Invited review article

Maintenance of pathogenic Th2 cells in allergic disorders

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ARTICLE INFO

Article history: Received 15 February 2017 Received in revised form 21 February 2017 Accepted 22 February 2017 Available online 5 April 2017

Keywords: Allergy IL-7 Inducible bronchus-associated lymphoid tissue (iBALT) Lymphatic endothelial cells (LECs) Pathogenic Th2 (Tpath2) cells

Abbreviations: APC, antigen-presenting cell; Tpath2, pathogenic Th2; AHR, airway hyperresponsiveness; Ig, immunoglobulin; IL, interleukin; iBALT, inducible bronchus-associated lymphoid tissue; Tcm, central memory T; Tem, effector memory T; Trm, tissue-resident memory T; CCR, CC chemokine receptor; CCL, CC chemokine ligand; ST2, suppression of tumorigenicity 2; MAPK, mitogen-activated protein kinase; BALF, bronchoalveolar lavage fluid; Tg, transgenic; ECRS, eosinophilic chronic rhinosinusitis; CRS, chronic rhinosinusitis; LEC, lymphatic endothelial cell; TCR, T-cell receptor; MHCI, major histocompatibility complex class II; OVA, ovalbumin; FDC, follicular dendritic cell; DC, dendritic cell; HEV, high endothelial venule; DTR, diphtheria toxin receptor

ABSTRACT

Immunological memory is an important protective mechanism that enables host organisms to respond rapidly and vigorously to pathogens that have been previously encountered. In addition to the protective function, memory CD4+ T helper (Th) cells play a central role in the pathogenesis of chronic inflammatory disorders, including asthma. Recently, several investigators have identified phenotypically and functionally distinct memory Th2 cell subsets that produce IL-5. These memory Th2 cell subsets play an important role in the pathology of allergic inflammation and function as memory-type “pathogenic Th2 (Tpath2) cells” both in mice and humans. We review the role of lung Tpath2 cells in the development of allergic inflammation and, in the context of recent findings, propose a mechanism by which Tpath2 cells not only survive but also continue to function at the sites where antigens were encountered. A greater understanding of the functional molecules or signaling pathways that regulate the inflammatory niche for Tpath2 cells may aid in the design of more effective treatments for chronic inflammatory disorders. Copyright © 2017, Japanese Society of Allergology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Immunological memory is generated following immunization or infection that activates the adaptive immune system. The key to long-term immune protection mediated by memory immune cells, is that they remember the differentiation instructions they received during the initial immune response. Memory CD4+ T helper (Th) cells direct and assist many other cell types and have the potential
to act as catalysts, enhancing the immune protection via several different pathways. Characteristics of immunological memory system are a rapid and robust response to major histocompatibility complex class II (MHCII) expressed on antigen-presenting cells (APCs), antigen-specific, and long-lived. It provides the basis for successful vaccines, as recurring infection with the same pathogen does not cause disease. Memory immune responses for bacterial and viral infections are important for survival. On the other hands, they are potentially harmful when directed against allergens. The mechanisms underlying the generation of memory immune system are still incompletely understood.

Most allergic disorders are characterized by Th2 cell-associated cellular processes. During the sensitization phase, allergen-specific CD4⁺ Th2 cells expand, acquire the capacity to express type 2 cytokines, and upregulate chemokine receptors and integrins associated with migration to various anatomical sites. The type 2 cytokines orchestrate multiple events associated with asthma, including eosinophil maturation and their survival, airway hyper-responsiveness (AHR), and B cell isotype switching to immunoglobulin E (IgE). After robust expansion and effector Th cell differentiation, approximately 90% of the effector Th cells die in contraction phase, and a small population of memory Th cells is retained.² There is evidence of persistent memory immune response in mice recovered from acute allergic airway inflammation, such as retained lung inflammation with infiltration of lymphocytes and macrophages, and elevated levels of allergen-specific IgG1 in the serum. In addition, secondary allergen exposure to respiratory organs induces rapid Th2 cell activation, high serum level of allergen-specific IgE, infiltration of eosinophils into the lung, cytokine secretion, differentiation of plasma cells, mucus hypersecretion, and AHR. Repetition of allergen challenges stimulate memory Th2 cells, exacerbate airway inflammation, and result in lung damage and remodeling.³⁻⁵

Recent studies on the characterization of memory Th2 cells have shown that certain interleukin (IL)-5-producing memory Th2 subpopulations at local sites induce allergic inflammation, including eosinophilic airway inflammation and chronic skin inflammation.⁶⁻⁸ In addition, a recent study showed that multiple rounds of cultivation were able to efficiently induce IL-5⁻producing Th2 cells among human cells.⁹ These populations appear to be responsible for the pathology of various Th2-type chronic inflammatory diseases, so we have termed these cells memory-type "pathogenic Th2 (Tpath2)" cells.¹⁰⁻¹²

We herein report the current understanding of the mechanisms underlying the induction and maintenance of Tpath2 cells and their roles in allergic disorders.

Memory-type Tpath2 cells are key drivers of allergic airway inflammation

Allergic asthma is a chronic airway inflammatory disease caused by an immune response against allergens. In both asthmatic patients and murine models, memory Th2 cells appear to regulate chronic inflammation.¹⁰⁻¹² However, it remains unclear how memory Th2 cells are induced and retained in vivo.¹³ Memory Th cell population is heterogeneous, consisting of circulating and non-circulating memory Th cells. In circulating memory T cells, there are two subsets: central memory T (Tcm) and effector memory T (Tem) cells.¹⁴ Tcm cells express CC chemokine receptor (CCR) 7 and CD62L, which regulate recirculation through lymphoid organs. Tem cells express receptors, which are required to migrate into non-lymphoid tissues, and have ability to produce high amount of cytokines, when they are stimulated with their relevant peptide. Non-circulating memory Th cells known as tissue-resident memory (Trm) cells has also been described; these cells are retained in the tissues.¹⁵ Studies primarily investigating CD8⁺ T cells have shown unique roles of Trm cells, including the direct rapid regulation of local infection and the indirect modification of the tissue microenvironment to induce inflammation.¹⁶ Recent investigations have shown that subsets of memory Th cells are also retained at specific peripheral sites as Trm cells, and may confer an effective in situ first line of defense against tissue-specific infections.¹⁷⁻¹⁹ Furthermore, allergen-specific memory Th2 cells reside within the lung for long periods of time after recovering from acute allergic asthma.¹¹ These results indicate that Trm cells may occupy a distinct compartment in the sites of infection compared to circulating memory Th cells. However, it remains unclear how these cells contribute to pathogenesis of inflammatory diseases because antigen-specific memory Th cells that are retained in nonlymphoid organs are rare.¹²⁻²⁵

Our group and others recently found that lung-resident antigen-specific memory Th2 cells are key drivers of lung allergic responses.²³⁻²⁴ Lung-resident antigen-specific memory Th2 cells appear to be sufficient to induce asthmatic symptoms, and IL-2 signaling is required for the differentiation and residency of these lung-resident memory Th2 cells. In addition, clinical studies show that lung transplantation from mildly asthmatic donor into non-asthmatic recipient cause airway diseases, while transplantation from a non-asthmatic donor into an asthmatic recipient can improve the diseases.²⁵

IL-5 is known to drive eosinophilia in lung tissue by supporting the development of eosinophils in the bone marrow, and by the

Lung (OVA i.n.):

![Image](image_url)

CD3ε KJ1 B220

Fig. 1. Memory Th2 cells preferentially localized in inducible bronchus-associated lymphoid tissue (iBALT). Effector Th2 cells were generated with splenic CD62L⁻CD4⁺ naive KJ1.26⁺ CD4⁺ T cells from DO11.10 ovalbumin (OVA)-specific αβTCR Tg mice. These cells were stimulated with an OVA peptide (Lys15, 0.3 μM) plus APCs (irradiated spleenocytes) under Th2-culture conditions (IL-2 [25 U/ml], IL-4 [100 U/ml] and anti-IFNγ mAb) for 6 days in vitro. Effector Th2 cells were transferred intravenously into BALB/c mice, which were subsequently challenged intra-nasally (i.n.) with OVA on days 1 and 3, and the immunofluorescent staining of lung tissue was performed on day 42. Representative confocal micrographs of lung tissue stained with anti-KJ1.26 (red), anti-CD3ε (blue), and anti-B220 (green) are shown. Scale bars, 100 μm. Antigen-specific memory Th2 cells (KJ1⁺) preferentially localized in iBALT.
recruitment of eosinophils to the lung mucosa and interstitium via the production of eotactic chemokines such as eotaxins 1, 2, and 3 (CC chemokine ligand [CCL] 11: CCL11, CCL24, and CCL26, respectively). Pathogenic Th2 cell subsets that induce allergic eosinophilic inflammation produce a large amount of IL-5, in addition to IL-4 and IL-13, and accumulate at the local sites of chronic inflammation. These IL-5-producing subpopulations can be phenotypically identified by the expression of cell surface molecules and their epigenetic status, both in mouse and human systems. Indeed, pathogenic IL-5-producing memory Th2 cells show high levels of histone H3 lysine 9 acetylation (H3K9-Ac) and H3 lysine 4 trimethylation (H3K4-Me3) at the Il5 gene locus and thus these cells possess permissive chromatin state and result in a large amount of IL-5 production upon antigen stimulation. Cumulative evidence shows that these population are key drivers of the pathology of various Th2-type chronic inflammatory diseases.

According to the recent genome-wide association studies, genetic polymorphisms in the gene encoding IL-33 and its receptor IL-1RL1 (suppression of tumorigenicity 2: ST2) are strongly correlated to asthma development.

![Fig. 2. The accumulation of lung-specific memory Th2 cells was dependent on the development of iBALT in the lungs.](image)
and ST2 are on different chromosomal locations, these two genes have consistently been identified in many studies, strongly suggesting that the IL-33-ST2 pathway is involved in asthma pathogenesis.47 Murine studies have revealed IL-33 as a key driver of acute and chronic allergic airway diseases.28 Indeed, a recent study in human subjects showed that the IL-33 levels in bronchoalveolar lavage fluid (BALF) were increased in asthmatic patients compared with control subjects. Furthermore, the IL-33 levels in BALF were negatively associated with the airway function, indicating that IL-33 is involved in asthma pathogenesis.47 In the context of the IL-33 signaling pathway, it was recently demonstrated that the IL-33-ST2-p38 mitogen-activated protein kinase (MAPK) axis is crucial for the induction and enhancement of the pathogenicity of memory Th2 cells in allergic airway inflammation in both mice and humans.50 In addition, IL-5–producing Tpath2 cells show selective and high ST2 expression, and the exposure of memory Th2 cells to IL-33 induces a significant increase in IL-5 production. Therefore, IL-33 is a critical factor in the induction and exacerbation of chronic airway inflammation through memory Th2 cells.

**Inducible bronchus-associated lymphoid tissue (iBALT) formation in chronic allergic airway inflammation**

The respiratory mucosal immune system functions as the first line of defense against pathogens and has a distinct role that initiates sequential immune response. It has been reported that bronchus-associated lymphoid tissue (BALT) develops as normal mucosal lymphoid tissue observed in the lung in certain mammalian species, including rabbits and rats, but not humans or mice.31 In humans and mice, BALT formation can be induced under a range of disease states characterized by chronic inflammation, infection, or autoimmunity, and these structures are now preferentially termed inducible BALT (iBALT).32,33 Recently, several groups have shown that iBALT structures could also be observed in Th2-associated immune response, such as HDM-driven airway inflammation model34 and ovalbumin (OVA)-induced chronic allergic airway inflammation model.43 iBALT is a classic example of a tertiary lymphoid tissue, since it does not develop in a pre-programed way and its occurrence, size, and number in the lung depends on the type and duration of antigenic exposure.35–37 The iBALT structure consists of separate B cell areas with the presence of follicular dendritic cells (FDCs), resident dendritic cells (DCs), high endothelial venules (HEVs) and lymphatics.32,38 Robust lymphangiogenesis was also observed in iBALT during sustained inflammation.39 Given that the structure of iBALT is similar to that of conventional secondary lymphoid organs, it is not too surprising that the chemokines and cytokines necessary for the development of secondary lymphoid organs are also important for the development of iBALT. It has been shown that antigen presentation and T cell priming can occur directly in iBALT in response to antigens derived from the airways.40 A recent study showed that influenza-specific memory CD8\(^+\) T cells can be maintained in iBALT and that these cells efficiently react to secondary influenza infections.41 In the lung under conditions of chronic inflammation, similar to memory CD8\(^+\) T cells, Tpath2 cells are preferentially localized in iBALT and exacerbate allergic airway inflammation upon secondary antigen exposure (Fig. 1).

Given that memory Th2 cells in the lung are preferentially localized in iBALT, we sought to investigate whether or not iBALT formation itself influences the number of memory Th2 cells maintained in the lung. Mice that lack expression of Ccl19 and Ccl21 (plt/plt) are known to have impaired iBALT formation.42 We used these mice to assess the accumulation of OVA-specific memory Th2 cells in the lung using our experimental system (Fig. 2A). *Plt/plt* mice had impaired iBALT formation (Fig. 2B) with a significantly decreased number of KJ1\(^+\) donor-derived memory Th2 cells in the lung (Fig. 2C). We next analyzed Ccr7-deficient mice that spontaneously develop organized BALT structures in the lung.43 OT-II OVA-specific TCR- and GFP-double transgenic (Tg) memory Th2 cells were transferred into Ccr7-deficient mice (Fig. 2D), and as

![Image](https://example.com/figure3.png)

**Fig. 3.** The disruption of iBALT structure reduced the number of memory Th2 cells in the lung. (A) Effectors Th2 cells from Thy1.1 OT-II Tg mice were transferred into CD11c-DTR Tg mice. Mice were challenged i.n. with OVA on days 1 and 3. PBS or Diphtheria toxin (DT) was i.n. administered on day 84, and mice were assessed on day 91. (B) On day 87, CD11c and MHC class II expression in the indicated organs of mice treated in A were analyzed by flow cytometry. (C) Representative confocal micrographs of iBALT in the lungs stained for anti-Thy1.1 (red), anti-B220 (green), and anti-GFP; CD11c (blue). (D) Representative staining profiles of Thy1.1 and CD4 (left), and absolute cell numbers of Thy1.1 ‘CD4’ (right; upper panel) and endogenous Thy1.1 ‘CD4’ cells (right; lower panel) in the spleen and the lungs. The mean values (six mice per group) are shown with the standard deviation. Scale bars, 40 μm. *p < 0.01. NS, not significant.
expected, the Ccr7-deficient mice showed highly organized BALT structures in the lung (Fig. 2E) with increased numbers of memory Th2 cells in iBALT (Fig. 2F). As DCs are required for the maintenance of iBALT structures, we used CD11c-diphtheria toxin receptor (DT) Tg mice to deplete CD11c+ DCs. The administration of diphtheria toxin to CD11c-DTR Tg mice after iBALT formation resulted in the efficient depletion of CD11c+ DCs (Fig. 3A, B) and reduced iBALT formation (Fig. 3C). Importantly, the number of Thy1.1+ donor-derived memory Th2 cells in the lung of diphtheria toxin-treated mice was significantly decreased compared to control mice (Fig. 3D). This was not the case for the Thy1.1+ memory Th2 cells in the spleen or for the number of endogenous Thy1.1+ CD4+ T cells (Fig. 3D). Collectively, these findings indicate that iBALT structures are required for the accumulation and efficient maintenance of memory Th2 cells in the lung.

Furthermore, antigen-specific memory Th2 cell-dependent allergic airway inflammation on antigen re-exposure was significantly exacerbated in the mice that have iBALT, suggesting that iBALT structures may act as environments regulating the pathogenesis of allergen-induced chronic airway inflammation.

**Lymphatic endothelial cells (LECs) as a niche for Tpath2 cells**

The maintenance of memory Th cells is still incompletely understood, and it is likely that multiple mechanisms differentially contribute to the homeostatic turnover and proliferative renewal of various memory populations. In secondary lymphoid organs, the maintenance of memory Th cells is dependent upon homeostatic T-cell receptor (TCR) signaling and multiple cytokines, including IL-7 and IL-15. Studies using mice Tg altered to allow inducible TCR signaling blockade have shown that homeostatic (non-specific) TCR signaling is dispensable for primary effector Th cells to transition to memory cells but is indispensable for memory Th cell homeostatic turnover and longevity in secondary lymphoid organs. It has been also reported that combined TCR signaling and IL-7 and IL-2 cytokine signaling suppress pro-apoptotic pathways during the transition to memory cells in human CD4 T cells. IL-7 is known to be required during the transition from effector to memory Th cells in secondary lymphoid organs as well as for the long-term maintenance of resting memory Th cells in the bone marrow. While multiple groups using several techniques have investigated the source of IL-7 signaling, the findings thus far are insufficient. However, the cumulative evidence suggests that IL-7 is produced by mesenchymal and epithelial cells, such as thymic epithelial cells, bone marrow stromal cells, lymph node fibroblastic reticular cells, epidermal keratinocytes, hepatocytes, and lymphatic endothelial cells (LECs) in the lymph nodes.

The mechanism underlying the maintenance of lung-resident memory Th cells is poorly understood. Memory Th cells are believed to reside in numerous peripheral tissues and actively translocate to secondary lymphoid organs for targeted interaction with IL-7-producing stromal cells. However, the direct evidence...
of memory Th cell retention in lung tissue was provided by the experiments of parabiosis, in which mouse pairs are surgically conjoined to create shared circulations.16

We have recently clarified that the maintenance of lung-resident antigen-specific memory Th2 cells is dependent on IL-7-producing LECs, which are localized within iBALT structures.23 Antigen-specific memory Th2 cells preferentially localized in iBALT and were maintained in an IL-7-dependent manner via an antigen-independent mechanism. Notably, IL-7-producing LECs express Thy1 (CD90) as a marker and increase in number under conditions of lung inflammation with the formation of iBALT, suggesting that the modification of the lung microenvironment promotes the survival of antigen-specific memory Th2 cells within iBALT.

Interestingly, an analysis of Thy1+ IL-7-producing LECs revealed the unique characteristic of this cell population as a producer of IL-5 (Fig. 4A), we analyzed Thy1+ memory Th2 cells generated in vivo and found that the number of Thy1+ memory Th2 cells was identical to that in control mice (Fig. 4B), and they preferentially localized and were maintained in iBALT (Fig. 4C). However, while in vitro-generated Il1rl1/− effector Th2 cells can produce type 2 cytokines (IL-4, IL-5, and IL-13) similar to wild-type control cells, Il1rl1/− memory Th2 cells showed a significantly reduced ability to produce type 2 cytokines upon anti-TCRβ antibody stimulation (Fig. 4D). Thus, the IL-33-ST2 signaling pathway is critical for memory-type Tpath2 cells to maintain their ability to produce type 2 cytokines in iBALT.

IL-7 may support the maintenance of memory Th2 cells, and IL-33 may confer their pathogenic function in terms of the ability to produce high amounts of IL-5 and induce eosinophilic inflammation in the airway. Interestingly, it was recently shown that IL-33 in combination with IL-7 stimulates peritoneal resident memory Th2 cells and produces significant amounts of IL-5 and IL-13 in a TCR-independent manner.66 Collectively, in the lung of chronic airway inflammatory diseases, it appears that Thy1+ IL-7+ IL-33+ LECs provide a niche for Tpath2 cells both for surviving and maintaining their pathogenicity (Fig. 5).

**Tpath2 cells and eosinophilic chronic rhinosinusitis (ECRS)**

Eosinophilic chronic rhinosinusitis (ECRS) is an inflammatory pathological condition of the nose and paranasal sinuses.68 ECRS is a subtype of chronic sinusitis (CRS) that is considered to occur secondarily to systemic eosinophil deregulation.69 Patients with CRS are classified into two subtypes: CRS with nasal polyps and CRS without nasal polyps. The pathogenesis of ECRS is poorly understood. These patients often have asthma, and ECRS shares many histologic and immunologic features with asthma, suggesting that ECRS and asthma may comprise the same immune process involving the upper and lower airways, respectively.68,69 Similar to eosinophilic asthma, ECRS is characterized by a high type 2

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**Fig. 5.** The induction and maintenance of memory-type pathogenic Th2 (Tpath2) cells in the airway. Allergen exposure induced IL-33 secretion from IL-33+ cells, including epithelial cells and endothelial cells in the airway. IL-33 induced a significant increase in the IL-5 production in memory Th2 cells in a ST2-p38 MAPK-dependent manner. Antigen restimulation induced greater amounts of IL-5 secretion from IL-33-activated memory-type Tpath2 cells than from conventional memory Th2 cells, thereby exacerbating chronic eosinophilic inflammation. iBALT formed in the lung during chronic inflammation, consisting of various type of immune cells, including T cells, B cells, dendritic cells (DCs), and follicular dendritic cells (FDCs). The selective localization and survival of memory-type Tpath2 cells within iBALT were supported by IL-7+ lymphatic endothelial cells (LECs). These IL-7+ LECs are also IL-33+ and may therefore increase the pathogenicity of memory Th2 cells. IL-7+IL-33+ LECs are considered to be the “niche” for the survival and maintenance of the function of Tpath2 cells at the sites of chronic inflammation.
cytokine milieu, i.e. a milieu enriched for IL-4, IL-5, and IL-13.70–72 The pathophysiological roles of Tpath2 cells in ectopic lymphoid structures observed in nasal polyps of patients with ECRS have also been confirmed. Furthermore, there is evidence showing the presence of local Th2 cells in nasal polyps expressing IL-17 receptor B.73 In addition, nasal polyps from patients with ECRS exhibit massive infiltration of CD45RO+ memory CD4 T cells within the ectopic lymphoid structures, which produce large amounts of IL-5 in response to IL-33 and IL-25.64,74 A more recent study has shown that the ectopic lymphoid structures in nasal polyps are involved in local IgE production in ECRS patients.75 In addition, the number of Thy1+IL-7−IL-33− LECs was increased in the ectopic lymphoid tissue of nasal polyps from ECRS patients; these cells are considered crucial for the local maintenance of memory Tpath2 cells.76 These recent results indicate that IL-33-induced memory-type Tpath2 cells are involved in shaping the pathology of ECRS. Further studies are needed to clarify the underlying molecular mechanisms in greater detail, in the context of the pathophysiological roles of Tpath2 cells in ECRS.

Concluding remarks

Animal models and clinical studies have clarified a central role for memory Th2 cells in chronic inflammatory disorders. The identification of memory-type Tpath2 cells and their functional roles during allergic asthma has had tremendous impact on the understanding of disease pathogenesis. The elimination of eosinophils from humans using an antibody to IL-5 (mepolizumab; GlaxoSmithKline, Brentford, UK) has helped reduce the exacerbation frequency in a subset of patients with high levels of circulating eosinophils in the blood and frequent exacerbations, even in those receiving inhaled steroids.70 This evidence supports the potential efficacy of Tpath2 cell-targeting treatments for chronic allergic disorders, including steroid-resistant and severe refractory asthma. The regulation of Tpath2 cells through the selective targeting of their cellular niche will provide new strategies for the treatment of inflammatory diseases. Further investigations to improve our understanding of the cell components that organize and contribute to ectopic lymphoid structure formation are required to achieve the successful regulation of Tpath2 cells, which these findings suggest would be an excellent method of treating chronic inflammatory disorders.

Acknowledgments

We would like to thank Dr. Andrew N. J. McKenzie (Medical Research Council, Cambridge) for kindly providing the Il1rl1−/− mice and Terutaka Kakiuchi (Toho University) for kindly providing the Il1rl1−/− mice. This work was supported by AMED-CREST, The Japan Agency for Medical Research and Development (AMED), and grants from the Ministry of Education, Culture, Sports, Science and Technology [MEXT Japan; Program for Leading Graduate School, Grants-in-Aid for Scientific Research (S) #26221305, (C) #24592083], and The Uehara Memorial Foundation.

Conflict of interest

The authors have no conflict of interest to declare.

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