

A T_H17-polarized cell population that has infiltrated the lung requires cells that convert to IFN- γ production in order to induce airway hyperresponsiveness

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Transmitting editor: T. Hamaoka

Received 23 January 2010, accepted 22 March 2010

Abstract

Adoptive cell transfer of an ovalbumin (OVA)-specific T_H17-polarized cell population from transgenic DO11.10 mice into BALB/c mice followed by OVA inhalation caused airway hyperresponsiveness (AHR) with severe neutrophilia. The transferred T_H17 cell population—previously polarized *in vitro* with IL-6, transforming growth factor- β and IL-23—contained negligible numbers of IFN- γ -producing cells; however, during T_H17-cell-dependent airway inflammation, significant numbers of IFN- γ -producing cells—including cells producing both IL-17 and IFN- γ and cells producing only IFN- γ —were detected in the lung in addition to cells producing only IL-17. Using T_H17-polarized cell populations derived from IL-17^{-/-} or IFN- γ ^{-/-} mice, it was demonstrated that IL-17 is essential for inducing neutrophilic airway inflammation and that IFN- γ is required for the AHR elevation. IFN- γ appeared to be derived from cells producing both IL-17 and IFN- γ and/or from cells producing only IFN- γ , which were converted from the transferred T_H17-polarized cell population. We also found that mAbs that neutralize IL-12 significantly suppressed the conversion of the T_H17-polarized cell population toward IFN- γ producers in the lung; concomitantly, with this decreased conversion, IL-12 neutralization also attenuated the AHR elevation in the lung. IL-12-dependent conversion of the transferred T_H17-polarized cell population into IFN- γ producers in the lung thus appeared to be a crucial process for inducing AHR elevation in T_H17-cell-dependent airway inflammation.

Keywords: airway inflammation, IFN- γ , IL-17, neutrophilia, severe asthma

Introduction

Bronchial asthma is generally identified as a heterogeneous syndrome associated with T_H2 responses, including allergen sensitization, IgE production, mast cell activation, recruitment of eosinophils into lung, mucus hypersecretion and airway hyperresponsiveness (AHR) (1–4). Our previous studies (5, 6) and other reports (7–9) demonstrated that recruitment of allergen-specific T_H2 cells that produce IL-4 and IL-13 was pivotal for the pathogenesis of eosinophilic asthma in mice and humans. There have been major advances in understanding the molecular mechanisms of T_H2-cytokine-associated asthma (10, 11); however, little has been evaluated concerning non-eosinophilic or refractory asthma.

We previously reported that T_H1 cells induced neutrophilic airway inflammation (12), and several researchers also confirmed that T_H1 cells critically contributed to inducing the elevation of AHR accompanied with neutrophilia using a T_H1-cell transfer model (13) or a model in which T_H1 cells plus LPS were administered (14, 15).

Recently, however, evidence has accumulated showing that a new subset of IL-17-producing CD4⁺ T cells (T_H17 cells)—a distinct population from T_H1 and T_H2 cells—was also involved in AHR elevation and neutrophilia (16–18). A high concentration of IL-17 was detected in biopsies from neutrophilic asthma patients (18–20); moreover, IL-17 levels

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have been shown to correlate with the incidence of AHR and the severity of disease (21, 22). Although IL-17 and/or IFN- γ —produced by T_h17 and T_h1 cells, respectively—were also detected during defense against infection (23–26), some clinical case studies suggested that pulmonary bacterial infection worsened asthma symptoms. Persistence or recurrence of infection raised the clinical stage of asthma from mild to severe; this severe asthma was resistant to steroids (27, 28). In addition, increased IFN- γ levels as well as neutrophilia were observed in biopsies from patients with severe asthma who exhibited respiratory impairment (29).

It remains unclear, however, which factors orchestrate the severity of T_h17 -cell-induced respiratory disease, including AHR elevation. We therefore evaluated the pathogenic role of T_h17 cells in the lung using our established T_h17 -dependent airway inflammation model.

In the present work, we demonstrated four major findings: (i) the T_h17 -polarized cell population induced neutrophilic airway inflammation, which is similar to T_h1 -cell-dependent diseases (12) but totally distinct from T_h2 -cell-dependent eosinophilic airway inflammation; (ii) the T_h17 -polarized cell population infiltrated into the lung in an IL-17-dependent manner and can convert into IFN- γ producers, which produce IFN- γ in addition to IL-17; (iii) the IFN- γ producers derived from the transferred T_h17 -polarized cell population were essential for inducing AHR elevation but not for the neutrophilia in T_h17 -dependent airway inflammation and (iv) IL-12, which was up-regulated in the lung tissue after ovalbumin (OVA) inhalation, was demonstrated to be a critical factor for the conversion of T_h17 -polarized cell population into IFN- γ producers in the lung and blockade of IL-12 inhibited the AHR elevation. These findings demonstrated that IL-12-induced conversion of the T_h17 -polarized cell population toward IFN- γ producers—such as IFN- γ -producing T_h1 -like cells and producers of both IL-17 and IFN- γ —was an important event for T_h17 -cell-associated AHR. Our work thus provides novel insight into severe respiratory diseases that depend on T_h17 cells.

Methods

Mice

Wild-type (WT) BALB/c mice were purchased from Charles River Breeding Laboratories (Kanagawa, Japan). OVA_{323–339}-specific I-A^d-restricted TCR-transgenic (DO11.10 Tg) mice were kindly donated by Dr K. Murphy (Washington University School of Medicine, St Louis, MO, USA). IFN- γ ^{−/−} and IL-17^{−/−} mice on a BALB/c background were kindly provided by Dr Y.I. (University of Tokyo, Tokyo, Japan). DO11.10 Tg/IFN- γ ^{−/−} and DO11.10 Tg/IL-17^{−/−} mice were bred in our facility. All mice were maintained in specific-pathogen-free conditions according to the guidelines of our Institute's Animal Department and used at 6–8 weeks.

Antibodies and reagents

Recombinant human (rh) IL-2 was kindly donated by Dr T. Sawada (Shionogi Pharmaceutical Institute Co., Osaka, Japan). rhIL-6 was kindly donated by Chugai Pharmaceutical Company Ltd (Shizuoka, Japan). Recombinant murine (rm) IL-4, rmIL-23 and recombinant human transforming

growth factor- β (rhTGF- β) were purchased from Pepro Tech EC Ltd (London, UK), R&D Systems, Inc. (Minneapolis, MN, USA) and Wako Pure Chemical Industries, Ltd (Osaka, Japan), respectively. PE-conjugated (PE-) anti-IL-4 mAb (11B11), PE-anti-IL-5 mAb (TRFK5), PE-anti-IL-17 mAb (TC11-18H10), allophycocyanin (APC)-conjugated anti-IFN- γ mAb (XMG1.2) and PE-Cy7-conjugated (PE-Cy7-) anti-CD4 mAb (GK1.5) were purchased from BD Biosciences (San Diego, CA, USA). The anti-OVA_{323–339}-specific TCR mAb was purified from ascites fluid of mice intra-peritoneally inoculated with KJ1.26 hybridoma cells in our laboratory and conjugated with FITC for some experiments. Neutralizing mAbs against IFN- γ (R4-6A2) and IL-12p40 (C15.1 and C15.6), donated by Dr G. Trinchieri (Wistar Institute of Anatomy and Biology, Philadelphia, PA, USA), and IL-4 (11B11), purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA), were purified from ascites fluids of mice inoculated with hybridomas in our laboratories. The mAb that was used to deplete neutrophils (Gr-1-positive cells) was purified from the ascites fluids of mice inoculated with the RB6-8C5 hybridoma, purchased from ATCC. Recombinant OVA_{323–339} peptide was kindly supplied by Dr H. Tashiro (Fujiya Co. Ltd, Hadano, Japan). OVA protein and β -methacholine chloride (Mch) were purchased from Sigma-Aldrich Japan (Tokyo, Japan).

In vitro generation of OVA-specific T_h2 - and T_h17 -polarized cell populations

CD4⁺CD45RB⁺ naive T_h cells were isolated from DO11.10 TCR-Tg mouse spleen cells using a cell sorter (FACS Aria; BD Biosciences, San Jose, CA, USA). Isolated naive T_h cells were cultured with rhIL-2 (100 U ml^{−1}), rmIL-4 (1 ng ml^{−1}), anti-IFN- γ mAb (10 μ g ml^{−1}) and anti-IL-12 mAbs (10 μ g ml^{−1}) for T_h2 differentiation, or with rhIL-6 (50 ng ml^{−1}), rhTGF- β (2 ng ml^{−1}), rmIL-23 (10 ng ml^{−1}), anti-IL-4 mAb (10 μ g ml^{−1}) and anti-IFN- γ mAb (10 μ g ml^{−1}) for T_h17 differentiation, in the presence of mitomycin-C-treated spleen cells and 5 μ g ml^{−1} OVA_{323–339} peptide. Each T_h -cell population was re-stimulated with OVA_{323–339} peptide under the same conditions at 48 h. For evaluation of T_h2 or T_h17 differentiation, these cells were analyzed by intracellular staining with APC-anti-IFN- γ , PE-anti-IL-17 and PE-anti-IL-4 mAb at day 8 as described previously (5, 6, 12). After washing out the factors at 8–10 days of culture, the generated T_h cells were transferred intravenously (i.v.) into syngeneic WT BALB/c mice.

Adoptive transfer model

A model of airway inflammation was established by the adoptive transfer of T_h cells as described previously (5, 6, 12). Briefly, OVA-specific T_h2 or T_h17 cells (2×10^7 cells in 0.2 ml PBS) were injected into the tail vein of normal recipient BALB/c mice. One day after the transfer, mice were exposed to aerosolized OVA protein (2 w/v% in PBS), which was generated by a nebulizer (OMRON NE-U07 nebulizer; Omron Corporation) driven at 1.0 ml min^{−1} atomization of OVA protein solution, for 30 min during consecutive 3 days. Twenty-four hours after the third OVA exposure, pulmonary function was tested and lung histological examinations were carried out for the mice. In separate experiments, IFN- γ ^{−/−}

and IL-17 $^{-/-}$ mice were i.v. injected with IFN- γ $^{-/-}$ T_h17 and IL-17 $^{-/-}$ T_h17 cells, respectively. In the antibody treatment experiments, intranasal injection (100 μ g in 25 μ l PBS per mouse) of anti-p35 mAb (C18.2; eBioscience), anti-p19 mAb (G23-8; eBioscience), anti-p40 mAbs (C15.1 and C15.6 donated by Dr G. Trinchieri) or control rat IgG were performed 30 min before each OVA inhalation.

Measurement of AHR

AHR was measured by Mch-induced airflow obstruction 24 h after final OVA inhalation as reported previously (5, 6, 12). Briefly, the subjected mice were placed into whole-body plethysmographs (Buxco Electronics, Troy, NY, USA) interfaced with computers using differential pressure transducers. Measurements were performed of respiratory rate, tidal volume and enhanced pause. Airway resistance was expressed as: $Penh = [(Te/0.3Tr) - 1] \times [2Pef/3Pif]$, where $Penh$ = enhanced pause, Te = expiratory time (seconds), Tr = relaxation time (seconds), Pef = peak expiratory flow (milliliters) and Pif = peak inspiratory flow (milliliters per second). Increasing doses of Mch were administered by nebulization (for 3 min), and $Penh$ were calculated over the subsequent 3 min.

Bronchoalveolar lavage and histological analysis

After measuring AHR, the lungs and tracheas were gently lavaged three times with 1 ml PBS containing 0.1% BSA (Sigma). Cytospins of the bronchoalveolar lavage (BAL) cells were prepared with Shandon Cytospin 3 (Thermo Electron) and stained with DAB/Tris-HCl (pH 7.25) solution and hematoxylin to evaluate the morphology differentials based on the staining characteristics. For evaluation of mucus hyperproduction, the lung tissues were perfused with 10% buffered formalin, and the sections were stained with hematoxylin and periodic acid-Schiff and observed using a microscope (BX50; OLYMPUS OPTICAL Co., Ltd, Tokyo, Japan).

Flow cytometric analysis

To analyze the number of OVA-specific T_h17 cells in the BAL fluid, the collected cells were stained with PE-Cy7-anti-CD4 mAb and FITC-conjugated anti-OVA₃₂₃₋₃₃₉-specific TCR mAb (KJ1.26). Then double-positive (CD4 $^+$ KJ1.26 $^+$) cells were detected by flow cytometry as transferred OVA-specific T_h17 cells. In addition, for evaluation of an ability to produce some cytokines, BAL cells were stimulated with anti-CD3 ϵ mAb (145-2C11; BD Biosciences) for 8 h and treated with Brefeldin-A (Sigma) for the last 2 h. The stimulated cells were stained with PE-Cy7-anti-CD4 and anti-OVA₃₂₃₋₃₃₉-specific TCR mAbs and then fixed with 4% paraformaldehyde. After permeabilization, the fixed cells were stained with APC-anti-IFN- γ and PE-anti-IL-17 mAbs (eBioscience and BD Biosciences, respectively). Data were acquired on a FACSCalibur (BD Biosciences) and analyzed using CellQuest software (BD Biosciences). Representative data, which are in individual dot plots from one mouse, are shown in the figures.

Enzyme-linked immunosorbent assay

The cytokine levels in the BAL fluid were measured by OptEIATM mouse IL-4, IFN- γ (BD Biosciences), Ready-SET-

GoTM mouse IL-17A (eBioscience) and Quantikine mouse IL-13 (R&D Systems, Inc.) ELISA kits according to the manufacturers' directions.

Real-time PCR

Total RNA was extracted from lung tissues of normal mice, OVA-exposed mice or T_h17 -cell-transferred and OVA-exposed mice using the Isogen RNA extraction kit (Nippon-gene) according to the manufacturer's instructions. cDNA was prepared from the total RNA with a reverse transcriptase (Invitrogen), oligo dT and dNTP mixture (Promega). The indicated cDNA was specifically amplified by thermal cycler (LightCycler; Roche Co.) using the corresponding primer pairs and probes for mouse IL-12p35 subunit, IL-23p19 subunit, IL-12/23p40 subunit, CCR6 and β -actin. The primer sequences and probe (indicated as number from list of Universal ProbeLibrary Set; Roche Co.) used were as follows: IL-12p35 (sense) 5'-CCATCAGCAGATCATTCTAGACA-A-3' and (anti-sense) 5'-CGCCATTATGATTCAGAGACTG-3' (probe number) #49; IL-23p19 (sense) 5'-TCCCTACTAG-GACTCAGCCAAC-3' and (anti-sense) 5'-TGGGCATCTGTT-GGGTCT-3' (probe number) #19; IL-12/23p40 (sense) 5'-GACTCCAGGGGACAGGCTA-3' and (anti-sense) 5'-GG-AGATGGTTAGCTTCTGAGGA-3' (probe number) #27; CCR6 (sense) 5'-TTCGCCACTCTAATCAGTAGGAC-3' and (anti-sense) 5'-TCTGGTGTAGAAAGGGAAGTGG-3' (probe number) #5 and β -actin (sense) 5'-AAGGCCAACCGT-GAAAAGAT-3' and (anti-sense) 5'-GTGGTACGACCAGAG-GCATAC-3' (probe number) #56. Sample signals were normalized to the housekeeping gene β -actin according to the $\Delta\Delta C_t$ method: $\Delta C_t = \Delta C_{t\text{sample}} - \Delta C_{t\text{reference}}$. Percentages against the control sample were then calculated for each sample.

Statistical analyses

All experiments were repeated at least three times. Mean values and standard deviation were calculated for data from representative experiments and shown in the figures. Significant differences in the results were determined by Student's *t*-test. A value of $P < 0.05$ or $P < 0.01$ was considered as significant in the present experiments.

Results

AHR and neutrophilia are induced in T_h17 -cell-dependent airway inflammation

T_h17 or T_h2 cells specific for OVA were generated under the well known conditions from DO11.10 Tg mice as described in Methods. BALB/c mice were i.v. transferred with 2×10^7 cells of T_h17 or T_h2 cells followed by OVA inhalation to induce T_h17 - or T_h2 -dependent airway inflammation. A significant elevation of AHR was induced in mice transferred with T_h17 cells, as was the case with T_h2 cells (Fig. 1A). The AHR elevation in T_h17 -cell-transferred mice was accompanied by a severe neutrophilia in the lung, while significant eosinophilia and mucus hyperproduction were not induced in contrast to T_h2 -cell-dependent airway inflammation (Fig. 1B and C). During T_h17 -cell-dependent AHR elevation, the levels of IL-17 and IFN- γ , but not IL-4 and IL-13, greatly

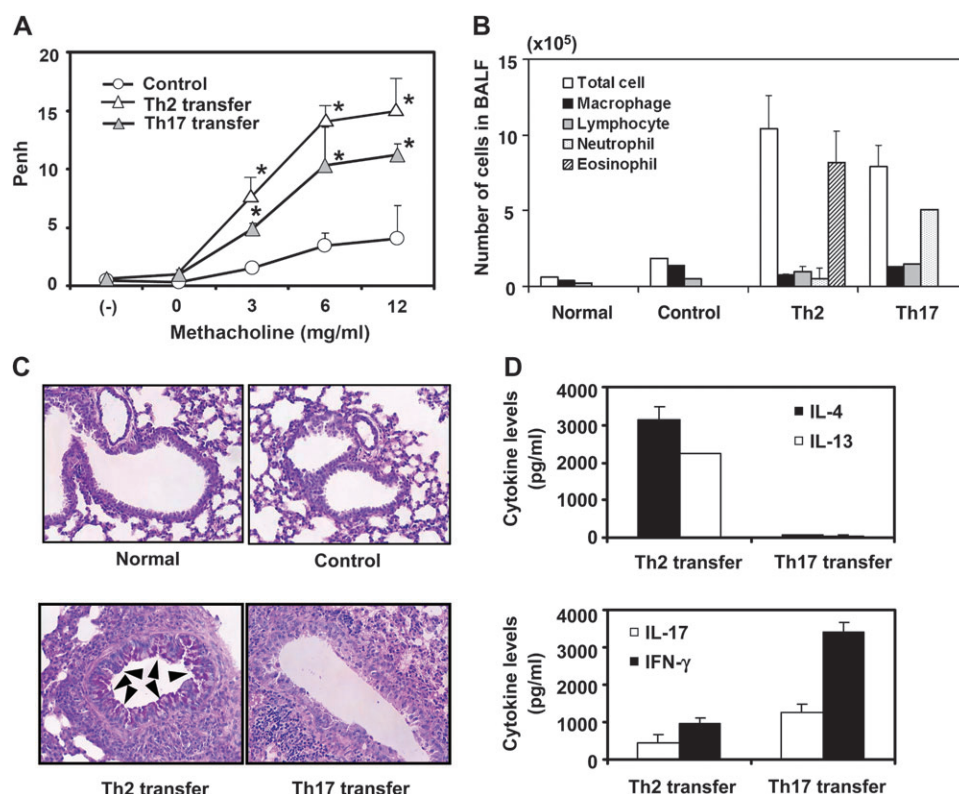


Fig. 1. AHR and neutrophilia are induced in T_H17 -cell-dependent airway inflammation. BALB/c mice were i.v. injected with OVA-specific T_H2 or T_H17 cells and exposed to OVA protein. (A) AHR of basal values (–) or the response to nebulized PBS (0) and to three concentrations of methacholine (3, 6 and 12 mg ml^{–1}) was measured with a whole-body plethysmograph. (B) Inflammatory cells collected from BAL fluid were stained with DAB/Tris–HCl (pH 7.25) solution and hematoxylin. Differentials of macrophages, lymphocytes, neutrophils and eosinophils were judged according to the morphology and staining characteristics. The number of each cell subset was counted and are indicated in the figure. (C) Lung tissues were perfused with 10% buffered formalin and stained with hematoxylin and periodic acid–Schiff. Representative sections ($\times 200$) are shown and arrowheads indicate mucus deposition on airway epithelium. (D) IL-4, IL-13, IL-17 and IFN- γ levels in BAL fluid were measured by ELISA at 6 h after the final OVA inhalation. One experimental group was composed of three mice ($n = 3$). Similar results were obtained in three separate experiments and representative data (mean $\pm$ SD) are shown in the figures. For control experiments, inhalation of OVA protein was done without transfer of T_H2 or T_H17 cells. Asterisk indicates significant differences from control mice ($P < 0.05$).

increased in the lung, which had a totally distinct cytokine profile in T_H2 -cell-transferred mice (Fig. 1D). Thus, the pathogenic features of T_H17 -cell-dependent airway inflammation appeared to be similar to our previously reported T_H1 -cell-dependent airway inflammation but totally different from that of T_H2 -cell-dependent inflammation (5, 6, 12).

The T_H17 -polarized cell population is converted into an IFN- γ -producing population after migration into the OVA-stimulated lung

The systemically transferred CD4⁺ KJ1.26⁺ T_H17 -polarized cells infiltrated into the OVA-stimulated lung and their numbers reached a plateau 24 h after the final inhalation of OVA (Fig. 2A). The cytokine profile of the T_H17 -polarized cell population before or after cell transfer into mice was determined after stimulation with anti-CD3 ϵ mAb. As shown in Fig. 2(B), the T_H17 -polarized cell population used for the experiment dominantly produced IL-17 but not IFN- γ before the cell transfer. Interestingly, however, some of the lung-infiltrating T_H17 -polarized cell population produced significant level of IFN- γ as well as IL-17. There were two subsets of IFN- γ producers in the lung: one comprised T_H1 -like cells that produced IFN- γ and the other one comprised cells that

produced both IL-17 and IFN- γ . The majority of IFN- γ producers expressed both CD4 and KJ1.26 cell surface markers, indicating that they were derived from the transferred T_H17 -polarized cell population (S. A. and T. N., unpublished results); moreover, it was demonstrated that IFN- γ producers were detected only in the lungs and sites local to them (i.e. lungs and lung-draining lymph nodes) but not other distal lymphoid tissues (Fig. 3). These results excluded the possibility that T_H17 -polarized cell spontaneously converted to IFN- γ producers without interacting with antigen (OVA)-sensitized host tissues. These data indicated that the transferred T_H17 -polarized cell population converted into IFN- γ producers after migration into the lung and interaction with OVA-sensitized lung tissue components during airway inflammation. These results prompted the next question: how do the two cytokines—IL-17 and IFN- γ —contribute to the AHR elevation and neutrophilia during airway inflammation?

IL-17 is a critical factor for the migration of pathogenic effector cells into the inflamed lung tissue

To evaluate the critical role of IL-17 in airway inflammation, a T_H17 -polarized cell population derived from IL-17^{–/–} DO11.10 Tg mice, which produced very little IFN- γ before

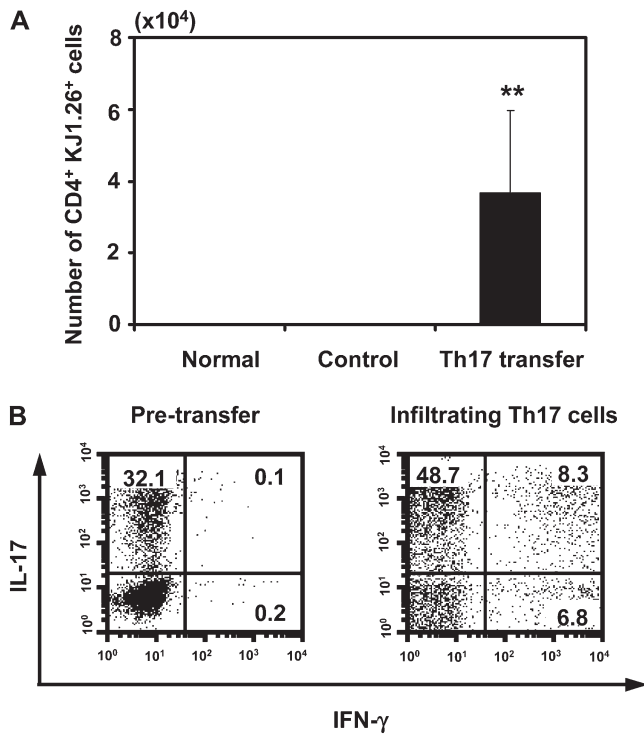


Fig. 2. The Th17-polarized cell population is converted into IFN- γ producers after migration into the OVA-exposed lung. BALB/c mice were i.v. injected with OVA-specific Th17 cells and exposed to OVA protein. At 24 h after final OVA inhalation, the mice were subjected to the following analysis. (A) OVA-specific Th17 (CD4⁺ KJ1.26⁺) cells in BAL fluid were detected by flow cytometry. The numbers of the infiltrating Th17 cells are shown in the figure. (B) Th17 cells were induced *in vitro*, and after transfer, lymphocytes collected from BAL fluid were analyzed by intracellular staining to detect cells producing IFN- γ and/or IL-17. FACS profiles and percentages gated on CD4⁺ KJ1.26⁺ cells are indicated. One experimental group was composed of three mice ($n = 3$). Similar results were obtained in three separate experiments and representative data (mean $\pm$ SD and individual experiments) are shown in the figures. In control experiments, Th17 cells transferred but there was no inhalation of OVA protein. The double asterisks indicate a significant difference from control mice ($P < 0.01$).

transfer, were transferred into IL-17^{-/-} BALB/c mice, which then inhaled OVA. The elevation of AHR and severe neutrophilia were significantly decreased in the mice transferred with IL-17^{-/-} Th17 cells in parallel with a great reduction of the numbers of lung-migrating CD4⁺ KJ1.26⁺ cells (Fig. 4 A–C); however, the ability of transferred CD4⁺ KJ1.26⁺ cells in the lung to produce IFN- γ was not abolished by the absence of IL-17 (Fig. 4D). Judging from Fig. 4(C and D), it appears that the absolute numbers of IFN- γ producers converted from the transferred IL-17^{-/-} Th17 cell population were also decreased to <30% of those from WT Th17-polarized cells. As a result, IFN- γ levels in the lungs of IL-17^{-/-} Th17-cell-transferred mice were reduced to nearly one-third of those in WT Th17-cell-transferred mice (S. A. and T. N, unpublished results).

Regarding the result that IL17^{-/-} Th17-polarized cells failed to migrate into the lung, we confirmed that messenger RNA (mRNA) expression levels of CCR6, which is a typical

chemokine receptor found on Th17 cells, were not changed between IL17^{-/-} Th17-polarized cells and WT-Th17 cells (Supplementary Figure 1 is available *International Immunology* Online). It is well known that IL-17 up-regulates many cytokines and chemokines that are essential for the development of inflammation (30). We therefore predict that various IL-17-dependent chemotactic mechanisms other than the CCR6-dependent pathway are involved in Th17 cell migration into the lung in our model.

At least, IL-17 is a key factor for the migration of inflammatory cells such as antigen-specific Th17 cells into the lung, which might be an important process for inducing airway inflammation.

IFN- γ derived from IFN- γ producers that have converted from the transferred Th17-polarized cell population is essential for inducing AHR elevation but not neutrophilia

Next, to evaluate the critical role of IFN- γ in addition to IL-17 during airway inflammation induced by the transfer of the Th17-polarized cell population, we examined whether IFN- γ ^{-/-} Th17 cells could induce AHR elevation and neutrophilia. IFN- γ ^{-/-} BALB/c mice were i.v. given OVA-specific IFN- γ ^{-/-} Th17 cells, which were derived from IFN- γ ^{-/-} DO11.10 Tg mice. After the final OVA inhalation, mice given WT Th17-polarized cells exhibited a great elevation of AHR as well as neutrophilia; however, mice given IFN- γ ^{-/-} Th17-polarized cells showed no significant elevation of AHR though they exhibited severe neutrophilia (Fig. 5A and B). Additionally, the numbers of lung-infiltrating IL-17-producing cells were greatly increased when mice were transferred with IFN- γ ^{-/-} Th17 cells rather than WT Th17 cells (Fig. 5C). Similarly, the ability of the infiltrating IFN- γ ^{-/-} Th17 cells to produce IL-17 was also augmented compared with WT Th17 cells (Fig. 5D). These results indicated that IL-17 alone was not enough to induce AHR elevation in the absence of IFN- γ even when its production was highly accelerated in the lung. From these results, we concluded that the AHR elevation required IFN- γ -producing cells converted from the Th17-polarized cell population; moreover, it was demonstrated that severe neutrophilia in addition to IL-17 production was not enough for AHR elevation, as shown in Fig. 5(B). Thus, we concluded that a cell population producing only IL-17 failed to induce AHR elevation; however, the co-existence of IFN- γ producers such as Th1-like cells and/or cells that produce IL-17 and IFN- γ —which might be converted in the lung from the transferred Th17-polarized cell population (Figs 2 and 3)—contributed to the AHR elevation.

IL-12 is a critical factor for conversion of the Th17-polarized cell population into IFN- γ producers in the lung

Recently, it has been demonstrated that IL-12 and/or IL-23 function as IFN- γ inducers that act on Th17 cells (31, 32), so we next examined the roles of these cytokines in the conversion of the Th17-polarized cell population toward IFN- γ producers in the lung. In mice given the Th17-polarized cell population, the mRNA expression of both IL-12 and IL-23 was enhanced in the lung at early times after OVA inhalation (Fig. 6A); however, such changes were not observed in mice that were treated with OVA inhalation but not with Th17 cell

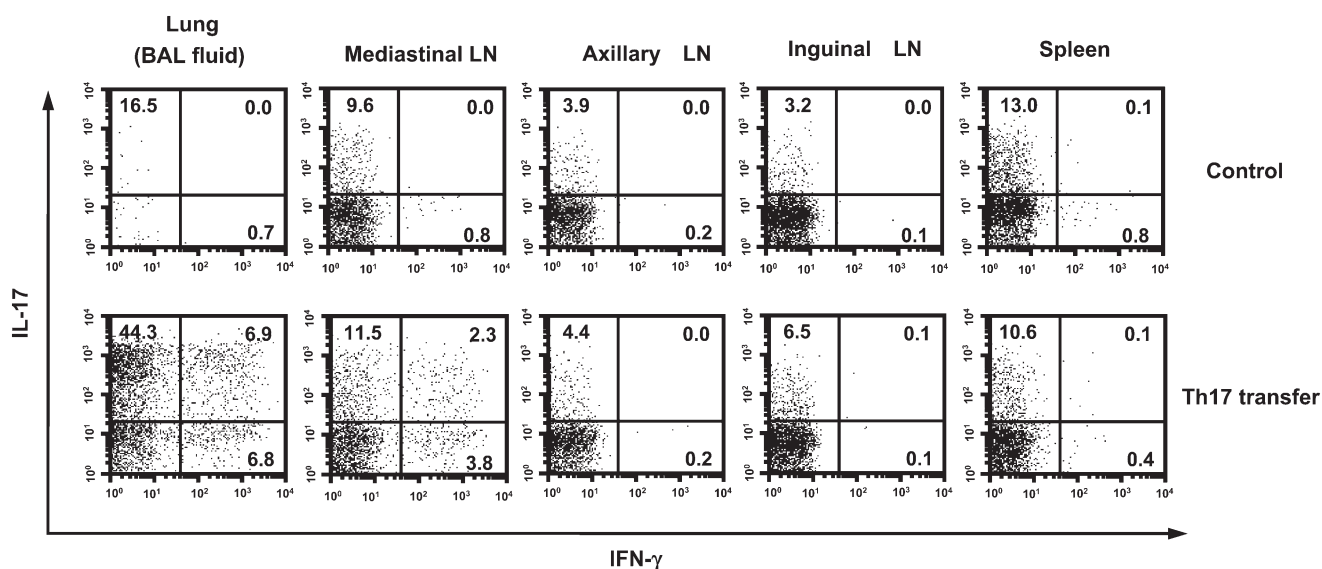


Fig. 3. Conversion of the T_H17 -polarized cell population into IFN- γ producers is induced only in the OVA-exposed sites local to the lung but not in other distal tissues. BALB/c mice were i.v. injected with an OVA-specific T_H17 -polarized cell population and exposed to OVA protein. At 24 h after final OVA inhalation, IFN- γ - and/or IL-17-producing cells among lymphocytes collected from BAL fluid, mediastinal lymph node (LN), axillary LN, inguinal LN and spleen were analyzed by intracellular staining. FACS profiles and percentages gated on CD4⁺ KJ1.26⁺ cells are indicated. One experimental group was composed of three mice ($n = 3$). Similar results were obtained in three separate experiments and representative FACS plots are shown in the figures. In control experiments, T_H17 cells were transferred but there was no inhalation of OVA protein.

transfer. IL-12 and/or IL-23 have been reported to be involved in the conversion of T_H17 cells toward T_H1 -like cells (31, 32). In our model, administration of mAb against the p35 subunit (which is unique to IL-12) or the p40 subunit (which is found in both IL-12 and IL-23) significantly blocked the conversion to IFN- γ -producing cells from the transferred T_H17 -polarized cell population, whereas mAb against p19 subunit (which is unique to IL-23) did not block the conversion to IFN- γ producers though it slightly inhibited the generation of IL-17-producing cells (Fig. 6B and C). These data indicate that IL-12 might be one of the important factors involved in the conversion of the T_H17 -polarized cell population toward IFN- γ producers in the lung during airway inflammation in our model.

Blockade of IL-12 inhibits the elevation of AHR in the T_H17 -cell-transferred airway inflammation model

We next examined the effect of anti-IL-12 mAbs (anti-p35 mAb or anti-p40 mAb) on AHR elevation. As shown in Fig. 7, it was clearly demonstrated that blockade of IL-12 prevented the T_H17 -cell-dependent AHR elevation concomitantly with a decrease in the numbers of IFN- γ -producing cells in the lung, which is shown in Fig. 6(B and C). We therefore concluded that IL-12-dependent conversion of the T_H17 -polarized cell population into IFN- γ producers was a critical process for inducing AHR elevation in airway inflammation caused by the transferred T_H17 -polarized cell population.

Discussion

In the present work, we initially demonstrated the critical role of IFN- γ in the elevation of AHR in our model of airway in-

flammation induced by the cell transfer of a T_H17 -polarized cell population. The T_H17 -polarized cell population induced the elevation of AHR and severe neutrophilia but not eosinophilia and mucus hyperproduction. We therefore conclude that the pathogenic feature of T_H17 -cell-induced airway inflammation is similar to T_H1 -cell-dependent airway inflammation but it is totally different from T_H2 -cell-dependent airway inflammation (5, 6, 12). Interestingly, the transferred T_H17 -polarized cell population, which exhibits no significant ability to produce IFN- γ before the cell transfer, converted into IFN- γ producers after migration into sites local to the lung following OVA inhalation; moreover, their production of IFN- γ in the lung is essential for AHR elevation. It was furthermore demonstrated that IL-12-dependent conversion of the T_H17 -polarized cell population into IFN- γ producers in the lung is a critical process for the elevation of AHR during T_H17 -cell-dependent airway inflammation.

It has been demonstrated that IL-17 plays a critical role for inducing various immune diseases such as airway inflammation, severe colitis, experimental autoimmune encephalomyelitis (EAE), graft-versus-host disease and contact dermatitis, which are mediated by IL-17-producing NKT cells, CD4⁺ T cells and CD8⁺ T cells (33–37). In our established OVA-specific T_H17 -cell-dependent model, we do not, however, detect the recruitment of host-derived IL-17-producing CD8⁺ T cells and IL-17-producing CD1d-restricted NKT cells into the lung after OVA inhalation (S. A. and T. N, unpublished results). In our model, therefore, the transferred T_H17 -polarized cell population mostly orchestrates the pathogenesis of airway inflammation.

Since T_H17 cells, a new subset of T_H cells, were identified, many investigators have characterized the various pathogenic

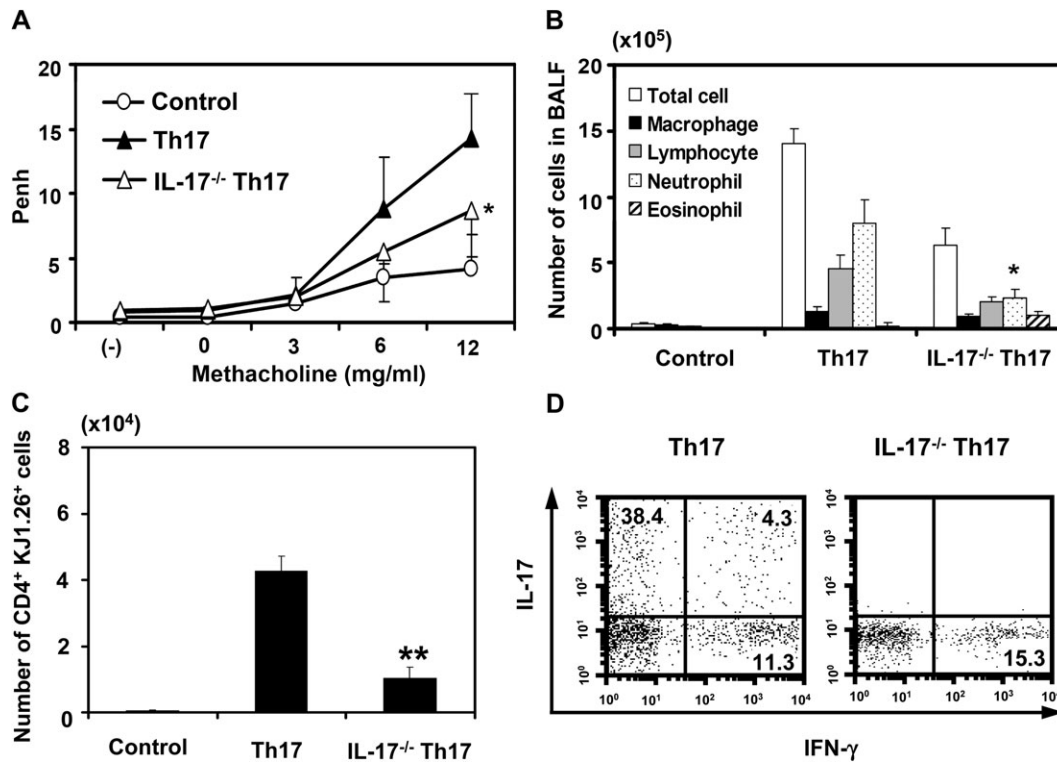


Fig. 4. IL-17 is a critical factor for the migration of pathogenic effector cells into inflamed lung tissue. IL-17^{-/-} BALB/c mice were i.v. injected with OVA-specific IL-17^{-/-} T_H17 cells and exposed to OVA protein. At 24 h after final OVA inhalation, the mice were subjected to the following analysis. (A) AHR of basal values (–) or the response to nebulized PBS (0) and to three concentrations of methacholine (3, 6 and 12 mg ml⁻¹) was measured with a whole-body plethysmograph. (B) Inflammatory cells collected from BAL fluid were stained with DAB/Tris-HCl (pH 7.25) solution and hematoxylin. Differentials of macrophages, lymphocytes, neutrophils and eosinophils were judged according to the morphology and staining characteristics. The numbers of each cell subset were counted and are indicated in the figure. (C) OVA-specific T_H17 cells or IL-17^{-/-} T_H17 (CD4⁺ KJ1.26⁺) cells in the BAL fluid were detected by flow cytometry. The numbers of infiltrating T_H17 cells or IL-17^{-/-} T_H17 cells are indicated. (D) IFN- γ -and/or IL-17-producing cells in BAL fluid were analyzed by intracellular staining. FACS profiles and percentages gated on CD4⁺ KJ1.26⁺ cells are indicated. One experimental group was composed of three mice ($n = 3$). In control experiments, OVA protein was inhaled but there was no transfer of T_H17 or IL-17^{-/-} T_H17 cells. Similar results were obtained in three separate experiments and representative data (mean $\pm$ SD) and FACS plots are shown in the figures. * $P < 0.05$ and ** $P < 0.01$ indicate significant differences from T_H17 -cell-transferred mice.

features of T_H17 -cell-dependent diseases such as colitis, EAE, arthritis and psoriasis and asthma (38–42).

Mckinley *et al.* (42) reported that the transfer of T_H17 -polarized cells induced AHR elevation. In that paper, they also demonstrated that intratracheal administration of an IL-17-expressing adenovirus caused the elevation of AHR; however, they did not investigate the effect of virus infection on inflammatory responses, including the elevation of IFN- γ production and the migration of immunoregulatory cells. The precise mechanisms underlying T_H17 -cell-dependent AHR elevation therefore remain unclear. Nakae *et al.* (43) clearly demonstrated the crucial role of IL-17 for inducing airway inflammation using IL-17-deficient mice. Consistent with their study (43) and another report (44), we also confirmed that IL-17 is surely required for the development of AHR in our T_H17 -dependent airway inflammation model because the transfer of an IL-17^{-/-} T_H17 cell population into recipient IL-17^{-/-} mice caused the reduction of AHR level concomitantly with a significantly decreased number of lymphocytes and neutrophils (Fig. 4). One explanation of this result is that IL-17 signaling appears to be required for the migration of inflammatory cells including T_H cells and neutrophils into the lung (43, 44). This is supported by our data (Fig. 4C) show-

ing that the absolute numbers of the transferred T_H17 cell population greatly decreased in the absence of IL-17 although the percentage of cells able to produce IFN- γ is similar in the lung. The total amount of IFN- γ in the lung of IL-17^{-/-} T_H17 -cell-transferred mice is thus much lower than levels in mice given a WT IL-17-producing T_H17 cell population. We therefore consider that the reduction of AHR elevation in IL-17-deficient mice may be derived from the decreased levels of IFN- γ in the lung. When IFN- γ ^{-/-} mice were transferred with IFN- γ ^{-/-} T_H17 cells followed by OVA inhalation, no significant AHR elevation was induced even though a large amount of IL-17 and severe neutrophilia are induced in the absence of IFN- γ (Fig. 5). Together with these observations, IFN- γ —which is produced by the converted IFN- γ producers from T_H17 -polarized cell population in the lung—appears to be required for AHR elevation.

To date, it has been generally accepted that IFN- γ negatively regulates the differentiation of naive T_H cells into T_H17 cells *in vitro* (45). Recently, however, it has been reported that T_H17 cells are able to become IFN- γ producers, as observed both *in vitro* and *in vivo* (31, 32). It has been reported that autoimmune diseases were established or mediated by the concurrent presence of T_H17 and T_H1 cells in mouse

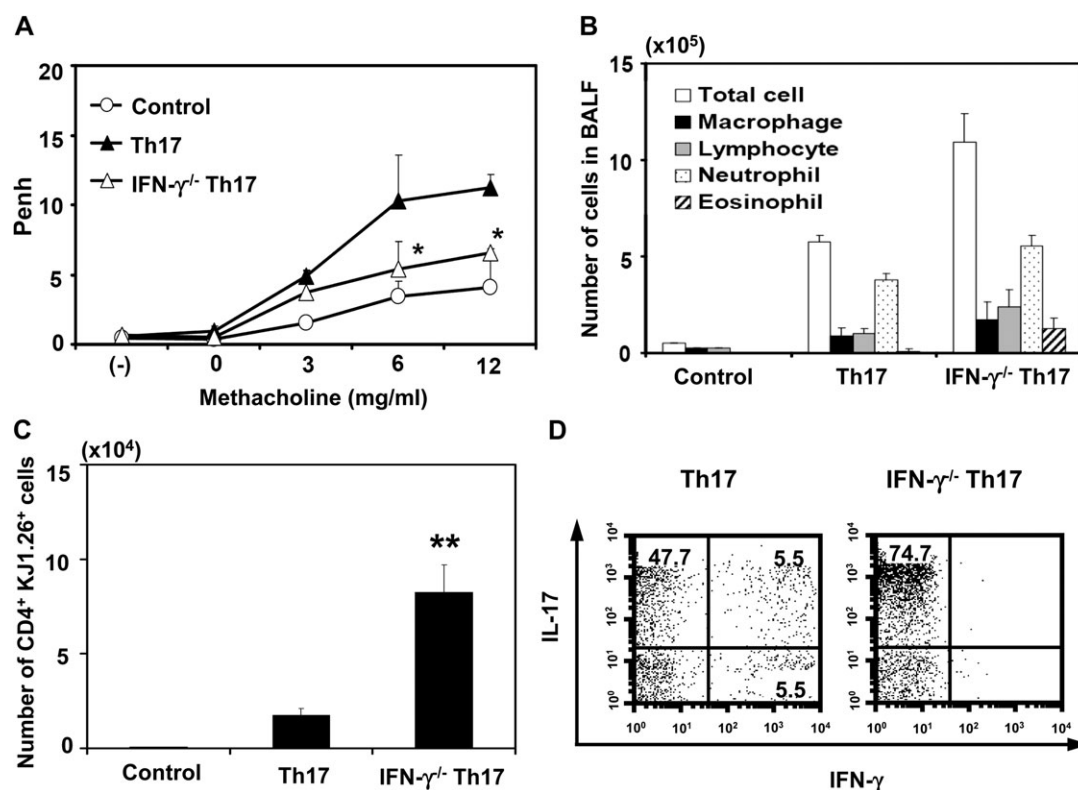


Fig. 5. IFN- γ derived from IFN- γ producers that have converted from the transferred T_H17-polarized cell population is essential for inducing AHR elevation but not neutrophilia. IFN- γ ^{-/-} BALB/c mice were i.v. injected with IFN- γ ^{-/-} T_H17 cells and exposed to OVA protein. At 24 h after final OVA inhalation, the mice were subjected to the following analysis. (A) AHR of basal values (–) or the response to nebulized PBS (0) and to three concentrations of methacholine (3, 6 and 12 mg ml⁻¹) was measured with a whole-body plethysmograph. (B) Inflammatory cells collected from BAL fluid were stained with DAB/Tris-HCl (pH 7.25) solution and hematoxylin. Differentials of macrophages, lymphocytes, neutrophils and eosinophils were judged according to the morphology and staining characteristics. The numbers of each cell subset were counted and indicated in the figure. (C) OVA-specific T_H17 cells or IFN- γ ^{-/-} T_H17 (CD4⁺ KJ1.26⁺) cells in the BAL fluid were detected by flow cytometry. The numbers of infiltrating T_H17 cells or IFN- γ ^{-/-} T_H17 cells are indicated. (D) IFN- γ ^{-/-} and/or IL-17-producing cells in BAL fluid were analyzed by intracellular staining. FACS profiles and percentages gated on CD4⁺ KJ1.26⁺ cells are indicated. One experimental group was composed of three mice ($n = 3$). In control experiments, OVA protein was inhaled but there was no transfer of T_H17 or IFN- γ ^{-/-} T_H17 cells. Similar results were obtained in three separate experiments and representative data (mean $\pm$ SD) and FACS plots are shown in the figures. * $P < 0.05$ and ** $P < 0.01$ indicate significant difference from T_H17 cell-transferred mice.

models (31, 46, 47). In addition, it has been also demonstrated that a T_H17 profile was converted into a T_H1-like profile under the influence of IL-12 and/or IL-23 (31, 32). In our airway inflammation model, mRNA expression of IL-12 and IL-23 was up-regulated at early times after OVA inhalation in the lung of T_H17-cell-transferred mice but not in mice treated with OVA inhalation alone (Fig. 6A). This result indicates that the interaction between the infiltrating T_H17-polarized cell population and lung cell components including epithelial cells, macrophages and dendritic cells may be essential for inducing IL-12 and IL-23. Although both cytokines have been reported to be involved in the ability of T_H17 cells to change into IFN- γ producers (31, 32), IL-12 but not IL-23 was demonstrated to be a key cytokine allowing the conversion of the T_H17-polarized cell population into IFN- γ producers—i.e. T_H1-like cells and cells producing IL-17 and IFN- γ —in our airway inflammation model. As shown in Fig. 6(B and C), mAbs against IL-12 subunits (p35 and p40) but not a mAb against the IL-23p19 subunit prevented the conversion of the T_H17-polarized cell population toward IFN- γ producers in OVA-inhaled lungs; furthermore, we also

demonstrated that administration of mAb against IL-12p35 or IL-12p40 subunit suppresses T_H17-cell-dependent AHR elevation (Fig. 7). The treatment with anti-p19 mAb slightly, but not significantly, decreased the AHR level and neutrophilia (S. A. and T. N., unpublished results). This result may suggest that neutrophilia is partially involved in the AHR elevation in our model. We also found that depletion of neutrophils decreased the AHR level as shown in Supplementary Figure 2, available at *International Immunology* Online. In addition, the IFN- γ level in BALF was not changed when neutrophils were depleted. IFN- γ might therefore influence infiltrating neutrophils to develop the AHR because it is reported that neutrophils are activated with IFN- γ (48); however, it remains unclear whether only stimulated neutrophils contribute to the AHR elevation because IFN- γ may also act on lung resident cells including airway smooth muscle cells for the development of AHR. Further investigations are required for the resolution of this issue.

In this study, at least, the AHR is enhanced by IFN- γ producers that have been converted from the T_H17-polarized cell population in the presence of IL-12 during T_H17-mediated

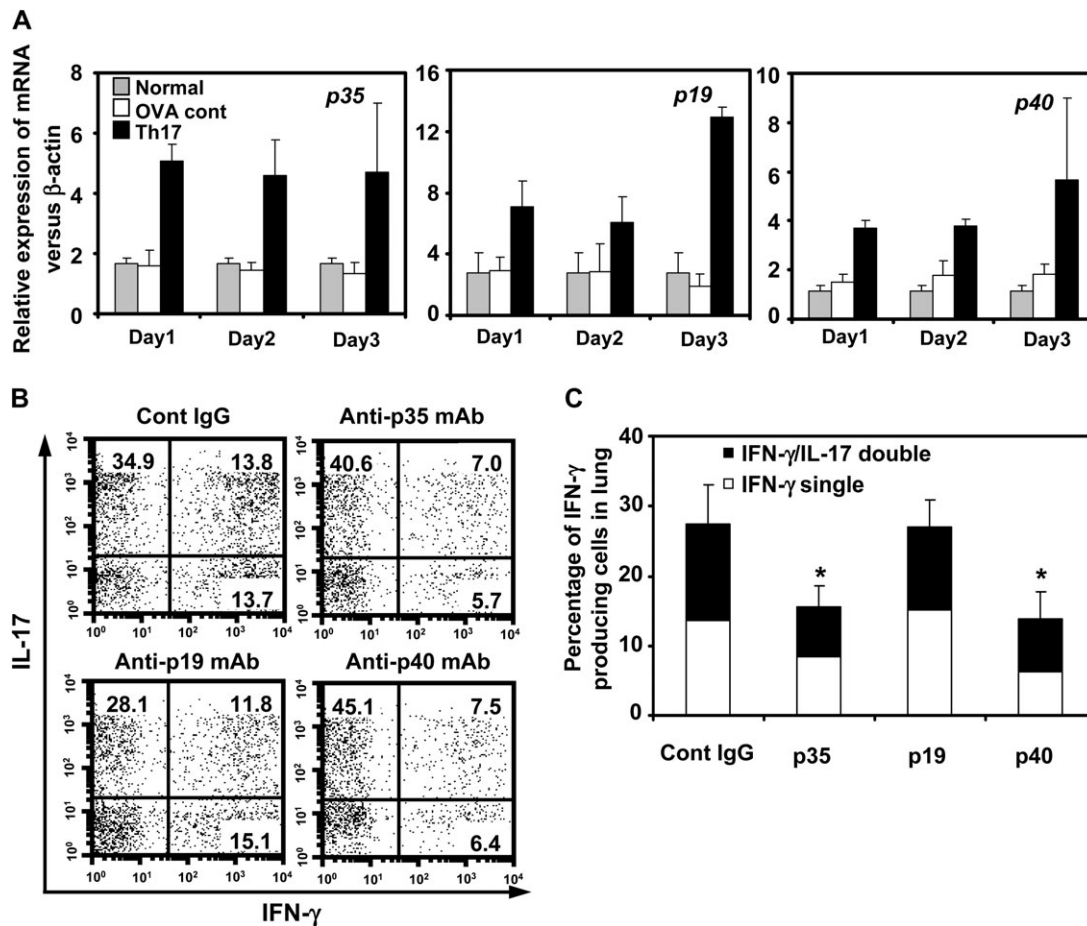


Fig. 6. IL-12 is critical factor for conversion of the T_h17 -polarized cell population into IFN- γ producers in the lung. BALB/c mice were i.v. injected with OVA-specific T_h17 cells and exposed to OVA protein. (A) At 1, 2 or 3 days during OVA inhalation, lung tissues were collected from normal mice, mice that inhaled OVA (OVA control) or mice that inhaled OVA and had been given T_h17 cells. IL-12p35, IL-23p19 and IL-12/23p40 subunit mRNA expression in collected lung tissues was evaluated by real-time PCR. (B) Anti-p35 mAb, anti-p19 mAb and anti-p40 mAb or control rat IgG was intranasally injected (100 μ g per mouse, respectively) at 30 min before the each OVA inhalation. At 24 h after final OVA inhalation, the mice were subjected to BAL analysis. IFN- γ - and/or IL-17-producing cells in BAL fluid were analyzed by intracellular staining. FACS profiles and percentages gated on CD4 $^+$ KJ1.26 $^+$ cells are indicated. (C) Percentages of total IFN- γ -producing T_h17 cells—including IFN- γ single (i.e. producing only IFN- γ) and IFN- γ /IL-17 double (i.e. producing IFN- γ and IL-17) producers—are indicated graphically. One experimental group was composed of three mice ($n = 3$). Similar results were obtained in three separate experiments and representative data (mean $\pm$ SD) and FACS plots are shown in the figures. * $P < 0.05$ indicate significant difference from control (IgG-treated) mice.

airway inflammation. Therefore, we conclude that IL-12 is a crucial factor for inducing IFN- γ producers, which are the cells responsible for development of AHR.

Two cytokines—IL-17 and IFN- γ —are necessary for limiting several pathogen infections (23, 24); however, some viral infections frequently raise the incidence of severe asthma that exhibits steroid-resistant characteristics (27, 28). Notably, it has been reported that IFN- γ contributed to the severity of respiratory dysfunction including AHR elevation, which is one of the most typical characteristic symptoms of severe asthma (29); therefore, IL-12-dependent conversion of the T_h17 -polarized cell population into IFN- γ producers may be also involved in the elevation of AHR in severe asthma.

In summary, we have demonstrated the following findings in our airway inflammation model caused by the transfer of a T_h17 -polarized cell population followed by OVA inhalation: (i) IL-17 itself can not induce AHR elevation though it is critical for neutrophilia and the migration of antigen-specific

T_h cells before the development of airway inflammation; (ii) the production of IFN- γ is essential for inducing AHR elevation but not for neutrophilia; (iii) IFN- γ , which is required for AHR elevation, comes from the converted IFN- γ producers that derive from the transferred T_h17 -polarized cell population in the lung; (iv) IL-12-dependent conversion of the T_h17 -polarized cell population toward IFN- γ -producing— T_h1 -like cells or cells that produce IL-17 and IFN- γ —is a key event for the elevation of AHR during airway inflammation. IL-17 producers therefore appear to be essential for developing neutrophilic airway inflammation, and IFN- γ producers are required for AHR elevation during airway inflammation.

Since there is no available treatment for T_h17 -cell-associated severe respiratory diseases, our findings will provide a novel insight to develop therapeutic strategies against T_h17 -dependent airway inflammation including concurrent pulmonary infection or steroid-resistant severe asthma.

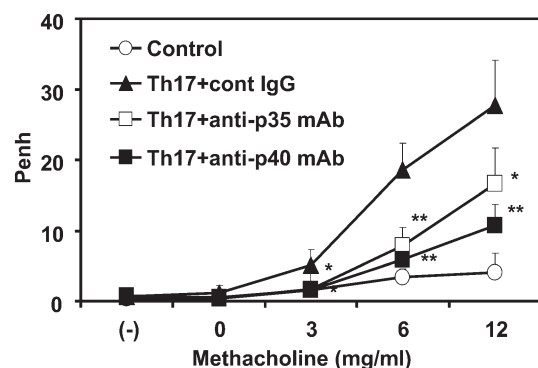


Fig. 7. Blockade of IL-12 inhibits the elevation of AHR in the Th17-cell-transferred airway inflammation model. BALB/c mice were i.v. injected with OVA-specific Th17 cells and exposed to OVA protein. Anti-p35 mAb and anti-p40 mAb or control rat IgG was intranasally injected (100 μ g per mouse, respectively) at 30 min before the each OVA inhalation. At 24 h after final OVA inhalation, the mice were subjected to AHR analysis. AHR of basal values (–) and the response to nebulized PBS (0) and to three concentrations of methacholine (3, 6 and 12 mg ml^{–1}) was measured with a whole-body plethysmograph 24 h after final OVA inhalation. One experimental group was composed of three mice ($n = 3$). Similar results were obtained in three separate experiments and representative data (mean $\pm$ SD) are shown in the figures. * $P < 0.05$ and ** $P < 0.01$ indicate significant difference from control (IgG-treated) mice.

Supplementary data

Supplementary Figures 1 and 2 are available at *International Immunology Online*.

Funding

National Project Sapporo Biocluster “Bio-S”, the Knowledge Cluster initiative of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Acknowledgements

We would like to thank Dr T. Sawada (Shionogi Pharmaceutical Institute Co., Osaka, Japan) and Dr H. Tashiro (Fujiya Co. Ltd, Hadano, Japan) for their kind donations of rhIL-2 and OVA_{323–339} peptide, respectively. We also thank the Chugai Pharmaceutical Company Ltd for their gift of rhIL-6. Conflict of Interest: The authors declare no competing financial interests.

References

- Barnes, P. J. 2008. Immunology of asthma and chronic obstructive pulmonary diseases. *Nat. Rev. Immunol.* 8:183.
- Bochner, B. S. and Busse, W. W. 2005. Allergy and asthma. *J. Allergy Clin. Immunol.* 115:953.
- Busse, W. W. and Lemanske, R. J. 2001. Asthma. *N. Engl. J. Med.* 344:350.
- Wills-Karp, M. 1999. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu. Rev. Immunol.* 17:255.
- Ashino, S., Wakita, D., Zhang, Y., Chamoto, K., Kitamura, H. and Nishimura, T. 2008. CpG-ODN inhibits airway inflammation at effector phase through down-regulation of antigen-specific Th2-cell migration into lung. *Int. Immunol.* 20:259.
- Hoshino, A., Tsuji, T., Matsuzaki, J. *et al.* 2004. STAT6-mediated signaling in Th2-dependent allergic asthma: critical role for the development of eosinophilia, airway hyper-responsiveness and mucus hypersecretion, distinct from its role in Th2 differentiation. *Int. Immunol.* 16:1497.

- Elias, J. A., Lee, C. G., Zheng, T., Ma, B., Homer, R. J. and Zhu, Z. 2003. New insights into the pathogenesis of asthma. *J. Clin. Invest.* 111:291.
- Durham, S. R., Till, S. J. and Corrigan, C. J. 2000. T lymphocytes in asthma: bronchial versus peripheral responses. *J. Allergy Clin. Immunol.* 106:S221.
- Brightling, C. E., Symon, F. A., Birring, S. S., Bradding, P., Pavord, I. D. and Wardlaw, A. J. 2002. TH2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis. *J. Allergy Clin. Immunol.* 110:899.
- Barnes, P. J. 2008. The cytokine network in asthma and chronic obstructive pulmonary diseases. *J. Clin. Invest.* 118:3546.
- Holgate, S. T. and Polosa, R. 2008. Treatment strategies for allergy and asthma. *Nat. Rev. Immunol.* 8:218.
- Takaoka, A., Tanaka, Y., Tsuji, T. *et al.* 2001. A critical role for mouse CXC chemokine(s) in pulmonary neutrophilia during Th type 1-dependent airway inflammation. *J. Immunol.* 167:2349.
- Cui, J., Pazdziorko, S., Miyashiro, J. S. *et al.* 2005. TH1-mediated airway hyperresponsiveness independent of neutrophilic inflammation. *J. Allergy Clin. Immunol.* 115:309.
- Hayashi, N., Yoshimoto, T., Izuhara, K., Matsui, K., Tanaka, T. and Nakanishi, K. 2007. T helper 1 cells stimulated with ovalbumin and IL-18 induce airway hyperresponsiveness and lung fibrosis by IFN- γ and IL-13 production. *Proc. Natl Acad. Sci. USA* 104:14765.
- Yang, M., Kumar, R. K. and Foster, P. S. 2009. Pathogenesis of steroid-resistant airway hyperresponsiveness: interaction between IFN- γ and TLR4/MyD88 pathways. *J. Immunol.* 182:5107.
- Lindén, A., Laan, M. and Anderson, G. P. 2005. Neutrophils, interleukin-17A and lung disease. *Eur. Respir. J.* 25:159.
- Dubin, P. J., McAllister, F. and Kolls, J. K. 2007. Is cystic fibrosis a TH17 disease? *Inflamm. Res.* 56:221.
- Pène, J., Chevalier, S., Preisser, L. *et al.* 2008. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. *J. Immunol.* 180:7423.
- Bullens, D. M., Truyen, E., Coteur, L. *et al.* 2006. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir. Res.* 7:135.
- Sun, Y. C., Zhou, Q. T. and Yao, W. Z. 2005. Sputum interleukin-17 is increased and associated with airway neutrophilia in patients with severe asthma. *Chin. Med. J. (Engl.)* 118:953.
- Barczyk, A., Pierzchała, W. and Sozańska, E. 2003. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir. Med.* 97:726.
- Chakir, J., Shannon, J., Molet, S. *et al.* 2003. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF- β , IL-11, IL-17, and type I and type III collagen expression. *J. Allergy Clin. Immunol.* 111:1293.
- Khader, S. A., Bell, G. K., Pearl, J. E. *et al.* 2007. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat. Immunol.* 8:369.
- Happel, K. I., Dubin, P. J., Zheng, M. *et al.* 2005. Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. *J. Exp. Med.* 202:761.
- Scriba, T. J., Kalsdorf, B., Abrahams, D. A. *et al.* 2008. Distinct, specific IL-17- and IL-22-producing CD4+ T cell subsets contribute to the human anti-mycobacterial immune response. *J. Immunol.* 180:1962.
- Acosta-Rodriguez, E. V., Rivino, L., Geginat, J. *et al.* 2007. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat. Immunol.* 8:639.
- Grissell, T. V., Powell, H., Shafren, D. R. *et al.* 2005. Interleukin-10 gene expression in acute virus-induced asthma. *Am. J. Respir. Crit. Care Med.* 172:433.
- Wos, M., Sanak, M., Soja, J., Olechnowicz, H., Busse, W. W. and Szczeklik, A. 2008. The presence of rhinovirus in lower airways of patients with bronchial asthma. *Am. J. Respir. Crit. Care Med.* 177:1082.
- Shannon, J., Ernst, P., Yamauchi, Y. *et al.* 2008. Differences in airway cytokine profile in severe asthma compared to moderate asthma. *Chest* 133:420.

- 30 Iwakura, Y., Nakae, S., Saijo, S. and Ishigame, H. 2008. The roles of IL-17A in inflammatory immune responses and host defense against pathogens. *Immunol. Rev.* 226:57.
- 31 Bending, D., De La Peña, H., Veldhoen, M. *et al.* 2009. Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. *J. Clin. Invest.* 119:565.
- 32 Lee, Y. K., Turner, H., Maynard, C. L. *et al.* 2009. Late developmental plasticity in the T helper 17 lineage. *Immunity* 30:92.
- 33 Michel, M. L., Keller, A. C., Paget, C. *et al.* 2007. Identification of an IL-17-producing NK1.1 (neg) iNKT cell population involved in airway neutrophilia. *J. Exp. Med.* 204:995.
- 34 Pichavant, M., Goya, S., Meyer, E. H. *et al.* 2008. Ozone exposure in a mouse model induces airway hyperreactivity that requires the presence of natural killer T cells and IL-17. *J. Exp. Med.* 205:385.
- 35 Kappel, L. W., Goldberg, G. L., King, C. G. *et al.* 2009. IL-17 contributes to CD4-mediated graft-versus-host disease. *Blood* 113:945.
- 36 Tajima, M., Wakita, D., Noguchi, D. *et al.* 2008. IL-6-dependent spontaneous proliferation is required for the induction of colitogenic IL-17-producing CD8+ T cells. *J. Exp. Med.* 205:1019.
- 37 He, D., Wu, L., Kim, H. K., Li, H., Elmetts, C. A. and Xu, H. 2006. CD8+ IL-17-producing T cells are important in effector functions for the elicitation of contact hypersensitivity responses. *J. Immunol.* 177:6852.
- 38 Noguchi, D., Wakita, D., Tajima, M. *et al.* 2007. Blocking of IL-6 signaling pathway prevents CD4+ T cell-mediated colitis in a T(h)17-independent manner. *Int. Immunol.* 19:1431.
- 39 Nakae, S., Nambu, A., Sudo, K. and Iwakura, Y. 2003. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J. Immunol.* 171:6173.
- 40 Komiyama, Y., Nakae, S., Matsuki, T. *et al.* 2006. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J. Immunol.* 177:566.
- 41 Ma, H. L., Liang, S., Li, J. *et al.* 2008. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. *J. Clin. Invest.* 118:597.
- 42 McKinley, L., Alcorn, J. F., Peterson, A. *et al.* 2008. Th17 cells mediate steroid-resistant airway inflammation and airway hyper-responsiveness in mice. *J. Immunol.* 181:4089.
- 43 Nakae, S., Komiyama, Y., Nambu, A. *et al.* 2002. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 17:375.
- 44 He, R., Oyoshi, M. K., Jin, H. and Geha, R. S. 2007. Epicutaneous antigen exposure induces a Th17 response that drives airway inflammation after inhalation challenge. *Proc. Natl Acad. Sci. USA* 104:15817.
- 45 Mangan, P. R., Harrington, L. E., O'Quinn, D. B. *et al.* 2006. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* 441:231.
- 46 Luger, D., Silver, P. B., Tang, J. *et al.* 2008. Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J. Exp. Med.* 205:799.
- 47 Kullberg, M. C., Jankovic, D., Feng, C. G. *et al.* 2006. IL-23 plays a key role in *Helicobacter hepaticus*-induced T cell-dependent colitis. *J. Exp. Med.* 203:2485.
- 48 Terri, N. and Beaman, B. L. 2004. Interferon- γ activation of polymorphonuclear neutrophil function. *Immunology* 112:2.