

REVIEW

T-cell subsets in allergy and tolerance induction

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Antigen-specific T lymphocytes are the central regulators of tolerance versus immune pathology against otherwise innocuous antigens and key targets of antigen-specific immune therapy. Recent advances in the understanding of T cells in tolerance and allergy resulted from improved technologies to directly characterize allergen-specific T cells by multiparameter flow cytometry or single-cell sequencing. This unravelled phenotypically and functionally distinct populations, such as Type 2a T helper cells (Th2a), follicular Th cells (Tfh), regulatory T cells (Treg), Type 1 regulatory T cells (Tr1), and follicular T regulatory cells. Here we will discuss the role of the different Th-cell subsets in the healthy state, during sensitization and development of allergy, and in tolerance induction by allergen immunotherapy (AIT). To date, the mechanisms of AIT as the only causal treatment of allergy are not completely understood. The analyses of allergen-specific T cells directly ex vivo during AIT support the concept of specific-Th2(a) cell deletion rather than an expansion of allergen-specific Tr1 or Treg cells as underlying mechanism.

Keywords: Allergy · Allergen immunotherapy · T cells · Th2a · Tfh13

Introduction

T lymphocytes play a crucial role in the maintenance of tolerance against innocuous antigens, and defective T-cell tolerance drives the development of allergy. Type I allergies are frequent chronic diseases characterized by an IgE-driven immune response towards environmental antigens and are manifesting in allergic rhinitis, allergic asthma, food allergy, and life-threatening anaphylaxis and can trigger atopic dermatitis. In this context, antigen-specific T cells mediate the key pathogenetic steps, that is the induction of allergen-specific IgE through cognate interaction with B lymphocytes, tissue infiltration, and damage [1, 2] but also tolerance induction [3], that is allergen immunotherapy (AIT). The key allergen-specific T-cell subpopulations include phenotypically and functionally diverse subsets, such as type 2a T helper cells (Th2a), follicular T helper cells (Tfh), regulatory T cells (Treg), type 1 regulatory T cells (Tr1) cells, and follicular T regulatory cells (Tfr). This review is focussed on the recent advances in the understanding of the allergen-specific T-cell biology during the onset of allergies and tolerance induction.

T cells in the maintenance of tolerance against allergens

In healthy individuals, different T-cell populations respond to allergens. The allergen-specific Th cells from healthy individuals are mostly in a naïve state and express IL-2 on allergen contact, and some cells with an antigen-experienced phenotype, able to produce cytokines, including IFN- γ and rarely IL-10, but not the Th2 cytokines IL-4 and IL-5 or IL-13 [4–6], were determined by short-term allergen restimulation (Fig. 1). Accordingly, mostly allergen-specific antibodies are absent; however, in some healthy individuals, isotype-switched antibodies of the IgG or IgA subclasses, but not IgE, are detectable in the sera or tears. In healthy individuals, the majority of T cells reacting against whole extracts from airborne allergens, such as plant pollen or house dust mite, are memory-type Treg expressing Foxp3, CD25^{high} and Helios [4] (Fig. 1). These findings support the hypothesis that Tregs effectively controls naïve T-cell activation and prevents T memory-cell differentiation. Genetic evidences further support that Foxp3⁺ Treg cells control Th2 cell differentiation. Loss of function mutations in the Foxp3 gene result in polyclonal Th2 polarization, hyper-IgE, and allergy in human (IPEX syndrome) and mice (scurfy mice) [7]. Treg responses as such seem not to

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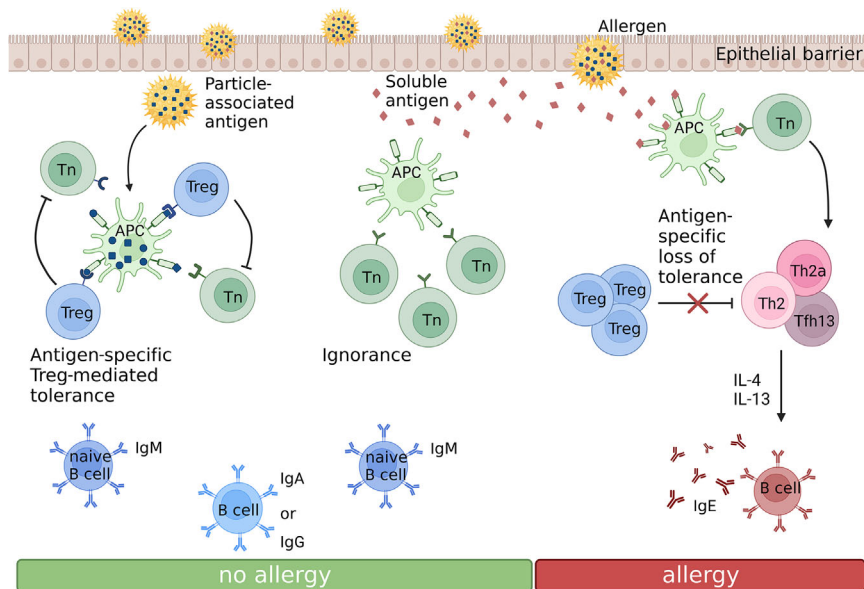


Figure 1. T cells in health and allergy. In the nonallergic state, the particulate fraction of allergens is presented by antigen-presenting cells (APC) to Tregs which are activated and efficiently suppress naive T cells (Tn) and consecutive T memory development. Soluble allergens are largely neglected, and no specific T memory response exists. Allergen-specific B-cell antibody responses are rare and of IgG or IgA isotype. In allergy, the allergen escaped the Treg control and sensitization results in allergen-specific Th2, Th2a, or Tfh13 cells, which secrete IL-4, IL-5, and IL-13. These cells instruct in B cells an IgE-isotype class switch and secretion of specific IgE.

be defective in allergy because the frequencies and phenotype of Tregs reactive to allergen extracts were not affected, as shown by short-term allergen restimulation analysis [4]. Foxp3 mutations also cause severe inflammatory autoimmunity, which is not occurring in atopic patients. Apparently, only a small number of allergens escape the Treg control followed by sensitization [7, 8]. As shown for birch pollen, after contact with aqueous solution, the particles dissociate into a soluble fraction containing the major allergenic proteins and a particle-attached protein fraction [4, 8]. In nonallergic individuals, soluble allergens appear to be ignored by the immune system, whereas particle-associated proteins appear to serve as important Treg targets (Fig. 1). Thus, the lack of antigen-specific Treg protection against soluble allergens may allow allergen-specific Th2 cell differentiation and allergy development [4] depending on environmental and genetic factors. To understand why this process is absent in healthy individuals is important and may be used to foster a nonallergic state, for example after tolerance induction.

Th2 cells and the onset of type I allergy

The presence of allergen-specific Th2 cells and the following specific IgE response is a hallmark of type I allergy [9]. Th2 cells are memory Th cells with stable phenotype over time and restimulations which express the transcription factor GATA3 as a lineage marker [9]. GATA3 is induced by IL-4, IL-13, and STAT5a-signalling [9, 10]. These cytokines are either secreted from Th2 cells or other cells such as innate lymphoid 2 cells [11]. Th2 cells secrete specific signature cytokines which include IL-4, IL-5, and IL-13 [9]. The functions of IL-4 and IL-13 in the onset of allergy are diverse and include the induction of IgE class switch in B cells and impairment of the epidermal barrier function [12]. IL-5 promotes eosinophilia as differentiation and survival factor and

chemotactic molecule. Recently, food allergen-specific Th2 cells were shown to expand Treg cells via IL-2 which suppresses Th2 cells in a negative feedback loop [13].

The sensitization and polarization of Th2 cells are complex and include antigen- and cytokine-dependent mechanisms, reviewed in Refs. [9, 14]. Initial reports showed that low antigen doses or long-term stimulated APCs with a reduced cellular fitness induce IL-4 and polarize Th2 cells, whereas high-antigen doses and vital APCs induce IFN- γ and Th1 cells [15, 16]. A weak antigen signal by allergens may result not only from a low amount, but also from a reduced epithelial barrier function or a modified antigen presentation in atopy as discussed recently [12, 17]. Additional to IL-4, cytokines from epithelial cells promote Th2 polarization such as IL-33 and thymic stromal lymphopoietin, either directly or indirectly through the induction of innate lymphoid 2 cells [9]. Of note, very strong antigen signals during helminth infection can also induce Th2 cells in an IL-4-independent manner, showing different mechanisms in type 2 reactions [14].

Th2 subsets in allergy

Recent data unravelled that Th2 cells are not a homogenous population but rather a functional group of distinct subpopulations. The distinct Th2-cell subpopulations discussed are summarized in Table 1.

Th2a cells

In 2017, Wambre et al. identified **Th2a** cells, which express a terminally differentiated CD4⁺ memory phenotype characterized by CD161+CD27-CrTh2+CD49d+ expression and appear to carry the Th2 memory cell function over a long time [18–20]. On

Table 1. Th cell subsets in allergy and tolerance induction.

T-cell subset	Marker	Function
Th2	CD3+CD4+Gata3+IL-4+	IL-4 secreting T cells, orchestrate type 2 immune answers
Th2a	CD4+CD161+CD27-CrTh2+CD49d+	Terminally differentiated T memory cells, secrete IL-4, IL-13, IL-9, and IL-5
Tfh13	CD4+Cxcr5+Bcl6+Gata3+Pd-1+IL-4+IL-13+	Tfh subset in allergic individuals, provide B cell help to generate high affinity IgE
Treg	CD4+FoxP3+CD25+CD127low	Suppressive T-cell subset
Tfr	CD4+Cxcr5+Pd-1+Bcl-6+Foxp3+	Suppressive counterpart of B-cell stimulating Tfh cells
Tr1	CD4+FoxP3-IL-10+TGF-β+	Suppressive T-cell population expressing IL-10

allergen stimulation, these Th2a cells secrete high amounts of IL-4, IL-13, IL-9, and IL-5 [18], which can be further increased by epithelium-derived IL-33 [21]. These cells were suggested to maintain the Th2 memory cell pool and affect tissues, but not IgE induction which depends on IL-4 and IL-13 from Tfh cells [22–25].

Tfh13 cells

Tfh13 were identified as a Tfh cell subset distinct from Th2 effector and Tfh2 cells by RNA single-cell sequencing of mouse CD4⁺ T cells and are characterized by the expression of CD4+Cxcr5+Bcl6+GATA3+Pd-1+IL-4+IL-13+ [24, 25]. In pre-clinical type 2 mouse models, Tfh13 cells were only found in allergen-sensitized mice and were required to generate high affinity IgE responses that can elicit anaphylaxis, whereas they were not found in mice without or low affinity IgE [25], supporting a distinct role of IL-13 in allergy and IgE regulation. They are discussed as Th2-cell derived [26]. Human Tfh13 cells are characterized by the same markers as mouse Tfh13 cells and single-cell RNAseq of allergen-specific Tfh13 cells confirmed the coexpression of IL-4, IL-5, and IL-13 [25, 27]. These cells circulate in the blood of allergic individuals [25, 27]. The signals required for the generation and survival of allergen-specific Th2a cells and Tfh13 cells are still unknown, but this knowledge is important to better understand type 2 immune responses and to enable better targeted treatment of allergy.

T follicular regulatory cells

A defect in Tregs in quantity or function was discussed as an underlying mechanism in allergy. As determined by single-cell flow cytometric analysis of viable allergen-specific Tregs, their number and function is comparable to healthy individuals [8, 28]. Thus, in allergy compared to the healthy state, not a functional Treg defect but rather the occurrence of additional Th2 effector cells is apparent [4, 29]. Of note, a novel regulatory T-cell subtype termed **Tfr cells** has been described as a suppressive counterpart of B-cell-stimulating Tfh cells recently [30]. These Tfr cells are characterized by the expression of CD4+Cxcr5+Pd-1+Bcl-

6+Foxp3+. Tfr cells differentiate from naïve Tregs in secondary lymphoid organs [31]. Patients with allergic rhinitisAR showed reduced frequencies of circulating Tfr cells compared to healthy individuals [30]. Tfr cells were proven the counterpart of Tfh13 cells as Tfr-deficient mice showed increased specific IgE and lung inflammation in a house dust mite-specific sensitization model [32]. However, the instructing signals and whether these can be targeted in a therapeutic manner to promote allergen-specific tolerance requires further research.

Tr1 T cells

Tr1 T cells are considered a suppressive CD4⁺ T-cell subpopulation that secretes IL-10 and TGF-β and lacks Foxp3 expression [33]. Allergen-specific T cells that express IL-10 after activation are described for food and aero allergens in allergic donors and healthy individuals [4, 29, 34, 35]. However, still no specific marker or lineage transcription factor exist to identify Tr1 cells with an epigenetically imprinted IL-10 gene [33], comparable with GATA3 in Th2 cells [9] or Foxp3 in Treg cells [7]. Mutation of the gene encoding the IL-10 or its receptor is not associated with allergy or hyper-IgE, other than Foxp3 deficiency, suggesting a minor function of Tr1 cells in the prevention of sensitization [36]. Moreover, IL-10 was excluded from the imprinted cytokine memory in human T cells, for example by promoter methylation and instable reexpression after restimulation [37]. Still, the validation that allergen-specific Tr1 cells exist facilitating repeated the reexpression of IL-10 and TGF-β on repeated stimulations, expansion and successful Tr1 cell therapy is pending. Thus, the data support the hypothesis that allergen-induced IL-10 expression in T cells is conditional on the stimulation. Thus, the existence of allergen-specific Tr1 cells and function in allergy remains to be established.

Induction of clinical tolerance

AIT is the only disease-modifying therapy of type I allergy with the aim to achieve long-term clinical remission [3]. It aims to turn the allergic state, with a low antigen threshold to elicit a clinical reaction, into a nonallergic state with increased allergen reaction

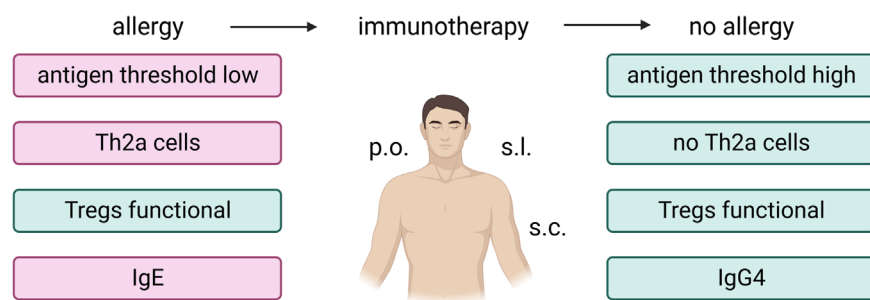


Figure 2. Tolerance induction by allergen immunotherapy. Before therapy, the allergic immune response is characterized by a low antigen dose to elicit symptoms, Th2a cell, and a humoral specific IgE response. Immunotherapy is administered commonly s.c., s.l., or p.o. After immunotherapy, the reaction antigen threshold is higher, not dominated by Th2a cells and humoral IgG4 induction. In both states, Tregs are functional.

threshold, and ideally to achieve a tolerant state without any clinical reactions (Fig. 2). Repeated high allergen doses over 3–5 years are required to achieve a long-lasting benefit of AIT [3]; however, a recurrence after 5–10 years is common. The only established AIT-response marker is the induction of antigen-specific serum IgG4, whereas specific IgE levels remain unchanged or decrease [38–42] (Fig. 2).

The data on T cells during AIT are heterogeneous, most likely due to a methodological heterogeneity, caused by different routes of application (mostly subcutaneous (s.c.), sublingual (s.l.), oral (p.o.), epicutaneous, intralymphatic) or the T-cell analysis technique (Fig. 2). It is not established whether the induction of clinical tolerance by AIT is mediated by T cells directly or via T-cell-mediated specific IgG4 induction in B cells. For example, the peptide immunotherapy with recombinant T-cell epitopes showed low efficacy against cat dander or house dust mite [43], but peptide immunotherapy using hydrolysed proteins against *Lolium perenne* induced clinical tolerance and specific IgG4 [44]. These data suggest that targeting T cells alone may not be sufficient to achieve clinical tolerance, which may be achieved by targeting T cells to promote specific IgG4 induction in B cells.

Beyond the different immunotherapy application routes and antigens, the detection of allergen-specific T cells is crucial and methodological differences are underlying causes of data heterogeneity. Three major technologies to directly detect specific T cells are currently used, that is activation-induced markers or antigen-reactive T-cell enrichment, MHC-tetramer staining, and single-cell sequencing [28]. The classical clonal *in-vitro* expansion method identifies specific T cells indirectly after allergen re-stimulation *ex vivo* and is discussed elsewhere [28]. Initially, it was assumed that Tregs are induced to promote IgG4 induction and AIT efficacy [45]. However, recent data obtained by antigen-reactive T-cell enrichment or tetramer methodology support the concept of a Th2(a)-cell depletion rather than Treg expansion during AIT [8, 29, 35]. The number of allergen-specific Th2(a) cells declines during AIT, which correlates with an increase in IgG4 levels [18, 20, 46, 47]. Also circulating Tfh cells were reduced by AIT while an increased number of Tfr cells were observed [44, 48, 49], accompanied by the induction of IgG4 [44]. This implies that Th2(a)-/Tfh-cell reduction rather than a Treg induction leads to IgG4 production. As Th2 cells are IL-4 inducible and a major target of AIT, the blockade of IL-4 signalling may improve the efficacy of AIT. Dupilumab blocks IL-4 and IL-13 signalling and its addition to a short-term AIT improved its tolerability, however, with little impact on the clinical symptoms

compared to AIT alone [50]. The new techniques for analysing the allergen-specific T cells during AIT *ex vivo* significantly impact the view on the mode of action during AIT: away from an induction of Tregs towards a reduction of TH2(a)/Tfh cells. Further research is warranted to identify how specific T cells interact with B cells during AIT to induce IgG4 in order to shorten the AIT treatment and increase the remission time.

In summary, the characterization of allergen-specific T cells using novel technologies has provided new insights into their function in health, allergy, and tolerance induction. Future research using these methods will reveal how these cells maintain survival and allergy, including recently discovered populations, such as Th2a, Tfh13, Tfr, and Treg cells. The profound knowledge of allergen-specific T-cell biology could enable curative treatment of otherwise chronic type I allergies.

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References

- 1 Roesner, L. M., Farag, A. K., Pospich, R., Traidl, S. and Werfel, T., T-cell receptor sequencing specifies psoriasis as a systemic and atopic dermatitis as a skin-focused, allergen-driven disease. *Allergy* 2022. 77: 2737–2747.
- 2 Faith, A., Singh, N., Farooque, S., Dimeloe, S., Richards, D. F., Lu, H., Roberts, D. et al., T cells producing the anti-inflammatory cytokine IL-10

- regulate allergen-specific Th2 responses in human airways. *Allergy* 2012. **67**: 1007–1013.
- 3 Durham, S. R. and Shamji, M. H., Allergen immunotherapy: past, present and future. *Nat. Rev. Immunol.* 2022. **23**: 317–328.
 - 4 Bacher, P., Heinrich, F., Stervbo, U., Nienen, M., Vahldieck, M., Iwert, C., Vogt, K. et al., Regulatory T cell specificity directs tolerance versus allergy against aeroantigens in humans. *Cell* 2016. **167**: 1067–1078.e1016.
 - 5 Weissler, K. A., Rasooly, M., DiMaggio, T., Bolan, H., Cantave, D., Martino, D., Neeland, M. R. et al., Identification and analysis of peanut-specific effector T and regulatory T cells in children allergic and tolerant to peanut. *J. Allergy Clin. Immunol.* 2018. **141**: 1699–1710.
 - 6 Seumois, G., Ramirez-Suastegui, C., Schmiedel, B. J., Liang, S., Peters, B., Sette, A. and Vijayanand, P., Single-cell transcriptomic analysis of allergen-specific T cells in allergy and asthma. *Sci. Immunol.* 2020. **5**: eaba6087.
 - 7 Rudensky, A. Y., Regulatory T cells and Foxp3. *Immunol. Rev.* 2011. **241**: 260–268.
 - 8 Bacher, P. and Scheffold, A., The effect of regulatory T cells on tolerance to airborne allergens and allergen immunotherapy. *J. Allergy Clin. Immunol.* 2018. **142**: 1697–1709.
 - 9 Nakayama, T., Hirahara, K., Onodera, A., Endo, Y., Hosokawa, H., Shinoda, K., Tumes, D. J. et al., Th2 cells in health and disease. *Annu. Rev. Immunol.* 2017. **35**: 53–84.
 - 10 Zhu, J., Yamane, H., Cote-Sierra, J., Guo, L. and Paul, W. E., GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. *Cell Res.* 2006. **16**: 3–10.
 - 11 Leyva-Castillo, J. M., Galand, C., Mashiko, S., Bissonnette, R., McGurk, A., Ziegler, S. F., Dong, C. et al., IL2 activation by keratinocyte-derived IL-25 drives IL-13 production at sites of allergic skin inflammation. *J. Allergy Clin. Immunol.* 2020. **145**: 1606–1614.e1604.
 - 12 Akdis, C. A., The epithelial barrier hypothesis proposes a comprehensive understanding of the origins of allergic and other chronic noncommunicable diseases. *J. Allergy Clin. Immunol.* 2022. **149**: 41–44.
 - 13 Lozano-Ojalvo, D., Tyler, S. R., Aranda, C. J., Wang, J., Sicherer, S., Sampson, H. A., Wood, R. A. et al., Allergen recognition by specific effector Th2 cells enables IL-2-dependent activation of regulatory T-cell responses in humans. *Allergy* 2022. **78**: 697–713.
 - 14 Paul, W. E., What determines Th2 differentiation, in vitro and in vivo? *Immunol. Cell Biol.* 2010. **88**: 236–239.
 - 15 Constant, S., Pfeiffer, C., Woodard, A., Pasqualini, T. and Bottomly, K., Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. *J. Exp. Med.* 1995. **182**: 1591–1596.
 - 16 Langenkamp, A., Messi, M., Lanzavecchia, A. and Sallusto, F., Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat. Immunol.* 2000. **1**: 311–316.
 - 17 Gheerbrant, H., Guillien, A., Vernet, R., Lupinek, C., Pison, C., Pin, I., Demenais, F. et al., Associations between specific IgE sensitization to 26 respiratory allergen molecules and HLA class II alleles in the EGEA cohort. *Allergy* 2021. **76**: 2575–2586.
 - 18 Wambre, E., Bajzik, V., DeLong, J. H., O'Brien, K., Nguyen, Q. A., Speake, C., Gersuk, V. H. et al., A phenotypically and functionally distinct human TH2 cell subpopulation is associated with allergic disorders. *Sci. Transl. Med.* 2017. **9**: eaam9171.
 - 19 Vandamme, C., Rytönen-Nissinen, M., Lönnberg, T., Randell, J., Harvima, R. J., Kinnunen, T. and Virtanen, T., Single-cell characterization of dog allergen-specific T cells reveals T(H)2 heterogeneity in allergic individuals. *J. Allergy Clin. Immunol.* 2022. **149**: 1732–1743.
 - 20 Monian, B., Tu, A. A., Ruiter, B., Morgan, D. M., Petrossian, P. M., Smith, N. P., Gierahn, T. M. et al., Peanut oral immunotherapy differentially suppresses clonally distinct subsets of T helper cells. *J. Clin. Invest.* 2022. **132**: e150634.
 - 21 Calise, J., Garabatos, N., Bajzik, V., Farrington, M., Robinson, D., Jeong, D., Londei, M. et al., Optimal human pathogenic T(H)2 cell effector function requires local epithelial cytokine signaling. *J. Allergy Clin. Immunol.* 2021. **148**: 867–875.
 - 22 Vijayanand, P., Seumois, G., Simpson, L. J., Abdul-Wajid, S., Baumjohann, D., Panduro, M., Huang, X. et al., Interleukin-4 production by follicular helper T cells requires the conserved IL4 enhancer hypersensitivity site V. *Immunity* 2012. **36**: 175–187.
 - 23 Harada, Y., Tanaka, S., Motomura, Y., Ohno, S., Yanagi, Y., Inoue, H. and Kubo, M., The 3' enhancer CNS2 is a critical regulator of interleukin-4-mediated humoral immunity in follicular helper T cells. *Immunity* 2012. **36**: 188–200.
 - 24 Kobayashi, T., Iijima, K., Dent, A. L. and Kita, H., Follicular helper T cells mediate IgE antibody response to airborne allergens. *J. Allergy Clin. Immunol.* 2017. **139**: 300–313.e307.
 - 25 Gowthaman, U., Chen, J. S., Zhang, B., Flynn, W. F., Lu, Y., Song, W., Joseph, J. et al., Identification of a T follicular helper cell subset that drives anaphylactic IgE. *Science* 2019. **365**: eaaw6433.
 - 26 Harada, Y., Sasaki, T., Wibisana, J. N., Okada-Hatakeyama, M., Liu, C., Ueno, H., Burrows, P. D. et al., Type 2 helper T cells convert into interleukin-13-expressing follicular helper T cells after antigen repriming. *Transl. Regul. Sci.* 2023. **5**: 1–12. In press.
 - 27 Wang, W., Lyu, S.-C., Ji, X., Gupta, S., Manohar, M., Dhondalay, G. K. R., Chinthrajah, S. et al., Transcriptional changes in peanut-specific CD4+ T cells over the course of oral immunotherapy. *Clin. Immunol.* 2020. **219**: 108568–108568.
 - 28 Bacher, P. and Scheffold, A., Antigen-specific regulatory T-cell responses against aeroantigens and their role in allergy. *Mucosal. Immunol.* 2018. **11**: 1537–1550.
 - 29 Berin, M. C., Agashe, C., Burks, A. W., Chiang, D., Davidson, W. F., Dawson, P., Grishin, A. et al., Allergen-specific T cells and clinical features of food allergy: lessons from CoFAR immunotherapy cohorts. *J. Allergy Clin. Immunol.* 2022. **149**: 1373–1382.
 - 30 Yao, Y., Wang, Z. C., Wang, N., Zhou, P. C., Chen, C. L., Song, J., Pan, L. et al., Allergen immunotherapy improves defective follicular regulatory T cells in patients with allergic rhinitis. *J. Allergy Clin. Immunol.* 2019. **144**: 118–128.
 - 31 Hao, H., Nakayamada, S. and Tanaka, Y., Differentiation, functions, and roles of T follicular regulatory cells in autoimmune diseases. *Inflamm. Regen.* 2021. **41**: 14.
 - 32 Clement, R. L., Daccache, J., Mohammed, M. T., Diallo, A., Blazar, B. R., Kuchroo, V. K., Lovitch, S. B. et al., Follicular regulatory T cells control humoral and allergic immunity by restraining early B cell responses. *Nat. Immunol.* 2019. **20**: 1360–1371.
 - 33 Gregori, S., Goudy, K. S. and Roncarolo, M. G., The cellular and molecular mechanisms of immuno-suppression by human type 1 regulatory T cells. *Front. Immunol.* 2012. **3**: 30.
 - 34 Akdis, M. B., Verhagen, J., Taylor, A., Karamloo, F., Karagiannidis, C., Cramer, R., Thunberg, S. et al., Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J. Exp. Med.* 2004. **199**: 1567–1575.
 - 35 Wambre, E., DeLong, J. H., James, E. A., Torres-Chinn, N., Pfutzner, W., Mobs, C., Durham, S. R. et al., Specific immunotherapy modifies allergen-specific CD4(+) T-cell responses in an epitope-dependent manner. *J. Allergy Clin. Immunol.* 2014. **133**: 872–879.e877.

- 36 Neumann, C., Scheffold, A. and Rutz, S., Functions and regulation of T cell-derived interleukin-10. *Semin. Immunol.* 2019. **44**: 101344.
- 37 Dong, J., Ivascu, C., Chang, H. D., Wu, P., Angeli, R., Maggi, L., Eckhardt, F. et al., IL-10 is excluded from the functional cytokine memory of human CD4⁺ memory T lymphocytes. *J. Immunol.* 2007. **179**: 2389–2396.
- 38 Heine, G., Francuzik, W., Doelle-Bierke, S., Drozdenko, G., Frischbutter, S., Schumacher, N., Radbruch, A. et al., Immunomodulation of high-dose vitamin D supplementation during allergen-specific immunotherapy. *Allergy* 2021. **76**: 930–933.
- 39 Gleich, G. J., Zimmermann, E. M., Henderson, L. L. and Yunginger, J. W., Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. *J. Allergy Clin. Immunol.* 1982. **70**: 261–271.
- 40 Scadding, G. W., Calderon, M. A., Shamji, M. H., Eifan, A. O., Penagos, M., Dumitru, F., Sever, M. L. et al., Effect of 2 years of treatment with sublingual grass pollen immunotherapy on nasal response to allergen challenge at 3 years among patients with moderate to severe seasonal allergic rhinitis: the GRASS randomized clinical trial. *JAMA* 2017. **317**: 615–625.
- 41 James, L. K., Shamji, M. H., Walker, S. M., Wilson, D. R., Wachholz, P. A., Francis, J. N., Jacobson, M. R. et al., Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J. Allergy Clin. Immunol.* 2011. **127**: 509–516.e505.
- 42 Renand, A., Shamji, M. H., Harris, K. M., Qin, T., Wambre, E., Scadding, G. W., Wurtzen, P. A. et al., Synchronous immune alterations mirror clinical response during allergen immunotherapy. *J. Allergy Clin. Immunol.* 2018. **141**: 1750–1760.e1751.
- 43 Ramchandani, R., Hossenbaccus, L. and Ellis, A. K., Immunoregulatory T cell epitope peptides for the treatment of allergic disease. *Immunotherapy* 2021. **13**: 1283–1291.
- 44 Sharif, H., Singh, I., Kouser, L., Mösges, R., Bonny, M. A., Karamani, A., Parkin, R. V. 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**: 738–749.
- 45 Akdis, M., Blaser, K. and Akdis, C. A., T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. *J. Allergy Clin. Immunol.* 2005. **116**: 961–968. quiz 969.
- 46 Luce, S., Batard, T., Bordas-Le Floch, V., Le Gall, M. and Mascarell, L., Decrease in CD38⁺ TH2A cell frequencies following immunotherapy with house dust mite tablet correlates with humoral responses. *Clin. Exp. Allergy* 2021. **51**: 1057–1068.
- 47 Ihara, F., Sakurai, D., Yonekura, S., Iinuma, T., Yagi, R., Sakurai, T., Ito, T. et al., Identification of specifically reduced Th2 cell subsets in allergic rhinitis patients after sublingual immunotherapy. *Allergy* 2018. **73**: 1823–1832.
- 48 Yao, Y., Chen, C. L., Wang, N., Wang, Z. C., Ma, J., Zhu, R. F., Xu, X. Y. 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**: 321–324.e310.
- 49 Sharif, H., Acharya, S., Dhondalay, G. K. R., Varricchi, G., Krasner-Macleod, S., Laisuan, W., Switzer, A. et al., Altered chromatin landscape in circulating T follicular helper and regulatory cells following grass pollen subcutaneous and sublingual immunotherapy. *J. Allergy Clin. Immunol.* 2021. **147**: 663–676.
- 50 Corren, J., Saini, S. S., Gagnon, R., Moss, M. H., Sussman, G., Jacobs, J., Laws, E. et al., Short-term subcutaneous allergy immunotherapy and dupilumab are well tolerated in allergic rhinitis: a randomized trial. *J. Asthma. Allergy* 2021. **14**: 1045–1063.

Abbreviations: AIT: allergen immunotherapy

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