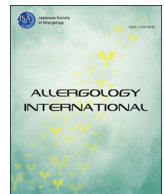




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Invited review article

T follicular helper and T_H2 cells in allergic responsesMasato Kubo ^{a, b, *}^a Division of Molecular Pathology, Research Institute for Biomedical Science, Tokyo University of Science, Noda, Japan^b Laboratory for Cytokine Regulation, Research Center for Integrative Medical Science (IMS), RIKEN Yokohama Institute, Yokohama, Japan

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Abbreviations:

AD, Atopic dermatitis; ASCL2, Achaete-scute homologue 2; BCL-6, B-cell lymphoma 6; BLIMP1, B lymphocyte-induced maturation protein 1; CCR6, CC chemokine receptor type 6; cDCs, Conventional dendritic cells; CNS, Conserved non-coding sequences; CXCR5, C-X-C chemokine receptor type 5; DAMP, Damage-associated molecular pattern; EBV2, EBV-induced G-protein coupled receptor 2; T_{FH}, Follicular helper T; GC, Germinal center; Cγ1, Germline the constant region gene for IgG1; HDM, House dust mite; IgE, Immunoglobulin E; ICOS, Inducible T cell co-stimulator; IRF4, Interferon regulatory factor 4; KLF4, Kruppel-like factor 4; LCR, Locus control region; LN, Lymph node; OVA, Ovalbumin; PCs, Plasma cells; PSGL1, P-selectin glycoprotein ligand-1; PD-1, Programmed cell death-1; RBP-J, Recombining binding protein suppressor of hairless; S1PR1, Sphingosine-1-phosphate receptor 1; TSLP, Thymic stromal lymphopoietin

ABSTRACT

IL-4 is a cytokine commonly secreted by T_H2 and follicular helper T (T_{FH}) cells after antigenic sensitization. T_H2 cells have been thought to be the major contributor of B cell help as a source of IL-4 responsible for class switch recombination to Immunoglobulin G1 (IgG1) and Immunoglobulin E (IgE). Importantly, there are some differences in transcriptional regulation between these two T cell subsets. The IL-4 production by T_H2 and T_{FH} cells is distinctively regulated by two pathways, GATA-3-mediated *IL4*-HS2 enhancer and Notch mediated *IL4*-CNS-2 enhancer. IgE and IgG1 antibody responses are mainly controlled by IL-4-secreting T_{FH} cells, but not by T_H2 cells. In this review, we discuss the role of T_H2 and T_{FH} cells in IgE production and allergic responses.

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Role of T_H2 cells in allergy

T_H2 cells regulate type 2 responses through the secretion of various type 2 cytokines, including IL-4, IL-5, IL-10, and IL-13. T_H2 cells mediate protection against helminthic infection but can cause several allergic diseases, including allergic rhinitis, anaphylaxis,

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atopic dermatitis (AD), and asthma. Classically, T_H2 cells have been thought to be the major contributor of B cell help as a source of IL-4, a cytokine that was originally identified as B cell stimulating factor^{1,2} responsible for class switch recombination to IgG1 and IgE through the induction of germline the constant region gene for IgG1 (C γ 1) and C ϵ transcripts.^{3–5} This conclusion was based on the results of *in vitro* experiments done in the late 1980's. The results from *in vitro* skewing culture conditions indicated that IL-4 promotes T_H2 differentiation from naive CD4 T cells.⁶ This depends on activation of STAT6 signaling through the IL-4 receptor, which induces expression of Gata3. STAT6 signaling also induces expression of the c-Maf transcription factor, which leads to high-level IL-4 expression. Together, these key transcription factors promote T_H2 cell differentiation and cytokine production. This network is known to be sensitive to the changing cytokine environment, the TCR signal strength, and the involvement of co-stimulation signals. The *Gata3* gene is controlled by distal and proximal promoters. During the differentiation process, the transcriptional autoactivation of *Gata3* provides a self-reinforcing feedback circuit.⁷ GATA-3 also antagonizes T_H1 differentiation by inhibiting expression of the IL-12 receptor β chain and of STAT4.

However, the initial source of IL-4 responsible for the differentiation of naive CD4⁺ T cells into T_H2 cells has been an undefined and long-standing question. Accumulating recent evidence has suggested that IL-4 does not have to be the trigger of T_H2 differentiation. Instead, an inhalation protocol using protease allergens such as house dust mite (HDM), indicated involvement of CD11b⁺ conventional dendritic cells (cDCs) in the regulation of T_H2 differentiation. Depletion of CD11c⁺ cells in Itgax-DTR mice significantly impairs T_H2 responses,⁸ and CD11b⁺ cDCs were rapidly recruited to lung airways and migrated to the draining lymph node (LN) to trigger T_H2 responses.⁹ In the initial response of T_H2 cell-mediated lung inflammation with protease allergens, group 2 innate lymphoid cells (ILC2)-derived IL-13 is critical to promote this migration of the CD11b⁺ cDCs into the draining LN, where they prime naive T cells to differentiate into T_H2 cells.¹⁰ Interferon regulatory factor 4 (IRF4) and Kruppel-like factor 4 (KLF4) transcription factors are also necessary for development of CD11b⁺ cDCs, because the IRF4⁺ cDC controls HDM-induced T_H2 responses,¹¹ and conditional deficiency of *Klf4* using CD11c-Cre reduced HDM-induced T_H2 responses.¹²

Development of follicular helper T (T_{FH}) cells

T_{FH} , a subset of CD4⁺ helper T cells, are involved in providing critical help for antibody maturation and germinal center (GC) formation. Since the presence of this unique CD4⁺ T cell subset migrating into B cell follicles and the GC after antigen priming was first described, T_{FH} cells have been defined as a specialized T cell subset that plays a crucial role in B cell responses by producing IL-4 and IL-21. T_{FH} cells are characterized by expression of B-cell lymphoma 6 (BCL-6), a zinc finger transcriptional repressor, and C-X-C chemokine receptor type 5 (CXCR5), which attracts the T_{FH} cells toward CXCL-13 present in the B cell follicle. Antigen presentation by DCs is necessary to induce BCL-6 and CXCR5 expression in the initial activation phase (Fig. 1). Inducible T cell co-stimulator (ICOS) signaling potentiates IL-21 production and antagonizes an inhibitory pathway, the KLF2-FOXO1 axis.^{13,14} IL-2- and IL-7-mediated STAT5 activation potently induce expression of B lymphocyte-induced maturation protein 1 (BLIMP1), which antagonizes BCL-6 and T_{FH} cell differentiation.¹⁵ STAT3 activation can potentially augment T_{FH} development through DC-derived IL-6 early in the T_{FH} developmental process.^{16,17} High mobility group box transcription factors, LEF-1 and TCF-1, contribute to T_{FH} differentiation through controlling the expression of the IL-6 receptor.¹⁸ Additional transcriptional factors, such as achaete-scute homologue 2 (ASCL2),

directly regulate the localization properties of T_{FH} cells. Interestingly, ASCL2 and KLF2 exclusively control the follicular localization of T cells. ASCL2 positively controls CXCR5 expression and targets expression of the IL-2 receptor. KLF2 positively controls the expression of CC chemokine receptor type 6 (CCR6), P-selectin glycoprotein ligand-1 (PSGL1), CCR7, and sphingosine-1-phosphate receptor 1 (S1PR1), and targets CXCR5 expression. In the initial phase of the T_{FH} program, the expression of BCL-6 and BLIMP-1 are mutually exclusive, because overexpression of either one exclusively drives the differentiation of T_{FH} or T_H1 cells.¹⁹ BCL-6 directly represses a gene set related to guidance and adhesion receptors that promote non-follicular positioning of T cells, such as CCR6, EBV-induced G-protein coupled receptor 2 (EBI2), PSGL1, CCR7, and S1PR1.²⁰ A recent broad-range transcriptional target study did not provide a single unique target of BCL-6 to explain the T_{FH} program, and the analysis suggested that BCL-6 might have a wide range, targeting the differentiation programs controlling T_H1 , T_H17 , T_H2 , and Treg cells, including T cell migration pathways.

Distinct regulation of IL-4 production in T_H2 and T_{FH} cells

IL-4 is a cytokine commonly secreted by T_H2 and T_{FH} cells after antigenic sensitization. Importantly, there are some differences in *Il4* gene transcriptional regulation between these two subsets. In the early 2000's, the elements controlling *Il4* gene transcription were extensively characterized using a combination of bioinformatics, biochemistry and reverse genetics. Conserved non-coding sequences (CNS) were identified as functionally critical regulatory elements, and classical hypersensitivity to digestion by DNase I and chromatin immune precipitation assays revealed the binding of protein factors typically found at regulatory elements. During the differentiation process, many epigenetic modifications are made to these regulatory regions. The mouse *Il4* locus is composed of multiple regulatory enhancer elements that map to conserved sequence 1 (HSS1 and HSS2), located between the *Il13* and *Il4* genes, HS0, located in the 5' region of *Il4*, HS1, located in the promoter region, HS2 and HS3 in the second exon, and HS5a and HS5, which is also called CNS2, located 3' of *Il4*.^{21–23} A T_H2 cytokine locus control region (LCR) has been characterized at several HS sites, Rad50 HS4 (RHS4), RHS5, RHS6, and RHS7, which are located between exon 22 and 25 of the *Rad50* gene (Fig. 2).^{24–27} HS2 is a critical GATA-3 binding site, and deletion of HS2 impaired IL-4 production in T_{FH} and T_H2 cells with loss of H3 acetylation and H3K4 methylation on the *Il4* locus.²⁸ Analysis of mice with an Ox40-Cre conditional *Gata3* knockout has demonstrated that GATA-3 is required for the initiation of *Il4* transcription during T_H2 differentiation.²⁹ The data from a tamoxifen inducible deletion system using *Gata3*^{flf} *CreERT2* mice demonstrate that *Gata3* deficiency greatly impaired IL-4 production but had less effect on T_{FH} development. Interestingly, *Gata3* rather increased IgG2 antibody responses, even though IL-4-dependent antibody responses, IgG1 and IgE, were greatly impaired.

In contrast, deletion of CNS2 impaired IL-4 production by T_{FH} , but not by T_H2 cells, indicating that CNS2 is a T_{FH} -specific enhancer (Fig. 2).^{30,31} Interestingly, Notch signaling directly controls CNS2 enhancer activity via specific binding of the transcription factor recombining binding protein suppressor of hairless (RBP-J), which is a transcriptional partner of the Notch intracellular domain (ICD), the cleaved active form of the receptor.^{32,33} T cell-specific conditional deficiency of *Rbpj* resulted in impaired IL-4 production by CD4⁺ T cells and decreased IL-4-dependent IgG1 and IgE antibody responses, but showed no or only a subtle effect on T_{FH} development.³⁰ In infection models with *Leishmania mexicana* or *Schistosoma mansoni* eggs, T cell-specific conditional deficiency of *Notch1/2* (N1N2lox/lox CD4-Cre mice) impaired IgG1 and IgE production

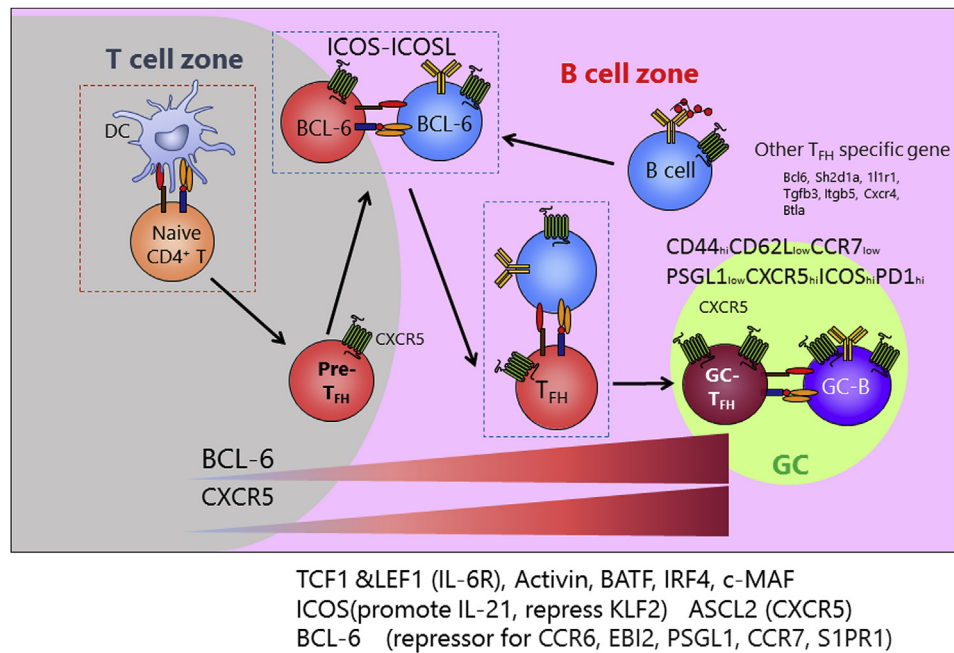


Fig. 1. Differentiation program of T_H cell. CD4 T cells differentiate into T_H cells, and antigen presentation by DCs induce BCL-6 and CXCR5 expression in the initial activation phase. ICOS signaling promotes IL-21 production and antagonizes an inhibitory pathway, the KLF2-FOXO1 axis. IL-2- and IL-7-mediated STAT5 activation potently induce expression of BLIMP1. LEF-1 and TCF-1 control the expression of the IL-6 receptor. ASCL2 positively controls CXCR5 expression and targets expression of the IL-2 receptor. In contrast, KLF2 positively controls the expression of CCR6, PSGL1, CCR7, and S1PR1, and targets CXCR5 expression. BCL-6 represses CCR6, EBI2, PSGL1, CCR7, and S1PR1.

along with reduction of T_H development.³⁴ Therefore, IL-4 production by T_H2 and T_H cells is distinctively regulated by two pathways, GATA-3-mediated HS2 enhancer and Notch-mediated CNS-2 enhancer. The GATA-3-mediated HS2 enhancer activation

is typically responsible for IL-4 expression in T_H and T_H2 cells, while Notch-mediated CNS-2 enhancer activation independently controls IL-4 expression in the T_H program.

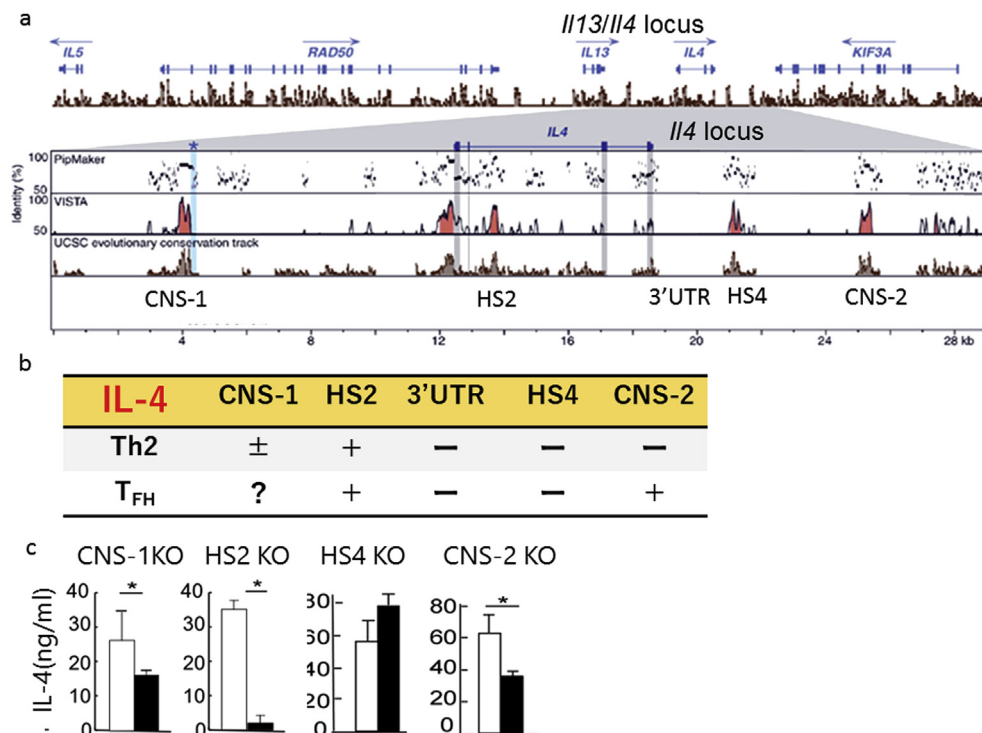


Fig. 2. Overview of cis-regulatory elements in the IL13/IL4 locus. (a) The IL4 locus contains several high homology regions. The middle rows in this figure display homology between human and mouse. These high homology regions well overlap with conserved non-coding sequences. The IL4 locus contains CNS1, HS2, 3' untranslated region (3' UTR), HS4, and CNS-2. (b), (c): CNS-2 enhancer has different activity in T_H2 and T_H. (b) provides a summary of each IL4 enhancer based on the results from deficient (Knock-out; KO) mice. (c) shows IL-4 expression in T_H cells from deletion mice of CNS-1, HS2, HS4, or CNS-2 enhancer.

The lineage relationship of T_{FH} and T_{H2} cells in allergic responses

T_{FH} cells comprise a major component of T_{H1}, T_{H2} or T_{H17} responses because of their potential to secrete IFN- γ , IL-4, and IL-21. Therefore, it is unclear whether T_{FH} cells are actually a distinct subset or rather the end result of the plasticity of other subsets that are imprinted by their follicular localization. The lineage relationship of T_{FH} cells to the other helper T cell subsets still remains unclear. A number of studies have identified T_{FH} responses under various T_{H2} inducing conditions and, in the context of T_{H2} immunity, T_{FH} cells might be the dominant source of IL-4 *in vivo*.^{30,31,35,36} During infection with parasitic helminths, IL-4 and IL-13 are crucial cytokines for promoting host survival, and helminths are potent inducers of the T_{H2} cytokines. IL-13 and the master transcription factor for T_{H2}, GATA-3, are present in large amounts in T_{H2} cells, in contrast to their small amounts in T_{FH} cells.³⁷ Thus, IL-13 and GATA-3 might be the segregation markers. In earlier work, T_{H2} cells producing both IL-4 and IL-13 were found within B cell follicles and B cell-deficient μ MT mice had reduced numbers of the double producing T cells. T_{FH} cells were found to develop from antigen-primed T_{H2} cells after antigen challenge *in vivo*, because the transferred CXCR5⁺ programmed cell death-1 (PD-1)⁺ T_{H2} cells became CXCR5⁺ T_{FH} cells, which also express IL-13 and GATA-3.³⁸ In contrast, T_{H2} cells can independently be the effector cells at the T-B cell border area of the priming LN, and this process might depend on CXCR5 expression on DCs, at least in the case of mice infected with the intestinal nematode *Heligmosomoides polygyrus*.³⁹

There are also other studies implying that T_{H2} and T_{FH} cells closely cooperate in T_{H2} immunity. Intranasal inhalation with HDM-induced T_{H2}-mediated allergic immunity, including mucus formation, eosinophilia, airway hyperresponsiveness, and IgE production. In the inhaled HDM model, *IL21R*^{-/-} mice developed less T_{H2} immunity and the IL-21 receptor (IL-21R) was needed to enhance T_{H2} cell function. Thus, IL-21 secreted by T_{FH} cells is needed for the acquisition of T_{H2} cell effector functions.⁴⁰ In contrast, T_{FH} cells have been reported to be a precursor of T_{H2} cells in the inhaled HDM mouse model. T_{H2} cell-mediated immunity after HDM re-challenge was impaired in B cell-deficient μ MT mice, because IL-4-producing and IL-13-producing CD4⁺ T cells were reduced in these mice. The accumulation of eosinophils in the lungs and bronchoalveolar lavage and the airway response in the sensitized and challenged mice indicated that T_{FH} cells are required for development of T_{H2} cells.⁴¹ There is some evidence implying that T_{FH} cells can maintain the capacity for developmental plasticity.⁴² In this context, re-sensitized IL-21⁺T_{FH} cells were observed to differentiate into other effector T cells and migrate into non-lymphoid tissues.⁴³

T_{H2} responses to airborne antigens, like HDM, uniquely require an initial phase of antigen sensitization that is not sufficient for T_{H2}-mediated airway inflammation, but is required for the development of T_{FH} cells and memory T_{H2} cells. In fact, a low dose of HDM administered by the skin or intranasal routes was sufficient for the induction of T_{FH} cells but not T_{H2} cells. Similarly, systemic immunization with ovalbumin (OVA) plus alum adjuvant preferentially induced IgE and IgG1 antibody responses, which are mainly controlled by IL-4-secreting T_{FH} cells, but not an IgG2 response.^{30,31} Protease allergens, including HDM, are known to cause barrier disruption in epithelial cells, leading to T_{H2} immunity through the elevation of multiple innate cytokines including IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) due to the stress of tissue injury. IL-33 is considered to function as an alarmin or a damage-associated molecular pattern (DAMP) molecule and is known to associate with T_{H2} immunity in allergic responses.^{44,45} A recent report demonstrated that IL-33 promoted IL-4 producing T_{FH} cells

and T_{H2} cells in LN when soluble antigen was exposed through the airway in conjunction with IL-33. Interestingly, the IL-33-induced T_{H2} and T_{FH} cells independently controlled eosinophilic airway inflammation and IgE antibody production.⁴⁶

In allergic human patients, T_{H2} immunity is often chronic. Studies in mice showed that re-sensitization caused a robust stimulation of memory T_{H2} cells in the draining LN. The memory T_{H2} cells acquired high level expression of T1/ST-2 and became a critical target of IL-33 in the pathogenesis of allergic airway inflammation. The activated memory OT-II T_{H2} cells secreted robust amounts of T_{H2} cytokines in response to OVA, and IL-5 production only required IL-33.⁴⁷ Allergen-nonspecific T_{H2} cells also make critical contributions to cysteine protease-induced eosinophilic inflammation as a result of relocating from the draining LN to the inflammatory site. These T_{H2} cells can produce high levels of IL-5 and IL-13 in response to IL-33, independent of antigen recognition, which is usually required for the initial T cell activation.¹

Role of T_{FH} cells in IgE production

IgE is the isotype of antibody tightly associated with type I allergic disease, including rhinitis, food allergy and anaphylaxis. IgE binds to the high-affinity Fc ϵ receptor on mast cells, which become activated after allergen crosslinking and release a variety of inflammatory chemical mediators, cytokines and chemokines though degranulation, leading to allergic inflammation. Germline transcription of C ϵ is a prerequisite to CSR to C ϵ , and the most important positive regulator of this process is the cytokine IL-4, primarily derived from CD4 T cells. Interestingly, although IgE transcripts could be detected in GC B cells, IgE-expressing B cells were barely detectable in the GC.⁴⁸ Using a tracing system using fluorescent IgE reporter mice (Verigem reporter mice) it was discovered that IgE-expressing B cells generated during primary antigen sensitization rapidly differentiated into both GC B cells and plasma cells (PCs). However, the IgE-expressing GC-B cells rapidly disappeared and other B cells become short-lived PC with less affinity maturation.⁴⁹ In this context, the major IL-4 source is T_{FH} cells^{30,31} which also secrete IL-21. IL-21 is a member of the common γ chain family of cytokines, which activate STAT3 and STAT5 signaling pathways,⁵⁰ and is known to be negative regulator of IgE production *in vivo* and *in vitro*. Mechanistically, IL-21 inhibits IL-4-induced germ line C ϵ transcription in B cells,⁵¹ and it has been shown that mice lacking IL-21R have higher production of IgE, but lower IgG1.⁵² IL-21 is reported to selectively kill the IgE-expressing B cells.⁵³ Recently, IL-21-expressing T_{FH} cells were identified in the GC dark zone, the site of hypermutation of GC B cells,⁵⁴ therefore, the rapid reduction of IgE-expressing GC B cells and decreased IgE affinity maturation might be due to the T_{FH}-derived IL-21 in the GC. In contrast, IL-21 is necessary for the generation of IgG1-expressing GC B cells. This differential effect of IL-21 on IgG1 and IgE would be a beneficial mechanism for CSR to IgG1 by excluding expansion of IgE antibody-producing cells.

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Conflict of interest

The authors have no conflict of interest to declare.

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