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## **T Follicular Helper (T<sub>fh</sub>) Cells in Normal Immune Responses and in Allergic Disorders**

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**Summary**

Follicular helper T cells ( $T_{fh}$ ) are located within germinal centers of lymph nodes. Cognate interaction between  $T_{fh}$ , B cells, and IL-21 drive B cells to proliferate and differentiate into plasma cells thereby leading to antibody production.  $T_{fh}$  cells and IL-21 are involved in infectious and autoimmune diseases, immunodeficiencies,

vaccination and cancer. Human peripheral blood CXCR5<sup>+</sup> CD4<sup>+</sup> T cells comprise different subsets of T<sub>fh</sub>-like cells. Despite the importance of the IgE response in the pathogenesis of allergic disorders, little is known about the role of follicular and blood T<sub>fh</sub> cells and IL-21 in human and experimental allergic disease. Here we review recent advances regarding the phenotypic and functional characteristics of both follicular and blood T<sub>fh</sub> cells and of the IL-21/IL-21R system in the context of allergic disorders.

## Introduction

Antibody production by plasma cells is a fundamental feature of the adaptive immune system. Soluble immunoglobulin can directly neutralize pathogens or participate in cellular immunity to provide protection against infections.<sup>1</sup> Antibodies can also play a significant role in disease. Notably, IgE produced in response to a wide spectrum of allergens can play a crucial role in the pathophysiology of allergic disorders.<sup>2</sup>

The type and the magnitude of an antibody response is heavily dictated by the immune environment. In particular, the differentiation of T cells into distinct lineages is thought to depend heavily on the local cytokine milieu and can greatly influence subsequent B cell responses. Follicular helper T cells (T<sub>fh</sub>) were initially described as a subset of CD4<sup>+</sup> cells found in human tonsils. They are localise within the B cell follicle and in particular the germinal centre (GC) and bear the chemokine receptor CXCR5.<sup>3-5</sup> The complex and interrelated processes of B cell affinity maturation (selection and hypermutation), class switch recombination, memory B cell differentiation and plasma cell differentiation all occur within GCs. GCs can therefore

play a central role in protective immunity against bacterial and viral pathogens and in the production of IgE. CXCR5 is highly expressed on the surface of B cells and binding its ligand CXCL13 allows for their migration to form follicles.<sup>6</sup> T<sub>fh</sub> surface expression of CXCR5 not only phenotypically distinguishes T<sub>fh</sub> cells from other effector T cells, but functionally drives T<sub>fh</sub> cell migration into B cell follicles, bringing them in to close proximity with B cells.<sup>5-7</sup> In addition, T<sub>fh</sub> cells express a diverse repertoire of other surface molecules (e.g. PD-1, CD40L, ICOS, OX40), which promote direct interaction between T<sub>fh</sub> and germinal center B cells and facilitate T cell dependent B cell help. T<sub>fh</sub> cells have been reported to express high levels of several cytokines including IL-21 and IL-4 which are critical in driving B cell proliferation, survival and isotype class switch. Since their initial identification the fundamental role of T<sub>fh</sub> cells in humoral immunity has been illustrated in infectious disease<sup>8</sup>, autoimmune disease<sup>9-10</sup> vaccination<sup>11</sup> immunodeficiencies<sup>12, 13</sup> and cancer.<sup>14</sup> However, little is known about the role of T<sub>fh</sub> cells and in particular IL-21 in various allergic disorders, In this review we discuss the phenotypic and functional characteristics of T<sub>fh</sub> and IL-21/IL-21R signaling and how they might impact on allergic disorders.

### **Initial T<sub>fh</sub> development (lessons learned from murine models)**

Despite the identification of T<sub>fh</sub> cells over a decade ago the processes that regulate T<sub>fh</sub> differentiation as a distinct CD4<sup>+</sup> T cell lineage has remained largely elusive. Recently, improvement and the proliferation of complex transgenic murine models have enabled the dissection of the T<sub>fh</sub> developmental pathway. Distinct CD4<sup>+</sup> T cells subsets have been traditionally identified by their expression of master regulator transcription factors, that are both necessary and sufficient for their development. In

murine models Bcl6 was identified as master regulator of T<sub>fh</sub> differentiation and acts as a transcriptional suppressor capable of preventing the development of other CD4<sup>+</sup> T cell subsets.<sup>15</sup> Similarly, studies in humans have confirmed an essential requirement for Bcl6, but also a requirement for the transcription factor Maf in driving full T<sub>fh</sub> development.<sup>16</sup>

The differentiation toward T<sub>fh</sub> cell like phenotype to a certain extent is dictated by the TCR affinity to its epitope, with different epitopes resulting in different T<sub>fh</sub> frequencies,<sup>17</sup> and higher affinity TCR interactions being preferentially observed in T<sub>fh</sub> populations compared to other CD4<sup>+</sup> T cell subsets<sup>18</sup> (Figure 1). After the initial T cell priming, T<sub>fh</sub> differentiation within the T cell zone of secondary lymphoid tissue requires cognate interactions between dendritic cells and T<sub>fh</sub> and ICOS:ICOSL signaling which causes the partial up-regulation of Bcl6 and CXCR5, and down-regulation of CCR7 (a T cell zone homing chemokine receptor), allowing initial migration from the T cell zone toward the B cell zone.<sup>19</sup> Recently, it was reported that the transcription factor Ascl2 is critical for the initial up-regulation of CXCR5 and down-regulation of CCR7 prior to Bcl6 induction.<sup>20</sup> Moreover, the E3 ubiquitin ligase Itch and the transcription factors Lef1 and Tcf7 also appear to be critical at early steps of T<sub>fh</sub> development.<sup>21-24</sup>

Subsequently ICOS- and SAP-dependent signaling provided by B cells within the marginal zone become essential for migration of T<sub>fh</sub> cells into the B cell follicle and T<sub>fh</sub> differentiation. Moreover, ICOS has been shown to provide critical migratory signals directing CD4<sup>+</sup> T cells toward the B cell follicle.<sup>25</sup> The importance of ICOS signaling has been highlighted by the influence of the Roquin-1 and -2, proteins that

degrade ICOS mRNA, in regulating  $T_{fh}$  differentiation to prevent autoantibody development.<sup>26</sup> In addition to TCR:MHC interactions and co-stimulatory signals, cytokines are also critical in  $T_{fh}$  development. IL-6 plays a role in the up-regulation and maintenance of Bcl6 expression within  $T_{fh}$ .<sup>16, 27-28</sup> IL-27 has been shown to drive  $T_{fh}$  differentiation and/or survival<sup>29-31</sup> as has type I interferon.<sup>32</sup> IL-12 is also thought to be capable of promoting  $T_{fh}$ -like cell differentiation.<sup>33</sup> Interestingly, IL-2 antagonizes  $T_{fh}$  differentiation through STAT5 signaling.<sup>34</sup> Overall these findings support a mechanism for T follicular helper cells that parallels the development of other  $CD4^+$  T cell lineages but promotes their development in the anatomically specific location of the B cell follicle.

#### **$T_{fh}$ cell migration and circulating $T_{fh}$ -like cells**

$T_{fh}$  cell interaction with B cells have previously been described for the GC  $T_{fh}$  residing in human tonsils and the vast majority of  $T_{fh}$  are found within the B cell follicles of secondary lymphoid tissues.<sup>3-5</sup> The limited access to secondary lymphoid tissue in humans has made it difficult to investigate the role  $T_{fh}$  cells. There is, however, now increasing evidence suggesting the presence of different subsets of  $CD4^+$  T cells in human blood with  $T_{fh}$ -like cells that may have enhanced capacity to promote B cell differentiation and function.<sup>11,35</sup> In addition, ectopic GCs can be found in the brain in multiple sclerosis,<sup>36</sup> in the synovium in rheumatoid arthritis (RA)<sup>37</sup> and in the kidney in systemic lupus erythematosus.<sup>38</sup> Although much progress has been made in understanding T-B cell migration in and out of the different compartments of secondary lymphoid organs, including GCs, little is known how these cells relate to  $T_{fh}$ -like cells in other locations. The relationship between tonsillar  $T_{fh}$  cells and blood  $CXCR5^+$   $CD4^+$  T cells has been actively investigated. It was suggested that blood

CXCR5<sup>+</sup> CD4<sup>+</sup> T cells were resting cells with a memory phenotype.<sup>4</sup> Unlike tonsillar T<sub>fh</sub> cells they showed limited capacity for providing B cell help *in vitro*<sup>5</sup> and multiple studies have found low level expression of Bcl6.<sup>39</sup>

Recent more rigorous studies have started to characterize human blood CXCR5<sup>+</sup> CD4<sup>+</sup> T cells, their relationship to T<sub>fh</sub> cells and their role in immune disorders. Morita *et al.* found that CXCR5<sup>+</sup> cells represent approximately 8% of CD4<sup>+</sup> T cells in the peripheral blood.<sup>40</sup> Consistent with previous findings<sup>4,41</sup> blood CXCR5<sup>+</sup> CD4<sup>+</sup> T cells expressed CCR7, and a small proportion of cells expressed the activation molecules (ICOS and CD69) expressed by conventional tonsillar T<sub>fh</sub> cells. When co-cultured with autologous naïve B cells (IgD<sup>+</sup> CD27<sup>+</sup> CD19<sup>+</sup> cells) and the staphylococcal superantigen enterotoxin B, CXCR5<sup>+</sup> CD4<sup>+</sup> T induced naïve B cells to produce immunoglobulins (Igs) (IgM, IgG, and IgA) suggesting that blood CXCR5<sup>+</sup> CD4<sup>+</sup> T cells are able to promote class switching and B cell differentiation into plasmablasts. It has been further demonstrated that, similar to tonsillar T<sub>fh</sub> cells,<sup>42</sup> blood CXCR5<sup>+</sup> CD4<sup>+</sup> T cells secrete IL-21 and that blocking IL-21 during these co-cultures inhibited antibody production. Conversely, the addition of IL-21 enhanced IgM, IgG, and IgA secretion. Blocking ICOS-ICOSL interaction also inhibited both IL-21 and Ig production. Collectively, these findings suggest that human blood CXCR5<sup>+</sup> CD4<sup>+</sup> T cells share some functional properties of follicular T<sub>fh</sub> cells. Other chemokine receptors can be used to define CD4<sup>+</sup> T cell subsets. CXCR3 is preferentially maintained by cells committed to the Th1 cell pathway,<sup>43</sup> whereas CCR6 is expressed by Th17 cells.<sup>44,45</sup> Morita *et al.* demonstrated that differential expression of CXCR3 and CCR6 define 3 major subsets within circulating blood CXCR5<sup>+</sup> CD4<sup>+</sup> T cells<sup>40</sup> (Figure 1). CXCR3<sup>+</sup> CCR6<sup>+</sup> cells secrete IFN- $\gamma$  but not Th2 or Th17 cytokines

and express T-bet. In addition to IL-21, CXCR3<sup>+</sup> CCR6<sup>+</sup> (Th2-like) cells produced Th2 cytokines (IL-4, IL-5, and IL-13) and express GATA-3. Whereas CXCR3<sup>+</sup> CCR6<sup>+</sup> (Th17-like) cells expressed Th17 cytokines (IL-17A and IL-22) and expressed ROR $\gamma$ t (Figure 1). When independently isolated both CXCR5<sup>+</sup> Th2- and Th17-like cells efficiently induced naïve B cells to proliferate and to differentiate into CD19<sup>lo</sup> CD38<sup>+</sup> plasmablasts. Th2-like cells induced naïve B cells to produce IgM, IgG, IgA, and IgE. Th17-like cells stimulated naïve B cells to produce IgM, IgG, and IgA, but not IgE. Finally, blocking IL-21 during co-culture of naïve B cells with CXCR5<sup>+</sup> Th2-like cells decreased IgM and IgG production, whereas blocking IL-4 inhibited IgE production.

Bentebibel and colleagues found that a small population of activated ICOS<sup>+</sup> CXCR3<sup>+</sup> CXCR5<sup>+</sup> CD4<sup>+</sup> T cells transiently appeared in blood after influenza vaccination and correlated with anti-influenza antibody titers.<sup>11</sup> He *et al.* (2013) supported the observation of increased circulating CXCR5<sup>+</sup> helper T cells upon influenza vaccination, finding they comprised CCR7<sup>hi</sup> PD-1<sup>lo</sup> and CCR7<sup>lo</sup> PD-1<sup>hi</sup> populations. CCR7<sup>lo</sup> PD-1<sup>hi</sup> CXCR5<sup>+</sup> helper T cells were the most potent, among circulating CD4<sup>+</sup> T cell subsets, at inducing plasma cell differentiation and antibody production. Furthermore, in SLE and RA patients increased frequencies of CCR7<sup>lo</sup> PD-1<sup>hi</sup> CD4<sup>+</sup> T cells correlated with elevated autoantibody profiles and more severe disease.<sup>35</sup> In parallel studies they found that in mice circulating T<sub>fh</sub> are generated in a SAP-independent fashion, unlike their follicle localized counterparts, suggesting the two populations may have common origins but distinct fates and developmental pathways. In a con-current paper Locci and co-workers undertook a detailed phenotypic characterization of human blood CXCR5<sup>+</sup> cells in comparison to GC resident T<sub>fh</sub> cells. Compared to GC T<sub>fh</sub> cells, human blood CXCR5<sup>+</sup> CD4<sup>+</sup> T cells

expressed intermediate levels of CXCR5.<sup>46</sup> The majority of these CXCR5<sup>+</sup> cells were PD-1<sup>-</sup>; however a substantial population (approximately 30%) of CXCR5<sup>+</sup> cells expressed PD-1 at moderate levels. Interestingly, PD-1 expression, as well as CXCR5 expression, was stable on the surface of the cells. The authors suggest that PD-1<sup>+</sup> CXCR5<sup>+</sup> CD4<sup>+</sup> T cells are quiescent with a stable phenotype, consistent with memory cells. Transcriptional analysis revealed that blood PD-1<sup>+</sup> CXCR3<sup>-</sup> CXCR5<sup>+</sup> cells most closely resembled GC T<sub>fh</sub> cells. They concluded that resting PD-1<sup>+</sup> CXCR3<sup>-</sup> CXCR5<sup>+</sup> CD4<sup>+</sup> T cells are a population of circulating memory T<sub>fh</sub> cells. Activation of PD-1<sup>+</sup> CXCR3<sup>-</sup> CXCR5<sup>+</sup> and PD-1<sup>+</sup> CXCR5<sup>+</sup> cells resulted in the production of high amounts of IL-21 and CXCL13. Moreover, PD-1<sup>+</sup> CXCR3<sup>-</sup> CXCR5<sup>+</sup> CD4<sup>+</sup> T cells were shown to have a cytokine profile comparable to that of active GC T<sub>fh</sub> cells upon re-stimulation. Importantly, in T:B co-cultures, PD-1<sup>+</sup> CXCR3<sup>-</sup> CXCR5<sup>+</sup> CD4<sup>+</sup> T cells were vastly superior at inducing memory B cell into plasma cell differentiation and IgG secretion. Thus, the authors concluded that the highly functional PD-1<sup>+</sup> CXCR3<sup>-</sup> CXCR5<sup>+</sup> CD4<sup>+</sup> T cells are resting memory T<sub>fh</sub> cells with a T<sub>fh</sub> gene signature and highest capacity for B cell help. Finally, they found that the ability of rare HIV-infected individuals to make antibodies against the virus is associated with the presence of highly functional T<sub>fh</sub> cells with PD-1<sup>+</sup> CXCR3<sup>-</sup> CXCR5<sup>+</sup> CD4<sup>+</sup> phenotype.

Collectively, the above findings greatly extend our knowledge of different subsets of human blood T<sub>fh</sub>-like cells. As summarized in Figure 1, while additional studies are required, the integration of PD-1, ICOS, CXCR3 and CCR7 and the application of new technologies to the analysis of CXCR5<sup>+</sup> cells will facilitate the identification of

functions of different subsets of blood T<sub>fh</sub> cells in health and in disease and their comparison to tissue-resident T<sub>fh</sub> cells<sup>47</sup>

### **T<sub>fh</sub>-like cells and follicular Treg cells**

In addition to blood T<sub>fh</sub> cells, “unconventional” subsets of T<sub>fh</sub> cells and follicular Treg cells have also been identified.<sup>43</sup> Natural killer T (NKT) cells express a semi-invariant TCR (V $\alpha$ 24J $\alpha$ 18 in humans) that recognizes glycolipid antigens ( $\alpha$ -galactosylceramide and simplexide) presented by CD1d expressed on APCs and monocytes.<sup>48</sup> Recent evidence indicates that a subset of mouse and human NKT cells are present in tonsils and express CXCR5. These cells are able to provide IL-21-dependent B cell help.<sup>49</sup> Some  $\gamma\delta$  T cells also express CXCR5 and but do not produce IL-21. These  $\gamma\delta$  T cells require extrinsic IL-21 to differentiate into  $\gamma\delta$  T<sub>fh</sub> cells and provide help to B cells.<sup>50</sup>

Perhaps of most interest are T follicular regulatory cells (T<sub>fr</sub>). Treg cells are a heterogeneous population of T cells with suppressive properties essential for maintaining tolerance and preventing misguided immune responses.<sup>43</sup> Treg cells can be classified into two main subsets: the thymus-derived naturally occurring CD4<sup>+</sup> CD25<sup>+</sup> Foxp3<sup>+</sup> Treg cells (nTreg) and the inducible Treg (iTreg) cells generated in the periphery.<sup>51</sup> Treg cells suppress the sensitization and effector phases of allergic reactions through the inhibition of allergen-induced degranulation of effector cells,<sup>52</sup> inhibition of Th2 cytokines,<sup>53</sup> induction of allergen-specific IgG<sub>4</sub>, and inhibition of IgE production.<sup>54</sup>

Increasing evidence indicates that T<sub>fh</sub> activities can be modulated by follicular Treg cells (T<sub>fr</sub> cells) which were originally described in human tonsils<sup>55</sup> These cells are thought to result from nTreg up regulation of CXCR5 and possess some characteristics of both T<sub>fh</sub> and Treg, express CXCR5, PD-1 and ICOS. T<sub>fr</sub> cells do not express B cell help molecules such as CD40L, IL-21, or IL-4.<sup>56</sup> T<sub>fr</sub> cells are present not only in lymph nodes but also in circulation and mediate its suppressive activity by contact-dependent mechanisms via CTLA-4 and have memory-like properties.<sup>57, 58</sup>

### **T<sub>fh</sub> function and IL-21**

Interleukin-21 (IL-21) was cloned as the ligand of the orphan receptor IL-21R.<sup>59</sup> The IL-21R mediates its effects through the interaction with the  $\gamma$  common chain ( $\gamma_c$ ), making IL-21 a member of the family of  $\gamma_c$  cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21). A key function of T<sub>fh</sub> cells is to provide IL-21-mediated help signals to B cells important for their survival. T<sub>fh</sub> cells, particularly GC T<sub>fh</sub> cells, can be distinguished from non-T<sub>fh</sub> cells by their pattern of cytokine production.<sup>61</sup> IL-21 is highly expressed, but not exclusively, by GC T<sub>fh</sub> cells.<sup>39, 61</sup> In human tonsils, T<sub>fh</sub> cells produce the highest amount of IL-21, but do not produce IL-17 or IL-22.<sup>62</sup> IL-21 is important for optimal GC B cell proliferation.<sup>63</sup> It is also the most potent driver of plasma cell differentiation in both mice<sup>64</sup> and humans<sup>42</sup> through the activation of STAT3.<sup>65</sup> In addition to its roles in promoting humoral immunity through signaling on B cells, IL-21 can also enhance the frequency of T<sub>fh</sub> both *in vitro* and *in vivo*.<sup>27</sup>

### **T<sub>fh</sub> and type-2 immunity**

T<sub>fh</sub> cells arising in the context of viral or helminth infections, can also produce IFN- $\gamma$  and IL-4,<sup>61,66</sup> though less than their canonical counterparts Th1 and Th2 cells.<sup>61,62</sup> T<sub>fh</sub> cells contribute to the IL-4-dependent B cell help in *in vivo* models of type 2 immunity.<sup>15</sup> GC T<sub>fh</sub> cells have been shown to produce IL-4 in a GATA3-independent

manner.<sup>5, 18, 61, 66</sup> the expression of IL-4 gene in T<sub>fh</sub> cells relies on the conserved *IL4* hypersensitivity site V located at the 3' enhancer in the *IL4* locus.,<sup>61, 67, 68</sup> IL-4 has an anti-apoptotic effect on B cells<sup>69</sup> crucial in apoptosis-prone GC B cells.<sup>70</sup> IL-4 is also a class switch factor for allergen-specific IgG<sub>1</sub> and IgE in both mice and humans. Expression of IL-4 by GC T<sub>fh</sub> cells is important for IgG synthesis.<sup>71</sup>

### **IL-21 and T<sub>fh</sub> in allergic disorders**

In the light of the known pleiotropic effects of IL-21 on the functions of different components of the innate and adaptive immune system, it was easy to predict the fundamental role(s) that IL-21 would play in autoimmune diseases,<sup>9</sup> in the response to bacterial and viral infections<sup>8</sup> and in tumor growth.<sup>14</sup> Evidence is accumulating that IL-21 and T<sub>fh</sub> cells might play a role in the pathogenesis of certain allergic diseases. It is well established that in allergic disorders, the allergen triggers a Th2-predominant immune response, leading to the generation of memory B cells that produce antigen specific IgE. Subsequent exposure to the allergen provokes cross-linking of IgE on mast cells and basophils which results in the release of pro-inflammatory mediators and chemokines/cytokines that are responsible for allergic symptoms.<sup>72</sup> In this context, IgE production by plasma cells is a crucial step in the immunopathological cascade of allergic disorders, as well as helminth immunity. However, the *in vivo* and *in vitro* biology of IgE-producing (IgE<sup>+</sup>) B cells and plasma cells, including the pathway(s) by which B cells differentiate from naïve to IgE memory B cells, remains poorly understood.<sup>2</sup> IgE is produced by both extrafollicular and GC pathways. Early IgE responses are of low affinity and originates from extrafollicular sources, whereas late IgE responses are of high affinity and arise from GCs.<sup>73</sup> Sequential class switch recombination (CSR) generates short-lived IgE

plasma cells. Direct CSR can also generate short-lived IgE plasma cells. A minority of long-lived, bone-marrow residing IgE plasma cells sustains IgE production. Other IgE<sup>+</sup> B cells differentiate into IgE<sup>+</sup> memory B cells.<sup>74</sup>

It is important to note that IgE-producing B cells may also undergo clonal selection and affinity maturation (resulting in IgE production) in the airway and gut-associated lymphoid tissue in patients with chronic allergic rhinitis,<sup>75, 76</sup> or asthma,<sup>77</sup> and food allergy,<sup>78</sup> respectively. These observations indicate IgE may be produced locally in lymphoid niches close to or within affected anatomical sites.

The role(s) of IL-21 and T<sub>fh</sub> cells in the regulation of IgE synthesis and other aspects of allergic diseases both in mice and in humans remain enigmatic since conflicting results have been reported so far. These discrepancies might be related to different experimental conditions (*in vitro* vs. *in vivo*, activating stimuli, presence of other cytokines, etc.) and species-specific differences. We have summarized the main evidence on IL-21 and its regulation of IgE responses in Table 1<sup>13,79-81</sup>. Of note, the recent identification of patients with loss-of-function mutations in *IL-21* and *IL-21R* genes has provided new insights into the role of IL-21 in IgE production in humans.<sup>13,</sup>

<sup>81</sup> Both mutations are associated with increased IgE levels, confirming the results obtained with IL-21R<sup>-/-</sup> mice<sup>82-83</sup>. IL-21R mutation is also associated with broad defects of the immune response that involves NK and T cells, while patients with IL-21 mutation present with an early-onset inflammatory bowel disease. Given the complex phenotypes associated with these mutations, it may be difficult to determine whether IL-21 acts only at the B cell level to modulate IgE production or other components of the immune system are involved. Nevertheless, *in vivo* results point

to a role for IL-21 in restraining the IgE response. Recently however it was found that IL-21, thought to at least be in part derived from  $T_{fh}$ , played a critical role in promoting Th2 immunity in a house dust mite driven model of allergic airway disease<sup>84</sup>. The therapeutic potential of IL-21 has also been evaluated in mouse models of IgE-mediated diseases.<sup>85, 86</sup> In addition, increased percentages of Th2-like  $T_{fh}$  ( $CD4^+ CXCR5^+ CXCR3^- CCR6^-$ ) have been reported in patients with allergic rhinitis with and without asthma<sup>78</sup> and patients that develop food allergy after liver transplantation.<sup>77</sup> Altogether, current observations suggest that a careful evaluation of experimental conditions, species-specific differences and also spatial and temporal contexts is required to understand the complex role(s) of IL-21/IL-21R in IgE production and IgE-mediated disorders. In addition, a cross-talk between IL-21 and the IgE-inducing cytokine IL-4 must also be taken into account since  $T_{fh}$  can express both cytokines during cognate interactions with GC B cells.<sup>87</sup>

### **Closing thoughts and open issues**

$T_{fh}$  cells and the functional molecules (e.g. IL-21 and IL-4) represent intriguing players in the initiation, progression and maintenance of allergic diseases. The studies performed so far on the possible role of  $T_{fh}$  cells in allergic disorders are predominantly indirect and focused on the role of IL-21 and on the response of IgE antibody. Multiple studies, in both humans and mice *in vitro* and *in vivo* show different and sometimes opposite results on the effect of IL-21 on IgE production. Although it has been shown that IL-21 inhibits the sequential switching of  $IgG_1^+$  B cells to IgE,<sup>88</sup> it is unclear whether IL-21 has a similar effect on direct switching to IgE. Most of the studies on  $T_{fh}$  cells in disease setting have been performed in animal models. Nevertheless, the establishment of circulating  $T_{fh}$ -like cells has

allowed several studies to highlight possible alterations of peripheral blood T<sub>fh</sub> in autoimmune disorders<sup>9</sup> and during bacterial and viral infections.<sup>4, 35</sup> To our knowledge few studies have been published in allergic disorders.<sup>77, 78</sup> Correlations and differences between the different subsets of blood T<sub>fh</sub> cells in various allergic disorders should be evaluated. These studies must not only evaluate which subset(s) of human blood and/or GC T<sub>fh</sub> cells are relevant to allergic diseases, but also whether these same cells or others may mediate the production of protective IgG<sub>4</sub> and IgA responses observed during allergen immunotherapy.

In conclusion, T<sub>fh</sub> cells are essential in generation of antibody-secreting plasma cells. As such, they are of potential importance in allergen-specific IgE production in allergic disease. As yet, evidence for a pro- or anti-allergic role is inconclusive and largely focused on the effects of IL-21, rather than T<sub>fh</sub> cells. Further studies of T<sub>fh</sub> subsets and IL-21 in human allergic disease are needed to indicate whether inhibiting or stimulating their role is a potential therapeutic target.

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## Figure Legends

Figure 1 Cellular and molecular interactions between dendritic cells (DCs), T follicular helper cells ( $T_{fh}$ ) and B cells in germinal centers (GC).

Naïve  $CD4^+$  T cells interact with DC in the T cell zones of lymph nodes or in the interfollicular zones. In humans, IL-12 is the most important cytokine produced by DCs capable to activate STAT4 in  $CD4^+$  T cells to produce IL-21. This cytokine then feedbacks to the same cells engaging the IL-21R which activates STAT3 which is responsible for the differentiation. These cells upregulate the expression of the transcription factor *Ascl2* and *BCL-6* to become early T follicular helper ( $T_{fh}$ ) cells. These transcription factors upregulate *CXCR5* and downregulate *CCR7*. *STAT5* is a negative regulator of  $T_{fh}$  differentiation through up-regulation of *Blimp-1*.  $T_{fh}$  cells migrate to the T:B cell border and differentiate into germinal center  $T_{fh}$  cells. Other cytokines produced by DCs (IL-27 and IL-6) and B cells (IL-6) contribute to the activation of *STAT3*. IL-21 also activates IL-21R on B cells increasing the expression of *BCL-6*. GC  $T_{fh}$  cells and certain subsets of peripheral blood  $T_{fh}$  cells can produce IL-4 which acts on IL-4R present on B cells. Cell-cell interactions between activated  $CD4^+$  T cells and DCs (ICOS-ICOSL) or between  $CD4^+$  T cells and B cells (CD28-CD86, ICOS-ICOSL, PD-1 - PD-1L, CD40-CD40L, etc.) also play a fundamental role in  $T_{fh}$  cell functions. Adapted from Tangye *et al.*<sup>102</sup>

## Figure 2 Human blood T<sub>fh</sub> subsets

Human blood T<sub>fh</sub> cells (CXCR5<sup>+</sup>) can be separated into four subsets based on the expression of CXCR3 and PD-1. The expression of CCR7 is inversely correlated with PD-1 expression. PD-1<sup>+</sup> cells expressing ICOS represent “activated” blood T<sub>fh</sub> cells. “Quiescent” blood T<sub>fh</sub> cells PD-1<sup>+</sup> CXCR3<sup>-</sup> are the most efficient cells providing help to B cells. CXCR3<sup>+</sup> cells provide help to B cells when activated (ICOS<sup>+</sup> PD-1<sup>+</sup> cells). However, their help ability is limited to memory B cells.

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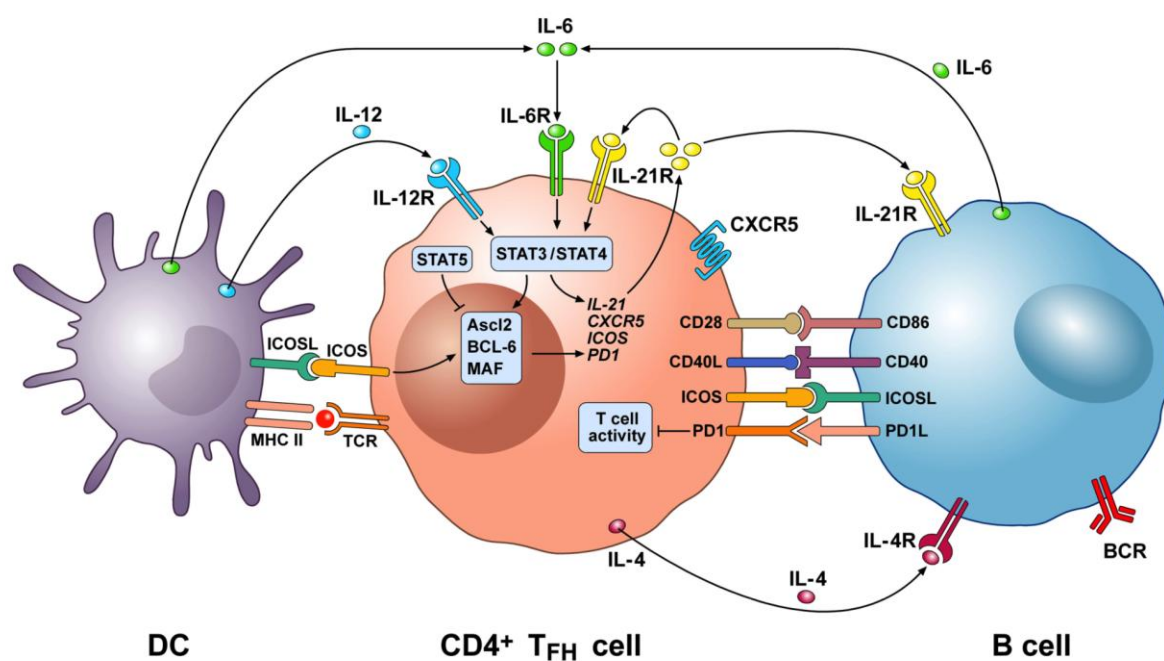
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**Table 1** *In vitro* and *in vivo* roles of IL-21 and T<sub>h</sub> in human and mouse IgE responses and allergic disorders

| Humans                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                               | Mice                                                                                                                                                                                                                      |                                                                                                                                                                                      |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>In vitro</i>                                                                                                                                                                                                                                     | <i>In vivo</i>                                                                                                                                                                                                                                                | <i>In vitro</i>                                                                                                                                                                                                           | <i>In vivo</i>                                                                                                                                                                       |
| IL-21 co-stimulates B cell proliferation induced by anti-CD40, whereas it inhibits the proliferation induced by IL-4 and anti-IgM <sup>88</sup>                                                                                                     | Homozygous loss-of-function mutations in <i>IL-21R</i> gene are associated with impaired T cell cytokine production, NK cell cytotoxicity, IL-21-induced B cell proliferation and CSR, and elevated serum IgM and IgE levels <sup>64</sup>                    | B cells isolated from IL-21R <sup>-/-</sup> and wild type mice produce comparable levels of IgG1 and IgE1 response to LPS or anti-CD40 and IL-4 <sup>91</sup>                                                             | IL-21R <sup>-/-</sup> mice have higher IgE levels at baseline <sup>92</sup> and after immunization <sup>91</sup>                                                                     |
| IL-21 enhances IgE production from and stimulates the proliferation of B cells stimulated with anti-CD40 and IL-4 or IL-13. However, it inhibits IgE production from unfractionated PBMCs, and only in response to IL-4 and not IL-13 <sup>93</sup> | Homozygous loss-of-function mutations in <i>IL-21</i> gene are associated with early-onset IBD, reduced numbers of CD19 <sup>+</sup> and IgG <sup>+</sup> class-switched memory B cells, serum IgG concentration but increased serum IgE levels <sup>17</sup> | IL-21 does not enhance the proliferation of anti-CD40 co-stimulated B cells, but does inhibit proliferation induced by anti-IgM and IL-4 and induces the apoptosis of primary resting and activated B cells <sup>94</sup> | i.p. injection of IL-21 inhibits antigen-specific IgE production <sup>95</sup>                                                                                                       |
| IL-21 induces class-switch recombination and plasma cell differentiation of B cells stimulated with anti-CD40 <sup>96</sup>                                                                                                                         | Patients with allergic rhinitis with and without asthma have higher levels of peripheral blood Th2-like T <sub>h</sub> (CD4 <sup>+</sup> CXCR5 <sup>+</sup> CXCR3 <sup>-</sup> CCR6 <sup>-</sup> ) cells <sup>64</sup>                                        | IL-21 inhibits IgE but not IgG1 production from B cells stimulated with LPS and IL-4 <sup>95</sup>                                                                                                                        | In a model of OVA-induced allergic rhinitis, intranasal administration of IL-21 reduces serum levels of OVA-specific IgE, eosinophil infiltration and number of sneeze <sup>85</sup> |
| IL-21 enhances IgE production when B cells are activated by anti-CD40 and IL-4. However, IL-21 inhibits                                                                                                                                             | Patients that develop food allergy after liver transplantation exhibit higher levels of peripheral blood Th2-like                                                                                                                                             | IL-21 inhibits IgE switch of IgG1 <sup>+</sup> B cells induced by anti-CD40 and IL-4 <sup>93</sup>                                                                                                                        | In a mouse model of systemic anaphylaxis induced by the administration of crude peanut extract, IL-21                                                                                |

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| <p>IgE secretion in PBMCs cultured by inducing IFN<math>\gamma</math>, whose production is dependent on a IL-21R polymorphism<sup>93</sup></p> <p>IL-21 drives the differentiation of CD40L-activated B cells into antibody producing cells. This effect is inhibited by IL-4 on naïve but not memory B cells<sup>42</sup></p> <p>Stimulation of CD40L-activated naïve B cells with IL-21 induces the differentiation of IgG<sup>+</sup> and IgA<sup>+</sup> cells. The concomitant presence of IL-4 inhibits IgA switch<sup>100</sup></p> <p>IL-21 enhances anti-CD40 plus IL-4-induced production of IgE by both purified B cells and PBMCs<sup>101</sup></p> | <p>T<sub>fh</sub> (CD4<sup>+</sup> CXCR5<sup>+</sup> CXCR3<sup>-</sup> CCR6<sup>r</sup>) cells<sup>97</sup></p> | <p>IL-21-mediated suppression of IgE switch induced by anti-CD40 and anti-IgM plus IL-4 is dependent on Bcl6<sup>97</sup></p> | <p>administration prevented the development of anaphylaxis following antigen challenge<sup>57</sup></p> <p>In a mouse model of IgE-mediated cutaneous hypersensitivity, i.p. administration of IL-21 during or after the sensitization procedure reduced the cutaneous reaction and serum concentrations of total IgE and OVA-specific IgE<sup>99</sup></p> |
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**Abbreviations:** CSR, class-switch recombination; IBD, inflammatory bowel disease; i.p., intraperitoneal; LPS, lipopolysaccharide; OVA, ovalbumin; PBMCs, peripheral blood mononuclear cells.



## Subsets of Human Blood T<sub>FH</sub> - like Cells

