not only by the ability of IL-21 to potently induce activation and differentiation of human and murine B cells in vitro, but also by the consequences of disrupted IL-21R/STAT3 signaling on humoral immunity in vivo in murine models and,

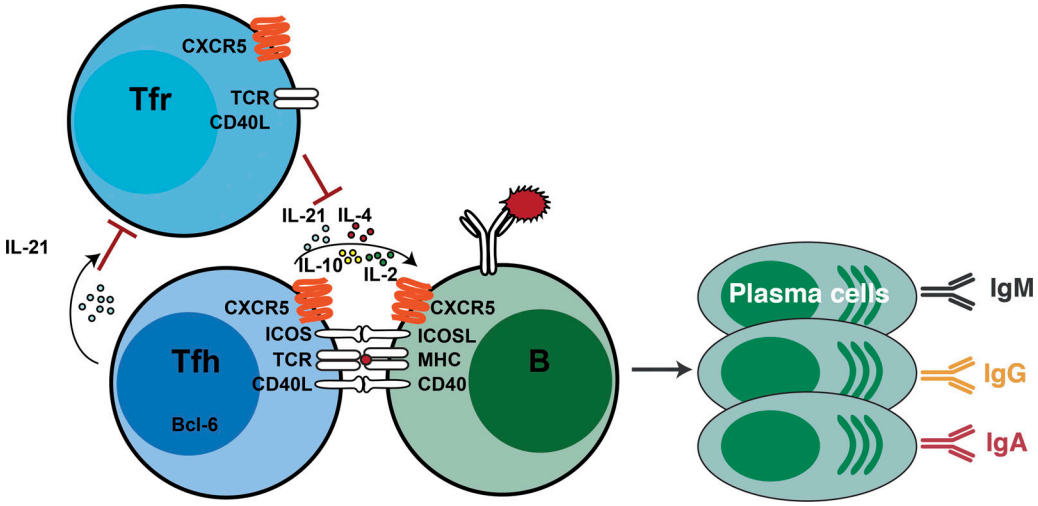

Figure 2. **The yin and yang of IL-21–mediated regulation of CD4⁺ T cell function during humoral immune responses.** Naive CD4⁺ T cells differentiate into Tfh cells, which directly promote the differentiation of cognate B cells into memory cells or PCs. This effect is largely mediated by the production by Tfh cells of IL-21. The magnitude of TD humoral immune responses is restrained by the actions of Tfr cells, which also arise from naive CD4⁺ T cells. Tfr cells limit the availability of B cell help by suppressing the production of IL-21, as well as IL-4, by Tfh cells. However, Tfh cell–derived IL-21 can also function to restrict the generation of Tfr cells. This dynamic balance presumably ensures the elicitation of optimal and effective immune responses without compromising the host by inducing deficient or autoreactive immune responses.

more importantly, humans. While analysis of development and function of immune cells in peripheral blood of healthy donors and patients with inborn errors of immunity may lack the granularity of studying kinetics of immune responses in vivo to known Ags in experimental mice, the parallels between impaired IL-21R/STAT3 signaling in human and murine B cells unequivocally reveals the fundamental role of this signaling axis in humoral immune responses. Thus, acquisition of IL-21 expression by Tfh cells and subsequent IL-21R–mediated B cell–intrinsic signaling are requisite and rate-limiting steps for generating robust and long-lasting protective humoral immunity in response to natural infection or immunization. Ongoing studies of IL-21/IL-21R biology and the discovery of additional patients with genetic variants impacting this pathway will reveal more mechanisms of action and functions for IL-21 and potentially identify strategies whereby modulating its effects could be clinically beneficial for treating immunodeficiency or allergy or enhancing humoral immunity in the setting of vaccination. Exciting times lie ahead for IL-21 enthusiasts!

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