

Increased elastase and myeloperoxidase activity associated with neutrophil recruitment by IL-17 in airways in vivo

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Background: A recent study demonstrated that intratracheal administration of the T-lymphocyte cytokine IL-17 recruits neutrophils into airways in vivo by C-X-C chemokine release. It is not known whether IL-17 may also activate airway neutrophils.

Objective: Our purpose was to evaluate whether IL-17 activates neutrophils in airways in vivo and, if so, whether the proinflammatory cytokine IL-1 β modulates this action of IL-17.

Methods: Intratracheal administration of human (h) IL-17 or rat (r) IL-1 β or hIL-17 plus rIL-1 β in anesthetized, spontaneously breathing rats was followed by bronchoalveolar lavage (BAL) 6 hours later. The BAL fluid was characterized in terms of neutrophil count, of the activity for myeloperoxidase (MPO), and in some cases of the activity for elastase (ELA). Isolated rat neutrophils were stimulated with hIL-17 in vitro, followed by characterization of MPO activity in the cell medium.

Results: hIL-17 (1 μ g) increased the ELA and the MPO activity, as well as the neutrophil count in BAL fluid, whereas the proinflammatory cytokine rIL-1 β (2.5 ng) did not. Pretreatment with rIL-1 β enhanced IL-17-induced ELA and MPO activity, without increasing the neutrophil count. The BAL ELA activity was inhibited by a specific inhibitor of neutrophil serine proteases. Stimulation with hIL-17 in vitro did not increase MPO activity in isolated neutrophils.

Conclusion: IL-17 can activate neutrophils in association with their recruitment into the airways in vivo and this effect is probably achieved through induced release of mediators from other airway cells. (*J Allergy Clin Immunol* 1999;104:143-9.)

Key words: Airway inflammation, IL-17, neutrophil activation

The recruitment and activation of neutrophils may play a pathogenetic role in the course of inflammatory airway diseases such as bronchial asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis.¹⁻³ It is known that neutrophil recruitment and activation can be stimulated in vitro by cytokines released from T lymphocytes, such as GM-CSF and TNF- α .⁴⁻⁶ However,

Abbreviations used

AEBSF:	4-(2-Aminoethyl)-benzensulfonylfluoride
BAL:	Bronchoalveolar lavage
COPD:	Chronic obstructive pulmonary disease
ELA:	Elastase
FMLP:	N-formyl-met-leu-phe
h:	Human
IT:	Intratracheal
m:	Mouse
MEOSAAPVNA:	Methoxysuccinyl-alanyl-alanyl-prolyl-valyl-paranitroanilide
MIP-2:	Macrophage inflammatory protein-2
MPO:	Myeloperoxidase
PLSD:	Protected least significant difference
r:	Rat
Suc-Ala ₃ -Na:	Succinyl-trialanine-nitroanilide

er, there is limited knowledge about how cytokines from T lymphocytes can contribute to the recruitment and activation of neutrophils in airways in vivo.

The T-lymphocyte cytokine, IL-17, can be produced by human CD4⁺ T cells from healthy subjects and subjects with contact dermatitis, as well as by CD4⁺ and CD8⁺ cells from mice.⁷⁻⁹ Human (h) IL-17 stimulates the secretion of cytokines, such as IL-6 in bronchial epithelial cells and IL-6 and IL-8 in fibroblasts,¹⁰ and hIL-17 probably acts by specific IL-17 receptors that are expressed in lung cells.¹¹ Hypothetically, hIL-17 released into airways should thus be capable of both mobilizing, recruiting, and activating neutrophils by the induced production of IL-6 and C-X-C chemokines in the mentioned cell types. In support of this hypothesis, we recently demonstrated that intratracheal (IT) administration of hIL-17 dose dependently and selectively recruits neutrophil into murine airways by C-X-C chemokine release.¹² It is not known, however, whether these neutrophils become activated in association with their recruitment by hIL-17 and, if so, whether the neutrophil priming cytokine IL-1 β may potentiate this type of activation.

The current study aimed to determine whether neutrophil recruitment by local administration of IL-17 in airways is associated with neutrophil activation and, if so, whether IL-1 β modulates the effect of IL-17. First, we compared the bronchoalveolar lavage (BAL) neutrophil count after IT instillation of hIL-17 with that after IT instillation of mouse (m) IL-17 to ascertain that species heterology is not a confounding factor for neutrophil recruitment in our rat airway model. Second, we

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assessed the effect of hIL-17 on neutrophil activation in the airways by determining the BAL fluid activity for elastase (ELA) and myeloperoxidase (MPO). Third, we compared the effect of hIL-17 and the species homologous cytokine rat (r) IL-1 β on BAL fluid neutrophil count and ELA and MPO activity. Fourth, we evaluated whether hIL-17 has a direct activating effect by determining MPO activity in isolated rat neutrophils in vitro.

METHODS

Animals

We used pathogen-free male Sprague-Dawley rats (range 300–400 g, B&K Universal AB, Stockholm, Sweden). Animals were maintained on a standard laboratory food with water ad libitum. The experiments were approved by the Ethical Committee for Animal Studies at Göteborg University (DNo 134/95).

IT instillation of cytokines in vivo

Rats were anaesthetized with 1 mL/kg of a 3:2 mixture of ketamine hydrochloride (50 mg/mL, Parke-Davis Scandinavia, Sweden) and xylazine chloride (20 mg/mL, Bayer Sverige AB, Göteborg, Sweden) intramuscularly. The trachea was intubated with a cannula (80 mm length and outer diameter 2.76 mm, Portex, Smiths Industries, Middlesex, UK). Rats were placed in a supine position with the head elevated. With use of a micropipette and an intravenous catheter inserted through the intubation cannula, 106 μ L of sterile PBS containing either hIL-17 (1 μ g, R&D systems Europe Ltd, UK), rIL-17 (1 μ g, R&D Systems Europe Ltd), or corresponding vehicle was instilled and flushed with 3 mL of air to deliver the solution to the lung. By use of this maneuver in separate experiments, we showed that an instilled dye solution is distributed evenly from the trachea down to small bronchi.

The dose of hIL-17 was chosen on the basis of separate dose-response experiments (data not shown) showing that 1 μ g of hIL-17 causes a significant and reproducible response in terms of neutrophil recruitment. The negative controls received the vehicle of the cytokines, consisting of sterile PBS with 0.21% BSA (grade V, Sigma Chemical, St Louis, Mo) and 0.1 ng of LPS (*Escherichia coli* serotype O55, B5, Sigma Chemical) because each cytokine per se contains LPS (less than 0.1 ng per 1 μ g of cytokine, according to the manufacturer). Separate experiment showed that 0.1 ng of LPS instilled IT does not significantly ($P = .83$, $n = 3-8$) increase the neutrophil count ($0.42 \pm 0.15 \times 10^6/\text{mL}$ vs $0.52 \pm 0.26 \times 10^6/\text{mL}$ for sterile PBS with 0.21% BSA). To determine a submaximal dose of rIL-1 β that recruits a similar number of neutrophils as does 1 μ g of hIL-17, we administered different doses of rIL-1 β (2.5, 5, 10.6, and 106 ng, R&D Systems Europe Ltd.) or the corresponding vehicle in another group of rats.

To assess the effect of neutrophil priming on the response to hIL-17 in vivo, rIL-1 β (2.5 ng) or the corresponding vehicle (see above) was instilled IT 15 minutes before the instillation of hIL-17 (1 μ g). After the IT instillation of the referred solutions, the animals regained consciousness and were returned to their cages.

BAL in vivo

BAL was performed 6 hours after the IT instillation of cytokines or corresponding vehicles. After a lethal dose of pentobarbital sodium was given (300 mg/kg intraperitoneally, Apoteksbolaget, Umeå, Sweden), a tracheal cannula (10 mm length and outer diameter 2.76 mm, Portex orange mount, Smiths Industries, Middlesex, UK) was inserted into the upper cervical trachea through a tracheotomy. The BAL fluid was collected in a plastic tube on ice after gentle, manu-

al instillation and withdrawal of five times 4 mL of sterile PBS with a syringe connected to the IT cannula (20 mL total volume). The BAL fluid was centrifuged at 200g during 10 minutes at 4°C (Eppendorf, Netheler, Germany), and the cell pellet was resuspended in washing buffer (sterile PBS containing 0.35% BSA and 0.1% glucose). Total cell counts were determined in hemocytometer with Türk solution. Differential cell counts were performed on cytospin preparations (Cytospin 3, Shandon Life Sciences, Astmor, UK) by May-Giemsa staining, according to standard morphologic criteria with use of oil immersion microscopy (original magnification $\times 1000$). Cell counts were carried out on 300 cells and the absolute number of each cell type was calculated.

For the measurement of ELA activity, a part of cell-free BAL fluid supernatant was concentrated 10-fold with Centricon-10 centrifugation filters (10-kD cutoff value, Amicon, Beverly, Mass) and stored at -80°C . The remaining unconcentrated supernatant was also pooled and stored at -80°C for the analysis of MPO activity.

ELA activity in BAL fluid supernatant

The ELA activity in the concentrated cell-free BAL fluid supernatant was measured with use of a synthetic elastase substrate, succinyl-trialanine-nitroanilide (Suc-[Ala₃]-Na, Sigma Chemical), according to the method of Bieth et al.¹³ Briefly, 0.5 mL of concentrated BAL fluid supernatant was added to an equal volume of 0.2 mol/L TRIS buffer (pH 8.0). This mixture was incubated with 20 μ L of 0.125 mol/L substrate at 37°C for 30 minutes. Optical density was measured at 410 nm by spectrophotometer (PU 8670 VUS/NIR, Phillips, Cambridge, UK) before and after the addition of the ELA substrate. A standard curve was constructed with known concentrations of porcine pancreatic ELA (pancreatopeptidase E, EC 3.4.21.36, Sigma Chemical). Results were expressed as nanomoles of substrate hydrolyzed per hour per milliliter of supernatant. Separate preliminary measurements with another ELA substrate, specific for human neutrophil ELA (methoxysuccinyl-alanyl-alanyl-prolyl-valyl-paranitroanilide [MEOSAAPVNA], Sigma Chemical),¹⁴ produced virtually the same results as did the (Suc-[Ala₃]-NA) ELA activity method. However, the reproducibility of the MEOSAAPVNA method was markedly weaker in comparison with the currently used method.

To determine whether the detected ELA activity is caused by serine proteases (derived mainly from neutrophils) or metalloproteases (derived mainly from macrophages), we examined inhibition of ELA activity in vehicle- and hIL-17-treated rats, respectively. Either a specific serine proteases inhibitor, 4-(2-aminoethyl)-benzenesulfonylfluoride (AEBSF) hydrochloride, 5 mmol/L (Sigma Chemical),^{15,16} or a metalloprotease inhibitor, EDTA (50 mmol/L, Sigma Chemical), was coincubated with BAL fluid supernatants for 30 minutes before addition of the synthetic ELA substrate (Suc-[Ala₃]-NA).

MPO activity in BAL fluid supernatant

MPO activity in BAL fluid supernatant was measured by use of a spectrophotometric assay.¹⁷ The MPO assay solution was made as follows: 0.1 mol/L sodium phosphate buffer at pH 7.0, 3.0 mL; 0.1 mol/L hydrogen peroxide (Sigma Chemical), 0.1 mL; guaiacol (Sigma Chemical), 0.048 mL; water, 26.9 mL. Two hundred microliters of unconcentrated BAL fluid supernatant was added to 800 μ L of assay solution and mixed rapidly. The absorbance was determined at 470 nm for 1 minute in a spectrophotometer, and the change of optical density per minute was calculated from the initial rate. MPO activity was expressed as milliunits per milliliter. One milliunit of enzyme is the amount that consumes 1 nmol/L of hydrogen peroxide per minute.

TABLE I. BAL total and differential cell counts in hIL-17- or mL-17-treated Sprague-Dawley rats

	BAL recovery volume (mL)	Total cell ($\times 10^6/\text{mL}$)	Macrophage ($\times 10^6/\text{mL}$)	Neutrophil ($\times 10^6/\text{mL}$)	Eosinophil ($\times 10^6/\text{mL}$)	Lymphocyte ($\times 10^6/\text{mL}$)
Vehicle (n = 4)	17.7 $\pm$ 0.20	1.87 $\pm$ 0.24	1.21 $\pm$ 0.21	0.65 $\pm$ 0.19	0.003 $\pm$ 0.002	0.015 $\pm$ 0.009
hIL-17 (n = 4)	18.0 $\pm$ 0.08	4.71 $\pm$ 1.10	1.98 $\pm$ 0.87	2.62 $\pm$ 0.39*	0.003 $\pm$ 0.002	0.003 $\pm$ 0.002
mIL-17 (n = 7)	17.9 $\pm$ 0.09	3.91 $\pm$ 0.49*	1.48 $\pm$ 0.39	2.41 $\pm$ 0.56*	0.006 $\pm$ 0.003	0.007 $\pm$ 0.006

Values are mean $\pm$ SEM.

* $P < .05$ compared with vehicle-treated group.

MPO activity in isolated rat neutrophils in vitro

Neutrophils were isolated from the blood of Sprague-Dawley rats anesthetized as described above. After cannulation of the carotid artery with an intravenous catheter, blood was drawn into glass tubes containing EDTA as an anticoagulant. Neutrophils were separated from whole blood as described previously.¹⁸ Briefly, whole blood was sedimented in the presence of 4.5% dextran T500 solution (Pharmacia, Uppsala, Sweden) to remove red blood cells, monocytes, and lymphocytes. The residual erythrocytes were lysed (0.8% ammonium chloride + 0.1% potassium carbonate), and the remaining neutrophils were washed twice with PBS. Finally, the neutrophils were brought to the final concentration of 5×10^6 cells/mL in PBS containing hIL-17 or rIL-1 β or the combination of hIL-17 plus rIL-1 β , N-formyl-met-leu-phe (FMLP), or vehicle (above). Neutrophils kept in 1-mL micro tubes (Sahstedt, Nümbrecht, Germany) were incubated in a shaking water bath at 37°C for 1 hour. After incubation, the neutrophils were spun down (5000 revolutions/min for 10 minutes) and cell supernatants were frozen at -80°C for later analysis of MPO levels (see above).

Statistical analysis

All data are expressed as mean with SEM. Data were analyzed with use of either the Student unpaired *t* test (2-way) or ANOVA followed by Fisher's protected least significant difference (PLSD) test. Fisher's PLSD was conducted only when $P < .05$ according to ANOVA. Each *P* value shown represent the *P* value for a single comparison with use of either Fisher's PLSD or the Student *t* test, as appropriate. The correlation between the dose of IL-1 β versus the BAL neutrophil count and between ELA and MPO activities was determined by use of linear regression. The value *n* equals the number of rats in each treatment group. A Macintosh computer (Apple Computer, Cupertino, Calif) equipped with standard statistical software (StatView, Abacus Concepts, Berkeley, Calif) was used.

RESULTS

hIL-17 and mL-17 versus BAL fluid neutrophil count

hIL-17 (1 μg) as well as mL-17 (1 μg) induced a significant and similar increase of the BAL fluid neutrophil count (Table I) 6 hours after IT instillation. Neither hIL-17 nor mL-17 caused any significant increase in the corresponding macrophage, eosinophil, or lymphocyte counts.

rIL-1 β versus BAL fluid neutrophil count

Similarly to hIL-17, rIL-1 β induced a significant increase of the BAL fluid neutrophil count 6 hours after IT instillation, and this increase was dose-dependent for rIL-1 β (Fig 1). The increase in neutrophils induced by

2.5 ng of rIL-1 β (14.7 pmol) and 1 μg of hIL-17 (6.3 nmol) displayed a similar order of magnitude.

hIL-17 and rIL-1 β versus ELA and MPO activity in BAL fluid supernatant

The ELA activity in the hIL-17-treated group was significantly higher than in the vehicle-treated group (Fig 2, A). In contrast, rIL-1 β alone did not increase the ELA activity at an equally effective dose (2.5 ng) in terms of neutrophil recruitment. Pretreatment with 2.5 ng of rIL-1 β significantly enhanced the hIL-17-induced ELA activity but not the BAL neutrophil count ($2.99 \pm 0.40 \times 10^6/\text{mL}$ for vehicle of rIL-1 β plus hIL-17, $3.33 \pm 0.51 \times 10^6/\text{mL}$ for rIL-1 β plus hIL-17, respectively, $P = .63$).

The MPO activity in the hIL-17-treated group was significantly higher than in the vehicle-treated group, but this was not the case in the IL-1 β -treated group (Fig 2, B). Pretreatment with 2.5 ng of IL-1 β significantly enhanced the hIL-17-induced MPO activity.

In the hIL-17-treated group the ELA activity correlated significantly with the MPO activity ($r = 0.83$, $P = .02$, $n = 7$).

In the vehicle-treated group, EDTA significantly attenuated the ELA activity, but this was not the case for AEBSF (Fig 3, A). In contrast, in the hIL-17-treated group both AEBSF and EDTA inhibited ELA activity significantly (Fig 3, B). The AEBSF-sensitive component of ELA activity also proved to be significantly higher in the hIL-17-treated group than in the vehicle group ($P = .02$). In contrast, we were unable to prove that the EDTA-sensitive component of ELA activity was different for these groups ($P = .07$).

In the hIL-17-treated group the AEBSF-sensitive ELA activity correlated significantly with MPO activity ($r = 0.95$, $P = .01$, $n = 5$). In contrast, there was no significant correlation between the EDTA-sensitive ELA activity and the MPO activity ($r = 0.125$, $P = .83$, $n = 5$).

MPO activity in isolated rat neutrophils in vitro

hIL-17 did not significantly increase MPO activity, whereas FMLP did (Fig 4).

DISCUSSION

This study on BAL from rat airways in vivo shows that the increased number of neutrophils in BAL that is induced by hIL-17 IT is associated with an increased

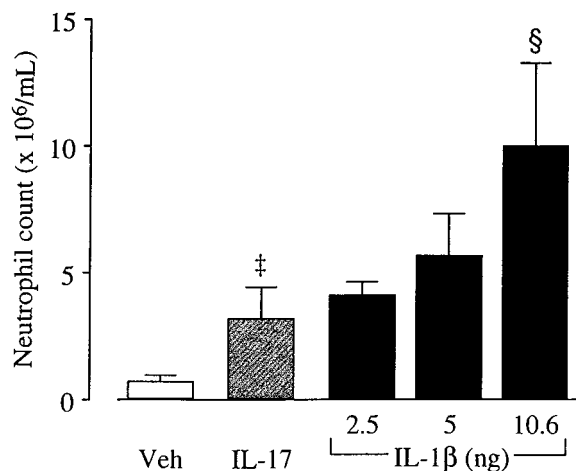


FIG 1. Effect of different doses of rIL-1 β compared with hIL-17 (1 μ g) and vehicle (see Methods) on BAL neutrophil count ($\times 10^6$ /mL) in SD rats 6 hours after IT instillation. Values shown are means with SEM bars. $n = 6$ all groups. Double dagger, $P < .01$ versus rats treated with vehicle of each cytokine (PBS containing 0.21% BSA and 1 ng/mL LPS); section symbol, $r = 0.76$, $P = .0001$.

activity for ELA and MPO. Pretreatment with rat IL-1 β enhances the hIL-17-induced ELA and MPO activity, without substantially increasing the BAL neutrophil count. Furthermore, hIL-17 exerts no direct activating effect on isolated rat neutrophils in vitro.

hIL-17 is a species heterologous cytokine that was used because recombinant rIL-17 protein was unavailable at the time of this study. We compared the effect of hIL-17 with that of mIL-17 to ascertain that there is interspecies cross-reactivity at the rIL-17 receptor, which was confirmed. This is not surprising because mIL-17 and rat IL-17 display a very high amino acid sequence homology (90%) and the glycosylation site is highly conserved for hIL-17, mIL-17, and rIL-17.⁹

Neutrophil ELA is important for killing gram-negative bacteria,¹⁹ and this protease is also a very potent secretagogue.²⁰ Neutrophil ELA may be responsible for hypersecretion in obstructive airway diseases because high levels of airway ELA are observed in asthma and COPD, diseases characterized by airway hypersecretion.²¹ Because azurophilic granules in neutrophils constitute the major source of ELA,²² an increased level of ELA activity in BAL fluid supernatant can be regarded as an indicator of neutrophil activation.²³

We now demonstrate that ELA activity in BAL fluid supernatant is increased in rats treated with hIL-17 (1 μ g) in vivo but not in rats treated with rIL-1 β (2.5 ng) in spite of the neutrophil count being increased to a similar degree by each of these different cytokines. To further characterize these cytokine-specific effects, we also measured ELA activity after treatment with rIL-1 β at a very high dose (106 ng), a dose that recruited approximately 8 times as many neutrophils as did the tested dose of hIL-17. We were unable, however, to detect any increased ELA or MPO activity after treatment with the high dose

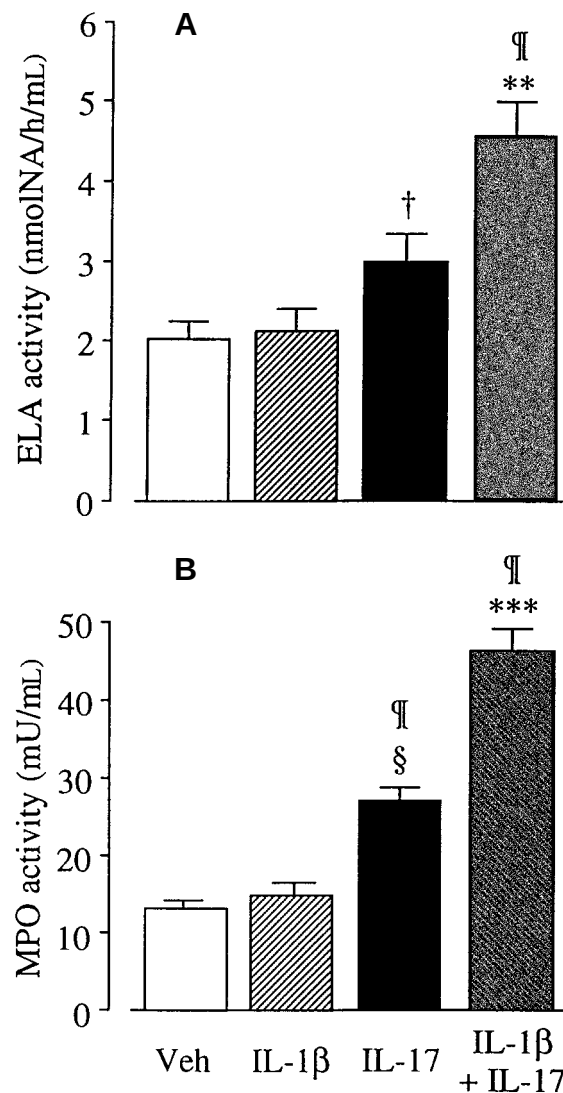


FIG 2. ELA activity (A) and MPO activity (B) in cell-free BAL fluid supernatants (see Methods) from Sprague-Dawley rats treated with either vehicle of cytokines (see Fig 1), rIL-1 β (2.5 ng), hIL-17 (1 μ g), or hIL-17 plus rIL-1 β (1 μ g and 2.5 ng, respectively) 6 hours after IT instillation. rIL-1 β was instilled 15 minutes before instillation of hIL-17. Values shown are means with SEM bars. $n = 6-7$. Dagger, $P < .05$ and paragraph symbol, $P < .01$ versus rats treated with vehicle of each cytokine. Two asterisk, $P < .005$ and three asterisks, $P < .001$ versus rats treated with hIL-17. Section symbol, $P < .001$ versus rats treated with rIL-1 β .

of rIL-1 β (data not shown). Thus, at the doses used in this study in vivo, rIL-1 β does not appear to activate neutrophils, which is compatible with previously published data.²⁴

In addition to neutrophils, macrophages may also release ELA under certain conditions.²⁵ We evaluated this hypothetical source of airway ELA in vehicle-treated rats and we found that the ELA activity was inhibited by the macrophage ELA inhibitor EDTA, but it was not inhibited by the neutrophil ELA inhibitor AEBSF. Thus

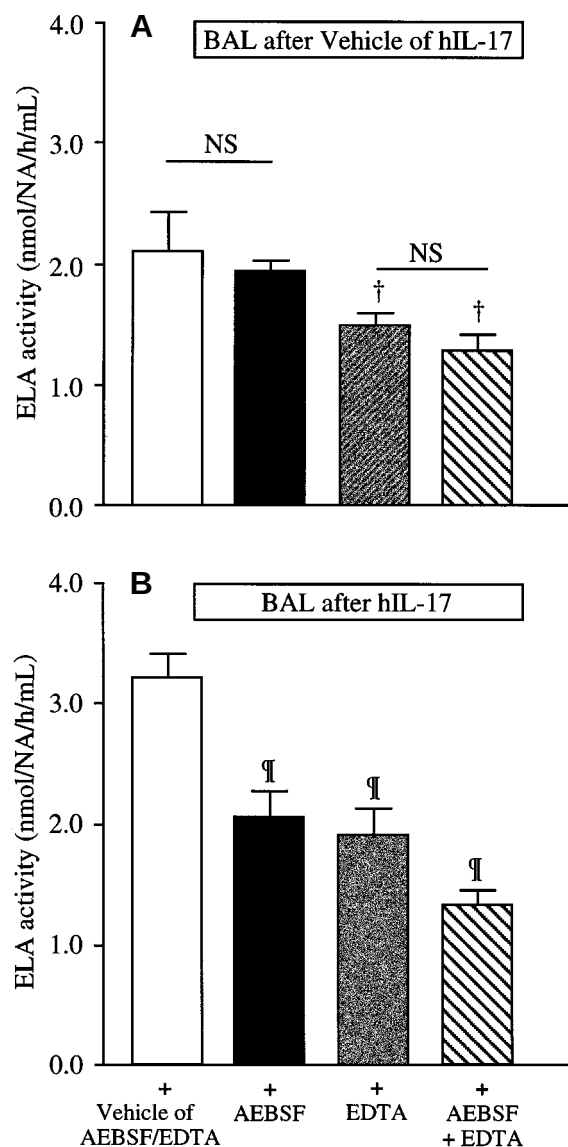


FIG 3. Inhibitory effect of serine protease inhibitor AEBSE (5 mmol/L) and metalloprotease inhibitor EDTA (50 mmol/L) or AEBSE plus EDTA (5 and 50 mmol/L, respectively) on ELA activity in cell-free BAL fluid supernatant. BAL fluid was harvested 6 hours after IT instillation of either vehicle of hIL-17 (**A**, see Fig 1) or hIL-17 (**B**). In hIL-17-treated rats, inhibition indexes (percent of baseline elastase activity in each group) of elastase activity were $-35.4\% \pm 6.44\%$ (AEBSE), $-40.9\% \pm 4.50\%$ (EDTA), and $-57.0\% \pm 5.70\%$ (AEBSE + EDTA), respectively. Values shown are means with SEM bars. $n = 5$. Dagger, $P < .05$; paragraph symbol, $P < .01$ versus cell-free BAL fluid supernatant treated with vehicle of AEBSE and EDTA. NS, Not significant.

macrophages account for the baseline ELA activity observed in the vehicle-treated rats. Possibly this baseline ELA activity is caused by the BSA and the LPS in the vehicle solution. In hIL-17-treated rats, however, ELA activity in BAL fluid supernatant was inhibited partially by the neutrophil ELA inhibitor AEBSE and partially by the macrophage ELA inhibitor EDTA. We also

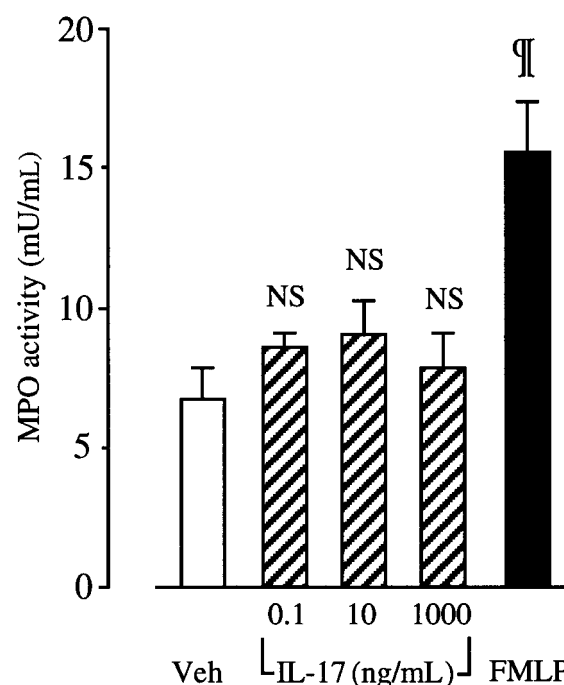


FIG 4. Effect of hIL-17 and FMLP (0.1 μ mol/L) on MPO activity in cell medium from Sprague-Dawley rat neutrophils in vitro. Values shown are means with SEM bars. $n = 6-9$. Paragraph symbol, $P < .01$. NS, Not significant.

found that the ELA activity was further depressed by cotreatment with AEBSE and EDTA in hIL-17-treated rats. Neutrophils therefore seem to account for the increased ELA activity in the hIL-17-treated rats.

MPO is another marker of neutrophil activation, although less specific than neutrophil ELA. MPO is a catalyzing enzyme that metabolizes superoxide radicals being produced during neutrophil activation.²⁶ In the current study the MPO activity in BAL fluid supernatant was increased in IL-17-treated rats only, and this MPO activity correlated significantly to ELA activity in BAL fluid supernatant. Noteworthy, we found an even stronger correlation between MPO activity and neutrophil ELA activity (the AEBSE-sensitive component), whereas there was no significant correlation between MPO activity and macrophage ELA activity (the EDTA-sensitive component). Taken together, these findings indicate that neutrophils can be activated by hIL-17 in airways in vivo; these neutrophils release ELA and probably MPO as well.

A recent clinical study shows that the level of the proinflammatory cytokine IL-1 β is increased in the airways during status asthmaticus.²⁷ This is of particular interest in view of the increased number of neutrophils in the airways during status asthmaticus² and the priming action of IL-1 β on neutrophil activation.^{28,29} In our study we now show that rIL-1 β primes for the release of neutrophil ELA and MPO caused by hIL-17 in the airways in vivo. Separate experiments showed no corresponding priming effect of rIL-1 β on neutrophil activation caused by macrophage inflammatory protein-2 (MIP-2) (data

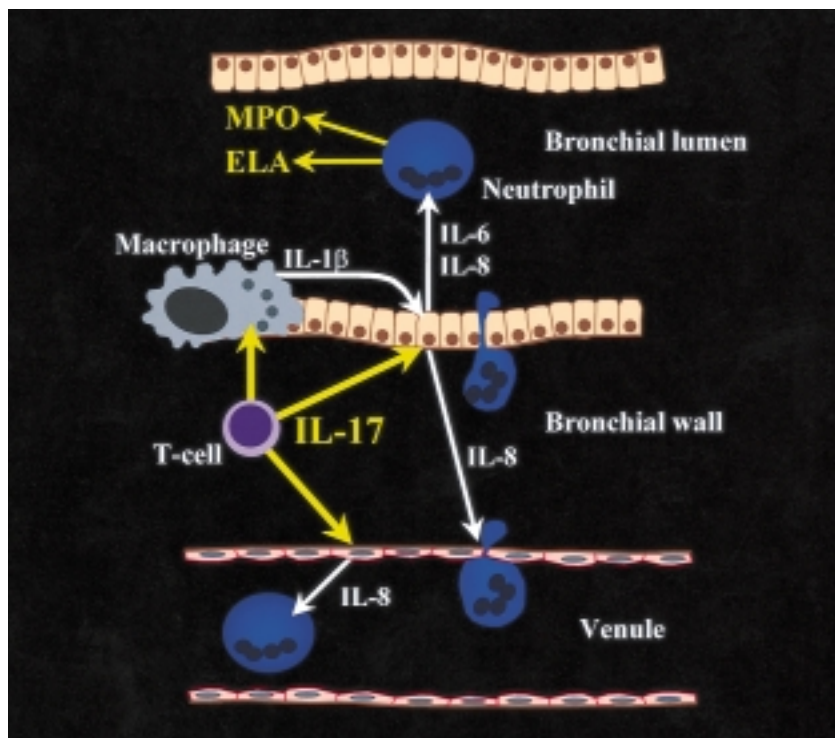


FIG 5. Hypothetic immunologic mechanisms behind the proinflammatory effect of IL-17 in airways.

not shown). Taken together, these findings suggest that the priming effect of IL-1 β is selective; IL-1 β can potentiate the neutrophil-activating effect of IL-17 but not that of a C-X-C chemokine (MIP-2) in the airways.

Finally, we were unable to demonstrate any significant increase of MPO activity in isolated rat neutrophils exposed to hIL-17 in vitro, whereas the bacterial neutrophil activator FMLP did increase this MPO activity in our experimental setup. This suggests that there is no direct effect exerted by IL-17 on neutrophil activation, in analogy with the lack of direct chemotactic effect of hIL-17 on human neutrophils in vitro.¹³ Because IL-17 facilitates the release of several potentially neutrophil-activating cytokines, such as IL-6 and IL-8, from the bronchial epithelium, fibroblasts, or other cell types present in the airways,¹⁰ it is likely that IL-17 activates airway neutrophils through the induced release of such cytokines. For example, it is possible that IL-17 stimulates airway macrophages to release IL-1 β ³⁰ and that this IL-1 β potentiates IL-17's increasing effect on the release of IL-6 and IL-8 in bronchial epithelial cells³¹ (see also hypothetical diagram in Fig 5).

In conclusion, if IL-17 is released in the airways as indicated in a recent preliminary report,³² this CD4⁺ cell cytokine can activate neutrophils in association with their recruitment into airways in vivo. This activating effect is potentiated by the neutrophil-priming cytokine IL-1 β and is probably mediated by local, induced release of neutrophil-activating cytokines other than IL-17.

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