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Overexpression of IL-15 In Vivo Enhances Tc1 Response, Which Inhibits Allergic Inflammation in a Murine Model of Asthma

Ryotaro Ishimitsu,† Hitoshi Nishimura,* Toshiki Yajima,* Taketo Watase,* Hideyuki Kawachi,† and Yasunobu Yoshikai‡

IL-15, a pleiotropic cytokine, is involved in the inflammatory responses in various infectious and autoimmune diseases. We have recently constructed IL-15-transgenic (Tg) mice, which have an increased number of memory-type CD8+ T cells in the peripheral lymphoid tissues. In the present study, we found that eosinophilia and Th2-type cytokine production in the airway were severely attenuated in OVA-sensitized IL-15-Tg mice following OVA inhalation. IL-15-Tg mice preferentially developed Tc1 responses mediated by CD8+ T cells after OVA sensitization, and in vivo depletion of CD8+ T cells by anti-CD8 mAb aggravated the allergic airway inflammation in IL-15-Tg mice following OVA inhalation. Adoptive transfer of CD8+ T cells from OVA-sensitized IL-15-Tg mice into normal mice before OVA sensitization suppressed Th2 response to OVA in the normal mice. These results suggest that overexpression of IL-15 in vivo suppresses Th2-mediated-allergic airway response via induction of CD8+ T cell-mediated Tc1 response. The Journal of Immunology, 2001, 166: 1991–2001.

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lergic asthma is a chronic inflammatory disease associated with a predominant Th2 response, IgE synthesis, airway infiltration by inflammatory cells, particularly eosinophils, and bronchial hyperreactivity (1, 2). The allergic responses are largely divided into two phases: systemic response in the induction phase and allergic inflammation in the eliciting phase (3, 4). Naive CD4+ T cells initially simulated with an allergen in the presence of IL-4 tend to develop into CD4+ Th2 cells, which secrete IL-4, IL-5, IL-6, and IL-13 for IgE isotype switching. When the same allergen is inhaled, the allergen cross-links performed bound IgE to the high affinity FcεR on mast cells lining the bronchial surface, which consequently release stored mediators by granule exocytosis and synthesize leukotrienes and cytokines. The allergic reaction is further promoted by the recruitment of Th2 cells, eosinophils, and basophils in the airway. Th1 cells, into which naive CD4+ T cells differentiate in the presence of IL-12 and IFN-γ, secrete IL-2, IFN-γ, and TNF-α not only for induction of cell-mediated immunity but also for inhibition of Th2 responses (5–7). Therefore, cytokines involved in Th1-biased response are thought to regulate Th2-mediated allergic responses. However, the basic mechanisms controlling Th2 responses in airway allergic responses are not clear.

IL-15 is a pleiotropic cytokine that promotes activation, proliferation, and cytokine release of various subsets of T cells (8–12), NK cells (13), mast cells (14), and B cells (15). With regard to Th1/Th2 cells, IL-15 is reported to be synergistic with IL-12 to induce proliferation of murine Th1 clones (16), whereas it induces IL-5 production by allergen-specific human Th2 clones (17). Thus, IL-15 may influence both Th1 and Th2 cells. Aberrant IL-15 expression was observed in patients with inflammatory autoimmune disease, such as rheumatoid arthritis (18, 19) and inflammatory bowel diseases (20, 21), suggesting that IL-15 is involved in the pathogenesis of inflammatory disease. However, the potential role of IL-15 in allergic responses in vivo remains to be elucidated.

We have recently constructed transgenic (Tg) mice expressing IL-15 cDNA encoding a secretable isoform of IL-15 precursor protein under the control of an MHC class I promoter, and we found that overexpression of IL-15 augmented the in vivo Th1 response to infection with Salmonella choleraesuis, an intracellular bacterium (22). In the present study, we investigated the in vivo roles of IL-15 in allergic responses in a murine model of asthma using IL-15-Tg mice. We found that the pulmonary eosinophilia and Th2 cytokine production in the airway were severely attenuated in OVA-sensitized IL-15-Tg mice following a challenge with inhalation of OVA. IL-15-Tg mice developed Tc1 responses mediated due to the action of CD8+ T cells against OVA, and in vivo depletion of CD8+ T cells by anti-CD8 mAb aggravated the allergic airway inflammation in the IL-15-Tg mice. Adoptive transfer of CD8+ T cells from OVA-sensitized IL-15-Tg mice revealed that the Tc1 cells suppressed Th2 response at induction phase. These results suggest that overexpression of IL-15 in vivo suppresses Th2-mediated allergic airway response via up-regulation of Th1 cells.

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2 Address correspondence and reprint requests to Dr. Yasunobu Yoshikai, Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya, Japan.
3 Abbreviations used in this paper: Tg, transgenic; BALF, bronchoalveolar lavage fluid; OVA-DOTAP, N-[1-e,2-dioleoyloxy]propyl-N,N,N-trimethylammonium methyl-sulfate; MMC, mytomycin C.
Materials and Methods

Tg gene construction and generation of Tg mice

IL-15-Tg mice, which were constructed using originally described IL-15 cDNA under the control of an MHC class I promoter, have been described previously (22). In brief, full-length cDNA encoding the murine IL-15 gene with the originally described exon 5 (710 bp; Ref. 23) was cloned between the BamHI and SalI sites of a Tg expression vector, pHSE-3⁹, which contains the H2-K promoter and Ig enhancer, and β-globin splice site and poly(A) signal. Transgene DNAs were microinjected into the male pronucleus of fertilized single-cell embryos of C57BL/6 mice. Microinjected eggs were transferred to pseudopregnant C57BL/6 foster mothers. IL-15-Tg mice were identified by digesting genomic DNA with PstI followed by Southern blot analysis using an IL-15-specific probe. Mice were maintained under specific pathogen-free conditions and offered food and water ad libitum. Age- and sex-matched C57BL/6 mice obtained from Japan SLC (Hamamatsu, Japan) were used as control mice. All mice were used at 6–8 wk of age.

Immunization and challenge

Mice were i.p. immunized with 100 μg chicken OVA (Grade IV; Sigma, St. Louis, MO) absorbed in 100 μl CFA on day 0. Seven days later, the mice were i.p. injected with 100 μg OVA in 100 μl IFA. The mice were then exposed to aerosolized PBS or OVA for 30 min/day on days 14, 16, 18, and 20. The aerosols were introduced into the chamber using a nebulizer. The concentration of OVA in the nebulizer was 1% w/v. The immunization protocol is shown in Fig. 1.

In vivo depletion of CD8⁺ cells

Mice were i.p. injected with 400 μg of anti-CD8 mAb 2 days before an initial immunization of OVA/CFA and on day 5 after the immunization. Control mice were i.p. injected with the same dose (400 μg) of rat IgG as an isotype control in the same schedule. The efficacy of CD8⁺ T cell depletion was monitored by flow cytometric analysis of spleen and bronchoalveolar lavage fluid (BALF) cells.

Adoptive transfer assays

Spleen cells of IL-15-Tg or non-Tg mice, which were sensitized twice with OVA/CFA and OVA/IFA at 7-day intervals, were recovered 14 days after the last sensitization and passed through nylon-wool columns. A CD8⁺ T cell subset was purified using a MACS column (Miltenyi Biotec, Auburn, CA). The purity of CD8⁺ T cell subset was confirmed to be more than 98% by FACS analysis. CD8⁺ T cells (1 × 10⁵ cells) were adoptively transferred into normal mice or mice that had been sensitized with OVA/CFA on day −14 and OVA/IFA on day −7. At 24 h after the adoptive transfer, the normal recipient mice were sensitized with OVA/CFA and OVA/IFA at 7-day intervals. In some experiments, 1 × 10⁴ nylon-wool column-passed T cells from normal mice sensitized with OVA/CFA and OVA/IFA at 7-day intervals. In some experiments, 1 × 10⁴ nylon-wool column-passed T cells from non-Tg mice sensitized with OVA/IFA and OVA/CFA were adoptively transferred into naive IL-15-Tg and non-Tg mice via tail vein inoculation. After 24 h, the mice were then exposed to aerosolized OVA every 2 days for 7 days.

In some experiments, 1 × 10⁷ nylon-wool column-passed T cells from non-Tg mice sensitized with OVA/IFA and OVA/CFA were adoptively transferred into naive IL-15-Tg and non-Tg mice via tail vein inoculation. After 24 h, the mice were then exposed to aerosolized OVA for 7 days.

Measurement of OVA-specific IgE, IgG1, and IgG2a

Levels of OVA-specific IgE, IgG1, and IgG2a were determined by ELISA. Sample wells of an ELISA plate were coated with OVA overnight and then blocked with 1% BSA in borate-buffered saline (0.05 M borate, 0.15 M NaCl, pH 8.6, 100 μM/well) at 37°C for 30 min. Diluted samples (100 μM/well) were incubated for 90 min at room temperature (Samples for IgE were diluted 1:100; IgG1, 1:1000; and IgG2a, 1:5). The plates were washed with borate-buffered saline with 0.05% Tween 20 and incubated with peroxidase-conjugated anti-mouse IgE, IgG1, or IgG2a (Nordic Immunology, Minneapolis, MN) for 90 min at room temperature. After further washing, plates were incubated for 20 min at room temperature with 100 μM/well of o-phenylenediamine solution (1 μg/ml with 3% H₂O₂) and read for OD at 492 nm.

Analysis of cytokines in BALF and serum

To obtain BALF, mice were anesthetized, a tracheal cannula was inserted via a midcervical incision, and the airways were lavaged three times with 1 ml PBS. BALF was immediately centrifuged (10 min, 4°C, 160 × g), and the supernatant was rapidly frozen. Commercial ELISA kits were used to measure the levels of IL-4 and IFN-γ (Genzyme, Cambridge, MA), IL-5 (Techne, AN’LYZA immunoassay system; Genzyme), and IL-13 (Quantikine M; R&D Systems Europe, Abingdon, U.K.) in the BALF. ELISA for mouse IL-15 in the BALF and serum was performed in triplicate using purified anti-mouse IL-15 mAb (capture mAb, G277-3588; PharMingen, San Diego, CA), biotin-conjugated anti-mouse IL-15 mAb (second mAb, G277-3960; PharMingen), and peroxidase-conjugated streptavidin (detection reagent; Genzyme) as described previously (22).

Staining of BALF cells

BALF cells were stained by the Pappenheim staining method using modified May-Grunwald’s solution (Merck, West Point, PA) and Giemsa’s stain solution (Katayama Chemical, Osaka, Japan), and the cells were identified as eosinophils, neutrophils, macrophages, and lymphocytes by standard morphology. At least 100 cells were counted, and the absolute number of each cell type was calculated.

Abs and reagents

PE-conjugated anti-CD8 (53-6.7), and FITC-conjugated anti-CD44 (IM7) and anti-CD3 (145-2C11) mAbs were purchased from PharMingen. Cy-Chrome-conjugated anti-CD4 (GK1.5) mAb and Cy-Chrome-conjugated streptavidin were obtained from PharMingen. Anti-H-2K²/D² (28-8-6, mouse IgG2a) mAb was purchased from PharMingen.

Flow cytometric analysis

The cells were stained with PE-, FITC-, and biotin-conjugated mAbs. To block FcR-mediated binding of the mAb, 2.4G2 (anti-FcR mAb) was added. All incubation steps were performed at 4°C for 30 min. To detect biotin-conjugated mAb, the cells were stained with Cy-Chrome-conjugated streptavidin after incubation with a primary mAb. The stained cells were analyzed by a FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA). Lymphocytes were gated by forward and side scattering.

Osmotic loading of Ag to APCs

Osmotic loading of OVA to APC was conducted as described previously (24). Briefly, OVA was dissolved in serum-free sterile PBS and mixed with a cationic lipid transfection reagent (Roche, Gipf-Oberfrick, Switzerland), N-[1-(2,3-diolloyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (OVA-DOTAP), to a final concentration of 200 μg/ml in a total volume 1 ml for 20 min at room temperature. Casein (casein enzymatic hydrolysate; Sigma) was used as control Ag (CASEIN-DOTAP). This mixture was then added to naive syngeneic splenocytes in RPMI 1640 medium at a concentration of 1 × 10⁷ cells/ml in a total volume of 30 ml and incubated overnight at 37°C in 5% CO₂. OVA-pulsed splenocytes were then removed and washed twice in PBS before metmycyn C (MMC)-treatment. After MMC-treatment for 30 min, the splenocytes were suspended in RPMI 1640 medium to a concentration of 2 × 10⁶ cells/ml, and used as APC.

Measurement of cytokine production of spleen cells

Spleen cells were incubated on a nylon-wool column at 37°C in 5% CO₂, for 60 min. The cell population eluted from the column contained >90% T cells, as determined by FACS analysis with anti-CD3 mAb. A CD4⁺ T cell or CD8⁺ T cell subset was purified from splenic T cells using MACS (Miltenyi Biotec). The purity of T cell subsets was confirmed to be more than 98% by FACS analysis. T cells (5 × 10⁵) and MMC-treated naive splenocytes (5 × 10⁵) were cultured in 96-cell culture plates (Falcon; Becton Dickinson) with 200 μg OVA. In some experiments, CD8⁺ T cells (5 × 10⁵ cells) were cultured in flat-bottom microtiter plates for 2 days with 5 × 10⁵ cells of OVA-DOTAP-treated or CASEIN-DOTAP-treated APC. To confirm whether CD8⁺ T cells recognize MHC class I with Ag loading osmotically, CD8⁺ T cells of IL-15-Tg mice were placed in culture with anti-mouse MHC class I mAb (anti-mouse H-2K²/D² mAb; 10 μg/ml) or the same amount of mouse IgG as an isotype control. After 48 h of culture, the cultured supernatants were collected and the amounts of secreted IL-4 and IFN-γ in the supernatants were determined by ELISA. For cellular proliferation, the cultures were pulsed with 1 μCi/well [³H]TdR for an additional 6 h after 48 h of culture. [³H]TdR incorporation was determined by liquid scintillation counting.

Statistical analysis

The statistical significance of the data was determined by Student’s t test. A value of p < 0.05 was taken as significant.
Results

Impaired allergic inflammation in the lungs of IL-15-Tg mice

The IL-15-Tg mice were i.p. sensitized with OVA/CFA followed by OVA/IFA 7 days later and then exposed to aerosolized OVA or PBS every 2 days from day 14 to day 20 after sensitization (Fig. 1A). The BALF was recovered 24 h after the last inhalation. As shown in Fig. 1B, the numbers of total cells and eosinophils in the BALF were markedly increased in OVA-sensitized mice after OVA inhalation, while the numbers were significantly lower in OVA-sensitized IL-15-Tg mice after OVA inhalation (p < 0.05). We next examined cytokine production in BALF after a challenge with OVA inhalation. We previously reported that a significant IL-15 level was detected in the serum of naive IL-15-Tg mice (22). An appreciable level of IL-15 protein was also detected in BALF even in naive IL-15-Tg mice (data not shown). As shown in Fig. 1C, a marginal level of IL-15 was detected in OVA-sensitized non-Tg mice after OVA inhalation, while a significant level of IL-15 was detected in OVA-sensitized IL-15-Tg mice irrespective of PBS or OVA inhalation. The levels of IL-5 and IL-13 were increased in the BALF of OVA-sensitized non-Tg mice after OVA inhalation. In contrast, the increases in IL-5 or IL-13 were only marginal, if any, in OVA-sensitized IL-15-Tg mice after OVA inhalation. IL-4 and IFN-γ levels in the BALF remained at undetectable levels after OVA inhalation in both groups of mice sensitized with OVA (data not shown). These results suggest that OVA-induced airway inflammation is severely impaired in OVA-sensitized IL-15-Tg mice.

Impaired IgE production in IL-15-Tg mice

To determine which type of T cell responses preferentially developed in IL-15-Tg mice by OVA sensitization, we first assessed OVA-specific IgE/IgG2a production in the serum on day 14 after i.p. injections with OVA/CFA on day 0 and with OVA/IFA on day 7. The non-Tg mice had the higher OVA-specific IgE level in the serum than did IL-15-Tg mice after OVA sensitization (Fig. 2; p < 0.05). On the contrary, the level of OVA-specific IgG2a was significantly higher in the IL-15-Tg mice than that in the non-Tg mice sensitized with OVA (Fig. 2; p < 0.05).

We next examined the proliferation and cytokine production by the splenic T cells from OVA-sensitized non-Tg or IL-15-Tg mice. We separated CD4+ T cells or CD8+ T cells from spleen cells of IL-15-Tg mice immunized with OVA by MACS. The purity of CD4+ or CD8+ T cells was confirmed to be >98% by FACS analysis. As shown in Fig. 3A, CD4+ T cells from OVA-sensitized non-Tg mice produced IL-4 in response to OVA, whereas the IL-4 production was significantly reduced in IL-15-Tg mice sensitized with OVA. The IFN-γ production in response to OVA was not increased in the CD4+ T cell population of the sensitized IL-15-Tg mice. Instead, CD8+ T cells from the OVA-sensitized IL-15-Tg mice produced an appreciable amount of IFN-γ in response to
OVA, and such production by CD8\(^+\) T cells was not detected in the OVA-sensitized non-Tg mice (Fig. 3B). IL-4 production in response to OVA was not detected in the CD8\(^+\) T cell population from either OVA-sensitized non-Tg or IL-15-Tg mice.

To further confirm the induction of Tc1 response mediated by CD8\(^+\) T cells in IL-15-Tg mice sensitized by OVA, we used OVA-DOTAP complex as Ag to osmotically load OVA peptide on the MHC class I in APC. As shown in Fig. 4A, CD8\(^+\) T cells of IL-15-Tg mice produced a significantly larger amount of IFN-\(\gamma\) in response to OVA-DOTAP-treated APC than those of non-Tg mice did. The production of IFN-\(\gamma\) by CD8\(^+\) T cells was almost completely inhibited by the addition of anti-MHC class I mAb to the culture (Fig. 4B). Thus, the increase in IFN-\(\gamma\) production in the IL-15-Tg mice is mainly due to the generation of OVA-specific Tc1 cells after sensitization with OVA.

**Effect of in vivo CD8\(^+\) T cell depletion on allergic inflammation in the lung**

Th2 response is inhibited by IFN-\(\gamma\)-produced by Th1 cells, raising the possibility that CD8\(^+\) T cell-mediated Tc1 responses may be responsible for an impaired allergen inflammation in the lung following OVA inhalation in IL-15-Tg mice. To test this hypothesis, CD8\(^+\) T cell-depleted mice were prepared by in vivo administration of anti-CD8 mAb. Both IL-15-Tg and non-Tg mice were i.p. injected with 400 \(\mu\)g of anti-CD8 mAb or an isotype control rat IgG 2 days (on day \(-2\)) before the first sensitization with OVA/CFA and 2 days (on day \(5\)) before the second sensitization with OVA/IFA and then exposed to OVA or PBS every 2 days from days \(14-20\) (Fig. 5A). To confirm whether CD8\(^+\) T cells are depleted by this method, FACS analysis for expression of CD8 in BALF recovered 24 h after the last inhalation was performed. Consistent with our previous findings in lymph node of naive IL-15-Tg mice (22), an increased number of CD44\(^+\)CD8\(^+\) T cells was detected in the BALF of the OVA-sensitized IL-15-Tg mice even after PBS inhalation, and the population was not increased in the IL-15-Tg mice after OVA inhalation (Fig. 5B). In contrast, the CD8\(^+\) T cells were almost completely depleted in the BALF of the IL-15-Tg mice exposed to OVA by in vivo administration of anti-CD8 mAb (Fig. 5B). As shown in Fig. 5C, the number of eosinophils was significantly increased in the lung of the IL-15-Tg mice treated with anti-CD8 mAb compared with those treated with control IgG (\(p < 0.05\)). The levels of IL-5 and IL-13 in BALF of the IL-15-Tg mice were also increased due to in vivo depletion of CD8\(^+\) T cells (Fig. 5D). In contrast, the count of eosinophils and the levels of IL-5 and IL-13 were not changed in the non-Tg mice treated with anti-CD8 mAb. Thus, in vivo depletion of CD8\(^+\) T cells aggravated the allergic inflammation in the lung of IL-15-Tg mice following OVA inhalation.

**Effect of in vivo CD8\(^+\) T cell depletion on IgE production following OVA sensitization**

We next examined the effects of in vivo treatment with anti-CD8 mAb on the induction of Th2 responses by sensitization with OVA/CFA and OVA/IFA. We confirmed that CD8\(^+\) T cells were almost completely depleted on day 14 in the lymph node and spleen of non-Tg and IL-15-Tg mice sensitized with OVA/CFA and OVA/IFA (data not shown). OVA-specific IgE and IgG1 in the sera were significantly increased in the IL-15-Tg mice following anti-CD8 mAb administration (Fig. 6A). In contrast, serum IgG2a level was decreased in IL-15-Tg mice due to CD8\(^+\) T cell depletion. In vivo depletion of CD8\(^+\) T cells did not affect the OVA-specific IgE, IgG1, or IgG2a level in the sera of non-Tg mice on day 14 after OVA sensitization.

The cell proliferation and cytokine production by splenic T cells on day 14 after i.p. injections with OVA/CFA on day 0 and with OVA/IFA on day 7 in response to OVA were next examined in mice depleted of CD8\(^+\) T cells. As shown in Fig. 6B, the proliferative response to OVA was increased in the IL-15-Tg mice treated with anti-CD8 mAb. IFN-\(\gamma\) production was significantly decreased in OVA-sensitized IL-15-Tg mice after in vivo treatment with anti-CD8 mAb, whereas IL-4 production was not affected in the IL-15-Tg mice by in vivo depletion of CD8\(^+\) T cells. These results suggest that CD8\(^+\) T cells are at least partly responsible for impaired IgE production by OVA sensitization and allergic airway inflammation following OVA inhalation in IL-15-Tg mice.
Effects of adoptive transfer with CD8<sup>+</sup> Tc1 cells on allergic inflammation and IgE production

To further determine whether the CD8<sup>+</sup> Tc1 cells generated in IL-15-Tg mice act on allergic inflammation at induction phase or at eliciting phase, we examined the effects of adoptive transfer with CD8<sup>+</sup> T cells derived from OVA-sensitized IL-15-Tg mice on the allergic inflammation. First, normal recipient mice were adoptively transferred with CD8<sup>+</sup> T cells from OVA-sensitized IL-15-Tg mice and then sensitized with OVA/CFA and OVA/IFA at 7-day intervals. Seven days after the last sensitization, mice were exposed to aerosolized OVA (Fig. 7A). As shown in Fig. 7, the eosinophilia and levels of IL-5 and IL-13 in BALF were significantly decreased in the recipients, whereas such suppressive effect was not evident in mice transferred with CD8<sup>+</sup> T cells from OVA-sensitized non-Tg mice. The suppressive effect on the allergic inflammation was not detected in mice transferred with CD8<sup>+</sup> T cells from naive IL-15-Tg mice or casein-sensitized IL-15-Tg mice (data not shown).

IgE production and T cell responses were examined in the recipients 14 days after sensitization. The levels of OVA-specific IgE and IgG1 in the sera of recipients were significantly lower in the mice transferred with CD8<sup>+</sup> T cells derived from OVA-sensitized IL-15-Tg mice compared with those from OVA-sensitized non-Tg mice (Fig. 8A). IL-4 production by splenic T cells of the recipients in response to OVA was significantly decreased in the mice transferred with CD8<sup>+</sup> T cells derived from OVA-sensitized IL-15-Tg mice, whereas the level of IFN-γ production was higher in these mice compared with recipients transferred with CD8<sup>+</sup> T cells from OVA-sensitized non-IL-15-Tg mice.

We next transferred CD8<sup>+</sup> T cells from OVA-sensitized IL-15-Tg mice into mice that had been sensitized with OVA/CFA on day −7 and OVA/IFA on day −0. Then the recipient mice were exposed to aerosolized OVA. An adoptive transfer with the CD8<sup>+</sup> T cells did not affect the allergic inflammation as assessed by eosinophilia and cytokines production in BALF (data not shown). These results suggest that CD8<sup>+</sup> Tc1 cells derived from IL-15-Tg mice suppress the allergic inflammation mainly via down-regulation of induction of Th2 response against OVA sensitization.

IL-15 overexpression did not directly suppress allergic inflammation

Although the impaired airway inflammation in IL-15-Tg mouse is mostly due to insufficient induction of Th2 responses for IgE production, it is not excluded that the possibility that overexpression of IL-15 directly suppress pulmonary inflammation in the lung
after OVA inhalation. To test this, we transferred splenic T cells derived from OVA-sensitized normal mice into IL-15-Tg or non-Tg mice and then exposed these mice to aerosolized OVA. As shown in Fig. 9B, the number of total cells in the BALF, especially macrophages, was increased in the IL-15-Tg mice. There was no difference in the number of eosinophils between the IL-15-Tg mice and non-Tg mice. Surprisingly, the level of IL-5 in the BALF was rather increased in the IL-15-Tg mice than in non-Tg mice (Fig. 9C). These results suggest that allergic inflammation may be exaggerated under the environment of IL-15 overproduction.

FIGURE 5. Effect of in vivo administration of anti-CD8 mAb on allergic inflammation in IL-15-Tg mice. A. Immunization protocol. On day −2 and day 5, mice were injected with anti-CD8 mAb or isotype control rat IgG Ab. All mice were sensitized with OVA on day 0 and day 7 and then exposed to aerosolized OVA for 30 min on days 14, 16, 18, and 20. B. Flow cytometry analysis of the BALF cells in IL-15-Tg mice sensitized with OVA after inhalation of OVA. IL-15-Tg mice or IL-15-Tg mice depleted of CD8+ T cells were sensitized with OVA and were then challenged with inhalation of OVA as described in the protocols in Fig. 1. The BALF cells from IL-15-Tg or non-Tg mice sensitized with OVA after inhalation of OVA or PBS (upper column), or OVA-sensitized IL-15-Tg or non-Tg mice injected with anti-CD8 mAb or isotype control rat IgG after inhalation of OVA (lower row) were stained with FITC-CD44 and PE-CD8α mAb, and then the cells were analyzed using a flow cytometer. The analysis gate was set on lymphocytes. Data are representative of three independent experiments using pooled cells from three IL-15-Tg or non-Tg mice and shown as typical two-color profiles. The cell composition (C) and cytokine production (D) in BALF of OVA-sensitized IL-15-Tg or non-Tg mice injected with anti-CD8 mAb (■) or isotype control rat IgG (□) after inhalation of OVA. The production level of IL-15 protein was expressed as net OD absorbance at 450 nm. The IL-15 level at the line in the figure was shown as the amount of the detection limit. Each bar represents data from at least three independent experiments using 6- to 8-wk-old mice. The values are expressed as means ± SD. *, p < 0.05.
Discussion

Airway inflammation in asthma and allergic diseases is a complex phenomenon driven predominantly by Th2-type cells (1–4). Asthma is characterized by overproduction of the Th2 cytokines IL-4, IL-5, and IL-13, which initiate and sustain the allergic asthmatic inflammatory responses by enhancing the production of IgE and the growth, differentiation, and recruitment of mast cells, basophils, and eosinophils. We found that eosinophilia and production of IL-5 and IL-13 in BALF were severely attenuated in OVA-sensitized IL-15-Tg mice following OVA inhalation. The impaired pulmonary inflammation observed in IL-15-Tg mice is at least partly a result of a predominant CD8\(^+\) Tc1 response to OVA in these mice. IL-15-Tg mice preferably developed CD8\(^+\) Tc1 responses, producing IFN-\(\gamma\), and generated a significantly lower level of IgE but a higher level of IgG2a specific for OVA after OVA sensitization. In vivo depletion of CD8\(^+\) T cells by anti-CD8 mAb aggravated the airway inflammation with eosinophilia in the IL-15-Tg mice. Furthermore, adoptive transfer with CD8\(^+\) Tc1 cells from IL-15-Tg mice suppressed IgE production with Th2 response against OVA. Overexpression of IL-15 may shed light on a novel role of CD8\(^+\) Tc1 cells in regulation of allergic diseases such as asthma and provide an insight into prophylactic and therapeutic approaches for controlling allergic diseases by IL-15.

One of the notable findings in the present study is that OVA-specific CD8\(^+\) Tc1 cells are generated in IL-15-Tg mice sensitized with OVA. IL-15 is known to be important for proliferation and maturation of memory-type CD8\(^+\) T cells (25–28). We previously reported that CD8\(^+\) T cells expressing memory markers, CD44\(^+\), Ly6C\(^-\), and CD69\(^+\) of the phenotype, were increased in naïve IL-15-Tg mice (22). Exogenous proteins such as OVA are usually present in association with MHC class II, whereas recent reports have suggested that there is a degree of degeneracy in the processing pathways and that Ags in the extracellular milieu can also be processed and presented in association with MHC class I, which is usually required for activation of CD8\(^+\) T cells (29–33). MacAry et al. (24) reported the existence of OVA-specific, MHC class I-restricted Tc1 cells in the rat i.p. immunized with OVA complexes to the adjuvant aluminum hydroxide. In the present study,
we detected significant levels of CD8$^+$ Tc1 responses in both non-Tg and IL-15-Tg mice sensitized with OVA/CFA, when we used OVA-DOTAP couples to load osmotically OVA-peptide on the MHC class I in APC. The CD8$^+$ Tc1 response, which was completely blocked by anti-MHC class I mAb, was significantly higher in the IL-15-Tg mice than in non-Tg mice. It could be speculated that OVA-specific CD4$^+$ and CD8$^+$ T cells may be generated following sensitization with OVA, but the CD8$^+$ Tc1 cells are maintained preferentially in the environment of IL-15 overexpression, resulting in dominant Tc1 responses in IL-15-Tg mice sensitized with OVA.

Our present results with adaptive transfer experiment reveal that CD8$^+$ Tc1 cells from IL-15-Tg mice significantly suppressed induction of Th2 response and IgE production. Th1 type cytokines such as IFN-$\gamma$ inhibit Th2 cell proliferation (34). Thus, production of IFN-$\gamma$ by predominant CD8$^+$ Tc1 response may be responsible for the impaired Th2 responses in IL-15-Tg mice. In contrast, in vivo treatment with anti-CD8 mAb did not completely restore Th2 response for IgE production to OVA sensitization and the allergic inflammation in the IL-15-Tg mice following OVA inhalation. This may be due to incomplete depletion of CD8$^+$ T cells by in vivo administration of anti-CD8 mAb. NK cells (13, 35), NK T cells (36, 37), and a significant fraction of $\gamma$6 T cells (26, 27, 38–42) are also reported to respond to IL-15. Thus, early production of IFN-$\gamma$ by these cells, besides CD8$^+$ T cells, may be also responsible for the impaired Th2 responses in IL-15-Tg mice.

Zuany-Amorim et al. (43) recently reported that early production of IL-4 by $\gamma$6 T cells is important for IgE response to OVA. It is reported that $\gamma$6 T cells express IL-12R$\beta$1 after stimulation with IL-15 and produce IFN-$\gamma$ in response to IL-12 (40). Therefore, it is also possible that $\gamma$6 T cells in IL-15-Tg mice differentiate into Th1-type cells, not Th2-type cells, resulting in impaired Th2 responses against OVA. This speculation warrants further examination of the characteristics of the $\gamma$6 T cells in IL-15-Tg mice.

Although the impaired airway inflammation is mostly due to insufficient induction of Th2 responses for IgE production in the periphery, the possibility that overexpression of IL-15 also affects pulmonary inflammation in the lung in the eliciting phase after OVA inhalation cannot be excluded. Lack et al. and other investigators (44–48) also suggested that nebulized IFN-$\gamma$ inhibits eosinophilic inflammation induced by allergen exposure in allergen-sensitized mice. In human subjects that underwent an allergen challenge, relative increases in CD8$^+$ T cells in BALF have been reported (49). Huang et al. (50) demonstrated that CD8$^+$ T cells from normal rats immunized with allergen play a protective role in the allergen-induced bronchial hyperresponsiveness and eosinophilia through activation of the Th1 cytokine response. Depletion of CD8$^+$ T cells with an mAb has been reported to enhance the airway hyperresponsiveness after OVA challenge in Sprague Dawley rats, a nonatopic strain that rarely develops airway hyperresponsiveness. These findings suggest that CD8$^+$ T cells have a

**FIGURE 7.** Effect of adoptive transfer of CD8$^+$ Tc1 cells from IL-15-Tg mice at induction phase on allergic inflammation. A, Immunization protocol. Splenocytes of IL-15-Tg or non-Tg mice sensitized with OVA/CFA on day −15 and OVA/IFA on day −8 were recovered on day −1 and passed through nylon-wool columns. Then, CD8$^+$ T cell subset ($1 \times 10^7$ cells) were purified by MACS and were adoptively transferred into normal mice. At 24 h after the adoptive transfer, the normal recipient mice were sensitized with OVA/CFA and OVA/IFA at 7-day intervals. These mice were then exposed to aerosolized OVA every 2 days for 7 days. BALF cells (B) and BALF cytokine (C) levels following OVA inhalation in mice transferred with CD8$^+$ T cell derived from OVA-sensitized IL-15-Tg (■) and non-Tg (□) at induction phase. Each bar represents value from three independent experiments using 6- to 8-wk-old mice. *, $p < 0.05$; **, $p < 0.001$. 

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suppress function in the airway hyperresponsiveness. The results of the present study showed increased levels of IL-15 protein and CD44+ CD8+ T cells derived from OVA-sensitized IL-15-Tg mice or non-Tg mice in BALF of OVA-sensitized IL-15-Tg mice after OVA inhalation. Therefore, IFN-γ production by CD8+ T cells from the spleen of IL-15-Tg or non-Tg mice sensitized with OVA were cultured with OVA in the presence of MMC-treated spleen cells for 48 h at 37°C. The proliferation activities of splenic T cells was assessed by incorporation of [3H]Tdr. IL-4 and IFN-γ productions by the T cells were assessed by ELISA. The data are representative of four independent experiments using pooled cells from three mice and are shown as the means of triplicate determinations ± SD. *p < 0.05.

FIGURE 8. Effect of adoptive transfer of CD8+ Tc1 cells from IL-15-Tg mice at induction phase on serum IgE and cytokine production. A, Serum levels of OVA-specific Igs in mice transferred with CD8+ T cells derived from OVA-sensitized IL-15-Tg mice (○) or non-Tg mice (□) were measured by ELISA. Per experimental group, three to four mice were used. The values are expressed as means ± SD. *, p < 0.05; **, p < 0.001. B, T cell response to OVA in mice transferred with CD8+ T cells derived from OVA-sensitized IL-15-Tg mice or non-Tg mice. The enriched T cells (5 × 10^5 cells) from the spleen of IL-15-Tg or non-Tg mice sensitized with OVA were cultured with OVA in the presence of MMC-treated spleen cells (5 × 10^5 cells) for 48 h at 37°C. The proliferation activities of splenic T cells was assessed by incorporation of [3H]Tdr. IL-4 and IFN-γ productions by the T cells were assessed by ELISA. The data are representative of four independent experiments using pooled cells from three mice and are shown as the means of triplicate determinations ± SD. *p < 0.05.

Clinical studies have demonstrated that reduced IFN-γ secretion in neonates is associated with the subsequent development of atopy (53–55). Furthermore, a predisposition toward the overproduction of Th1 cytokines may protect against atopy, because patients with multiple sclerosis (56), rheumatoid arthritis (57), or tuberculosis (58), conditions associated with increased production of Th1 cytokines, have a reduced predisposition toward the development of atopy. Thus, the results of these studies suggest that methods to enhance IFN-γ production might be clinically useful in the treatment of allergic asthma. In the present study, we found that the early production of IFN-γ by CD8+ Tc1 cells in IL-15-Tg mice successfully reversed ongoing Ag-specific Th2-dominated responses toward Th1-dominated responses. This finding indicates possible therapeutic effects of IL-15 via up-regulation of Th2 responses in the treatment of asthma and allergic diseases. However, because IL-15 is a pleiotropic cytokine with effects on a number of cell types, it must be carefully used for patients with apparent allergic inflammation.

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the mice were exposed to aerosolized OVA every 2 days for 7 days. BALF cells (B) and BALF cytokine (C) levels following OVA-inhalation in the IL-15-Tg mice (■) and non-Tg mice (□) transferred with splenic T cells derived from OVA-sensitized mice. Each bar represents data from at least three independent experiments using 6- to 8-wk-old mice. *p < 0.05; **p < 0.001.

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