

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Implications of nasopharynx-associated lymphoid tissue (NALT) in the development of allergic responses in an allergic rhinitis mouse model

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Abstract

Background: Nasopharynx-associated lymphoid tissue (NALT) serves as an important inductive site for mucosal immunity in the upper respiratory tract. Despite its importance in the mucosal immune system, little is known regarding the role of NALT in airway allergic immune responses. We aimed to elucidate the role of NALT in the induction of upper airway allergic responses in a mouse model.

Methods: Inhibitor of DNA binding/differentiation 2 (Id2)^{-/-} and Id2^{+/-} mice was exposed to the ovalbumin (OVA)-induced allergic rhinitis model, because the former resulted in the NALT deficiency. The allergic parameters, such as allergic symptoms, serum OVA-specific immunoglobulin E (IgE) levels, eosinophil infiltration, and cytokine profiles in the nasal mucosa, were compared between Id2^{-/-} and Id2^{+/-} groups.

Results: NALT-null, Id2^{-/-} mice displayed significantly lower allergic responses compared with Id2^{+/-} mice, as demonstrated by lower levels of allergic symptoms, serum OVA-specific IgE, eosinophilic infiltration, and local Th2 cytokine transcriptions. To determine which of two factors, that is, the absence of NALT or the alteration of immunocompetent cell populations caused by the Id2 deficiency, has a larger effect on the attenuated allergic immune responses in Id2^{-/-} mice, lethally irradiated Id2^{-/-} mice were engrafted with C57BL/6 wild-type bone marrow cells and showed still significantly lower allergic immune responses compared with equally treated Id2^{+/-} mice. In addition, IgE class switch recombination-associated molecules, such as ϵ immunoglobulin heavy-chain germline gene transcript, ϵ mRNA, and activation-induced cytidine deaminase mRNA, were detected in NALT from OVA-sensitized wild-type mice.

Conclusion: These results show the critical role of NALT for the induction of allergic responses in the upper airway at least in part by means of class switching to IgE *in situ*.

Abbreviations:

AID, activation-induced cytidine deaminase; BM, bone marrow; CLN, cervical lymph node; CSR, class switch recombination; GFP, green fluorescence protein; i.n., intranasal; i.p., intraperitoneal; Id2, inhibitor of DNA binding/differentiation 2; IgE, immunoglobulin E; NALT, nasopharynx-associated lymphoid tissues; OVA, ovalbumin; ϵ mRNA, mRNA encoding the heavy chain of IgE; ϵ GLT, ϵ immunoglobulin heavy-chain germline gene transcript.

Nasopharynx-associated lymphoid tissue (NALT) is an organized lymphoid structure that is present on both sides of the nasopharyngeal duct, dorsal to the cartilaginous soft palate of mice, which is considered to be equivalent to Waldeyer's ring in humans (1). NALT is an important inductive site for the induction and regulation of mucosal immunity in the upper respiratory tract, similar to Peyer's patch (PP) in the intestinal immune system (2). Nasal immunization with an antigen and cholera toxin as a mucosal adjuvant has been shown to generate antigen-specific IgA-producing cells in the nasal passages and distal mucosal sites, including the genitourinary and intestinal tracts (3, 4).

Despite our knowledge on the importance of NALT in the mucosal immune system, little is known regarding its role in upper airway allergic immune responses. There has been only one study to date on the kinetics of antigen presentation in the lymphoid structures of the upper respiratory tract. Following intranasal application, auto-fluorescent dye was detected in the NALT and cervical lymph nodes (CLN) within 4 and 8 h, respectively. The uptake of dye in the NALT was more rapid and persisted for a longer period of time, compared with the uptake in the CLN, suggesting a prominent role for NALT in allergen processing of the upper airways (5). However, no direct relationship between NALT and airway allergic immune responses has been uncovered.

The major goal of our study was to elucidate the role of NALT in allergic immune responses in the upper airway, using inhibitor of DNA binding/differentiation 2 ($Id2^{-/-}$) mice, which lack NALT, as an allergic rhinitis model, and directly demonstrating class switch recombination (CSR) to immunoglobulin E (IgE) and IgE synthesis in NALT of allergen-sensitized mice by RT-PCR.

Methods

Animals

57BL/6 mice were purchased from SLC (Shizuoka, Japan). The $Id2^{-/-}$ mice (129/Sv) were generated previously by Yoshifumi Yokota (6). $Id2^{-/-}$ mice lack lymph nodes, PPs, and NALT and show a greatly reduced population of natural killer (NK) cells, which is because of an intrinsic defect in

NK cell precursors. By contrast, $Id2^{+/-}$ mice have NALT and normal population of immune cells.

Allergen sensitization and nasal challenge

On days 0, 7, and 14, the mice were sensitized by intraperitoneal (i.p.) injection of 25 μ g of ovalbumin (OVA) and 1 mg of alum in 300 μ l of phosphate-buffered saline (PBS). One week after the last OVA sensitization (Day 21), the mice received a series of seven daily intranasal (i.n.) OVA (500 μ g) challenges (Fig. 1).

Allergic symptoms

For 20 min after the final nasal challenge with OVA, sneezing frequencies and nasal rubbing events were recorded for each mouse by blinded observers, over a 5-min interval. The mice were then killed 24 h after the last nasal challenge for further analyses (7).

Nasal histology

Twenty-four hours after the final OVA nasal challenge, nasal passage tissues were surgically removed from the mice, fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Under high magnification ($\times 400$), infiltrating eosinophils were counted in the nasal septal mucosa.

OVA-specific immune parameters

To measure total and OVA-specific IgE, serum samples collected from the mice at the time of killing were serially diluted and added to 96-well plates coated with purified anti-mouse IgE mAb (clone R35-72; BD PharmingenTM, San Jose, CA, USA). A purified mouse IgE isotype (27-74; BD PharmingenTM) was used as a standard. To detect total IgE, HRP-conjugated anti-mouse IgE (23G3; Southern Biotechnology, Birmingham, AL, USA) was added to the plate. To detect OVA-specific IgE, biotin-labeled OVA was added, followed by HRP-labeled anti-biotin (Vector Laboratories, Burlingame, CA, USA). The reactions were developed using 3,3',5,5'-tetramethylbenzidine (Moss Inc., Belfast, ME, USA)

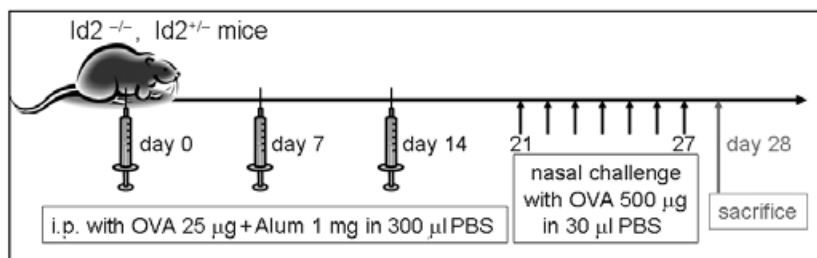


Figure 1 Allergic rhinitis model. On days 0, 7, and 14, inhibitor of DNA binding/differentiation 2 mice received an intraperitoneal (i.p.) injection of 25 μ g of ovalbumin (OVA) and 1 mg of alum in 300 μ l of phosphate-buffered saline. One week after the last OVA

sensitization (day 21), a series of seven daily intranasal (i.n.) OVA (500 μ g) challenges were administered. The mice were killed 24 h after the last nasal challenge for further analyses.

and were terminated by adding 2 N H₂SO₄. The optical density (OD) was recorded by a luminometer (iEMS Reader; Labsystems, Helsinki, Finland) set at 450 nm. The end-point titers of OVA-specific IgE are expressed as the reciprocal log₂ of the last dilution of a sample that resulted in an OD value 0.1 higher than background.

Real-time RT-PCR for IL-4, IL-5, IL-13, and IFN- γ in the nasal mucosa

Total RNA was prepared from the nasal mucosa with TriZol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized using superscript reverse transcriptase (Invitrogen) and oligo(dT) primers (Fermentas, Burlington, ON, Canada). For analysis of IL-4 (Mm00445258_g1), IL-5 (Mm00439646-m1), IL-13 (Mm4331182-m1), IFN- γ (Mm99999071-m1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Mm03302249_g1), PDAR (Pre-Developed Assay Reagent) kits of primers and probes were purchased from Applied Biosystems (Foster City, CA, USA). Amplification of IL-4, IL-5, IL-13, IFN- γ , and GAPDH cDNA was carried out in MicroAmp optical 96-well reaction plates (Applied Biosystems). The reaction was performed using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The average transcript levels of genes were then normalized to GAPDH.

Irradiation and bone marrow (BM) transfer

For radiation chimera experiments, a previously reported protocol was adopted (8). Recipient mice (Id2 or C57BL/6) were lethally irradiated with 1100 rad (Gamma cell; Nordion International Inc., Kanata, ON, Canada). The recipient mice were anesthetized immediately following irradiation and were transplanted with 2×10^6 BM cells derived from donor mice (C57BL/6 or Id2). Specifically, the BM cells in 100 μ l of medium were transplanted by retro-orbital intravenous injection with a 26-gauge needle. At 6 week after transplantation, flow cytometric analysis from C57BL/6 mice, which were irradiated and engrafted with BM cells derived from green fluorescence protein (GFP)-transgenic mice, revealed that 90–95% of the immune system of the recipient mice had been reconstituted with GFP-positive donor hematopoietic cells (data not shown).

Allergic sensitization began 6 week after transplantation. Allergic symptoms and nasal histology were assessed, and total IgE and OVA-specific IgE were measured as described above.

RNA isolation and RT-PCR

Total RNA was isolated from NALT of OVA-sensitized and PBS-administered mice using the Trizol Reagent (Invitrogen) following the manufacturer's instructions. RNA was suspended in RNase-free water and quantified by spectrophotometry at 260 nm. RNA was reverse transcribed to cDNA, and ϵ immunoglobulin heavy-chain germline gene transcript (ϵ GLT), mRNA encoding the heavy chain of IgE (ϵ mRNA), and activation-induced cytidine deaminase (AID) were PCR-amplified as described elsewhere (9). The primers used for

PCR were as follows: the oligonucleotide primers specific for ϵ GLT (sense, 5'-TGGGATCAGACGATGGAGAATAG-3'; antisense, 5'-CCAGGGTCATGGAAGCAGTG-3'); the oligonucleotide primers specific for ϵ mRNA (sense, 5'-TACGACGAGAACGGGTTTGCTTAC-3'; antisense, 5'-AGTTCACAGTGCTCATGTTTCAG-3'); the oligonucleotide primers specific for AID (sense, 5'-GGCTGAGGTTAGGGTTCCATCTCAG-3'; antisense, 5'-GAGGGAGTCAAGAAAGTCACTGGA-3'); the oligonucleotide primers specific for GAPDH (sense, 5'-ACCACAGTCCATGCCATCAC-3'; antisense, 5'-TCCACCACCCTGTTGCTGTA-3').

Statistical analysis

Data are expressed as means $\pm$ SD. All data were analyzed by a Mann–Whitney *U*-test. *P* values <0.05 were considered to be statistically significant.

Results

Decreased antigen-specific allergic immune responses in Id2-deficient mice

After the nasal challenge of sensitized mice, Id2^{-/-} mice sneezed and rubbed their noses significantly less frequently than Id2^{+/-} mice did (Fig. 2A,B). Moreover, the serum level of OVA-specific IgE was significantly lower in Id2^{-/-} mice than in Id2^{+/-} mice (Fig. 2D), while Id2^{-/-} mice had a significantly higher total IgE levels as described previously (6) (Fig. 2C). The numbers of eosinophils infiltrating the nasal mucosa per high-magnification field were significantly lower in Id2^{-/-} mice than in Id2^{+/-} mice (Fig. 2E–G).

mRNA expressions of the Th2 cytokines, IL-4 and IL-13, significantly decreased in the nasal mucosa of Id2^{-/-} mice as compared to Id2^{+/-} mice (*P* < 0.05, Fig. 2H,J). On the contrary, mRNA expressions of IL-5 and the Th1 cytokine, IFN- γ , did not differ significantly between Id2^{-/-} and Id2^{+/-} mice (Fig. 2I,K).

Critical role of NALT for the induction of upper airway allergic responses

Adoptive transfer of C57BL/6 wild-type BM cells into lethally irradiated 129/Sv Id2^{-/-} mice did not lead to the formation of NALT, although the immunocompetent cells were replaced by wild type (WT) cells (data not shown). When the engrafted Id2 mice were sensitized with OVA, clinical symptoms of sneezing and nose rubbing were significantly less frequent in the reconstituted Id2^{-/-} mice than similarly treated Id2^{+/-} mice (Fig. 3A,B). The reconstituted Id2^{-/-} mice also had a significantly lower serum OVA-specific IgE level (Fig. 3D) and fewer eosinophils infiltration in the nasal mucosa than similarly treated Id2^{+/-} mice (Fig. 3E). By contrast, the reconstituted Id2^{-/-} mice still had a significantly higher total serum IgE level than Id2^{+/-} mice (Fig. 3C).

Lethally irradiated C57BL/6 wild-type mice that were transplanted with BM cells from Id2^{-/-} or Id2^{+/-} mice did not show any significant differences in the frequencies of

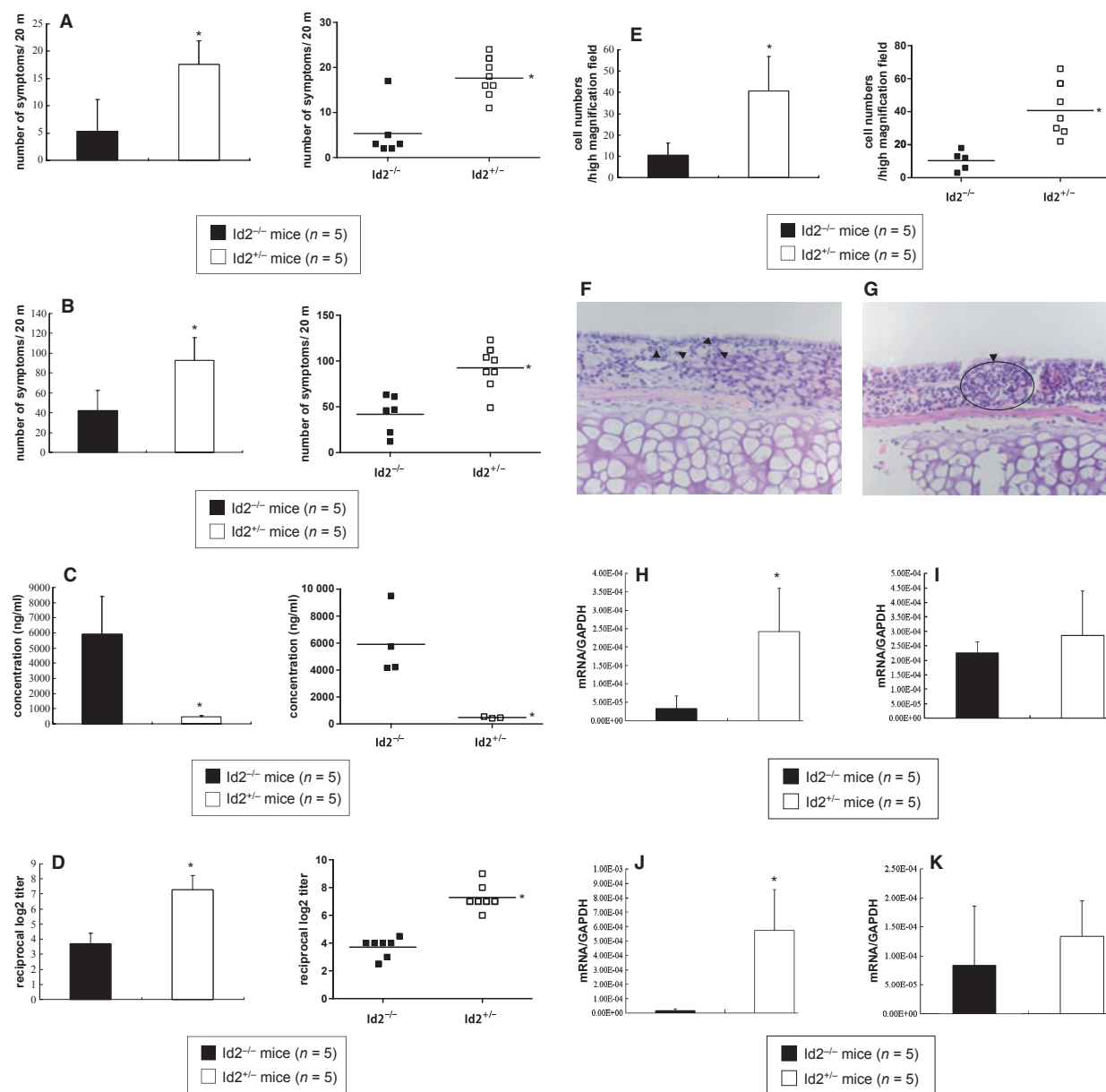


Figure 2 Comparison of nasal symptoms, serum immunoglobulin E (IgE) levels, eosinophilic infiltration, and local cytokine expression levels between $\text{Id2}^{-/-}$ and $\text{Id2}^{+/+}$ mice. A, sneezing; B, nasal rubbing; C, total serum IgE level; D, serum ovalbumin-specific IgE level; E, eosinophilic infiltration (A–E, the graph on the left represents the mean values with error bars. The graph on the right represents individual data with median values); F, G, The representative H

& E staining pictures under 400-fold magnification field demonstrate that a few eosinophils (arrow heads) are seen in the nasal submucosal tissue of $\text{Id2}^{-/-}$ mice (F), whereas a lot of eosinophils (in the circle) congregate in $\text{Id2}^{+/+}$ mice submucosal tissue (G); H–K, IL-4 (H), IL-5 (I), IL-13 (J), and IFN- γ (K) mRNA expression levels in the nasal mucosa. * $P < 0.05$. Id2 , inhibitor of DNA binding/differentiation 2.

sneezing and nasal rubbing (Fig. 4A,B), and in the levels of serum OVA-specific IgE antibodies (Fig. 4D). The total serum IgE levels were significantly higher in the WT mice transplanted with $\text{Id2}^{-/-}$ mice marrow cells than those from $\text{Id2}^{+/+}$ mice (Fig. 4C), while the wild-type mice transplanted with $\text{Id2}^{+/+}$ mice marrow cells had a significantly more severe eosinophilic infiltration (Fig. 4E).

Expression of class switch recombination molecules to IgE in NALT

ϵ Germline transcription is shown to be the first step for the commitment of B cells to become IgE-producing cells (10). When the ϵ GLT expression was examined in NALT, its expression was detected in the tissue from OVA-sensitized

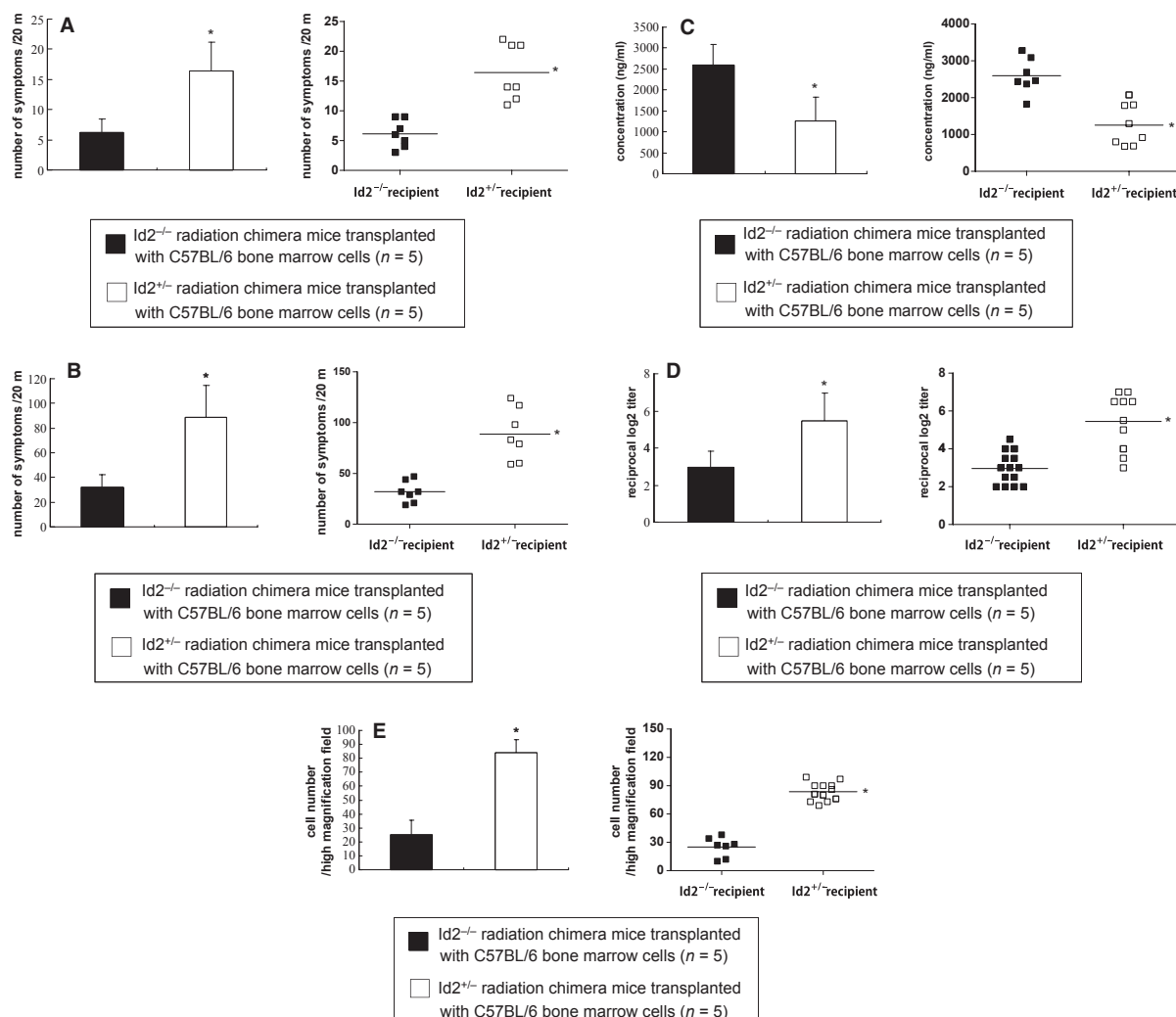


Figure 3 Comparison of nasal symptoms, serum immunoglobulin E (IgE) level, and eosinophilic infiltration between lethally irradiated Id2^{-/-} and Id2^{+/-} mice, transplanted with bone marrow cells from C57BL/6 wild-type mice. A, sneezing; B, nasal rubbing; C, total serum IgE level; D, serum ovalbumin-specific IgE level; E, eosinophilic

infiltration (A–E, the graph on the left represents the mean values with error bars. The graph on the right represents individual data with median values). **P* < 0.05. Id2, inhibitor of DNA binding/differentiation 2.

WT mice (Fig. 5A). IgE synthesis by B cells is the final step in the production of IgE. When NALT of WT mice with allergic rhinitis was examined, the presence of ϵ heavy-chain-specific mRNA was detected in OVA-sensitized mice, but not those from negative control (Fig. 5B). To further support for the induction of IgE commitment in NALT of OVA-sensitized WT mice, mRNA expression of isotype CSR molecule of AID was markedly increased in mice with allergic rhinitis when compared with control mice (Fig. 5C).

Discussion

NALT contains all the necessary lymphoid cells, including T cells, B cells, and antigen-presenting cells, for the induction and regulation of inhaled antigen-specific mucosal immune

responses (11, 12). Additionally, these tissues are rich in Th0-type CD4⁺ T cells, which can develop into either Th1- or Th2-type cells (13). NALT also provides a molecular and cellular environment that allows for a CSR of μ to α genes, leading to the generation of IgA-committed B cells and the induction of memory B cells (14, 15). It is thus widely accepted that NALT is a key player in the uptake of nasally delivered antigens and leads to the subsequent induction of antigen-specific IgA immune responses (11).

It is reasonable to hypothesize that allergic immune responses to inhaled allergens can be induced by NALT in a similar manner to the induction of mucosal immune responses. Our group reported that Th2-inducer-type CD8 α ⁻ CD11b⁺ myeloid dendritic cells (m-DCs) increased in NALT after nasal challenge with OVA. We also showed that lymphoid

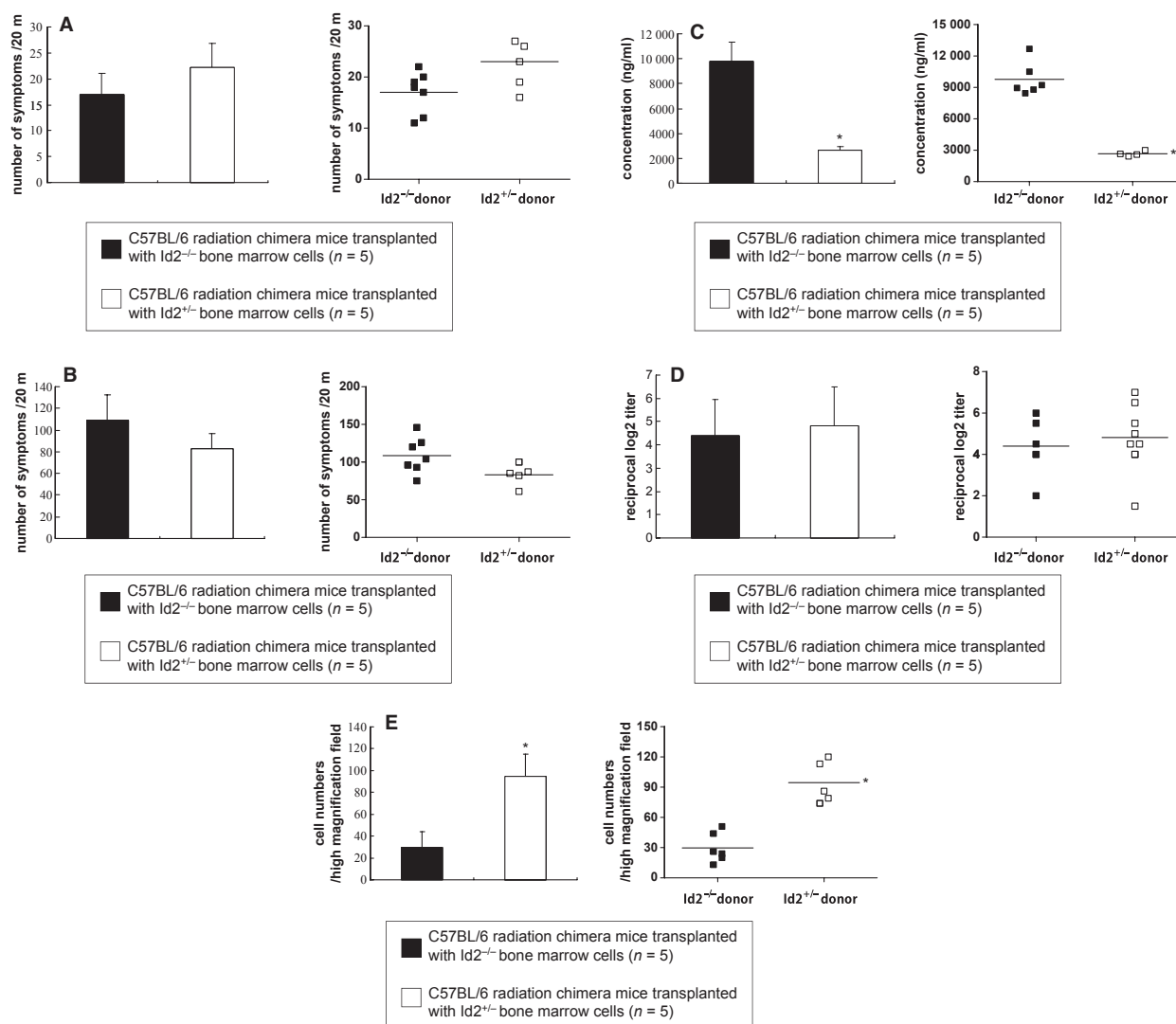


Figure 4 Comparison of nasal symptoms, serum immunoglobulin E (IgE) level, and eosinophilic infiltration between lethally irradiated C57BL/6 wild-type mice, transplanted with bone marrow cells from $Id2^{-/-}$ and $Id2^{+/+}$ mice. A, sneezing; B, nasal rubbing; C, total serum IgE level; D, serum ovalbumin-specific IgE level; E, eosinophilic

infiltration (A–E, the graph on the left represents the mean values with error bars. The graph on the right represents individual data with median values). * $P < 0.05$. $Id2$, inhibitor of DNA binding/differentiation 2.

chemokines CCL19 and CCL21 are involved in the recruitment of CCR7 expressing naturally occurring Tregs in NALT and help suppress the pathological Th2 environment induced by m-DCs during the development of allergic rhinitis (16). However, the direct role of NALT in the induction of upper airway allergic immune responses has not been elucidated.

Inhibitor of DNA binding/differentiation 2 is a transcription factor inhibitor with a basic helix-loop-helix motif that has been shown to be crucial for various cell differentiation processes. As a result, $Id2^{-/-}$ mice lack secondary lymphoid organs such as peripheral lymph nodes, PPs, and NALT. The $Id2^{-/-}$ mice are known to be the only organism that lacks NALT (6). It has also been reported that $Id2^{-/-}$ mice

are in a Th2-dominant state and have a selective defect in the $CD8\alpha^{+}$ dendritic cell (DC) subset (17). Several studies lead us to consider that the Th2 dominance observed in $Id2^{-/-}$ mice is caused, at least in part, by the defect in $CD8\alpha^{+}$ DCs. It has been demonstrated that distinct DC subsets participate in Th1 and Th2 cell development (18–22). In particular, $CD8\alpha^{+}$ DCs produce a large amount of IL-12, a crucial cytokine for Th1 development, and prime naïve $CD4^{+}$ T cells, leading to Th1 differentiation (20–22). In addition, $Id2^{-/-}$ mice exhibit a greatly reduced number of NK cells (14), which produce a large amount of IFN- γ in an IL-12-dependent manner. Furthermore, IFN- γ is a key factor directing the differentiation of naïve $CD4^{+}$ T cells into Th1

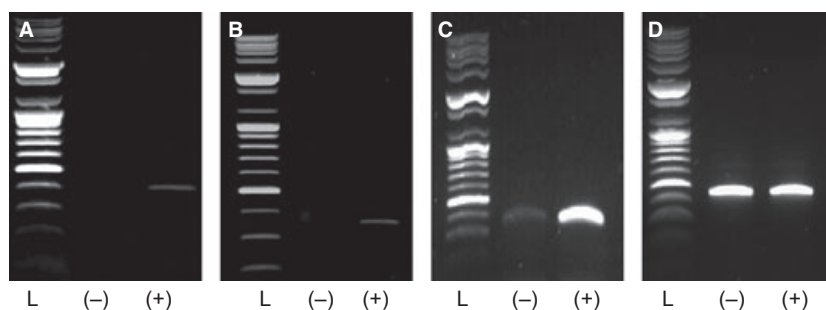


Figure 5 Expressions of ϵ immunoglobulin heavy-chain germline gene transcript (ϵ GLT), mRNA encoding the heavy chain of immunoglobulin E (ϵ mRNA), and activation-induced cytidine deaminase (AID) mRNA in nasopharynx-associated lymphoid tissues (NALT). ϵ GLT (A), ϵ mRNA (B), and AID mRNA (C) were PCR-amplified from NALT of mice with and without ovalbumin (OVA) sensitization. Glyc-

eraldehyde-3-phosphate dehydrogenase (D) was amplified to control cDNA loading. Lanes marked L (-) and (+) indicate DNA ladder, NALT without OVA sensitization, and NALT with OVA sensitization, respectively. The results presented are representative of at least three reproducible independent PCR amplifications.

cells (23). Thus, a decreased NK cell population is one potential cause of skewed Th2 development in $Id2^{-/-}$ mice. Upon B cell activation, $Id2$ acts as a negative regulator to prevent potentially harmful effects brought about by excessive immunological reactions. One specific role is to maintain a low serum concentration of IgE (24). Taken together with Th2 dominance, this can adequately explain the higher levels of total IgE in $Id2^{-/-}$ mice compared with $Id2^{+/+}$ mice.

Interestingly, despite higher levels of total IgE, $Id2^{-/-}$ mice produced significantly lower levels of antigen-specific IgE compared with $Id2^{+/+}$ mice in the allergic rhinitis model. Furthermore, allergic symptoms, eosinophilic infiltration, and Th2 cytokine production in the nasal mucosa were significantly lower in $Id2^{-/-}$ mice than in $Id2^{+/+}$ mice. As a whole, the major points of difference between $Id2^{-/-}$ and $Id2^{+/+}$ mice are the existence of NALT and defects in immune cells. To determine which of these two factors has a larger effect on attenuated allergic immune responses in $Id2^{-/-}$ mice, we created radiation chimera mice with transplanted donor BM cells. Lethally irradiated 129/Sv $Id2^{-/-}$ or $Id2^{+/+}$ mice and C57BL/6 mice were engrafted with C57BL/6 wild-type and $Id2^{-/-}$ or $Id2^{+/+}$ BM cells, respectively. The $Id2^{-/-}$ radiation chimera mice transplanted with C57BL/6 wild-type BM cells lacked NALT but had normal immune cell populations. On the contrary, C57BL/6 radiation chimera mice transplanted with $Id2^{-/-}$ BM cells had normal NALT but defective immune cell populations. Lethally irradiated $Id2^{-/-}$ mice engrafted with C57BL/6 wild-type BM cells had significantly lower allergic immune responses compared with equally treated $Id2^{+/+}$ mice. However, there were no statistically significant differences in allergic immune responses between C57BL/6 radiation chimera mice transplanted with $Id2^{-/-}$ and $Id2^{+/+}$ BM cells, except for infiltrating eosinophil counts that were significantly higher in C57BL/6 radiation chimera mice transplanted with $Id2^{+/+}$ BM cells than those with $Id2^{-/-}$ BM cells. This finding might be attributable to the fact that $Id2$ protein is important for the differentiation of eosinophils (25). On the other hand, $Id2^{-/-}$ radiation chimera

mice transplanted with C57BL/6 BM cells still showed significantly higher total serum IgE levels than equally treated $Id2^{+/+}$ mice (Fig. 3C). It is speculated that there might be possibilities of another mechanisms involved in Th2 dominance and hyper-IgE in $Id2^{-/-}$ mice, except for immune cell defects as mentioned above.

Activation-induced cytidine deaminase expression is required for CSR (10), thus occurs in lymphoid tissue (26), and has been observed in the nasal mucosa of patients with allergic rhinitis (9, 27). We have demonstrated expression of the enzyme AID as well as ϵ GLT expression in NALT B cells of OVA-sensitized mice. The expression of ϵ GLT and AID demonstrates that NALT is primed for class switching to IgE. Our data also show the evidence of mature ϵ heavy-chain mRNA production in NALT by OVA sensitization. It is reasonable to assume that this reflects ongoing, elevated synthesis of mature IgE in allergen-sensitized NALT by local B cells.

Taken altogether, these results suggest that NALT plays a critical role in the induction of allergic immune responses in the upper respiratory tract at least in part by means of class switching to IgE *in situ*.

Author contributions

D.Y.K. participated in experimental design, data analysis, and manuscript writing. S.F., T.N., K.T., and I.G.K. assisted with data acquisition and analysis. Y.Y. provided $Id2$ mice. C.H.L. provided intellectual guidance. H.K. established the initial scientific questions, provided continuing intellectual guidance, and participated in experimental design and manuscript writing.

Declaration

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, see: <http://www.textcheck.com/cgi-bin/certificate.cgi?id=SMP32A>.

Conflict of interest

No conflicts of interest declared.

References

- Kuper CF, Hamelers DM, Bruijntjes JP, van der Ven I, Biewenga J, Sminia T. Lymphoid and non-lymphoid cells in nasal-associated lymphoid tissue (NALT) in the rat. An immuno- and enzyme-histochemical study. *Cell Tissue Res* 1990;**259**:371–377.
- Wu HY, Nguyen HH, Russell MW. Nasal lymphoid tissue (NALT) as a mucosal immune inductive site. *Scand J Immunol* 1997;**46**:506–513.
- Asakura K, Saito H, Hata M, Kataura A. Antigen-specific IgA response of NALT and cervical lymph node cells in antigen-primed rats. *Acta Otolaryngol* 1998;**118**:859–863.
- Yanagita M, Hiroi T, Kitagaki N, Hamada S, Ito HO, Shimauchi H, et al. Nasopharyngeal-associated lymphoreticular tissue (NALT) immunity: fimbriae-specific Th1 and Th2 cell-regulated IgA responses for the inhibition of bacterial attachment to epithelial cells and subsequent inflammatory cytokine production. *J Immunol* 1999;**162**:3559–3565.
- Vinke JG, Fokken WJ. The role of the adenoid in allergic sensitisation. *Int J Pediatr Otorhinolaryngol* 1999;**49**(Suppl 1):S145–S149.
- Yokota Y, Mansouri A, Mori S, Sugawara S, Adachi S, Nishikawa S, et al. Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. *Nature* 1999;**397**:702–706.
- Rhee CS, Libet L, Chisholm D, Takabayashi K, Baird S, Bigby TD, et al. Allergen-independent immunostimulatory sequence oligodeoxynucleotide therapy attenuates experimental allergic rhinitis. *Immunology* 2004;**113**:106–113.
- Rennert PD, Hochman PS, Flavell RA, Chaplin DD, Jayaraman S, Browning JL, et al. Essential role of lymph nodes in contact hypersensitivity revealed in lymphotoxin- α -deficient mice. *J Exp Med* 2001;**193**:1227–1238.
- Takhar P, Smurthwaite L, Coker HA, Fear DJ, Banfield GK, Carr VA, et al. Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. *J Immunol* 2005;**174**:5024–5032.
- Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 2000;**102**:553–563.
- Kiyono H, Fukuyama S. NALT- versus Peyer's-patch-mediated mucosal immunity. *Nat Rev Immunol* 2004;**4**:699–710.
- Fukuyama S, Hiroi T, Yokota Y, Rennert PD, Yanagita M, Kinoshita N, et al. Initiation of NALT organogenesis is independent of the IL-7R, LT β R, and NIK signaling pathways but requires the Id2 gene and CD3 (–)CD4(+)CD45(+) cells. *Immunity* 2002;**17**:31–40.
- Hiroi T, Iwatani K, Iijima H, Kodama S, Yanagita M, Kiyono H. Nasal immune system: distinctive Th0 and Th1/Th2 type environments in murine nasal-associated lymphoid tissues and nasal passage, respectively. *Eur J Immunol* 1998;**28**:3346–3353.
- Shikina T, Hiroi T, Iwatani K, Jang MH, Fukuyama S, Tamura M, et al. IgA class switch occurs in the organized nasopharynx- and gut-associated lymphoid tissue, but not in the diffuse lamina propria of airways and gut. *J Immunol* 2004;**172**:6259–6264.
- Shimoda M, Nakamura T, Takahashi Y, Asanuma H, Tamura S, Kurata T, et al. Isotype-specific selection of high affinity memory B cells in nasal-associated lymphoid tissue. *J Exp Med* 2001;**194**:1597–1607.
- Takamura K, Fukuyama S, Nagatake T, Kim DY, Kawamura A, Kawauchi H, et al. Regulatory role of lymphoid chemokine CCL19 and CCL21 in the control of allergic rhinitis. *J Immunol* 2007;**179**:5897–5906.
- Kusunoki T, Sugai M, Katakai T, Omatsu Y, Iyoda T, Inaba K, et al. TH2 dominance and defective development of a CD8⁺ dendritic cell subset in Id2-deficient mice. *J Allergy Clin Immunol* 2003;**111**:136–142.
- Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000;**18**:767–811.
- Maldonado-Lopez R, Moser M. Dendritic cell subsets and the regulation of Th1/Th2 responses. *Semin Immunol* 2001;**13**:275–282.
- Maldonado-Lopez R, De Smedt T, Michel P, Godfroid J, Pajak B, Heirman C, et al. CD8 α ⁺ and CD8 α [–] subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J Exp Med* 1999;**189**:587–592.
- Pulendran B, Smith JL, Caspary G, Brasel K, Pettit D, Maraskovsky E, et al. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc Natl Acad Sci U S A* 1999;**96**:1036–1041.
- Maldonado-Lopez R, Maliszewski C, Urbain J, Moser M. Cytokines regulate the capacity of CD8 α ⁺ and CD8 α [–] dendritic cells to prime Th1/Th2 cells in vivo. *J Immunol* 2001;**167**:4345–4350.
- Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu Rev Immunol* 1995;**13**:251–276.
- Sugai M, Gonda H, Nambu Y, Yokota Y, Shimizu A. Role of Id proteins in B lymphocyte activation: new insights from knockout mouse studies. *J Mol Med* 2004;**82**:592–599.
- Buitenhuis M, van Deutekom HW, Verhagen LP, Castor A, Jacobsen SE, Lammers JW, et al. Differential regulation of granulopoiesis by the basic helix-loop-helix transcriptional inhibitors Id1 and Id2. *Blood* 2005;**105**:4272–4281.
- Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson NO, et al. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *J Biol Chem* 1999;**274**:18470–18476.
- Coker HA, Durham SR, Gould HJ. Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. *J Immunol* 2003;**171**:5602–5610.