International Immunology, Vol. 22, No. 1, pp. 7–12
doi:10.1093/intimm/dxp112
Advance Access publication 23 November 2009

IL-21 and T follicular helper cells

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Received 25 September 2009, *accepted* 30 October 2009

REVIEW

Abstract

Upon encounter with antigen, CD4$^+$ T cells differentiate into effector T_h subsets with distinctive functions that are related to their unique cytokine profiles and anatomical locations. One of the most important T_h functions is to provide signals to developing B cells that induce specific and appropriate antibody responses. The major CD4$^+$ T cell subset that helps B cells is the T follicular helper (T_{FH}) cell, whose expression of the chemokine receptor CXCR5 [chemokine (C–X–C motif) receptor 5] serves to localize this cell to developing germinal centers (GCs) where it provides instructive signals leading to Ig class switching and somatic mutation. T_{FH} cells produce high levels of IL-21, a cytokine that is critical for GC formation and also for the generation of T_{FH} cells. Although T_{FH} cells have been found to produce cytokines characteristic of other T_h subsets, they represent a distinct lineage whose development is driven by the transcription factor B-cell CLL lymphoma-6 (BCL6). Consistent with their critical role in the generation of antibody responses, dysregulated T_{FH} function has been associated with the development of systemic autoimmunity. Here, we review the role of IL-21 in the regulation of normal T_{FH} development and function as well as in progression of autoimmune responses.

Keywords: autoimmunity, BCL6, germinal center, T_h subsets

Introduction

Specific CD4$^+$ T cell effector responses can be delegated to distinct subsets characterized as T_h1, T_h2, T_h17 or regulatory T cell (Treg) cells, with the development of each of these subsets being driven, respectively, by the master lineage-specific transcription factors T-bet [now also denoted as T-box 21 (TBX21)], GATA binding protein 3 (GATA-3), retinoid-related orphan receptor γt (RORγt) or forkhead box protein P3 (FOXP3) (1). T-cell-dependent antibody production is a critical component of the normal immune response, and T_h2 cells were originally believed to be the predominant source of B cell help because of their production of IL-4, a cytokine known to be involved in B cell proliferation as well as Ig class switching (1).

Subsequently, IL-21 was identified as a T_h-derived, type I, four-α-helical bundle cytokine that was critical for plasma cell generation as well as isotype switching (2) and normal Ig production (3), consistent with IL-21 being a T_h2-specific cytokine (4); however, other data indicated that IL-21 had T_h1-like properties as well (5).

More recently it became clear that the CD4$^+$ T cells involved in germinal center (GC) formation and function—denoted T follicular helper cells (T_{FH} cells)—were distinct from any of these previously identified subsets. These T_{FH} cells expressed high levels of the chemokine receptor CXCR5 [chemokine (C–X–C motif) receptor 5], allowing them to home to and be retained by the lymphoid follicle, where contact with antigen-primed B cells led to B cell proliferation, isotype switching and somatic mutation of the Ig repertoire (6, 7). Gene microarray analysis revealed that follicle-localized CXCR5$^+$ T_h cells had a very distinctive transcriptional profile that distinguished these cells from T_h1 or T_h2 cells, with high-level IL-21 and B-cell CLL lymphoma-6 (BCL6) messenger RNAs (mRNAs) (5), both of which are now considered hallmarks of T_{FH} cells (6).

IL-21 is a type I cytokine that signals via a specific receptor protein, IL-21R (8, 9), and the common cytokine receptor γ chain, γ_c, which is shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 (10); γ_c is mutated in humans with X-linked SCID (11). IL-21 signals in part via STAT3 (signal transducer and activator of transcription 3) (12), with actions on a wide range of lineages, including T cells, B cells, NK cells and dendritic cells (10). Specifically, IL-21 can promote the expansion of CD8$^+$ T cells, is critical for normal Ig production by B cells, can inhibit dendritic cell function and, interestingly, can be pro-apoptotic for B cells and NK cells (10). Whereas IL-21 was first identified as

a cytokine produced by activated peripheral blood T cells (9), it is now known that it can be produced by a range of differentiated T_h populations (10) as well as by NKT cells (13).

In this review, we discuss the role of IL-21 in the generation of T_{FH} cells as well as in the functional interaction of these cells with B cells within the GC. The relationship between T_{FH} and other T_h subsets will also be discussed, as more evidence mounts in support of flexibility of cytokine profiles within the T_{FH} cells. In addition, the role played by IL-21 in the interaction between the T_{FH} master transcription factor BCL6 and other lineage-specific transcription factors will be discussed. Finally, elevated levels of IL-21 and T_{FH}-specific surface molecules have been implicated in the development of systemic autoimmune responses, and the mechanisms for disease development will be addressed.

Identification of a T_{FH} profile

Early molecular profiling of human T_{FH} cells revealed that although a large number of transcripts were shared by T_{FH}, T_h1 and T_h2 effector cells, a subset of genes were preferentially expressed by T_{FH} cells, including those encoding CXCR5, IL-21, BCL6 and ICOS (inducible co-stimulatory molecule) (5). Although these genes were transiently expressed to some degree by activated T_h1 or T_h2 cells, the spatial and temporal expression pattern of these proteins in T_{FH} cells was coincident with their functions. Interaction between CXCR5 and chemokine (C–X–C motif) ligand 13 (CXCL13), which is produced in the follicle, was required for the localization of T_{FH} to the GC (14), whereas ICOS co-stimulatory interactions with inducible co-stimulatory molecule ligand (ICOSL) on B cells were found to be important for GC development (15). BCL6 was already known to be highly expressed within the GC and to be required for GC formation (16). Finally, accumulating evidence has revealed that IL-21 is a key determinant of B-cell differentiation (2, 3, 10).

Both *in vivo* and *in vitro* evidence demonstrates that IL-21 is a T-cell-derived cytokine that is very important for B-cell proliferation and differentiation (10). Mice that constitutively expressed IL-21 were found to have higher levels of plasma cells as well as higher levels of serum Igs (2). *In vitro* stimulation of both murine and human B cells with IL-21 induced the plasma-cell-associated transcription factor BLIMP1 (B-lymphocyte-induced maturation protein 1) [now termed PR domain containing 1, with ZNF domain (PRDM1)] and subsequent plasma cell differentiation as well as the accumulation of isotype-switched Igs (2, 17). Interestingly, Ozaki *et al.* also showed that IL-21 is a potent inducer of BCL6 (2), a transcription factor that negatively regulates expression of BLIMP1, just as BLIMP1 represses BCL6 (18), suggesting that these two transcription factors are induced in distinct populations of cells.

When a population of $CXCR5^+$ tonsillar T_{FH} cells was cultured with naive human B cells, the accumulation of Ig-secreting B cells was decreased dramatically if IL-21 action was blocked, demonstrating that T_{FH} secretion of IL-21 was critical for the differentiation of naive B cells (19). Interestingly, in both murine and human *in vitro* systems, IL-4 was shown to antagonize the actions of IL-21 on plasma cell differentiation (2, 19), even though analysis of *Il21r* or *Il4* single-knockout (KO) mice and *Il21r* plus *Il4* double-KO mice revealed that cooperative actions of these cytokines were required for Ig production (2). In addition, even though IL-21 is a potent inducer of IL-10 (20), IL-21 was found to be much more potent than IL-10 for the induction of Ig secretion from both naive and memory human B cells *in vitro*, suggesting that IL-21 plays a major role in both primary and memory responses to T-cell-dependent antigens (19).

Although microarray analysis and hierarchical clustering revealed that the overall transcriptional profile of T_{FH} cells was distinct from that of T_h17 cells as well as those of T_h1 or T_h2 cells (21), both T_{FH} and T_h17 subsets share high-level expression of IL-21 (21). IL-21 was found to be required in part for the *in vitro* differentiation of T_h17 cells, but it was not necessary for their *in vivo* differentiation (22). These data suggested certain similarities but also important differences between T_{FH} cells and T_h17 cells, worthy of further investigation.

IL-21 in the GC: B-cell versus T_{FH}-cell action

GC development is impaired in mice deficient for IL-21 signaling (3). As producers of high levels of IL-21, T_{FH} cells are important regulators of the GC reaction because of their close association with $CXCR5^+$ B cells localized to the follicle that undergo differentiation in response to both soluble and cell-mediated signals and produce high-affinity Ig (6, 7) (Fig. 1).

A recent study investigated whether this IL-21 requirement for the GC reaction reflected the action of IL-21 on either the B cells or the T_{FH} cells (23). Immunization of IL-21-KO mice with a T-cell-dependent antigen showed that CXCR5 surface expression on $CD4^+$ T cells was greatly reduced in the absence of IL-21, suggesting that IL-21 has an autocrine role for proper T_{FH} development. Consistent with this, IL-21R expression was significantly higher on $CXCR5^+CD4^+$ than on

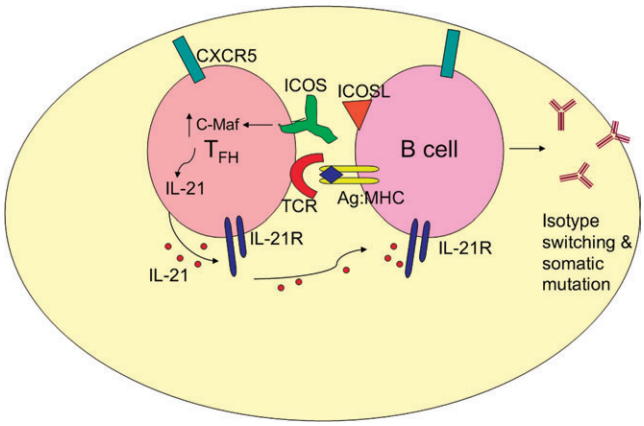

Fig. 1. The GC reaction is facilitated by the interaction between surface molecules on T_{FH} cells and developing B cells, both of which use CXCR5 to localize there. Interactions include the binding of ICOSL to ICOS, which leads to the up-regulation of c-Maf and subsequent production of high levels of IL-21 by T_{FH} cells. IL-21 then acts on T_{FH} in an autocrine manner to promote its function in GC B-cell development and antibody production. T_{FH}-cell-derived IL-21 can also bind to the IL-21R on B cells.

$CXCR5^{-}CD4^{+}$ T cells (23). Adoptive transfer of wild-type (WT) $CD4^{+}$ T cells into *Il21r*-KO recipients followed by immunization rescued GC formation and partially rescued Ig production. However, the transfer of WT B cells into *Il21r*-KO mice could not rescue Ig production, indicating that the defect in these mice was intrinsic to the $CD4^{+}$ T cells (23).

Surface expression of ICOS is required for efficient T_{FH} development, as ICOS-KO mice have reduced numbers of $CXCR5^{+}$ CD4 T cells (24). In addition, ICOSL-deficient mice had reduced expression of IL-21 (21). Specific deletion of ICOSL in B cells resulted in a significant reduction in both the number of $CXCR5^{+}CD4^{+}$ T cells as well as the level of IL-21 after immunization, demonstrating that the interaction of ICOS on T_{FH} cells with ICOSL on GC B cells is required for maximal IL-21 production (21).

Analogous to T_{FH} cells, T_h17 cells also express ICOS on their cell surface. An investigation of the requirement of ICOS for T_{FH} and T_h17 development revealed that although ICOS was required for GC formation, ICOS was not required for T_h17 differentiation but instead was required for the maximal expansion of both T_h17 cells and T_{FH} cells (25). T_{FH} and T_h17 cells were also found to share high expression of the transcription factor c-Maf, and deletion of c-Maf led to reduced numbers of both T_{FH} and T_h17 cells. This was related to the direct regulation of IL-21 transcription by c-Maf subsequent to the induction of c-Maf by ICOS–ICOSL interaction (25, 26).

T_{FH} cells producing alternative cytokines

Although it is clear that T_{FH} cells are critical for directing the development of an antibody response by GC B cells, the above studies (21, 23) reported that IL-21 acts primarily in an autocrine manner on the T_{FH} population, even though it is well known that IL-21 receptors are expressed on B cells and B cells can also respond to this cytokine by undergoing differentiation and producing Ig (2, 3, 10). Interestingly, gene microarray analysis suggested that T_{FH} cells lacked expression of most cytokines or transcription factors associated with the T_h1, T_h2 or T_h17 subsets (5, 21), including IL-4 and IFN-γ, making it unclear how and where these cytokines could influence B-cell Ig isotype switching if they were not produced by T_{FH} cells. Importantly, however, *in vitro* T_h activity for B-cell Ig production has also been detected in T_h1, T_h2 and T_h17 polarized populations, indicating that this helper activity is not specific only to T_{FH} populations (27).

Several reports have challenged the notion that T_{FH} cells lack the expression of cytokines associated with these other T_h subsets, including three studies that used infection with helminths that elicit strong T_h2 responses (28–30).

The first study monitored IL-4 expression by employing a 'dual-reporter' mouse model in which IL-4 transcription and protein production could be distinguished. After infection with *Schistosoma mansoni*, the majority of IL-4-producing cells in lymph node and spleen expressed the T_{FH} markers CXCR5, ICOS and programmed death-1 (PD-1), although the IL-4-producing cells within liver granulomas did not express these markers (28). These IL-4-producing T_{FH} cells expressed amounts of IL-4 and GATA-3 transcripts that were similar to the level found in T_h2 cells that did not express T_{FH} markers and also produced the high levels of BCL6 characteristic of T_{FH} cells. Experiments in which $CXCR5^{-}PD\text{-}1^{-}$ cells that expressed green fluorescent protein-labeled IL-4 (this marks IL-4 protein in this dual-reporter system) were transferred into naive mice and then antigen challenged demonstrated that 20% of these cells could become $CXCR5^{+}PD\text{-}1^{+}$ *in vivo*, confirming that at least a minority of T_h2 cells can develop into T_{FH} cells (28).

Consistent with this, the second study reported that IL-4-producing T_{FH} cells were present in the mesenteric lymph nodes after infection with *Heligmosomoides polygyrus*. Using the IL-4 transcription/protein dual-reporter mice described above, $CD4^{+}$ T cells that were committed to the T_h2 lineage were found throughout the follicle, but those T_h2 cells that produced IL-4 protein were found only near B cells in the GC region (29). These IL-4 protein-producing cells expressed high levels of CXCR5, ICOS, PD-1, IL-21 and BCL6, as is typical of T_{FH} cells.

The third study elegantly addressed the role of T_{FH} cytokine production in isotype switching and somatic mutation. Here, conjugates consisting of B cells plus T cells were isolated from GCs of IL-4/IFNγ dual-reporter mice that had been infected with *Leishmania major* (30). These T cells expressed high levels of IL-21, BCL6 and CXCR5 characteristic of T_{FH} cells. B-cell–T-cell conjugates with IL-4-expressing cells were shown to contain IgG1 transcripts, whereas conjugates that contained IFNγ-expressing cells contained mainly IgG2a transcripts, demonstrating that the specific cytokine produced by the T_{FH} was critical in the regulation of isotype switching in B cells. Purified IL-4-producing cells were analyzed for IL-21 production by enzyme-linked immunosorbent spot, and $IL\text{-}4^{+}$ T_{FH} cells from lymph nodes produced significantly higher amounts of IL-21 than did IL-4-producing cells from lungs. The IL-4-producing cells from the lungs but not the IL-4-producing T_{FH} from the GC could function as classical T_h2 cells in that they could elicit eosinophil recruitment when transferred to IL-4-deficient mice, suggesting important differences between these populations (30).

In addition to evidence supporting the existence of T_{FH} cells expressing either IL-4 or IFNγ, studies in mice prone to autoimmune lupus revealed the existence of T_{FH} cells that produce both IL-17 and IL-21 (31).

The most surprising example of lineage switching involves the conversion of $FOXP3^{+}$ Treg cells to T_{FH} cells in the Peyer's patches of the gut, where GCs are critical for the production of IgA (32). When $FOXP3^{+}$ cells were transferred into T-cell-deficient CD3ε-KO mice, 80% of these were found to down-regulate FOXP3 expression, migrate into the B-cell follicles of Peyer's patches and express high levels of IL-21 and BCL6 (32). Although FOXP3 down-regulation was not dependent on B cells as it could occur in B-cell-deficient mice, the acquisition of CXCR5, PD-1 and IL-21 required interaction with B cells in Peyer's patches (32).

IL-21 in combination with transforming growth factor-β1 (TGFβ1) produced by T_{FH} cells can also contribute to the differentiation of IgA-secreting plasmablasts in the gut (33). In this scenario, the combination of TGFβ1 with IL-21 up-regulated chemokine (C–C motif) receptor 10 (CCR10) and down-regulated CXCR5, thus allowing migration of the B cells out of the GC and toward the mucosal surface.

Collectively, the above studies show that T_{FH} cells can produce IL-4, IFNγ or IL-17 and that these cells are potentially derived from a range of lineages, including T_h1, T_h2, T_h17 or Treg cells (Fig. 2).

BCL6 as a master regulator of T_{FH} cells

Although early gene expression analysis established a correlation between BCL6 transcription factor expression and T_{FH} cells, only recently have experimental approaches verified the causal nature of this expression. Consistent with the reduced T_{FH} cell numbers in *Il21r*-KO mice and with the original demonstration by Ozaki *et al.* (2) in 2004 that IL-21 is a potent inducer of BCL6, *Il21*-KO mice showed diminished expression of BCL6, and either IL-6 or IL-21 could induce BCL6 expression in naive $CD4^+$ T cells (34).

While over-expression of BCL6 was shown to up-regulate endogenous BCL6, IL-21R, IL6R and CXCR5 mRNA, it also inhibited IL-17 expression by blocking the functional activity of RORγt without affecting RORγt mRNA expression (34). BCL6 over-expression also can inhibit the expression of T_h1-associated genes and T_h2-associated genes (34). Experiments employing mixed bone marrow chimeras showed that BCL6 expression was required in both T and B cells for normal GC formation.

BCL6 repression of the development of other T_h lineages involves direct binding and suppression of the promoters of the *Tbx21* and *Rorc* genes in T_{FH} cells (34). BCL6 can also repress expression of microRNAs that otherwise suppress expression of T_{FH}-specific genes such as those encoding CXCR5 and PD-1 (35). Thus, over-expression of BCL6 led to a global down-regulation of microRNAs that targeted sites in the 3′ untranslated regions of CXCR5 and PD-1 mRNAs, thereby promoting expression of the T_{FH} program (35).

Constitutive BCL6 expression induced expression of a panel of T_{FH} markers but it also inhibited the expression of BLIMP1 (36), known to be an inhibitor of BCL6 function (18). Over-expression of BLIMP1 in $CD4^+$ T cells significantly reduced the number of T_{FH} cells, without effecting the expression of T_h2-specific, T_h17-specific or Treg-specific transcription factors (36). Thus, just as BCL6 and BLIMP1 can function as mutually inhibitory cross-regulators of B cell differentiation pathways (18, 37), so can they regulate the CD4 T-cell differentiation pathway, amplifying the T_{FH} responses that will then induce strong BCL6-dependent GC reactions. It is interesting that IL-21 is a potent inducer of both BLIMP1 and BCL6 (2), but because of their cross-inhibition, presumably one of these factors will dominate within a particular cell.

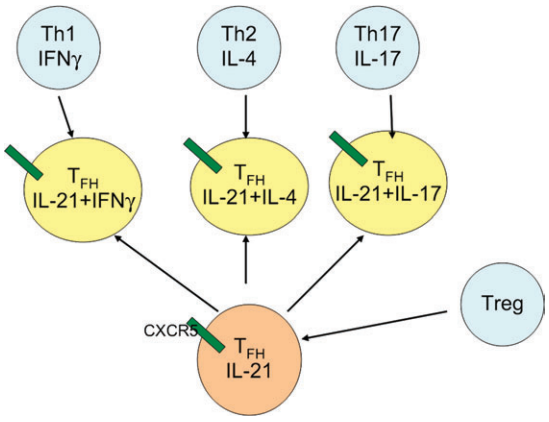

Fig. 2. T_{FH} cells are developmentally related to other $CD4^+$ T cell subsets. Although initially characterized as producing IL-21 but not cytokines associated with the T_h1, T_h2 and T_h17 subsets, evidence now indicates that T_{FH} cells localizing to the follicle through their expression of CXCR5 can also express IFNγ, IL-4 and IL-17. Treg cells are capable of undergoing conversion to T_{FH} cells in Peyer's patches. The green bars designate CXCR5.

T_{FH} cells and IL-21 in autoimmunity

GCs are a critical site for selection of appropriate antibody specificities and T_{FH} cells play an important role in the regulation of autoantibody production, as shown by the systemic autoimmune responses that result from over-expression of T_{FH} signaling proteins such as ICOS or IL-21.

The first suggestion of a connection of IL-21 to autoimmune disease came from the BXSB-*Yaa* mouse model of systemic lupus erythematosus, which was found to have elevated levels of IL-21 mRNA and serum protein (2) that temporally correlated with development of disease. In this model, IL-21 signaling was shown to be essential to the development of the disease phenotype, as when the BXSB-*Yaa* mice were crossed onto the *Il21r*-KO background, there was no hypergammaglobulinemia, autoantibody production or renal disease (38). Interestingly, these BXSB-*Yaa*/*Il21r*-KO mice had greatly reduced numbers of $CXCR5^+ICOS^+CD4^+$ T cells, and the excessive IL-21 production did not derive from this population of conventional T_{FH} cells, but rather from an extrafollicular population of $ICOS^+$ $CD4^+$ T cells (38).

Mice homozygous for the *sanroque* allele of Roquin also develop a lupus-like disease accompanied by the accumulation of excessive numbers of both GCs and T_{FH} cells with high levels of ICOS and IL-21 expression (39), and lupus-like symptoms were dependent on enhanced GC formation as they could be reduced by deletion of even one allele of the BCL6 (40). Interestingly, however, T_{FH} formation in this disease may be more dependent on ICOS than IL-21 (40).

In the MRL^{lpr} mouse, which also develops a lupus-like disease, an extrafollicular population of $ICOS^{hi}CD4^+$ T cells that have down-regulated P-selectin glycoprotein ligand 1 (PSGL-1) were the primary source for IL-21 production and subsequent extrafollicular development of IgG^+ plasmablasts (41). These extrafollicular PSGL-1^{lo} $CD4^+$ cells also occurred at a high frequency in several other autoimmune models. In contrast to the originally defined T_{FH} population, these PSGL-1^{lo} $CD4^+$ T cells also secreted IL-4 and IFNγ, which could instruct appropriate isotype switching in this extrafollicular setting (41). The relationship between these extrafollicular $CD4^+$ T cells and the T_{FH} population remains to be determined.

It is important to recognize that IL-21 can contribute to other autoimmune diseases as well, ostensibly by B-cell-independent mechanisms. For example, in the non-obese diabetic (NOD) mouse model of type I diabetes, disease no longer develops when the NOD mouse is crossed to the *Il21r*-KO background (42, 43). Thus, whereas the relationship

of IL-21 to T_{FH} is very important, one must be cognizant that it may not dominate all types of autoimmune responses.

Conclusions

T_{FH} cells are now recognized as a distinct helper subset whose differentiation requires the transcription factor BCL6, leads to a GC-localized population of cells that are critical in the development of a strong Ig response. Naive T_{FH} cells produce abundant IL-21, which then acts as an autocrine factor for the functional activity of these cells. In addition, it is now clear that T_{FH} also have the capacity to produce cytokines characteristic of T_h1, T_h2 and T_h17 cells, emphasizing the developmental and functional plasticity of all these subsets. Future studies are needed to further unravel mechanisms for controlling the production and activation of T_{FH} cells, as these cells and the IL-21 they produce play important roles in a number of systemic autoimmune diseases.

Funding

Division of Intramural Research, National Heart, Lung, and Blood Institute, National Institutes of Health.

Acknowledgements

We thank Drs Irina Rochman, Edwin Wan and Jian-Xin Lin, National Heart, Lung and Blood Institute, National Institutes of Health for critical comments.
Conflict of Interest: The authors are inventors on patents and patent applications related to IL-21.

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