

REVIEW

Open Access



Maintenance of memory-type pathogenic Th2 cells in the pathophysiology of chronic airway inflammation

Kiyoshi Hirahara^{1*}, Kenta Shinoda¹, Yusuke Endo¹, Tomomi Ichikawa¹ and Toshinori Nakayama^{1,2}

Abstract

Background: Immunological memory is critical for long-standing protection against microorganisms; however, certain antigen-specific memory CD4⁺ T helper (Th) cells drive immune-related pathology, including chronic allergic inflammation such as asthma. The IL-5-producing memory-type T_{path2} subset is important for the pathogenesis of chronic allergic inflammation. This memory-type pathogenic Th2 cell population (T_{path2}) can be detected in various allergic inflammatory lesions. However, how these pathogenic populations are maintained at the local inflammatory site has remained unclear.

Methods: We performed a series of experiments using mice model for chronic airway inflammation. We also investigated the human samples from patients with eosinophilic chronic rhinosinusitis.

Results: We recently reported that inducible bronchus-associated lymphoid tissue (iBALT) was shaped during chronic inflammation in the lung. We also found that memory-type T_{path2} cells are maintained within iBALT. The maintenance of the T_{path2} cells within iBALT is supported by specific cell subpopulations within the lung. Furthermore, ectopic lymphoid structures consisting of memory CD4⁺ T cells were found in nasal polyps of eosinophilic chronic rhinosinusitis patients, indicating that the persistence of inflammation is controlled by these structures.

Conclusion: Thus, the cell components that organize iBALT formation may be therapeutic targets for chronic allergic airway inflammation.

Keywords: Memory-type pathogenic Th2 cells, Interleukin-33, Inducible bronchus-associated lymphoid tissue (iBALT), Chronic inflammation

Background

Asthma is characterized by chronic airway inflammation, mucus hyperproduction, airway hyperresponsiveness, and variable airway obstruction. The pathophysiology of chronic airway inflammation involves in various types of immune cells such as CD4⁺ T cells, B cells, innate lymphoid cells, and eosinophils. In particular, T helper (Th) 2 cells and type 2 innate lymphoid cells play central roles in the pathogenesis of allergic airway inflammation.

Recent studies have identified “epithelial cytokines” such as IL-25, IL-33, and TSLP as key modulators of type 2 immune responses. IL-33 is constitutively expressed on

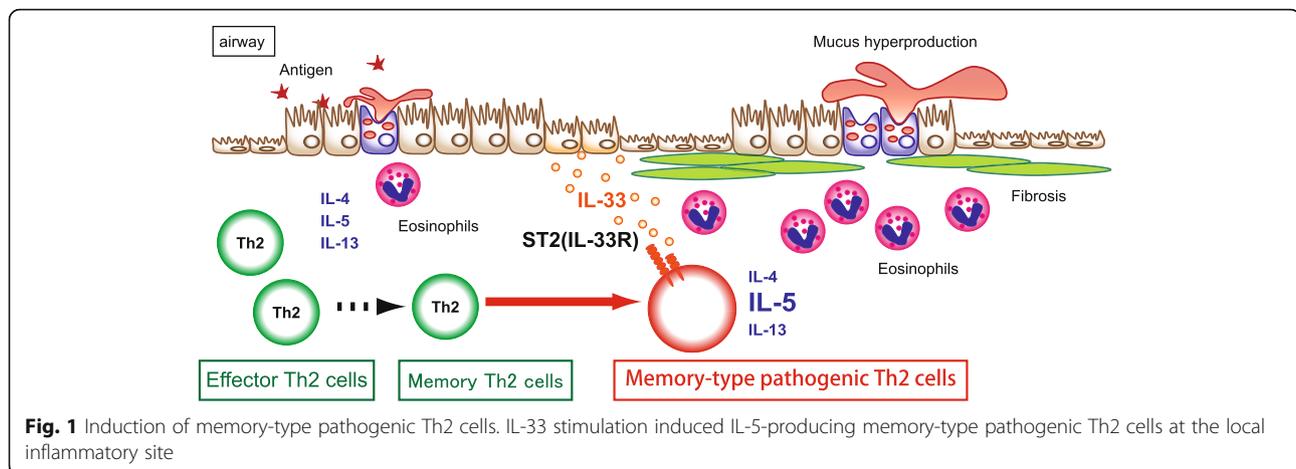
epithelial cells in mucosal barrier organs [1]. Chronic repeated exposure to various exogenous allergens or pathogens, such as tobacco smoke or inhaled irritant particles, prompts epithelial cells to release their stored IL-33, which is involved in chronic allergic inflammatory diseases such as asthma, eosinophilic chronic rhinosinusitis (ECRS), pollen allergy, and eosinophilic pneumonia. IL-33 was originally identified as a ligand for the ST2 receptor (also known as IL1RL1) [2]. Effector Th2 cells, regulatory T cells, mast cells, and ILC2s are known to be target cells of IL-33. We found that memory-type pathogenic Th2 (T_{path2}) cells, which produce large amounts of IL-5, expressed high levels of ST2 [3, 4] (Fig. 1). The expression of ST2 on memory-type T_{path2} cells was higher than that on effector Th2 cells, which suggested that memory-type T_{path2} cells were novel targets of IL-33 in vivo.

* Correspondence: hiraharak@chiba-u.jp

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

Full list of author information is available at the end of the article





In humans, it has been shown that bronchus-associated lymphoid tissue (BALT) is shaped in the lung in response to inflammatory states caused by infectious organisms, smoking, and auto-immune diseases; under these conditions, this tissue known as inducible BALT (iBALT) [5, 6]. For example, patients with chronic obstructive pulmonary disease (COPD) showed ectopic lymphoid structures in the lungs [5]. However, whether or not iBALT is involved in the pathophysiology of chronic allergic diseases, such as asthma, and how memory-type T cells are maintained in the local inflammatory tissues has been unclear.

Results

To determine whether or not iBALT was induced in chronic allergic inflammation, we generated OVA-specific effector Th2 cells in vitro and then adoptively transferred them to syngeneic mice that were intranasally administered OVA twice. We analyzed these mice 42 days after the adoptive transfer. Hematoxylin and eosin (HE) staining of the mouse lungs showed that massive infiltration of inflammatory cells had been induced and persisted even 42 days after the intra-nasal administration of OVA (Fig. 2a). Immuno-histochemical staining showed the formation of iBALT structures containing donor-derived memory Th2 cells that were detected by KJ-1.26 (KJ1), which is a monoclonal antibody that recognizes OVA-specific TCR DO11.10, MHC class-II-positive cells, B220-positive cells, CD11c-positive cells, stromal cells, and CD21-positive follicular dendritic cells (Fig. 2b). These results indicate that the iBALT detected in our experimental model was comparable to that noted in previous reports [6]. Notably, the memory Th2 cells showed greater accumulation in iBALT than in non-lymphoid areas (Fig. 2c).

We noted no difference in the number of memory Th2 cells in the spleen with and without intranasal administration of OVA. In sharp contrast, we observed a

significant increase in the number of memory Th2 cells in the lung following the intranasal administration of OVA. These memory Th2 cells in the lung produced increased levels of IL-5 (Fig. 2d). Taken together, these findings show that the adoptive transfer of effector Th2 cells followed by the intra-nasal administration of OVA resulted in iBALT formation and the accumulation of memory-type T_{path2} cells in the lung.

We then assessed the patho-physiological role of memory-type T_{path2} cells maintained in iBALT. The OVA-induced airway inflammatory responses were assessed using the mice with iBALT formation. iBALT-induced mice showed enhanced infiltration of inflammatory cells in the BALF compared with the control animals. Consistent with this result, the airway hyperresponsiveness and mucus production were enhanced in the mice with iBALT. Thus, the memory-type T_{path2} cells in the mice with iBALT were involved in the pathogenicity of eosinophilic airway inflammation.

IL-7 is a key cytokine involved in the maintenance of T cells in vivo [7]. We therefore wanted to determine whether or not IL-7 was involved in the maintenance of memory Th2 cells in iBALT using IL-7 GFP knock-in mice (collaboration with Professor Ikuta in Kyoto University). We found that a main population of IL-7-producing cells was accumulated in iBALT in the lung (Fig. 2e). Within the iBALT, most memory Th2 cells were co-localized with IL-7-producing cells. When we analyzed the PECAM-1-positive endothelial cells, *Pdpn* and *Prox1*, which are specific markers for lymphatic endothelial cells, were highly expressed in the isolated PECAM1⁺IL-7-GFP⁺ cells. Interestingly, PECAM1⁺IL-7-GFP⁺ cells also expressed *Il33* mRNA. A FACS analysis revealed that the PECAM1⁺IL-7-GFP⁺ cells expressed Lyve-1 and podoplanin. Taken together, these results suggest that lymphatic endothelial cells in iBALT produce IL-7. We also found that PECAM1⁺IL-7-GFP⁺ cells showed high expression of Thy1. We generated *Il-7^{fllox/}*

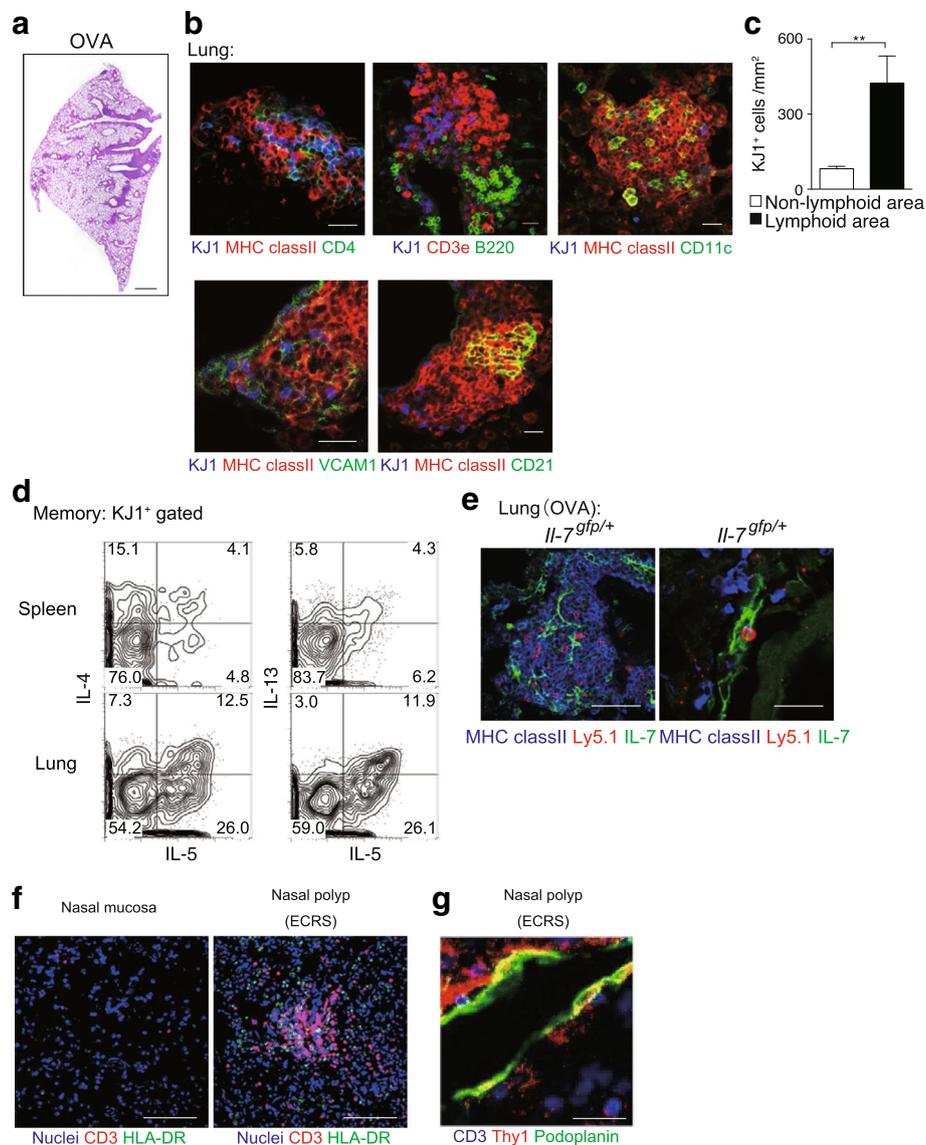


Fig. 2 iBALT is induced under conditions of chronic allergic inflammation in both mice and humans. **a** The intra-nasal administration of the antigen resulted in iBALT formation in the lungs of mice. **b** iBALT included memory Th2 cells, MHC-class II⁺ cells, B220⁺ cells, CD11c⁺ cells, VCAM1⁺ cells, and CD21⁺ cells. **c** The memory Th2 cells showed greater accumulation in the lymphoid areas than in the non-lymphoid areas. **d** Memory Th2 cells in the lung produced more Th2 cytokines compared to those from memory Th2 cells in the spleen. **e** IL-7-producing cells and Ly5.1⁺ memory Th2 cells were detected in mice iBALT. **f** Ectopic lymphoid structures were generated in the polyps of patients with ECRS. **g** Podoplanin-positive lymphatics were increased in the polyps of patients with ECRS. (Shinoda et al. *PNAS*(2016) Copyright (2016) National Academy of Sciences). KJ: OVA-specific T cell receptor

fllox mice crossed with *Tie2-Cre* transgenic mice, in which the mouse endothelial-specific receptor tyrosine kinase (*Tie2*) promoter directs expression of Cre recombinase, to investigate the role of IL-7 produced by LECs. When iBALT was induced using *Il-7^{fl/fl}Tie2-Cre⁺* Tg mice as hosts, iBALT formation was impaired in the lung of *Il-7^{fl/fl}Tie2-Cre⁺* Tg mice. We also detected decreased numbers of memory Th2 cells in the lung. Taken together, these findings indicate that Thy1⁺IL-7⁺ lymphatic endothelial cells (LECs) support the memory Th2 cell survival in iBALT in vivo.

IL-5-producing Tpath2 cells have been detected in the PBMCs of patients with eosinophilic gastrointestinal disease [8]. However, whether or not Tpath2 cells are maintained in the local inflammatory tissue in humans has been unclear. ECRS is a chronic upper respiratory airway allergic disease characterized by the formation of nasal polyps and the infiltration of massive eosinophils in the polyps [9]. We analyzed local inflammatory tissues from the polyps of patients with ECRS. Very little T cell infiltration and few lymphoid structures were detected

in the nasal mucosa of control subjects. However, in sharp contrast, the nasal polyps of patients with ECRS showed massive infiltration of CD3⁺ T cells accompanied by elevated numbers of ectopic lymphoid structures (Fig. 2f). The majority of accumulated CD3⁺ T cells were memory-type CD4⁺ T cells, as they expressed CD4 together with CD45RO. Furthermore, podoplanin-positive lymphatics were increased in the nasal polyps of patients with ECRS compared to the control nasal mucosa (Fig. 2g). *IL7* and *IL33* were expressed more strongly in CD45⁻PECAM1⁺Thy1⁺ cells than in CD45⁻PECAM1⁺Thy1⁻ cells.

Discussion

Our research highlighted that Thy1⁺IL-7⁺ lymphatic endothelial cells (LECs) support memory Th2 cell survival in iBALT in the chronic inflamed lung from mice [10]. Moreover, we found that memory-type CD4⁺ T cells and IL-7⁺IL-33⁺ LECs accumulated in polyps from ECRS patients. These results indicate that Thy1⁺IL-7⁺ LECs produce IL-33 and may confer the pathogenicity on T_H2 cells. The major IL-7-producing cells in the iBALT are the LECs that are co-localized with memory Th2 cells in the lung. A set of experiments by using IL-7 conditional knockout mice (*Tie2-Cre⁺IL-7^{fl/fl}* mice) verified the importance of IL-7-production from LECs on the maintenance of memory Th2 cells in iBALT. Thus, these cells likely provide a survival niche for memory Th2 cells at local inflammatory sites in the airway. Further study is needed to investigate the contribution of IL-7 to T cell-mediated chronic inflammatory diseases such as steroid-resistant asthma.

Conclusion

In summary, our findings showed that the iBALT structure supports the T_H2 cell survival in chronic airway inflammation. The cell components and or functional molecules that organize iBALT formation may be therapeutic targets for chronic allergic airway inflammation.

Abbreviations

BALF: Bronchoalveolar lavage fluid; COPD: Chronic obstructive pulmonary disease; iBALT: Inducible bronchus-associated lymphoid tissue; IL: Interleukin; ILC2s: Type 2 innate lymphoid cells; T_H2 cells: Pathogenic Th2 cells

Acknowledgements

We would like to sincerely thank our collaborators, Professor Koichi Ikuta (Kyoto University) and Professor Yoshitaka Okamoto (Chiba University).

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KH, KS, YE, TI, and TN prepared the manuscript and figures. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Animal care was conducted in accordance with the guidelines of Chiba University. All animal experiments were approved by the Chiba University Review Board for Animal Care. All patients signed informed consent forms, and the study was approved by Ethics Committee of the Chiba University Graduate School of Medicine and each participating hospital.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Department of Immunology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba 260-8670, Japan. ²AMED-CREST, AMED, 1-8-1 Inohana Chuo-ku, Chiba 260-8670, Japan.

Received: 23 January 2018 Accepted: 9 April 2018

Published online: 20 June 2018

References

- Pichery M, et al. Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues: in situ analysis using a novel IL-33-LacZ gene trap reporter strain. *J Immunol.* 2012;188:3488–95. <https://doi.org/10.4049/jimmunol.1101977>.
- Schmitz J, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity.* 2005;23:479–90. <https://doi.org/10.1016/j.immuni.2005.09.015>.
- Endo Y, et al. The interleukin-33-p38 kinase axis confers memory T helper 2 cell pathogenicity in the airway. *Immunity.* 2015;42:294–308. <https://doi.org/10.1016/j.immuni.2015.01.016>.
- Nakayama T, et al. Th2 cells in health and disease. *Annu Rev Immunol.* 2016; <https://doi.org/10.1146/annurev-immunol-051116-052350>.
- Hogg JC, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med.* 2004;350:2645–53. <https://doi.org/10.1056/NEJMoa032158>.
- Rangel-Moreno J, et al. Inducible bronchus-associated lymphoid tissue (iBALT) in patients with pulmonary complications of rheumatoid arthritis. *J Clin Invest.* 2006;116:3183–94. <https://doi.org/10.1172/JCI28756>.
- Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. *Nat Rev Immunol.* 2011;11:330–42. <https://doi.org/10.1038/nri2970>.
- Mitson-Salazar A, et al. Hematopoietic prostaglandin D synthase defines a proeosinophilic pathogenic effector human TH2 cell subpopulation with enhanced function. *J Allergy Clin Immunol.* 2016;137:907–918 e909. <https://doi.org/10.1016/j.jaci.2015.08.007>.
- Hamilos DL. Drivers of chronic rhinosinusitis: inflammation versus infection. *J Allergy Clin Immunol.* 2015;136:1454–9. <https://doi.org/10.1016/j.jaci.2015.10.011>.
- Shinoda K, et al. Thy1+IL-7+ lymphatic endothelial cells in iBALT provide a survival niche for memory T-helper cells in allergic airway inflammation. *Proc Natl Acad Sci U S A.* 2016;113:E2842–51. <https://doi.org/10.1073/pnas.1512600113>.