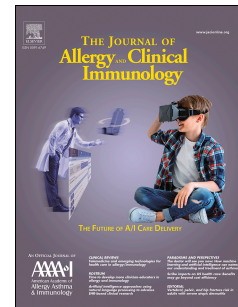


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Kiyoshi Hirahara, MD. PhD., Ami Aoki, MD. PhD., Masahiro Kiuchi, DVM. PhD.,
Toshinori Nakayama, MD. PhD.



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Memory-type pathogenic Th2 cells and ILC2s in type 2 allergic inflammation

Kiyoshi Hirahara MD. PhD.^{1, 2*}, Ami Aoki MD. PhD.¹, Masahiro Kiuchi DVM. PhD.¹, Toshinori Nakayama MD. PhD.^{1, 3*}

¹Department of Immunology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba, 260-8670 Japan.

²AMED-PRIME, AMED, 1-8-1 Inohana Chuo-ku, Chiba 260-8670, Japan.

³AMED-CREST, AMED, 1-8-1 Inohana Chuo-ku, Chiba 260-8670, Japan.

*Correspondence to: Drs. Kiyoshi Hirahara and Toshinori Nakayama

Department of Immunology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba, 260-8670 Japan.

Phone number: +81-43-226-2200, FAX number: +81-43-227-1498

E-mail: hiraharak@chiba-u.jp, tnakayama@faculty.chiba-u.jp

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20 **Key words**

21 Memory-type pathogenic Th2 cells, Tissue-resident memory T cells, ILC2s, epithelial
22 cytokines, IL-33

23

24 **Abbreviations**

25 Th2: T helper type 2

26 T_{path2} cells: pathogenic Th2 cells

27 ILC: innate lymphoid cells

28 GWAS: genome-wide association study

29 T_{RM} cells: tissue-resident memory T cells

30

Main manuscript

Type 2 immunity is characterized by the production of signature cytokines, such as IL-4, IL-5, and IL-13, which regulate a variety of biological responses¹. Type 2 immunity is important for the host defense response against parasitic infection¹. Type 2 immunity is also involved in shaping the pathogenesis of allergic diseases, such as bronchial asthma and atopic dermatitis¹. In bronchial asthma, type 2 immunity is involved in not only IL-4-induced IgE class switching, IL-5-induced induction and activation of eosinophils, and IL-13-induced eosinophilic airway inflammation but also amphiregulin-induced peri-airway fibrosis and aseptic inflammation induced by Charcot-Leyden crystals^{2,3}. Furthermore, the pathogenic interaction between the nervous system and immune cells in type 2 inflammation causes various allergic diseases, such as bronchial asthma and atopic dermatitis⁴.

Besides the induction of allergic inflammation, type 2 immunity regulates tissue homeostasis and tissue repair responses³. It is induced by both the acquired and innate immune systems. In the acquired immune system, T helper type 2 (Th2) and B cells are major cell populations that produce type 2 cytokines, whereas group 2 innate lymphoid cells (ILC2s) are the main source of type 2 cytokines in the innate immune system.

The organ-specific environment affects the function of both memory-type Th2 cells and ILC2s because subpopulations of memory-type Th2 cells and ILC2s are known to reside in specific mucosal organs, such as the lung and skin³. For example, the respiratory tract is covered by the tracheal mucosa formed from goblet cells, clara cells, ciliated cells, and tuft cells to prevent the invasion of various foreign antigens. On exposure to various stimuli, the

airway epithelial cells release epithelial cytokines, such as IL-25, IL-33, and TSLP, which activate tissue-resident immune cells in the airway mucosa and elicit a type 2 immune response in the local inflammatory sites³. Epithelial cells and chemosensory epithelial cells called tuft cells produce IL-25 and TSLP³. When the mucosal barrier is broken down and the epithelial cells that form the mucosa are destroyed, IL-33 is released from the cell and transmits signals to other neighboring cells. Indeed, multiple genome-wide association studies (GWAS) have shown that *IL33* and *IL1RL1* are genes associated with the pathogenesis of bronchial asthma¹.

Regarding the main target cell populations for epithelial cytokines *in vivo*, certain subpopulations of memory Th2 cells express high levels of ST2, which is a receptor of IL-33, and produce inflammatory cytokines, such as IL-5 and Amphiregulin, following IL-33 stimulation². Because the ST2^{hi} memory-type Th2 cells are involved in the pathogenesis of various allergic inflammatory diseases via producing large amounts of inflammatory cytokines, this subpopulation is referred to as memory-type pathogenic Th2 (Tpath2) cells¹. Memory-type Tpath2 cells, with their unique expression of cell surface molecules, TCRβ⁺CD44^{hi}CD62L^{low}IL-7Rα^{hi}ST2^{hi}IL-17RB^{hi}, are involved in the induction of antigen-specific allergic inflammatory responses and the pathogenesis of various human diseases, such as eosinophilic sinusitis, hay fever, and peanut allergy¹ (**Table 1 left**). ILC2s are another target cell population for epithelial cytokines, as ILC2s express high levels of ST2 and other cell surface molecules, including IL-7Rα, CD90.2, and ICOS^{5,6} (**Table 1 right**). In fact, Tpath2 cells and ILC2s share various important characteristics, including the expression

of lineage-determining transcription factors (e.g. GATA3); the production of proinflammatory cytokines, such as Amphiregulin, IL-5 and IL-13; and the expression of cell surface molecules (e.g. ST2 and IL-7R α)^{1, 5, 7}. Furthermore, an assay for transposase-accessible chromatin using sequencing (ATAC-Seq) revealed similarities in the chromatin landscapes between Tpath2 cells and ILC2s in the lung⁸. However, despite these similarities, ILC2s can be directly activated by IL-33 and IL-25 stimulation and can rapidly produce pro-inflammatory cytokines, such as IL-5 and IL-13. In contrast, memory-type Tpath2 cells have little ability to produce IL-5 or IL-13 by epithelial cytokine stimulation alone. Interestingly, in memory Tpath2 cells, dual-specificity phosphatase (DUSP) 10, a MAP kinase phosphatase, inhibits IL-33-induced p38-MAPK phosphorylation and the reduction in the phosphorylation of p38-MAPK that confers the inability of memory-type Tpath2 cells to produce cytokines in an innate-like fashion⁷. Another important difference between these two cell population is that ILC2s respond to a variety of external stimuli, including neuronal biomolecules, to regulate immune responses^{5, 6}. For example, the neuropeptide NMU induces the activation of ILC2s and exacerbates allergic lung inflammation⁴. In the case of Tpath2 cells, whether or not Tpath2 cells anticipate neuronal-derived factors is unclear.

ILC2s reside in non-lymphoid mucosal tissues from the neonatal period. Disruption of the airway epithelial cells followed by the release of IL-33 causes the activation of local tissue-resident immune cells, including ILC2s. Activated ILC2s induce local inflammation by producing type 2 cytokines. However, ILC2s have no ability to respond to antigenic stimulation because they do not express receptors for antigen recognition, which is the fundamental

difference between ILC2s and memory-type T_{path2} cells. In contrast, naïve CD4⁺ T cells are largely absent in non-lymphoid tissues. During the initiation phase of inflammation, antigen-specific naïve CD4⁺ T cells are activated by certain antigens for priming and expansion in the lymph nodes followed by exposure of type 2 cytokines and migration into the inflammatory tissue (**Figure 1 left**). Therefore, in the early phase of inflammation, ILC2s and Th2 cells show distinct responses with unique kinetics and magnitude via different stimuli, although ILC2s and Th2 cells share a similar effector functional program.

Once inflammation occurs in the mucosal tissue, a subpopulation of memory T cells known as tissue-resident memory T (T_{RM}) cells is induced and resides in the post-inflammatory tissues (**Figure 1 right**). At the same time, inflammation causes the induction of ectopic lymphoid tissue, such as inducible bronchus-associated lymphoid tissue (iBALT) and conjunctiva-associated lymphoid tissue (CALT), both of which have separate B cell areas, containing follicular dendritic cells (FDCs), dendritic cells (DCs), high endothelial venules (HEVs) and lymphatics. T_{RM} cells are maintained within iBALT in the non-lymphoid tissue¹. Of note, the subpopulation of CD4⁺ T_{RM} cells shows an enhanced expression of ST2 upon IL-33 stimulation. Under conditions of chronic allergic inflammation, these subpopulations of ST2^{high} CD4⁺ T_{RM} cells are involved in the pathogenesis of chronic inflammation and are maintained in the ectopic lymphoid tissue. Through IL-33-ST2 signaling accompanied by TCR stimulation, these memory-type T_{path2} cells can produce large amounts of IL-5, which recruits eosinophils to the inflamed tissue. Another subpopulation of memory-type T_{path2} cells has the ability to produce Amphiregulin, an epithelial growth factor

(EGF) family member, via IL-33-ST2 signaling accompanied by TCR stimulation. Amphiregulin induces the activation of eosinophils, which secrete osteopontin, an extracellular matrix component; its deposition causes fibrotic responses in both mice and humans (**Figure 1 right**). In the eosinophils, amphiregulin upregulates the expression of CD101, which is a cell surface marker of activated eosinophils². CD4⁺ T_{RM} cells induce antigen-specific secondary immune responses in the mucosal tissue. Thus, CD4⁺ T_{RM} cells in the airway mucosa are involved in the pathogenesis of chronic allergic airway inflammation and fibrosis of lung tissue induced by specific antigen stimulation, such as house dust mite and *Aspergillus fumigatus*⁹.¹⁰. In fact, CD103-negative CD4⁺ T_{RM} cells produce various types of inflammatory cytokines, including IL-4, IL-5 and IL-13, in the lungs of mice with repeated exposure to *Aspergillus fumigatus*¹⁰. Thus, memory-type T_{path2} cells reside in the mucosal tissue as CD4⁺ T_{RM} cells and are involved in the pathogenesis of allergic airway inflammation.

Given the above, both memory-type T_{path2} cells and ILC2s are shown to be involved in the pathogenesis of type 2 inflammation, but they contribute to shaping the pathogenesis in different phases via different stimuli (**Figure 1**). The discussion of the similarities and differences between CD4⁺ T cells and ILCs is interesting from an evolutionary perspective. To develop new therapeutic strategies for intractable allergic diseases, it will become increasingly important for immunologists and clinicians to understand the precise features of T_{path} cells and ILCs.

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Figure Legends

Figure 1. Cellular and molecular interaction of ILC2s and T_{path2} cells in the pathogenesis of allergic airway inflammation. (Left) In the acute phase of airway inflammation, IL-33 from the epithelial cells directly activates ILC2s and other tissue-resident innate immune cells. Activated ILC2s produce type 2 cytokines to initiate type 2 inflammation. Dendritic cells (DCs) in the airway engulf antigen and migrate rapidly into draining lymph nodes (LNs). In the draining LNs, antigen-presenting DCs stimulate antigen-specific naïve CD4 T cells to differentiate into functionally distinct effector helper T (Th) cell subsets, Th2 cells. Antigen-specific Th2 cells infiltrate into the inflamed lung and produce IL-4, IL-5, and IL-13 upon antigen stimulation. (Right) Cellular and molecular interaction of memory-type T_{path2} cells in chronic inflammation. A lymphoid structure called induced bronchus-associated lymphoid tissue (iBALT) is formed, where antigen-specific memory-type T_{path2} cells are maintained. These memory-type T_{path2} cells reside in the mucosal tissue as CD4⁺ T_{RM} cells. Antigen-specific memory-type T_{path2} cells are involved in various pathologies of chronic airway inflammation accompanied by eosinophilic inflammation, airway remodeling, fibrosis, and smooth muscle hyperplasia. IL-5-producing T_{path2} cells (red) recruit eosinophils to the inflammatory sites. Amphiregulin-producing T_{path2} cells (orange) activate the recruited eosinophils to produce osteopontin.

Table 1

Antigen	Memory-type T _{path2} cells ¹	ILC2s ⁶
	Dependent	Independent
Cell surface markers (mouse)	CD4 ⁺ CD44 ^{hi} CD62L ^{lo} CXCR3 ^{lo} CD127 ^{hi} ST2 ^{hi} (Lung tissues from mice induced chronic airway inflammation)	CD45 ⁺ Lineage ⁻ Sca1 ⁺ CD90 ⁺ CD127 ⁺ and ST2 ^{hi} IL17RB ^{lo} IL18R1 ^{+/-} KLRG1 ^{+/-} (Lung) ST2 ^{lo} IL17RB ^{lo} IL18R1 ^{hi} KLRG1 ⁺ (Skin) ST2 ^{hi} IL17RB ^{lo} IL18R1 ⁻ KLRG1 ⁺ (Fat) ST2 ^{lo} IL17RB ^{hi} IL18R1 ⁻ KLTG1 ^{hi} (Gut)
	CD4 ⁺ CD45RO ^{hi} CD69 ^{hi} ST2 ^{hi} IL17RB ^{hi} (nasal polyps from patients with ERCS) CD4 ⁺ CD45RO ^{hi} CRTH2 ^{hi} CCR4 ^{hi} CXCR3 ^{lo} CCR6 ^{lo} CD27 ^{lo} (PBMCs from patients with pollen allergy)	CD45 ⁺ Lineage ⁻ CD127 ⁺ CD161 ⁺ CRTH2 ⁺ and ICOS ⁺ CD69 ⁺ (Skin, Tonsil, Lung from healthy subjects)
Cell surface marker (human)	CD4 ⁺ CRTH2 ^{hi} CD161 ^{hi} hPGDS ^{hi} (PBMCs from patients with EGID and AD)	ICOS ⁻ CD69 ⁻ (PBMCs from healthy subjects)
	CD4 ⁺ CD45RO ^{hi} GPR15 ^{hi} (PBMCs and colons from patients with UC)	elevated expressed ST2, IL17RB, TSLPR, KLRG1 (Skin tissues from patients with AD)

Abbreviations

CD62L: CD62 ligand, CXCR3: C-X-C motif chemokine receptor 3, CD127: Interleukin-7 receptor subunit α , ST2: IL-1 receptor-like1 (IL-33 receptor), IL17RB: IL-17 receptor B (IL-25 receptor), IL18R1: IL-18 receptor 1, Sca1: Stem cells antigen-1, KLRG1: Killer cell lectin-like receptor G1 hi: highly expressed, lo: low expression, +: positive, +/-: heterogeneous expression, ERCS: eosinophilic chronic rhinosinusitis, CRTH2: Chemoattractant receptor-homologous molecule on Th2 cells,

PBMC: Peripheral blood mononuclear cells, hPGDS: Hematopoietic prostaglandin D synthase, EGID: eosinophilic gastrointestinal disease, AD: atopic dermatitis, GPR15: G protein-coupled 15, UC: Ulcerative colitis, TSLPR: Thymic stromal lymphopoietin receptor

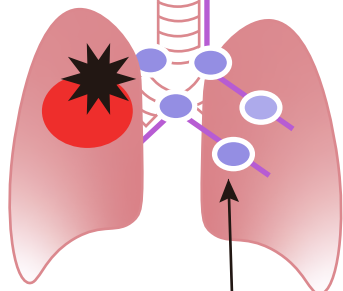
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Figure 1

Lung

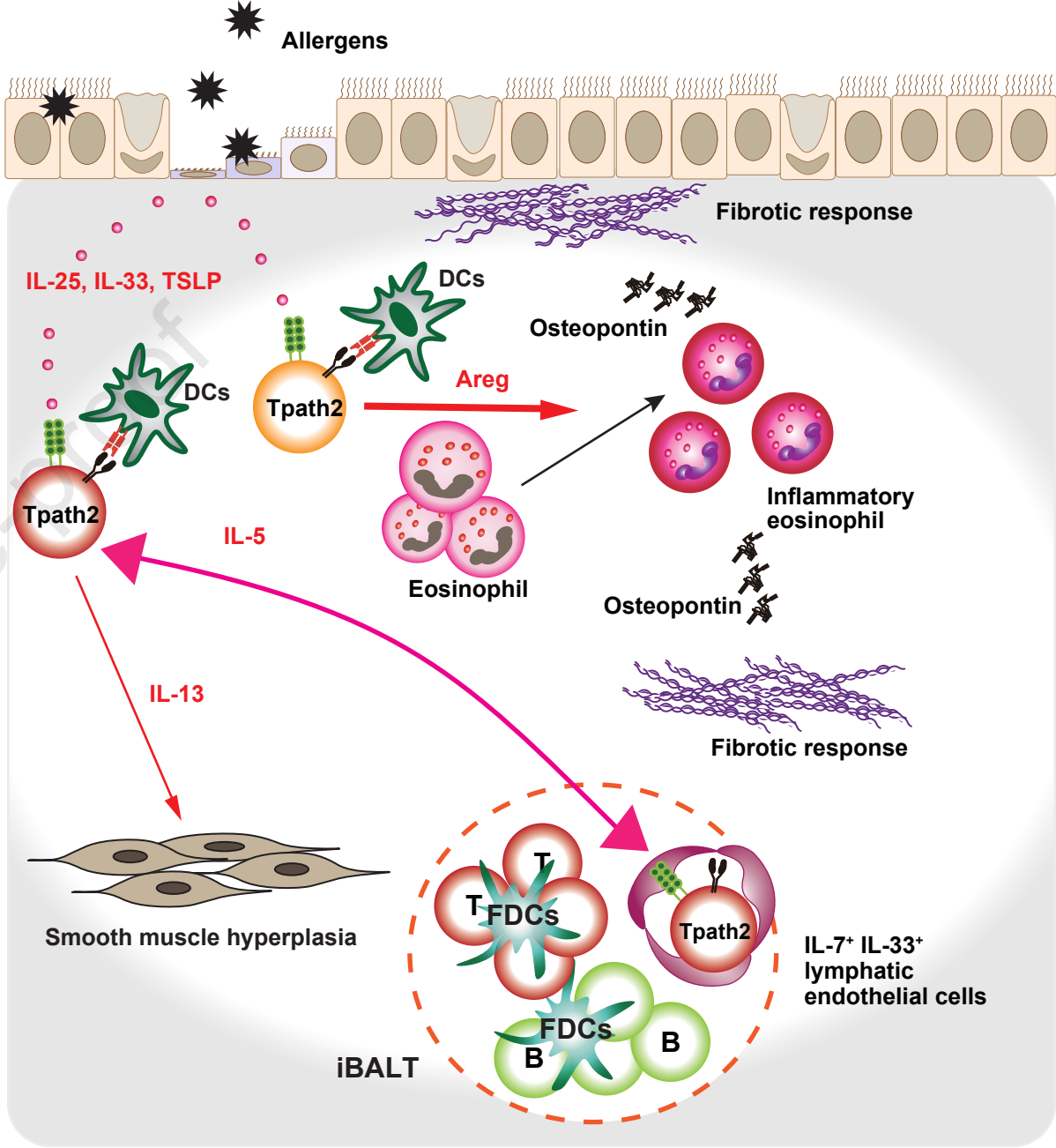
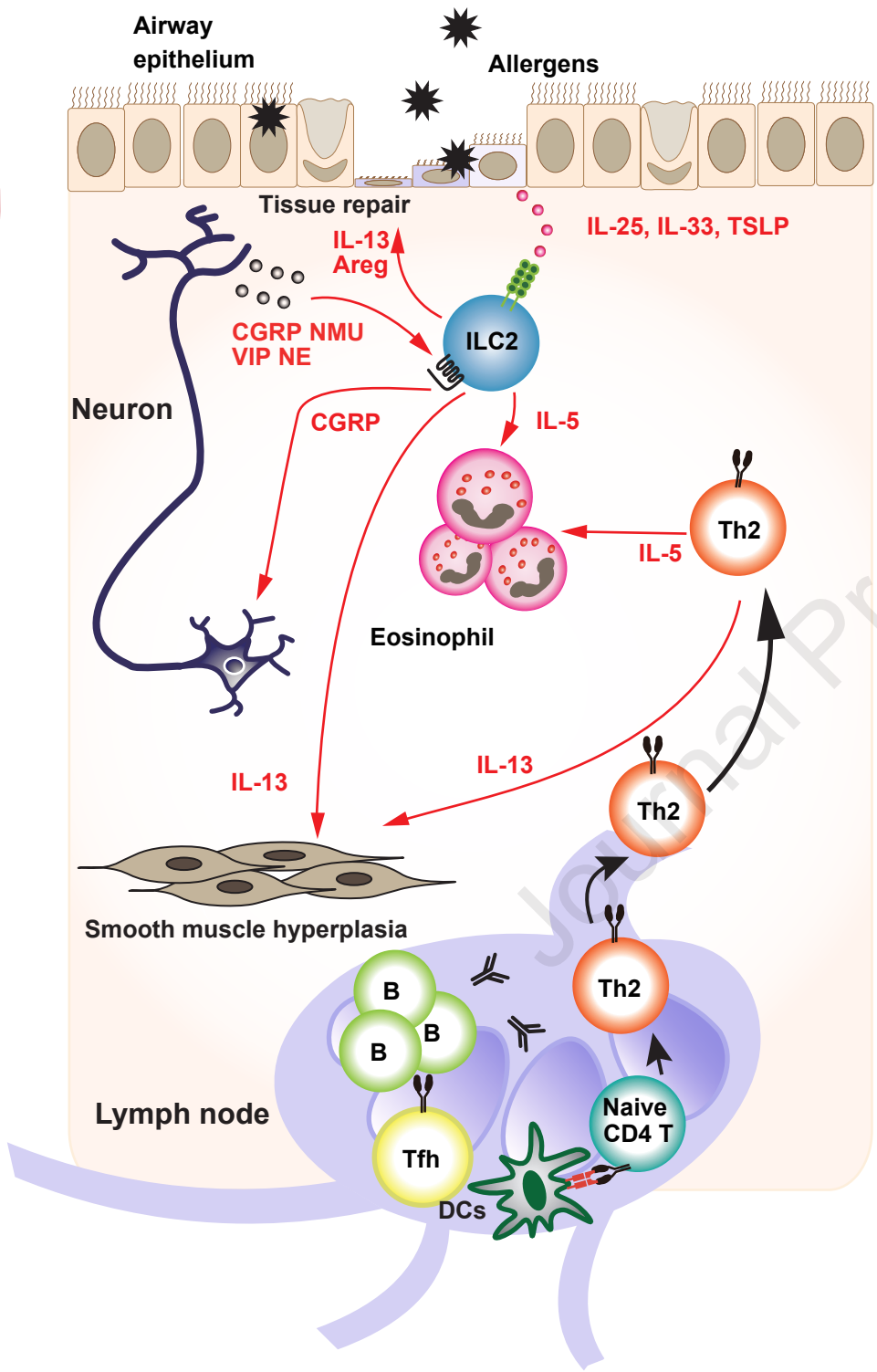
Allergens



Lymph node

Acute phase

Chronic phase



Cytokine/Chemokine receptor (IL-33 receptor etc.) MHC Class II TCR: T cell receptor

ILC2: Type 2 innate lymphoid cell
CGRP: Calcitonin gene-related peptide
NMU: Neuromedin U
VIP: Vasoactive intestinal peptide
NE: Norepinephrine
Areg : Amphiregulin
Tpath2: Memory-type pathogenic type2 T helper cell
B: B cell
T: T cell
FDCs: Follicular dendritic cells
iBALT: Inducible bronchus associated lymphoid tissue