

# Interleukin-17 Orchestrates the Granulocyte Influx into Airways after Allergen Inhalation in a Mouse Model of Allergic Asthma

Peter W. Hellings, Ahmad Kasran, Zhanju Liu, Philippe Vandekerckhove, Anja Wuyts, Lutgart Overbergh, Chantal Mathieu, and Jan L. Ceuppens

Laboratory of Experimental Immunology, Department of Otorhinolaryngology-Head and Neck Surgery, Laboratory of Hematology, and Laboratory of Molecular Immunology, Rega Institute, Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Department of Internal Medicine, University Hospitals, Faculty of Medicine, University of Leuven, Leuven, Belgium

Interleukin (IL)-17 is produced by activated memory CD4<sup>+</sup> cells and induces cytokines and chemokines that stimulate neutrophil generation and recruitment. Here, we investigated the involvement of IL-17 in the bronchial influx of neutrophils in experimental allergic asthma. Inhalation of nebulized ovalbumin (OVA) by sensitized mice with bronchial eosinophilic inflammation resulting from chronic OVA exposure induced early IL-17 mRNA expression in inflamed lung tissue, concomitant with a prominent bronchial neutrophilic influx. Anti-IL-17 monoclonal antibodies (mAb) injected before allergen inhalation strongly reduced bronchial neutrophilic influx, in a manner equally as potent as the anti-inflammatory dexamethasone. Remarkably, anti-IL-17 mAb significantly enhanced IL-5 levels in both BAL fluid and serum, and aggravated allergen-induced bronchial eosinophilia. In another series of experiments, anti-IL-17 mAb were given repeatedly during the inhalatory challenge phase with OVA of sensitized mice. This treatment regimen abated bronchial neutrophilia in parallel with reduction of bone marrow and blood neutrophilia. In addition, anti-IL-17 mAb treatment elevated eosinophil counts in the bone marrow and bronchial IL-5 production, without alteration of allergen-induced bronchial hyperresponsiveness. In summary, our results demonstrate that IL-17 expression in airways is upregulated upon allergen inhalation, and constitutes the link between allergen-induced T cell activation and neutrophilic influx. Because neutrophils may be important in airway remodeling in chronic severe asthma, targeting IL-17 may hold therapeutic potential in human asthma.

In allergic asthma, inhalation of allergens leads to an inflammatory cascade in which T lymphocytes of the CD4<sup>+</sup> subset play a central role. Activated CD4<sup>+</sup> cells release so-called T helper 2 (Th2) cytokines that contribute substantially to the classic triad of allergic asthma, i.e., allergen-specific

IgE production, bronchial hyperresponsiveness (BHR), and eosinophilic inflammation (1). Besides eosinophils, recent evidence is emerging for neutrophil participation in different features of human asthma: airway gland hypersecretion, BHR, and airway wall remodeling (2). Indeed, bronchial neutrophilia has been reported in severe asthma attacks (3–6), and after bronchial allergen challenge in patients with asthma (7). Experimental data in a rat model of allergic asthma suggest that T lymphocytes are essential for the induction of bronchial neutrophilia (8). However, factors responsible for T lymphocyte-mediated bronchial neutrophilic influx remain to be identified. We here hypothesized that interleukin (IL)-17, a T cell-derived cytokine whose biologic functions are beginning to be elucidated (9–11), may represent the link between activated CD4<sup>+</sup> cells and bronchial neutrophilic influx in allergic asthma.

IL-17 is a 20- to 30-kD protein that is primarily secreted by memory CD4<sup>+</sup> T cells and that shares homology with a protein encoded by the open reading frame 13 of *Herpesvirus saimiri* (12). Neither IL-17 nor its receptor have sequence similarity with any known cytokine or cytokine receptor (11). Due to the ubiquitous distribution of its receptor (11), IL-17 exerts pleiotropic biologic activities. Ligation of its receptor initiates the transcription of nuclear factor- $\kappa$ B (NF- $\kappa$ B) (13) and c-Jun NH<sub>2</sub>-terminal kinase (14) via tumor necrosis factor (TNF) receptor-associated factor 6 (15). Recent reports illustrate that IL-17 plays a pivotal proinflammatory role in several inflammatory responses like experimental autoimmune neuritis (16), organ allograft rejection (17), tumorigenicity of cervical tumors (18), and bacterial pneumonia (19). In patients, expression of IL-17 is found in inflamed sites of rheumatoid arthritis (20, 21), multiple sclerosis (22), psoriasis (23), allergic contact dermatitis (24), and allergic asthma (2).

Increasing evidence suggests that IL-17, acting either directly or indirectly, significantly stimulates neutrophil maturation, migration, and function. Overexpression of IL-17 results in massive peripheral neutrophilia associated with increased levels of granulocyte colony-stimulating factor (G-CSF) and enhanced granulopoiesis (25). Alternatively, impaired IL-17 receptor signaling leads to delayed bronchial recruitment of neutrophils and impaired clearance of *Klebsiella pneumoniae* infection (19). In several cell systems, IL-17 induces the release of C-X-C chemokines (10, 24, 26) and upregulates interferon (IFN)- $\gamma$ -mediated ICAM-1 expression (23, 24). These proinflammatory effects of IL-17 are further potentiated by the induction of TNF- $\alpha$  and IL-1

(Received in original form February 4, 2002 and in revised form July 25, 2002)

Address correspondence to: Jan Ceuppens, Laboratory of Experimental Immunology, Onderwijs en Navorsing, U.Z. Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium. E-mail: Jan.Ceuppens@med.kuleuven.ac.be

Abbreviations: bronchoalveolar lavage, BAL; bronchial hyperresponsiveness, BHR; bone marrow, BM; enzyme-linked immunosorbent assay, ELISA; eosinophil peroxidase, EPO; experimental units, EU; granulocyte macrophage colony-stimulating factor, G-CSF; hematoxylin and eosin, H&E; interferon- $\gamma$ , IFN- $\gamma$ ; interleukin, IL; monoclonal antibodies, mAb; methacholine, MCh; May-Grünwald-Giemsa, MGG; myeloperoxidase, MPO; ovalbumin, OVA; enhanced pause, Penh; T helper 2, Th2; tumor necrosis factor, TNF.

Am. J. Respir. Cell Mol. Biol. Vol. 28, pp. 42–50, 2003

DOI: 10.1165/rcmb.4832

Internet address: www.atsjournals.org

(27), which are involved in upregulating cellular adhesion molecules and hence migration of inflammatory cells (28). In addition to favoring the recruitment of neutrophils, IL-17 has been reported to activate neutrophils within airways (29). Whether increased immunoreactivity for IL-17 in asthmatic bronchi (2) reflects allergen-induced stimulation of IL-17 secretion, and/or the release of biologically active IL-17, remains hypothetical. Therefore, we studied the relevance of IL-17 in a mouse model of allergic asthma. The mouse model we use here is characterized by production of allergen-specific IgE and induction of nonspecific BHR and bronchial eosinophilic inflammation (30, 31). To mimic allergen exposure of patients with asthma and chronically inflamed airways, we extended the routinely used biphasic protocol for induction of experimental allergic asthma, i.e., allergen sensitization and airway exposure, with an acute provocation phase. This provocation is performed by exposure of BALB/c mice to nebulized allergen and induces bronchial IL-17 expression and an early neutrophilic influx in airways. In the first part of this study, we investigated the effects of neutralization of IL-17 by administration of anti-IL-17 monoclonal antibodies (mAb), before provocation, on the inflammatory response following allergen provocation. Second, we investigated the effects of prolonged neutralization of IL-17 on bronchial inflammation, cytokine production, and bone marrow (BM) biology by administration of anti-IL-17 mAb during the inhalatory challenge phase of sensitized mice.

## Materials and Methods

### mAbs and Reagents

Ovalbumin (OVA; grade V), bovine serum albumin (BSA), o-dianisidine dihydrochloride, o-phenylenediamine, and metacholine (Mch) were purchased from Sigma (St Louis, MO). Neutralizing mAb against IL-17 (50,104.11, rat IgG) and recombinant mouse IL-17 were purchased from R&D Systems (Minneapolis, MN). Control rat IgG was purchased from Rockland (Gilbertsville, PA). Türk's solution was purchased from Merck Diagnostica (Darmstadt, Germany) and RPMI 1640 was purchased from BioWhittaker (Walkersville, MD). Enzyme-linked immunosorbent assay (ELISA) reagents for mouse IL-5 and IFN- $\gamma$  quantification were purchased from PharMingen (San Diego, CA). An ELISA kit from Biosource International (Fleurus, Belgium) was used for measurement of IL-4 levels.

### Establishment of a Chronic Asthma Model and Anti-IL-17 mAb Treatment

Male BALB/c mice (Harlan, Horst, The Netherlands) were kept under conventional pathogen-free conditions and were actively sensitized by seven intraperitoneal injections of 10  $\mu$ g OVA in 0.5 ml of pyrogen-free saline on alternate days from Days 1–13 as described previously (30) (Figure 1). Mice were then exposed daily for 5 min to nebulized OVA (10 mg/ml; PARI TurboBOY, Starnberg, Germany) or saline from Days 33–40. On Day 41, eosinophilic airway inflammation was found with typical features reminiscent of human allergic asthma (30). After an allergen-free interval, an acute allergen provocation was performed on Day 50 by inhalation of nebulized OVA (200 mg/ml) or saline for 10 min. To study the early influx of white blood cells after acute allergen exposure, groups of 12 mice were killed before and at 3, 6, and 12 h after allergen inhalation. As neutrophilic influx was maximal at 3 h after provocation, this time-point was chosen for further analyses. Neutralizing mAb against IL-17 or control rat IgG were injected intraperitoneally at a dosage of 50  $\mu$ g 30 min before allergen provocation ( $n = 12$  per group). To compare the effects of blocking the proinflammatory cytokine IL-17 with the effects of the anti-inflammatory dexamethasone, we injected dexamethasone (3 mg/kg) or an equal volume of saline intraperitoneally 30 min before the allergen inhalation on Day 50 in another group of mice ( $n = 6$  per group).

In addition to single neutralization of IL-17 before allergen provocation, we investigated the effects of prolonged neutralization of IL-17 during the development of allergic airway inflammation in a second series of experiments. To this purpose, anti-IL-17 mAb or control rat IgG were injected (50  $\mu$ g intraperitoneally) from Day 32 on alternate days during the OVA inhalation phase (Days 33–40,  $n = 6$  per group; Figure 1). Mice were killed on Day 41, i.e., 24 h after the eighth inhalation of OVA. Experimental procedures were approved by the local Ethical Committee of Animal Experiments.

### Evaluation of Eosinophilia in Peripheral Blood, Bronchoalveolar Lavage Fluid, and Bone Marrow

Mice were anaesthetized with intraperitoneal injection of urethane (2.1 g/kg) at 3 h after allergen provocation (Day 50) or at 24 h after chronic allergen inhalation (Day 41). After retro-orbital bleed, blood smears were stained with the classic May-Grünwald-Giemsa (MGG) stain, and differential cell counts were performed. The lungs were lavaged five times with 1 ml of phosphate-buffered saline (PBS) supplemented with BSA 5% through a tracheal polyethylene catheter (inner diameter 0.85 mm). The first lavage was centrifuged at  $1400 \times g$  for 5 min and the supernatant stored at  $-20^{\circ}\text{C}$  until measurement of cytokines. The pellet was added to the subsequent four lavages. After centrifugation ( $1400 \times g$ , 5 min),

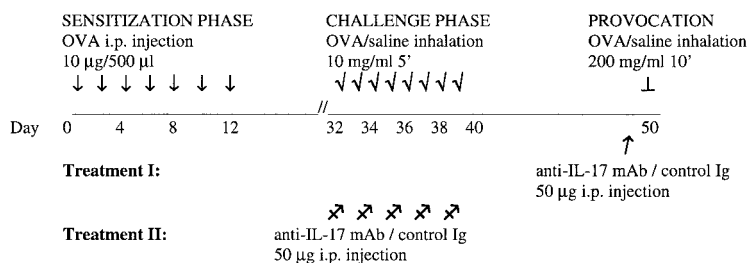


Figure 1. Experimental protocol for induction of experimental asthma. First, all BALB/c mice were sensitized to OVA by repeated injections of OVA (10  $\mu$ g/500  $\mu$ l, intraperitoneally) on alternate days from Days 1–13. Secondly, sensitized mice were repeatedly exposed to nebulized OVA (10 mg/ml) or saline for 5 min daily from Days 33–40. Finally, an acute provocation was performed by exposure of mice to nebulized OVA (200 mg/ml) or saline for 10 min on Day 50. In addition to induction of allergic airway inflammation, mice ( $n = 12$  per group) were treated with anti-IL-17 mAb or control Ig (50  $\mu$ g, intraperitoneally) 30

min before provocation on Day 50 and killed at 3 h after provocation. In a second series of experiments, anti-IL-17 or control Ig were injected on alternate days from Days 32–40 ( $n = 6$  per group), i.e., during the induction of allergic inflammation, and killed at 24 h after the eighth inhalatory OVA challenge.

cells were washed and resuspended in 200  $\mu$ l of PBS. For cell counting, 10  $\mu$ l of cell suspension were added to 90  $\mu$ l of Türk's solution and the number of cells was calculated in a Bürker chamber. After dissection of one femur, BM was prelevated by flushing the femoral shaft with RPMI 1640, and smears were made. Differential cell counts were performed on MGG-stained smears of peripheral blood and BM, and cytospin preparations of bronchoalveolar lavage (BAL) fluid cells.

### Histologic Analysis

After performing BAL, lungs were dissected and fixed overnight in buffered formalin (5%). After dehydration and embedding in paraffin, 5- $\mu$ m sections were stained with the classic hematoxylin and eosin (H&E). On H&E-stained sections, eosinophils could be easily recognized by their polyglobular nucleus and bright cytoplasmic granules. For assessment of neutrophilic influx, a classic myeloperoxidase (MPO) stain was performed as described previously (32). The maximal thickness of the inflammatory infiltrates around two bronchioli and two arterioli in the lung were measured using an eyepiece graticule at a magnification of  $\times 25$ , and the average of these four values was calculated for each mouse.

### Measurement of IL-5, IFN- $\gamma$ , and IL-4 Levels

IL-5, IL-4, and IFN- $\gamma$  levels in BAL fluid (1:2 diluted) and serum (undiluted) were measured by sandwich ELISA using paired matched Ab according to the manufacturer's instructions. The sensitivity of these assays was 4.5, 2.0, and 1.2 pg/ml for IL-5, IL-4, and IFN- $\gamma$ , respectively.

### MPO and Eosinophil Peroxidase Assay

MPO activity in BAL fluid was measured using a method described by Bradley and coworkers (33). In brief, 100  $\mu$ L of undiluted BAL fluid were incubated for 15 min with 2.9 ml of PBS containing 0.0005% of H<sub>2</sub>O<sub>2</sub> and 0.167 mg/ml of o-dianisidine dihydrochloride at pH 6.0. The reaction was stopped by adding 100  $\mu$ L NaN<sub>3</sub> 1% and the optical density was measured at a wavelength of 460 nm. Eosinophil peroxidase (EPO) activity in BAL fluid was assessed by incubation of undiluted BAL fluid (100  $\mu$ l) with Tris-HCl buffer (50  $\mu$ l) for 10 min. Then, 100  $\mu$ l of substrate solution, containing 2 mg/ml of o-phenylenediamine and 1.3  $\mu$ l/ml of H<sub>2</sub>O<sub>2</sub> in Tris-HCl buffer, were added. The reaction was stopped by adding H<sub>2</sub>SO<sub>4</sub>, and the optical density was measured at 492 nm.

### RT-PCR for IL-17 and Other Cytokines

Part of the left lung was dissected at 3 h after acute allergen provocation, immediately frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$ . Total RNA was extracted using TRIzol (Life Technologies, Gaithersburg, MD). A constant amount of 1  $\mu$ g of target RNA was reverse transcribed using 100 U Superscript II RT (Life Technologies) at  $42^{\circ}\text{C}$  for 80 min in the presence of 5  $\mu$ M Oligo(dT)<sub>16</sub>. Real-time quantitative RT-PCR was performed for IL-17, IL-4, IL-5, IL-10, IL-13, IFN- $\gamma$ , IL-12 p40, and IL-1 $\beta$  mRNA in the ABI prism 7,700 Sequence detector (Applied Biosystems, Foster City, CA) as described before (34). The primer and probe for mouse IL-17 were designed based on the published sequence (35), and are as follows: IL-17 forward primer, 5' GCTCCAGAAGGCCCT CAGA 3'; IL-17 reverse primer, 5' AGCTTTCCTCCGCAT TGA 3'; IL-17 Taqman probe, 5' FAM-CTCTCCACCGCAAT GAAGACCCTGA-TAMRA 3'. The amplified PCR-fragment is 142 bp in length, located from bp 229 to 370. The primer and probe sequences used for IL-4, IL-5, IL-10, IL-13, IFN- $\gamma$ , IL-12, and IL-1 $\beta$  were as previously published (34). Each PCR amplification was performed in triplicate wells, using the following condi-

tions: 10 min at  $94^{\circ}\text{C}$ , followed by a total of 40 or 45 two-temperature cycles (15 s at  $94^{\circ}\text{C}$  and 1 min at  $60^{\circ}\text{C}$ ).

### Measurements of Nonspecific BHR

On Day 41, bronchial responsiveness to inhaled metacholine (Mch) was measured using whole body plethysmography (Buxco; EMKA Technologies, Paris, France) as described previously (30). In brief, mice that had been treated with either control Ig or anti-IL-17 mAb during the phase of challenges with nebulized OVA (Days 33–40), were exposed to incremental doses of nebulized Mch (0, 5, 10, 20, and 50 mg/ml) for 1 min, and mean Penh values were recorded during 3 min after each dose of Mch.

### Data Analyses

Data are expressed as means  $\pm$  SEM. Statistical analyses were performed using the Mann-Whitney test. A difference was considered to be significant when  $P < 0.05$ .

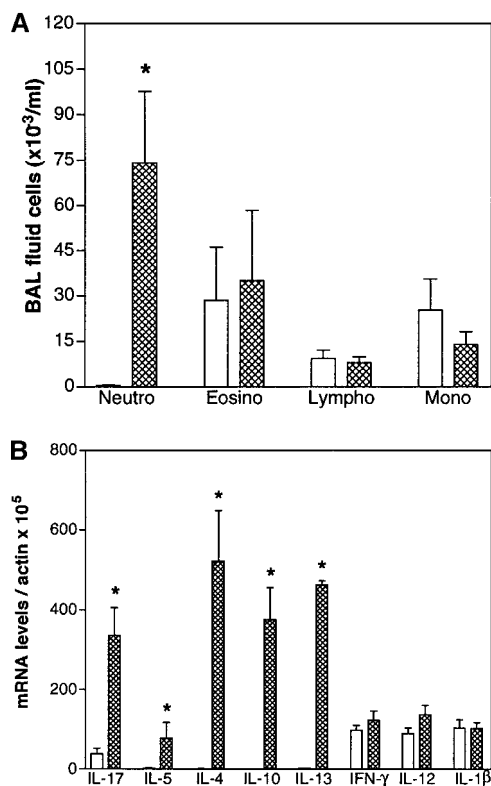
## Results

### Allergen Provocation of Mice with Eosinophilic Airway Inflammation Induces Bronchial Neutrophilic Influx and IL-17 Expression

All mice in this study were sensitized by i.p. injection of OVA (Days 1–13) and then exposed daily (Days 33–40) to aerosols containing OVA or saline as described previously (30) (Figure 1). In this way, peribronchial eosinophilic inflammation and airway hyperresponsiveness were induced in OVA-challenged mice (30). After an allergen-free interval of 10 d, during which (peri-)bronchial inflammation partially resided, an acute allergen provocation was performed by means of inhalation of nebulized OVA (200 mg/ml) or saline for 10 min. At 3 h after provocation with OVA, bronchial cellular influx was maximal and constituted primarily of neutrophils (51.2% of cells in BAL fluid; Figure 2A). As neutrophils were hardly present before provocation ( $0.4 \pm 0.1 \times 10^3$ /ml of BAL fluid), their prominent influx in the bronchoalveolar lumen at 3 h after provocation with OVA ( $74.3 \pm 23.6 \times 10^3$ /ml of BAL fluid,  $P < 0.001$ ; Figure 2A) was remarkable. In contrast to the significant neutrophilic influx, the numbers of eosinophils and lymphocytes were not altered by allergen provocation as early as 3 h after provocation (Figure 1A). It was only at 12 and 24 h after provocation with OVA that a significant increase in bronchial eosinophil and lymphocyte counts was observed (data not shown).

Figure 2B illustrates that IL-17 mRNA levels in lung tissue were significantly increased by allergen provocation on Day 50 ( $P < 0.005$ ). Furthermore, mRNA of Th2-type cytokines, i.e., IL-5, IL-4, IL-10, and IL-13, were also induced in inflamed airways after allergen provocation (Figure 1B). It is interesting to notice that allergen inhalation did not affect mRNA levels of IFN- $\gamma$ , IL-12 p40, or IL-1 $\beta$  (Figure 2B). Provocation with nebulized saline on Day 50 did not induce any cellular influx nor mRNA for IL-17 or Th2 cytokines in lung tissue (data not shown).

The OVA-mediated nature of the observed bronchial neutrophilic influx and IL-17 mRNA induction after provocation with OVA of mice with experimental asthma was illustrated by extensive pilot studies. Negligible bronchial neutrophilic influx and minute amounts of IL-17 mRNA



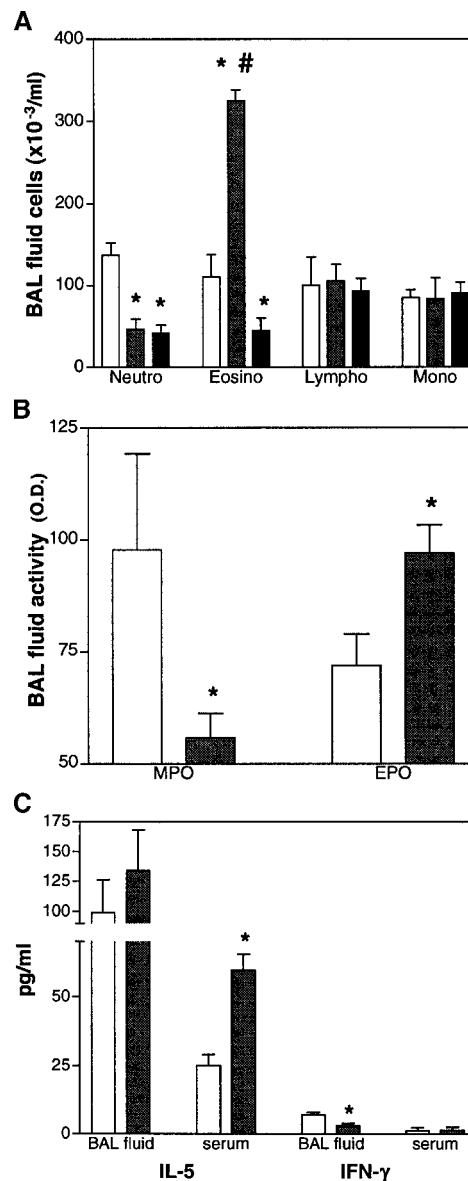
**Figure 2.** Bronchial responses in mice after acute allergen provocation. Mice were first sensitized (Days 1–13) and chronically exposed to nebulized OVA (Days 33–40). On Day 50, an acute provocation was performed by inhalation of nebulized OVA. (A) Cell counts on MGG-stained cytospin preparations of BAL fluid of mice killed before (*open bars*) and at 3 h after provocation (*filled bars*). Neutrophils (Neutro), eosinophils (Eosino), lymphocytes (Lympho), and monocytes (Mono) were differentiated. (B) mRNA levels for IL-17, IL-4, IL-5, IL-10, IL-13, IL-12 p40, IFN- $\gamma$ , and IL-1 $\beta$  were measured in lung tissue using real time quantitative RT-PCR before (*open bars*) and at 3 h after provocation (*filled bars*). Data were expressed as means  $\pm$  SEM for each group ( $n = 12$ ). \* $P < 0.005$  versus mice killed before provocation.

were induced by OVA inhalation (200 mg/ml for 10 min) in nonsensitized mice, as well as after inhalation of nebulized BSA (200 mg/ml for 10 min) by mice with OVA-induced eosinophilic airway inflammation (data not shown).

To exclude a major contribution of endotoxins contaminating the OVA solution to the bronchial neutrophilic influx and IL-17 mRNA induction, experiments were performed in which sensitized and OVA-challenged mice were exposed on Day 50 to nebulized LPS for 10 min at 5  $\mu$ g/ml, i.e., the concentration of endotoxins present in an OVA solution of 200 mg/ml (measured with the Limulus Amebocyte Lysate test from BioWhittaker, Walkersville, MD). Exposure to this concentration of nebulized endotoxin did not induce neutrophilic influx nor IL-17 mRNA in the airways (data not shown).

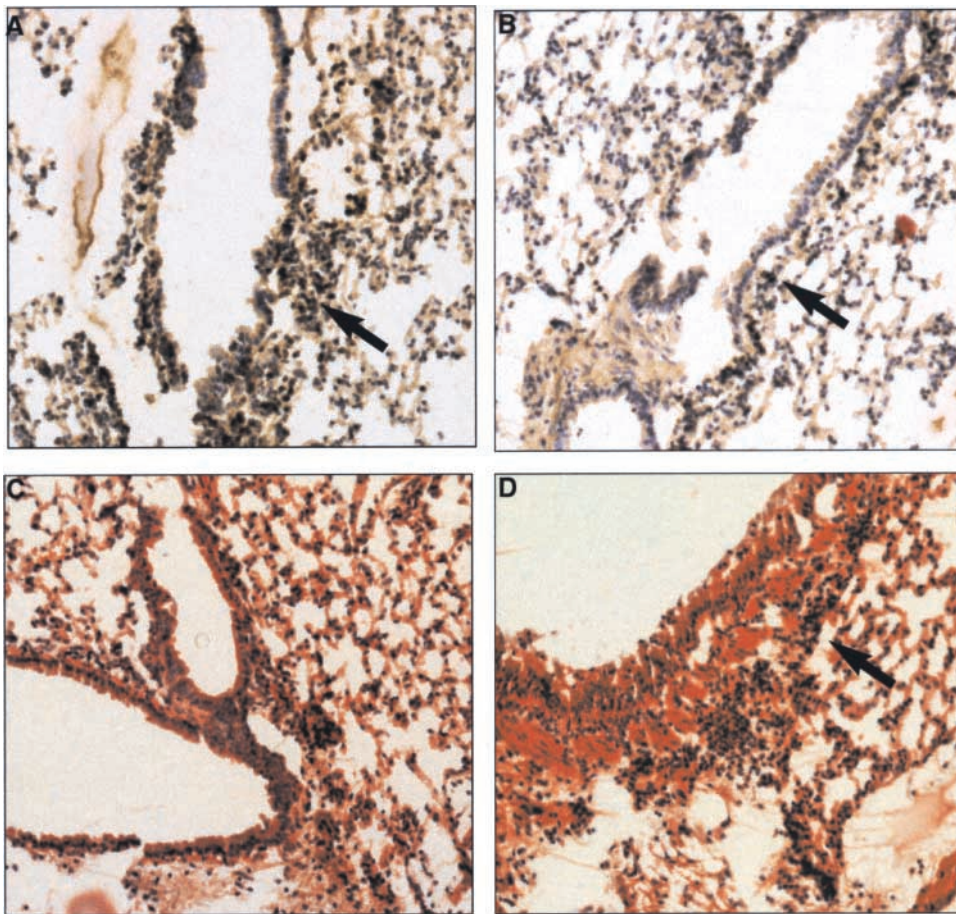
**Effect of Anti-IL-17 mAb Administration before Acute Provocation on Bronchial Responses after Provocation**

When anti-IL-17 mAb were injected intraperitoneally 30 min before OVA provocation on Day 50, this treatment



**Figure 3.** Effects of anti-IL-17 therapy on bronchial inflammation after OVA provocation. Mice that had been sensitized (Days 1–13) and challenged with OVA (Days 33–40) were injected with control Ig (*open bars*), anti-IL-17 mAb (*gray bars*), or dexamethasone (*black bars*) 30 min before inhalation of nebulized OVA on Day 50 and killed at 3 h after provocation. (A) Neutrophils (Neutro), eosinophils (Eosino), lymphocytes (Lympho), and monocytes (Mono) were counted on MGG-stained cytospin preparations of BAL fluid. (B) The activity of MPO and EPO were measured in BAL fluid of both groups of mice. (C) Levels of IL-5 and IFN- $\gamma$  were measured in BAL fluid and serum using sandwich ELISA in both groups. \* $P < 0.05$  compared with control mice ( $n = 10$ –12 mice/group); one representative experiment out of two is shown.

markedly altered the bronchial influx of granulocytes following allergen provocation. Neutrophilic influx into the bronchial lumen was significantly reduced by anti-IL-17 mAb compared with the control ( $P < 0.05$ ; Figure 3A). Interestingly, anti-IL-17 mAb inhibited the bronchial neu-



**Figure 4.** Histologic sections of inflamed bronchi. Mice that had been sensitized (Days 1–13) and challenged with OVA (Days 33–40) were acutely exposed to nebulized OVA on Day 50. Lungs were removed for histologic examination of bronchi at 3 h after provocation. Bronchial sections of control Ig (A and C) and anti-IL-17-treated mice (B and D) were stained with the classic MPO stain (A and B) and H&E stain (C and D), for visualization of bronchial subepithelial neutrophils (A and B) and eosinophils (C and D) respectively, as indicated with *black arrows*.

trophilic influx in a manner equally as potent as did dexamethasone therapy ( $46.3 \pm 12.9$  and  $40.1 \pm 10.1 \times 10^4/\text{ml}$  of BAL fluid, respectively; Figure 3A). In line with the observation of reduction of bronchial neutrophilic influx by anti-IL-17 mAb, we found less MPO activity in BAL fluid ( $55.8 \pm 5.5$  versus  $97.8 \pm 21.4$  OD; Figure 3B) and smaller MPO-positive infiltrates in bronchial tissue ( $25.3 \pm 5.2 \times 10^{-2}$  versus  $75.3 \pm 7.9 \times 10^{-2}$  mm,  $P < 0.05$ ; Figures 4A and 4B). Also in the circulation, percentages of neutrophils in the white cell population were reduced after administration of anti-IL-17 mAb ( $44.4 \pm 1.2\%$  versus  $50.2 \pm 1.3\%$ ,  $P < 0.05$ ).

To our surprise, OVA-induced eosinophilic airway influx was enhanced by anti-IL-17 mAb ( $P < 0.05$ ; Figure 3A). This observation was consistent with elevated EPO activity in BAL fluid ( $P < 0.05$ ; Figure 3B) and thicker peribronchial eosinophilic infiltrates in anti-IL-17-treated mice compared with controls ( $69.3 \pm 9.3 \times 10^{-2}$  versus  $28.3 \pm 7.3 \times 10^{-2}$  mm,  $P < 0.05$ ; Figures 4C and 4D). Anti-IL-17 mAb did not affect blood eosinophilia following provocation (data not shown).

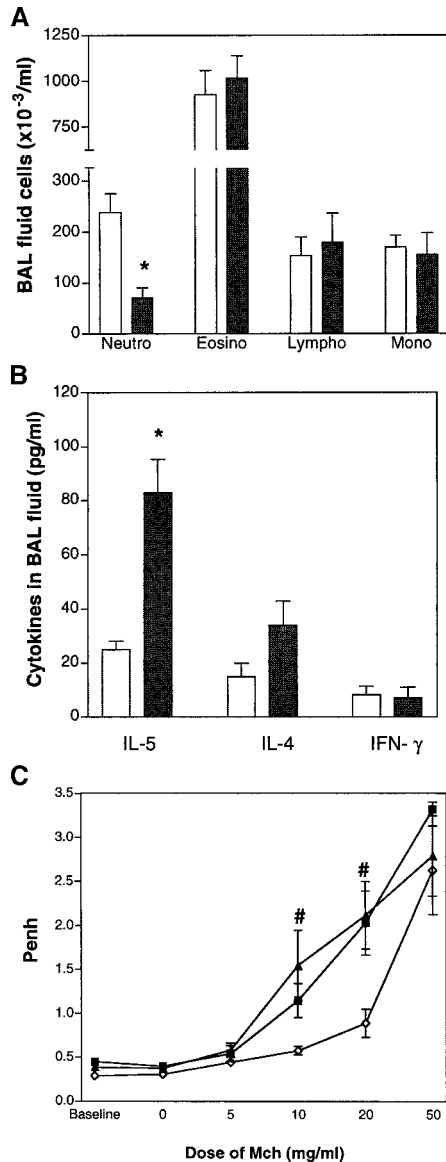
#### Effect of Anti-IL-17 mAb on IL-5 Production *In Vivo*

To explain the remarkable effect of neutralizing IL-17 on bronchial eosinophilia after OVA provocation on Day 50, IL-5 levels were measured. Levels of circulating IL-5 were elevated at 3 h after OVA provocation in anti-IL-17-

treated mice compared with controls ( $59.7 \pm 5.8$  versus  $25.0 \pm 4.1$  pg/ml, respectively,  $P < 0.05$ ; Figure 3C). Also in BAL fluid, higher levels of IL-5 were found in mice injected with anti-IL-17 mAb before OVA provocation, without reaching levels of significance ( $P > 0.05$ ; Figure 3C). As far as IL-4 production is concerned, anti-IL-17 mAb enhanced IL-4 levels in serum ( $19.8 \pm 4.1$  versus  $12.3 \pm 2.1$  pg/ml) and BAL fluid ( $42.2 \pm 5.0$  versus  $32.4 \pm 2.4$  pg/ml) in a nonsignificant way ( $P > 0.05$ ). In contrast to Th2 cytokines, IFN- $\gamma$  levels in BAL fluid were reduced by anti-IL-17 mAb ( $2.9 \pm 1.1$  versus  $7.0 \pm 1.3$  pg/ml,  $P < 0.05$ ) and systemic levels of IFN- $\gamma$  were under the detection limit (1.2 pg/ml) in both groups of mice. In OVA-sensitized and OVA-challenged mice, exposure to nebulized saline on Day 50 did not induce detectable production of IL-4, IL-5, and IFN- $\gamma$  in BAL fluid, nor in serum of either anti-IL-17 mAb or control mice (data not shown).

#### Effects of Prolonged Neutralization of IL-17 during Repeated Allergen Inhalation on the Manifestation of Allergic Airway Inflammation

In addition to the effects of acute neutralization of IL-17 before allergen provocation, we studied the effects of prolonged neutralization of IL-17 on bronchial inflammation and BM biology. To this purpose, anti-IL-17 mAb or control Ig were administered to sensitized mice on alternate



**Figure 5.** Effects of repeated anti-IL-17 mAb administration during the challenge phase on the development of allergic airway inflammation. Mice that had been sensitized to OVA (Days 1–13) were treated with anti-IL-17 mAb (filled bars) or control IgG (open bars) before and during the phase of repeated inhalations of nebulized OVA (Days 33–40). Mice were killed on Day 41 and differential cell counts performed on MGG-stained cytospin preparations of BAL fluid (A). Protein levels of IL-5, IL-4, and IFN- $\gamma$  were measured in BAL fluid with sandwich ELISA (B). Using whole body plethysmography, airway responsiveness to incremental doses of MCh was evaluated by measuring Penh values (C) in sensitized mice that had inhaled nebulized OVA or saline (Sal). \* $P < 0.05$  versus control; # $P < 0.05$  versus Sal;  $n = 6$ –12 mice/group; one representative experiment out of two is shown.

days from Days 32–40, i.e., during the inhalatory challenge phase with OVA or saline (Days 33–40, Figure 1). On Day 41, less neutrophils were found in BAL fluid of OVA-challenged mice treated with anti-IL-17 mAb (Figure 5A), whereas the number of eosinophils was equally high in both

groups (Figure 5A). Neutralization of IL-17 led to higher production of IL-5 ( $83.1 \pm 12.3$  versus  $25.0 \pm 3.2$  pg/ml,  $P < 0.05$ ; Figure 5B) and to a lesser extent of IL-4 in inflamed bronchi ( $34.1 \pm 9.0$  versus  $15.0 \pm 5.3$  pg/ml,  $P < 0.05$ ; Figure 5B). IFN- $\gamma$  levels in BAL fluid were low and not affected by anti-IL-17 mAb (Figure 5B). Serum levels of IL-5, IL-4, and IFN- $\gamma$  were below detection limit at the time of analysis (data not shown) and bronchial responsiveness to inhaled Mch was not affected by chronic administration of anti-IL-17 mAb during the inhalatory challenge phase (Figure 5C). In bronchi of saline-challenged mice treated with anti-IL-17 mAb or control Ig, no inflammation was present and no cytokine level was above the detection limit (data not shown).

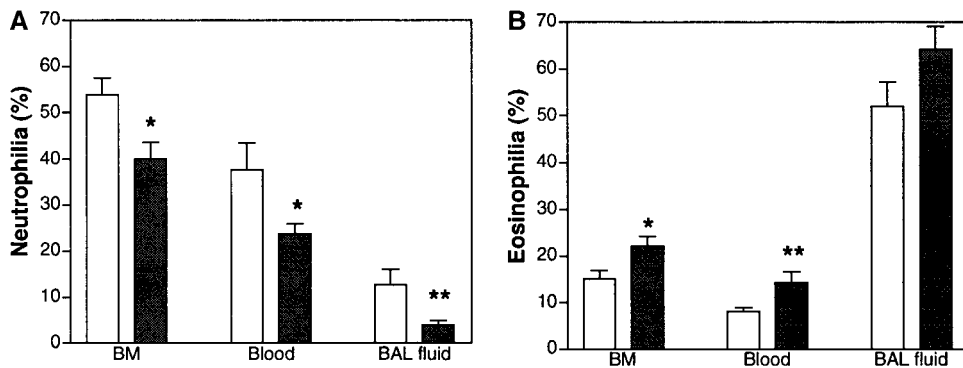
To explain the effects of chronic anti-IL-17 mAb on granulocyte biology, we analyzed granulocyte counts in different compartments of mice killed on Day 41. BM analysis showed that repeated administration of anti-IL-17 mAb significantly decreased the percentage of neutrophils in BM (Figure 6A). Reduced neutrophil counts in BM corresponded with lower neutrophilia in blood and in BAL fluid (Figure 6A). In contrast, eosinophil percentages (Figure 6B) were significantly enhanced in the BM and peripheral blood of mice given anti-IL-17 mAb (Figure 6B), whereas eosinophil percentages in BAL fluid were equally high in both groups (Figure 6B).

## Discussion

The purpose of this study was to investigate the role of IL-17 in the induction of CD4<sup>+</sup> cell-dependent bronchial neutrophilic influx in a mouse model of allergic asthma. We demonstrated that IL-17 mRNA expression is upregulated in inflamed bronchi after allergen inhalation, and that IL-17 differentially regulates the allergen-induced bronchial influx of granulocytes, both after single as well as after repeated allergen inhalation by sensitized mice. Anti-IL-17 mAb administered before allergen provocation abrogated the bronchial neutrophilic influx in a manner equally as potent as did dexamethasone. At the same time, anti-IL-17 mAb enhanced bronchial IL-5 production and eosinophilic airway inflammation. In addition, repeated administration of anti-IL-17 mAb during the inhalatory challenge phase decreased the bronchial neutrophilia and neutrophil numbers in the BM, whereas the opposite trend was found for eosinophils.

We here report for the first time upregulation of IL-17 mRNA in inflamed airways after allergen inhalation. The allergen-specific nature of this observation has been investigated in pilot studies. To exclude a major contribution of endotoxins to the observed IL-17 mRNA induction and bronchial neutrophilic influx after allergen provocation, OVA-sensitized and -challenged mice were exposed on Day 50 to nebulized endotoxins, at a concentration present in the OVA solution used for provocation (*see RESULTS*). As this exposure did not induce any IL-17 mRNA production or bronchial neutrophilia, the observed responses following the OVA provocation are likely allergen-mediated.

Activated T lymphocytes have been regarded to be the principal source of IL-17 (10, 36). Our finding of rapid induction of mRNA for IL-17 after allergen provocation suggests the presence of an IL-17-producing memory T cell



**Figure 6.** Effects of chronic anti-IL-17 therapy on granulocyte biology in different compartments. Mice that had been sensitized to OVA (Days 1–13) were treated with anti-IL-17 mAb (filled bars) or control IgG (open bars) before and during the phase of repeated inhalations of nebulized OVA (Days 33–40). Mice were killed on Day 41 and percentages of neutrophils (A) and eosinophils (B) were counted in BM, peripheral blood, and BAL fluid. \* $P < 0.05$  and \*\* $P < 0.005$  versus control ( $n = 6$  per group); one representative experiment out of two is shown.

population in inflamed airways. Flow cytometric analysis of CD4<sup>+</sup> lymphocytes obtained from peribronchial lymph nodes of mice with experimental allergic asthma for intracellular IL-17 showed that these CD4<sup>+</sup> cells represent a significant source of IL-17 (unpublished observation). From existing literature, IL-17 production does not segregate into a distinct subset of CD4<sup>+</sup> cells, as IL-17-producing T cell clones belong to the Th1 phenotype in rheumatoid arthritis (37), and both Th1 and Th2 phenotypes after priming in the presence of *Borrelia burgdorferi* (38) and in allergic contact dermatitis (39). Here, the simultaneous induction of mRNA for IL-17 and Th2, and not Th1 cytokines, suggests that activated Th2 lymphocytes are involved in IL-17 secretion. In addition to T cells, eosinophils in bronchial mucosa of patients with asthma have recently been reported to express IL-17 (2), suggestive of a multicellular origin of IL-17 in allergic airway inflammation.

After demonstration of enhanced expression of IL-17 by OVA inhalation, we studied the functional relevance of IL-17 in experimental allergic asthma *in vivo*. We first demonstrated that neutralization of IL-17 before acute allergen provocation severely impairs the neutrophilic influx into inflamed airways following allergen inhalation. Therefore, these data illustrate that IL-17 secretion is primarily involved in the allergen-mediated bronchial neutrophilic influx. Previously, Laan and coworkers (26) reported that IL-17 is capable of inducing neutrophil recruitment toward the airways. The study by Ye and colleagues (19) and our study are the first to show that endogenous IL-17 is critical for lung polymorphonuclear cell recruitment in response to a gram-negative pathogen and an allergen, respectively. Taken together, IL-17 plays a pivotal role in the neutrophil migration toward inflamed airways.

Several mechanisms of action may underlie our observation of inhibition of neutrophilic recruitment by anti-IL-17 mAb. First of all, we demonstrated that absence of functional IL-17 resulted in lower neutrophil counts in the BM and less circulating neutrophils, suggestive of reduced allergen-mediated stimulation of neutropoiesis. This sequence of events may result from less IL-17-mediated production of G-CSF and stem cell factor in anti-IL-17 mAb-treated mice (11, 25, 40) and highlights the key role of IL-17 in

regulating stress-induced neutropoiesis. As BM responses in mice can occur as early as 2 h after mucosal allergen encounter (41), one could assume that modulation of BM biology by anti-IL-17 mAb may have an impact on the early bronchial cellular infiltrate after allergen provocation. In addition, anti-IL-17 mAb may have impaired the migration of neutrophils toward bronchi due to lower production of chemokines like KC (42), macrophage inflammatory protein-2 (19, 26), and/or granulocyte chemotactic protein-2 (GCP-2), a C-X-C chemokine that is the most potent murine neutrophil-chemoattractive protein (43) and is nearly as effective as human IL-8 in attracting neutrophils (44). We could not, however, detect any change in bronchial production of granulocyte chemotactic protein-2 on protein level after anti-IL-17 therapy (unpublished observation). Alternatively, decreased production of other factors like pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  (28), or impaired upregulation of ICAM-1 (23, 24, 27), may explain the impaired bronchial influx of neutrophils in anti-IL-17-treated mice as well.

Unexpectedly, we observed that anti-IL-17 mAb significantly aggravated bronchial eosinophilic inflammation after allergen provocation. We attribute this phenomenon to enhanced production of IL-5 in serum as well as in bronchi of anti-IL-17 mAb-treated mice. Indeed, circulating IL-5 is one of the major stimuli for eosinopoiesis (41) and migration of eosinophils toward airways (45). In addition to more IL-5 production, absence of IL-17 may interfere with the production of other mediators involved in the chemotaxis of eosinophils toward airways, like RANTES (24).

In view of the upregulation of IL-5 and to a lesser extent of IL-4 by neutralization of IL-17, we tend to speculate that IL-17 is an immunomodulatory cytokine involved in dampening allergic airway inflammation through inhibition of Th2 cytokine secretion. However, we were unable to demonstrate any direct effect of recombinant mouse IL-17 on IL-5 production by lymphocytes *in vitro* studies (unpublished observation). Therefore, we think that the mechanism underlying enhanced IL-5 production by administration of anti-IL-17 mAb most likely represents an indirect mechanism.

Interestingly, the reduction of bronchial neutrophilia in

anti-IL-17 mAb-treated mice was not associated with beneficial effects on airway hyperresponsiveness. This observation is consistent with data obtained in rat models of allergic asthma (8) and acute respiratory distress syndrome (46), where a reduction of bronchial neutrophilia did not alter bronchial function. We should, however, mention that reduction of neutrophilic inflammation by anti-IL-17 mAb was not absolute, allowing no firm conclusion on the role of neutrophils in the development of BHR. Another reason for the lack of effect of anti-IL-17 mAb on BHR may be that the bronchial eosinophilia, which is still considered to play a role in the manifestation of BHR (1), was equally high in both groups of mice studied.

Whether targeting IL-17 may have therapeutic potential in human airway disease in future remains speculative. It is, however, interesting to mention that blocking anti-IL-17 mAb decreased the bronchial influx of neutrophils in a manner equally as potent as did dexamethasone, a potent anti-inflammatory agent. In view of the nonspecific mode of action of dexamethasone, targeting IL-17 may represent a new, more specific, therapeutic approach for airway diseases with major neutrophilic inflammation like acute severe asthma attacks and chronic obstructive pulmonary diseases (47). However, studies are warranted to dissect the role of neutrophils in allergic airway disease *in vivo*. *In vitro* studies show that neutrophilic proteases are important mucin secretagogues (48), mediate activation of epithelial cells, heighten vascular permeability (49), and even activate eosinophils (50). Bronchial neutrophils may also be involved in airway remodeling in asthma, as they represent a potentially important source of transforming growth factor- $\beta$  (51). However, it remains to be determined to what extent acute and/or prolonged reduction of bronchial neutrophilia may lead to decreased mucus secretion, bronchial remodeling, and/or symptom relief. Therefore, better insight into the pathophysiologic role of bronchial neutrophils is required before one can delineate the potentially beneficial effect of anti-IL-17 mAb in human airway disease.

In conclusion, we here show for the first time that IL-17 is produced in experimental allergic asthma following allergen inhalation, and that IL-17 orchestrates T cell-dependent granulocyte influx into inflamed airways. Our data reveal that IL-17 is critical for the bronchial recruitment of neutrophils, and may hence contribute to the chronic inflammatory changes associated with allergic asthma.

**Acknowledgments:** The authors thank E. Dewil, H. Devijvere, and the staff of the animal housing department for taking care of the mice. This work was supported by Grants G.0227.01 and 7.0029.00 (Levenslijn) of the FWO (Fund for Scientific Research) Vlaanderen. P.H. is the recipient of a research fellowship of the FWO Vlaanderen.

## References

- Busse, W. W., and R. F. Lemanske, Jr. 2001. Asthma. *N. Engl. J. Med.* 344:350–362.
- Molet, S., O. Hamid, F. Davoine, E. Nutku, R. Taha, N. Page, R. Olivenstein, J. Elias, and J. Chakir. 2001. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J. Allergy Clin. Immunol.* 108:430–438.
- Sur, S., T. B. Crotty, G. M. Kephart, B. A. Hyma, T. V. Colby, C. E. Reed, L. W. Hunt, and G. J. Gleich. 1993. Sudden-onset fatal asthma: a distinct entity with few eosinophils and relatively more neutrophils in the airway submucosa? *Am. Rev. Respir. Dis.* 148:713–719.
- Lamblin, C., P. Gosset, I. Tillie-Leblond, F. Saulnier, C. H. Marquette, B. Wallaert, and A. B. Tonnel. 1998. Bronchial neutrophilia in patients with noninfectious status asthmaticus. *Am. J. Respir. Crit. Care Med.* 157:394–402.
- Carroll, N., S. Carello, C. Cooke, and A. James. 1996. Airway structure and inflammatory cells in fatal attacks of asthma. *Eur. Respir. J.* 9:709–715.
- Ordóñez, C. L., T. E. Shaughnessy, M. A. Matthay, and J. V. Fahy. 2000. Increased neutrophil numbers and IL-8 levels in airway secretions in acute severe asthma: clinical and biologic significance. *Am. J. Respir. Crit. Care Med.* 161:1185–1190.
- Montefort, S., C. Gratziau, D. Goulding, R. Polosa, D. O. Haskard, P. H. Howarth, S. T. Holgate, and M. P. Carroll. 1994. Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules 6 hours after local allergen challenge of sensitized asthmatic airways. *J. Clin. Invest.* 93:1411–1421.
- Renzi, P. M., J. P. Yang, T. Diamantstein, and J. G. Martin. 1996. Effects of depletion of cells bearing the interleukin-2 receptor on immunoglobulin production and allergic airway responses in the rat. *Am. J. Respir. Crit. Care Med.* 153:1214–1221.
- Rouvier, E., M. F. Luciani, M. G. Mattei, F. Denizot, and P. Golstein. 1993. CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. *J. Immunol.* 150:5445–5456.
- Yao, Z., S. L. Painter, W. C. Fanslow, D. Ulrich, B. M. Macduff, M. K. Spriggs, and R. J. Armitage. 1995. Human IL-17: a novel cytokine derived from T cells. *J. Immunol.* 155:5483–5486.
- Fossiez, F., O. Djossou, P. Chomarat, L. Flores-Romo, S. Ait-Yahia, C. Maat, J. J. Pin, P. Garrone, E. Garcia, S. Saeland, D. Blanchard, C. Gaillard, B. Das Mahapatra, E. Rouvier, P. Golstein, J. Banchereau, and S. Lebecque. 1996. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J. Exp. Med.* 183:2593–2603.
- Yao, Z., W. C. Fanslow, M. F. Seldin, A. M. Rousseau, S. L. Painter, M. R. Comeau, J. I. Cohen, and M. K. Spriggs. 1995. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity* 3:811–821.
- Awane, M., P. G. Andres, D. J. Li, and H. C. Reinecker. 1999. NF-kappa B-inducing kinase is a common mediator of IL-17-, TNF-alpha-, and IL-1 beta-induced chemokine promoter activation in intestinal epithelial cells. *J. Immunol.* 162:5337–5344.
- Karin, M. 1996. The regulation of AP-1 activity by mitogen-activated protein kinases. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 351:127–134.
- Schwandner, R., K. Yamaguchi, and Z. Cao. 2000. Requirement of tumor necrosis factor receptor-associated factor (TRAF)6 in interleukin 17 signal transduction. *J. Exp. Med.* 191:1233–1240.
- Pelidou, S. H., L. P. Zou, G. Deretzi, C. Oniding, E. Mix, and J. Zhu. 2000. Enhancement of acute phase and inhibition of chronic phase of experimental autoimmune neuritis in Lewis rats by intranasal administration of recombinant mouse interleukin 17: potential immunoregulatory role. *Exp. Neurol.* 163:165–172.
- Antonyamsy, M. A., W. C. Fanslow, F. Fu, W. Li, S. Qian, A. B. Troutt, and A. W. Thomson. 1999. Evidence for a role of IL-17 in organ allograft rejection: IL-17 promotes the functional differentiation of dendritic cell progenitors. *J. Immunol.* 162:577–584.
- Tartour, E., F. Fossiez, J. Joyeux, A. Galinha, A. Gey, E. Claret, X. Sastre-Garau, J. Couturier, V. Mosseri, V. Vives, J. Banchereau, W. H. Fridman, J. Wijdenes, S. Lebecque, and C. Sautes-Fridman. 1999. Interleukin 17, a T-cell-derived cytokine, promotes tumorigenicity of human cervical tumors in nude mice. *Cancer Res.* 59:3698–3704.
- Ye, P., F. H. Rodriguez, S. Kanaly, K. L. Stocking, J. Schurr, P. Schwarzenberger, P. Oliver, W. Huang, P. Zhang, J. Zhang, J. E. Shellito, G. J. Bagby, S. Nelson, K. Charrier, J. J. Peschon, and J. K. Kolls. 2001. Requirement of interleukin 17 receptor signaling for lung cxc chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J. Exp. Med.* 194:519–528.
- Chabaud, M., F. Fossiez, J. L. Taupin, and P. Miossec. 1998. Enhancing effect of IL-17 on IL-1-induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritis synoviocytes and its regulation by Th2 cytokines. *J. Immunol.* 161:409–414.
- Ziolkowska, M., A. Koc, G. Luszczkiewicz, K. Ksiezopolska-Pietrzak, E. Klimczak, H. Chwalinska-Sadowska, and W. Maslinski. 2000. High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. *J. Immunol.* 164:2832–2838.
- Matusevicius, D., P. Kivisakk, B. He, N. Kostulas, V. Ozenci, S. Fredrikson, and H. Link. 1999. Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. *Mult. Scler.* 5:101–104.
- Teunissen, M. B., C. W. Koomen, R. de Waal Malefyt, E. A. Wierenga, and J. D. Bos. 1998. Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J. Invest. Dermatol.* 111:645–649.
- Albanesi, C., A. Cavani, and G. Girolomoni. 1999. IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and che-

- mokine production in human keratinocytes: synergistic or antagonist effects with IFN-gamma and TNF-alpha. *J. Immunol.* 162:494-502.
25. Schwarzenberger, P., V. La Russa, A. Miller, P. Ye, W. Huang, A. Zieske, S. Nelson, G. J. Bagby, D. Stoltz, R. L. Mynatt, M. Spriggs, and J. K. Kolls. 1998. IL-17 stimulates granulopoiesis in mice: use of an alternate, novel gene therapy-derived method for in vivo evaluation of cytokines. *J. Immunol.* 161:6383-6389.
  26. Laan, M., Z. H. Cui, H. Hoshino, J. Lotvall, M. Sjostrand, D. C. Gruenert, B. E. Skoogh, and A. Linden. 1999. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J. Immunol.* 162:2347-2352.
  27. Jovanovic, D. V., J. A. Di Battista, J. Martel-Pelletier, F. C. Jolicoeur, Y. He, M. Zhang, F. Mineau, and J. P. Pelletier. 1998. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. *J. Immunol.* 160:3513-3521.
  28. Thomas, P. S. 2001. Tumour necrosis factor-alpha: the role of this multifunctional cytokine in asthma. *Immunol. Cell Biol.* 79:132-140.
  29. Hoshino, H., M. Laan, M. Sjostrand, J. Lotvall, B. E. Skoogh, and A. Linden. 2000. Increased elastase and myeloperoxidase activity associated with neutrophil recruitment by IL-17 in airways in vivo. *J. Allergy Clin. Immunol.* 105:143-149.
  30. Hellings, P. W., E. M. Hessel, J. Van den Oord, A. Kasran, P. Van Hecke, and J. L. Ceuppens. 2001. Eosinophilic rhinitis accompanies the development of lower airway inflammation and hyperreactivity in sensitized mice exposed to aerosolized allergen. *Clin. Exp. Allergy* 31:782-790.
  31. Hellings, P. W., P. Vandenberghe, A. Kasran, L. Coorevits, L. Overbergh, C. Mathieu, and J. L. Ceuppens. 2002. Blockade of CTLA-4 enhances allergic sensitization and eosinophilic airway inflammation in genetically predisposed mice. *Eur. J. Immunol.* 32:585-594.
  32. Denis, M., L. Guojian, M. Widmer, and A. Cantin. 1994. A mouse model of lung injury induced by microbial products: implication of tumor necrosis factor. *Am. J. Respir. Cell Mol. Biol.* 10:658-664.
  33. Bradley, P. P., D. A. Priebat, R. D. Christensen, and G. Rothstein. 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J. Invest. Dermatol.* 78:206-209.
  34. Overbergh, L., D. Valckx, M. Waer, and C. Mathieu. 1999. Quantification of murine cytokine mRNAs using real time quantitative reverse transcriptase PCR. *Cytokine* 11:305-312.
  35. Kennedy, J., D. L. Rossi, S. M. Zurawski, F. Vega, Jr., R. A. Kastelein, J. L. Wagner, C. H. Hannum, and A. Zlotnik. 1996. Mouse IL-17: a cytokine preferentially expressed by alpha beta TCR + CD4-CD8-T cells. *J. Interferon Cytokine Res.* 16:611-617.
  36. Chung, D. R., T. Chitnis, R. J. Panzo, D. L. Kasper, M. H. Sayegh, and A. O. Tzianabos. 2002. CD4+ T cells regulate surgical and postinfectious adhesion formation. *J. Exp. Med.* 195:1471-1478.
  37. Aarvak, T., M. Chabaud, P. Miossec, and J. B. Natvig. 1999. IL-17 is produced by some proinflammatory Th1/Th0 cells but not by Th2 cells. *J. Immunol.* 162:1246-1251.
  38. Infante-Duarte, C., H. F. Horton, M. C. Byrne, and T. Kamradt. 2000. Microbial lipopeptides induce the production of IL-17 in Th cells. *J. Immunol.* 165:6107-6115. (In Process Citation)
  39. Albanesi, C., C. Scarponi, A. Cavani, M. Federici, F. Nasorri, and G. Girolomoni. 2000. Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon-gamma- and interleukin-4-induced activation of human keratinocytes. *J. Invest. Dermatol.* 115:81-87.
  40. Cai, X. Y., C. P. Gommoll, Jr., L. Justice, S. K. Narula, and J. S. Fine. 1998. Regulation of granulocyte colony-stimulating factor gene expression by interleukin-17. *Immunol. Lett.* 62:51-58.
  41. Gaspar Elsas, M. I., D. Joseph, P. X. Elsas, and B. B. Vargaftig. 1997. Rapid increase in bone-marrow eosinophil production and responses to eosinopoietic interleukins triggered by intranasal allergen challenge. *Am. J. Respir. Cell Mol. Biol.* 17:404-413.
  42. Witowski, J., K. Pawlaczyk, A. Breborowicz, A. Scheuren, M. Kuzlan-Pawlaczyk, J. Wisniewska, A. Polubinska, H. Friess, G. M. Gahl, U. Frei, and A. Jorres. 2000. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GROalpha chemokine from mesothelial cells. *J. Immunol.* 165:5814-5821. (In Process Citation)
  43. Wuyts, A., A. Haelens, P. Proost, J. P. Lenaerts, R. Conings, G. Opdenakker, and J. Van Damme. 1996. Identification of mouse granulocyte chemotactic protein-2 from fibroblasts and epithelial cells: functional comparison with natural KC and macrophage inflammatory protein-2. *J. Immunol.* 157:1736-1743.
  44. Wuyts, A., A. D'Haese, V. Cremers, P. Menten, J. P. Lenaerts, A. De Loof, H. Heremans, P. Proost, and J. Van Damme. 1999. NH2- and COOH-terminal truncations of murine granulocyte chemotactic protein-2 augment the in vitro and in vivo neutrophil chemotactic potency. *J. Immunol.* 163:6155-6163.
  45. Wang, J., K. Palmer, J. Lotvall, S. Milan, X. F. Lei, K. I. Matthaei, J. Gauldie, M. D. Inman, M. Jordana, and Z. Xing. 1998. Circulating, but not local lung, IL-5 is required for the development of antigen-induced airways eosinophilia. *J. Clin. Invest.* 102:1132-1141.
  46. Pauwels, R. A., J. C. Kips, R. A. Peleman, and M. E. Van Der Straeten. 1990. The effect of endotoxin inhalation on airway responsiveness and cellular influx in rats. *Am. Rev. Respir. Dis.* 141:540-545.
  47. Nadel, J. A. 2000. Role of neutrophil elastase in hypersecretion during COPD exacerbations, and proposed therapies. *Chest* 117(5 Suppl. 2):386S-389S.
  48. Kim, K. C., J. Nassiri, and J. S. Brody. 1989. Mechanisms of airway goblet cell mucin release: studies with cultured tracheal surface epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 1:137-143.
  49. Persson, C. G. 1986. Role of plasma exudation in asthmatic airways. *Lancet* 2:1126-1129.
  50. Liu, H., S. C. Lazarus, G. H. Caughey, and J. V. Fahy. 1999. Neutrophil elastase and elastase-rich cystic fibrosis sputum degranulate human eosinophils in vitro. *Am. J. Physiol.* 276:L28-34.
  51. Chu, H. W., J. B. Trudeau, S. Balzar, and S. E. Wenzel. 2000. Peripheral blood and airway tissue expression of transforming growth factor beta by neutrophils in asthmatic subjects and normal control subjects. *J. Allergy Clin. Immunol.* 106:1115-1123.