ORIGINAL ARTICLE

https://doi.org/10.4110/in.2017.17.3.163

Immune Network

pISSN 1598-2629 · eISSN 2092-6685

Clonal Expansion of Allergen-specific CD4$^+$ T Cell in the Lung in the Absence of Lymph Nodes

Garam Choi[1,2], Byung-Seok Kim[1,2], Young-Jun Park[1], Inbo Shim[1] and Yeonseok Chung[1,2]

[1]Laboratory of Immune Regulation, Research Institute of Pharmaceutical Sciences, [2]BK21 Plus Program, College of Pharmacy, Seoul National University, Seoul 08826, Korea

The expansion of allergen-specific CD4$^+$ T cells is a critical step in inducing airway inflammation during allergic asthma. Such clonal expansion of T cells is initiated through the interaction between allergen-bearing dendritic cells and allergen-specific naïve T cells in the draining lymph nodes. Whether such T cell clonal expansion also occurs in the lung, the primary organ encountering inhaled allergens, remains unclear. Compared with wild-type mice, we found similar frequencies of CD4$^+$ T cells in the lung of lymph node-deficient *Rorgt*$^{gfp/gfp}$ mice after repeated exposure to inhaled allergens. In addition, we observed an evident population of CD4$^+$ T cells that underwent clonal expansion in the lung of allergen-challenged mice treated with an S1P antagonist FTY720 in an *in vivo* proliferation study with CFSE-labeled OT-II T cells. Moreover, the expansion of allergen-specific CD4$^+$ T cells was significantly enhanced in the lungs of *Rorgt*$^{gfp/gfp}$ mice in comparison to that of wild-type mice. These results together demonstrate that the clonal expansion of allergen-specific CD4$^+$ T cells occurs in the absence of the lymph nodes, indicating that the lung can act as a primary site of the clonal expansion of CD4$^+$ T cells in response to inhaled allergens.

[Immune Network 2017;17(3):163-170]

Keywords: Allergens, Lung, RORγt, CD4$^+$ T cell, Proliferation

INTRODUCTION

T cell clonal expansion is an explosive increase of antigen-specific T cells which are essential for efficient adaptive immunity against invading foreign antigens (1,2). The clonal expansion initiates when antigen-specific naive T cells engage dendritic cells (DCs) bearing a cognate antigen in the secondary lymphoid organs such as lymph nodes (3,4). The recognition of an antigenic peptide in the context of MHC molecules by a T cell receptor delivers antigen-driven signals into T cells, termed 'signal 1'. Costimulatory molecules on the antigen-presenting DCs provide additional signals (signal 2) that lead to clonal expansion by inducing autocrine IL-2 production from T cells. In addition, cytokines from the DCs further stimulate the activated T cells to differentiate into a certain lineage of effector T cells (signal 3) (5-7).

It is well-established that the clonal expansion of naïve T cells occurs in the draining lymph nodes since antigen-bearing DCs migrate into the T cell zone of the lymph nodes by upregulating C-C chemokine receptor type 7 (8,9). On the other hand, T cell-DC interaction can also occur in the tertiary lymphoid structures in non-lymphoid organs such as the lung. For instance, induced bronchus-

Received on March 19, 2017. Revised on May 3, 2017. Accepted on May 8, 2017.

*Corresponding Author. Yeonseok Chung, College of Pharmacy, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Building 29 (Suite 210), Seoul 08826, Korea. Tel: 82-2-880-7874; E-mail: yeonseok@snu.ac.kr

Abbreviations: DC, dendritic cell; iBALT, induced bronchus-associated lymphoid tissue; LTα, lymphotoxin α; LTβ, lymphotoxin β; RORγt, retinoic acid-related orphan receptor gamma t; LTi, lymphoid tissue inducer; T_H17, T helper 17; S1P, sphingosine-1-phosphate; PAO, protease from *Aspergillus oryzae*; BMDC, bone marrow-derived dendritic cell; mLN, mediastinal lymph node

associated lymphoid tissue (iBALT) is one type of tertiary lymphoid structures in the lung. Recent studies highlighted a crucial role of this tertiary lymphoid structure in the priming of T cell responses during influenza A or herpesvirus infection (10,11). However, whether inhaled allergens can also trigger the clonal expansion of naïve T cells in the lung remains unknown.

Lymph node-deficient mice are useful tools in studying the requirement of the lymph node in inducing immune responses. Mice deficient in lymphotoxin α (LTα) exhibit abnormal development of peripheral lymphoid organs. These mice do not have detectable popliteal, inguinal, para-aortic, mesenteric, axillary, or cervical lymph nodes (12). LTα-deficient mice show normal counts of $CD4^+$ and $CD8^+$ T cells in peripheral blood. On the other hand, Lymphotoxin β (LTβ) -deficient mice have mesenteric and cervical lymph nodes (13). Tumor necrosis factor (TNF) is a member of TNF ligand family and is structurally similar to LTα and LTβ. *Lta/Ltb/Tnf* triple knock-out mice also have abnormal lymphoid development similar to that of the phenotype of LTα- or LTβ-deficient mice. Unlike single mutant mice, these mice have additional disruption to the spleen microarchitecture and exhibit impaired antibody responses to a T cell-dependent antigen (14). The retinoid acid-related orphan receptor gamma t (RORγt) is required for the development of lymphoid tissue inducer (LTi) cells and for the generation of T helper 17 (T_H17) cells (15). The *Rorgt*$^{gfp/gfp}$ mice are defective of all lymph nodes and Peyer's patches. The mice are protected from the experimental autoimmune encephalomyelitis due to the lack of tissue-infiltrating T_H17 cells (16,17).

In this study, we aimed to investigate whether the clonal expansion of allergen-specific T cells can occur in the lung by using lymph node-deficient *Rorgt*$^{gfp/gfp}$ mice and sphingosine-1-phosphate (S1P) antagonist FTY720 in an intranasal allergen-induced animal model of lung inflammation *in vivo*. Our findings demonstrate that the clonal expansion of naïve $CD4^+$ T cells can occur in the lung in response to the exposure of inhaled allergens.

MATERIALS AND METHODS

Mice

C57BL/6 mice were purchased from Orient. *Rorgt*$^{gfp/gfp}$, OT-II and B6.SJL ($CD45.1^+$) mice were purchased from Jackson Laboratory. OT-II mice were crossed with B6.SJL for adoptive transfer study. All mice were maintained under the semi-specific-pathogen-free facility in an animal center (Seoul National University). All animal experiments were performed using a protocol approved by the Institutional Animal Care and Use Committee Seoul National University (SNU-140602-2-10).

Allergen-induced lung inflammation

Mice were anesthetized with isoflurane and were intranasally administered with a mixture of 7 μg of proteinase from *Aspergillus oryzae* and 20 μg of ovalbumin (PAO/Ova) in 50 μL PBS every other day for a total of four times (day 0, 2, 4, 6). Sixteen hours after the last challenge, all mice were euthanized and the mediastinal lymph node, the lung and blood were collected for further analysis.

CFSE-labeled OT-II adoptive transfer

OVA-specific CD4 T cells were isolated from the lymph nodes and the spleens of B6.SJL OT-II mice by using a $CD4^+$ T cell Isolation Kit. The isolated $CD4^+$ T cells were labeled with 2 μM of CFSE and intravenously transferred into C57BL/6 mice. Next day, the recipients were intranasally injected with PAO/Ova (50 μg/7 μg). For kinetic analysis experiments, the mediastinal lymph node and the lung were dissected from mice after 36, 48, 60, 72 hours from challenge and the $CFSE^+$ cells were analyzed using flow cytometry.

FTY720 treatment

For T cell migration inhibition experiments, CFSE-labeled OT-II cells (5×10^6 cells/transfer) were transferred into C57BL/6 mice and then the mice were treated with intraperitoneal injection of FTY720 (1 mg/kg) or vehicle 6 hours after intranasal administration. Forty-eight hours after the intranasal administration, all mice were euthanized and the mediastinal lymph node, the lung and blood were obtained and analyzed by flow cytometry.

Isolation of lymphocytes from the lung and blood

For lymphocyte isolation from mouse lung, the lung was dissected into single lobes and cut into small pieces using the Gentle MACS Dissociator. These lobes were digested in RPMI 1640 medium containing 10% FBS, 0.5 mg/mL of collagenase IV, 2 mg/mL of dispase, and 2.5 μg/mL of DNase I for 30 minutes at 37ºC. The lung cells were filtered (100 μm) and then washed with PBS containing 1.5% FBS. The lymphocytes were isolated from whole lung cells or heparinized blood using Lymphocyte Separation Medium.

Flow cytometry

For the flow cytometry analysis, cells were stained with PE-Cyanine7-conjugated anti-mouse CD4 (RM4-5), PerCP/Cy5.5-conjugated anti-mouse CD45.2 (104), Pacific Blue-conjugated anti-mouse CD45.1 (A20) and APC-conjugated anti-mouse TCR Vα2 (B20.1). These cells were analyzed by FACSVerse and obtained data were analyzed using a software called Flowjo.

CD4+ T cell proliferation *in vitro*

$CD4^+$ cells were isolated from lymph nodes and spleen of wild-type and *Rorgt*$^{gfp/gfp}$ mice with $CD4^+$ T cell Isolation Kit and then $CD4^+CD62L^+CD25^-CD44^-$ cells were sorted using FACSAria III cell sorter. These isolated naïve T cells were labeled with 2 μM of CFSE. The CFSE-labeled naïve T cells (1×10^5/well) were co-cultured with bone marrow-derived dendritic cells (BMDC) (1×10^4/well) in RPMI-1640 supplemented with 10% FBS and soluble anti-CD3 (2C11, 0.5 μg/mL) for 72 hours.

Statistics

Data were analyzed with GraphPad Prism 7. Statistics were calculated with the two-tailed Student's t-test. p-values are presented within the figures or in the figure legend.

RESULTS

Analysis of CD4+ T cells in the lung of lymph node-deficient *Rorgt*$^{gfp/gfp}$ mice challenged with intranasal allergens

Draining lymph nodes harboring antigen-captured DCs act as primary sites of the proliferation of T cells specific to the antigen, termed clonal expansion. As a first step to determine if lymph nodes are necessary to initiate allergen-specific $CD4^+$ T cell responses in the airway, we employed *Rorgt*$^{gfp/gfp}$ mice in which all peripheral lymph nodes and Peyer's patches are absent due to the lack of LTi cells (Fig. 1A) (15). Intranasal challenges with proteinase from *Aspergillus oryzae* in combination with ovalbumin (PAO/Ova) have been used to induce allergic airway inflammation in mice (18-20). Total number of immune cells as well as the frequency of $CD4^+$ T cells in the lung of unchallenged mice was comparable between *Rorgt*$^{+/gfp}$ and *Rorgt*$^{gfp/gfp}$ mice (Fig. 1B and 1C). We intranasally challenged wild-type or *Rorgt*$^{gfp/gfp}$ mice with PAO/Ova every other day for a total of four times (Fig. 2A). One day after the last challenge, we analyzed immune cell

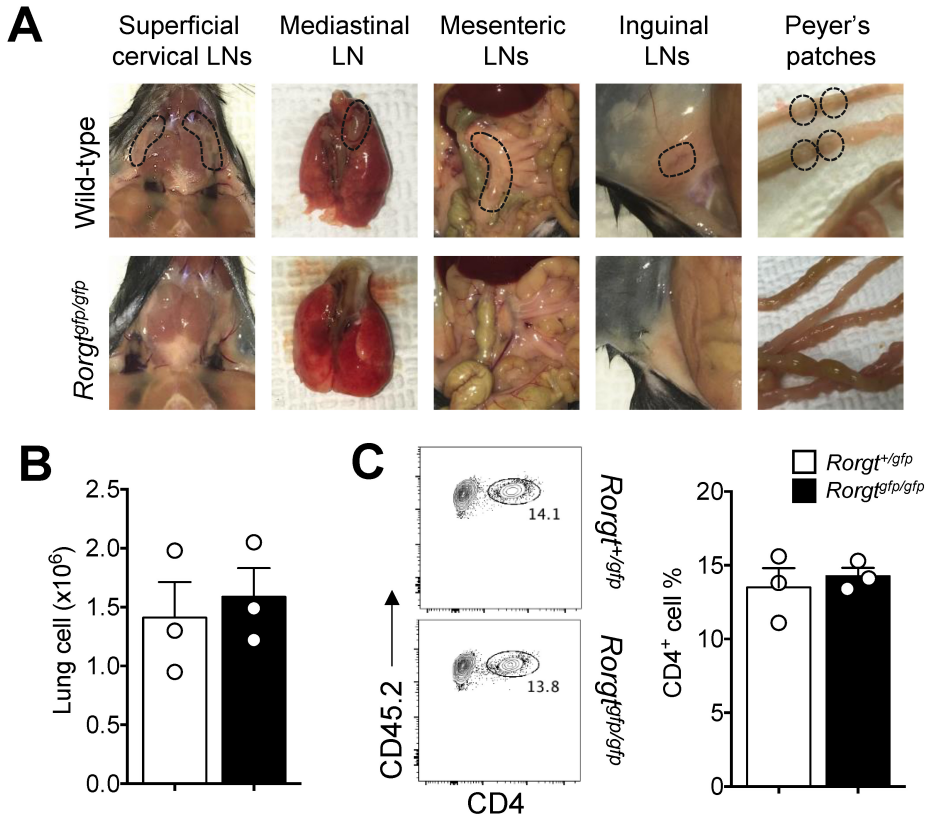

Figure 1. $CD4^+$ T cell population in the lung of *Rorgt*$^{gfp/gfp}$ mice in steady-state. (A) Representative pictures of the superficial cervical lymph nodes, the mLN, the mesenteric lymph nodes, the inguinal lymph nodes and the Peyer's patches of wild-type mice and *Rorgt*$^{gfp/gfp}$ mice. (B) The number of $CD45.2^+$ immune cells in the lungs of *Rorgt*$^{+/gfp}$ and *Rorgt*$^{gfp/gfp}$ mice and (C) the proportion of $CD4^+$ cells among $CD45.2^+$ cells in the lung of *Rorgt*$^{+/gfp}$ and *Rorgt*$^{gfp/gfp}$ mice before intranasal challenge. n=3 mice per group (B and C).

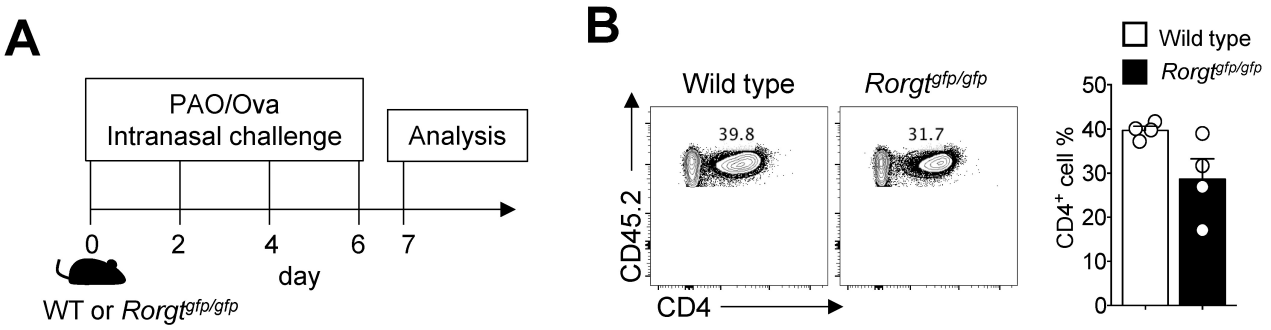

Figure 2. CD4$^+$ T cells are major immune cells in the lung of RORγt-deficient mice after intranasal challenges with allergens. (A) Experimental scheme. Wild-type mice and *Rorgt*$^{gfp/gfp}$ mice were intranasally injected with PAO/Ova every other day for 4 times and analyzed one day after the last challenge. (B) The proportion of CD4$^+$ cells among CD45.2$^+$ cells in the lung of wild-type mice and *Rorgt*$^{gfp/gfp}$ mice after intranasal challenges with PAO/Ova. Data are means±SEM. n=4 mice per group. Data are representative of two independent experiments.

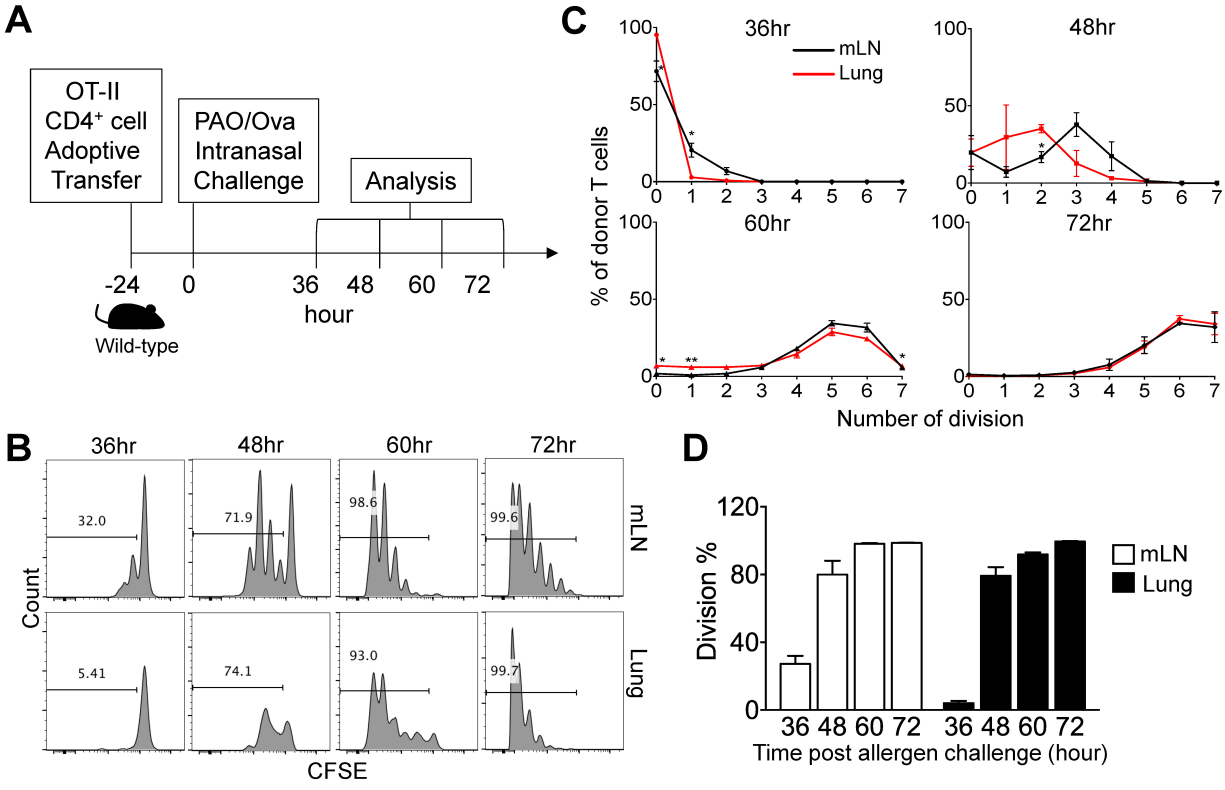

Figure 3. Kinetics of allergen-specific CD4$^+$ T cell proliferation in the BALT in response to intranasal allergen. (A) Experimental scheme. (B-D) Proliferation of OT-II T cells after intranasal PAO/Ova in the indicated tissue. CD45.1$^+$ donor T cells were gated and analyzed for CFSE dilution by flow cytometry. Data are means±SEM. *p<0.05 and **p<0.01 in comparison with the mLN. Data are representative of three independent experiments.

population in the airway and found that the majority of CD45.2$^+$ cells in the lung of *Rorgt*$^{gfp/gfp}$ mice were CD4$^+$ T cells. Although the result was not statistically significant, the frequency of CD4$^+$ T cells in the lung of *Rorgt*$^{gfp/gfp}$ mice was slightly lower in comparison to that of wild-type mice (Fig. 2B). These results suggest that the CD4$^+$ T cell responses against allergen challenges could occur in the airway even in the absence of lymph nodes.

Kinetic analysis of allergen-specific CD4$^+$ T cell division in the BALT upon intranasal allergen

To characterize the expansion of CD4$^+$ T cells in the bronchus-associated lymphoid tissues (BALT) against intranasally-challenged allergens, we performed kinetic

analysis of allergen-specific T cell proliferation to intranasal allergen. We adoptively transferred CFSE-labeled $CD4^+$ T cells from B6.SJL OT-II mice ($CD45.1^+CD45.2^+$) into the C57BL/6 recipients ($CD45.2^+$) before the recipients were challenged with a single dose of intranasal PAO/Ova. The dilution of CFSE in the donor T cells in the mediastinal lymph node (mLN) and the lung of the recipients was analyzed in 36, 48, 60 and 72 hours after the intranasal challenge (Fig. 3A). As shown in Fig. 3B, after 36 hours the evident division of OT-II T cells was observed in the mLN, but not in the lung. By 48 hours, the majority of donor OT-II T cells in the mLN as well as in the lung underwent at least one division (Fig. 3C and 3D). Interestingly, we observed no difference in the percentage of donor T cells that divided more than once between the mLN and the lung at 48 hours and thereafter. These data suggest that the expansion of allergen-specific $CD4^+$ T cells occurred primarily in the mLN, but the division number of donor cells in the mLN and in the lung was appeared to be comparable by 48 hours after allergen challenge.

Expansion of allergen-specific $CD4^+$ T cells in the lung of FTY720-treated mice

The observation in Fig. 2 and Fig. 3 suggested that the clonal expansion of allergen-specific $CD4^+$ T cells might occur in the lung in the absence of lymph nodes. To explore this hypothesis, we utilize an antagonist of S1P receptor, FTY720, which blocks the S1P-dependent egression of lymphocytes from the lymph nodes. FTY720 does not affect the proliferation of antigen-specific T cells (21,22). CFSE-labeled OT-II T cells ($CD45.1^+CD45.2^+$) were intravenously injected into C57BL/6 recipients and the recipients were intranasally challenged with PAO/Ova. Since FTY720 can also affect the migration of DCs (23-25), we injected FTY720 or vehicle into the recipients 6 hours after the intranasal challenge. Forty-eight hours after the challenge, we analyzed the dilution of the CFSE in the donor T cells (Fig. 4A). As depicted in Fig. 4B, the percentage of donor T cells in the mLN and the lung was found to be comparable between vehicle- and FTY720-treated groups. However, the frequency of donor T cells in the blood was profoundly lower in the FTY720-treated mice compared to that of vehicle-treated mice, indicating that such treatment efficiently inhibited the egression of allergen-specific T cells from the mLN.

Notably, we observed an evident donor OT-II T cell population that underwent at least one division in the lung of FTY720-treated recipients, and that the percentage of these dividing donor OT-II T cells was slightly, but significantly, higher in the FTY720-treated recipients than that of vehicle-treated recipients (Fig. 4C and 4D). Similar

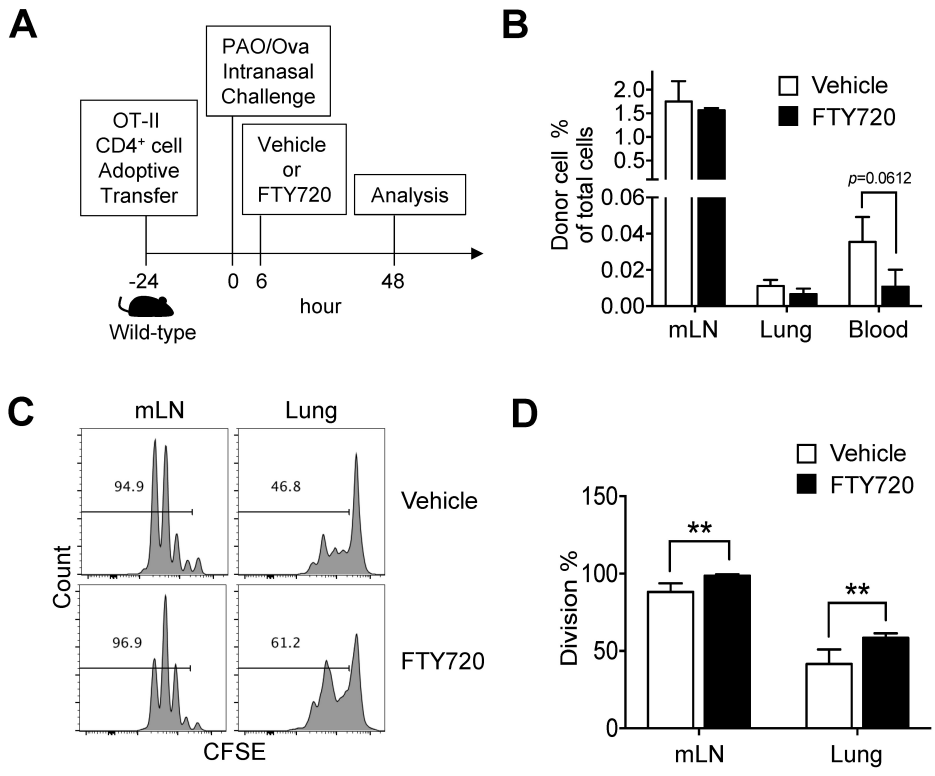

Figure 4. Expansion of allergen-specific $CD4^+$ T cell in the lung in mice treated with FTY720. (A) Experimental scheme. CFSE-labeled $CD45.1^+$OT-II T cells were transferred into wild-type mice ($CD45.2^{+/+}$) and the recipients were intranasally challenged with PAO/Ova. Six hours after the intranasal challenge, the mice were intraperitoneally injected with 1 mg/kg FTY720 or vehicle (n=3). (B-D) Forty-eight hours later, the frequency and the dilution of CFSE of donor T cells were analyzed in the indicated lymphoid tissues or blood. Data are means±SEM. **p<0.01 in comparison with vehicle-treated group. Data are representative of two independent experiments.

results were observed in the mLN of the recipients. These results demonstrate that the clonal expansion of allergen-specific CD4$^+$ T cells in response to intranasal allergens can occur simultaneously in the lung and in the mLN in mice treated with FTY720.

Expansion of allergen-specific CD4$^+$ T cells in the lung of *Rorgt*$^{gfp/gfp}$ mice

The observed division of OT-II T cells in the lung of FTY720-treated mice in Fig. 4 prompted us to hypothesize that the clonal expansion of allergen-specific CD4$^+$ T cells could be initiated in the absence of egression of activated T cells from the mLN *in vivo*. To explore this possibility in a definitive *in vivo* system, we asked if the expansion of allergen-specific CD4$^+$ T cells in the lung could occur in *Rorgt*$^{gfp/gfp}$ mice lacking lymph nodes. We performed a similar adoptive transfer study with CFSE-labeled OT-II T cells (CD45.1$^+$CD45.2$^+$) into wild-type or *Rorgt*$^{gfp/gfp}$ mice, and the recipients were intranasally challenged with PAO/Ova (Fig. 5A).

Consistent with the observation in Fig. 4, we observed an evident division of the donor OT-II T cells in the lung of *Rorgt*$^{gfp/gfp}$ mice (Fig. 5B). Unexpectedly, the frequency of donor T cells that underwent at least one division in the lung of *Rorgt*$^{gfp/gfp}$ mice was significantly higher than that of wild-type mice (Fig. 5B). We observed no difference in the proliferation of wild-type and *Rorgt*$^{gfp/gfp}$ CD4$^+$ T cells *in vitro* (Fig. 5C). This indicates that the division of OT-II T cells in the lung was accelerated in the *Rorgt*$^{gfp/gfp}$ mice, probably due to the lack of mLN. Collectively, these results imply that the clonal expansion of allergen-specific CD4$^+$ T cells occurred in the lung independently of the lymph nodes *in vivo*.

DISCUSSION

DCs residing in the lung capture inhaled allergens and migrate into the draining lymph nodes. The allergen-specific adaptive immune responses are initiated by the interaction between these allergen-bearing DCs and allergen-specific CD4$^+$T cells in the lymph nodes, which results in an expansion of the allergen-specific T cell clones. However, whether lung can act as a primary site for the clonal expansion of allergen-specific CD4$^+$ T cell has been unclear. Our findings in the present study collectively indicate that the clonal expansion of allergen-specific CD4$^+$ T cells could occur in the lung in the absence of the lymph nodes, since the division of allergen-specific CD4$^+$ T cell takes place in the lung of mice treated with an S1P receptor antagonist FTY720 as well as in the lung of *Rorgt*$^{gfp/gfp}$ mice lacking all peripheral lymph nodes. Hence, although the draining lymph nodes are the primary sites of clonal expansion of T cells under steady-state, allergen-specific clonal expansion can also occur in the absence of the lymph nodes *in vivo*.

Immature DCs residing in the lung capture invading antigens in the airway and migrate into the draining lymph nodes through chemotaxis where they differentiate antigen-specific naïve T cells into effector T cells by

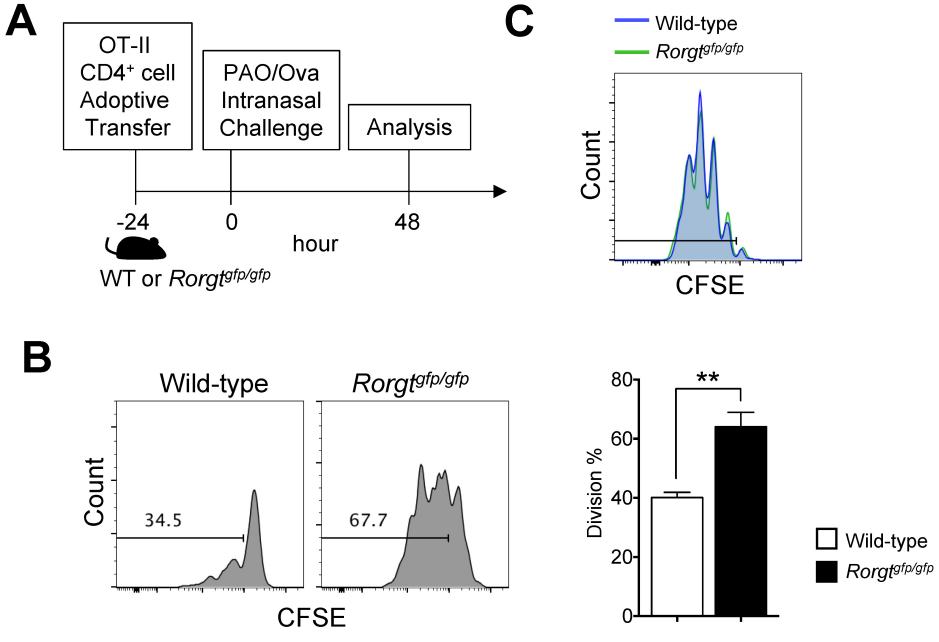

Figure 5. Expansion of allergen-specific CD4$^+$ T cell in the lung of *Rorgt*$^{gfp/gfp}$ mice. (A) Experimental scheme (n=3). (B) Forty-eight hours after the challenge, the proportion of divided cells among CFSE-labeled donor T cells was analyzed by flow cytometry. Numbers on the line indicate the percentage of divided cells. (C) Seventy-two hours after co-culture of the indicated T cells with BMDC in the presence of anti-CD3, CFSE-dilution was measured to determine the proliferation of the T cells. Data are means±SEM. **p<0.01 in comparison with wild-type mice. Data are representative of two independent experiments.

providing signal 1, 2 and 3. Effector T cells then leave the lymph nodes and migrate into the periphery to mediate adaptive immunity (26). Aside from the lymph nodes, tertiary lymphoid structures can be developed in the lung in case of chronic inflammation, infection and cancer. These tertiary lymphoid structures are ectopic lymphoid-like structures that consist of T cells and B cells (27,28). Like the lymph nodes, antigen-specific T cell responses are known to be induced in these tertiary lymphoid structures (27). In this regard, it is noteworthy that secondary lymphoid organs are not essential for the maintenance of immunologic memory and the utility of iBALT in a murine model of herpesvirus MHV-68 infection (11). In line with this study, our results in the present study showed that the expansion of allergen-specific T cell occurs in the lung in the absence of lymph nodes in *Rorgt*$^{gfp/gfp}$ mice and in the lung of FTY720-treated mice after intranasal challenges with allergens. It has been suggested that CD11c$^+$ DCs are essential for the efficient priming of T cell responses against influenza A infection in the tertiary lymphoid structure. By contrast, it has been also shown that CD11c$^+$ DCs are not necessary for the maintenance of tertiary lymphoid structures in an allergic airway inflammation model (10,11). The type(s) of antigen presenting cells that trigger clonal expansion of naïve CD4$^+$ T cells in the lung in the present study is unclear at the moment.

Treatment with FTY720 has been shown to suppress the T helper 1- and T helper 2-driven lung inflammation by reducing migration of T cells from the mLN into the lung and by reducing the migration of lung DCs to the mLN (29,30). Although these prior findings seem to contradict the observed expansion of allergen-specific CD4$^+$ T cells in the lung of FTY720-treated mice and *Rorgt*$^{gfp/gfp}$ mice, the question of whether the clonal expansion of allergen-specific CD4$^+$ T cells can occur in the lung in the absence of the lymph nodes has not been addressed. Similar to our findings, it has been also shown that RORγt-deficient mice harbored an increased number of iBALT in response to influenza A virus infections (31). Taken together, these prior studies and the present study suggest that, when the lymph nodes are absent, allergen-bearing DCs in the lung could trigger T cell clonal expansion in the lung, probably by interacting circulating T cells. Further studies are necessary to dissect the exact cellular and spatial mechanisms of the interaction between DCs and naïve T cells in the lung.

In summary, the present study unveils that the clonal expansion of CD4$^+$ T cells occurs in the lung in response to intranasal allergens in the absence of the lymph nodes *in vivo*. The contribution of this lymph node-independent expansion of allergen-specific T cells on the development of allergic airway inflammation needs to be determined.

ACKNOWLEDGEMENTS

The work is supported by a research grant HI14C2282 from the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea.

CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

REFERENCES

1. Mueller, D. L., M. K. Jenkins, and R. H. Schwartz. 1989. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu. Rev. Immunol.* 7: 445-480.
2. Mittler, J. N., and W. T. Lee. 2004. Antigen-specific CD4 T cell clonal expansion and differentiation in the aged lymphoid microenvironment. II. The memory T cell response is diminished. *Mech. Ageing Dev.* 125: 59-68.
3. Bousso, P. 2008. T-cell activation by dendritic cells in the lymph node: lessons from the movies. *Nat. Rev. Immunol.* 8: 675-684.
4. Gunzer, M., C. Weishaupt, A. Hillmer, Y. Basoglu, P. Friedl, K. E. Dittmar, W. Kolanus, G. Varga, and S. Grabbe. 2004. A spectrum of biophysical interaction modes between T cells and different antigen-presenting cells during priming in 3-D collagen and *in vivo*. *Blood* 104: 2801-2809.
5. Boyman, O., and J. Sprent. 2012. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat. Rev. Immunol.* 12: 180-190.
6. Chen, L., and D. B. Flies. 2013. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat. Rev. Immunol.* 13: 227-242.
7. Corthay, A. 2006. A three-cell model for activation of naive T helper cells. *Scand. J. Immunol.* 64: 93-96.
8. Britschgi, M. R., A. Link, T. K. Lissandrin, and S. A. Luther. 2008. Dynamic modulation of CCR7 expression and function on naive T lymphocytes in vivo. *J. Immunol.* 181: 7681-7688.
9. MartIn-Fontecha, A., S. Sebastiani, U. E. Hopken, M. Uguccioni, M. Lipp, A. Lanzavecchia, and F. Sallusto. 2003. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. *J. Exp. Med.* 198:

615-621.
10. GeurtsvanKessel, C. H., M. A. Willart, I. M. Bergen, L. S. van Rijt, F. Muskens, D. Elewaut, A. D. Osterhaus, R. Hendriks, G. F. Rimmelzwaan, and B. N. Lambrecht. 2009. Dendritic cells are crucial for maintenance of tertiary lymphoid structures in the lung of influenza virus-infected mice. *J. Exp. Med.* 206: 2339-2349.
11. Halle, S., H. C. Dujardin, N. Bakocevic, H. Fleige, H. Danzer, S. Willenzon, Y. Suezer, G. Hammerling, N. Garbi, G. Sutter, T. Worbs, and R. Forster. 2009. Induced bronchus-associated lymphoid tissue serves as a general priming site for T cells and is maintained by dendritic cells. *J. Exp. Med.* 206: 2593-2601.
12. De, T. P., J. Goellner, N. H. Ruddle, P. R. Streeter, A. Fick, S. Mariathasan, S. C. Smith, R. Carlson, L. P. Shornick, J. Strauss-Schoenberger, and. 1994. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. *Science* 264: 703-707.
13. Koni, P. A., R. Sacca, P. Lawton, J. L. Browning, N. H. Ruddle, and R. A. Flavell. 1997. Distinct roles in lymphoid organogenesis for lymphotoxins alpha and beta revealed in lymphotoxin beta-deficient mice. *Immunity* 6: 491-500.
14. Kuprash, D. V., M. B. Alimzhanov, A. V. Tumanov, S. I. Grivennikov, A. N. Shakhov, L. N. Drutskaya, M. W. Marino, R. L. Turetskaya, A. O. Anderson, K. Rajewsky, K. Pfeffer, and S. A. Nedospasov. 2002. Redundancy in tumor necrosis factor (TNF) and lymphotoxin (LT) signaling in vivo: mice with inactivation of the entire TNF/LT locus versus single-knockout mice. *Mol. Cell. Biol.* 22: 8626-8634.
15. Eberl, G., S. Marmon, M. J. Sunshine, P. D. Rennert, Y. Choi, and D. R. Littman. 2004. An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. *Nat. Immunol.* 5: 64-73.
16. Ivanov, I. I., B. S. McKenzie, L. Zhou, C. E. Tadokoro, A. Lepelley, J. J. Lafaille, D. J. Cua, and D. R. Littman. 2006. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 126: 1121-1133.
17. Sun, Z., D. Unutmaz, Y. R. Zou, M. J. Sunshine, A. Pierani, S. Brenner-Morton, R. E. Mebius, and D. R. Littman. 2000. Requirement for RORgamma in thymocyte survival and lymphoid organ development. *Science* 288: 2369-2373.
18. Choi, G., and Y. Chung. 2016. Blockade of STAT3 in T cells inhibits germinal center reactions against intranasal allergens. *Biomol. Ther. (Seoul.)* 24: 244-251.
19. Lim, H., M. Cho, G. Choi, H. Na, and Y. Chung. 2015. Dynamic control of Th2 cell responses by STAT3 during allergic lung inflammation in mice. *Int. Immunopharmacol.* 28: 846-853.
20. Kheradmand, F., A. Kiss, J. Xu, S. H. Lee, P. E. Kolattukudy, and D. B. Corry. 2002. A protease-activated pathway underlying Th cell type 2 activation and allergic lung disease. *J. Immunol.* 169: 5904-5911.
21. Matloubian, M., C. G. Lo, G. Cinamon, M. J. Lesneski, Y. Xu, V. Brinkmann, M. L. Allende, R. L. Proia, and J. G. Cyster. 2004. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 427: 355-360.
22. Brinkmann, V., A. Billich, T. Baumruker, P. Heining, R. Schmouder, G. Francis, S. Aradhye, and P. Burtin. 2010. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat. Rev. Drug Discov.* 9: 883-897.
23. Ciabattini, A., E. Pettini, F. Fiorino, G. Prota, G. Pozzi, and D. Medaglini. 2011. Distribution of primed T cells and antigen-loaded antigen presenting cells following intranasal immunization in mice. *PLoS One* 6: e19346.
24. Reines, I., M. Kietzmann, R. Mischke, T. Tschernig, A. Luth, B. Kleuser, and W. Baumer. 2009. Topical application of sphingosine-1-phosphate and FTY720 attenuate allergic contact dermatitis reaction through inhibition of dendritic cell migration. *J. Invest. Dermatol.* 129: 1954-1962.
25. Lan, Y. Y., C. A. De, B. L. Colvin, M. Abe, V. Brinkmann, P. T. Coates, and A. W. Thomson. 2005. The sphingosine-1-phosphate receptor agonist FTY720 modulates dendritic cell trafficking *in vivo*. *Am. J. Transplant.* 5: 2649-2659.
26. Krummel, M. F., F. Bartumeus, and A. Gerard. 2016. T cell migration, search strategies and mechanisms. *Nat. Rev. Immunol.* 16: 193-201.
27. Pitzalis, C., G. W. Jones, M. Bombardieri, and S. A. Jones. 2014. Ectopic lymphoid-like structures in infection, cancer and autoimmunity. *Nat. Rev. Immunol.* 14: 447-462.
28. Germain, C., S. Gnjatic, and M. C. eu-Nosjean. 2015. Tertiary lymphoid structure-associated B cells are key players in anti-tumor immunity. *Front. Immunol.* 6: 67.
29. Sawicka, E., C. Zuany-Amorim, C. Manlius, A. Trifilieff, V. Brinkmann, D. M. Kemeny, and C. Walker. 2003. Inhibition of Th1- and Th2-mediated airway inflammation by the sphingosine 1-phosphate receptor agonist FTY720. *J. Immunol.* 171: 6206-6214.
30. Idzko, M., H. Hammad, N. M. van, M. Kool, T. Muller, T. Soullie, M. A. Willart, D. Hijdra, H. C. Hoogsteden, and B. N. Lambrecht. 2006. Local application of FTY720 to the lung abrogates experimental asthma by altering dendritic cell function. *J. Clin. Invest* 116: 2935-2944.
31. Lochner, M., C. Ohnmacht, L. Presley, P. Bruhns, M. Si-Tahar, S. Sawa, and G. Eberl. 2011. Microbiota-induced tertiary lymphoid tissues aggravate inflammatory disease in the absence of RORgamma t and LTi cells. *J. Exp. Med.* 208: 125-134.