Contribution of IL-18 to eosinophilic airway inflammation induced by immunization and challenge with *Staphylococcus aureus* proteins


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Abstract

We previously reported that intranasal challenge with ovalbumin (OVA) plus IL-18 induces airway hyperresponsiveness (AHR) and eosinophilic airway inflammation in mice with OVA-specific Th1 cells. These two conditions can be prevented by neutralizing anti-IFN-γ and anti-IL-13 antibodies, respectively. The mice develop AHR and eosinophilic airway inflammation after challenge with OVA plus LPS instead of IL-18 and endogenous IL-18 is known to be involved. In contrast, IL-18 does not facilitate these changes in mice possessing OVA-specific Th2 cells. Here, we investigated whether IL-18 is involved in the development of asthma in mice immunized and challenged with bacterial proteins. Upon intranasal exposure to protein A (SpA) derived from *Staphylococcus aureus*, mice immunized with SpA exhibited AHR and peribronchial eosinophilic inflammation if IFN-γ or IL-13 were present, respectively. The CD4⁺ T cells from draining lymph nodes (DLNs) of the SpA-immunized and -challenged mice produced a robust IFN-γ and IL-13 in response to immobilized anti-CD3 antibodies. Treatment with neutralizing anti-IL-18 antibodies prevented asthmatic inflammation concomitant with their impaired potential to express IFN-γ and IL-13. Furthermore, naive mice that received the CD4⁺ T cells from DLNs of SpA-immunized mice developed airway inflammation depending upon the presence of IL-18. Immunodeficient mice that received human PBMCs, which had been stimulated with SpA in vitro, developed dense peribronchial accumulation of human CD4⁺ T cells upon SpA challenge. Neutralizing anti-human IL-18 antibodies protected against this airway inflammation. These results suggest the importance of IL-18 for the development of asthmatic inflammation associated with airway exposure to bacterial proteins.

Keywords: airway hyperresponsiveness, asthma, eosinophilic inflammation, IL-18, *Staphylococcus aureus*

Introduction

Bronchial asthma is complex syndrome characterized by airway hyperresponsiveness (AHR) and reversible airflow obstruction with airway inflammation and mucus formation (1–8). Bronchial asthma is believed to be mediated by Th2 cells and their cytokines. IL-13 produced by the Th2 cells can principally account for almost all the above pathogenic responses (2, 9). However, other subsets of CD4⁺ T cells, such as Th1, Th17, regulatory T (Treg) and CD1d-restricted NKT cells, are now recognized to play a role in the modulation of airway allergic inflammation (10). Th2 cell-directed therapy has limited...
efficacy (11), suggesting that bronchial asthma develops by diverse immunological mechanisms. Respiratory infections caused by bacteria frequently activate the T\textsubscript{H}1-cell response through activation of Toll-like receptors (12, 13) and are associated with the initiation and/or exacerbation of bronchial asthma in humans (14, 15). These clinical studies strongly suggest that some types of bronchial asthma may be explained by the activation of T\textsubscript{H}1-cell responses. However, intranasal challenge with ovalbumin (OVA) alone cannot evoke asthma in mice carrying OVA-specific T\textsubscript{H}1 cells (16, 17), indicating that the T\textsubscript{H}1-cell response alone is not sufficient enough to induce these pathological alterations. We have demonstrated that intranasal challenge with exogenous IL-18 or bacterial LPS induces IL-18 production in mice. In conjunction with OVA, this can induce robust asthma in mice immunized with OVA and the T\textsubscript{H}1 adjuvant, CFA (16–18). OVA initiates OVA-specific T\textsubscript{H}1 cells to produce IFN-\gamma but not IL-13, whereas OVA with IL-18 is capable of activating these T\textsubscript{H}1 cells to produce larger amounts of IFN-\gamma, as well as IL-13, IL-9 and various chemokines that recruit eosinophils and other leukocytes (16). Persistent stimulation with IL-18 and the antigen alters the T\textsubscript{H}1 cells, resulting in them producing both IFN-\gamma and IL-13 (19). With respect to their potential to produce both pro-inflammatory and pro- atopic cytokines/chemokines, we designated T\textsubscript{H}1 cells that were re-stimulated with antigen and IL-18 as super T\textsubscript{H}1 cells (16, 18, 19). In asthmatic mice possessing super T\textsubscript{H}1 cells, IL-13 is responsible for the eosinophilic airway inflammation and remodeling. AHR is caused by IFN-\gamma, but not IL-13 (16, 17), which is in contrast to T\textsubscript{H}2-cell-initiated asthmatic alterations where IL-13 plays a common and critical role (20, 21). Thus, IL-18 is likely to be involved in the development of bacterial infection-associated asthma. However, it is entirely unknown whether bacteria or their products by themselves can trigger super T\textsubscript{H}1 cell type bronchial asthma.

The site of a pathogenic infection often determines the phenotype of infection-associated atopic diseases presumably by recruiting and activating pathogen-specific effector T cells and by inducing IL-18 release from the site. Infection with bacteria such as \textit{Staphylococcus aureus} sometimes exacerbates atopic dermatitis in humans (22). We recently observed that consecutive and topical application of \textit{S. aureus} protein A (SpA) (23) induces atopic dermatitis-like skin alterations in naive NC/Nga mice that have a genetically impaired skin barrier (19). The CD4\textsuperscript{+} T cells prepared from the DLNs of mice with SpA-induced dermatitis express a cytokine profile characteristic of super T\textsubscript{H}1 cells. Administration of neutralizing anti-IL-18 antibodies protects against dermatitis as well as super T\textsubscript{H}1-cell development (19). Based on these observations, we assumed that the mice carrying SpA-specific T\textsubscript{H}1 cells were highly vulnerable to asthma upon intranasal challenge with SpA. To test this hypothesis, we generated a novel asthmatic inflammation mouse model to determine the requirement of IL-18 in the development of SpA-induced asthma. Severely immunodeficient mice that had been inoculated with SpA-stimulated human PBMCs exhibited airway inflammation following intranasal challenge with SpA. Treatment with neutralizing anti-human IL-18 antibodies prevented this airway inflammation. Thus, IL-18 could be a potential target for the treatment of asthmatic inflammation associated with bacterial infection.

Methods

Animals and reagents

Female BALB/c mice and BALB/c nu/nu mice were purchased from CLEA Japan (Osaka, Japan). C57BL/6 background Rag2\textsuperscript{-/-}C57BL/6 mice were from Taconic Farms (Hudson, NY, USA). All animals were bred and/or maintained in specific pathogen-free conditions at the animal facilities of Hyogo College of Medicine and were used at 6–10 weeks of age. Animal experiments were performed in accordance with the guidelines of the National Institutes of Health, as specified by the animal care policy of Hyogo College of Medicine. SpA from \textit{S. aureus} Cowan I was purchased from CalbioChem (La Jolla, CA, USA). Recombinant murine IL-18 was purchased from MBL (Nagoya, Japan). Anti-mouse CD3e mAb (2C11), anti-mouse CD4 mAb (GK1), anti-human CD4 mAb (RPA-T4) and anti-human CD45 mAb (HI30) were from BD Biosciences Pharmingen (San Diego, CA, USA). Neutralizing anti-IFN-\gamma mAb was partly purified from the ascites fluid collected from BALB/c nu/nu mice inoculated intraperitoneally with hybridoma 6A2 purchased from the American Type Culture Collection (Manassas, VA, USA) (17, 19). Soluble IL-13Ra2-Fc was purchased from R&D Systems (San Diego, CA, USA) (17, 19). Rabbit polyclonal anti-mouse IL-18 antibodies were prepared in our laboratory (19). We generated a neutralizing anti-human IL-18 mAb as described previously (24).

Induction of asthma

The experimental protocol for asthma induction was the same as described in our previous report except we used SpA instead of OVA (17) (Supplementary Figure 1 is available at \textit{International Immunology} Online). Briefly, BALB/c mice were immunized with SpA (500 \textmu g) in CFA, followed by a boost with SpA in incomplete Freund’s adjuvant (IFA) at day 14. For the adoptive cell transfer study, CD4\textsuperscript{+} T cells isolated from DLNs of the immunized and boosted mice were labeled with 5-carboxyfluorescein diacetate succinimidyl ester (CFSE) and 6-CFSE. The CFSE-labeled cells (1 \times 10^7) were administered intravenously into naive BALB/c mice (17). Two weeks following the SpA boost or after CD4\textsuperscript{+} T cell transfer, mice were exposed intranasally to 50 \mu l of SpA (250 \mu g) in PBS for three consecutive days. In some experiments, neutralizing anti-mouse IL-18 antibodies (500 \mu g) were injected intraperitoneally into the mice at 1 day before and 1 day after intranasal exposure to SpA (17, 19). Anti-IFN-\gamma mAb (100 \mu g) or IL-13Ra2-Fc (20 \mu g) was intranasally administered as outlined previously (17). Mice were sacrificed at 24 h after the final intranasal exposure of SpA.

Invasive measurement of AHR

Invasive measurement of AHR was assessed as an increase in pulmonary resistance (RLung) in response to aerosolized \textbeta-methacholine as described previously (17). RLung was measured by Pulmos-II (MIPS, Osaka, Japan) hardware and software (MIPS).

Preparation of CD4\textsuperscript{+} lymph node cells and lung homogenate

CD4\textsuperscript{+} T cells from the DLN were purified by magnetic-activated cell sorting (17). Lungs were homogenized with
PBMCs from healthy volunteers (26) were cultured with Preparation of human PBMCs (XMG1.2) and PE-anti-IL-13 mAb (eBio13A). intravenously into \( \text{Rag2}^{-/-} \) mice. Establishment of mice implanted with human PBMCs The use of human PBMCs were approved by the College Review Board of Hyogo College of Medicine. Preparation of human PBMCs PBMCs from healthy volunteers (26) were cultured with 100 \( \mu \text{g ml}^{-1} \) of SpA for 4 days. Experimental protocols for the use of human PBMCs were approved by the College Review Board of Hyogo College of Medicine. Establishment of mice implanted with human PBMCs SpA-stimulated human PBMCs \( (1 \times 10^6) \) were transplanted intravenously into \( \text{Rag2}^{-/-} \text{C57BL/6}^{-/-} \) mice (27). One-week post-transplantation, we isolated lymphocytes from the peripheral blood and spleen of the recipient mice and analyzed proportions of human CD45\(^+\) cells in each preparation by flow cytometry. We used mice that contained >5% human CD45\(^+\) cells in their peripheral blood because they also contained > 10% dual CD45\(^+\)/CD4\(^+\) cells in their spleen (described below). The mice that received human SpA-stimulated PBMCs were then exposed intranasally to SpA for three consecutive days. In order to block the action of human IL-18, anti-human IL-18 mAb \( (300 \text{ng ml}^{-1}) \) was intranasally administered 1 h before SpA exposure. Twenty-four hours following the final administration of SpA, lungs were sampled for histological and confocal microscopic studies. Preparation of bronchoalveolar lavage fluid Bronchoalveolar lavage fluid (BALF) was collected (17) and total cell number was determined in each sample. Cytospin preparations of BALF were stained with Dif-Quik (Baxter Healthcare Corp., Miami, FL, USA). Eosinophils and neutrophils were distinguished from each other by their difference in staining. Histology Lung specimens were fixed in 10% buffered formalin and sections were stained with hematoxylin and eosin (17). Fields of view on a microscope were selected at random and printed in large scale to distinguish eosinophils from other cell types. Eosinophils and the total number of nucleated cells in each field of view were counted. The mean \( \pm \) SD of 10 fields of view per sample were calculated. Confocal laser microscopic analysis Frozen sections were fixed and incubated with FITC- or PE-conjugated mAb, followed by evaluation using a laser confocal microscope (model IX81; Olympus, Tokyo, Japan) (19). Detection of cytokines and chemokines Concentrations of IL-4, IL-13, tumor necrosis factor-\( \alpha \) and IFN-\( \gamma \) in culture supernatants were determined with appropriate ELISA kits (Genzyme, Cambridge, MA, USA). Mouse IL-18 was measured by an ELISA kit from MBL. The concentrations of various mouse chemokines were measured with a Bio-Plex Cytokine assay kit (Bio-Rad, Hercules, CA, USA). Statistics Three to five mice were used for each experimental group. Data are expressed as the mean \( \pm \) SD of triplicate samples. Significance between experimental and control groups was determined via an unpaired Student’s \( t \)-test. A \( P \) value <0.05 was considered significant. Two to three experiments were performed per assay, and the representative data were shown. Results SpA-induced asthmatic inflammation We examined whether intranasal challenge with SpA induces asthma-like airway inflammation in SpA-immunized mice. We immunized BALB/c mice subcutaneously with SpA in the T\(_1\) adjuvant, CFA, followed by a booster with SpA in IFA 2 weeks later. Twenty-eight days after the initial immunization, we administered SpA through a nasal tract for three consecutive days and examined the severity of asthmatic inflammation by measuring AHR, analyzing BALF preparations and lung histology (Supplemental Figure 1 is available at International Immunology Online). Invasive measurement of AHR revealed that SpA-immunized mice exhibited substantial AHR upon intranasal SpA challenge (Fig. 1A). None of the mice exhibited AHR after treatment with PBS (Fig. 1A) and naive mice were free from AHR even after SpA challenge (Fig. 1A). Thus, SpA immunization and SpA challenge are both required for the development of AHR. Following intranasal challenge with SpA, SpA-immunized mice demonstrated an increase in the number of eosinophils and neutrophils in BALF (Fig. 1B). These increases were not observed after treatment with PBS (Fig. 1B). The severity of AHR, consistent with our previous observations (16, 17), coincided with the cell numbers of eosinophils in BALF. This was also the case for the density of eosinophilic inflammation around the airway. Histological analysis revealed that only SpA-immunized mice developed severe inflammation around the airway following challenge with SpA, but not with PBS (Fig. 1C, E and F). Furthermore, intranasal challenge with SpA, but not PBS or OVA, induced eosinophilia (Supplemental Figure 2 is available at International Immunology Online), suggesting that SpA works in an antigen-specific manner. Naive mice exhibited only modest lung inflammation, if any, after intranasal SpA challenge (Fig. 1D). Eosinophils accumulated around the airway of the SpA-immunized and -challenged mice but not in mice treated with the other combinations of immunogens (Fig. 1J). Taken together, these results indicate that SpA-immunized and -challenged mice fulfill the clinical signs of asthma-like inflammation and are a suitable mouse model for bacterial infection-associated asthma-like inflammatory illnesses.
IL-18 blockade prevents staphylococcal asthma

Fig. 1. Requirement of endogenous IL-18 for SpA-induced bronchial asthma. SpA-immunized mice ‘I(+)’ or naive mice ‘I(−)’ were intranasally challenged with SpA (250 μg/50 μl) ‘C(+)’ or PBS (50 μl) ‘C(−)’. Neutralizing anti-IL-18 antibodies (500 μg) (A, B, G and J), soluble IL-13Rα2-Fc (sIL-13Rα-Fc) (20 μg) (H) or neutralizing anti-IFN-γ (αIFNγ antibody) (100 μg) was administered twice into SpA-immunized mice intravenously, (I) 1 day before or after 3-day intranasal challenge with SpA (αIL-18 antibody). Twenty-four hours after the last SpA challenge, invasive measurement of AHR (A), cellular analysis of BALF (B) and histological analysis of lung specimens (C–I) were performed. (C–I) Upper panels are at low magnifications, and the lower panels are high magnification images of the areas indicated by a red-dotted square in the corresponding
Requirement of IL-18 for SpA-induced asthma

As previously reported, SpA-induced atopic dermatitis develops in an IL-18-dependent manner (19). We investigated whether IL-18 plays a pivotal role in the development of SpA-induced asthmatic inflammation. To test this, we administered neutralizing anti-IL-18 antibodies into the SpA-immunized mice 1 day before and 1 day after the initial intranasal SpA challenge (Supplementary Figure 1 is available at International Immunology Online). Administration of neutralizing anti-IL-18 antibodies profoundly reduced AHR (Fig. 1A) and significantly hampered respiratory inflammation and eosinophilia (Fig. 1B, G and J). Consistently (2, 9, 16), blocking the action of IL-13, but not IFN-γ, protected against eosinophilia in the airway (Fig. 1H–J). Conversely and consistently, blockade of IFN-γ, but not of IL-13, prevented AHR (16) (Supplementary Figure 3 is available at International Immunology Online). It would appear that endogenous IL-18 seems to be important in the development of SpA-induced asthmatic airway inflammation due to IL-13 production.

Super Tₘ₁-cell differentiation

As IL-13 was profoundly involved in inflammation of the airways (Fig. 1H and J), we examined whether this experimental immunization/challenge protocol induces the development of CD4⁺ DLN cells into super Tₘ₁ cells or into IL-13-secreting Tₐ₂ cells. We stimulated CD4⁺ DLN cells with immobilized anti-CD3 mAb and measured the concentrations of Th1 and Th2 cytokines. CD4⁺ DLN cells from SpA-immunized and -challenged mice produced larger amounts of IFN-γ and IL-13, but little IL-4, compared with SpA-immunized mice without SpA challenge (Fig. 2A), indicating their development into super Tₘ₁ cells, but not Tₐ₂ cells, during intranasal SpA challenge. Furthermore, we examined whether both IL-13 and IFN-γ are produced by a single CD4⁺ cell isolated from SpA-challenged and SpA-immunized mice. We isolated cells from mediastinal lymph nodes of SpA-challenged and SpA-immunized mice and incubated the cells with plate-bound anti-CD3 in the presence or absence of exogenous IL-18. We found a very small proportion of IL-13⁺IFN-γ⁺ CD4⁺ T cells after TCR stimulation alone (Fig. 2B). However, upon TCR and IL-18 stimulation, the proportion of IL-13⁺IFN-γ⁺ CD4⁺ T cells was significantly elevated (Fig. 2B), suggesting that super Tₘ₁-cell differentiation occurs in SpA-immunized mice during intranasal challenge with SpA. At the same, this stimulation induced an increase in two other populations: IL-13-producing cells and IFN-γ-producing cells (Fig. 2B). Thus, three populations, consisting of IL-13-producing cells, IFN-γ-producing cells and IL-13 plus IFN-γ-producing cells, contribute to induction of SpA-induced bronchial asthma.

Next, we investigated the roles of endogenous IL-18 in super Tₘ₁-cell development. CD4⁺ DLN cells prepared from the mice additionally treated with anti-IL-18 antibodies produced much less IFN-γ and IL-13 than those from SpA-induced asthmatic mice (Fig. 2A), suggesting the possibility that IL-18 release during SpA challenge participates in super Tₘ₁-cell differentiation. To test this possibility, we examined whether IL-18 is produced in the asthmatic lung. SpA immunization alone failed to induce significant increase in IL-18 concentration within the lung tissue (Fig. 2C). SpA immunization and challenge seemed to increase IL-18 levels significantly in the lung (Fig. 2C). These results suggest that airway constituents such as respiratory epithelial cells and/or alveolar macrophages might release IL-18 in response to SpA.

Induction of chemokines attracting eosinophils and neutrophils in the lungs

As IL-18 is capable of inducing chemokine production from epithelial cells, Tₐ₁ cells and super Tₘ₁ cells (16, 18), we examined whether IL-18 could induce expression of chemokines in the lung, particularly chemokines recruiting eosinophils and neutrophils, during intranasal SpA challenge. Lung homogenates from mice immunized with SpA only contained almost basal amounts of chemokines attracting eosinophils, including CCL5 (RANTES) and CCL11 (Eotaxin), and neutrophils, such as CXCL1 (KC) and CCL2 (MCP-1), when compared with naive mice (Fig. 3). However, it was only after SpA challenge that pulmonary levels of CCL5, CCL11, CCL2 and CXCL1 were significantly elevated (Fig. 3). This was also the case for the chemokines attracting diverse types of leukocytes, such as, CCL3 (MIP-1α) and CCL4 (MIP-1β), as well as the pro-inflammatory cytokines, IL-1β and IL-6 (Fig. 3). In contrast, Tₐ₂ cytokines, IL-4 and IL-5 were not induced after SpA challenge (Fig. 3). As expected, treatment with neutralizing anti-IL-18 antibodies during intranasal exposure to SpA significantly reduced chemokine expression levels (Fig. 3). Thus, the expression of these chemokines could be induced by IL-18.

Importance of SpA-activated super Tₘ₁ cells in the development of airway inflammation

We examined whether super Tₘ₁ cells are effector cells of SpA-induced asthmatic inflammation. To test this, we transferred CD4⁺ DLN cells from SpA-immunized mice into naive mice, followed by intranasal administration of SpA for three consecutive days. Upon daily exposure to PBS, mice receiving the CD4⁺ DLN cells demonstrated an intact response to methacholine treatment and evaded airway inflammation (Figs 1A and C and 4A and C). Upon exposure to SpA, these mice exhibited obvious AHR (Fig. 4A) and airway inflammation (Fig. 4C), prompting us to investigate whether donor CD4⁺ DLN cells migrated into the airway as a response to SpA challenge in order to exert their effector functions. We labeled the donor cells with CFSE, injected them into naive recipient mice and analyzed their localization in the recipient lung after SpA challenge. Many CFSE-labeled cells had migrated into the lung (Fig. 4B). Most of the
pulmonary CD4+ T cells co-expressed CFSE (Fig. 4B), indicating that the donor CD4+ T cells but few of the recipient cells, accumulated in the lung. Upon exposure to PBS, few CFSE-labeled cells or CD4+ T cells were observed in the recipient lung (data not shown). These results demonstrate that SpA-specific CD4+ DLN cells migrate and are fully activated after being exposed to SpA, eventually leading to the development of asthmatic inflammation. Blocking the action of IL-18 protected the mice from AHR and airway inflammation (Fig. 4A and C), suggesting that SpA-induced IL-18 in the airway in combination with administered SpA might differentiate the donor cells toward super Th1 cells, thereby becoming highly pathogenic effector cells.

Involvement of human IL-18 in SpA-induced airway inflammation in transiently humanized mice

Finally, we investigated whether IL-18 is a therapeutic target for the treatment of airway inflammation in humans, associated with bacterial infection. First, we tried to generate mice transiently carrying human immune competent cells. We incubated PBMCs from healthy donors with SpA in vitro, transferred them into immunodeficient mice without T cells, B cells and NK cells and investigated whether human PBMCs settled in the recipient mice by calculating proportions of human CD45+ hematopoietic cells (27) in peripheral immune tissues. Human CD45+ cells were robustly observed in the spleen and peripheral blood of the recipient mice at day 7 after PBMC transfer (Fig. 5). About one-third of human CD45+ cells co-expressed the human CD4 marker (Fig. 5).

Like the CD4+ DLN cell-transplanted mice (Fig. 4), the SpA-stimulated humanized mice developed airway inflammation upon intranasal challenge with SpA, concomitant with dense accumulation of human CD4+ T cells around the airway (Fig. 6C and D). This result indicated that SpA-stimulated human CD4+ T cells migrated into the airway and presumably evoked pulmonary inflammation in response to exogenous SpA and endogenous IL-18. Upon PBS exposure, however, the host mice showed weak airway inflammation with modest but apparent accumulation of human CD4+ T cells (Fig. 6A and B). We investigated the role of human IL-18 and confirmed that anti-human IL-18 mAb (24) potently neutralized human IL-18 in these mice by attenuating airway accumulation of human CD4+ T cells (Fig. 6E and F). This mAb prevented SpA-induced airway inflammation in these mice by attenuating airway accumulation of human CD4+ T cells (Fig. 6E and F). Therefore, human cell-derived IL-18 might fully activate human SpA-specific T<sub>H1</sub> cells to become pathogenic effector cells. Indeed, human PBMCs could release super T<sub>H1</sub> cell-inducing cytokines, such as IL-12 and IL-18, and super T<sub>H1</sub> cytokines, such as IL-13 and IFN-γ, in response to SpA in vitro (Supplementary Figure 5 is available at International Immunology Online).
Discussion

This study showed that endogenous IL-18 is critical for the development of SpA-induced asthmatic inflammation in mice. Upon intranasal exposure to SpA, mice immunized with SpA developed bronchial asthma-like airway inflammation (Fig. 1), concomitant with super Th1-cell development and elevation of lung pro-inflammatory cytokine/chemokine production (Figs 2A–B and 3). Notably, lung IL-18 levels were significantly elevated in the SpA-induced asthmatic mice (Fig. 2C), and all these responses were prevented by inhibiting the action of IL-18 (Figs 1–3). Thus, IL-18 released from the lung exposed to SpA, together with SpA-presenting APC, might enter into mesenteric lymph node, in which SpA-specific Th1 cells might develop toward super Th1 cells. Furthermore, SpA-activated CD4+ T cells, when transferred into naive mice, prepared the host mice to be highly responsive to intranasal administration of SpA, inducing bronchial asthma-like symptoms dependent upon endogenous IL-18 (Fig. 4). Finally, the humanized mice developed airway inflammation in a manner dependent on human IL-18 after intranasal SpA challenge (Fig. 6). IL-18 is important for *S. aureus*-associated asthmatic inflammation in mice and perhaps in humans.

We generated an airway inflammation model of temporally humanized mice by intranasal challenge with the bacterial protein, SpA. *Rag2<sup>−/−</sup>*<sup>C57Bl6</sup> mice injected with human PBMC transiently possessed human hematopoietic cells in their peripheral lymphoid organs (Fig. 5). Due to their lack of T, B and NK cells, the recipient mice could not recognize human donor cells as antigens. In contrast, human donor cells consisting of those types of lymphocytes had the potential to be activated by recognizing xenogeneic recipient cells in host mice. In fact, mice having received human PBMCs spontaneously exhibited non-specific inflammatory changes in their lungs and livers as compared with control *Rag2<sup>−/−</sup>*<sup>C57Bl6</sup> mice (Figs 1C and 6A; Supplementary Figure 6 is available at International Immunology Online). Despite apparent infiltration with human hematopoietic cells in the steady state (Fig. 6B), the basal lung inflammatory change was minimal (Fig. 6A), and the host mice survived without ill effects until sacrificed. This may be partly due to the inability of host cells to respond to IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 signals. Therefore, it could be said that these transiently humanized mice have limited inflammatory responses without SpA challenge. However, intranasal SpA challenge induced severe airway inflammation in the mice that received SpA-stimulated PBMCs (Fig. 6C and D). Human CD4<sup>+</sup> T cells were densely recruited into airway after SpA challenge (Fig. 6C). Administration of neutralizing anti-human IL-18 significantly inhibited the development of airway inflammation by diminishing the accumulation of the donor cells (Fig. 6E and F). SpA-specific human CD4<sup>+</sup> T cells activated by both SpA and IL-18 likely induced the development of airway inflammation by releasing human cytokines and chemokines that recruit human PBMCs. Additionally, some of human chemokines [e.g. CCL5 (RANTES)] might act on murine cells to migrate as well. Further study is required to identify the human factors involved in this airway inflammation. Nonetheless, our results strongly suggest that IL-18 is a potent clinical target for the treatment of bronchial asthma associated with *S. aureus* colonization or infection.

Fig. 3. IL-18 involvement in the induction of chemokines for neutrophils and eosinophils. Lung homogenates were prepared from the mice of three experimental groups (five mice per group) as shown in the legend to Fig. 1A and naive mice (five mice per group). The concentration of CCL2 (MCP-2), CCL3 (MIP1α), CCL4 (MIP1β), CCL5 (RANTES), CCL11 (Eotaxin), CXCL1 (KC), IL-1β, IL-4, IL-5 and IL-6 were measured by BioPlex®. Data are representative of three independent experiments.
It is unclear how human T cells recognize SpA in the transiently humanized mice. After in vitro stimulation with SpA, human PBMCs produced IFN-γ and IL-13 (Supplementary Figure 3 is available at International Immunology Online), and the CD4+ T-cell population expanded (data not shown), suggesting that human APCs possess the potential to present SpA to the CD4+ T cells in order to activate them. It was assumed that APCs included in the human donor cell preparation might serve as APCs for the SpA-specific human effector CD4+ T cells in humanized mice as well. Alternatively, SpA-specific human effector cells might recognize SpA presented by xenogeneic mouse APCs by the mechanisms currently poorly understood.

We did not measure AHR of transiently humanized mice for a number of reasons. First, the recipients are Rag2-/-C0/C0 mice, which lack responsiveness to IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Second, in general, mice are poor responders to human cytokines. Third, C57BL/6 mice, a background of Rag2-/-C0/C0 mice, are resistant to Th2 type and super Th1 cell type asthma (16). Fourth, is that transiently humanized mice are not homogeneous in terms of the degree of repopulation with human CD4+ T cells.

Bronchial asthma is now recognized to have diverse immunopathogenesis. Recently, we demonstrated that intranasal challenge with OVA, plus the IL-18-inducible bacterial LPS, initiates robust bronchial asthma in mice immunized with OVA/CFA. Neutralization of IL-18 during OVA plus LPS challenges inhibits AHR in OVA-specific T(1) cell-bearing mice, suggesting that OVA plus LPS activates T(1) cells via endogenous IL-18. In contrast, the role of endogenous IL-18 in allergic T(2)/IgE-dependent asthma is not clearly defined. One report demonstrated only partial contribution of IL-18 to OVA inhalation-induced chronic allergic airway inflammation with remodeling (20). Another report demonstrated that IL-18 does not affect AHR and airway inflammation in allergic bronchial asthma (21), contrasting strikingly to the clear pathological role of endogenous IL-18 in non-allergic super T(1) cell-dependent asthma (17).

Various types of cells, including macrophages and epithelial cells, can produce IL-18 (28). In this study, we observed elevation of lung IL-18 in the SpA-immunized mice only after intranasal SpA challenge (Fig. 2B), suggesting that IL-18 might be derived from airway constituents, such as respiratory epithelial cells and/or alveolar macrophages. Despite our intensive efforts, we could not observe obvious release of IL-18 from either type of cells after in vitro stimulation with SpA.

Recent reports have clearly shown that respiratory epithelial cells play a pivotal role in the development of T(2) type murine asthma induced by airway exposure to the house dust mite, a common allergen of human asthma (29–31). It was believed that an antigen-specific T(2) cell response develops only under the limited condition of immunization with the protein in combination with T(1) cell adjuvant. Beyond this dogma, it was shown that multiple intra-tracheal challenges with house dust mite alone could trigger a T(2) cell response without prior immunization with antigen/ T(2) adjuvant complex. Mice lacking TLR4 expression on their respiratory epithelial cells, but not hematopoietic cells, were able to evade T(2) type asthma. Because house dust mites possess intrinsic TLR4 agonists, TLR4 on respiratory...
epithelial cells might recognize the agonist and produce Th2-activating cytokines, such as IL-25, TSLP and IL-33 (29, 32, 33), eventually resulting in the development of Th2 type asthma. As previously reported, daily topical application of SpA without Th1 adjuvant can induce atopic dermatitis, in which super Th1-cell development plays a critical role (19). Intriguingly, murine epidermal cells can release super Th1 cell-activating IL-18 in response to SpA (34). Thus, SpA, like house dust mites in the airway, seems to exert dual actions as a T-cell antigen and adjuvant in the skin. These observations together with our present results suggest that respiratory mucosa and skin, particularly respiratory epithelial cells and epidermal cells, respectively, are the sites required for activation of Tn2 and/or super Tn1 cells. A similar mechanism might also be responsible for the airway asthmatic inflammation induced by SpA.

Accumulated evidence suggests the involvement of IL-18 in atopic diseases in humans. Patients with atopic dermatitis and bronchial asthma have higher levels of serum IL-18 than healthy volunteers (35, 36). In particular, serum IL-18 levels are shown to coincide with the disease severity of atopic dermatitis. Furthermore, gain-of-function polymorphisms of IL-18 are observed in patients with atopic dermatitis and bronchial asthma (37–39). This implies that IL-18 is preferentially produced after airway and/or dermal colonization with microbes in those patients, eventually leading to the development of exposure site-specific super Tn1 cell-dependent allergic diseases.

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IL-18 blockade prevents staphylococcal asthma

References