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Involvement of GATA-3-dependent Th2 lymphocyte activation in airway hyperresponsiveness

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Yamashita, Naomi, Hiroyuki Tashimo, Hirofumi Ishida, Yukiko Matsuo, Hidekazu Tamauchi, Masazumi Terashima, Ikuo Yoshiwara, Sonoko Habu, and Ken Ohta. Involvement of GATA-3-dependent Th2 lymphocyte activation in airway hyperresponsiveness. *Am J Physiol Lung Cell Mol Physiol* 290: L1045–L1051, 2006. First published February 17, 2006; doi:10.1152/ajplung.00195.2005.—The pathophysiological characteristics of bronchial asthma consist of chronic inflammation of airways, airway hyperresponsiveness, and bronchoconstriction. Studies have shown that T helper type 2 (Th2) cytokines produced by both T cells and mast cells in the airway contribute substantially to the initiation of inflammation in both experimental and human bronchial asthma. GATA-3 is a transcription factor essential to the production of Th2 cytokines by T lymphocytes. To clarify the role of GATA-3-expressing T cells in the pathophysiology of bronchial asthma, we utilized transgenic (Tg) mice carrying the GATA-3 gene and the ovalbumin (OVA)-specific T cell receptor gene (GATA-3-Tg/OVA-Tg). Mice were intranasally administered OVA without systemic immunization. Airway responses were analyzed with noninvasive and invasive whole body plethysmographs. GATA-3-Tg/OVA-Tg mice exhibited significantly higher IL-13 and IL-4 protein expression in the airway. Although there were no differences in the types of infiltrating cells between GATA-3-Tg/OVA-Tg and GATA-3-non-Tg/OVA-Tg mice and no significant increase in IgE level in either group compared with nontreated mice, the response after ACh inhalation was significantly elevated in GATA-3-Tg/OVA-Tg on the seventh day of intranasal treatment with OVA. This hyperresponsiveness was inhibited by 5-lipoxygenase inhibitor and IL-13 neutralization, suggesting that airway responses were induced through IL-13 and leukotriene pathway. In conclusion, airway hyperresponsiveness, a characteristic of bronchial asthma, is regulated at the level of GATA-3 transcription by T lymphocytes in vivo.

T helper type 2 cytokines; transgenic mice; allergic inflammation

CHRONIC INFLAMMATION OF AIRWAYS, airway hyperresponsiveness, and bronchoconstriction are key pathophysiological findings of asthma. Eosinophils are prevalent in inflamed airways in bronchial asthma. Studies have shown that T helper type 2 (Th2) cytokines play principal roles in the initiation of eosinophilic inflammation in both experimental and human bronchial asthma (22, 24, 32). It has been reported that the number of cells producing IL-4, IL-5, and IL-13 is increased in the airways of asthmatics (12, 19). Because IgE-cross-linked mast cells also produce Th2 cytokines (6), some investigators be-

lieve that mast cells are responsible for production of Th2 cytokines in the initiation of asthmatic responses (5). However, others have shown that T cell transfer can initiate an asthmatic response in the absence of IgE production (4, 15).

Th2 and Th1 cells arise through the differentiation of naïve CD4⁺ T cells, and certain transcription factors promote Th2 or Th1 lineage differentiation. GATA-3 is indispensable for directing Th2 differentiation and was shown to play an important role in the gene expression of Th2 cytokines in mice and humans (20, 25, 33, 36, 38). GATA-3 mRNA-positive cells have been shown to be increased in bronchoalveolar lavage fluid (BALF) and airway biopsy specimens from bronchial asthmatic patients (1, 17, 21).

In this report, we focused on GATA-3 and aimed to clarify the role of GATA-3-expressing T cells in the pathogenesis of IgE-independent bronchial asthma. Double transgenic mice carrying the GATA-3 gene and ovalbumin (OVA)-specific T cell receptor gene were used to clarify whether it is possible to induce an asthmatic response when Th2 cells are stimulated by allergen independent of mast cell involvement. Our data indicate that GATA-3 hyperexpressive T cells can initiate airway responsiveness in vivo.

METHODS

Animals. All experiments conformed to the Laboratory Animal Research Guide for Care and Use of Laboratory Animals. Experiments using transgenic mice were approved by Teikyo University School of Medicine. Double transgenic (Tg) mice carrying the OVA-T cell receptor (TCR) gene and GATA-3 gene (GATA-3-Tg/OVA-Tg) were used. As controls, single transgenics expressing either the OVA-TCR transgenic mice (GATA-3-non-Tg/OVA-Tg) or GATA-3 transgenic mice (GATA-3-Tg/OVA-non-Tg) were used. Murine GATA-3 cDNA was kindly provided by Dr. M. Yamamoto (Tsukuba Univ. School of Medicine, Tsukuba, Japan). The generation of GATA-3 transgenic mice used in this study was described previously (27). Mouse GATA-3 cDNA was cloned into *Bam*HI sites of vector pw120 containing the potent lymphocyte-specific protein tyrosine kinase (lck) distal promoter and human growth factor poly(A) sequence. The hemizygous GATA-3 transgenic mice were crossed with another type of transgenic BALB/c mice homozygous for a TCR recognizing the OVA_{323–339} peptide in the context of MHC Class II (I-A^d) restriction (23). For genotyping the pups, tail DNA was extracted by a DNA Isolation kit (GENTRA, Minneapolis, MN) and examined by PCR using the following primers: GATA-3 sense,

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5'-TCTCACTCTCGAGGCAGCATGA-3' and GATA-3 antisense, 5'-GGTACCATCTCGCCGCCACAG-3' as previously described (27). Wild-type BALB/c mice were obtained from Charles River Laboratories Japan (Yokohama, Japan).

Treatment of mice. Seven- to 10-wk-old GATA-3-Tg/OVA-Tg, GATA-3-non-Tg/OVA-Tg, GATA-3-Tg/OVA-non-Tg, and BALB/c mice were anesthetized by ether and were sensitized intranasally with 800 μ g of OVA (40 μ l of 10 mg/ml OVA in normal saline, twice). This procedure was repeated every day for 7 days. To explore the role of 5-lipoxygenase, mice were intraperitoneally injected with the 5-lipoxygenase inhibitor AA-861 (30 mg/kg, solved in saline; Wako Pure Chemical, Osaka, Japan) 30 min before each administration of nasal OVA. To analyze the effects of IL-13, mice were treated with recombinant mouse IL-13 R α 2/Fc chimera (R&D Systems, Minneapolis, MN), which inhibits the effects of IL-13 (7, 32). Mice were injected with IL-13 R α 2/Fc chimera (20 μ g/mouse) or PBS twice a week. To compare the response, OVA-immunized mice were established. The mice were immunized with OVA alum (10 μ g of OVA + 2 mg of aluminum hydroxide gel) at days 0 and 7, and subsequently OVA-specific IgE antibodies were induced.

RNA extraction and cDNA synthesis. Lungs were frozen in liquid nitrogen immediately after isolation and were used for RNA extraction. Lung tissue was homogenized at 4°C, and total RNA was extracted using Isogen, a modified acid guanidium-phenol-chloroform method (Nippon Gene, Tokyo, Japan) (2). RNA was treated with 10 units DNase (Qiagen, Hilden, Germany) as per the manufacturer's instructions. After quantifying total RNA, cDNA synthesis was performed as follows. Five micrograms of total RNA were incubated with 5 mM MgCl₂, 1 mM dNTP mixture, 0.25 units reverse transcriptase, 1 unit RNase inhibitor, and 0.125 μ M oligo(dT) (Takara Biochemicals, Tokyo, Japan) at 42°C for 15 min, 99°C for 5 min, and then at 5°C for 5 min using GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA).

The level of OVA-TCR mRNA was examined by RT-PCR. The resultant cDNA was added to a PCR mixture containing 200 mM dNTP, 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 3 mM MgCl₂, 0.001% gelatin, 2 units *Taq* polymerase (Promega, Madison, WI), and 1 μ M oligonucleotide primer. The specific primers for the mouse OVA-TCR gene were 5'-CAGGCAACTCCGCTAGTAGCTTGGTATC-3' and 5'-ACTGTCTAGCTTTGAGCCTTCACCAA-3'. The expected size of the amplified product was 300 bp. The mixture was amplified using a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). Denaturation was performed at 94°C for 1 min with annealing at 60°C for 2 min and primer extension at 72°C for 3 min. These steps were repeated for 35 cycles. The amplified products were separated by electrophoresis on 2% agarose gels and visualized by staining with ethidium bromide.

Quantification of mRNA. The levels of GATA-3 and 5-lipoxygenase mRNA were examined by real-time PCR using the Light Cycler-Fast Start DNA Master SybrGreen I kit (Roche Diagnostics, Mannheim, Germany). In this system, double-stranded DNA is labeled with SybrGreen I and then detected. The reaction was undertaken in 20 μ l, containing 3 mM MgCl₂, 1 μ M primers, FastStart *Taq* DNA polymerase, dNTP mix, and SybrGreen I (Light Cycler-Fast Start DNA Master SybrGreen I kit). Quantification was performed on the basis of the standard curve obtained using five dilutions of cDNA. Results are shown as relative expression of mRNA (% of GATA-3-non-Tg) after normalization to the level β -actin mRNA. The primers used were as follows and were synthesized by Nihon Gene Research Laboratories (Sendai, Japan): β -actin 5'-CCTGTATGCCTCTGGTCGTA-3', 5'-CCATCTCTGCTCGAAGTCT-3' 260 bp, GATA-3 5'-AGTGTGTGAAGTGC GG-3', 5'-CCCCATTAGCGTTTCCT-3' 219 bp, and 5-lipoxygenase 5'-CTCCAACATATGCGGGC-3', 5'-CTTGCGGAATCGGATCA-3' 215 bp.

Western blot analysis. Protein was extracted from interphase and organic phase of lung homogenates using Isogen following the manufacturer's description (2). Briefly, the interphase and organic phase

were mixed with ethanol. The supernatant containing the protein was precipitated using isopropanol. Protein fractions from the lung were eluted with SDS sample buffer and separated by SDS-PAGE. Then they were electrophoretically transferred onto Hybond-P membranes. The membranes were blocked in buffer containing 5% BSA and incubated with goat polyclonal anti-GATA-3 antibody (1:100; Santa Cruz Biotechnologies, Santa Cruz, CA) and then with horseradish peroxidase (HRP)-conjugated rabbit anti-goat Ig (1:2,000, Dako). For control, anti- β -actin antibody (1:200, Santa Cruz Biotechnologies) was used. The immunoblotted proteins were visualized by using an enhanced chemiluminescence kit (ECL, Amersham Pharmacia Biotech, Piscataway, NJ), quantified using an image analyzer (LAS 3000; Fuji Film, Kanagawa, Japan), and expressed as relative expression of GATA-3-non-Tg after normalization to the level β -actin.

Measurement of airway responsiveness. Twenty-four hours after the final intranasal sensitization with OVA, airway responsiveness was evaluated. Mice inhaled 100 and then 200 mg/ml of ACh for 3 min each using an ultrasonic nebulizer (Omron, Tokyo, Japan) at a flow rate of 1.5 l/min. Airway response was evaluated in nonanesthetized mice by enhanced pause (Penh) using a pulmonary function analyzer (Buxco Electronics, Troy, NY). Data are expressed as %Penh, which is defined by the formula [(Penh after inhalation of ACh/Penh before inhalation) $\times$ 100 (%)].

Invasive measurements were also undertaken as previously reported (34). Briefly, anesthetized mice were tracheostomized and injected with pancuronium bromide. The animals were connected to a Harvard ventilator with 0.3 ml of tidal volume and a respiratory frequency of 120 beats/min. Next, they were placed in a whole body plethysmograph (Buxco Electronics) to measure airway resistance (Raw).

To obtain BALF, infusion and collection of saline through the intratracheal catheter were repeated until 5 ml of BALF was obtained (34). BALF was centrifuged at 1,500 rpm for 10 min at 4°C. Pellets were dissolved in 1 ml of PBS, and the number of cells were counted. A cytospin specimen was obtained by rotation at 640 rpm for 2 min. Then, cells were stained with Diff Quick (International Reagents, Osaka, Japan), and cell fractions were examined by microscopy. BALF was concentrated 10-fold using Amicon Ultra 4 (Millipore, Bedford, MA). The levels of IL-4, IL-13, and IFN- γ in the BALF were evaluated by ELISA Quantikine kit (R&D Systems).

Heparinized blood obtained by cardiac puncture was centrifuged at 15,000 rpm for 10 min at 4°C, and the serum was obtained. Serum IgE level was determined using a mouse IgE quantification kit (Morinaga), a model 450 Microplate Reader, and a Microplate Manager Program (Bio-Rad Laboratories, Richmond, CA).

To measure OVA-specific IgE, we coated the plate with OVA using the AlaSTAT system (Diagnostics Products, Los Angeles, CA) with some modifications as previously reported (35). After being incubated with 10-fold-diluted samples, HRP-labeled rat anti-murine IgE-specific antibody (Morinaga, Kanagawa, Japan) was added to the plate as the second antibody. Color was developed and measured at 450-nm optical density.

Histological analysis. After evaluation of airway responsiveness, mice were exsanguinated and lungs were extracted. The lungs were fixed overnight with intratracheal infusion of 10% formalin, maintaining the airway pressure at 10 cmH₂O, and were embedded in paraffin. Embedded sections were deparaffinized. Hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) staining were performed.

Statistical analysis. Data were statistically analyzed by Student's *t*-test and ANOVA. Statistical significance was set at *P* < 0.05.

RESULTS

Expression of GATA-3 mRNA and protein. First, we examined whether intranasal administration of OVA induced functional GATA-3 gene expression in GATA-3-Tg mice. The lung tissues were prepared after seven nasal administrations of

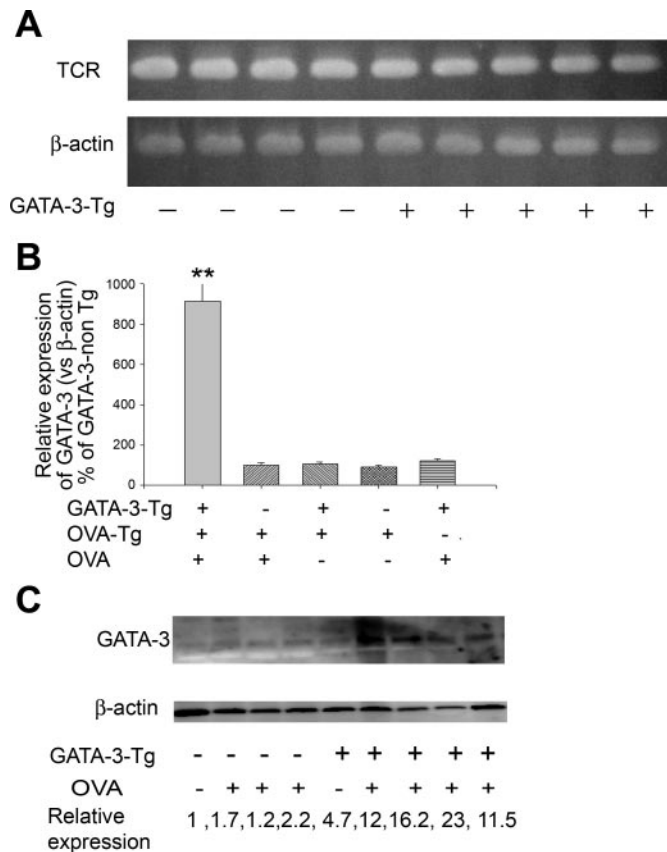


Fig. 1. Ovalbumin (OVA)-T cell receptor (TCR) and GATA-3 mRNA and protein expression. **A**: OVA-TCR expression examined by RT-PCR in lung cells. **B**: GATA-3 mRNA expression was examined by real-time PCR. Data are means $\pm$ SE from 4–8 mice. Data are expressed as relative % expression of GATA-3-non-Tg/OVA-Tg mice (second bar from left, 100%) after normalization to β -actin. **C**: GATA-3 protein expression in the lung. GATA-3 protein level was determined by Western blot. Lungs were homogenized and used for Western blot as described in METHODS. OVA+, mice with OVA intranasal administration; OVA–, mice with saline administration. Relative expression was fold increase compared with intensity of GATA-3 band of unstimulated GATA-3-non-Tg/OVA-Tg mice (first band from left) after normalization to intensity of β -actin band. ** $P < 0.01$.

OVA. To confirm equal levels of TCR expression between GATA-3-Tg/OVA-Tg and GATA-3-non-Tg/OVA-Tg mice, TCR mRNA expression was examined by RT-PCR. There was no significant difference in TCR mRNA expression between the two groups of mice (Fig. 1A). As shown in Fig. 1B, expression of GATA-3 mRNA was greater in GATA-3-Tg/OVA-Tg compared with GATA-3-non-Tg/OVA-Tg mice. In addition, induction of GATA-3 mRNA expression was not observed in GATA-3-Tg/OVA-non-Tg mice after OVA administration (Fig. 1B). Figure 1C shows protein expression of GATA-3. Because anti-GATA-3 antibody for Western blot recognized GATA-3 protein from both transgenic and endogenous GATA-3 genes, we observed a weak protein band in GATA-3-non-Tg/OVA-Tg mice (Fig. 1C). On average, GATA-3 protein expression was 10-fold greater in OVA-stimulated GATA-3-Tg/OVA-Tg mice than that in OVA-stimulated GATA-3-non-Tg/OVA-Tg mice. The data confirmed the GATA-3 transgene induced GATA-3 protein expression in the lung.

Histological examination and BALF cell analysis. Histological examination by HE staining revealed significant infiltration of lymphocytes in the submucosal area in both GATA-3-Tg/OVA-Tg and GATA-3-non-Tg/OVA-Tg groups (Fig. 2A). Although bronchial epithelial shedding was not prominent in either group, PAS staining revealed more PAS-positive mucus-secreting cells in GATA-3-Tg/OVA-Tg than in GATA-3-non-Tg/OVA-Tg groups (Fig. 2B). In addition, neither BALB/c mice nor GATA-3-Tg/OVA-non-Tg mice exhibited inflammation after OVA administration, suggesting that the infiltration of lymphocytes was induced in an OVA antigen-specific manner (Fig. 2A).

BALF cell analysis was performed at 24 h after the final intranasal treatment with OVA. The total number of cells in BALF was significantly increased in OVA intranasally treated GATA-3-Tg/OVA-Tg and GATA-3-non-Tg/OVA-Tg mice compared with the nontreated groups ($P < 0.01$; Fig. 3A). In addition, OVA-treated GATA-3-Tg/OVA-non-Tg mice exhibited no increase of BALF cells. Macrophages were dominant in nontreated groups and OVA-treated GATA-3-Tg/OVA-non-Tg mice. In contrast, an increase in lymphocytes and

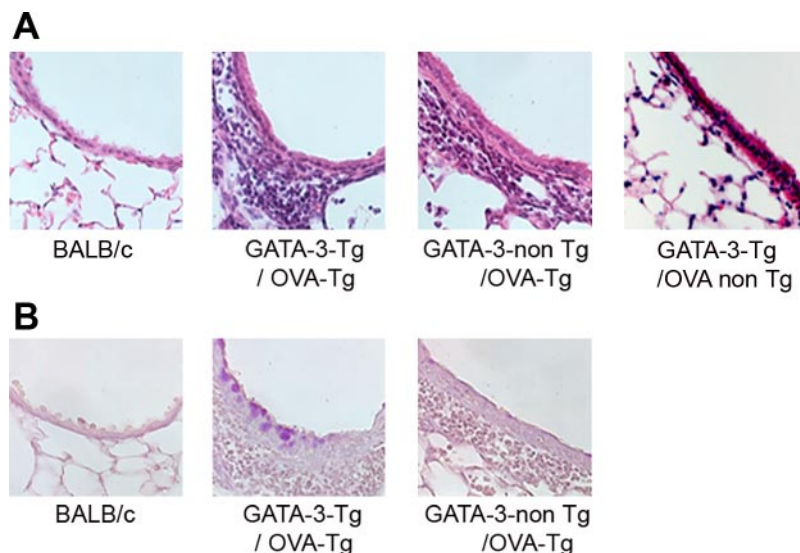


Fig. 2. Histological analysis. Hematoxylin-eosin (A) and periodic acid-Schiff staining (B) were performed on paraffin-embedded sections. After 7 nasal administrations of OVA, lungs were extracted and fixed overnight with intratracheal infusion of 10% formalin maintaining the airway pressure at 10 cmH₂O.

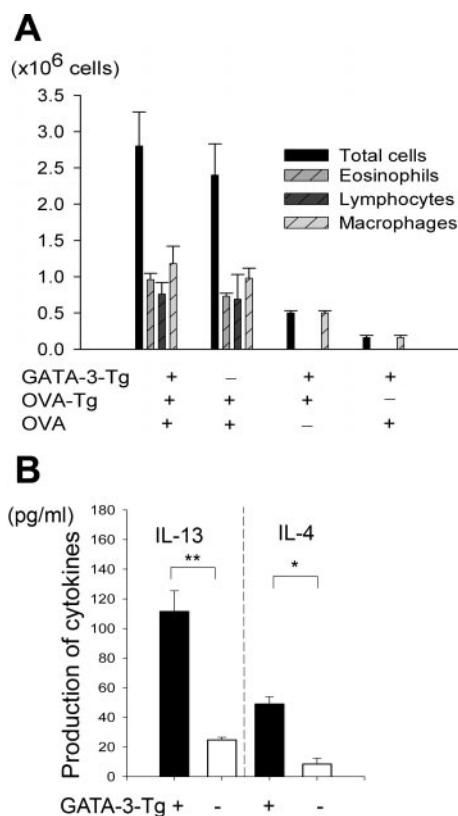


Fig. 3. Bronchoalveolar lavage fluid (BALF) cell analysis. A: lungs were subjected to lavage through intubation until 5 ml of BALF were obtained. Cells present in the BALF were pelleted, resuspended in 1 ml of saline, and placed on glass slides, where they were counted and fixed by cytospin. Slides were then stained with Diff Quik, and cell differentiation was assessed microscopically. Each bar indicates means $\pm$ SE of 5 mice. Similar experiments were undertaken at least 3 times. OVA+, mice with OVA intranasal administration; OVA-, mice with saline administration. B: cytokine protein expression by ELISA. Data are means $\pm$ SE of BALF from 4 mice. ** P < 0.01; * P < 0.05.

eosinophils was observed in intranasally treated groups. The total number of cells or cell fractions did not differ significantly between GATA-3-Tg/OVA-Tg and GATA-3-non-Tg/OVA-Tg mice. Eosinophils were slightly increased in GATA-3-Tg/OVA-Tg mice compared with GATA-3-non-Tg/OVA-Tg mice.

To examine cytokine profiles, BALF was analyzed for the cytokine levels. The level of IL-13 and IL-4 in BALF of GATA-3-Tg/OVA-Tg mice was significantly higher than that of GATA-3-non-Tg/OVA-Tg mice (Fig. 3B). We examined the level of IFN- γ and found no differences between the two groups [39.7 ± 17.5 (pg/ml) for GATA-3-Tg/OVA-Tg and 93.7 ± 19.4 for GATA-3-non-Tg/OVA-Tg]. These results indicate that the infiltrating cells in the airways of GATA-3-Tg mice exhibited Th2 cytokine protein production.

Serum IgE level. Serum IgE levels as determined by ELISA were 131.1 ± 24.0 (ng/ml) for nontreated mice, 48.9 ± 8.60 for nasally OVA-treated GATA-3-Tg/OVA-Tg mice, and 49.0 ± 18.44 for nasally OVA-treated GATA-3-non-Tg/OVA-Tg mice. There was no difference between GATA-3-Tg and GATA-3-non-Tg, nor was the IgE level increased in nasally OVA-treated groups.

When mice were immunized with OVA alum, serum IgE levels were $1,436 \pm 414.6$ for GATA-3-Tg/OVA-Tg and

424.6 ± 99.7 for GATA-3-non-Tg/OVA-Tg, with values significantly higher in GATA-3-Tg (P < 0.05), which is comparable to findings of a previous report (27). The serum IgE levels of mice immunized with OVA alum were significantly increased compared with those of intranasally OVA-treated groups (P < 0.05).

OVA-specific IgE exhibited the same tendency as total IgE. Nasally treated mice were 2.2 ± 0.44 vs. 1.7 ± 0.47 units (GATA-3-Tg vs. GATA-3-non-Tg). Immunized mice with OVA alum were 50.2 ± 4.2 vs. 34.8 ± 5.4 units (GATA-3-Tg vs. GATA-3-non-Tg).

Measurement of airway responsiveness. Next, airway responsiveness was examined in Tg and non-Tg mice to examine the functional consequence of enforced GATA-3 expression. On the seventh day of intranasal treatment, the %Penh values after ACh treatment at 100 mg/ml were 456.7 ± 123.82 for GATA-3-Tg/OVA-Tg and 160.7 ± 14.02 for GATA-3-non-Tg/OVA-Tg mice. After ACh treatment at 200 mg/ml, the %Penh values were $1,376.1 \pm 402.68$ for GATA-3-Tg/OVA-Tg and 250.4 ± 39.78 for GATA-3-non-Tg/OVA-Tg mice. Airway response was significantly increased in intranasally treated GATA-3-Tg/OVA-Tg mice (P < 0.05; Fig. 4A). Intranasally treated GATA-3-Tg mice with no elevation of

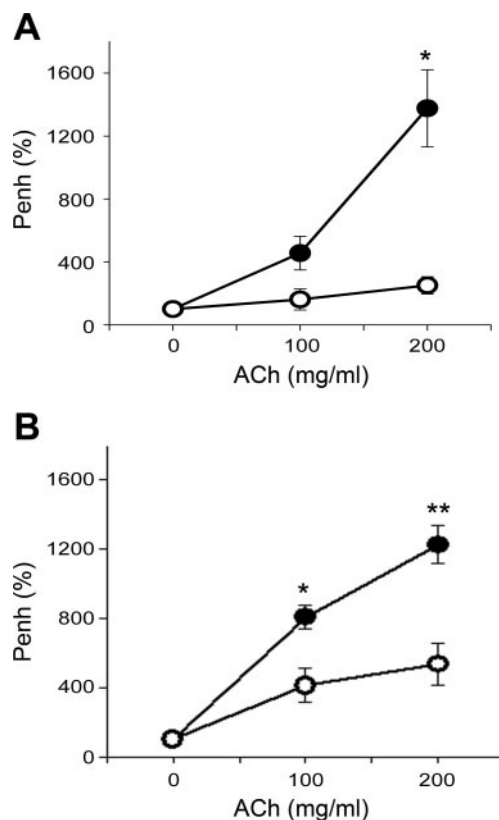


Fig. 4. Airway responsiveness. Airway responses to increasing doses of ACh were determined in mice treated intranasally only (A) and treated intranasally with OVA after systemic immunization with OVA alum (B). Each of the indicated concentrations of ACh was inhaled for 3 min. Enhanced pause (Penh) was measured for 5 min after the inhalation of each dose. Baselines of Penh were 0.46 ± 0.02 vs. 0.37 ± 0.09 (A) and 0.46 ± 0.01 vs. 0.49 ± 0.01 (B) (GATA-3-Tg vs. GATA-3-non-Tg). There were no differences among baselines of Penh. Data are expressed % Penh as described in METHODS. Each bar represents means $\pm$ SE of 5 different mice. * P < 0.05; ** P < 0.01 GATA-3-Tg/OVA-Tg (●) vs. GATA-3-non-Tg/OVA-Tg (○).

total IgE and OVA-specific IgE showed a comparable airway response to 200 mg/ml of ACh as GATA-3-Tg/OVA-Tg mice with elevated IgE level by immunization with OVA alum (Fig. 4).

To confirm the airway response of GATA-3-Tg mice with only intranasal treatment and without systemic immunization, we also examined airway response in tracheotomized mice. Airway resistance was analyzed before and after 5 mg/ml of ACh inhalation. Before ACh inhalation, there was no difference among GATA-3-Tg/OVA-Tg, GATA-3-non-Tg/OVA-Tg, and GATA-3-Tg/OVA-non-Tg mice (1.60 ± 0.042 cmH₂O·ml⁻¹·s⁻¹, 1.53 ± 0.0159 cmH₂O·ml⁻¹·s⁻¹, and 1.64 ± 0.017 cmH₂O·ml⁻¹·s⁻¹, respectively). After ACh treatment, Raw of GATA-3-Tg/OVA-Tg (4.63 ± 0.64 cmH₂O·ml⁻¹·s⁻¹) was significantly increased over that of GATA-3-non-Tg/OVA-Tg (2.91 ± 0.07 cmH₂O·ml⁻¹·s⁻¹) and GATA-3-Tg/OVA-non-Tg (3.05 ± 0.11 cmH₂O·ml⁻¹·s⁻¹; $P < 0.01$, $n = 6$ /group). These data suggest that higher expression of the OVA-TCR-specific GATA-3 transgene induction is responsible for higher airway responsiveness.

Mechanisms of elevated airway response in GATA-3-Tg mice. To explore the mechanisms of elevated airway responsiveness in GATA-3-Tg mice treated only intranasally with OVA, we examined the roles of IL-13 and 5-lipoxygenase. IL-13 has been reported to induce airway hyperresponsiveness directly through the induction of 5-lipoxygenase synthesis, followed by greater production of leukotriene (LT) (10, 29). LT plays central roles in airway hyperresponsiveness and airway smooth muscle proliferation (3, 11). Baseline levels of LT expression were not different between genotypes. Upon nasal OVA challenge, however, a significantly higher level of 5-lipoxygenase expression was induced in GATA-3-Tg/OVA-Tg than in GATA-3-non-Tg/OVA-Tg mice (Fig. 5A).

To explore the role of IL-13 and LT in airway hyperresponsiveness in GATA-3-Tg/OVA-Tg mice, we examined the effects of IL-13 Rα2/Fc chimera and 5-lipoxygenase inhibitor (AA-861) in this model. As shown in Fig. 5B, treatment with IL-13 Rα2/Fc chimera and AA-861 inhibited the hyperresponsiveness in GATA-3-Tg/OVA-Tg, suggesting a critical role of IL-13 and LT in airway responsiveness in GATA-3-Tg mice.

DISCUSSION

We induced a dominance of Th2 cells by cross-breeding GATA-3 overexpressing mice with OVA-specific TCR transgenic mice. Intranasal OVA treatment without systemic sensitization resulted in airway hyperresponsiveness that was significantly augmented in the double transgenic mice. This airway hyperresponsiveness was present even when mice had no elevated serum IgE and OVA-specific IgE. It has been reported that IL-4 and IL-13, which are produced by mast cells and lymphocytes, are responsible for airway hyperresponsiveness (30, 31). Our study revealed that airway hyperresponsiveness can be caused by activation of Th2 cells without induction of IgE antibodies through activation of IL-13 production and 5-lipoxygenase pathways.

Naïve CD4⁺ T lymphocytes can differentiate into Th1 or Th2 cells that secrete distinct sets of cytokines. The zinc finger-type transcription factor GATA-3 is a transcription factor essential for Th2 differentiation and production of Th2 cytokines (12, 16). GATA-3 is weakly expressed in naïve T cells and becomes strongly expressed upon Th2 differentiation

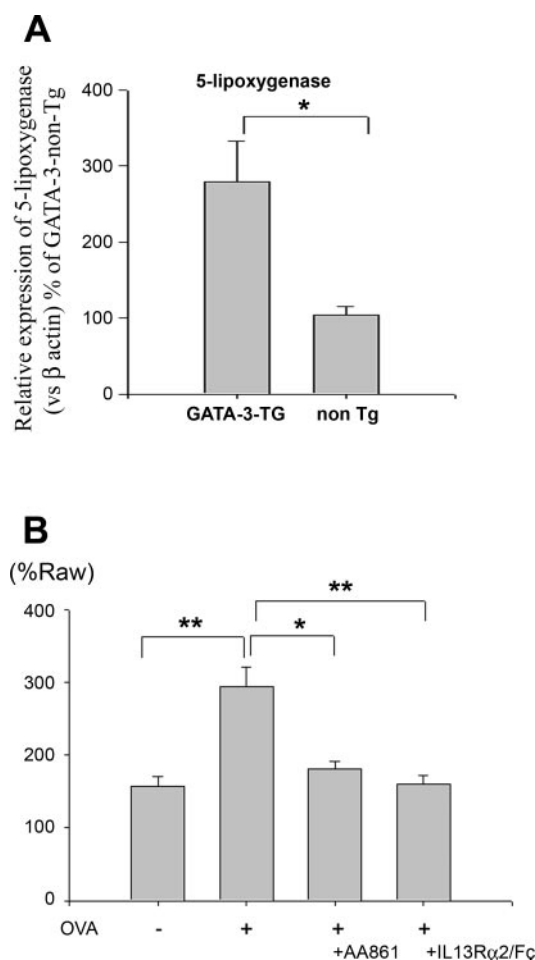


Fig. 5. Role of IL-13 and 5-lipoxygenase pathways in GATA-3-Tg mice. A: 5-lipoxygenase mRNA expressions. mRNA expression of 5-lipoxygenase was determined by real-time PCR. RNA was extracted from whole lung of intranasally OVA-treated mice. Data are shown as means $\pm$ SE of 3 different mice. Data are expressed as relative % expression of GATA-3-non-Tg after normalization to β -actin. $*P < 0.05$. B: effects of IL-13 Rα2/Fc chimera and 5-lipoxygenase inhibitor on airway responsiveness of GATA-3-Tg/OVA-Tg. Mice were treated with IL-13 Rα2/Fc chimera and 5-lipoxygenase inhibitor (AA-861). Airway responsiveness was measured at 24 h after final treatment with OVA. The airway resistance (Raw) was measured before and after inhalation of 5 mg/ml ACh. Data are shown as % Raw compared with that before ACh inhalation and means $\pm$ SE of 4 different mice. Baseline Raw of each column was 1.70 ± 0.06 , 2.04 ± 0.11 , 2.05 ± 0.38 , and 1.69 ± 0.08 cmH₂O·ml⁻¹·s⁻¹, respectively. $*P < 0.05$; $**P < 0.01$.

but becomes shut off in Th1 cell differentiation (12, 26). GATA-3 binds to promoter regions of IL-5 and IL-13 genes and directly regulates the mRNA level of IL-5 and IL-13 (33). Although GATA-3 does not enhance IL-4 promoter activity directly, it is known that GATA-3 elevates the IL-4 mRNA level through remodeling of chromatin (13). Nawijn et al. (18, 19) generated transgenic mice with the GATA-3 gene under the control of the CD2 promoter to examine the function of GATA-3 in vivo. In these mice, Th1 differentiation was repressed and the number of Th2 cells was increased.

In our study, the expression of GATA-3 was controlled by lck. Therefore, induction of GATA-3 and an increase in the number of Th2 cells were observed after antigen-specific activation. When OVA was applied intranasally, the number of lymphocytes in the airway wall increased. Because the GATA-

3-Tg and non-Tg both carry the OVA-TCR transgene, infiltration of T cells was observed in both mice. Protein expression of IL-4 and IL-13 was increased in GATA-3-Tg compared with non-Tg, indicating airways of GATA-3-Tg were infiltrated primarily with Th2 cells.

In our study, airway hyperresponsiveness was significantly increased in GATA-3-Tg compared with non-Tg without induction of IgE by OVA alum systemic immunization (Fig. 4A). However, a slight increase in infiltration of eosinophils was observed in GATA-3-Tg compared with non-Tg. Although eotaxin has been reported to be regulated by IL-13 (14), we observed a slight increase in the eotaxin level (data not shown). Administration of IL-13 and IL-4 has been reported to directly induce mucus cells and airway hyperresponsiveness (29, 30). IL-13 also has been reported to induce 5-lipoxygenases on resident cells and cysteinyl leukotriene 1 receptor on smooth muscle cells (8, 10, 28, 29, 31). IL-13-induced airway hyperresponsiveness is ascribed to the increase of 5-lipoxygenase (10, 29). In GATA-3-Tg mice, we observed higher mRNA expression of 5-lipoxygenase. In addition, we found that antagonism of IL-13 and inhibition of the 5-lipoxygenase pathway abolished airway responsiveness. Thus augmentation of airway response in GATA-3-Tg was ascribed to the increase of IL-13 and LT. Although interaction of lymphocytes, mast cells, eosinophils, and other cells in the airway may take place in asthmatic airways, our data reveal that infiltration of Th2-dominant T lymphocytes alone causes airway hyperresponsiveness.

When the expression of GATA-3 was inhibited in transgenic mice carrying the dominant negative GATA-3 transgene, production of IL-4, IL-5, IL-13, and IgE, subsequent infiltration of eosinophils, and hypersecretion of mucus cells were strongly repressed (37). Similarly, blockade of GATA-3 expression by antisense oligonucleotides did not increase airway responsiveness and did not induce infiltration of eosinophils (9). Bronchial asthma is known as a Th2-dependent disease, and expression of GATA-3 is increased in airway biopsy specimens of human asthmatics (1, 17). Airway hyperresponsiveness, a characteristic of bronchial asthma, was induced by controlling lymphocytes at the transcriptional level in vivo. These facts suggest that GATA-3, essential for the differentiation of Th2 cells, plays an important role in pathophysiology of both atopic and nonatopic bronchial asthma and can be a target molecule for the therapy of bronchial asthma.

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