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IL-17 is a pivotal proinflammatory molecule in asthmatics. However, the cellular source of IL-17 in asthma has not been identified to date. In this study, we report that macrophages rather than Th17 cells are the main producer of IL-17 in allergic inflammation related to asthma. After OVA challenge in a mouse model mimicking allergic asthma, the increased IL-17+ cells in the lung were mainly CD11b+F4/80+ macrophages, instead of T cells or others. Importantly, IL-17+ alveolar macrophages (AMs), but not IL-17+ interstitial macrophages, were significantly increased after allergen challenge. The increase of IL-17+ AMs was not due to the influx of IL-17+ macrophages from circulation or other tissues, but ascribed to the activation of AMs by mediator(s) secreted by IgE/OVA-activated mast cells. Depleting alveolar macrophages or neutralizing IL-17 prevented the initiation of OVA-induced asthma-related inflammation by inhibiting the increase of inflammatory cells and inflammatory factors in bronchoalveolar lavage fluid. Th2 cytokine IL-10 could down-regulate IL-17 expression in alveolar macrophages. The increased IL-17 and the decreased IL-10 in bronchoalveolar lavage fluid were further confirmed in asthmatic patients. These findings suggest that IL-17 is mainly produced by macrophages but not Th17 cells in allergic inflammation related to asthma. Mast cell-released mediators up-regulate the expression of IL-17 by macrophages, whereas IL-10 down-regulates IL-17 expression. The Journal of Immunology, 2008, 181: 6117–6124.

Allergic asthma is a chronic inflammatory disorder of the lung characterized by a prevailing Th2 immune response and allergic airway inflammation (1). The balance between Th2 response and Th1 response has been thought to determine the allergic airway inflammation. It has been generally accepted that Th2 cytokines such as IL-4, IL-5, IL-9, and IL-13 play critical roles in orchestrating and amplifying allergic inflammation in asthma. Th1 cytokine IFN-γ was thought to prevent this process (2, 3), however, recent studies indicate that IFN-γ secreted by Th1 cells may cause severe airway inflammation under certain conditions (4, 5).

In addition to Th1/Th2 cytokines, a critical proinflammatory cytokine IL-17 has attracted great attention recently. IL-17 acts on a broad range of cells to induce the expression of cytokines (IL-6, IL-8, GM-CSF, G-CSF), chemokines, and metalloproteinases (6–9). It also cooperates with TLR ligands, IL-1β, and TNF-α to enhance inflammatory reactions and stimulate the production of β-defensins and other antimicrobial peptides (10). The important roles of IL-17 in allergic asthma have also been noticed. Although it was speculated that IL-17 may have a negative effect on the established allergic asthma (11), IL-17/IL-17R signaling has been proved to be critically required to develop allergic asthma (12). The concentration of IL-17 is significantly increased in bronchoalveolar lavage fluid (BALF), sputum, and blood from patients with asthma (13, 14). The overexpression of IL-17 in lung epithelium causes chemokine production and leukocyte infiltration in vivo (15). IL-17 promotes the recruitment and expansion of neutrophils (16). In a mouse model of allergic airway inflammation, systemic blockade of IL-17 inhibits the allergen-induced accumulation of neutrophils in the airway (17). These findings suggest that IL-17 plays an important role during the pathophysiological process of allergic asthma. Despite the important role of IL-17 in allergic asthma, the cellular source of IL-17 in allergic asthma has not yet been identified, hindering the further elucidation of the mechanisms underlying the onset of allergic asthma.

Although Th17, the third subset of Th cells, has been considered as the primary producer of IL-17 (18, 19), other cell types can also be the source of IL-17, including CD8+T cells, NK cells, and γδ T cells (20–23). In this study, we intended to clarify whether Th17 is the main source of IL-17 in allergic inflammation related to asthma. Unexpectedly, we found that IL-17 was mainly produced by CD11b+ macrophages, rather than Th17, NK cells, or others. Such macrophage-derived IL-17 was required for the initiation of allergic inflammation in a mouse model mimicking allergic asthma. Allergen-induced allergic inflammation is concomitant with a significant increase of IL-17-producing alveolar macrophages but not interstitial macrophages. This process was negatively regulated by Th2 cytokine IL-10 and positively regulated by mast cell-released mediator(s).
Materials and Methods

**Mice**

Eight-week-old virgin female BALB/c mice were purchased from the Center of Medical Experimental Animals of Hubei Province (Wuhan, China) for studies approved by the Animal Care and Use Committee of Tongji Medical College.

**Allergic asthma patients and control subjects**

Six patients (aged 35 ± 16 years old, range 26–54) with asthmatic symptom were recruited in the Department of Allergy of Tongji Hospital, Tongji Medical College. The diagnosis was based on the asthma guidelines proposed by the American Thoracic Society (24). The atopic status of patients and control subjects was ascertained by skin prick allergen tests and positive plasma-specific IgE assays to house dust mites, cat, dog, mixed cockroaches, and mixed molds. Six age-matched healthy individuals (aged 36 ± 11 years old, range 28–46) were recruited as normal control. All subjects were nonsmokers and free from upper respiratory tract infection for 4 wk preceding the study. This study was approved by the Ethical Committee of the Medical Faculty of Tongji Medical College. Informed consent was obtained from all subjects.

**Sensitization and allergen challenge in mouse model**

Mice were sensitized by i.p. injection of 0.3 ml solution containing 100 μg of OVA (Sigma-Aldrich) bound to Inj ectum Alum (Pierce) on days 0, 7, and 14. The sensitized mice were challenged by the administration of aerosolized 1% OVA for 25 min on days 17, 18, and 19.

**Preparation of BALF**

Six days after sensitization or 1 day after the last challenge in mouse model, bronchoalveolar lavage (BAL) was performed four times by instillation of 1 ml of PBS through the tracheal cannula. After lysis of RBC, the cell numbers in BALF were determined using standard hematologic procedures. Cytospin of BALF was prepared and stained with the Wright-Giemsa method (Fisher Scientific). Differential cell counting was performed using standard morphological criteria.

**Assay for cytokines**

For mouse cytokine detection, BALF was performed by instillation of PBS. The cell-free BALF supernatants of first 0.25-ml aliquot were collected after spinning down the cells. The amounts of IL-4, IL-5, IL-10, IL-13, and IL-17 in the BALF supernatants or IL-17 (IL-17A) and IL-17F in culture supernatants were determined by ELISA kits (R&D Systems). The minimum detectable dose was 2, 7, 4, 1.5, 5, and 4 pg/ml, respectively.

For human cytokines detection, the cell-free supernatants of the first 30-ml aliquot from the lavage were used for IL-10 and IL-17 detection by ELISA kit (R&D Systems).

**Isolation of immune cells from lung**

Lung tissue of mouse was digested with collagenase and hyaluronidase and minced. After lysis of RBC, the dissociated cells were underlaid with 5 ml RPMI 1640 supplemented with 10% FBS, 2 mM l-glutamine, 1 mM sodium pyruvate, 1 mM HEPES, and 50 μM 2-ME. The cells were cultured in the presence of IL-3 and SCF (10 ng/ml each, PeproTech), and the nonadherent cells were passaged every 3 days. After 4 wk later, 98% of the cells were c-Kit+ mast cells, confirmed by flow cytometry.

**RT-PCR and real-time RT-PCR**

Cells were lysed with TRIZol reagent (Invitrogen) and total RNA was extracted according to manufacturer’s instructions. The primers were designed with the Oligo Primer Analysis 4.0 software and the sequences were blasted (http://www.ncbi.nlm.nih.gov/BLAST/). One-step RT-PCR kit (Qiagen) was used for RT-PCR. The primer sequences were as follows: IL-17, sense 5'-CTGTGTCGTCTGACGTGTTG-3', antisense 5'-ATGTGGTGGTCCAGCTTTC-3'; IL-17F, sense 5'-CTGTGTCGTCTGACGTGTTG-3', antisense 5'-GTTCATGGTGCTGTCTTCCTG-3'; IL-17G, sense 5'-CTGTGTCGTCTGACGTGTTG-3', antisense 5'-GTTCATGGTGCTGTCTTCCTG-3'; GAPDH, sense 5'-GTGGAGATTGTTGCACAAGC-3', antisense 5'-CTGTGTCGTCTGACGTGTTG-3'.

For real-time RT-PCR assays, 100 ng of total RNA was used for reverse transcription using Superscript II RNase H reverse transcriptase (Invitrogen) in a volume of 25 μl. Then 2 μl of cDNA was amplified with SYBR Green Universal PCR Mastermix (Bio-Rad) in duplicate. For sample analysis, the threshold was set based on the exponential phase of products, and CT value for samples was determined. The resulting data were analyzed

anti-mouse DX5, allophyocytan-conjugated anti-mouse c-Kit, and PE-conjugated anti-mouse F4/80 Abs. After cellular surface staining, cells were washed, fixed, and permeabilized with Fix-Perm solution for intracellular staining with FITC-conjugated anti-mouse IL-17 Ab (clone eBio17B7, eBioscience). In the same way, the cells from BALF of human subjects were stained with PE-conjugated anti-human F4/80 and FITC-conjugated anti-human IL-17 Abs. All fluorophore-conjugated Abs and the corresponding isotypes were purchased from eBioscience. The stained cells were used for flow cytometric analysis (BD LSRII).

**Alveolar macrophage depletions**

In mouse model, alveolar macrophages were depleted by administering 2-chloroadenosine (2-CA) (25). Sensitized mice were given the aerosolized 5 mM of 2-CA (Sigma-Aldrich) for 20 min, driven at a rate of 0.75 ml/min. Aerosolized 2-CA was administered twice, 12 h before and 4 h after the first OVA challenge. More than 75% of alveolar macrophages were depleted, evaluated by counting the macrophages in BALF from treated and untreated mice, but other type of cells were not influenced.

**Generation of bone marrow-derived mast cells**

Bone marrow cells were harvested from femurs of mice and cultured in RPMI 1640 supplemented with 10% FBS, 2 mM l-glutamine, 1 mM sodium pyruvate, 1 mM HEPES, and 50 μM 2-ME. The cells were cultured in the presence of IL-3 and SCF (10 ng/ml each, PeproTech), and the nonadherent cells were passaged every 3 days. After 4 wk later, 98% of the cells were c-Kit+ mast cells, confirmed by flow cytometry.

**Western blot**

Cell lysates and prestained m.w. markers were separated by SDS-PAGE followed by transfer onto nitrocellulose membranes. The membranes were blocked in TBST (Tris-buffered saline with 0.5% of Triton X-100) containing 5% nonfat milk, and probed with anti-IL-17 or IL-17F Abs (R&D Systems). After incubation with the secondary Ab conjugated with HRP, membranes were extensively washed, and the immunoreactivity was visualized by ECL, according to the manufacturer’s protocol (ECL kit, Santa Cruz Biotechnology).

**Determination of airway responsiveness**

Airway responsiveness was assessed as changes in airway function after challenge with aerosolized methacholine (Sigma-Aldrich) as described previously (26). Briefly, mice were anesthetized, tracheostomized, mechanically ventilated. Ventilation was achieved at 160 breaths per minute at a tidal volume of 0.15 ml with a positive end-expiratory pressure of 2–4 cm H2O. Lung resistance was continuously computed by fitting flow, volume, and pressure.

Mice were challenged with methacholine aerosol in increasing concentrations (6.25–50 mg/ml in saline). Aerosolized agent was administered for 10 s with a tidal volume of 0.5 ml. From 20 s up to 3 min after each aerosol challenge, the data of lung resistance was continuously collected. Maximum values of lung resistance were selected to express changes in airway function, which was represented as a percentage change from baseline after administration of the saline aerosol.
with the comparative CT method for relative gene expression quantification against housekeeping gene GAPDH.

**In vitro treatment of alveolar macrophages**

Two × 10⁶ mast cells were incubated overnight at 37°C with 1 μg/ml anti-OVA IgE (Uscnlife Company), washed, and treated with 10 μg/ml of OVA for 48 h. The untreated mast cells were used as control.

Bone marrow-derived dendritic cells (DCs) were incubated with OVA (0.2 mg/ml) for 45 min at 37°C, washed three times, and then plated in round-bottom 96-well plates at 1 × 10⁶ cells/well. Five × 10⁶ T cells isolated from spleen were added to the DCs for 72 h coculture.

Two × 10⁵ alveolar macrophages were cultured in 24-well plates in the absence or presence of the supernatants from OVA/IgE-stimulated mast cells or control mast cells, and/or in the presence of IL-4 (20 ng/ml), IL-5 (20 ng/ml), IL-10 (20 ng/ml), or IL-13 (20 ng/ml). Anti-TNF-α Ab (R&D Systems) was used to block TNF-α in the supernatants from OVA/IgE-stimulated mast cells as indicated. The supernatants from OVA/DC-stimulated T cells or control T cells were also used to stimulate macrophages. Twelve hours later, the expression of IL-17 gene was detected by real-time RT-PCR. Forty-eight hours later, the left cells were used for flow cytometric analysis of intracellular IL-17, and IL-17 in supernatants was detected by ELISA as indicated.

**Intranasal administration of IL-10**

Two hours after each OVA challenge, mice were lightly anesthetized with diethyl ether. In brief, 0.2 g IL-10 in 40 μl PBS was administered into nasal cavities, 20 μl to each.

**Statistics**

Data were expressed as mean value ± SD and interpreted by nonparametric ANOVA test. Differences were considered to be statistically significant when p < 0.05.

**Results**

CD11b⁺ F4/80⁺ macrophages are major source of IL-17 in murine acute allergic inflammation in lung

To identify whether Th17 is the main producer of IL-17 in allergic asthma, we used a murine model of OVA-induced acute allergic inflammation in lung. CD11b⁺ F4/80⁺ macrophages are major source of IL-17 in pulmonary allergic inflammation. The data shown are representatives of FACS profiles (n = 5).

**FIGURE 1.** CD11b⁺ F4/80⁺ macrophages are major source of IL-17 in allergic inflammation in lung. A–C, T, B, NK, and mast cells are not the main producer of IL-17 after allergen challenge. The mononuclear cells isolated from the lung tissues of mice were stained for the analysis of CD3, CD19, DX5, c-Kit, and IL-17 by flow cytometry. The data shown are representatives of FACS profiles (n = 5).

A, Low scatter cells were gated and analyzed. B, IL-17⁺ T cells (CD3⁺) in mononuclear cells. C, IL-17⁺ B cells (CD19⁺) in mononuclear cells. D, NK (DX5⁺) and mast cells (c-Kit⁺) in CD3⁺ IL-17⁺ cells. The data shown are from the gated CD3⁺ IL-17⁺ cells. E, Macrophages are a major source of IL-17 after allergen challenge. The above mononuclear cells were stained for the analysis of CD3, CD11b, F4/80, and IL-17. The CD3⁺ cells were gated. Among them, the CD11b⁺ IL-17⁺ cells were further gated for the analysis of F4/80. The data shown are representatives of FACS profiles (n = 5). F, Macrophages express IL-17. The gated CD3⁺ CD11b⁺ cells were sorted and used for Wright-Giemsa staining and for the analysis of IL-17 and IL-17F expressions by real-time PCR and Western blot. The cells were also cultured for 48 h and IL-17 and IL-17F in the supernatants were detected by ELISA. G, Analysis of IL-17 in AMs and IMs. The percentage of IL-17⁺ macrophages in AMs and IMs was shown (n = 6). *p < 0.05, compared with control. H, IL-17-producing cells in spleen macrophages (SM), PMs, and blood monocytes (BM). For SM and PM, the results were shown as the percentage of F4/80⁺ IL-17⁺ cells in total F4/80⁺ cells. For BM, the result was shown as the percentage of CD14⁺ IL-17⁺ cells in total CD14⁺ cells.
inflammation in the lung. The expression of IL-17 in mononuclear cells isolated from the lungs of mice was analyzed by flow cytometry. The results from the analysis of low scatter cells (Fig. 1A) showed that IL-17-producing cells in the mononuclear cells was around 6% in both control and OVA-sensitized groups, and reached as high as 15% in the challenge group (Fig. 1B). Surprisingly, CD3$^+$IL-17$^+$ cells were <1% in mononuclear cells in all of three groups, although they were slightly increased after sensitization and challenge with allergen (Fig. 1B). Similarly, the percentage of CD19$^+$IL-17$^+$ cells in mononuclear cells was also very low, and only slightly changed after sensitization and challenge with allergen (Fig. 1C). These data suggest that neither Th17 cells nor B cells are the main producer of IL-17 in murine lung with allergic inflammation.

We then analyzed the expression of IL-17 in CD3$^+$ cells. The result showed that DX5$^+$ NK cells and c-Kit$^+$ mast cells accounted for only a low percentage in CD3$^+$IL-17$^+$ cells, and were not increased after allergen challenge (Fig. 1D).
IL-17+ cells in neutrophils after sensitization and allergen challenge (data not shown). The increase of IL-17+ cells after allergen challenge was mainly contributed by CD3+ CD11b+ cells, and most of CD3+ CD11b+ IL-17+ cells were F4/80 positive (Fig. 1E). Therefore, we sorted CD3+ CD11b+ cells for further analysis. The results showed that they have macrophage morphological characteristics, and that they indeed produced IL-17 at both the mRNA and protein levels (Fig. 1F). Thus, we identified that the main producer of IL-17 in the lung of mice with allergic inflammation were macrophages, which were strikingly increased after OVA challenge (Fig. 1E).

Next, we analyzed IL-17 expression in alveolar macrophages (AMs) and interstitial macrophages (IMs). The percentages of IL-17+ cells in these two populations were low, and not significantly influenced by sensitization with OVA (Fig. 1G). In the challenged mice, the percentage of IL-17+ cells in IM was decreased, whereas that in AM was increased by 6-fold (Fig. 1G). We then analyzed spleen macrophages, peritoneal macrophages, and peripheral blood monocytes. A low proportion of cells (around 2%) in each case expressed IL-17, and the expression pattern was not influenced by allergen challenge after sensitization (Fig. 1H). Therefore, the recruitment of peripheral monocyte-macrophage will not increase IL-17+ macrophages in the alveolus. The increase of IL-17-producing macrophages in the alveolus after allergen challenge is due to either the activation of AM or the migration of IL-17+ IM into the alveolus.

**Mast cell-released mediator(s) up-regulates IL-17 expression in murine macrophages**

Mast cells play an essential role in the development of airway hyperresponsiveness (27–29). Therefore, we tested whether mast cells may up-regulate IL-17 expression in alveolar macrophages. The bone marrow-derived mast cells were stimulated with OVA/IgE. The supernatants were then used to stimulate peritoneal macrophages (PMs) or AMs from naive mice. IL-17 transcripts were significantly increased in either the PM or AM group in the presence of the supernatant from OVA/IgE-stimulated mast cells (Fig. 2A). However, the direct stimulation of macrophages with OVA/IgE or with the supernatant from control mast cells did not increase IL-17 expression (Fig. 2A). Further analysis revealed that IL-17-producing macrophages and IL-17 in the supernatants were significantly increased after 48-h stimulation with the supernatant of OVA/IgE-stimulated mast cells (Fig. 2A). IL-17 expression of the macrophages stimulated by mast cell-derived mediator(s) was not affected by blocking TNF-α (Fig. 2B), although most cell-derived TNF-α has been demonstrated to be at the center in the OVA-induced asthma model (30, 31). Furthermore, IL-23, a critical cytokine for IL-17 production was not detectable in the supernatant of IgE/OVA-stimulated mast cells (data not shown). On the other hand, the supernatant from the OVA/DC-stimulated T cells did not induce macrophage to express IL-17 (Fig. 2B). These data indicate that OVA/IgE-activated mast cells release mediator(s) to induce IL-17 expression in alveolar macrophages, which explains the mechanism underlying the increase of IL-17-producing macrophages in the alveolus. The data also indicate that mast cells may stimulate macrophages to express IL-17 by releasing other mediator(s), but not TNF-α or IL-23.

**Macrophage-derived IL-17 is necessary for acute allergic inflammation in the lung**

Next, we investigated the role of alveolar macrophage-derived IL-17 in the murine acute allergic inflammation model mimicking asthma. Using aerosolized 2-CA, 75% of alveolar macrophages, but not other cell types, were depleted, evaluated by counting the cells in BALF (data not shown). Thus, sensitized mice were challenged by OVA-aerosol with or without macrophage depletion. Alternatively, the sensitized mice without macrophage depletion were treated twice with IL-17-neutralizing Ab (clone eBioMM17F3, IL-17A-specific, eBioscience), 1 h after the first and third OVA-aerosol challenge, respectively. The onset of asthma was well observed in the OVA-challenge group. Either macrophage depletion or treatment with IL-17 neutralizing Ab suppressed the increase of inflammatory cells (Fig. 3A) and cytokine IL-4, IL-5, IL-13, and IL-17 in BALF (Fig. 3B) inhibited the
infiltration of inflammatory cells in the lung (Fig. 3C) and reduced the airway hyperresponsiveness (Fig. 3D). Thus, these data suggest that IL-17-producing macrophages in alveoli are responsible for the initiation of asthma-related inflammation in the lung.

**IL-10 down-regulates IL-17 expression by murine macrophages**

Given that cytokines IL-4, IL-5, IL-10, and IL-13 play important roles in allergic asthma, in this study, we wondered whether they could regulate IL-17 expression by macrophages. For this purpose, alveolar macrophages were stimulated with these cytokines, respectively. The transcriptional activity of the IL-17 gene was not significantly influenced by IL-4, IL-5, and IL-13, whereas IL-10 significantly down-regulated the expression of IL-17 (Fig. 4A). More convincingly, intranasal administration of IL-10 effectively suppressed the increase of IL-17-producing alveolar macrophages in OVA-challenged mice (Fig. 4B), whereas the other three cytokines did not influence the amount of IL-17-producing macrophages (data not shown). These data suggest that IL-10 is a negative regulator for IL-17 expression by macrophages in acute allergic inflammation in the lung.

We then further analyzed the expression of IL-10 in BALF after OVA challenge. The result showed that the transcriptional activity of IL-10 gene in the cells from BALF was significantly reduced 24 h after OVA challenge (Fig. 4C). Consistently, IL-10 protein level in BALF was also significantly decreased (Fig. 4C). The expression of IL-10 was recovered 7 days after challenge (Fig. 4C), which was correlated to the decrease of both the percentage of IL-17+ AMs and IL-17 level in BALF 7 days after challenge (Fig. 4D). Therefore, the increase of IL-17-producing AMs in allergic asthma may be partially explained by the decrease of IL-10 after the challenge with allergen.

**IL-17 expression is increased in human asthmatic alveolar macrophages**

To interpret the above data in human asthmatics, we further analyzed the expression of IL-17 by alveolar macrophages from patients with allergic asthma. The results showed that the percentage of IL-17+ cells in AMs and the IL-17 level in the BALF from patients with allergic asthma was much higher than those from control subjects (Fig. 5A–C). However, the IL-10 level in the BALF from patients was much lower than that from control subjects (Fig. 5D). We therefore cultured the alveolar macrophages from patients and treated the cells with IL-10 in vitro. The result showed that the expression of IL-17 was down-regulated by IL-10 (Fig. 5E). These data suggested that IL-10 down-regulates IL-17 expression by human alveolar macrophages.

**Discussion**

In the present study, we demonstrate that lung macrophages rather than Th17 or other types of immune cells are the main producer of IL-17 in the OVA-induced allergic inflammation in the lung. Moreover, it is alveolar macrophages rather than interstitial macrophages that are the main source of IL-17 in this situation. Either depleting alveolar macrophages or neutralizing IL-17 in lung effectively inhibits OVA-induced allergic inflammation related to asthma, which is consistent with previous reports on the role of IL-17 in OVA-induced airway hypersensitivity response (12). Enhanced macrophage recruitment during asthma-related processes may result in the accumulation of tissue macrophages, which are activated by Th2 cytokines, leading to Th2 polarized reaction and inflammation (32, 33). Thus, the recruited lung macrophages have the potential to promote asthmatic development. However, the inconsistent results have been obtained from different studies. Several reports suggest that macrophages are crucial for down-regulating the initiation and progression of allergic asthma (34–36). Such regulatory role is partly due to the inhibitory effect of macrophages on Th2 by activating Th1 and producing regulatory factors such as NO, PGE₂, and IL-10 (37). On the other hand, other reports suggest the proinflammatory effects of macrophages, since macrophages can promote eosinophilic airway inflammation (38) and can be stimulated by allergen/IgE immune complexes to produce proinflammatory cytokines (39). The difficulty in elucidating the role of lung macrophages in asthma may mainly be ascribed to the heterogeneity and plasticity of macrophages. Consistent with this, our data in this report show that alveolar macrophages, but not interstitial macrophages, play an important role in allergic inflammation related to asthma. Before and after sensitization, IL-17-producing macrophages are kept at a low percentage in alveolar macrophages, but the challenge with allergen increased the percentage by about 6-fold in alveolar macrophages. In contrast, the challenge with allergen did not increase the percentage of IL-17-producing macrophages in lung interstitial macrophages. Taken
together, we propose that lung macrophages may play different roles in allergic asthma, depending on the microenvironment and the progressive stage of allergic asthma. The final proportion of different macrophage subsets may determine the final onset of allergic asthma.

AMs are the predominant immune effector cells resident in the alveolar spaces and conducting airways (40, 41). AMs have a distinctive phenotype compared with other resident macrophages in the body. They express high levels of mannose receptor and scavenger receptor-A, and constitutively secrete large quantities of lysozyme (32, 42). After stimulation, they produce a range of proinflammatory cytokines (43). Therefore, AMs are considered to be responsible for activating inflammatory responses sufficient to eliminate the pathogens (44). However, the role of AM in asthma is largely unexplored, compared with other airway inflammatory cell types (37). In this study, we found that AM promotes asthma-related inflammation in the mouse model by producing proinflammatory cytokine IL-17. The allergen challenge following the sensitization mainly increased the IL-17-producing macrophages in AM rather than in interstitial macrophages. In line with this, both IL-$^+$ cells in alveolar macrophages and IL-17 level in BALF from patients with asthmatic symptom were much higher than those from control subjects. Taken together, these data suggest that AM is an important population of macrophages responsible for the initiation of asthmatic symptoms.

The present study demonstrated that IL-17 expression by alveolar macrophages from mice can be up-regulated by soluble mediators derived from the activated mast cells. Because mast cells are located in the respiratory tract, alveolar macrophages are easily accessible to mast cell mediators. This may explain why IL-17$^+$ AMs are increased after OVA challenge. Meanwhile, this study also demonstrated that IL-17 expression by alveolar macrophages from mouse and asthmatic patients can be negatively regulated by the Th2 cytokine IL-10, even though other Th2 cytokines are considered as an asthmatic mediator. The decrease of IL-10 expression has been found in asthmatic AM (45–47). Our data further show that IL-10 level was significantly reduced in BALFs from both mice and patients with asthmatic symptom, possibly involving the decrease of IL-10 expression in both AM and Th2 cells. Thus, shifting the balance between IL-10 and mast cell-derived mediators in asthma potentiates the increased production of IL-17 by AMs.

Our findings disclose a new role of alveolar macrophage in the pathogenesis of allergic asthma by producing IL-17, and imply a potential strategy for asthma prevention and treatment by blockade of IL-17. However, asthma is an extremely complex disease, involving both innate and adaptive immune responses. The infiltration of different immune cells results in different pathological processes, depending on the immune and inflammatory microenvironment at the moment. Thus, cell populations playing a dominant role in asthma may be different. In this regard, IL-17 may target different cell types and produce different consequences. For instance, if eosinophils are dominant, IL-17 may be a negative regulator of established allergic asthma (11). However, under neutrophil infiltration condition, IL-17 may play a pro-asthmatic role. Therefore, although IL-17 may represent an interesting therapeutic target in asthma, the types of asthma involving different cell populations must be taken into consideration.

Disclosures

The authors have no financial conflict of interest.