Contribution of IL-18-induced innate T cell activation to airway inflammation with mucus hypersecretion and airway hyperresponsiveness

Yuriko Ishikawa1,2, Tomohiro Yoshimoto1,2 and Kenji Nakanishi1,2

1Department of Immunology and Medical Zoology, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501, Japan
2Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Saitama 332-0012, Japan

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Abstract

Human bronchial asthma is characterized by airway hyperresponsiveness (AHR), eosinophilic airway inflammation, mucus hypersecretion and high serum level of IgE. IL-18 was originally regarded to induce Th1-related cytokines from Th1 cells in the presence of IL-12. However, our previous reports clearly demonstrated that IL-18 with IL-2 promotes Th2 cytokines production from T cells and NK cells. Furthermore, IL-18 with IL-3 stimulates basophils and mast cells to produce Th2 cytokines. Thus, we examined the capacity of IL-2 and IL-18 to induce AHR, airway eosinophilic inflammation and goblet cell metaplasia. Intranasal administration of IL-2 and IL-18 induces AHR, mucus hypersecretion and eosinophilic inflammation in the lungs of naive mice. CD4+ T cells are prerequisite for this IL-2 plus IL-18-induced bronchial asthma, because CD4+ T cells-depleted or Rag-2-deficient (Rag-2/C255/C255) mice did not develop bronchial asthma after IL-2 plus IL-18 treatment. Both STAT6/C255/C255 mice and IL-13-neutralized wild-type mice failed to develop AHR, goblet cell metaplasia and airway eosinophilic inflammation, while IL-4/C255/C255 mice almost normally developed, suggesting that IL-13 is a major causative factor and IL-4 mainly enhances the degree of AHR and eosinophilic inflammation. Both IL-4 and IL-13 equally induce eotaxin in mouse embryonic fibroblasts. However, only IL-13 blockade inhibited asthma symptoms, suggesting that IL-13 but not IL-4 is produced abundantly and plays a critical role in the pathogenesis of bronchial asthma in this model. As airway epithelial cells store robust IL-18, IL-18 might be critically involved in pathogen-induced bronchial asthma, in which pathogens stimulate epithelial cells to produce IL-18 without IL-12 induction.

Introduction

Bronchial asthma is characterized by airway hyperresponsive-ness (AHR), eosinophilic airway inflammation, airway remodeling, airway mucus hypersecretion and high serum levels of IgE (1–4). AHR and mucus oversecretion are often linked to asthma symptoms and morbidity. Although the mechanisms underlying these features are complex, it is widely accepted that Th2 cells, which produce a limited repertoire of cytokines including IL-4, IL-5, IL-9 and IL-13, are responsible for inducing these characteristic features of bronchial asthma (5–7). Indeed, CD4+ T cells producing IL-4, IL-5, IL-9 and IL-13 were identified in the bronchoalveolar lavage (BAL) and airway biopsy (8,9) of asthma patients. Experimental animal studies also revealed that Th2 cells induce airway eosinophilia, goblet cell metaplasia with mucus oversecretion and AHR (10). Among the Th2-related cytokines, IL-13 is suggested to play a critical role in induction of AHR and mucus oversecretion (11–14). Indeed, blockade of IL-13 markedly inhibits allergen-induced AHR, eosinophilic inflammation and goblet cell metaplasia in animal models (11,12). Furthermore, direct intranasal administration of IL-13 into naive mice induces mucus hypersecretion, airway eosinophilic infiltration and AHR (11,12).

In contrast, Th1 cells had generally been regarded to protect against bronchial asthma by damping the activity of Th2 cells via IFN-γ (15,16). However, our recent studies revealed that naive mice transferred with antigen-specific Th1 cells exhibit bronchial asthma after challenge with both antigen and IL-18 but not with antigen alone (17). We found that Th1 cells...
become very pathological, when they are stimulated with Ag and IL-18, possibly by production of IL-13 and IFN-γ. Our study substantiated further the previous study that administration of a combination of IL-13 and IFN-γ induces severe airway inflammation independently of antigenic challenge (18).

IL-18 was originally identified as a factor that enhances IFN-γ production from T<sub>h</sub>1 cells in the presence of anti-CD3 and IL-12 (19–21). However, our recent studies and those of others demonstrated that IL-18 directly promotes T<sub>h</sub>2 cytokines production from T cells, NK cells, basophils and mast cells, resulting in IgE production (22–27). Usually, secretion of cytokines by T cells is the result of antigen-stimulation. Therefore, cytokines can play a critical role in regulation of an acquired immune response. However, T cells stimulated with IL-12 plus IL-18 exhibit T<sub>h</sub>1 response (28). Furthermore, T cells also show T<sub>h</sub>2 response when stimulated with IL-2 and IL-18 in the absence of Ag stimulation (25,26). Since IL-18 induces T<sub>h</sub>1 or T<sub>h</sub>2 cytokines production by T cells without TCR engagement, we could designate these TCR-independent T cell activation pathways as innate type 1 or type 2 T cell activation, respectively (28–30). As airway epithelial cells store robust IL-18 (31), we could assume the possibility that some types of infectious agents might stimulate bronchial epithelial cells to produce IL-18. Thus, it is very important to determine the biological relevance of IL-18-induced innate T cell activation pathway in the pathogenesis of bronchial asthma.

Here, we have demonstrated that intranasal administration of IL-2 and IL-18 into naive mice induces them to express various mRNAs for cytokines (IL-4, -5, -9, -13, -17) and chemokines (MIP-2, GCP-2 and eotaxin) in their lungs and to develop dense infiltration of eosinophils and neutrophils in the peribronchial and perivenular region, goblet cell metaplasia with mucus hypersecretion and AHR, which are prominent features of bronchial asthma. These data suggest the biological relevance of IL-18 in antigen-independent bronchial asthma following viral or bacterial airway infection.

**Methods**

**Animals and reagents**

Specific pathogen-free (SPF) female BALB/c mice and BALB/c-background STAT6-deficient (STAT6<sup>-/-</sup>) mice were purchased from Jackson Laboratory. BALB/c IL-4<sup>-/-</sup> and Rag-2<sup>-/-</sup> mice were obtained from Taconic (Germantown, NY, USA). All mice were bred under SPF condition at the animal facilities of Hyogo College of Medicine (Nishinomiya, Japan) and were used at 8–10 weeks of age. Animal experiments were conducted according to the Guideline for Animal Experiments at Hyogo College of Medicine. Recombinant human IL-2 was kindly provided by Ajinomoto Co. Inc. (Kawasaki, Japan). Recombinant mouse IL-5, IL-13 and IL-18 were purchased from Genetics Institute Inc. (Cambridge, MA, USA) and MBL (Nagoya, Japan), respectively. Recombinant mouse IL-4 was obtained and purified from a recombinant baculovirus (AcMNPV/IL-4) prepared in our laboratory.

**In vitro culture**

Splenic CD<sub>4<sup>+</sup></sub> T cells from BALB/c mice were purified by MicroBeads (anti-mouse CD4, clone RM4-5; Miltenyi Biotec, Bergisch Gladbach, Germany). The enriched CD<sub>4<sup>+</sup></sub> T cells (10<sup>6</sup>/0.2 ml well<sup>-1</sup>) were cultured with medium alone or various combinations of IL-2 (200 pM) and IL-18 (50 ng ml<sup>-1</sup>) for 4 days in RPMI 1640 supplemented with 10% FBS, 2-ME (50 µM), γ-glutamine (2 mM), penicillin (100 U ml<sup>-1</sup>) and streptomycin (100 µg ml<sup>-1</sup>) and PBS for 1–4 days. Control mice were exposed to PBS alone. Mice were analyzed at 24 h after the final exposure to PBS alone or cytokines. To deplete CD<sub>4<sup>+</sup></sub><sup>+</sup> T cells, BALB/c mice were intraperitoneally injected four times (−14, −10, −7, −4 days before cytokine treatment) with mAb to CD4 (clone, GK1.5; 0.5 mg day<sup>-1</sup>). For the blockade of IL-13 in vivo, 20 µg of sIL-13Rα2-Fc or 20 µg of control human IgG (Genetics Institute Inc.) were daily administered intranasally as the mixed form with IL-2 and IL-18 for 4 days.

**Measurement of AHR**

We measured AHR to β-methacholine (Mch) inhalation in mice by using Pulmos-l (MIPS, Osaka, Japan) hardware and software as described in our previous report (17). We placed a mouse in a chamber and exposed it to aerosols of saline (baseline) first and then to increased concentrations of Mch (5, 10 and 20 mg ml<sup>-1</sup>). After each 2 min exposure, we measured enhanced pause, a dimensionless index that reflects changes in amplitude of pressure waveform and expiratory time, for 3 min.

**BAL**

BAL was performed with three aliquots of 1.0 ml of PBS per mouse. Total cell counts were performed. Cytospin preparations of BAL fluid (BALF) were stained with Diff-Quik (Baxter Healthcare Corp., Miami, FL, USA), and differentials were performed based on morphology and staining characteristics.

**Histology**

Lungs were prepared for histology by perfusion of the animal via the right ventricle with 10 ml of PBS, then fixed in 10% buffered formalin, cut into 3-µm sections and stained with hematoxylin and eosin or predigested periodic acid Schiff.

**Preparation of embryonic fibroblasts**

Mouse embryonic fibroblasts were prepared from E14 embryos generated by BALB/c mice. Briefly, the heads were removed, and the carcasses were finely minced with scissors and digested by incubation in PBS containing 0.1% trypsin (GIBCO-BRL, Grand Island, NY, USA) and 0.02% EDTA (NACALAI TESQUE, INC., Kyoto, Japan) for 20 min at 37°C. Trypsin was inactivated by washing the cells twice using DMEM supplemented with 10% FBS, 2-ME (50 µM), γ-glutamine (2 mM), penicillin (100 U ml<sup>-1</sup>) and streptomycin (100 µg ml<sup>-1</sup>) and the cells were plated onto tissue culture dishes. After 3–4 days, when the growth of fibroblasts was established, embryo fragments were removed. Embryonic fibroblasts were maintained as a monolayer culture, and cells from the second or third passage were used.

**In vivo treatment of mice**

Mice were daily exposed intranasally to various combinations of IL-18 (0–1 µg day<sup>-1</sup>) and IL-2 (500 U day<sup>-1</sup>) in 50 µl of PBS for 1–4 days. Control mice were exposed to PBS alone. Mice were analyzed at 24 h after the final exposure to PBS alone or cytokines. To deplete CD<sub>4<sup>+</sup></sub><sup>+</sup> T cells, BALB/c mice were intraperitoneally injected four times (−14, −10, −7, −4 days before cytokine treatment) with mAb to CD4 (clone, GK1.5; 0.5 mg day<sup>-1</sup>). For the blockade of IL-13 in vivo, 20 µg of sIL-13Rα2-Fc or 20 µg of control human IgG (Genetics Institute Inc.) were daily administered intranasally as the mixed form with IL-2 and IL-18 for 4 days.
For amplification of IL-17 cDNA, PCR assays were performed as follows: IL-4, 5'-AGA GAT CC, 3'; IL-13, 5'-AGA GAG ATT CCC, 3'; IL-17, 5'-GTA CGA CCA GAG GCA TAC AGG, 3'-GAT GAC CAT TTC CCA GAA AC; β-actin, 5'-GTA CGA CCA GAG GCA TAC AGG, 3'-GAT GAC CAT TTC CCA GAA AC.

Homogenized lungs

BALB/c mice were daily exposed intranasally to IL-18 (1 µg day⁻¹) and IL-2 (500 U day⁻¹) for 1–4 days. Lungs were removed at 8 h after the final exposure to IL-2 and IL-18 and total RNA was extracted using Trizol reagent, treated with DNase I and reverse transcribed using Superscript II RT and oligo (dT)₁₂–₁₈ primer (all four reagents from Invitrogen, Carlsbad, CA, USA). For amplification of IL-4 and IL-13 cDNA, after initial denaturation step at 94°C for 2 min, 34 cycles were performed at 94°C for 1 min followed by 55°C for 1 min and 68°C for 1 min, and then further extension at 68°C for 7 min. For amplification of IL-9 cDNA, PCR assays were performed for 34 cycles (94°C for 1 min followed by 58°C for 1 min and 68°C for 1 min). For amplification of IL-5, MIP-2, eotaxin-2, RANTES and GCP-2 cDNA, PCR assays were performed for 34 cycles (94°C for 1 min followed by 60°C for 1 min and 68°C for 1 min). For amplification of eosinax and β-actin cDNA, PCR assays were performed for 30 cycles (94°C for 1 min followed by 62°C for 1 min and 68°C for 1 min). For amplification of IL-17 cDNA, PCR assays were performed for 34 cycles (94°C for 15 s followed by 58°C for 30 s and 68°C for 1 min). At the end of cycles, samples were stored at 4°C until analyzed. After amplification, PCR products were separated by electrophoresis in 1.7% agarose gels and visualized by UV light illumination. Primer sequences were as follows: IL-4, 5'-GAA TGG TAC GGC AGG CAT ATC, 3'-CTC AGT ACT AGC AGT AAT CCA; IL-5, 5'-ATG GAG ATT CCC ATG AGC AC, 3'-GTC TCT CCT CGC CAC ACT TC; IL-9, 5'-TAC ATC CTT GCC TCT GTT TT, 3'-CAG AAA TGA CAG TGT GTT GC; IL-13, 5'-ACA GCT CCC TGG TTC TCT CA, 3'-GCT ACT TCG ATT TGT GGA TCG G; IL-17, 5'-TCT CTC GCA AGA GAT CC, 3'-AGT TTG GGA CCC CCT TTC TAC AC; eotaxin, 5'-CTC CAC AGC GCT TCT ATT CC, 3'-GTT ATT TGT GGG GTG AGC AC; GCP-2, 5'-CCA CCC AGG GAT TTT TTC TA, 3'-GGT CCC CAT TTC ATG AGA GA; eotaxin-2, 5'-CTG TGA CCA TCC CCT CAT CT, 3'-ATT TCT TGG GTT TGG TTC GC; MIP-2, 5'-GAA GTC ATA GCC ACT CTC AAG GGG C, 3'-CAA CTC ACC CTC TCC CCA GAA AC; RANTES, 5'-GTC CCC AGC TCA AGG AT, 3'-ATT TCT TGG GTT TGG TTC GC; MIP-2, 5'-GAA GTC ATA GCC ACT CTC AAG GGG C, 3'-CAA CTC ACC CTC TCC CCA GAA AC; streptomycin (100 µg ml⁻¹). Supernatants were harvested and tested for IL-4 and IL-13 contents by ELISA. Mouse embryonic fibroblasts (5 × 10⁵/0.2 ml well⁻¹) were cultured with complete DMEM alone, various combinations of IL-4 (103 U ml⁻¹) and IL-13 (10 ng ml⁻¹) or IL-5 (10 ng ml⁻¹) for 2 days. Supernatants were harvested and tested for eotaxin contents by ELISA.

Results

IL-2 plus IL-18-induced mucus hypersecretion, airway inflammation and AHR

We first examined the effect of IL-2 and IL-18 treatment on airway inflammation and AHR. We daily treated naive BALB/c mice with intranasal administration of IL-2 and/or IL-18 for 4 days. Histopathological analysis of the lung revealed that administration of IL-18 (1 µg day⁻¹) and IL-2 (500 U day⁻¹) strongly induced peribronchial and perivascular infiltration with eosinophils and mononuclear cells, and goblet cell metaplasia with mucus hypersecretion in the airways (Fig. 1A and B). However, treatment with each component alone did not induce or only weakly induced these changes (Fig. 1A). Titration study indicated that IL-18, in the presence of IL-2 (500 U day⁻¹), was not sufficient to induce airway inflammation and goblet cell metaplasia. However, 3 or 4 days treatment induced overt airway inflammation and goblet cell metaplasia (data not shown). Furthermore, mice received IL-2 and IL-18 >3 days through their nasal tracts exhibited AHR after nasal exposure to various concentrations of Mch, whereas mice received PBS, IL-2 or IL-18 alone did not exhibit (Fig. 1C), suggesting that intranasal administration of IL-2 and IL-18 induces naive mice to develop mucus hypersecretion, airway inflammation and AHR. To understand the mechanisms underlying IL-2 and IL-18-induced AHR, we examined the numbers of inflammatory cells infiltrating lung tissues of mice after their exposure to PBS, IL-2 and/or IL-18 for 4 days (Fig. 1D). Analysis of BALF revealed marked increases in the numbers of eosinophils and neutrophils after administration of both IL-2 and IL-18 (Fig. 1D), suggesting that intranasal administration of IL-2 and IL-18 induces naive mice to develop mucus hypersecretion, airway inflammation and AHR.

CD4⁺ T cell-dependent bronchial asthma in IL-2 plus IL-18-treated mice

To determine whether this IL-2 plus IL-18-induced bronchial asthma is dependent on the function of CD4⁺ T cells, we administrated IL-2 and IL-18 into BALB/c wild-type mice depleted of CD4⁺ T cells by the pretreatment with anti-CD4 antibody or Rag-2⁻²⁻ mice, lacking both T cells and B cells. Anti-CD4 treatment significantly impaired IL-2 plus IL-18-induced goblet cell metaplasia with mucus production (Fig. 2A), AHR upon Mch challenge (Fig. 2B) and the number of eosinophils and neutrophils in BALF (Fig. 2C). IL-2 plus IL-18-administered Rag-2⁻²⁻ mice showed no mucus production, AHR and airway inflammation (Fig. 2). These results clearly indicated that IL-2 plus IL-18-induced bronchial asthma by activation of CD4⁺ T cells.
To determine which cytokines mediate IL-2 plus IL-18-induced goblet cell metaplasia with mucus hypersecretion, airway infiltration with eosinophils and neutrophils and AHR, we intra-nasally administered IL-2 and IL-18 into STAT6−/− mice, IL-4−/− mice or BALB/c wild-type mice given a soluble IL-13Rα2-human Fc fusion protein (sIL-13Rα2-Fc), which selectively binds to and neutralizes IL-13 (17,26). As shown in Fig. 3, STAT6−/− mice completely evaded IL-2 and IL-18-induced bronchial asthma, suggesting the critical involvement of IL-4 and/or IL-13 in bronchial asthma. However, IL-4−/− mice normally developed goblet cell metaplasia (Fig. 3A), whereas wild-type mice treated with sIL-13Rα2-Fc failed to do so (Fig. 3A), suggesting that IL-13 is indispensable for inducing mucus overproduction. IL-4−/− mice diminished but still exhibited AHR and airway eosinophilic infiltration (Fig. 3B and C), whereas wild-type mice treated with sIL-13Rα2-Fc did not exhibit AHR and airway eosinophilic infiltration (Fig. 3B and C), suggesting the importance of IL-13 in the pathogenesis of bronchial asthma. In Fig. 3(D), we demonstrated that splenic CD4+ T cells become highly IL-13-producing cells when they are stimulated with IL-2 and IL-18 in vitro. Compared with IL-13 production (29.8 ± 6.8 ng ml−1), IL-2 plus IL-18-stimulated T cells produced IL-4 poorly (94.5 ± 7.5 pg ml−1). Furthermore, compared with the degree of IL-4 induction, IL-2 and IL-18 more strongly induced IL-13 in the lungs (Fig. 3E). These results suggest the possibility that IL-2 plus IL-18 induces goblet cell metaplasia, AHR and eosinophilic infiltration principally by strong IL-13 induction and only partly by IL-4 due to poor IL-4 induction.

Expression of mRNAs for cytokines and chemokines in lung of IL-2 plus IL-18-treated mice

To evaluate further which cytokines and/or chemokines regulate IL-2 plus IL-18-induced airway eosinophilic and neutrophilic inflammation, lungs were tested for the expression of mRNA for cytokines and chemokines at various time points after intranasal administration of IL-2 and IL-18. mRNAs for cytokines (IL-4, IL-5, IL-9, IL-13 and IL-17) and chemokines (MIP-2, GCP-2, eotaxin and eotaxin-2) were measured by RT-PCR. Normally these cytokines/chemokines mRNAs are below the detection level in the lungs of naive mice. However, mRNAs for IL-4, IL-5, IL-9 and IL-13 became detectable at day 1 and gradually increased in the lungs of both wild-type and STAT6−/− mice after intranasal administration of IL-2 and IL-18 (Fig. 4). IL-5 and IL-9 mRNAs were apparently expressed in STAT6−/− mice. Nevertheless, these mice did not develop AHR and eosinophilic airway inflammation (Fig. 3B and C), suggesting that these two cytokines are not involved in IL-2 plus IL-18-induced asthma. mRNAs for IL-17 and CXC chemokines (MIP-2 and GCP-2) also became detectable at
day 1 and sustained their expressions until day 4 in both wild-type and STAT6−/− mice treated with intranasal administration of IL-2 and IL-18 (Fig. 4). MIP-2 and GCP-2 are potent chemoattractants for neutrophils (32,33). IL-17 is produced principally by CD4+ T cells and bears the potential to increase neutrophil recruitment into the airways by releasing several CXC chemokines, including GCP-2 and MIP-2 in bronchial epithelial cells (34,35). Thus, IL-2 and IL-18-induced airway neutrophilic infiltration even in STAT6−/− mice could be explained by the function of IL-17 and/or CXC chemokines (MIP-2 and GCP-2). Most striking difference in the pattern of expression of mRNAs between wild-type and STAT6−/− mice is the expression of eotaxin, which is the most potent chemoattractant for eosinophils (Fig. 4). Eotaxin mRNA expression became detectable at day 1 and are persistently high up to day 4. STAT6−/− mice failed to increase this message, suggesting that IL-2 and IL-18 induce this message by STAT6 activation (Fig. 4). In addition to eotaxin, we have measured mRNAs for eotaxin-2 and RANTES, which are also known to recruit eosinophils. Like eotaxin, eotaxin-2 mRNA expression was promptly and STAT6-dependently induced and its expression was persistently high up to day 4. However, mRNA for RANTES was constitutively and highly expressed in the lungs of naive mice and was not up-regulated by IL-2 plus IL-18 treatment (Fig. 4). These results explain why STAT6−/− mice treated with IL-2 plus IL-18 lacked airway eosinophilic infiltration, even though they express IL-5 mRNA expression in their lungs.

We finally compared the activity of IL-4, IL-5 or IL-13 to induce eotaxin expression in cultured fibroblasts. As reported (36,37), IL-4 and IL-13 showed comparable effect on eotaxin induction from cultured fibroblasts but failed to exhibit additive inducing effect (IL-4, 605.9 ± 37.9 pg ml−1; IL-13, 467.7 ± 33.4 pg ml−1; IL-4 + IL-13, 599.5 ± 36.0 pg ml−1). In contrast, IL-5 could not induce this chemokine expression. These results substantiated further the observation that IL-2 and IL-18 induce eosinophilic infiltration in the lungs by dominant IL-13 induction with poor IL-4 in lung-infiltrating CD4+ T cells.

**Discussion**

Patients with extrinsic bronchial asthma develop AHR when challenged with corresponding allergen. Thus, allergen acts as a causative factor in Th2 cell-mediated bronchial asthma. However, bronchial asthma patients often exhibit AHR following infection with bacteria or viruses (38). It is well-known evidence that infection with *Mycoplasma pneumoniae* or influenza A often induces asthma-like AHR even in the non-allergic patients (39–41). Therefore, it is very important to reveal the mechanism of how these infections exacerbates bronchial asthma independently of Ag. Since IL-18 is deeply associated with induction or exacerbation of bronchial asthma and is also markedly induced following several infections, in this report we tested the pathological effect of IL-18 on AHR and airway inflammation.

IL-18 is a unique cytokine that regulates both Th1 and Th2 responses (23). IL-18 and IL-12 act synergistically to promote IFN-γ production (19,28,42). However, IL-18 with IL-2 shows the capacity to induce production of Th2 cytokines (IL-4, IL-5, IL-9 and IL-13) from CD4+ T cells (25,26). Thus, IL-18 induces Th1 or Th2 cytokines production from T cells dependently of its
cytokine milieu. In general, T<sub>h1</sub> or T<sub>h2</sub> cells require TCR engagement to produce Th1- or Th2-related cytokines, respectively. As noted above, IL-18 plus IL-12- or IL-18 plus IL-2-stimulated T cells produce T<sub>h1</sub>- or T<sub>h2</sub>-related cytokines, respectively, without TCR engagement. Therefore, we previously proposed that there are two types of T cell activation pathway, acquired type (TCR-dependent) activation and innate type (TCR-independent) activation pathways (29). It is well accepted that acquired T cell activation pathway plays a critical role in an acquired immune response, particularly bronchial asthma. In contrast, it is still unclear whether the innate T cell activation pathway is indeed involved in the pathogenesis of bronchial asthma. Therefore, we sought to determine the pathological effect of IL-18-mediated innate T cell activation pathway for induction of bronchial asthma.

In this study, we have shown that daily intranasal administrations of IL-2 and IL-18 into naive mice for four consecutive days induce them to develop goblet cell metaplasia with mucus hypersecretion, airway eosinophilic as well as neutrophilic infiltration and AHR, which are prominent features of bronchial asthma (Figs. 1 and 2). Then, we tried to elucidate the underlying mechanism of how this treatment induces...
bronchial asthma. We found that IL-2 and IL-18 act on CD4+ T cells to produce IL-13, which in turn induces goblet cell metaplasia with mucus hypersecretion and eosinophilic infiltration in the lungs. Mucus hypersecretion is completely inhibited by IL-13-neutralization. Furthermore, IL-4−/− mice normally developed goblet cell metaplasia and eosinophilic infiltration in response to intranasal administration of IL-2 and IL-18. Thus, IL-2 and IL-18 induce goblet cell metaplasia and eosinophilic inflammation principally by IL-13 induction. Indeed and importantly, in vitro CD4+ T cell activation with IL-2 and IL-18 induces IL-13 production strongly and IL-4 production weakly (Fig. 3D). In contrast to the poor IL-4 induction by IL-2 and IL-18, this combination strongly induced IL-13 in the lungs (Fig. 3E). Thus, IL-2 and IL-18 mainly induced bronchial asthma via IL-13. However, IL-4−/− mice partially reduced AHR (Fig. 3B), suggesting that IL-2 plus IL-18-stimulated T cells partly induce AHR via IL-4.

In animal models of allergic asthma, blockade of IL-13 markedly inhibits allergen-induced AHR (11,12). IL-13−/− mice sensitized and challenged with antigen fail to develop AHR, although they develop severe airway inflammation (43). Furthermore, IL-13 delivery to the airway of naive mice causes AHR without antigen (11,12). All of these reports demonstrate the essential role of IL-13 in the development of AHR. However, other studies suggest that IL-4 by itself can induce AHR. Indeed, IL-4 delivery to the airway of naive Rag-2−/− mice causes AHR (12). Like IL-13, IL-4 affects epithelial cells and smooth muscle cells to induce AHR (44,45). Furthermore, in lung epithelial cells, both IL-4 and IL-13 inhibit the activity of inducible nitric oxide synthesis and decrease production of nitric oxide, a bronchodilator (46). Our data indicated the strong contribution of IL-13 to bronchial asthma. However, contribution of IL-4 to IL-2 plus IL-18-induced asthma seems to be very weak, perhaps due to the low ability of IL-2 plus IL-18-stimulated T cells to produce IL-4.

We also found that intranasal administration of IL-2 and IL-18 induces various mRNAs for cytokines (IL-4, IL-5, IL-9, IL-13 and IL-17) and chemokines (MIP-2 and GCP-2) in lungs of wild-type and STAT6−/− mice (Fig. 4). Wild-type mice developed eosinophilic infiltration, which was inhibited by neutralization of IL-13. STAT6−/− mice did not develop eosinophilic infiltration, even though they normally expressed mRNAs for IL-5 and RANTES, suggesting that STAT6 activation is responsible for inducing eosinophilic infiltration. Wild-type mice expressed eotaxin and eotaxin-2, while STAT6−/− mice did not (Fig. 4), suggesting that induction of eotaxin and eotaxin-2 is dependent upon STAT6 activation. IL-13 or IL-4 equally induced eotaxin in cultured fibroblasts. In contrast, IL-5 failed to induce this chemokine. Beside this, IL-4 or IL-13 can induce the endothelial expression of vascular cell adhesion molecule 1 (VCAM-1) (47), which binds to integrin α4β1 (VLA-4) expressed on eosinophils (48). Thus, IL-13 induces eosinophilic infiltration by induction of eotaxin and VCAM-1 in a STAT6-dependent manner. IL-4 also has the same potential. Nevertheless, neutralization of IL-13 alone almost completely inhibited AHR, eosinophilic infiltration and mucus secretion (Fig. 3). Based on these evidences, we concluded that IL-2 and IL-18 induce eosinophilic infiltration by strong promotion of IL-13 induction from T cells.

IL-2 and IL-18 also induced neutrophilic infiltration. In contrast to airway eosinophilia, IL-2 plus IL-18-induced airway neutrophilia is not dependent on STAT6 (Fig. 3). It has been reported that IL-17 bears the potential to increase neutrophil recruitment into the airways by releasing several different CXC chemokines including GCP-2, Gro-α and IL-8 in human bronchial epithelial cells or MIP-2 in mouse bronchial epithelial cells, respectively (34,35). All of these CXC chemokines are potent neutrophil chemotaxants (33). In this study, IL-2 plus IL-18-administered STAT6−/− mice showed airway neutrophilia (Fig. 3) and increased expression of mRNAs for IL-17, MIP-2 and GCP-2 (Fig. 4). Taken together, IL-2 plus IL-18 induced airway neutrophilia by induction of IL-17 and/or CXC chemokines (MIP-2 and GCP-2).

Recently, we have demonstrated that Tn1 cells have the capacity to produce both Tn1 cytokines and Tn2 cytokines when stimulated with Ag and IL-18 and become very pathological cells when they show such phenotype in the lung (17). Mice transferred with Ag-specific Tn1 cells exhibit neither AHR nor respiratory tract eosinophilic inflammation when challenged with intranasal administration of Ag. However, they suffer from severe bronchial asthma upon challenge.
with Ag plus IL-18, with comparable levels of AHR and eosinophilic inflammation to those in mice transferred with T_{H}2 cells and challenged with Ag later (17). The former type of asthma is categorized as acquired type Th1 asthma to distinguish this from T_{H}2-induced bronchial asthma. Consistent with previous reports, neutralization of IL-13 inhibits AHR and eosinophilic infiltration in T_{H}2-induced bronchial asthma. In contrast, blockade of IL-13 does not reduce IL-18-induced T_{H}1 asthma, although this treatment markedly reduces eosinophilic infiltration in the airway (17), suggesting that molecules other than IL-13 contribute to the development of Ag plus IL-18-induced AHR in T_{H}1 mice. In the present study, we have revealed that neutralization of IL-13 almost completely inhibited IL-2 plus IL-18-induced bronchial asthma (Fig. 3). However, it is important to know the effect of endogenous IFN-γ. Thus, we administered IL-2 plus IL-18 into wild-type BALB/c and BALB/c-background IFN-γ{−/−} mice. Both wild-type and IFN-γ{−/−} BALB/c mice showed similar degree of mucus production in the lungs. Furthermore, they showed comparable level of AHR upon Mch challenge. However, IL-2 plus IL-18-administered IFN-γ{−/−} mice exhibited ~1.5-fold increase in the number of eosinophils in their BALF (data not shown). These results taken together indicate that compared with strong IL-12 plus IL-18 effect on IFN-γ production from T cells, IL-2 plus IL-18 poorly induced T cell IFN-γ production, resulting in little effect on AHR, mucus production and eosinophilic infiltration. Thus, IL-2 plus IL-18 mainly induces AHR via IL-13 but not IFN-γ. Importantly, intranasal administration of IL-2 plus IL-18 into mice induces bronchial asthma independently of antigenic challenge. Therefore, IL-18 is critically involved in the pathogenesis of both acquired type and innate type bronchial asthma, suggesting IL-18 as a therapeutic target for the treatment of both types of bronchial asthma.

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Abbreviations
AHR airway hyperresponsiveness
Mch methacholine
BAL bronchoalveolar lavage
BALF bronchoalveolar lavage fluid
IL-13Rα2-Fc IL-13Rα2-human Fc fusion protein

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