

DR. ZHENG LIU (Orcid ID : 0000-0002-4168-6702)

Article type : Review

## **Roles of follicular helper and regulatory T cells in allergic diseases and allergen immunotherapy**

Yin Yao, M.D., Ph.D.<sup>1, 2</sup>, Cai-Ling Chen, M.D.<sup>1</sup>, Di Yu, Ph.D.<sup>2</sup>, Zheng Liu, M.D., Ph.D.<sup>1</sup>

<sup>1</sup>Department of Otolaryngology-Head and Neck Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P.R. China

<sup>2</sup>The University of Queensland Diamantina Institute, Faculty of Medicine, The University of Queensland, Brisbane, Australia

### **Corresponding authors:**

Zheng Liu, M.D., Ph.D.

Department of Otolaryngology-Head and Neck Surgery

Tongji Hospital, Tongji Medical College

Huazhong University of Science and Technology

No. 1095 Jiefang Avenue

Wuhan 430030, P.R. China

Tel: 86-27-83663529

E-mail: zhengliuent@hotmail.com

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ALL.14639](#)

This article is protected by copyright. All rights reserved

---

and

Di Yu, Ph.D.

The University of Queensland Diamantina Institute

Faculty of Medicine

The University of Queensland

Translational Research Institute (TRI)

37 Kent Street, Woolloongabba, QLD 4102, Brisbane, Australia

Tel: 61- 07-34436954

E-mail: di.yu@uq.edu.au

**Short title:** T<sub>FH</sub> and T<sub>FR</sub> cells in allergy and immunotherapy

**Word count:** 5,563

## **Abstract**

Allergic diseases are characterized by overactive type 2 immune responses to allergens and immunoglobulin E (IgE)-mediated hypersensitivity. Emerging evidence suggests that follicular helper T (T<sub>FH</sub>) cells, rather than type 2 T-helper (T<sub>H2</sub>) cells, play a crucial role in controlling IgE production. However, follicular regulatory T (T<sub>FR</sub>) cells, a specialized subset of regulatory T

---

(T<sub>REG</sub>) cells resident in B-cell follicles, restricts T<sub>FH</sub> cell-mediated help in extrafollicular antibody production, germinal center (GC) formation, immunoglobulin affinity maturation, and long-lived, high-affinity plasma and memory B-cell differentiation. In mouse models of allergic asthma and food allergy, CXCR5<sup>+</sup> T<sub>FH</sub> cells, not CXCR5<sup>-</sup> conventional T<sub>H</sub>2 cells, are needed to support IgE production, otherwise exacerbated by CXCR5<sup>+</sup> T<sub>FR</sub> cell deletion. Upregulation of T<sub>FH</sub> cell activities, including a skewing toward type 2 T<sub>FH</sub> (T<sub>FH</sub>2) and IL-13 producing T<sub>FH</sub> (T<sub>FH</sub>13) phenotypes, and defects in T<sub>FR</sub> cells have been identified in patients with allergic diseases. Allergen immunotherapy (AIT) reinstates the balance between T<sub>FH</sub> and T<sub>FR</sub> cells in patients with allergic diseases, resulting in clinical benefits. Collectively, further understanding of T<sub>FH</sub> and T<sub>FR</sub> cells and their role in the immunopathogenesis of allergic diseases creates opportunities to develop novel therapeutic approaches.

**KEYWORDS:** allergen immunotherapy, allergy, follicular helper T cell, follicular regulatory T cell, immunoglobulin E

---

## 1. INTRODUCTION

Allergic diseases are a collection of disorders caused by the hypersensitivity of the immune system to innocuous environmental antigens, called allergens. This disease group including allergic rhinitis (AR, also known as hay fever), allergic asthma, food allergy, and atopic dermatitis (AD, also known as eczema) is estimated to affect more than 30% of the global population with an ever-increasing prevalence.<sup>1,2-4</sup> Allergies can dramatically impair the quality of life of patients and some may trigger life-threatening reactions, thus, causing a significant socioeconomic burden.<sup>2,5</sup>

Immunoglobulin E (IgE), the least abundant class of immunoglobulins, is the key driver of allergic responses.<sup>4,6,7</sup> The high-affinity receptor for IgE, FcεRI, is highly expressed by mast cells and basophils in both humans and mice, and cross-linking of FcεRI through allergen-IgE binding efficiently sensitizes the mast cells and basophils to release biologically active mediators including histamine, tryptases, and various cytokines and chemokines, causing a cluster of typical allergic symptoms.<sup>8</sup> In humans, FcεRI is also expressed by dendritic cells (DCs) and monocytes,<sup>9</sup> which mediates IgE-dependent allergen uptake and presentation, and subsequently promotes type 2 T-helper (T<sub>H</sub>2) cell polarization.<sup>9</sup> The low-affinity receptor FcεRII is mainly expressed by B cells in human and mice and facilitates antigen presentation and IgE synthesis.<sup>8</sup> For decades, allergen-specific IgE has been widely used in the clinical diagnosis of allergic diseases.<sup>10</sup> Recently, anti-IgE antibodies have shown considerable clinical efficacy in treating allergic asthma, AR, AD, and food allergy,<sup>11</sup> supporting the pathogenic role of IgE in allergic diseases.

B-cell class-switch recombination to the IgE isotype is induced by interleukin (IL)-4 and IL-13, the signature cytokines abundantly produced by T<sub>H</sub>2 cells.<sup>6,12</sup> In contrast to the historic view that T<sub>H</sub>2 cells control IgE production, recent studies have identified a new type of T<sub>H</sub> cell localized in B-cell follicles in the secondary lymphoid organs, which is responsible for regulating antibody isotype switching, affinity maturation and B-cell memory generation.<sup>13-16</sup> This population, named follicular helper T (T<sub>FH</sub>) cell, is characterized by the high expression of CXC chemokine receptor 5 (CXCR5), programmed cell death protein 1 (PD-1), B-cell lymphoma 6 (Bcl-6), and IL-21 in both mice and humans.<sup>13-16</sup> The significance of T<sub>FH</sub> cells in health and disease was initially revealed by their pathogenic role in promoting lupus and rheumatic diseases,

and protective role underpinning vaccine responses.<sup>13-19</sup> Recent studies on mice and humans have revealed that IL-4<sup>+</sup> T<sub>FH</sub> cells, but not CXCR5<sup>-</sup> T<sub>H2</sub> cells, are required for IgE production.<sup>18,20,21</sup> More recently, it has been shown that IL-13-producing T<sub>FH</sub> cells, named as T<sub>FH</sub>13 cells and having both IL-4 and IL-13 production, are responsible for the production of high- but not low-affinity IgE in house dust mite (HDM)-sensitized mice, though as a rare population.<sup>22</sup> The increased circulating T<sub>FH</sub>13 cells were observed in humans with IgE-mediated allergy.<sup>22</sup> Furthermore, a subset of regulatory T (T<sub>REG</sub>) cells has been identified localizing within B-cell follicles and co-expressing T<sub>REG</sub> and T<sub>FH</sub> cell markers, CD3<sup>+</sup>CD4<sup>+</sup>CXCR5<sup>+</sup>Bcl-6<sup>+</sup>Foxp3<sup>+</sup>, in both mice and humans.<sup>23-25</sup> These follicular regulatory T (T<sub>FR</sub>) cells counteract T<sub>FH</sub> cells and suppress IgE production in mice and humans, thus, preventing allergic responses.<sup>16,26</sup>

Allergen immunotherapy (AIT) is a common and effective treatment for allergic diseases, aiming to re-establish allergen-specific immune tolerance.<sup>27-31</sup> Despite the alteration of various immune cells and immunoglobulins, which has been implicated in AIT-induced immune tolerance, the precise mechanisms underlying AIT are far from being fully understood.<sup>27-29,32</sup> In the clinic, one of the major challenges is to identify specific and powerful biomarkers to guide the proper selection of patients and monitor the response to AIT.<sup>33,34</sup> Several recent studies have shown a re-establishment in the balance of T<sub>FH</sub> and T<sub>FR</sub> cells after AIT, which associates with AIT efficacy.<sup>35-37</sup> Yao et al. reported that the mean ratio of T<sub>FR</sub> to T<sub>FH2</sub> cells increased from 0.20 to 0.24 in AR patients after AIT, indicating an improvement but not full reinstatement of T<sub>FR</sub> and T<sub>FH2</sub> cell balance as compared to that in healthy controls (T<sub>FR</sub> to T<sub>FH2</sub> cell ratio: 0.28).<sup>35</sup> These new advances underline a potential value of using T<sub>FH</sub> and T<sub>FR</sub> cells as therapeutic targets and biomarkers in AIT.

In this review, we summarize the recent progress in our understanding of how T<sub>FH</sub> and T<sub>FR</sub> cells regulate allergic diseases, particularly their roles in controlling IgE production. We also discuss the modulation of T<sub>FH</sub> and T<sub>FR</sub> cells associated with efficient AIT and provide a perspective view on targeting T<sub>FH</sub> and T<sub>FR</sub> cells as a therapeutic strategy against allergic diseases.

## **2. DEVELOPMENT, PHENOTYPE, AND ROLE OF T<sub>FH</sub> CELLS**

---

Identification of the CD4<sup>+</sup> T<sub>FH</sub> lineage-specific transcription factor, Bcl-6, in 2009<sup>38-40</sup> led to the broad acceptance of these cells as a distinct lineage from T<sub>H</sub>1, T<sub>H</sub>2, T<sub>H</sub>17, and T<sub>REG</sub> cells. As the development of T<sub>FH</sub> cells has already been comprehensively reviewed,<sup>13-15</sup> herein we aim to summarize only the key processes. T-cell differentiation initiates when naïve CD4<sup>+</sup> T cells encounter antigen-presenting DCs at the T-cell zones of lymphoid tissues.<sup>14,41,42</sup> In mice, it has been found that in addition to antigen presentation and co-stimulation, DC-derived cytokines, in particular IL-6, activate signal transducer and activator of transcription (STAT)3 to increase Bcl-6 expression in T cells.<sup>14</sup> IL-6 also potentiates murine T cells to produce IL-21, which in turn activates STAT3 and further enhances Bcl-6 expression.<sup>14</sup> In contrast, in humans, IL-12 but not IL-6 potently induces IL-21-expressing CD4<sup>+</sup> T<sub>FH</sub>-like cells by activating STAT4.<sup>41</sup> Additionally, transforming growth factor beta (TGF-β) also promotes human T<sub>FH</sub> cell differentiation by acting in synergy with STAT4-activating cytokines.<sup>43</sup> By mouse studies, it has been found that T cells primed at T-cell zones rapidly downregulate the CC-chemokine receptor 7 (CCR7);<sup>44</sup> a fraction of cells upregulate CXCR5 to become precursor T<sub>FH</sub> (pT<sub>FH</sub>) cells.<sup>45</sup> This dynamic change of chemokine receptors facilitates the migration of pT<sub>FH</sub> cells to the chemokine (CXC motif) ligand 13 (CXCL13)-enriched T–B-cell border, where antigen-specific B cells present class II MHC-antigen to cognate pT<sub>FH</sub> cells.<sup>46,47</sup> Although the exact mechanisms that guide the migration of T<sub>FH</sub> cells into the center of B-cell follicles and the full maturation of germinal center (GC)-T<sub>FH</sub> cells are not entirely clear, mouse and human studies indicated that the stable B–T<sub>FH</sub> cell interaction mediated by SLAM-associated protein (SAP)-dependent signaling is critical for completing and sustaining this final step of T<sub>FH</sub> cell differentiation.<sup>48,49</sup> Bcl-6, CD40 ligand (CD40L), and ICOS expressed by T<sub>FH</sub> cells are essential for the GC formation in both humans and mice.<sup>14,50</sup> Although CXCR5 is not absolutely necessary for T<sub>FH</sub> cell differentiation and GC formation, the acquisition and maintenance of high CXCR5 expression on T<sub>FH</sub> cells promote their proper localization in GCs.<sup>14</sup> In the GC, T<sub>FH</sub> cells not only orchestrate the selection of high-affinity B cells but also promote the differentiation of B cells into immunoglobulin-producing plasma cells and memory B cells by secreting cytokines and expressing surface effector molecules.<sup>13-15</sup> IL-21 produced by T<sub>FH</sub> cells is necessary for optimal GC response and plasma cell differentiation in mice

---

and humans.<sup>13,51,52</sup> IL-4 may act in synergy with IL-21 to support GC response as shown by the severer impairment of GC response in mice lack of both cytokines than that in mice with single cytokine deficiency.<sup>53</sup> IL-9 produced by T<sub>FH</sub> cells has been implicated in supporting memory B cell generation in mouse models as well.<sup>54</sup> Besides cytokine signals, the interaction of CD40 ligand (CD40L) on T<sub>FH</sub> cells with CD40 on B cells is also important for GC B cells to differentiate into memory B cells and plasma cells as revealed by mouse studies.<sup>13,55</sup>

Advancement in understanding the origin and function of T<sub>FH</sub> cells is largely attributed to research on murine secondary lymphoid organ models.<sup>13-15</sup> Because obtaining human samples of secondary lymphoid organs such as tonsil, spleen, and lymph node is extremely challenging, circulating CXCR5<sup>+</sup>CD4<sup>+</sup> T<sub>FH</sub> (cT<sub>FH</sub>) cells in human peripheral blood samples have been studied as an alternative.<sup>21,45,56,57</sup> Although flow cytometric analysis showed that cT<sub>FH</sub> cells express largely undetectable Bcl-6 and lower levels of ICOS and PD-1 in comparison with *bone fide* T<sub>FH</sub> cells in GCs,<sup>21,45</sup> they share functional similarity with GC T<sub>FH</sub> cells and are able to induce antibody production from B cells (Table 1).<sup>21</sup> cT<sub>FH</sub> cells contain long-lived memory cells, which are considered to represent a circulating memory compartment of T<sub>FH</sub> cells.<sup>21,45</sup> Detailed analysis of cT<sub>FH</sub> cells has identified several subsets with distinct phenotypic and functional features.<sup>21,45</sup> To date, there are several different combinations of surface markers that define cT<sub>FH</sub> cell subsets (Table 1).<sup>21,45,58</sup> He et al. demonstrated that cT<sub>FH</sub> cells comprise activated CCR7<sup>low</sup>PD-1<sup>high</sup> and resting CCR7<sup>high</sup>PD-1<sup>low</sup> subsets (Table 1).<sup>45</sup> Resting cT<sub>FH</sub> cells are reported to recirculate through blood, similar to central memory T cells, whereas activated cT<sub>FH</sub> cells may originate from pT<sub>FH</sub> cells but not T<sub>FH</sub> cells in GCs.<sup>45</sup> An increase of activated cT<sub>FH</sub> cells correlated with the abundance of newly generated T<sub>FH</sub> cells in secondary lymphoid organs in both mice and humans,<sup>45</sup> suggesting that cT<sub>FH</sub> cells are indicative of active T<sub>FH</sub> cell differentiation in lymphoid tissues. Based on the expression of CXCR3 and CCR6, Morita et al. classified cT<sub>FH</sub> cells into three major subsets: CXCR3<sup>+</sup>CCR6<sup>-</sup> type 1 T<sub>FH</sub> (cT<sub>FH</sub>1), CXCR3<sup>-</sup>CCR6<sup>-</sup> type 2 T<sub>FH</sub> (cT<sub>FH</sub>2), and CXCR3<sup>-</sup>CCR6<sup>+</sup> type 17 T<sub>FH</sub> (cT<sub>FH</sub>17) cells (Table 1).<sup>21</sup> These cT<sub>FH</sub> cell subsets highly resemble their equivalent T<sub>H</sub> cell subsets. cT<sub>FH</sub>1 cells express T-bet and interferon gamma (IFN- $\gamma$ ); cT<sub>FH</sub>2 cells express Gata3, IL-4, IL-5, and IL-13; and cT<sub>FH</sub>17 cells express Ror $\gamma$ t and IL-17A.<sup>21</sup> IL-21 is the signature cytokine of

T<sub>FH</sub> cells, and it promotes B-cell proliferation and the differentiation into plasma cells.<sup>14,53,59,60</sup> T-cell cytokines also play a decisive role in isotype class switching with IL-4 directing IgG4 and IgE, IL-13 directing IgE, and IL-21 directing IgG and IgA class-switch recombination.<sup>14,53,59-61</sup> However, several studies reported that IL-21 is a negative regulator of IgE class switch recombination in mouse and human B cells.<sup>62,63</sup> T<sub>FH</sub>2 cells, but not T<sub>FH</sub>1 and T<sub>FH</sub>17 cells, have been identified to promote IgE secretion from B cells.<sup>21</sup> It is now coming to the surface that the antigen type impacts the differentiation of T<sub>FH</sub> cells.<sup>22,53,64,65</sup> For example, T<sub>FH</sub>1 cells are generated during human immunodeficiency virus and *Ehrlichia muris* infection,<sup>64</sup> whereas T<sub>FH</sub>2 and T<sub>FH</sub>13 cells are induced during allergen-sensitization.<sup>22</sup> The discovery of dual transcription factor-expressing or cytokine-producing T<sub>FH</sub> cells indicates the plasticity and functional complexity of T<sub>FH</sub> cells.<sup>22,53,64,65</sup> These dual transcription factor-expressing or cytokine-producing T<sub>FH</sub> cells play a specific role in dictating selective features of GC reaction. For example, Gata3 and Bcl-6 double positive T<sub>FH</sub> cells drive high-affinity IgE production by secreting IL-21, IL-4, and IL-13 simultaneously in mice during allergic sensitization.<sup>22</sup>

### 3. DEVELOPMENT, PHENOTYPE, AND ROLE OF T<sub>FR</sub> CELLS

A population of CXCR5<sup>+</sup> and Foxp3-expressing T<sub>REG</sub> cells was identified in B-cell follicles of secondary lymphoid organs by both mouse and human studies and coined as T<sub>FR</sub> cells by three independent research groups in 2011.<sup>23-25</sup> T<sub>FR</sub> cells restrain T<sub>FH</sub> cell-induced GC responses by repressing T<sub>FH</sub> cell activation and its B cell-helper function.<sup>23-25,66</sup> Accumulating evidence suggests that T<sub>FR</sub> cells likely develop from thymic T<sub>REG</sub> cells in a Bcl-6-dependent differentiation process, which is akin but also different to the development of T<sub>FH</sub> cells.<sup>23-25,67-70</sup> Notably, in contrast to the positive role of IL-21 in the development of T<sub>FH</sub> cells, IL-21 signaling inhibits mouse T<sub>FR</sub> cell differentiation.<sup>71,72</sup>

Phenotypically, T<sub>FR</sub> cells co-express the typical markers of T<sub>FH</sub> and T<sub>REG</sub> cells. Similar to T<sub>FH</sub> cells, T<sub>FR</sub> cells express high levels of CXCR5, PD-1, ICOS, and Bcl-6, which are required for migrating into B-cell follicles and maintaining the follicular residence.<sup>23-25</sup> Meanwhile, T<sub>FR</sub> cells also express high levels of T<sub>REG</sub>-associated suppressive molecules, such as glucocorticoid-induced

TNF receptor-related protein, cytotoxic T lymphocyte-associated protein 4 (CTLA-4), and IL-10.<sup>23-25</sup> T<sub>FR</sub> cells were initially defined as CD4<sup>+</sup>CXCR5<sup>+</sup>PD-1<sup>+</sup>Bcl-6<sup>+</sup>Foxp3<sup>+</sup> T cells.<sup>23-25</sup> However, recent studies have highlighted a heterogeneity of T<sub>FR</sub> cells, such as the observation of CD25<sup>-</sup> T<sub>FR</sub> cells in both mice and humans.<sup>73-75</sup> Since T<sub>FR</sub> cell differentiation is suppressed by IL-2, downregulation of CD25 is likely to be essential for the full maturation of T<sub>FR</sub> cells as revealed by mouse studies.<sup>76</sup> Using highly multiplexed immunofluorescence imaging, Sayin et al. discovered T<sub>FR</sub> cells with low PD-1 expression levels, which are enriched in T-B-cell and GC-mantle borders in human lymph nodes.<sup>73</sup> Therefore, the definition of T<sub>FR</sub> cells should be considered with caution. The different definitions of mouse and human T<sub>FR</sub> cells as determined by flow cytometric studies is summarized in Table 2.

The relationship between blood circulating T<sub>FR</sub> (cT<sub>FR</sub>) cells and T<sub>FR</sub> cells in B-cell follicles has been established by several studies (Figure 1).<sup>35,69,77</sup> In mice, cT<sub>FR</sub> cells have memory-like properties and can recirculate through the blood and home toward B-cell follicles after reactivation.<sup>69</sup> Yao et al. reported that T<sub>FR</sub> cell frequencies in tonsil and peripheral blood were closely correlated in a cohort of 21 subjects ( $r = 0.590$ ,  $p = 0.005$ ) (Table 3).<sup>35</sup> Compared to GC T<sub>FR</sub> cells, human cT<sub>FR</sub> cells express substantially lower levels of CCR5, ICOS, PD-1, and Bcl-6 and significantly higher levels of CCR7 and CD62L,<sup>35,69,77,78</sup> highlighting a recirculating property of cT<sub>FR</sub> cells. Moreover, Yao et al. observed that CXCR5<sup>+</sup>CD45RA<sup>low</sup>CD25<sup>high</sup>CD127<sup>low</sup> cT<sub>FR</sub> cells from healthy subjects were able to suppress T<sub>FH</sub> cell-induced IgM, IgA, IgG, and IgE production by B cells, *in vitro* (Table 3).<sup>35</sup> Consistently, Dhaeze et al. reported that human CXCR5<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>PD-1<sup>+</sup> cT<sub>FR</sub> cells had comparable capacity as tonsil T<sub>FR</sub> cells in inhibiting the proliferation of CXCR5<sup>+</sup> T cells *in vitro*.<sup>78</sup> However, Fonseca et al. reported that human CXCR5<sup>+</sup>CD25<sup>+</sup>CD127<sup>-</sup> cT<sub>FR</sub> cells lacked B-cell suppressive capacity.<sup>77</sup> Using a model of NP-OVA immunization, Sage et al. found that CD4<sup>+</sup>ICOS<sup>+</sup>CXCR5<sup>+</sup>GITR<sup>+</sup> cT<sub>FR</sub> cells demonstrated weaker capacity to suppress T<sub>FH</sub>-B-cell responses than GC T<sub>FR</sub> cells.<sup>69</sup> These discrepancies may be caused by the different T<sub>FR</sub> cell isolation methods in these studies and emphasize the heterogeneity of T<sub>FR</sub> cells (Table 2).

CTLA-4, IL-10, and TGF- $\beta$ , the key molecules for T<sub>REG</sub> cell-mediated suppression,<sup>79-81</sup> have

---

also been implicated in the suppressive activity of T<sub>FR</sub> cells.<sup>82-84</sup> Deletion of CTLA-4 on T<sub>FR</sub> cells led to heightened antigen-specific antibody responses in mice.<sup>82,83</sup> Consistently, patients with CTLA-4 haploinsufficiency displayed higher frequencies of cT<sub>FH</sub> cells with concomitantly lower frequencies of cT<sub>FR</sub> cells than healthy subjects.<sup>84</sup> Although several studies have shown that T<sub>FR</sub> cell-derived IL-10 promoted GC responses and IgE production in mice,<sup>85,86</sup> IL-10-producing follicular T cells appear to inhibit IgE production in humans.<sup>87,88</sup> Despite these progresses, the molecular mechanisms underlying the suppressive function of T<sub>FR</sub> cells requires further investigation, particularly in comparison to conventional T<sub>REG</sub> cells.

#### **4. T<sub>FH</sub> AND T<sub>FR</sub> CELLS AND IgE AFFINITY**

High-affinity IgE is more pathogenic at inducing allergic responses than low-affinity IgE.<sup>89</sup> Nevertheless, how T<sub>FH</sub> cells instruct high-affinity IgE production remains largely unknown. There are two main pathways of class switching for IgE: a direct pathway from IgM to IgE and a sequential pathway from IgM to IgG1 and then to IgE.<sup>8,22</sup> In a mouse study, Xiong et al. reported that the sequential class switching directed high-affinity IgE production, whereas the direct class switching typically generated low-affinity IgE.<sup>90</sup> Sequential class switching is involved in the generation of long-lived IgE<sup>+</sup> plasma cells.<sup>91</sup> The antigen-specific IgG1<sup>+</sup> B cells have been reported to predate the IgE responses in a murine model of epicutaneous sensitization to foods.<sup>92</sup> Similar findings were observed by human studies whereby IgE-producing plasma cells showed the evidence of sequential switching from affinity-matured IgG1<sup>+</sup> B cells during allergic sensitization.<sup>93,94</sup> Very recently, a rare T<sub>FH</sub> cell population producing IL-13 (T<sub>FH</sub>13) has been identified in HDM and peanut-induced allergic mouse models.<sup>22</sup> Single-cell RNA-sequencing analysis of murine T cells revealed that T<sub>FH</sub>13 cells are distinct from T<sub>H</sub>2 and T<sub>FR</sub>2 cells, characterized by concomitant high expression of IL-4, IL-5, IL-13, Bcl-6, and Gata3.<sup>22</sup> Conditional deletion of T<sub>FH</sub>13 cells specifically abrogated the production of high-affinity IgE while leaving low-affinity IgE intact and only slightly reducing high-affinity IgG1 in *Alternaria*-sensitized mice.<sup>22</sup> Notably, the frequency of IL-4<sup>+</sup> T<sub>FH</sub> cells was comparable between T<sub>FH</sub>13-deficient and control mice.<sup>22</sup> These results suggest that production of high-affinity IgE

critically depends on T<sub>FH</sub>13 cells, whereas IL-4<sup>+</sup> T<sub>FH</sub> cells are more likely involved in low-affinity IgE production. Consistently, the IL-13 receptor, alpha 1 (IL-13R $\alpha$ 1) were highly expressed by IgE<sup>+</sup> and IgG1<sup>+</sup> GC B cells in allergen-sensitized mice, supporting a role of IL-13 and IgG1<sup>+</sup> GC B cells in the sequential switching to high-affinity IgE (Table 1).<sup>22</sup> Nevertheless, a recent study indicated that class-switch recombination to IgE occurred primarily during the initial T-cell:B-cell interaction rather than in GCs by using several mouse models.<sup>95</sup> Future studies with novel technologies, such as intravital imaging, are required to delineate the location of IL-4<sup>+</sup> T<sub>FH</sub> and T<sub>FH</sub>13 cells and their interaction with IgM<sup>+</sup> and IgG1<sup>+</sup> B cells.

Using a specific and inducible T<sub>FR</sub> cell-deleter mouse model, Clement et al. found that T<sub>FR</sub> cells regulated antigen-specific antibody production and B-cell memory at the incipient stages of the GC reaction.<sup>96</sup> Deleting T<sub>FR</sub> cells before GC initiation resulted in heightened HDM-specific IgE production and increased lung inflammation in mice with HDM-induced allergic asthma.<sup>96</sup> However, the deletion of T<sub>FR</sub> cells did not change the frequencies of total T<sub>FH</sub> cells, GC B cells, IgG1<sup>+</sup> B cells, IgE<sup>+</sup> B cells, and plasma cells in mice with HDM-induced allergic asthma, suggesting a selective role of T<sub>FR</sub> cells in controlling antigen-specific IgE production *in vivo*.<sup>96</sup> *In vitro*, co-cultures of purified T<sub>FH</sub> cells and total B cells from HDM-sensitized mice resulted in robust IL-4, IL-5, and IL-13 production, IgG1 and IgE class-switching, and plasma cell differentiation, which was significantly suppressed by T<sub>FR</sub> cells.<sup>96</sup> These data suggest that murine T<sub>FR</sub> cells are able to suppress T<sub>FH</sub> cell, most likely T<sub>FH</sub>13 cell, -mediated IgE responses.

## 5. T<sub>FH</sub> AND T<sub>FR</sub> CELLS IN ALLERGIC DISEASES

### 5.1. Allergic asthma

In a mouse model of allergic asthma induced by house dust mites (HDM), IL-21-producing follicular helper-like T (T<sub>FH</sub>-like) cells were revealed to amplify the function of T<sub>H</sub>2 cells.<sup>97</sup> André et al. reported that sensitization to HDM elicited IL-4-committed T<sub>FH</sub> cells, but not effector T<sub>H</sub>2 cells, in draining lymph nodes that, after the HDM challenge, further differentiated into HDM-specific T<sub>H</sub>2 cells and homed to the lungs in mice.<sup>17</sup> Noble et al. reported that abrogation of T<sub>FH</sub> cells expansion by disrupting IL-6 receptor signaling diminished the production of

antigen-specific IgE and IgG1; however, no effects were observed in lung T<sub>H</sub>2 responses in a mouse model of HDM-induced asthma.<sup>98</sup> Similarly, Kobayashi et al. found that the deficiency of Bcl-6<sup>+</sup> T<sub>FH</sub> cells caused a marked reduction in IgE levels, but not T<sub>H</sub>2 cell responses, in the airways of an allergen-induced asthma model.<sup>18</sup> Taken together, it appears that T<sub>FH</sub> and T<sub>H</sub>2 cells execute distinct predominant roles in allergic airway responses in mice, with T<sub>FH</sub> cells mainly supporting IgE production (Figure 1). This notion is also supported by the findings that T<sub>FH</sub> cells, but not T<sub>H</sub>2 cells, are required for IgE production in helminth models.<sup>99,100</sup>

Consistently, Uwadiae et al. discovered that the administration of anti-ICOS ligand antibody specifically disrupted T<sub>FH</sub> cell responses, accompanied by reduced lung inflammation and airway hyper-responsiveness in a mouse asthma model induced by HDM.<sup>101</sup> In a similar model, He et al. reported that the deletion of Blimp-1 upregulated Bcl-6 expression in CD4<sup>+</sup> T cells, leading to the suppression of Gata3 expression and T<sub>H</sub>2 cell differentiation.<sup>102</sup> Their results suggest distinct mechanisms underpinning T<sub>FH</sub> and T<sub>H</sub>2 cell differentiation in response to allergens and Blimp-1 as a rheostat oppositely determining T<sub>FH</sub> v.s. T<sub>H</sub>2 fate commitment in the murine model of allergic asthma.

Consistent with the pathogenic role of T<sub>FH</sub> cells in murine allergic asthma, increased cT<sub>FH</sub> cells has been noted in patients with asthma.<sup>103</sup> Gong et al. found that cT<sub>FH</sub> cells from patients with asthma were more effective at inducing IgE production from autologous B cells than those from control subjects; the frequency of cT<sub>FH</sub> cells was also positively correlated with serum IgE levels (Table 3).<sup>103,104</sup> Moreover, the skewing of cT<sub>FH</sub> cells toward type 2 subsets was observed in patients with asthma,<sup>104-107</sup> and the frequency of cT<sub>FH</sub>2 cells correlated with fractional exhaled nitric oxide and blood eosinophil levels (Table 3).<sup>105</sup> Miyajima et al. reported that inhaled corticosteroid treatment significantly reduced the frequencies of cT<sub>FH</sub>2 and ICOS<sup>+</sup> cT<sub>FH</sub> cells, accompanied by an improvement in symptoms in patients with allergic asthma.<sup>107</sup> Although T<sub>FR</sub> cells have been found to suppress HDM-specific IgE production and lung inflammation in mice with HDM-induced allergic asthma,<sup>96</sup> there is no study addressing the involvement of T<sub>FR</sub> cells in human asthmatic patients at present.

---

## 5.2. AR

Although studies of total CXCR5<sup>+</sup>CD4<sup>+</sup> cT<sub>FH</sub> cells in patients with AR have shown conflicting results, the increased frequency of CXCR3<sup>+</sup>CCR6<sup>+</sup> cT<sub>FH</sub>2 cells has been consistently demonstrated in patients with AR relative to that in healthy subjects (Table 3).<sup>105,108-111</sup> Moreover, Yao et al. found that AR-derived cT<sub>FH</sub>2 cells produced larger amounts of IL-4 and had an enhanced capacity to induce IgE production than those from controls (Table 3).<sup>111</sup> In contrast, no differences were observed in cT<sub>FH</sub>1, cT<sub>FH</sub>17, or CXCR5<sup>+</sup> conventional cT<sub>H</sub>2 cells between patients with AR and healthy subjects.<sup>111</sup> The frequencies of Der p 1-specific IL-4<sup>+</sup> cT<sub>FH</sub>2 cells, but not CXCR5<sup>+</sup> T<sub>H</sub>2 cells, positively correlated with the level of Der p-specific IgE (Table 3);<sup>111</sup> however, both cell types increased in patients with AR who are allergic to HDM, suggesting that in AR, T<sub>FH</sub>2 cells orchestrate IgE production in an antigen-specific manner. In this study, antigen-specific T<sub>FH</sub> or non-T<sub>FH</sub> cell subsets were defined by cytokine production. Although cytokine detection allows the identification of functional T cell subsets, cytokine-producing populations represent only a small fraction of total antigen-specific T cells.<sup>112</sup> Moreover, the phenotypic properties of the reactive cells may be altered after antigen stimulation *in vitro*.<sup>112</sup> Therefore, further studies employing other techniques are required to identify antigen-specific T<sub>FH</sub> or non-T<sub>FH</sub> cell subsets in allergic patients, such as MHC-peptide multimers method.

Chemokines and their receptors control immune cell migration. Der p 1-specific cT<sub>FH</sub>2 cells in patients with AR expressed CXCR5, whereas CXCR5<sup>+</sup> conventional cT<sub>H</sub>2 cells expressed CCR2 and CCR5,<sup>111</sup> indicating a preferential migration of CXCR5<sup>+</sup> cT<sub>H</sub>2 cells to peripheral inflammatory sites to produce local inflammation (Figure 1). The higher expression of CCR7 and CD62L on human cT<sub>FH</sub> cells than on cT<sub>H</sub>2 cells indicates that allergen-specific cT<sub>FH</sub> cells may recirculate between the blood and secondary lymphoid organs (Figure 1).

To study T<sub>FR</sub> cells in AR, Yao et al. examined the tonsillar T<sub>FR</sub> cells in subjects undergoing routine tonsillectomy due to tonsillar hypertrophy and who had or did not have concomitant AR.<sup>35</sup> Tonsillar Foxp3<sup>+</sup> T<sub>FR</sub> cells were dramatically lower in patients with AR than in subjects without AR (Table 3).<sup>35</sup> Furthermore, after establishing a correlation between cT<sub>FR</sub> cell and tonsillar T<sub>FR</sub> cell frequencies, Yao et al. determined a significant reduction of cT<sub>FR</sub> cells, defined as

CD3<sup>+</sup>CD4<sup>+</sup>CXCR5<sup>+</sup>CD45RA<sup>low</sup>CD25<sup>high</sup>CD127<sup>low</sup> or CD3<sup>+</sup>CD4<sup>+</sup>CXCR5<sup>+</sup>CD45RA<sup>low</sup>Foxp3<sup>+</sup> T<sub>FR</sub> cells, in patients with AR compared to that in control subjects (Table 3).<sup>35</sup> Nevertheless, the percentages of total T<sub>REG</sub> cells and CD45RA<sup>low</sup>CXCR5<sup>-</sup> T<sub>REG</sub> cells were comparable between patients with AR and control subjects.<sup>35</sup> The selective reduction of T<sub>FR</sub> cells highlights a specific role of T<sub>FR</sub> cells in preventing the development of AR. Furthermore, cT<sub>FR</sub> cells from patients with AR displayed a selective defect in suppressing the production of IgE but not that of other types of immunoglobulins.<sup>35</sup> This raised the possibility that distinctive mechanisms may underlie the function of T<sub>FR</sub> cells in controlling the production of different immunoglobulin isotypes. Recently, a subset of IL-10-enriched follicular T cells in human tonsils was defined as CD25<sup>+</sup>CXCR5<sup>high</sup>PD-1<sup>high</sup> cells.<sup>87</sup> These cells were able to restrain class switching to IgE, but not IgG, via IL-10 *in vitro*.<sup>87</sup> In addition, cT<sub>FR</sub> cells derived from patients with AR had a compromised IL-10-producing capacity relative to those obtained from healthy subjects.<sup>88</sup>

Using unsupervised hierarchical clustering and correlation analysis, additional negative correlations have been identified in the cT<sub>FR</sub> cell frequencies, with symptom scores and allergen-specific IgE levels in patients with AR.<sup>35,88</sup> Notably, cT<sub>FR</sub>/cT<sub>FH2</sub> cell ratios show a better correlation with symptom severity than the frequencies of T<sub>FR</sub> cells ( $r = -0.516$  for cT<sub>FR</sub>/cT<sub>FH2</sub> cell ratio *vs*  $r = -0.427$  for T<sub>FR</sub> cells),<sup>35,88</sup> emphasizing the importance of T<sub>FH</sub> and T<sub>FR</sub> cell balances in the pathogenesis of AR.

### 5.3. Chronic rhinosinusitis with nasal polyps (CRSwNP)

Although there is continuous debate as to whether allergy is related to the pathogenesis of CRSwNP, local hyper-production of immunoglobulins, even in the absence of systemic atopy, has been documented in patients with CRSwNP.<sup>113-120</sup> Elevated IgE and IgD levels were observed predominantly in eosinophilic nasal polyps, whereas IgG and IgA levels increased in both eosinophilic and non-eosinophilic nasal polyps.<sup>113,116</sup> IgE and IgD are suggested to activate mast cells to drive local eosinophilic inflammation, and IgG can contribute to the activation of classical complement pathways in nasal polyps.<sup>116,121,122</sup> Xiao et al. and Calus et al. reported a higher frequency of IL-21<sup>+</sup>CD4<sup>+</sup> T cells locally in patients with CRSwNP than in control subjects.<sup>114,123</sup>

Calus et al. further revealed that polyp CXCR5<sup>+</sup>CD4<sup>+</sup> T cells could induce the differentiation of naïve B cells into plasmablasts *in vitro*.<sup>123</sup> Zhang et al. observed a significant expansion of CXCR5<sup>+</sup> T<sub>FH</sub> cells in nasal polyps in comparison to those in the inferior turbinate mucosa from control subjects.<sup>113</sup> T<sub>FH</sub> cells were mainly identified in lymphoid follicle-like structures in nasal polyps.<sup>113,115</sup> Based on cytokine expressions, the total T<sub>FH</sub>, IFN- $\gamma$ <sup>+</sup> T<sub>FH</sub>1, IL-17A<sup>+</sup> T<sub>FH</sub>17, and IL-21<sup>+</sup> T<sub>FH</sub> cell counts were significantly higher in both eosinophilic and non-eosinophilic polyps than in the inferior turbinate mucosa from control subjects, whereas IL-4<sup>+</sup> T<sub>FH</sub>2 cell counts were higher only in eosinophilic polyps (Tables 1 and 3).<sup>113</sup> In T–B-cell cocultures, polyp CXCR5<sup>+</sup> T<sub>FH</sub> cells, but not CXCR5<sup>–</sup> T<sub>H</sub> cells, were shown to have a potent ability to promote immunoglobulin production.<sup>113</sup> CXCR5<sup>+</sup> T<sub>FH</sub> cells from eosinophilic polyps had a higher capacity to induce IgE production from B cells than those from non-eosinophilic polyps, which was dependent on IL-4 and IL-21 (Tables 1 and 3).<sup>113</sup> These results indicate that IL-4<sup>+</sup> T<sub>FH</sub>2 cells are essential for local IgE production in nasal polyps. In addition, skewed B-cell composition toward GC, memory, and antibody-secreting B cells was observed in nasal polyps and positively correlated with T<sub>FH</sub> cell frequencies in nasal polyps.<sup>113</sup> These findings suggest that T<sub>FH</sub> cells may drive local B-cell proliferation and differentiation and immunoglobulin production in nasal polyps.<sup>113</sup>

#### 5.4. Other allergic diseases

Food allergy is a growing public health concern with peanut allergy as the best characterized example of food-induced fatal anaphylaxis.<sup>124,125</sup> Inhalation of peanut flour induced the expansion of T<sub>FH</sub> cells in the draining lymph nodes of mice, which promoted peanut-specific IgE production more efficiently than T<sub>H</sub>2 cells.<sup>126</sup> Genetic depletion of T<sub>FH</sub> cells compromised IgE production and protected mice from anaphylaxis, without affecting T<sub>H</sub>2 cells, in a mouse model for peanut allergy.<sup>126</sup> Intriguingly, food allergens induced the generation of T<sub>FH</sub>2 cells in gut-associated lymphoid tissues and drove the spontaneous IgE elevation, even in mice without commensal microbiota (germ-free housing).<sup>127</sup> In contrast to T<sub>FR</sub> cells in allergic asthma and rhinitis as described above, Xie et al. recently reported a surprising result in that the loss of T<sub>FR</sub> cells reduced peanut-specific IgE titers but increased non-specific IgE levels in a peanut-induced food allergy

model.<sup>86</sup> They further revealed that the promotion of antigen-specific IgE production by T<sub>FR</sub> cells is dependent on IL-10.<sup>86</sup> However, this conflicted with the reported suppressive role of IL-10 in IgE production,<sup>128</sup> thereby warranting further investigation. In human, significantly higher frequencies of IL-4<sup>+</sup> cT<sub>FH</sub>2 and IL-4<sup>+</sup>IL13<sup>+</sup> cT<sub>FH</sub>13 cells were observed in patients with allergies to peanut than in non-sensitized individuals.<sup>22</sup>

Regarding AD, the expansion of CXCR5<sup>+</sup>ICOS<sup>+</sup>PD-1<sup>+</sup> cT<sub>FH</sub> cells with enhanced IL-21-production was discovered in children with AD and the frequency of CXCR5<sup>+</sup>ICOS<sup>+</sup>PD-1<sup>+</sup> cT<sub>FH</sub> cells correlated with disease severity (Table 3).<sup>129</sup> Although Trichot et al. did not find significant differences in the frequency of CXCR5<sup>+</sup> T<sub>FH</sub> cells and their subsets between patients with AD and healthy subjects, higher frequencies of total T<sub>FH</sub>, T<sub>FH</sub>1, and T<sub>FH</sub>2 cells were noted in the intermediate/high responders than in low responders after a 12-month dupilumab treatment.<sup>130</sup>

## 6. T<sub>FH</sub> AND T<sub>FR</sub> CELLS IN AIT

AIT not only confers disease-modifying effects but also induces lasting clinical benefits for many years even after discontinuing therapy.<sup>28,29,131,132</sup> Despite the modulation of various immune cells and immunoglobulins being implicated in AIT-induced immune tolerance, the precise mechanisms underlying AIT are far from understood.<sup>133-135</sup> Advances in our understanding of the critical function of T<sub>FH</sub> and T<sub>FR</sub> cells in controlling allergic diseases has enabled researchers to dissect the role of T<sub>FH</sub> and T<sub>FR</sub> cells in AIT-induced immune tolerance. Schulten et al. described a lower frequency of CD45RO<sup>+</sup> cT<sub>FH</sub> cells after subcutaneous AIT in patients allergic to timothy grass than in those not receiving AIT (Table 3).<sup>36</sup> They observed that *in vitro* T-cell receptor and IL-2 stimulation, which mimicked AIT, induced the differentiation of T<sub>FH</sub> cells into CXCR5<sup>+</sup>FOXP3<sup>+</sup>Helios<sup>+</sup> T<sub>FR</sub> cells, as shown by the increase of T<sub>FR</sub> cells by about 6-fold compared to those without IL-2 stimulation.<sup>36</sup> In a randomized, double-blind, placebo-controlled trial, Sharif et al. reported that a short-course of *Lolium perenne* peptide subcutaneous immunotherapy dramatically reduced the frequencies of IL-4<sup>+</sup>, IL-21<sup>+</sup>, and IL-4<sup>+</sup>IL-21<sup>+</sup> CXCR5<sup>+</sup>PD-1<sup>+</sup> cT<sub>FH</sub> cells, whereas increased frequency of IFN-γ<sup>+</sup> cT<sub>FH</sub> cells in patients with grass pollen-induced AR, suggesting an immune switch from T<sub>FH</sub>2 to T<sub>FH</sub>1 during AIT (Table 3).<sup>37</sup>

Furthermore, when investigating patients with AR who were allergic to HDM, Yao et al. noted that the frequencies of both Der p 1-specific IL-4<sup>+</sup> cT<sub>FH</sub> and CXCR5<sup>+</sup> T<sub>H</sub>2 cells significantly reduced after subcutaneous AIT (Figure 2 and table 3). Nevertheless, the reduction of Der p 1-specific IL-4<sup>+</sup> cT<sub>FH</sub>2 cells was more pronounced than that of Der p 1-specific IL-4<sup>+</sup> cT<sub>H</sub>2 cells, underlining a dominant effect of T<sub>FH</sub>2 cells in AIT.<sup>111</sup> Moreover, Yao et al. found that subcutaneous AIT not only increased the abundance of cT<sub>FR</sub> cells (Figure 2)<sup>35</sup> but also restored the defected suppressive capacity of cT<sub>FR</sub> cells in patients with AR.<sup>35</sup> Similarly, Sharif et al. observed a significant increase of CD4<sup>+</sup>CXCR5<sup>+</sup>PD-1<sup>+</sup>FoxP3<sup>+</sup> cT<sub>FR</sub> cells and CTLA-4<sup>+</sup> cT<sub>FR</sub> cells in *Lolium perenne* peptide-treated patients with AR (Table 3).<sup>37</sup>

As a “blocking” antibody, IgG4 can compete with IgE for allergen binding, and thus, may be protective against allergic reactions.<sup>34,135</sup> The induction of allergen-specific IgG4 has been associated with successful AIT.<sup>34</sup> Yao et al. showed that the increase of Der p-specific IgG4 levels positively correlated with the reduction of Der p-specific IgE levels in patients with AR undergoing subcutaneous AIT.<sup>88</sup> These data raised a notion that AIT may induce a shift from allergen-specific IgE to IgG4, which is potentially mediated by IL-10-secreting T<sub>FR</sub> cells.<sup>85</sup> Indeed, Ito et al. observed a significantly high frequency of IL-10-producing cT<sub>FR</sub> cells in patients with IgG4-related disease, which positively correlated with serum IgG4 levels.<sup>136</sup>

When analyzing the association of T<sub>FH</sub> and T<sub>FR</sub> cell modulation with AIT efficacy, Yao et al. observed that the reduction of Der p 1-specific IL-4<sup>+</sup> T<sub>FH</sub>2 cells and increase of T<sub>FR</sub> cells, but not their corresponding CXCR5<sup>+</sup> T<sub>H</sub>2 and T<sub>REG</sub> cells, positively correlated with the improvement of symptoms in patients undergoing subcutaneous AIT (Table 3).<sup>111</sup> It is noteworthy that the ratio of T<sub>FR</sub>/T<sub>FH</sub>2 cells appeared to be more closely correlated with the clinical benefits of AIT than T<sub>FR</sub> cells alone ( $r = -0.451$  for cT<sub>FR</sub>/cT<sub>FH</sub>2 cell ratio vs  $r = -0.325$  for T<sub>FR</sub> cells),<sup>35</sup> emphasizing a greater contribution of the T<sub>FR</sub> and T<sub>FH</sub>2 cell rebalance in improving the efficiency of AIT. To date, the mechanisms underlying the re-establishment of T<sub>FH</sub> and T<sub>FR</sub> cell balance during AIT remain poorly understood. Botta et al. reported that the kinetics of T<sub>FR</sub> cell generation was slower than that of T<sub>FH</sub> cells;<sup>76</sup> thus, it is conceivable that prolonged allergen stimulation of AIT favors T<sub>FR</sub> cell generation.

---

Taken together, the changes in  $T_{FH}$  and  $T_{FR}$  cells after AIT and their association with AIT efficacy highlights a potential value of  $T_{FH}$  and  $T_{FR}$  cells as therapeutic targets and biomarkers for AIT. However, the variations of  $T_{FH}$  and  $T_{FR}$  definitions may limited clinical approaches of targeting  $T_{FH}$  and  $T_{FR}$  cells. Therefore, the heterogeneity of  $T_{FH}$  and  $T_{FR}$  cells should be carefully considered in further studies. Given that the correlation between  $T_{FH}$  and  $T_{FR}$  cells and AIT has been established in the context of subcutaneous AIT, it is necessary to assess the changes of  $T_{FH}$  and  $T_{FR}$  cells in other forms of AIT, such as the sublingual form. In addition, randomized trials are required to further validate the value of using  $T_{FH}$  and  $T_{FR}$  cells as a biomarker for predicting or monitoring the efficacy of AIT.

Low-dose IL-2 therapy has been shown to substantially inhibit the generation of  $T_{FH}$  cells, resulting in marked reductions of disease activity in patients with systemic lupus erythematosus.<sup>58</sup> In addition, IL-2/anti-IL-2 complexes efficiently expanded peripheral  $T_{REG}$  cells in mice in the presence or absence of allergens.<sup>137</sup> Therefore, administration of low-dose of IL-2 during AIT may be a potential strategy to improve the efficacy of AIT. In future, it is very interesting to seek adjunctive medicines, chemical reagents, and nutrients, and even gene editing methods that can modify the function of  $T_{FH}$  and  $T_{FR}$  cells to enhance the efficacy of AIT.<sup>138</sup>

## 7. CONCLUSIONS AND PERSPECTIVES

A tilted balance in the  $T_{FH}$ – $T_{FR}$  cell regulatory axis, caused by aberrant  $T_{FH2}$  and  $T_{FH13}$  cell activation and  $T_{FR}$  cell deficiency, may represent a common feature of allergic diseases. AIT can re-establish this balance, and therefore, a change in the  $T_{FH}/T_{FR}$  cell ratio may be applied as candidate biomarkers to guide and optimize AIT. Despite new knowledge gained in past decade on the role of  $T_{FH}$  and  $T_{FR}$  cells and the advancement in our understanding of the pathogenesis of allergic diseases, several key questions remain to be answered, such as why are  $T_{FR}$  cells from patients with AR selectively defective at inhibiting IgE production but do not affect other antibodies isotypes? Additionally, what are the molecular mechanisms underlying the functions of  $T_{FH}$  and  $T_{FR}$  cells in controlling high-affinity IgE production? Moreover, the allergen specificity of  $T_{FH}$  and  $T_{FR}$  cells in allergic diseases remain to be elucidated in detail. Addressing these questions

---

will help in the development of future novel therapeutic interventions for allergic diseases.

**ACKNOWLEDGMENTS:** The authors thank Anna Globinska for the figures.

**FUNDING:** This work was supported by the National Natural Science Foundation of China (NSFC) grants 81920108011 and 81630024.

**CONFLICT OF INTEREST:** Y.Y. has nothing to disclose. C.L.C. has nothing to disclose. D.Y. has nothing to disclose. Z.L. reports grants from National Natural Science Foundation of China (81920108011 and 81630024) during the conduct of the study.

**AUTHOR'S CONTRIBUTIONS:** Z.L. and D.Y. searched literature for this article and made critical discussions of content. Y.Y. and C.L.C. wrote the article. All authors reviewed and/or edited the article before submission.

## REFERENCES

1. Agache I, Akdis CA. Precision medicine and phenotypes, endotypes, genotypes, regiotypes, and theratypes of allergic diseases. *J Clin Invest* 2019;129(4):1493-1503.
2. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organ J* 2014;7(1):12.
3. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. *Lancet* 2018;391(10122):783-800.
4. Akdis CA, Arkwright PD, Bruggen MC, et al. Type 2 immunity in the skin and lungs. *Allergy* 2020;75(7):1582-1605.
5. Bousquet J, Devillier P, Arnavielhe S, et al. Treatment of allergic rhinitis using mobile technology with real-world data: The MASK observational pilot study. *Allergy* 2018;73(9):1763-1774.
6. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012;18(5):693-704.
7. Zellweger F, Eggel A. IgE-associated allergic disorders: recent advances in etiology, diagnosis, and treatment. *Allergy* 2016;71(12):1652-1661.
8. Wu LC, Zarrin AA. The production and regulation of IgE by the immune system. *Nat Rev Immunol* 2014;14(4):247-259.
9. Shin JS, Greer AM. The role of FcεpsilonRI expressed in dendritic cells and monocytes. *Cell Mol Life Sci* 2015;72(12):2349-2360.
10. Bousquet J, Khaltayev N, Cruz AA, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63(suppl 86):8-160.
11. Guntern P, Eggel A. Past, present, and future of anti-IgE biologics. *Allergy* 2020. doi: 10.1111/all.14308.
12. Gould HJ, Ramadani F. Peanut allergen-specific antibodies go public. *Science* 2018;362(6420):1247-1248.
13. Vinuesa CG, Linterman MA, Yu D, MacLennan ICM. Follicular helper T cells. *Annu Rev Immunol* 2016;34:335-368.

- 
14. Qi H. T follicular helper cells in space-time. *Nat Rev Immunol* 2016;16:612-625.
15. Crotty S. T follicular helper cell biology: a decade of discovery and diseases. *Immunity* 2019;50(5):1132-1148.
16. Deng J, Wei Y, Fonseca VR, Graca L, Yu D. T follicular helper cells and T follicular regulatory cells in rheumatic diseases. *Nat Rev Rheumatol* 2019;15(8):475-490.
17. Ballesteros-Tato A, Randall TD, Lund FE, Spolski R, Leonard WJ, León B. T follicular helper cell plasticity shapes pathogenic T helper 2 cell-mediated immunity to inhaled house dust mite. *Immunity* 2016;44(2):259-273.
18. Kobayashi T, Iijima K, Dent AL, Kita H. Follicular helper T cells mediate IgE antibody response to airborne allergens. *J Allergy Clin Immunol* 2017;139(1):300-313.
19. Su C, Johnson ME, Torres A, et al. Mapping effector genes at lupus GWAS loci using promoter Capture-C in follicular helper T cells. *Nat Commun* 2020;11(1):3294.
20. Varricchi G, Harker J, Borriello F, Marone G, Durham SR, Shamji MH. T follicular helper (Tfh) cells in normal immune responses and in allergic disorders. *Allergy* 2016;71(8):1086-1094.
21. Morita R, Schmitt N, Bentebibel SE, et al. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* 2011;34(1):108-121.
22. Gowthaman U, Chen JS, Zhang BY, et al. Identification of a T follicular helper cell subset that drives anaphylactic IgE. *Science* 2019;365(6456):eaaw6433.
23. Wollenberg I, Agua-Doce A, Hernandez A, et al. Regulation of the germinal center reaction by Foxp3<sup>+</sup> follicular regulatory T cells. *J Immunol* 2011;187(9):4553-4560.
24. Linterman MA, Pierson W, Lee SK, et al. Foxp3<sup>+</sup> follicular regulatory T cells control the germinal center response. *Nat Med* 2011;17(8):975-982.
25. Chung Y, Tanaka S, Chu F, et al. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat Med* 2011;17(8):983-988.
26. Cretney E, Kallies A, Nutt SL. Differentiation and function of Foxp3(+) effector regulatory T cells. *Trends Immunol* 2013;34(2):74-80.

27. Pfaar O, Agache I, de Blay F, et al. Perspectives in allergen immunotherapy: 2019 and beyond. *Allergy* 2019;74 Suppl 108:3-25.
28. Shamji MH, Durham SR. Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers. *J Allergy Clin Immunol* 2017;140(6):1485-1498.
29. Berings M, Karaaslan C, Altunbulakli C, et al. Advances and highlights in allergen immunotherapy: On the way to sustained clinical and immunologic tolerance. *J Allergy Clin Immunol* 2017;140(5):1250-1267.
30. Agache I, Lau S, Akdis CA, et al. EAACI Guidelines on Allergen Immunotherapy: House dust mite-driven allergic asthma. *Allergy* 2019;74(5):855-873.
31. Huang Y, Wang C, Wang X, et al. Efficacy and safety of subcutaneous immunotherapy with house dust mite for allergic rhinitis: A Meta-analysis of Randomized Controlled Trials. *Allergy* 2019;74(1):189-192.
32. Bacher P, Scheffold A. The effect of regulatory T cells on tolerance to airborne allergens and allergen immunotherapy. *J Allergy Clin Immunol* 2018;142(6):1697-1709.
33. van Zelm MC, McKenzie CI, Varese N, et al. Recent developments and highlights in immune monitoring of allergen immunotherapy. *Allergy* 2019;74(12):2342-2354.
34. Shamji MH, Kappen JH, Akdis M, et al. Biomarkers for monitoring clinical efficacy of allergen immunotherapy for allergic rhinoconjunctivitis and allergic asthma: an EAACI Position Paper. *Allergy* 2017;72(8):1156-1173.
35. Yao Y, Wang ZC, Wang N, et al. Allergen immunotherapy improves defective follicular regulatory T cells in patients with allergic rhinitis. *J Allergy Clin Immunol* 2019;144(1):118-128.
36. Schulten V, Tripple V, Seumois G, et al. Allergen-specific immunotherapy modulates the balance of circulating Tfh and Tfr cells. *J Allergy Clin Immunol* 2018;141(2):775-777.
37. Sharif H, Singh I, Kouser L, et al. Immunologic mechanisms of a short-course of *Lolium perenne* peptide immunotherapy: A randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2019;144(3):738-749.
38. Nurieva RI, Chung Y, Martinez GJ, et al. Bcl6 mediates the development of T follicular

- helper cells. *Science* 2009;325(5943):1001-1005.
39. Johnston RJ, Poholek AC, DiToro D, et al. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* 2009;325(325):1006-1010.
40. Yu D, Rao S, Tsai LM, et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* 2009;31(3):457-468.
41. Schmitt N, Morita R, Bourdery L, et al. Human dendritic cells induce the differentiation of interleukin-21-producing T follicular helper-like cells through interleukin-12. *Immunity* 2009;31(1):158-169.
42. Klechevsky E, Morita R, Liu M, et al. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. *Immunity* 2008;29(3):497-510.
43. Schmitt N, Liu Y, Bentebibel SE, et al. The cytokine TGF- $\beta$  co-opts signaling via STAT3-STAT4 to promote the differentiation of human TFH cells. *Nat Immunol* 2014;15(9):856-865.
44. Hardtke S, Ohl L, Förster R. Balanced expression of CXCR5 and CCR7 on follicular T helper cells determines their transient positioning to lymph node follicles and is essential for efficient B-cell help. *Blood* 2005;106(6):1924-1931.
45. He J, Tsai LM, Leong YA, et al. Circulating precursor CCR7(lo)PD-1(hi) CXCR5+CD4+T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity* 2013;39(4):770-781.
46. Pepper M, Pagan AJ, Igyarto BZ, Taylor JJ, Jenkins MK. Opposing signals from the Bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. *Immunity* 2011;35(4):583-595.
47. Yeh CH, Nojima T, Kuraoka M, Kelsoe G. Germinal center entry not selection of B cells is controlled by peptide-MHCII complex density. *Nat Commun* 2018;9(1):928.
48. Hatzi K, Nance JP, Kroenke MA, et al. BCL6 orchestrates Tfh cell differentiation via multiple distinct mechanisms. *J Exp Med* 2015;212(4):539-553.
49. Qi H, Cannons JL, Klauschen F, et al. SAP-controlled T-B cell interactions underlie

- germinal centre formation. *Nature* 2008;455(7214):764-769.
50. Shi J, Hou S, Fang Q, et al. PD-1 controls follicular T helper cell positioning and function. *Immunity* 2018;49(2):264-274.
51. Zotos D, Coquet JM, Zhang Y, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. *J Exp Med* 2010;207(2):365-378.
52. Linterman MA, Beaton L, Yu D, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *J Exp Med* 2010;207(2):353-363.
53. Weinstein JS, Herman EI, Lainez B, et al. TFH cells progressively differentiate to regulate the germinal center response. *Nat Immunol* 2016;17(10):1197-1205.
54. Wang Y, Shi J, Yan J, et al. Germinal-center development of memory B cells driven by IL-9 from follicular helper T cells. *Nat Immunol* 2017;18(8):921-930.
55. Wan Z, Lin Y, Zhao Y, Qi H. TFH cells in bystander and cognate interactions with B cells. *Immunol Rev* 2019;288(1):28-36.
56. Locci M, Havenar-Daughton C, Landais E, et al. Human circulating PD-1<sup>+</sup>CXCR3-CXCR5<sup>+</sup> memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. *Immunity* 2013;39(4):758-769.
57. Bentebibel SE, Lopez S, Obermoser G, et al. Induction of ICOS<sup>+</sup>CXCR3<sup>+</sup>CXCR5<sup>+</sup> TH cells correlates with antibody responses to influenza vaccination. *Sci Transl Med* 2013;5(176):176ra132.
58. He J, Zhang X, Wei Y, et al. Low-dose interleukin-2 treatment selectively modulates CD4(+) T cell subsets in patients with systemic lupus erythematosus. *Nat Med* 2016;22(9):991-993.
59. Zotos D, Coquet JM, Zhang Y, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. *J Exp Med* 2010;207(2):365-378.
60. Ozaki K, Spolski R, Feng CG, et al. A critical role for IL-21 in regulating immunoglobulin production. *Science* 2002;298(5598):1630-1634.
61. Moens L, Tangye SG. Cytokine-Mediated Regulation of Plasma Cell Generation: IL-21 Takes Center Stage. *Front Immunol* 2014;5:65.

62. Yang Z, Wu CAM, Targ S, Allen CDC. IL-21 is a broad negative regulator of IgE class switch recombination in mouse and human B cells. *J Exp Med* 2020;217(5):e20190472.
63. Suto A, Nakajima H, Hirose K, et al. Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. *Blood* 2002;100(13):4565-4573.
64. Levack RC, Newell KL, Popescu M, et al. CD11c(+) T-bet(+) B cells require IL-21 and IFN-gamma from type 1 T follicular helper cells and intrinsic Bcl-6 expression but develop normally in the absence of T-bet. *J Immunol* 2020;15;205(4):1050-1058.
65. Carpio VH, Aussenac F, Puebla-Clark L, et al. T helper plasticity is orchestrated by STAT3, Bcl6, and Blimp-1 balancing pathology and protection in Malaria. *iScience* 2020;23(7):101310.
66. Sage PT, Ron-Harel N, Juneja VR, et al. Suppression by TFR cells leads to durable and selective inhibition of B cell effector function. *Nat Immunol* 2016;17(12):1436-1446.
67. Leavenworth JW, Verbinnen B, Yin J, Huang H, Cantor H. A p85  $\alpha$ -osteopontin axis couples the receptor ICOS to sustained Bcl-6 expression by follicular helper and regulatory T cells. *Nat Immunol* 2015;16(1):96-106.
68. Fonseca VR, Ribeiro F, Graca L. T follicular regulatory (Tfr) cells: Dissecting the complexity of Tfr-cell compartments. *Immunol Rev* 2019;288(1):112-127.
69. Sage PT, Alvarez D, Godec J, et al. Circulating T follicular regulatory and helper cells have memory-like properties. *J Clin Invest* 2014(12);124:5191-5204.
70. Xu L, Huang Q, Wang H, et al. The Kinase mTORC1 Promotes the Generation and Suppressive Function of Follicular Regulatory T Cells. *Immunity* 2017;47(3):538-551.
71. Jandl C, Liu SM, Canete PF, et al. IL-21 restricts T follicular regulatory T cell proliferation through Bcl-6 mediated inhibition of responsiveness to IL-2. *Nat Commun* 2017;8:14647.
72. Ding YN, Hsu HC, Li J, et al. IL-21 inhibited follicular regulatory T cells to promote autoreactive germinal center development in autoimmune BXD2 mice. *Arthritis Rheumatol* 2013;65:S1159-S1159.
73. Sayin I, Radtke AJ, Vella LA, et al. Spatial distribution and function of T follicular

- regulatory cells in human lymph nodes. *J Exp Med* 2018;215(6):1531-1542.
74. Ritvo PG, Churlaud G, Quiniou V, et al. Tfr cells lack IL-2R $\alpha$  but express decoy IL-1R2 and IL-1Ra and suppress the IL-1-dependent activation of Tfh cells. *Sci Immunol* 2017;2(15):eaan0368.
75. Wing JB, Kitagawa Y, Locci M, et al. A distinct subpopulation of CD25(-) T-follicular regulatory cells localizes in the germinal centers. *Proc Natl Acad Sci* 2017;114(31):E6400-E6409.
76. Botta D, Fuller MJ, Marquez-Lago TT, et al. Dynamic regulation of T follicular regulatory cell responses by interleukin 2 during influenza infection. *Nat Immunol* 2017;18(11):1249-1260.
77. Fonseca VR, Agua-Doce A, Maceiras AR, et al. Human blood Tfr cells are indicators of ongoing humoral activity not fully licensed with suppressive function. *Sci Immunol* 2017;2(14):eaan1487.
78. Dhaeze T, Peelen E, Hombrouck A, et al. Circulating Follicular Regulatory T Cells Are Defective in Multiple Sclerosis. *J Immunol* 2015;195(3):832-840.
79. Sakaguchi S, Mikami N, Wing JB, Tanaka A, Ichiyama K, Ohkura N. Regulatory T Cells and Human Disease. *Annu Rev Immunol* 2020;38:541-566.
80. Klocke K, Sakaguchi S, Holmdahl R, Wing K. Induction of autoimmune disease by deletion of CTLA-4 in mice in adulthood. *Proc Natl Acad Sci* 2016;113(17):E2383-2392.
81. Pierau M, Lingel H, Vogel K, et al. CTLA-4-competent conventional T cells back up regulatory T cells to restrain memory T-helper type 2 cell responses. *Allergy* 2020;75(3):684-687.
82. Sage PT, Paterson AM, Lovitch SB, Sharpe AH. The coinhibitory receptor CTLA-4 controls B cell responses by modulating T follicular helper, T follicular regulatory, and T regulatory cells. *Immunity* 2014;41(6):1026-1039.
83. Wing JB, Ise W, Kurosaki T, Sakaguchi S. Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4. *Immunity* 2014;41(6):1013-1025.

84. Kucuk ZY, Charbonnier LM, McMasters RL, Chatila T, Bleesing JJ. CTLA-4 haploinsufficiency in a patient with an autoimmune lymphoproliferative disorder. *J Allergy Clin Immunol* 2017;140(3):862-864.
85. Laidlaw BJ, Lu Y, Amezquita RA, et al. Interleukin-10 from CD4<sup>+</sup> follicular regulatory T cells promotes the germinal center response. *Sci Immunol* 2017;2(16):eann4767.
86. Xie MM, Chen Q, Liu H, et al. T follicular regulatory cells and IL-10 promote food antigen-specific IgE. *J Clin Invest* 2020;130(7):3820-3832.
87. Cañete PF, Sweet RA, Gonzalez-Figueroa P, et al. Regulatory roles of IL-10-producing human follicular T cells. *J Exp Med* 2019;216(8):1843-1856.
88. Yao Y, Wang N, Chen CL, et al. CD23 expression on switched memory B cells bridges T-B cell interaction in allergic rhinitis. *Allergy* 2020. doi: 10.1111/all.14288.
89. Gowthaman U, Chen JS, Eisenbarth SC. Regulation of IgE by T follicular helper cells. *J Leukoc Biol* 2020;107(3):409-418.
90. Xiong H, Dolpady J, Wabl M, et al. Sequential class switching is required for the generation of high affinity IgE antibodies. *J Exp Med* 2012;209(2):353-364.
91. Asrat S, Kaur N, Liu X, et al. Chronic allergen exposure drives accumulation of long-lived IgE plasma cells in the bone marrow, giving rise to serological memory. *Sci Immunol* 2020;5(43):eaav8402.
92. Jimenez-Saiz R, Ellenbogen Y, Koenig JFE, et al. IgG1(+) B-cell immunity predates IgE responses in epicutaneous sensitization to foods. *Allergy* 2019;74(1):165-175.
93. Hoof I, Schulten V, Layhadi JA, et al. Allergen-specific IgG(+) memory B cells are temporally linked to IgE memory responses. *J Allergy Clin Immunol* 2020;146(1):180-191.
94. Looney TJ, Lee JY, Roskin KM, et al. Human B-cell isotype switching origins of IgE. *J Allergy Clin Immunol* 2016;137(2):579-586.
95. Roco JA, Mesin L, Binder SC, et al. Class-switch recombination occurs infrequently in germinal centers. *Immunity* 2019;51(2):337-350.
96. Clement RL, Daccache J, Mohammed MT, et al. Follicular regulatory T cells control

- 
- humoral and allergic immunity by restraining early B cell responses. *Nat Immunol* 2019;20(10):1360-1371.
97. Coquet JM, Schuijs MJ, Smyth MJ, et al. Interleukin-21-producing CD4(+) T cells promote type 2 immunity to house dust mites. *Immunity* 2015;43(2):318-330.
98. Noble A, Zhao J. Follicular helper T cells are responsible for IgE responses to Der p 1 following house dust mite sensitization in mice. *Clin Exp Allergy* 2016;46(8):1075-1082.
99. Liang HE, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat Immunol* 2011;13(1):58-66.
100. Meli AP, Fontes G, Soo CL, King IL. T follicular helper cell-derived IL-4 is required for IgE production during intestinal helminth infection. *J Immunol* 2017;199(1):244-252.
101. Uwadiae FI, Pyle CJ, Walker SA, et al. Targeting the ICOS/ICOS-L pathway in a mouse model of established allergic asthma disrupts T follicular helper cell responses and ameliorates disease. *Allergy* 2019;74(4):650-662.
102. He K, Hettinga A, Kale SL, et al. Blimp-1 is essential for allergen-induced asthma and Th2 cell development in the lung. *J Exp Med* 2020;217(7):e20190742.
103. Gong F, Su Q, Jiang D, et al. High frequency of circulating follicular helper T cells in patients with bronchial asthma. *Clin Lab* 2014;60(6):963-968.
104. Gong F, Zhu HY, Zhu J, et al. Circulating CXCR5<sup>+</sup>CD4<sup>+</sup>T cells participate in the IgE accumulation in allergic asthma. *Immunol Lett* 2018;197:9-14.
105. Kamekura R, Shigehara K, Miyajima S, et al. Alteration of circulating type 2 follicular helper T cells and regulatory B cells underlies the comorbid association of allergic rhinitis with bronchial asthma. *Clin Immunol* 2015;158(2):204-211.
106. Gong F, Qian C, Zhu H, et al. Circulating follicular T-helper cell subset distribution in patients with asthma. *Allergy Asthma Proc* 2016;37(6):154-161.
107. Miyajima S, Shigehara K, Kamekura R, et al. Activated circulating T follicular helper cells and skewing of T follicular helper 2 cells are down-regulated by treatment including an inhaled corticosteroid in patients with allergic asthma. *Allergol Int* 2020;69(1):66-77.

108. Wang XQ, Ke X, Shen Y, et al. Changes in circulating follicular helper T-cells in Chinese patients with allergic rhinitis. *Acta Otolaryngol* 2016;136(2):199-204.
109. Akiyama M, Suzuki K, Yamaoka K, et al. Number of circulating follicular helper 2 T cells correlates with IgG4 and interleukin-4 levels and plasmablast numbers in IgG4-related disease. *Arthritis Rheumatol* 2015;67(9):2476-2481.
110. Kim AS, Doherty TA, Karta MR, et al. Regulatory B cells and T follicular helper cells are reduced in allergic rhinitis. *J Allergy Clin Immunol* 2016;138(4):1192-1195.
111. Yao Y, Chen CL, Wang N, et al. Correlation of allergen-specific T follicular helper cell counts with specific IgE levels and efficacy of allergen immunotherapy. *J Allergy Clin Immunol* 2018;142(1):321-324.
112. Bacher P, Scheffold A. New technologies for monitoring human antigen-specific T cells and regulatory T cells by flow-cytometry. *Curr Opin Pharmacol* 2015;23:17-24.
113. Zhang YN, Song J, Wang H, et al. Nasal IL-4(+)CXCR5(+)CD4(+) T follicular helper cell counts correlate with local IgE production in eosinophilic nasal polyps. *J Allergy Clin Immunol* 2016;137(2):462-473.
114. Xiao L, Wei Y, Zhang YN, et al. Increased IL-21 expression in chronic rhinosinusitis with nasal polyps. *Clin Exp Allergy* 2015;45(2):404-413.
115. Song J, Wang H, Zhang YN, et al. Ectopic lymphoid tissues support local immunoglobulin production in patients with chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol* 2018;141(3):927-937.
116. Zhai GT, Wang H, Li JX, et al. IgD-activated mast cells induce IgE synthesis in B cells in nasal polyps. *J Allergy Clin Immunol* 2018;142(5):1489-1499.
117. Lau A, Lester S, Moraitis S, et al. Tertiary lymphoid organs in recalcitrant chronic rhinosinusitis. *J Allergy Clin Immunol* 2017;139(4):1371-1373.
118. Gevaert P, Holtappels G, Johansson SGO, et al. Organization of secondary lymphoid tissue and local IgE formation to *Staphylococcus aureus* enterotoxins in nasal polyp tissue. *Allergy* 2005;60(1):71-79.
119. Tomassen P, Vandeplas G, Van Zele T, et al. Inflammatory endotypes of chronic

- rhinosinusitis based on cluster analysis of biomarkers. *J Allergy Clin Immunol* 2016;137(5):1449-1456.
120. Gevaert P, Nouri-Aria KT, Wu H, et al. Local receptor revision and class switching to IgE in chronic rhinosinusitis with nasal polyps. *Allergy* 2013;68(1):55-63.
121. Van Zele T, Gevaert P, Watelet JB, et al. Staphylococcus aureus colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J Allergy Clin Immunol* 2004;114(4):981-983.
122. Van Roey GA, Vanison CC, Wu J, et al. Classical complement pathway activation in the nasal tissue of patients with chronic rhinosinusitis. *J Allergy Clin Immunol* 2017;140(1):89-100.
123. Calus L, Derycke L, Dullaers M, et al. IL-21 is increased in nasal polyposis and after stimulation with staphylococcus aureus enterotoxin B. *Int Arch Allergy Immunol* 2017;174(3-4):161-169.
124. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *J Allergy Clin Immunol* 2018;141(1):41-58.
125. Eiwegger T, Hung L, San Diego KE, et al. Recent developments and highlights in food allergy. *Allergy* 2019;74(12):2355-2367.
126. Dolence JJ, Kobayashi T, Iijima K, et al. Airway exposure initiates peanut allergy by involving the IL-1 pathway and T follicular helper cells in mice. *J Allergy Clin Immunol* 2018;142(4):1144-1158.
127. Hong SW, O E, Lee JY, et al. Food antigens drive spontaneous IgE elevation in the absence of commensal microbiota. *Sci Adv* 2019;5(5):eaaw1507.
128. Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. *Allergy* 2008;63(11):1455-1463.
129. Szabo K, Gaspar K, Dajnoki Z, et al. Expansion of circulating follicular T helper cells associates with disease severity in childhood atopic dermatitis. *Immunol Lett*

- 
- 2017;189:101-108.
130. Trichot C, Fauchoux L, Karpf L, et al. T helper cell diversity and response to Dupilumab in atopic dermatitis patients. *J Allergy Clin Immunol* 2020; S0091-6749(20):30807-1.
131. Pfaar O, Alvaro M, Cardona V, et al. Clinical trials in allergen immunotherapy: current concepts and future needs. *Allergy* 2018;73(9):1775-1783.
132. Jutel M, Bruggenjurgen B, Richter H, Vogelberg C. Real-world evidence of subcutaneous allergoid immunotherapy in house dust mite-induced allergic rhinitis and asthma. *Allergy* 2020;75(8):2046-2054.
133. Ihara F, Sakurai D, Yonekura S, et al. Identification of specifically reduced Th2 cell subsets in allergic rhinitis patients after sublingual immunotherapy. *Allergy* 2018;73(9):1823-1832.
134. Heeringa JJ, McKenzie CI, Varese N, et al. Induction of IgG2 and IgG4 B-cell memory following sublingual immunotherapy for ryegrass pollen allergy. *Allergy* 2020;75(5):1121-1132.
135. Jutel M, Akdis CA. Immunological mechanisms of allergen-specific immunotherapy. *Allergy* 2011;66(6):725-732.
136. Kamekura R, Takahashi H, Ichimiya S. New insights into IgG4-related disease: emerging new CD4<sup>+</sup> T-cell subsets. *Curr Opin Rheumatol* 2019;31(1):9-15.
137. Kohler C, Smole U, Kratzer B, et al. Allergen alters IL-2/alphaIL-2-based Treg expansion but not tolerance induction in an allergen-specific mouse model. *Allergy* 2020;75(7):1618-1629.
138. Dorofeeva Y, Shilovskiy I, Tulaeva I, et al. Past, presence, and future of allergen immunotherapy vaccines. *Allergy* 2020. doi: 10.1111/all.14300.
139. Bentebibel SE, Schmitt N, Banchereau J, Ueno H. Human tonsil B-cell lymphoma 6 (BCL6)-expressing CD4<sup>+</sup> T-cell subset specialized for B-cell help outside germinal centers. *Proc Natl Acad Sci* 2011;108(33):E488-497.
140. Vaeth M, Muller G, Stauss D, et al. Follicular regulatory T cells control humoral autoimmunity via NFAT2-regulated CXCR5 expression. *J Exp Med* 2014;211(3):545-561.

- 
141. Rookhuizen DC, DeFranco AL. Toll-like receptor 9 signaling acts on multiple elements of the germinal center to enhance antibody responses. *Proc Natl Acad Sci* 2014;111(31):E3224-3233.
142. Aloulou M, Carr EJ, Gador M, et al. Follicular regulatory T cells can be specific for the immunizing antigen and derive from naive T cells. *Nat Commun* 2016;7:10579.
143. Maceiras AR, Almeida SCP, Mariotti-Ferrandiz E, et al. T follicular helper and T follicular regulatory cells have different TCR specificity. *Nat Commun* 2017;8:15067.
144. Kawamoto S, Maruya M, Kato LM, et al. Foxp3(+) T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. *Immunity* 2014;41(1):152-165.
145. Fu WW, Liu XD, Lin X, et al. Deficiency in T follicular regulatory cells promotes autoimmunity. *J Exp Med* 2018;215(3):815-825.
146. Sage PT, Francisco LM, Carman CV, Sharpe AH. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. *Nat Immunol* 2013;14(2):152-161.
147. Wang S, Charbonnier LM, Rivas MN, et al. MyD88 adaptor-dependent microbial sensing by regulatory T cells promotes mucosal tolerance and enforces commensalism. *Immunity* 2015;43(2):289-303.
148. Sage PT, Tan CL, Freeman GJ, et al. Defective TFH cell function and increased TFR cells contribute to defective antibody production in aging. *Cell Reports* 2015;12(2):163-171.
149. Miles B, Miller SM, Folkvord JM, et al. Follicular regulatory T cells impair follicular T helper cells in HIV and SIV infection. *Nat Commun* 2015;6:8608.
150. Foster WS, Grime CJ, Tan HL, et al. Enhanced frequency and function of follicular T cells in the tonsils of house dust mite-sensitized children. *Allergy* 2020;75(5):1240-1243.
151. Wallin EF, Jolly EC, Suchánek O, et al. Human T-follicular helper and T-follicular regulatory cell maintenance is independent of germinal centers. *Blood* 2014;124(17):2666-2674.
152. Wen Y, Yang B, Lu J, Zhang J, Yang H, Li J. Imbalance of circulating CD4(+)CXCR5(+)FOXP3(+) Tfr-like cells and CD4(+)CXCR5(+)FOXP3(-) Tfh-like cells

---

in myasthenia gravis. *Neurosci Lett* 2016;630:176-182.

153. Zhang CJ, Gong Y, Zhu WL, et al. Augmentation of circulating follicular helper T cells and their impact on autoreactive B cells in myasthenia gravis. *J Immunol* 2016;197(7):2610-2617.
154. Wei Y, Feng J, Hou Z, Wang XM, Yu D. Flow cytometric analysis of circulating follicular helper T (Tfh) and follicular regulatory T (Tfr) populations in human blood. *Methods Mol Biol* 2015;1291:199-207.

---

## Figure legend

**Fig. 1 Differentiation and migration of  $T_{FH}$ ,  $T_{H2}$ , and  $T_{FR}$  cells.** DCs prime naïve  $CD4^+$  T cells in the T-cell zones of lymphoid tissues and initiate the differentiation of  $T_{FH}$  or  $T_{H2}$  cells. Some precursor  $T_{FH}$  ( $pT_{FH}$ ) cells acquire CXC chemokine receptor type 5 ( $CXCR5$ )<sup>+</sup> phenotype and enter into the circulation, whereas  $pT_{FH}$  cells with downregulated C-C chemokine receptor ( $CCR$ )7 expression traffic to the B-cell follicle to complete their full differentiation.  $T_{FH}$  cells promote immunoglobulin E (IgE) production and plasma cell differentiation by secreting interleukin (IL)-4, IL-13, and IL-21. Naïve  $T_{REG}$  cells differentiate into  $T_{FR}$  cells in the process akin to  $T_{FH}$  cells.  $T_{H2}$  cells with high expression of  $CCR2$  or  $CCR5$  preferentially migrate to inflamed nonlymphoid tissues, such as nasal polyps, and mediate local inflammatory responses by producing IL-4, IL-5, IL-9, and IL-13. In addition,  $CXCR5^+$   $T_{FH}$  cells and B cells are organized to form ectopic lymphoid tissues (eLTs) in inflammatory tissues, which can support local immunoglobulin production. Upon re-challenge, circulating memory  $T_{FH}$  ( $cT_{FH}$ ) and  $T_{FR}$  ( $cT_{FR}$ ) cells rapidly return to lymphoid tissues to accelerate GC formation. Abbreviations: ASC, antibody secreting cells;  $CXCR5$ , CXC chemokine receptor type 5;  $CCR$ , C-C chemokine receptor; DCs, dendritic cells; eLTs, ectopic lymphoid tissues; EOS, eosinophils; GC, germinal center; IgE, immunoglobulin E; IL, interleukin; MC, mast cells; T, naïve T;  $T_{FH}$ , follicular helper T;  $cT_{FH}$ , circulating  $T_{FH}$ ;  $pT_{FH}$ , precursor  $T_{FH}$ ;  $T_{H2}$ , type 2 T helper;  $T_{FR}$ , follicular regulatory T;  $cT_{FR}$ , circulating  $T_{FR}$ ;  $pT_{FR}$ , precursor  $T_{FR}$ .

**Fig. 2 AIT re-establishes the balance between  $T_{FH}$  and  $T_{FR}$  cells in patients with AR.** Compared to healthy controls, patients with AR demonstrate increased frequencies of circulating  $T_{FH2}$  cells and reduced frequencies of circulating  $T_{FR}$  cells. Circulating  $T_{FH2}$  cells from patients with AR display a greater capacity to induce IgE production than those from healthy controls, whereas AR-derived  $T_{FR}$  cells showed a defective function of suppressing IgE production. AIT reduces the number of allergen-specific circulating  $T_{FH2}$  cells and improves the frequencies and function of circulating  $T_{FR}$  cells. Abbreviations: AR, allergic rhinitis; AIT, allergen immunotherapy;  $T_{FH2}$ , type 2 follicular helper T;  $T_{FR}$ , follicular regulatory T.

**Table 1 Definition and function of T<sub>FH</sub> cell subsets in humans.**

| Location     | Cell type                          | Definition by flow cytometry                                                                        | Major function                                                                                                              | Ref.     |
|--------------|------------------------------------|-----------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|----------|
| Blood        | Memory cT <sub>FH</sub>            | CD4 <sup>+</sup> CD45RA <sup>-</sup> CXCR5 <sup>+</sup>                                             | Promote IgM, IgG, and IgA production by autologous naïve B cells <i>in vitro</i>                                            | 21       |
|              | cT <sub>FH</sub> 1                 | CD4 <sup>+</sup> CD45RA <sup>-</sup> CXCR5 <sup>+</sup><br>CXCR3 <sup>+</sup> CCR6 <sup>-</sup>     | Unable to induce immunoglobulin production from autologous naïve B cells <i>in vitro</i>                                    | 21       |
|              | cT <sub>FH</sub> 2                 | CD4 <sup>+</sup> CD45RA <sup>-</sup> CXCR5 <sup>+</sup><br>CXCR3 <sup>-</sup> CCR6 <sup>-</sup>     | Promote IgM, IgG, IgA, and IgE production by autologous naïve B cells <i>in vitro</i>                                       | 21       |
|              | cT <sub>FH</sub> 17                | CD4 <sup>+</sup> CD45RA <sup>-</sup> CXCR5 <sup>+</sup><br>CXCR3 <sup>-</sup> CCR6 <sup>+</sup>     | Promote IgM, IgG, and IgA production by autologous naïve B cells <i>in vitro</i>                                            | 21       |
|              | Activated cT <sub>FH</sub>         | CD4 <sup>+</sup> CD45RA <sup>-</sup> CXCR5 <sup>+</sup><br>CCR7 <sup>low</sup> PD-1 <sup>high</sup> | Promote IgG production by autologous memory B cells <i>in vitro</i>                                                         | 45       |
|              | Resting cT <sub>FH</sub>           | CD4 <sup>+</sup> CD45RA <sup>-</sup> CXCR5 <sup>+</sup><br>CCR7 <sup>high</sup> PD-1 <sup>low</sup> | Promote IgG production by autologous memory B cells in a capacity less than activated T <sub>FH</sub> cells <i>in vitro</i> | 45       |
|              |                                    |                                                                                                     |                                                                                                                             |          |
| Tonsil       | GC T <sub>FH</sub>                 | CD4 <sup>+</sup> CXCR5 <sup>high</sup> ICOS <sup>high</sup>                                         | Promote IgM, IgA, and IgG production by memory and GC B cells <i>in vitro</i>                                               | 139      |
|              | T <sub>FH</sub> outside GC         | CD4 <sup>+</sup> CXCR5 <sup>low</sup> ICOS <sup>low</sup>                                           | Promote IgM, IgA, and IgG production by naïve and memory B cells <i>in vitro</i>                                            | 139      |
| Nasal polyps | T <sub>FH</sub>                    | CD4 <sup>+</sup> CXCR5 <sup>+</sup>                                                                 | Induce IgM, IgG1, IgG2, IgG3, IgG4, IgA, and IgE production by autologous naïve B cells <i>in vitro</i>                     | 113, 123 |
|              | T <sub>FH</sub> 1                  | CD4 <sup>+</sup> CXCR5 <sup>+</sup> IFN- $\gamma$ <sup>+</sup>                                      | Unknown                                                                                                                     | 113      |
|              | T <sub>FH</sub> 2                  | CD4 <sup>+</sup> CXCR5 <sup>+</sup> IL-4 <sup>+</sup>                                               | Induce IgE production by autologous naïve B cells <i>in vitro</i>                                                           | 113      |
|              | T <sub>FH</sub> 17                 | CD4 <sup>+</sup> CXCR5 <sup>+</sup> IL-17A <sup>+</sup>                                             | Unknown                                                                                                                     | 113      |
|              | IL-21 <sup>+</sup> T <sub>FH</sub> | CD4 <sup>+</sup> CXCR5 <sup>+</sup> IL-21 <sup>+</sup>                                              | Induce IgG, IgE, and IgA production by                                                                                      | 113      |

GC, germinal center; CCR, C-C chemokine receptor; CXCR, C-X-C chemokine receptor; cT<sub>FH</sub>, circulating follicular helper T; ICOS, inducible T-cell costimulator; IFN- $\gamma$ , interferon gamma; Ig, immunoglobulin; IL, interleukin; PD-1, programmed cell death protein 1; T<sub>FH</sub>, follicular helper T.

**Table 2 Definition of T<sub>FR</sub> cells by flow cytometry.**

| Species | Location         | Definition                                                                                                             | Ref.                 |
|---------|------------------|------------------------------------------------------------------------------------------------------------------------|----------------------|
| Mice    | Lymphoid tissues | CD4 <sup>+</sup> CXCR5 <sup>high</sup> PD-1 <sup>high</sup> Foxp3 <sup>+</sup>                                         | 24, 67, 140-144      |
|         |                  | CD4 <sup>+</sup> CXCR5 <sup>+</sup> Bcl-6 <sup>+</sup> Foxp3 <sup>+</sup>                                              | 25, 76, 82, 145      |
|         |                  | CD4 <sup>+</sup> ICOS <sup>+</sup> CXCR5 <sup>+</sup> Foxp3 <sup>+</sup> CD19 <sup>-</sup>                             | 66, 69, 96, 146, 147 |
|         |                  | CD4 <sup>+</sup> CXCR5 <sup>+</sup> Foxp3 <sup>+</sup>                                                                 | 70                   |
|         |                  | CD4 <sup>+</sup> Ly6C <sup>-</sup> PSGL-1 <sup>low</sup> CXCR5 <sup>high</sup> PD-1 <sup>high</sup> Foxp3 <sup>+</sup> | 85                   |
|         | Blood            | CD4 <sup>+</sup> ICOS <sup>+</sup> CXCR5 <sup>+</sup> Foxp3 <sup>+</sup> CD19 <sup>-</sup>                             | 69, 146, 148         |
| Human   | Lymphoid tissues | CD4 <sup>+</sup> CXCR5 <sup>+</sup> Bcl-6 <sup>+</sup> Foxp3 <sup>+</sup>                                              | 25                   |
|         |                  | CD4 <sup>+</sup> CXCR5 <sup>high</sup> PD-1 <sup>high</sup> Foxp3 <sup>+</sup>                                         | 74                   |
|         |                  | CD4 <sup>+</sup> CXCR5 <sup>+</sup> Foxp3 <sup>+</sup>                                                                 | 77                   |
|         |                  | CD4 <sup>+</sup> CD45RA <sup>-</sup> CXCR5 <sup>high</sup> Foxp3 <sup>+</sup>                                          | 35                   |
|         |                  | CD3 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>high</sup> CD127 <sup>-</sup> CXCR5 <sup>+</sup>                           | 149                  |
|         |                  | CD3 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>high</sup> CD127 <sup>-</sup> CXCR5 <sup>high</sup> PD-1 <sup>high</sup>   | 149                  |
|         |                  | CD4 <sup>+</sup> CXCR5 <sup>int/high</sup> PD-1 <sup>int/high</sup> Foxp3 <sup>int/high</sup> CD25 <sup>high</sup>     | 150                  |
|         | Blood            | CD4 <sup>+</sup> CXCR5 <sup>+</sup> CD57 <sup>+</sup> CD127 <sup>-</sup> CD25 <sup>+</sup>                             | 151                  |
|         |                  | CD4 <sup>+</sup> CXCR5 <sup>+</sup> Foxp3 <sup>+</sup>                                                                 | 77, 152, 153         |
|         |                  | CD4 <sup>+</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup> CD25 <sup>high</sup> CD127 <sup>low</sup>                      | 35, 88, 111, 154     |
|         |                  | CD4 <sup>+</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup> CD25 <sup>high</sup> CD127 <sup>low</sup> Foxp3 <sup>+</sup>   | 35                   |
|         |                  | CD4 <sup>+</sup> CXCR5 <sup>+</sup> FOXP3 <sup>+</sup> Helios <sup>+</sup>                                             | 36                   |
|         |                  | CD4 <sup>+</sup> CD25 <sup>+</sup> CD127 <sup>+</sup> CXCR5 <sup>+</sup> PD-1 <sup>+</sup>                             | 78                   |

BCL-6, B-cell lymphoma 6; CCR, C-C chemokine receptor; CXCR, C-X-C chemokine receptor; Foxp3, forkhead box P3; ICOS, inducible T-cell costimulator; PD-1, programmed cell death protein 1; PSGL-1, P-selectin glycoprotein ligand-1; T<sub>FR</sub>, follicular regulatory T.

**Table 3 T<sub>FH</sub> and T<sub>FR</sub> cells in allergic diseases and immunotherapy in humans.**

| Disease         | Sample size | Cell type                                                                                                                                                                                                                                                                                                       | Major finding                                                                                                                                                                                                                                                      | Ref. |
|-----------------|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Allergic asthma | 26          | cT <sub>FH</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup>                                                                                                                                                                                                                                                          | Frequencies of cT <sub>FH</sub> cells are increased in patients with allergic asthma                                                                                                                                                                               | 103  |
| Allergic asthma | 30          | cT <sub>FH</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup><br>cT <sub>FH</sub> 1: CXCR3 <sup>+</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 2: CXCR3 <sup>-</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 17: CXCR3 <sup>-</sup> CCR6 <sup>+</sup> cT <sub>FH</sub>                  | Frequencies of cT <sub>FH</sub> 2 cells are increased in patients with allergic asthma; frequencies of T <sub>FH</sub> 2 and T <sub>FH</sub> 2/T <sub>FH</sub> 1 ratio positively correlate with the total IgE level in the blood in patients with allergic asthma | 106  |
| Allergic asthma | 25          | cT <sub>FH</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup>                                                                                                                                                                                                                                                          | Frequencies of cT <sub>FH</sub> and IL-4 <sup>+</sup> and IL-21 <sup>+</sup> cT <sub>FH</sub> cells are increased in asthmatic patients; cT <sub>FH</sub> cells from asthmatic patients promote IgE production <i>in vitro</i>                                     | 104  |
| Asthma + AR     | 19          | cT <sub>FH</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR5 <sup>+</sup><br>cT <sub>FH</sub> 1: CXCR3 <sup>+</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 2: CXCR3 <sup>-</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 17: CXCR3 <sup>-</sup> CCR6 <sup>+</sup> cT <sub>FH</sub> | Frequencies and numbers of cT <sub>FH</sub> 2 cells are increased in patients with concomitant asthma and AR; fractional exhaled nitric oxide and blood eosinophil levels positively correlate with T <sub>FH</sub> 2/B <sub>REG</sub> ratio                       | 105  |
| AR              | 23          | cT <sub>FH</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR5 <sup>+</sup><br>cT <sub>FH</sub> 1: CXCR3 <sup>+</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 2: CXCR3 <sup>-</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 17: CXCR3 <sup>-</sup> CCR6 <sup>+</sup> cT <sub>FH</sub> | Frequencies and numbers of cT <sub>FH</sub> 2 cells are increased in patients with AR                                                                                                                                                                              | 105  |
| AR              | 10          | cT <sub>FH</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup> PD-1 <sup>+</sup>                                                                                                                                                                                                                                        | Frequencies of cT <sub>FH</sub> cells are reduced in patients with AR                                                                                                                                                                                              | 110  |
| AR              | 32          | cT <sub>FH</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup> ,<br>CD4 <sup>+</sup> PD-1 <sup>+</sup> CXCR5 <sup>+</sup> , and                                                                                                                                                                                         | Frequencies of cT <sub>FH</sub> cells are increased in patients with AR                                                                                                                                                                                            | 108  |

CD4<sup>+</sup>CXCR5<sup>+</sup> ICOS<sup>+</sup>

|                                |    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |     |
|--------------------------------|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| AR (allergic to timothy grass) | 25 | cT <sub>FH</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CD45RO <sup>+</sup><br>CXCR5 <sup>+</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Frequencies of cT <sub>FH</sub> cells are comparable between patients with AR and healthy controls                                                                                                                                                                                                                                                                                                                                                                                                                                               | 36  |
| AR (allergic to HDM only)      | 27 | cT <sub>FH</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>-/low</sup> CD127 <sup>-/+</sup> CD45RA <sup>low</sup> CXCR5 <sup>+</sup><br>cT <sub>FH</sub> 1: CXCR3 <sup>+</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 2: CXCR3 <sup>-</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 17: CXCR3 <sup>-</sup> CCR6 <sup>+</sup> cT <sub>FH</sub>                                                                                                                                                                                                                                                                                                                                                                                                    | Frequencies of cT <sub>FH</sub> cells are comparable between patients with AR and healthy controls; frequencies of cT <sub>FH</sub> 2 cells are increased in patients with AR; cT <sub>FH</sub> 2 cells from patients with AR robustly promote IgE production <i>in vitro</i> ; frequencies of allergen-specific IL-4 <sup>+</sup> T <sub>FH</sub> cells are increased and positively correlated allergen-specific IgE levels in patients with AR                                                                                                | 111 |
| AR (allergic to HDM only)      | 44 | cT <sub>FH</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>-/low</sup> CD127 <sup>-/+</sup> CD45RA <sup>low</sup> CXCR5 <sup>+</sup><br>cT <sub>FH</sub> 1: CXCR3 <sup>+</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 2: CXCR3 <sup>-</sup> CCR6 <sup>-</sup> cT <sub>FH</sub><br>cT <sub>FH</sub> 17: CXCR3 <sup>-</sup> CCR6 <sup>+</sup> cT <sub>FH</sub><br>cT <sub>FR</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>high</sup> CD127 <sup>low</sup> CD45RA <sup>low</sup> CXCR5 <sup>+</sup> , and<br>CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>high</sup> CD127 <sup>low</sup> CD45RA <sup>low</sup> CXCR5 <sup>+</sup> Foxp3 <sup>+</sup><br>GC T <sub>FR</sub> : CD4 <sup>+</sup> CXCR5 <sup>high</sup> Foxp3 <sup>+</sup><br>CD25 <sup>-</sup> | Frequencies and numbers of tonsillar T <sub>FR</sub> cells are reduced in patients with AR; frequencies of T <sub>FR</sub> cells in tonsils correlate with those in bloods; frequencies and numbers of cT <sub>FR</sub> cell are reduced in patients with AR; cT <sub>FR</sub> cell frequency and cT <sub>FR</sub> /cT <sub>FH</sub> 2 ratio negatively associate with disease severity and allergen-specific IgE level in patients with AR; cT <sub>FR</sub> cells from AR patients are defective in suppressing IgE production <i>in vitro</i> | 35  |
| AR (allergic to HDM only)      | 38 | cT <sub>FH</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>-/low</sup> CD127 <sup>-/+</sup> CD45RA <sup>low</sup> CXCR5 <sup>+</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Frequencies of cT <sub>FH</sub> 2 cells are increased and frequencies of cT <sub>FR</sub> cells are decreased in patients with AR; frequencies of cT <sub>FR</sub> cells and                                                                                                                                                                                                                                                                                                                                                                     | 88  |

cT<sub>FR</sub>: CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>CD45RA<sup>low</sup>CXCR5<sup>+</sup> cT<sub>FR</sub>/cT<sub>FH</sub>2 ratio negatively correlate with CD23 expression on switched memory B cells in patients with AR; cT<sub>FR</sub> cells from patients with AR are defective in suppressing CD23 expression on switched memory B cells *in vitro*

|                                     |     |                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                              |     |
|-------------------------------------|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Aeroallergen-sensitized individuals | 13  | cT <sub>FH</sub> 13: CD4 <sup>+</sup> CXCR5 <sup>+</sup> CD40L <sup>+</sup> IL-4 <sup>+</sup> IL-13 <sup>+</sup>                                                                                                                                                         | Frequencies of cT <sub>FH</sub> 13 cells are increased in aeroallergen sensitized allergic patients                                                                                                                                                                                                                                                                                                                          | 22  |
| Nasal polyps                        | 152 | T <sub>FH</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup>                                                                                                                                                                                                                    | Frequencies of nasal T <sub>FH</sub> cells and its subsets are increased in nasal polyps; nasal IL-4 <sup>+</sup> T <sub>FH</sub> cells may contribute to local IgE production in eosinophilic nasal polyps                                                                                                                                                                                                                  | 113 |
| Peanut allergy                      | 6   | cT <sub>FH</sub> 13: CD4 <sup>+</sup> CXCR5 <sup>+</sup> CD40L <sup>+</sup> IL-4 <sup>+</sup> IL-13 <sup>+</sup>                                                                                                                                                         | Frequencies of cT <sub>FH</sub> 13 cells are increased in patients with peanut allergy                                                                                                                                                                                                                                                                                                                                       | 22  |
| AD                                  | 29  | cT <sub>FH</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup> ICOS <sup>+</sup> PD-1 <sup>+</sup>                                                                                                                                                                               | Frequencies of cT <sub>FH</sub> cells are increased and frequencies of IL-21 <sup>+</sup> cT <sub>FH</sub> cells associate with disease severity in children with AD                                                                                                                                                                                                                                                         | 129 |
| AD                                  | 29  | cT <sub>FH</sub> : CD4 <sup>+</sup> CD45RO <sup>+</sup> CXCR5 <sup>+</sup>                                                                                                                                                                                               | Frequencies of cT <sub>FH</sub> cells are comparable between patients with AD and healthy controls                                                                                                                                                                                                                                                                                                                           | 125 |
| AIT (AR patients)                   | 32  | cT <sub>FH</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CD45RO <sup>+</sup> CXCR5 <sup>+</sup><br>T <sub>FH</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup> FOXP3 <sup>+</sup> Helios <sup>+</sup><br>cT <sub>FR</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup> FOXP3 <sup>+</sup> | Frequencies of cT <sub>FH</sub> cells, especially CXCR5 <sup>high</sup> cT <sub>FH</sub> cells, are reduced after AIT; T <sub>FR</sub> cells are enriched in the CXCR5 <sup>low</sup> cT <sub>FH</sub> cells, and frequencies of CXCR5 <sup>low</sup> cT <sub>FH</sub> cells are increased after AIT; anti-IL-2 reduce CXCR5 expression in cT <sub>FH</sub> cells in the presence of anti-CD3/28 stimulation <i>in vitro</i> | 36  |

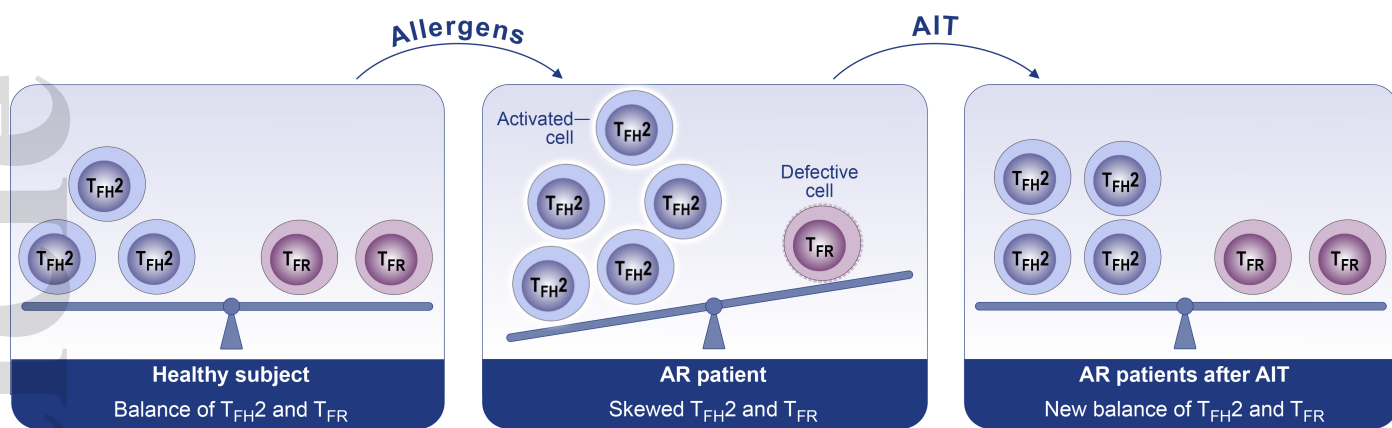
Helios<sup>+</sup>

|                   |    |                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                              |     |
|-------------------|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| AIT (AR patients) | 22 | T <sub>FH</sub> :<br><br>CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>-/low</sup> CD127 <sup>-/+</sup> CD45RA <sup>low</sup> CXCR5 <sup>+</sup>                                                                                                                                                                                | Frequencies of allergen-specific IL-4 <sup>+</sup> T <sub>FH</sub> cells are reduce after semidepot HDM allergen extracts treatment; reduction of allergen-specific IL-4 <sup>+</sup> T <sub>FH</sub> cells correlates with clinical efficacy after 12 months of AIT                                                                                                         | 111 |
|                   | 27 | cT <sub>FH2</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>-/low</sup> CD127 <sup>-/+</sup> CD45RA <sup>low</sup> CXCR5 <sup>+</sup> CXCR3 <sup>-</sup> CCR6 <sup>-</sup><br><br>cT <sub>FR</sub> : CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>high</sup> CD127 <sup>low</sup> CD45RA <sup>low</sup> CXCR5 <sup>+</sup> | Frequencies of cT <sub>FR</sub> cells and cT <sub>FR</sub> /cT <sub>FH2</sub> ratio are increased after semidepot HDM allergen extracts treatment; cT <sub>FR</sub> cells display stronger function to inhibit IgE production after AIT; the increase of cT <sub>FR</sub> cells and cT <sub>FR</sub> /cT <sub>FH2</sub> ratio correlate with clinical after 12 months of AIT | 35  |
|                   | 21 | cT <sub>FR</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup> PD-1 <sup>+</sup><br><br>cT <sub>FR</sub> : CD4 <sup>+</sup> CXCR5 <sup>+</sup> PD-1 <sup>+</sup> FoxP3 <sup>+</sup>                                                                                                                                                   | Frequencies of IL-4 <sup>+</sup> and IL-4 <sup>+</sup> IL-21 <sup>+</sup> T <sub>FH</sub> cells are reduced after <i>Lolium perenne</i> peptide immunotherapy; frequencies of cT <sub>FR</sub> cells are increased after AIT                                                                                                                                                 | 37  |

---

AD, atopic dermatitis; AIT, allergy immunotherapy; AR, allergic rhinitis; B<sub>REG</sub>, regulatory B cells; CCR, C-C chemokine receptor; CXCR, C-X-C chemokine receptor; cT<sub>FH</sub>, circulating follicular helper T; cT<sub>FR</sub>, circulating follicular regulatory T; Foxp3, forkhead box P3; HDM, house dust mite; ICOS, inducible T-cell costimulator; Ig, immunoglobulin; IL, interleukin; PD-1, programmed cell death protein 1; T<sub>FH</sub>, follicular helper T; T<sub>FR</sub>, follicular regulatory T.

This article is protected by copyright. All rights reserved



all\_14639\_f2.jpg