The Journal of Immunology

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J Immunol 1999; 162:2347-2352; ;
http://www.jimmunol.org/content/162/4/2347

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Neutrophil Recruitment by Human IL-17 Via C-X-C Chemokine Release in the Airways[1]

Martti Laan,* Zhi-Hua Cui,* Hiroshi Hoshino,* Jan Lötvall,* Margareta Sjöstrand,* Dieter C. Gruenert,† Bengt-Eric Skoogh,* and Anders Lindén[2]*

IL-17 is a recently discovered cytokine that can be released from activated human CD4$^+$ T lymphocytes. This study assessed the proinflammatory effects of human (h) IL-17 in the airways. In vitro, hIL-17 increased the release of IL-8 in human bronchial epithelial and venous endothelial cells, in a time- and concentration-dependent fashion. This effect of hIL-17 was inhibited by cotreatment with an anti-hIL-17 Ab and was potentiated by hTNF-α. In addition, hIL-17 increased the expression of hIL-8 mRNA in bronchial epithelial cells. Conditioned medium from hIL-17-treated bronchial epithelial cells increased human neutrophil migration in vitro. This effect was blocked by an anti-hIL-8 Ab. In vivo, intratracheal instillation of hIL-17 selectively recruited neutrophils into rat airways. This recruitment of neutrophils into the airways was inhibited by an anti-hIL-17 Ab and accompanied by increased levels of rat macrophage inflammatory protein-2 (rMIP-2) in bronchoalveolar lavage (BAL) fluid. The BAL neutrophilia was also blocked by an anti-rMIP-2 Ab. The effect of hIL-17 on the release of hIL-8 and rMIP-2 was also inhibited by glucocorticoids, in vitro and in vivo, respectively. These data demonstrate that hIL-17 can specifically and selectively recruit neutrophils into the airways via the release of C-X-C chemokines from bronchial epithelial cells and suggest a novel mechanism linking the activation of T-lymphocytes to recruitment of neutrophils into the airways. ***The Journal of Immunology*****, 1999, 162: 2347–2352.**

Measurements in sputum and/or bronchoalveolar lavage (BAL)[3] fluid demonstrate that the level of the human C-X-C chemokine, human IL-8 (hIL-8), is increased in inflammatory airway diseases that are characterized by high neutrophil numbers, such as acute asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (1–3). There appears to be a strong correlation between the level of hIL-8 and the increase in neutrophil numbers (2). A causal role of hIL-8 in neutrophil recruitment is also indicated by the inhibitory effect of an anti-hIL-8 Ab on neutrophil chemotaxis induced by sputum from patients with COPD or cystic fibrosis (3). As a consequence of its neutrophil-recruiting capacity, hIL-8 may increase the levels of neutrophil-derived inflammatory mediators such as elastase and myeloperoxidase per se, or in conjunction with other mediators (1, 2). Several studies now indicate that bronchial epithelial and venous endothelial cells constitute two important sources of hIL-8 and may thereby control neutrophil influx in the airways (4, 5). Thus, mediators causing hIL-8 release in bronchial epithelial and/or endothelial cells may play a crucial role in modulating neutrophil-related airway inflammation.

Activated CD4$^+$ T lymphocytes may play a central role in airway inflammation (6–9). This is indicated by the ability of anti-CD4$^+$ Abs to inhibit the influx of eosinophils and neutrophils into murine airways (9). In the case of eosinophils, IL-5 is considered to link the activation of CD4$^+$ to the influx of eosinophils (10). However, until now, there has been little evidence indicating which cytokine might mediate recruitment of neutrophils induced by activated CD4$^+$ cells.

hIL-17 was recently discovered and is a 16-kDa protein that can be released from activated human CD4$^+$ T lymphocytes in vitro (11). Rat (r) and mouse (m) IL-17 display a 60–70% homology with hIL-17 (12). All three of these forms of mammalian IL-17 have a highly conserved site for glycosylation, suggesting that interspecies cross-reactivity is possible (12). IL-17 has specific receptors in several types of cells, including lung cells (13). In response to hIL-17, human fibroblasts release IL-8 in vitro (14). However, at present, it is not known whether human airway cells, such as bronchial epithelial cells and venous endothelial cells, release hIL-8 in response to hIL-17, or whether hIL-17, via the release of a C-X-C chemokine, can induce significant neutrophil recruitment into the airways in vivo.

To evaluate whether hIL-17 can induce neutrophil recruitment via C-X-C chemokine production, the current study characterized the effect of hIL-17 on hIL-8 protein release and mRNA levels in human bronchial epithelial and venous endothelial cells in vitro. The chemotactic activity of conditioned medium from the bronchial epithelial cells was tested using human neutrophils in vitro, with and without an anti-hIL-8 Ab. A rat model was utilized to evaluate the effect of hIL-17 on neutrophil recruitment into the airways in vivo. This model was also used to study the effect of hIL-17 on airway release of the rat C-X-C chemokine correlate to hIL-8, macrophage inflammatory protein-2 (rMIP-2) (15). The

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Received for publication September 29, 1998. Accepted for publication November 5, 1998.

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[1] This study was supported by the Herman Krefting's Foundation, the Swedish Medical Research Council (K97-04X-09-04-8-08A, U1268), the Vårdal Foundation, and The Swedish Heart Lung Foundation. D.C.G. was supported by National Institutes of Health Grants DK47766 and DK46002.

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[3] Abbreviations used in this paper: BAL, bronchoalveolar lavage; h, human; r, rat; m, mouse; MIP-2, macrophage inflammatory protein-2; HPF, high power field; i.t., intratracheally.

0022-1767/99/$02.00

study also examined the effect of an anti-rMIP-2 Ab on neutrophil recruitment into rat airways induced by hIL-17.

Materials and Methods

Materials

Transformed human bronchial epithelial (16HBE14_o-, abbreviated as 16HBE) cells (16) were utilized because of their ability to release hIL-8 in response to inflammatory stimuli similar to that observed for primary human bronchial epithelial cells (17). Another human airway epithelial-like cell line (Calu-3) (18), obtained from the American Type Culture Collection (Manassas, VA), was also used. HUVEC cells (19) were also utilized for their ability to release hIL-8 in response to inflammatory stimuli (5). HUVEC cells, EGM Bulletkit (endothelial cell basal medium and supplements), trypsin-EDTA solution, trypsin neutralizing solution, and HBSS were obtained from Clonetics (San Diego, CA). Recombinant hIL-17, goat neutralizing anti-hIL-17 Ab, recombinant hIL-8, mouse neutralizing anti-hIL-8 Ab, recombinant hTNF-α, and hIL-8 ELISA kits were obtained from R&D Systems (Minneapolis, MN). Penicillin-streptomycin, L-glutamine, PBS, amphotericin B, BSA, and hydrocortisone were purchased from Sigma (St. Louis, MO). Fibronectin (human) and collagen (bovine, type I) were obtained from Becton Dickinson Labware (Bedford, MA), and MEM Earle-Eagle was from Life Technologies (Inchinnan, Scotland, U.K.). Ketamine hydrochloride (Park-Davis, Barcelona, Spain), xylazine chloride (Bayer Sverige, Göteborg, Sweden), dexamethasone (Sigma), pentobarbitone (Apoteksbolaget, Umeå, Sweden), rabbit anti-rMIP-2 Ab (Serotec, Oxford, U.K.), and rMIP-2 ELISA kit (BioSource International, Camarillo, CA) were also obtained commercially.

In vitro experiments

Cell culture conditions. 16HBE cells were grown in MEM Earle-Eagle medium with FCS (10%), 2 mM L-glutamine, 100 U/ml penicillin, 100 g/ml streptomycin, and 5 μg/ml amphotericin B on collagen and fibronectin-coated dishes (20). Calu-3 cells were grown in MEM Eagle, supplemented with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml amphotericin B. HUVEC cells were grown in endothelial cell basal medium, supplemented with 6% FCS, 1 μg/ml hydrocortisone, 0.01 μg/ml recombinant human epidermal growth factor, 25 μg/ml gentamicin, 0.025 μg/ml amphotericin B, and 12 μg/ml bovine brain extract. All cells were grown to confluence in 6-well plastic plates (Becton Dickinson, Mountain View, CA). At 18 h before the experiments, the concentration of FCS was reduced to 1% in 16HBE and Calu-3 cells and to 2% in HUVEC cells to minimize the basal (inherent) hIL-8 release. Before the addition of stimuli, the cells were washed twice with PBS and placed in fresh medium with 1% (16HBE and Calu-3) or 2% (HUVEC) of FCS. PBS supplemented with 0.35% BSA was used to deliver hIL-17 and hTNF-α. The corresponding concentration of BSA (total concentration including BSA in cytokines equal to 0.45%) was used as a vehicle.

Effect of hIL-17 on hIL-8 protein release. Conditioned media were removed from cells and frozen at the end of each experiment. Thawed samples were centrifuged (4000 rpm for 10 min.) to pellet cells and cell debris. The supernatants were analyzed using an ELISA for hIL-8 according to the manufacturer's instructions.

Effect of hIL-17 on hIL-8 mRNA levels. Total RNA was isolated and analyzed using RT-PCR. Simultaneously, amplification of β-actin was done as an internal standard for RT-PCR (21). 16HBE cells were grown to confluence in 6-well plates and treated with hIL-17 or vehicle. Two hours later cells were harvested by scraping, were washed with PBS, and then suspended in RNA STAT-60 (Tel-Test B, Friendswood, TX).

Total RNA was isolated from 16HBE cells by a single-step method with the RNA STAT-60 kit according to the manufacturer's instructions. The isolate was treated with RQ1 RNase-free DNase (Promega, Madison, WI) and then extracted. The concentration and purity of isolated RNA was determined spectrophotometrically.

Reverse transcription involved incubation of 2 μg of total RNA and random hexamer (Pharmacia, Uppsala, Sweden) with dNTPs (Pharmacia), RNase inhibitor (Promega), superscript reverse transcriptase (Life Technologies, Gaithesburg, MD, USA), first strand buffer (Life Technologies), and diethyl pyrocarbonate-treated water (DEPC-H_2O) for 1 h at 42°C. The reaction was terminated by heating at 70°C for 10 min.

PCR amplification utilized commercially available primers to hIL-8 and β-actin (Clontech Laboratories, Palo Alto, CA) and was performed in the presence of AmplyTaq Gold DNA Polymerase, PCR buffer, and 2.0 mM $MgCl_2$ (all from Perkin-Elmer Cetus, Norwalk CT), dNTP mix (Pharmacia), and sterile water. The PCR was conducted in a Perkin-Elmer Cetus GeneAmp PCR System 9600 starting with a 12-min incubation at 95°C, followed by 24 cycles of denaturation at 94°C for 45 s, annealing at 65°C for 45 s, and extension at 72°C for 2 min. The final extension was at 72°C for 7 min. The PCR products were separated on a 3% agarose gel (SeaKem and NuSieve, FMC BioProducts, Rockland, MA) containing ethidium bromide. ϕX147/*Hae*III-digested DNA (Life Technologies) was used as a molecular size marker. Gels were photographed under UV light and images were scanned (StudioScan II/si; Agfa, Mortcel, Belgium) for densitometric analysis, i.e., bands were quantified in terms of position, height and area in two dimensions. This "volume" analysis was performed with IPLab Gel software for Macintosh (BioSystematica, Plymouth, U.K.). Results were expressed as a ratio calculated from the volume of the amplified cytokine mRNA product divided by the volume of the amplified housekeeping (β-actin) mRNA.

Effect of hIL-17 on human neutrophil chemotaxis. Neutrophils were isolated from peripheral blood of healthy, adult volunteers. Whole blood was sedimented in the presence of 4.5% dextran T500 solution (Pharmacia) to remove RBC, monocytes, and lymphocytes (22). The residual erythrocytes were lysed (0.8% NH_4Cl + 0.1% $KHCO_3$), and the remaining neutrophils were washed twice with HBSS. The neutrophils were brought to the final concentration of 3×10^6 cells/ml in HBSS containing 1% BSA. To analyze the chemotactic activity of conditioned medium from the 16HBE cells, neutrophils were suspended in MEM Earle-Eagle. The chemotaxis assay was performed in a 48-well microchemotaxis chamber (Neuroprobe, Cabin John, MD) as previously described (23). Briefly, the solutions and equipment were brought to 37°C before onset of the experiment. The bottom wells of the chamber were filled with 25 μl of fluid containing either the chemotactic stimulus, the control solution, or conditioned medium from 16HBE cells. A polycarbonate filter with pore size of 3 μm (Nucleopore, Pleasanton, CA) was placed over the bottom wells. The silicon gasket and upper piece of the chamber were applied, and 50 μl of neutrophil suspension were pipetted into upper wells as triplicate samples. The chamber was incubated in humidified air with 5% CO_2 at 37°C for 20 min, then disassembled, and the filter was removed. The filter was then fixed, stained with Diff-Quick dyes (Svenska Labex, Helsingborg, Sweden), and mounted on a glass slide. Neutrophils that completely migrated through the filter were counted in five random, high power fields (HPF) (×1000) from each well. Triplicate wells were used for each data point. The chemotactic response was defined as the mean number of migrating cells per HPF.

The negative control used was HBSS with 1% BSA which caused migration of 8.7 ± 2.7 neutrophils/HPF ($n = 4$). Pure hIL-8 (1×10^{-9}) and FMLP (1×10^{-7}) were utilized as positive "technical" controls and caused migration of 148 ± 21.2 and 168.5 ± 26.9 neutrophils/HPF respectively ($n = 4$). If no response to these stimuli was observed, the experiment was excluded. Inhibition of hIL-8-mediated neutrophil recruitment involved 10 μg/ml of anti-hIL-8 Ab added to the stimuli 15 min before experiment. This concentration of Ab showed 89 ± 3% ($n = 4$) inhibition of neutrophil recruitment caused by 1×10^{-9} M of hIL-8 and had no effect on chemotaxis induced by 1×10^{-7} of FMLP. In the experiments with conditioned media from 16HBE cells, the conditioned medium from vehicle-treated 16HBE cells served as a negative control. This kind of stimulus resulted in migration of 17.3 ± 2.7 neutrophils/HPF and was not affected by adding an anti-hIL-8 Ab (17.8 ± 1.8 neutrophils/HPF, Student's paired t test, two-way: $p = 0.9$, $n = 4$).

In vivo experiments

Animals. Brown Norway rats (male, 250–300 g, Harlan, Oxon, U.K.) were used under conditions approved by the animal ethics committee in Göteborg University (Dno 134/95).

BAL. Animals were anesthetized with ketamine (50 mg/kg i.m.) and xylazine (5 mg/kg i.m.). The trachea was intubated with a cannula (OD 2.8 mm Portex, Smiths Industries, Middlesex, U.K.), and the solution with hIL-17 (1 μg in 50 μl of vehicle) was instilled intratracheally (i.t.). An equivalent volume of vehicle (PBS containing 0.45% BSA) was administered correspondingly in the control animals. After the administration of hIL-17 or vehicle, the animals regained consciousness.

For time course experiment, animals were treated i.t. with hIL-17 or vehicle. BAL was collected at 2, 4, 6, and 8 h after the administration of IL-17 or vehicle.

To evaluate whether the effect of IL-17 is specific in vivo (i.e., not due to unspecific protein deposition), hIL-17 protein solution (1 μg in 50 μl of vehicle) was coincubated with an anti-hIL-17 Ab (30 μg in 50 μl of vehicle) at 37°C for 15 min and installed i.t. An equivalent volume of corresponding vehicle (100 μl of PBS containing 0.45% BSA and 1 ng LPS) or IL-17 (1 μg in 100 μl of PBS with BSA and LPS) was utilized as negative and positive control, respectively. BAL was collected 6 h later.

To establish whether 6 h of incubation with hIL-17 increases the release of MIP-2 in rat airways, rMIP-2 protein level were determined in BAL fluid by ELISA.

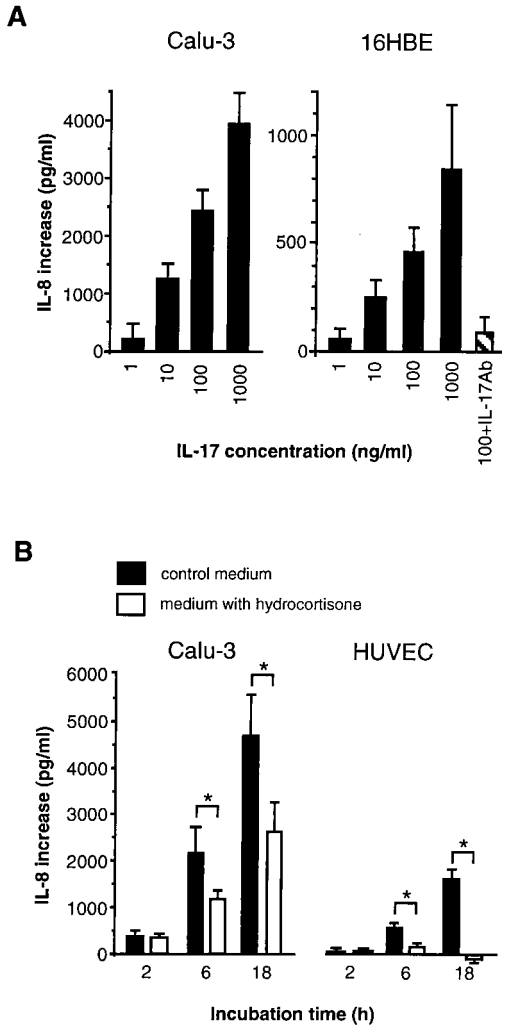

FIGURE 1. *A*, Release of hIL-8 in Calu-3 cells (Spearman's rank correlation: $r = 0.9$, $p < 0.001$, $n = 6$) and 16HBE cells ($r = 0.8$, $p < 0.01$, $n = 6$) caused by 18-h treatment with hIL-17 at various concentrations. Coincubation with an anti-hIL-17 Ab (3 μg/ml, 15 min.) blocked the effect of hIL-17 (Student's unpaired, two-way *t* test: $p < 0.001$, $n = 5$) in 16HBE cells. *B*, Release of hIL-8 caused by hIL-17 (100 ng/ml) at various time points in Calu-3 cells (Spearman's rank correlation: $r = 0.9$, $p < 0.01$, $n = 5$) and HUVEC cells ($r = 0.9$, $p < 0.05$, $n = 4$). Hydrocortisone (1 μg/ml for 3 days) significantly (Student's paired, two-way *t* test: $*$, $p < 0.05$, $n = 4$–5) inhibited hIL-17-mediated hIL-8 release in both cell lines. Data are mean values with SEM and refer to an increase in hIL-8 above basal levels in vehicle treated cells. This basal secretion was 7010 ± 521 pg/ml in Calu-3 and 592 ± 67 pg/ml in 16HBE cells (*A*), and 1640 ± 387, 6621 ± 1425, 15035 ± 2592, 736 ± 138, 2437 ± 316, 6210 ± 500 in Calu-3 cells and 368 ± 79, 956 ± 141, 3996 ± 912 pg/ml and 97 ± 14, 290 ± 40, 1323 ± 184 pg/ml in HUVEC cells after 2, 6 and 18 h, in control and glucocorticoid treated cells, respectively (*B*).

To determine whether endogenous MIP-2 released by hIL-17 (1 μg/rat i.t.) contributes to neutrophil recruitment in rat airways, anti-rMIP-2 Ab was instilled i.t. before administration of hIL-17. Animals were pretreated with: 1) anti-rMIP-2 Ab (100 μg/rat i.t.) 15 min before administration of hIL-17, 2) an equivalent volume of vehicle for MIP-2 (PBS) before administration of hIL-17, and 3) vehicles of both anti-rMIP-2 and hIL-17. BAL was performed 6 h after administration of hIL-17 or vehicle. In separate experiments, an anti-MIP-2 Ab (100 μg/rat i.t.) had no effect on "basal" neutrophil recruitment compared with animals treated with vehicle for the anti-MIP-2 Ab (0.1 ± 0.05 vs 0.08 ± 0.03 × 10^6/animal, respectively, Student's *t* test, two-way: $p = 0.7$, $n = 4$).

To test whether a glucocorticoid inhibits hIL-17-induced neutrophil recruitment, animals were treated with: 1) the vehicle for dexamethasone (2-hydroxypropyl-β-cyclodexin) 1 h before addition of the vehicle for hIL-17, 2) the vehicle for dexamethasone prior hIL-17 administration, and 3) dexamethasone (3 mg/kg i.p.) 1 h before hIL-17 administration. BAL was performed 8 h after administration of hIL-17 or vehicle.

At the indicated time-points (see Figs. 5–7), animals were euthanized by pentobarbitone (100 mg/kg i.p.). The airway was cannulated through a tracheostoma and lavaged with 5 × 4 ml of PBS at room temperature. BAL fluid was centrifuged (200 × *g* for 10 min at 4°C), and the cell pellet was resuspended in washing buffer (sterile PBS containing 0.35% BSA and 0.1% glucose). Total BAL cell counts were determined in haemocytometer using Türks staining. For differential BAL cell counts, cytospin preparations were made and stained using the May-Giemsa method. Differential cell counts were conducted according to standard morphology criteria using oil immersion microscopy (magnification, ×1000). Cell counts were conducted on 300 cells and the absolute number of each cell type was calculated. The cell-free BAL fluid was collected and stored at −80°C.

rMIP-2 protein levels. rMIP-2 in cell-free BAL fluid was assessed using an ELISA for murine MIP-2 according to the manufacturer's instructions.

Results

In vitro experiments

Effect of hIL-17 on hIL-8 protein release. In the concentration range of 1–1000 ng/ml, hIL-17 increased hIL-8 levels in conditioned medium from Calu-3 and 16HBE cells in a concentration-dependent fashion (Fig. 1*A*). Coincubation with an anti-hIL-17 Ab blocked the hIL-17-induced hIL-8 increase in medium from 16HBE cells (Fig. 1*A*). In Calu-3 and HUVEC cells, there was a time-dependent effect of hIL-17 on the increase in hIL-8 during 2–18 h (100 ng/ml) (Fig. 1*B*). At a hIL-17 concentration of 100 ng/ml, hydrocortisone significantly inhibited hIL-8 increase in both Calu-3 and HUVEC cells (Fig. 1*B*). Cotreatment of cells with a concentration of hIL-17 (100 ng/ml) that caused a submaximum response plus hTNF-α (20 ng/ml) resulted in a potentiated increase in hIL-8 in conditioned medium from 16HBE cells when compared with the sum of the increase observed with hIL-17 alone or hTNF-α alone (Fig. 2). By comparison, the hIL-8 increase caused by cotreatment with hIL-17 (100 ng/ml) plus hTNF-α was significantly higher (Student;s *t* test, paired, two-way: $p < 0.05$, $n = 6$) than that observed after treatment with a maximum-effective concentration of hTNF-α alone (100 ng/ml) (4957 ± 605 and 3574 ± 406 pg/ml, respectively). In addition, hydrocortisone inhibited the increase in hIL-8 levels caused by hIL-17 in the conditioned medium from 16HBE cells (Fig. 2).

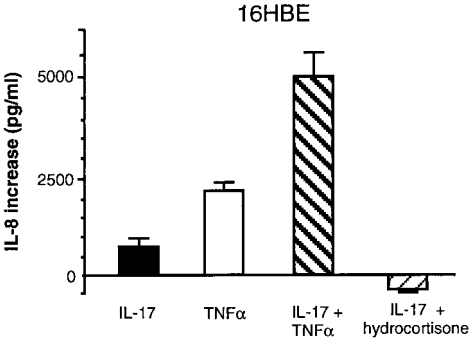

FIGURE 2. Release of hIL-8 in 16HBE cells caused by 18-h treatment with hIL-17 (100 ng/ml), hTNF-α (20 ng/ml), or hIL-17 plus hTNF-α (100 and 20 ng/ml, respectively). hIL-8 levels were measured in cell-free conditioned medium using ELISA. Cotreatment with hIL-17 and hTNF-α resulted in a potentiating effect on hIL-8 release (Student's paired, two-way *t* test: $p < 0.005$, compared with the sum of hIL-17 alone plus hTNF-α alone, $n = 6$). Pretreatment with hydrocortisone (1 μg/ml for 3 days) abolished the increase in hIL-8 caused by hIL-17 (100 ng/ml) (Student's paired, two-way *t* test: $p < 0.01$, $n = 6$). Data are mean values with SEM and refer to increase in hIL-8 above basal release in vehicle-treated cells. This basal secretion was 618 ± 48 pg/ml.

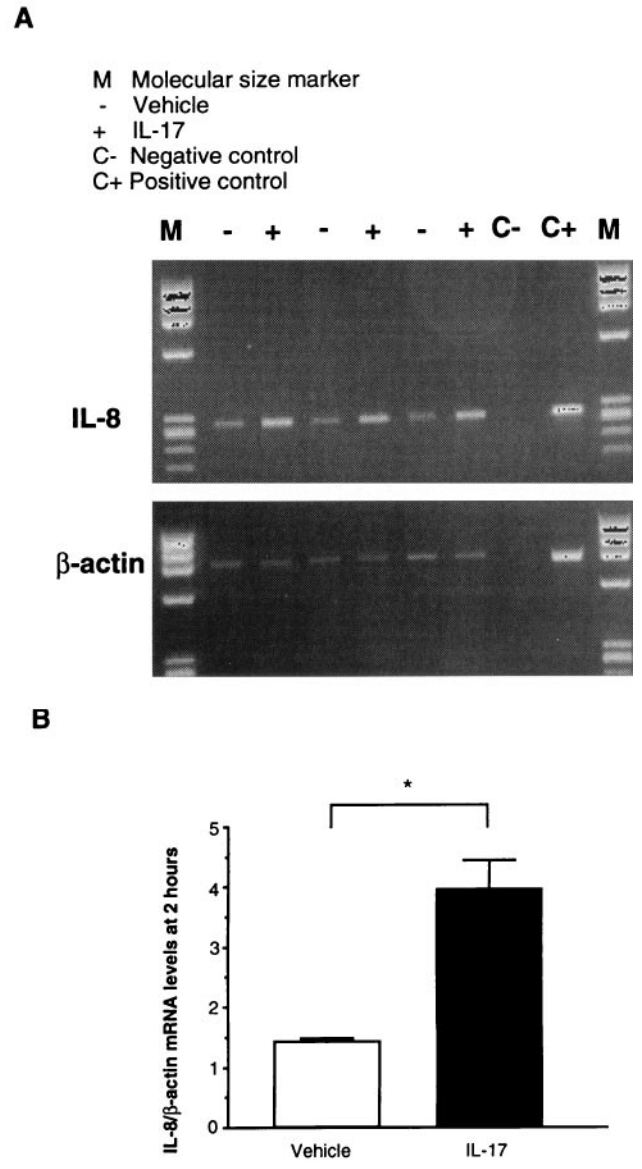

FIGURE 3. Expression of hIL-8 mRNA with and without 2-h treatment with hIL-17 (1000 ng/ml) in 16HBE cells. RT-PCR was performed as described in *Materials and Methods*. *A*, hIL-8 and β-actin mRNA expression in gel from three independent experiments. *B*, hIL-8/β-actin mRNA ratio as measured by photodensitometry. In *B*, data are mean values with SEM (Student's paired, two-way *t* test: $*$, $p < 0.05$; $n = 3$).

Effect of hIL-17 on hIL-8 mRNA levels. Treatment with hIL-17 (1000 ng/ml) for 2 h increased the hIL-8/β-actin mRNA ratio as determined by RT-PCR in 16HBE cells (Fig. 3).

Effect of hIL-17 on neutrophil chemotaxis. Conditioned medium from hIL-17 treated 16HBE cells significantly increased the migration of human neutrophils (Fig. 4). An anti-hIL-8 Ab blocked this effect on neutrophil migration (Fig. 4). By contrast, human IL-17 had no significant direct chemotactic effect on neutrophil migration in the concentration range of 1–1000 ng/ml (peak migration with 10 ng/ml: 146 $\pm$ 56% of migration induced by vehicle, Student's *t* test, paired, two-way: $p = 0.5$, $n = 4$). In addition, hIL-17 (10 ng/ml) had no additive effect (115 $\pm$ 23%) on neutrophil chemotaxis caused by 3×10^{-10} M of hIL-8 (Student's *t* test, paired, two-way: $p = 0.5$, $n = 4$, compared with hIL-8 alone).

In vivo experiments

Neutrophil recruitment induced by hIL-17. hIL-17 significantly and selectively (Fig. 5) increased the absolute number of neutrophils in rat BAL fluid at 4, 6, and 8 h after instillation i.t. (Fig. 6). No such increase in neutrophils was detected after 2 h. The effect of hIL-17 on neutrophil recruitment was significantly inhibited by an anti-hIL-17 Ab at 6 h after instillation (Fig. 7*A*).

Pretreatment of animals with an anti-rMIP-2 Ab blocked the increase in neutrophil numbers in BAL fluid from rats given IL-17 i.t. (Fig. 7*B*). Also, a significant increase in the level of rMIP-2 was detected in BAL fluid after exposure to hIL-17 (136 $\pm$ 31 ng/ml for vehicle and 565 $\pm$ 162 ng/ml for hIL-17, $p < 0.05$ according to Student's unpaired, two-way *t* test, $n = 9$).

Pretreatment with dexamethasone significantly decreased the number of neutrophils in BAL fluid from rats given hIL-17 i.t. as well (Fig. 7*C*).

Discussion

Not only the fact that anti-CD4$^+$ Abs (9), but also that anti-IL-2 receptor Abs (24) inhibit allergen-induced neutrophil recruitment in airways, provide strong evidence that CD4$^+$ T-lymphocytes are functionally important for inflammatory cell recruitment into the airways. It has been suggested that T lympocytes indirectly recruit neutrophils by releasing proinflammatory cytokines, such as TNF-α or IL-1β, which subsequently increase adhesion molecule expression and chemokine release (25, 26). However, T cells are not the dominating source of TNF-α or IL-1β (25), and therefore other cytokines may be involved in mediating the effect of T cells on neutrophils.

The current study now demonstrates that hIL-17, a cytokine derived from activated CD4$^+$ cells, increases the release of the major human neutrophil chemoattractant, the C-X-C chemokine, IL-8, in human bronchial epithelial and venous endothelial cells in vitro. The inhibition of hIL-8 release caused by cotreatment with hIL-17 plus an anti-hIL-17 Ab indicates a specific effect exerted by hIL-17 (i.e., not an effect due to protein deposition). The fact that hIL-8 mRNA expression is increased by hIL-17 in epithelial cells strongly indicates that hIL-17 induces de novo synthesis of hIL-8, and this is in line with recent data showing that hIL-17 activates

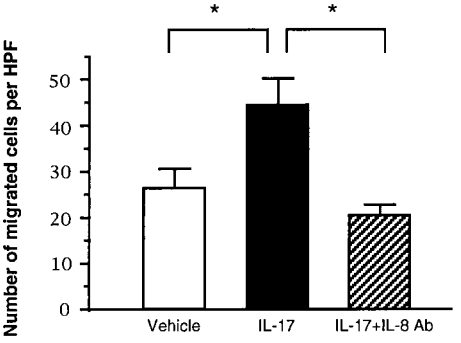

FIGURE 4. Human neutrophil migration in chemotaxis multiwell chamber caused by 20-min stimulation with conditioned medium from 16HBE cells treated with hIL-17. Conditioned media were obtained from 16HBE cells treated for 18 h with hIL-17 (1000 ng/ml) or vehicle. Conditioned medium from hIL-17-treated cells caused a significant increase (ANOVA, $F = 0.003$) in neutrophil migration vs vehicle (Fischer's PLSD: $p < 0.01$; $n = 6$). Fifteen minutes of coincubation of supernatants from hIL-17-treated cells with an anti-hIL-8 Ab (10 μg/ml) completely abolished this increase in neutrophil migration (Fischer's PLSD: $p < 0.01$; $n = 6$). Data are mean values with SEM and refer to the number of neutrophils per light microscope HPF.

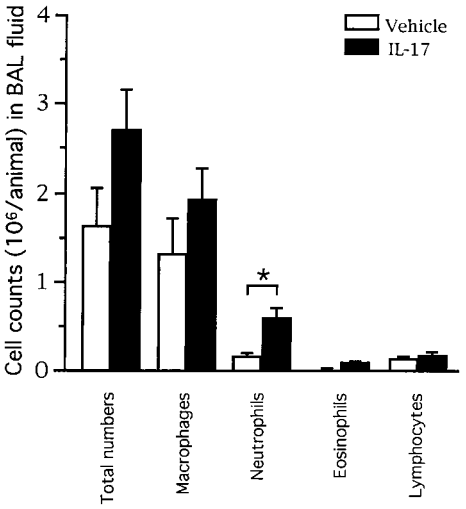

FIGURE 5. Effect of hIL-17 (1 μg/rat i.t.) on cell number in rat BAL fluid in vivo 8 h after administration. Data show the absolute number of cells (10^6 cells/rat) in BAL for each cell type and are expressed as mean values with SEM. The differential counts at other time points (2, 4, and 6 h) were similar to that at 8 h. hIL-17 significantly increased the absolute number of neutrophils alone (Student's unpaired, two-way *t* test: *, $p < 0.05$; $n = 9$–12).

NF-κB, a transcription factor initiating C-X-C chemokine expression (27). The functional relevance of the hIL-8 production in hIL-17 induced neutrophil recruitment in vitro is confirmed by the observation that conditioned medium from airway epithelial cells treated with hIL-17 causes neutrophil chemotaxis, whereas hIL-17 alone does not.

The current study also demonstrates that, in vivo, i.t. installation of hIL-17 causes selective recruitment of neutrophils into rat airways. This recruitment is inhibited by an anti-hIL-17 Ab, thus proving the specific effect of hIL-17 in vivo. The IL-17-induced neutrophil recruitment is accompanied by an increased level of the rat functional analogue of hIL-8, rMIP-2, in BAL fluid. In addition, an anti-rMIP-2 Ab inhibits this hIL-17-induced neutrophil recruitment. The time course of neutrophil recruitment in vivo induced by hIL-17 supports an indirect effect of hIL-17 via chemokine release. The 4-h lag in recruitment is consistent with the time required for de novo synthesis and release of C-X-C chemokines and subsequent neutrophil recruitment in the airways (28). We do

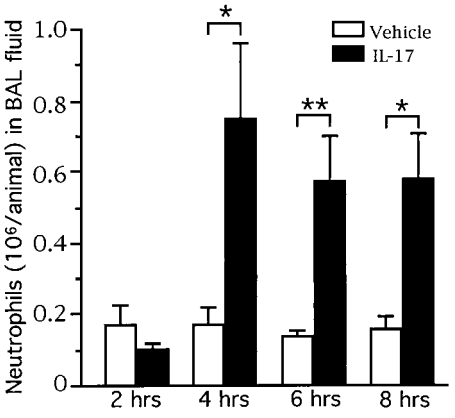

FIGURE 6. Time course of neutrophil number in rat BAL fluid after treatment with hIL-17 (1 μg/rat i.t.) in vivo. Data show the absolute number of neutrophils (10^6 cells/rat) and are presented as mean values with SEM (Student's unpaired, two-way *t* test: *, $p < 0.05$, and **, $p < 0.01$; $n = 4$–12).

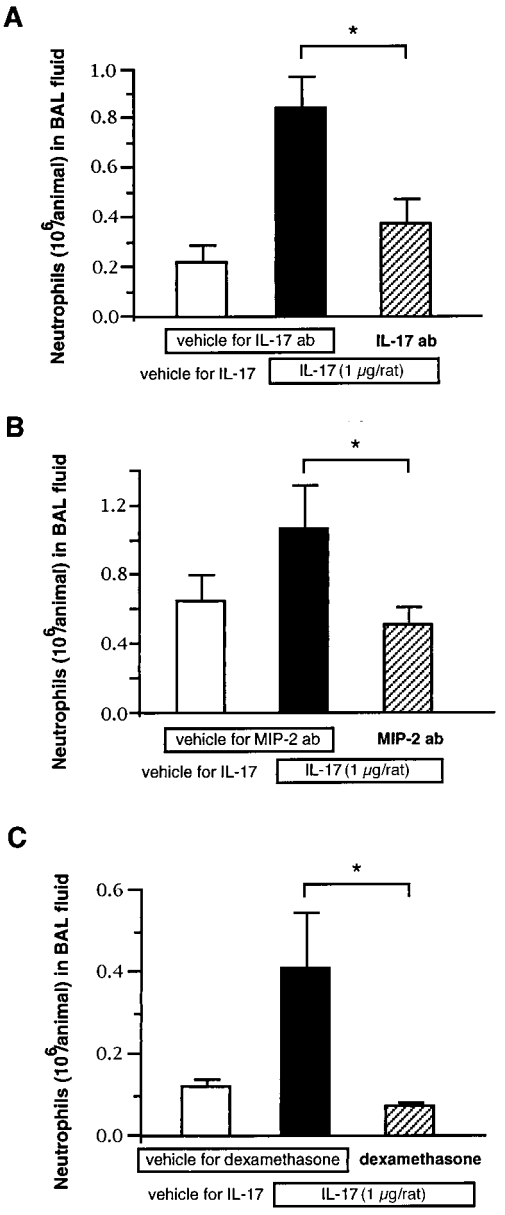

FIGURE 7. Effect of anti-hIL-17 Ab (*A*), anti-rMIP-2 (*B*), or dexamethasone (*C*) on hIL-17 induced neutrophil recruitment into airways in BN rat. Experiments were performed as described in *Materials and Methods*, and BAL was collected at 6 (*A* and *B*) or 8 (*C*) h, respectively. Cotreatment with an anti-hIL-17 Ab or pretreatment with anti-rMIP-2 Ab as well as pretreatment with dexamethasone significantly decreased the number of neutrophils in BAL recruited by hIL-17 (Student's unpaired t test, one-way: *, $p < 0.05$; $n = 6$–10).

not find it likely that there is a problem with interspecies cross-reactivity for hIL-17 and rat cells because separate experiments have demonstrated that mIL-17, given i.t. to Sprague Dawley rats, produces virtually the same level of selective neutrophil recruitment as does hIL-17 (unpublished data), and rIL-17 and mIL-17 display a 90% homology. Also, mIL-17 and rIL-17, as well as hIL-17 induce cytokine release in mouse stromal cells (29), which provides further support of interspecies cross-reactivity at the IL-17 receptor.

The notion that hIL-17 can recruit neutrophils via C-X-C chemokine release is consistent with the recent in vitro finding that hIL-17 releases hIL-8 from fibroblasts (14). Furthermore, a recent study has demonstrated the release of the neutrophil-activating

protein, hIL-6, caused by hIL-17 in airway epithelial cells (13). It is thus possible that IL-17 is indirectly involved in both chemotaxis and activation of neutrophils in the airways.

The level of the proinflammatory cytokine, hTNF-α, is increased in sputum of patients with airway inflammation (30). In addition to its ability to increase the expression of ICAM-1 in endothelial cells, hTNF-α also increases the production of other cytokines such as hIL-6 and hIL-8 (26). In the current study, the potentiating effect on the release of hIL-8 in airway epithelial cells caused by cotreatment with hIL-17 plus hTNF-α implies another potential mechanism for neutrophil recruitment. In fact, the effect of this combined treatment with hIL-17 and hTNF-α by far exceeded the effect caused by the maximally effective concentration of hTNF-α alone. The two pro-inflammatory cytokines IL-17 and TNF-α may therefore cooperate in causing C-X-C chemokine release from airway cells and, as a result, potentiate the subsequent neutrophil recruitment.

Glucocorticoids are widely utilized to inhibit inflammation in the airways. One important anti-inflammatory mechanism for this class of drugs is inhibition of cytokine production (31). In this study, a glucocorticoid (hydrocortisone) potently inhibited the release of IL-8 caused by hIL-17 in human bronchial epithelial and venous endothelial cells in vitro. Correspondingly, in vivo, another glucocorticoid (dexamethasone) also attenuated the neutrophil influx into rat airways caused by hIL-17. These observations indicate that glucocorticoids down-regulate C-X-C chemokine release and are consistent with previous findings using stimuli other than hIL-17 (31, 32). However, it cannot be excluded that glucocorticoids also reduce the number of IL-17 receptors present on bronchial epithelial or endothelial cells.

In conclusion, this study demonstrates that hIL-17, a cytokine released from activated $CD4^+$ cells, exerts a specific, pro-inflammatory effect by increasing C-X-C chemokine release in airway cells. As indicated by the neutrophil recruitment induced by hIL-17 both in vitro and in vivo, the IL-17-induced release of C-X-C chemokines is functionally significant in airways. In the case of hIL-8, the in vitro data indicate that the hIL-17 induced release is due to de novo synthesis. The in vitro data also indicate that hIL-17 potentiates the effect of the proinflammatory cytokine hTNF-α on hIL-8 release. Thus, if released in airways, IL-17 may link T-lymphocyte activation to the recruitment of neutrophils in airway inflammation.

Acknowledgments

The technical assistance of Mirjana Hahn-Zoric, Ph.D., is gratefully acknowledged.

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