Interleukin-17 Stimulates the Expression of Interleukin-8, Growth-Related Oncogene-α, and Granulocyte–Colony-Stimulating Factor by Human Airway Epithelial Cells

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Interleukin (IL)-17 is a recently discovered cytokine, which is proposed to play a role in neutrophilic airway inflammation via the release of proinflammatory cytokines and chemokines. To evaluate the role of IL-17 in inflammatory protein production from the airway epithelium, we have analyzed the effects of IL-17 on primary human bronchial epithelial cells (HBECs). Using gene arrays, changes in gene expression in response to IL-17 stimulation were investigated and only IL-8, growth-related oncogene (Gro)α, and granulocyte colony-stimulating factor (G-CSF) were found to be upregulated. Secretion of IL-8, Groα, and G-CSF in response to IL-17 was measured in HBEC cell culture supernatants by enzyme-linked immunosorbent assay. Upregulation of Groα, IL-8, and G-CSF was observed to be 8-, 5-, and 8-fold, respectively, after 48 h stimulation with IL-17. When tested at equivalent concentrations, IL-17 was found to be 2- to 3-fold more potent than tumor necrosis factor (TNF)-α in stimulating release of Groα and G-CSF from HBECs. In addition, IL-17 was found to synergistically enhance TNF-α–induced production of IL-8, Groα, and G-CSF. It is proposed that IL-17 may play an important role in neutrophil recruitment via stimulating the release of IL-8, Groα, and G-CSF from airway epithelial cells.

Interleukin (IL)-17 is a recently described cytokine which may play a role in the pathogenesis of respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD) (1, 2). Airway neutrophilia is a prominent feature of these diseases, but the mechanisms resulting in the observed neutrophilia are not well characterized. Elevation of the neutrophil chemoattractant IL-8 in bronchoalveolar lavage of patients with COPD, as well as in patients with acute severe asthma, has been correlated with increased neutrophil numbers (3, 4). In addition to elevated neutrophil numbers, the presence of markers of neutrophil activation myeloperoxidase and human neutrophil lipocalin have been observed (5). Tumor necrosis factor (TNF)-α is known to stimulate the release of IL-8 from airway epithelial cells (6), and it is proposed that IL-17 may also play an important role because it has recently been demonstrated to be upregulated in asthmatic airways (7). However, there are no studies reported yet which have examined the IL-17 levels in patients with COPD.

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Abbreviations: base pair(s), bp; enzyme-linked immunosorbent assay, ELISA; glyceraldehyde-3-phosphate dehydrogenase, GAPDH; granulocyte colony-stimulating factor, G-CSF; growth-related oncogene-α, Groα; human bronchial epithelial cell, HBEC; interleukin, IL; reverse transcription–polymerase chain reaction, RT-PCR; tumor necrosis factor-α, TNF-α.

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IL-17 is released only from activated human CD4+ T-lymphocytes (8) or by CD4+ and CD8+ cells in mice (9). However, more recently IL-17 has been reported to be released from peripheral blood eosinophils (7), particularly those isolated from individuals with asthma. In contrast, the IL-17R is widely distributed and is found expressed on a wide range of cells, including an A549 lung epithelial cell line, peripheral blood mononuclear cells, a THP-1 monocytic cell line, and a 293 human embryonic kidney cell line (10). IL-17 has been reported to act on a wide range of cells to induce the production of a variety of proinflammatory mediators and mediators of hematopoiesis (11). These include the IL-17–induced release of IL-1β and TNF-α from human macrophages (12); IL-6, IL-8, and Groα production and intercellular adhesion molecule (ICAM)-1 expression by human fibroblasts (7, 8); and IL-8 release from a human airway epithelial cell line (13). IL-17 has also been shown to be involved in the production of other inflammatory mediators, including G-CSF, nitric oxide, COX-2, and prostaglandin E2 from a variety of cell types (11, 14–16). In addition to these effects *in vitro*, a role for IL-17 in regulating the inflammatory response *in vivo* has been reported. The intratracheal installation of hIL-17 in an *in vivo* rat model has been shown to result in neutrophil recruitment into the airways via the release of the hIL-8 homolog MIP-2 (13). IL-1β has also been demonstrated to play a role in neutrophil recruitment to the airways in a rat model but, unlike IL-17, did not result in neutrophil activation as measured by the release of MPO and elastase (17).

The airway epithelium is an important source of inflammatory mediators and is believed to be involved in regulating airway inflammation (18). We report here an analysis of the effects of IL-17 on primary human bronchial epithelial cells (HBECs). IL-17–induced expression of IL-8, Groα, and G-CSF mRNA was demonstrated and IL-17 was also shown to potently induce production of IL-8, Groα, and G-CSF proteins. The combined synergistic effect of IL-17 and TNF-α was also examined. These results together suggest that IL-17 may play an important role in stimulating the release of IL-8, Groα, and G-CSF from the airway epithelium resulting in neutrophilic infiltration into the airways.

Materials and Methods

Materials

Human TNF-α, human IL-17, and anti-human IL-17 antibody were all purchased from R&D Systems (Abingdon, Oxfordshire, UK). All enzyme-linked immunosorbent assay (ELISA) antibodies and standards were purchased from R&D Systems except the avidin-peroxidase (HRP) conjugate which was purchased from Sigma (Poole, UK). Dexamethasone was purchased from Sigma.

Culture of Primary HBECs

Primary normal HBECs and media were purchased from Biowhittaker (Wokingham, UK). Cells were maintained as described by the manufacturer in bronchial epithelial (BEBM) growth medium supplemented with 52 μg/ml bovine pituitary extract, 0.5 μg/ml hydrocortisone, 0.5 ng/ml human recombinant epidermal growth factor, 0.5 μg/ml epinephrine, 10 μg/ml transferrin, 5 μg/ml insulin, 6.5 ng/ml retinoic acid, 50 μg/ml gentamicin, 50 μg/ml amphotericin-B, and 6.5 μg/ml triiodothryonine. Cells were grown at 37°C in an atmosphere of 5% CO_2 and used between passages 3 and 5. Cells were seeded at a density of 3,500 cells/cm^2 and fed with growth medium until 80–90% confluent. For all IL-17 or TNF-α stimulations cells were grown to 50–60% confluence before serum starvation overnight.

RNA Preparation and Reverse Transcriptase–Polymerase Chain Reaction

HBECs were grown to ~ 90% confluence in T175-cm^2 flasks for RNA preparations. Cells were serum-starved overnight then stimulated with either IL-17 (200 ng/ml) or TNF-α (20 ng/ml), or were left unstimulated. Cells were harvested and lysed in 1 ml Trizol (Gibco Ltd., Paisley, UK). Total RNA preparations were performed using the protocol described by Gibco Ltd. The final RNA pellet was resupended in 25 μl RNase-free water. For reverse transcriptase (RT)-polymerase chain reaction (PCR) and hybridizations, RNA was treated with DNase to remove any residual genomic DNA contamination. RNA (25–50 μg) was treated with 20 U of RNase-free DNase I (Promega, Southampton, UK) in the presence of RNasin (40 U) at 37°C for 30 min. The RNA was then phenol/chloroform-extracted and ethanol-precipitated before use.

For RT-PCR analysis, first-strand cDNA was synthesized from 1 μg of total RNA in a total reaction volume of 20 μl using random primers, reagents, and conditions supplied in the first-strand cDNA synthesis kit for RT-PCR (AMV) from Roche Molecular Biochemicals (Lewes, UK). For PCR, each reaction mixture contained 0.2 mM dNTPs, 1× PCR buffer containing 1.5 mM $MgCl_2$, 0.5 U Taq DNA polymerase (Roche Molecular Biochemicals), 50 pmol each primer, 2 μl of first-strand cDNA, and deionized water in a 50-μl reaction volume. TNF-α, G-CSF, and IL-8 RT-PCR primers were purchased from Stratagene (Amsterdam Zuidoost, the Netherlands) and gave PCR products of expected sizes 354 bp, 471 bp, and 200 bp, respectively. Other primers were designed from published sequences as follows: ICAM-1 primer forward: 5′ AAA GTC ATC CTG CCC CGG GG 3′, and reverse: 5′ AGG GCA GTT TGA ATA GCA CA 3′. The expected size of the ICAM-1 PCR product was 189 bp. Groα primer forward: 5′ ACT CAA GAA TGG GCG GAA AG 3′, and reverse: 5′ TGG CAT GTT GCA GGC TCC T 3′. The expected size of the Groα PCR product was 468 bp. Cycling conditions were as follows: 94°C for 2 min, annealing for 15 s, 72°C for 30 s for 1 cycle, followed by 34 cycles of 94°C for 15 s, annealing for 15 s, 72°C for 30 s. Annealing for all the primer pairs was performed at 55°C except for TNF-α where an annealing temperature of 50°C was used. Reactions were analyzed on 2% agarose gels and stained with ethidium bromide. Identity of PCR products was confirmed either by DNA sequencing or by restriction enzyme digestion of purified PCR products. Control RT-PCR reactions were performed with primers specific to the housekeeping gene glyceraldehyde-3 phosphate dehydrogenase (GAPDH) with primers purchased from Stratagene and using conditions suggested by the manufacturer.

Hybridizations

Total RNA which had been DNase-treated was used in filter hybridization experiments. Custom made Atlas nylon filters which contained cDNAs from 219 genes arrayed on a nylon membrane were obtained from Clontech (Basingstoke, UK). These filter cDNAs included 9 housekeeping genes, 3 negative controls, and 207 genes which were mainly inflammatory genes. RNA probes were prepared using the Atlas kit (Clontech) according to the manufacturer's instructions. In brief, total RNA (2–5 μg) was reverse transcribed in the presence of [α-^{32}P]dATP to generate a labeled probe. The probe was purified using Clontech NucleoSpin extraction columns, according to the manufacturer's instructions, and denatured before use. Hybridizations were performed in ExpressHyb (Clontech) at 68°C in the presence of 0.1 mg/ml denatured herring sperm DNA. After hybridization, the membrane was washed four times at 68°C, each wash for 30 min, in 2× SSC, 1% SDS. The membrane was then washed once with 0.1× SSC, 0.5% SDS for 30 min at 68°C followed by a final 5-min wash with 2× SSC at room temperature. The filter was exposed to a phosphorimager screen for 5 d. Images were processed using a STORM 840 Phosphorimager (Molecular Dynamics, Amersham Place, UK) and quantified using ImageQuant 5.0 software (Molecular Dynamics).

Measurement of IL-8, Groα, G-CSF, and IL-6 in Cell Culture Media

For protein measurements in cell culture supernatants, HBECs were grown in 12-well plates and serum-starved overnight before stimulation. Immediately before stimulation, the medium was replaced with fresh serum-free medium and cells stimulated for 48 h with TNF-α or IL-17, or were not stimulated. Controls consisted of unstimulated cells with an equal volume of medium added to the cells in place of cytokine. For cells treated with compound, compound dissolved in dimethyl sulfoxide was added to a final concentration of 0.1–20 μM and controls were performed with dimethyl sulfoxide only added to a final concentration of 0.2%. For experiments with an anti–IL-17 blocking antibody, the antibody was premixed with IL-17 and incubated 1 h at 37°C before addition to the cells. The final concentration of IL-17 in the assay was 100 ng/ml and of antibody 0–4 μg/ml. G-CSF and IL-6 concentrations in cell culture supernatants were measured by sandwich ELISA using a G-CSF or IL-6 ELISA kit purchased from R&D Systems. For measurement of IL-8, a 96-well immunosorb plate was coated overnight with 100 μl/well of an anti–hIL-8 monoclonal antibody diluted to 5 μg/ml in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6). Plates were washed three times with wash buffer (PBS, pH 7.4, 0.05% tween-20) then incubated with hIL-8 standards (0–10 μg) or cell culture supernatants diluted in Buffer A (wash buffer containing 2% FCS). After incubation at 37°C for 2 h the plate was washed as described previously. To each well was added 100 μl of biotinylated anti-human IL-8 polyclonal antibody (0.05 μg/ml) diluted in Buffer A. This was then incubated at 37°C for 2 h. The plate was again washed before adding 100 μl/well of avidin peroxidase conjugate diluted 1:50,000 in Buffer A followed by incubation at 37°C for 1 h. The plate was again washed before adding 100 μl of BM Blue POD substrate (Roche Molecular Biochemicals) for 10 min at room temperature. The reaction was stopped by adding 50 μl of 1 M H_2SO_4. The absorbance at 450 nm, corrected for background measured at 650 nm, was measured spectrophotometrically. Readings were corrected for a blank, which contained no IL-8. The amount of IL-8 in each sample calculated from the standard curve. For measurement of Groα concentrations in cell culture supernatants, the assay was performed as described for IL-8 except that Groα standards and anti-human Groα antibodies were used in place of the IL-8 standard and antibodies.

Statistical Analysis

The data presented are the means ± SEM of at least three experiments. Two sample *t* tests were performed to determine if there

were significant differences between control and treatment groups (*$P < 0.05$, **$P < 0.001$).

Results

IL-17 Stimulates Expression of Groα, IL-8, and G-CSF mRNA in HBECs

To establish the effects of IL-17 on gene expression in primary HBECs, total RNA was isolated from cells stimulated for 6 h with either IL-17 or TNF-α or from unstimulated cells. Nylon filters containing immobilized cDNAs from 207 genes coding for many cytokines, chemokines, proteins, and enzymes involved in the inflammatory response, were hybridized with cDNA probes prepared from the total RNA. The results are summarized in Table 1. IL-17 induced a greater than 2-fold upregulation of only 3 out of the 207 genes on the filter. In contrast, six genes were induced greater than 2-fold by TNF-α treatment (Table 1). IL-8 gene expression was found to be upregulated by both IL-17 and TNF-α by 2.5- and 3.2-fold, respectively. Groα and G-CSF were found to be potently induced by IL-17 by 8.5- and 17.8-fold, respectively. Groα and G-CSF were also upregulated by TNF-α but to a much lesser extent. In contrast, ICAM-1, TNF-α, and superoxide manganese dismutase (SOD-2) were upregulated greater than 2-fold by TNF-α; however, no such induction was observed in the case of IL-17.

To confirm the observed effects of IL-17 on mRNA expression, RT-PCR analysis was performed using primers specific for Groα, G-CSF, IL-8, and ICAM-1. A similar upregulation of IL-8 mRNA was observed with both IL-17 and TNF-α compared with unstimulated cells, as can be seen in Figure 1. In contrast, IL-17 was found to dramatically induce Groα and G-CSF mRNA expression, to a greater extent than TNF-α, confirming our findings using the gene arrays. A GAPDH control indicated the presence of similar mRNA levels in all samples. When analyzed by RT-PCR, TNF-α potently induced expression of ICAM-1, whereas IL-17 had no effect on ICAM-1 expression. The effects observed for IL-17 on IL-8, Groα, G-CSF, and ICAM-1 gene expression were also confirmed in total RNA isolated from HBECs from a second donor (Figure 1).

TABLE 1

Effect of IL-17 and TNF-α on gene expression in HBECs

	-Fold Induction	
Gene Name	IL-17	TNF-α
IL-8	2.5	3.2
Groα	8.5	2.1
G-CSF	17.8	1.9
ICAM-1	0.3	8.3
TNF-α	0.2	2.2
SOD-2	1.0	2.9

HBECs were stimulated with IL-17 (200 ng/ml) or TNF-α (20 ng/ml) for 6 h before total RNA isolation. cDNA probes were prepared from the total RNA and hybridized to nylon filters with immobilized cDNAs from 219 genes as described in MATERIALS AND METHODS. Genes upregulated > 2-fold by either TNF-α or IL-17 stimulation are shown. -Fold induction is relative to the housekeeping gene GAPDH.

Effect of IL-17 on Groα, IL-8, G-CSF, and IL-6 Production from HBECs

To establish the effects of IL-17 on Groα, IL-8, G-CSF, and IL-6 protein release, ELISAs were used to analyze cell culture supernatants from IL-17–stimulated HBECs. IL-17 induced a time-dependent increase of IL-8, Groα, and G-CSF production between 0 and 48 h (data not shown). To analyze the concentration-dependent effects of IL-17, HBECs were treated with IL-17 at concentrations between 0 and 1,000 ng/ml for 48 h. IL-17 was found to stimulate a dose-dependent and significant increase in IL-8, Groα, and G-CSF production (Figure 2). A 4.6-, 7.5-, and 8.1-fold induction of IL-8, Groα, and G-CSF, respectively, over basal levels were observed with 10 ng/ml IL-17 at 48 h. A concentration of 10 ng/ml IL-17 induced levels of IL-8, Groα, and G-CSF of 3.98 ± 0.33, 26.67 ± 4.76, and 4.91 ± 1.00 ng/ml, respectively. IL-17 stimulated HBEC cell culture supernatants were also analyzed for IL-6 protein levels by ELISA and the results are shown in Figure 3. A 3.6-fold upregulation of IL-6 was seen after IL-17 stimulation for 48 h, with levels of 0.63 ± 0.04 ng/ml measured.

The effect of an anti–hIL-17 antibody on IL-17–induced chemokine production was evaluated. The antibody was tested at concentrations of 0–4 μg/ml and cells stimulated with the antibody/cytokine mixture for 48 h. The blocking anti–hIL-17 antibody was found to significantly inhibit the IL-17–induced release of IL-8 and Groα at concentrations of 2 and 4 μg/ml (Figure 4). At these antibody concentrations, production of IL-8 and Groα was reduced to near basal levels. IL-17, which had been heat-inactivated at 100°C for 10 min, was also examined for its effect on IL-8 release from HBECs. The IL-8 protein levels were found to be similar to basal levels (data not shown), indicating that IL-17, and not trace amounts of endotoxin in the IL-17 preparation, is responsible for the observed mediator release.

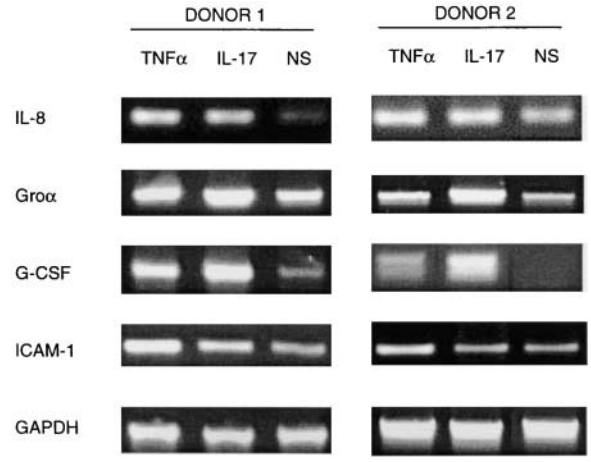

Figure 1. RT-PCR analysis of IL-17– or TNF-α–stimulated IL-8, Groα, G-CSF, and ICAM-1 expression in HBECs. HBECs were stimulated with TNF-α (20 ng/ml) or IL-17 (200 ng/ml) for 6 h or unstimulated (NS), total RNA was isolated, and RT-PCR analysis performed. Representative gels indicating IL-8, Groα, G-CSF, ICAM-1, and GAPDH expression are shown. Cells from two donors were analyzed.

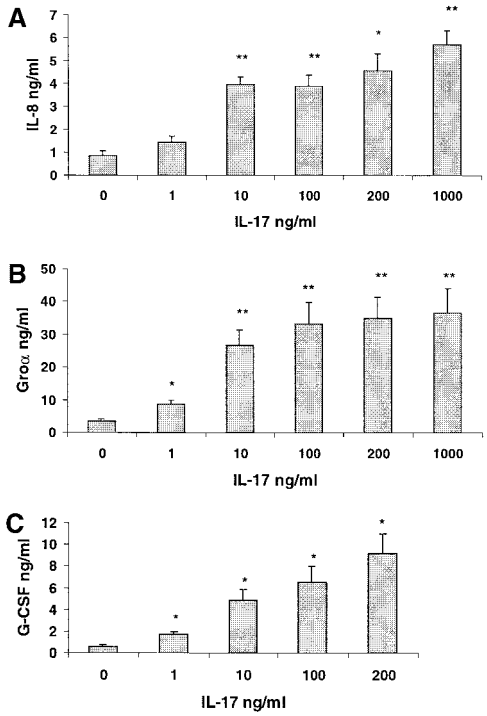

Figure 2. Effect of increasing concentrations of IL-17 on IL-8, Groα, and G-CSF production from HBECs. HBECs were stimulated with IL-17 at 0, 1, 10, 100, 200, and 1,000 ng/ml for 48 h. IL-8 (*A*), Groα (*B*), and G-CSF (*C*) concentrations in cell culture supernatants determined by ELISA are shown. Data shown are for experiments performed on cells isolated from at least two donors and are expressed as mean ± SEM for $n = 5$–9, where $^{*}P < 0.05$ and $^{**}P < 0.001$.

IL-17 Synergistically Enhances TNF-α–induced IL-8, Groα, and G-CSF Release from HBECs

The effects of IL-17 (10 ng/ml) and TNF-α (10 ng/ml) alone and in combination on the release of IL-8, Groα, and G-CSF were evaluated. IL-17 was found to be more potent than an equivalent concentration of TNF-α at stimulating release of Groα. Whereas IL-17 stimulates an 8.8-fold increase in Groα production over basal levels, TNF-α induces only a 4.3-fold induction. The combination of TNF-α and IL-17 induced a statistically significant 2.0-fold greater increase in Groα production than the calculated additive value, indicating synergy between the two cytokines (Figure 5). In addition, the level of Groα released by the combination of IL-17 and TNF-α (61.61 ± 3.13 ng/ml) was greater than the maximum level which could be induced by IL-17 alone (21.27 ± 1.85 ng/ml).

The combination of TNF-α and IL-17 on G-CSF production from HBECs produced a similar synergistic effect to that observed with Groα (Figure 5). The combination of TNF-α and IL-17 gave rise to a significantly higher level of G-CSF (19.22 ± 1.98 ng/ml) than the calculated additive value for the two cytokines alone (4.42 ± 1.12 ng/ml). The production of IL-8 was also synergistically enhanced by a combination of TNF-α and IL-17. Once again, the combination of TNF-α and IL-17 gave rise to a significantly higher level of IL-8 (24.94 ± 2.12 ng/ml) than the calculated additive value for the two cytokines alone (6.25 ± 1.04 ng/ml) and is shown in Figure 5.

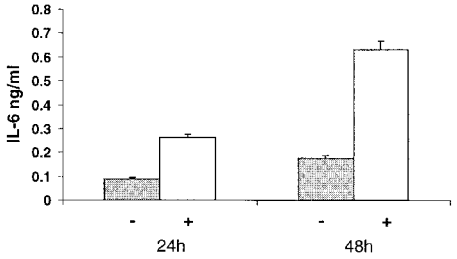

Figure 3. Effect of IL-17 on IL-6 production from HBECs. HBECs were either unstimulated or stimulated with IL-17 at 50 ng/ml for 24 or 48 h. IL-6 concentrations in cell culture supernatants determined by ELISA are shown. Data is expressed as mean ± SEM for $n = 4$.

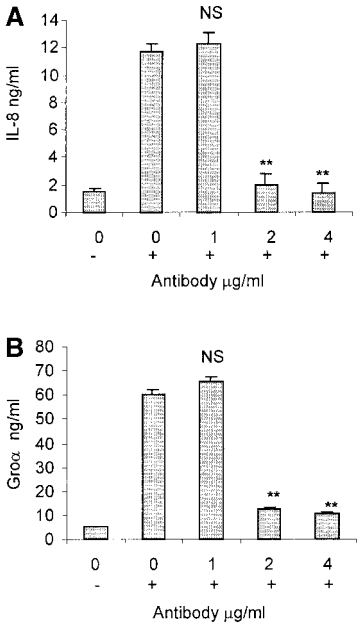

Figure 4. Effect of a blocking anti-human-IL-17 antibody. Human IL-17 (100 ng/ml) was co-incubated with an anti-hIL-17 antibody at 0, 1, 2, and 4 μg/ml and used to treat HBECs for 48 h. Amounts of IL-8 (*A*) and Groα (*B*) in cell culture supernatants were analyzed by ELISA. (−) indicates no stimulation and (+) indicates IL-17 stimulation. Data shown are for experiments performed on cells isolated from at least two donors and are expressed as mean ± SEM for $n > 3$, where $^{**}P < 0.001$ and NS is nonsignificant compared with no antibody.

Effects of Dexamethasone and MG132 on IL-17–stimulated IL-8 Release from HBECs

The effects of the steroid dexamethasone and the proteasome inhibitor MG132 on IL-17–mediated IL-8 release from HBECs were evaluated. The proteasome inhibitor MG132 has previously been shown to inhibit TNF-α–induced nuclear factor-κB activation in a human epithelial cell line (5). To examine the effects of the MG132 on IL-17–stimulated IL-8 release, HBECs were pretreated with compound before cytokine stimulation. Although dexamethasone caused a slight reduction of IL-8 release at 20 μM, this effect was not statistically significant (Figure 6). In contrast, dexamethasone significantly inhibited TNF-α–induced IL-8 release (data not shown). The proteasome inhibitor MG132 had a slight, but not statistically significant, effect on IL-17–induced IL-8 release at a concentration of 10 μM (Figure 6).

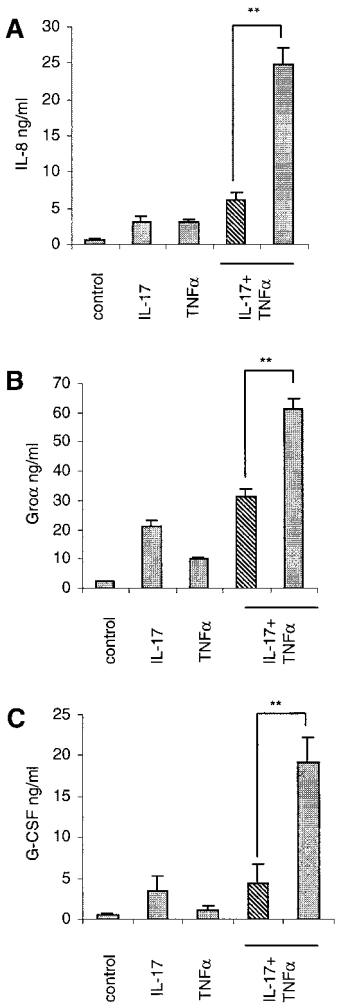

Figure 5. Effect of a combination of IL-17 and TNF-α on IL-8, Groα, and G-CSF production. IL-17 (10 ng/ml) and TNF-α (10 ng/ml) alone and in combination were used to stimulate HBECs for 48 h. IL-8 (*A*), Groα (*B*), and G-CSF (*C*) were measured in cell culture supernatants by ELISA. The *hatched area* indicates the calculated additive value for TNF-α and IL-17 stimulation. Data shown are for experiments performed on cells isolated from at least two donors and are expressed as mean ± SEM for $n = 8$–9, where $^{**}P < 0.001$.

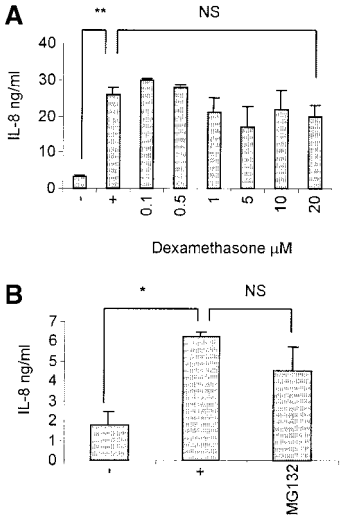

Figure 6. Effects of dexamethasone and MG132 on IL-17–stimulated IL-8 release. HBECs were pretreated for 1 h with dexamethasone (0–20 μM) or MG132 (10 μM) before stimulation with IL-17 (100 ng/ml) for 48 h. IL-8 ELISAs were used to measure the effects of dexamethasone (*A*) or MG132 (*B*). Data is expressed as mean ± SEM for three independent experiments, where $^{*}P < 0.05$, $^{**}P < 0.001$, and NS is non-significant.

Discussion

There is increasing evidence to suggest that IL-17 plays an important role in neutrophil maturation, recruitment, and activation. In the current study we have analyzed the effects of IL-17 on inflammatory mediator release from primary HBECs. We have shown using gene array studies that IL-17 potently induces mRNA expression for the chemokines IL-8 and Groα and the hematopoietic cytokine G-CSF. These mRNAs were strongly induced upon IL-17 treatment, suggesting rapid *de novo* synthesis, and it is interesting to note that of the 207 cDNAs on the array, only these 3 were found to be upregulated upon IL-17 stimulation. Although TNF-α and IL-17 were both independently able to induce Groα, IL-8, and G-CSF mRNA expression, they differed in their profiles. IL-17 induced Groα and G-CSF expression with a greater potency than TNF-α, but, unlike TNF-α, was unable to stimulate ICAM-1 and SOD-2 expression. Several studies have shown that IL-17 induces IL-6 release from multiple cell types including fibroblasts, stromal cells, and endothelial cells (8, 11). Analysis of the data from our array experiments revealed only low levels of IL-6 message, with no induction by IL-17. In contrast, IL-6 protein was released from IL-17–stimulated HBECs. The inability to detect changes in IL-6 message levels may reflect the limit of sensitivity of the array hybridization methods used.

We have also demonstrated the IL-17–induced release of IL-8, Groα, and G-CSF at the protein level, and concentrations of IL-17 used to induce protein release are similar to those reported previously (12, 13). When tested at equivalent concentrations, IL-17 was found to be 2- to 3-fold more potent than TNF-α in stimulating the release of Groα and G-CSF from HBECs. In contrast, IL-17 and TNF-α induced similar levels of IL-8 these cells. Both IL-8 and Groα are potent neutrophil chemoattractants (19) and have been proposed to play an important role in asthma and COPD (20). In support of this proposal, IL-8 levels have been found to be upregulated in the induced sputum of patients with COPD, correlating with elevated levels of neutrophils (21). We have shown that IL-17 can potently stimulate Groα production from airway epithelial cells and therefore Groα, in addition to IL-8, may play an important role in the observed IL-17–induced neutrophil recruitment and activation *in vivo*. Furthermore, we have shown in this study that IL-17 is more potent than TNF-α in the production of Groα from HBECs.

Steroids are commonly used to treat airways inflammation of patients with asthma and are thought to act in part by inhibiting inflammatory cytokine production (22, 23). However, although we have observed that dexamethasone inhibits TNF-α–induced IL-8 release from HBECs, we saw no effect by dexamethasone on IL-17–mediated chemokine production. This is in contrast to the reported inhibitory effects of hydrocortisone on IL-17–mediated IL-8 release from a HBEC line (13). This discrepancy may in part be due to the difference in cell types used in the two studies. In our experiments we have not used polarized cells, and it is possible that the IL-17 receptor may be distributed in a non-uniform fashion, giving rise to the observed effects.

IL-17 was found to synergistically enhance TNF-α–stimulated release of IL-8, Groα, and G-CSF from HBECs. IL-17 has previously been reported to synergize with TNF-α in the release of IL-8 from a 16HBE epithelial cell line (13) and in the release of Groα from human peritoneal mesothelial cells (24). However, this is the first report describing

the synergistic enhancement of Groα and G-CSF production from HBECs. G-CSF is a pleiotrophoic cytokine, which plays a role in hematopoiesis, selectively stimulating the proliferation of bone marrow stem cells to neutrophils. Whereas TNF-α and IL-8 have been shown to be compartmentalized to the lung, for G-CSF this is not the case (25, 26). The lung may be an important source of G-CSF (27) and IL-17 may play a key role in stimulating G-CSF release from lung-derived cells, including airway epithelial cells as used in our study. IL-17, therefore, may act indirectly as a signal from the lung to the bone marrow to promote an ongoing supply of neutrophils, via stimulation of G-CSF release. Evidence for a role of IL-17 in G-CSF release *in vivo* has recently been reported. Overexpression of IL-17 using a recombinant adenovirus *in vivo* has been shown to induce TNF-α, IL-1β, MIP-2, and G-CSF production in the airways and enhance bacterial clearance and survival after challenge with *Klebsiella pneumoniae* (28).

Undifferentiated HBECs are commonly used as *in vitro* models for studying the effects of cytokines on gene expression in the airway epithelium (29, 30) and in this study we have used primary cultures of human airway epithelial cells to study the effects of IL-17. This approach allows us to assign any changes observed to those mediated via IL-17, but it is possible that there will be some differences to those that would be observed if the same cells were examined *in vivo*. Differentiated primary HBECs are believed to more closely model the *in vivo* situation, but this model has the disadvantage of being composed of several cell types, including goblet cells and ciliated cells. Therefore, it is difficult to predict what differences in gene expression might be expected if differentiated HBECs had been used for this study.

In summary, we have shown that IL-17 activates only a small subset of genes in primary HBECs. The mRNA expression and protein levels of IL-8, Groα, and G-CSF are potently induced by IL-17. The cytokine IL-17 also synergizes with TNF-α in the release of IL-8, Groα, and G-CSF, and consequently IL-17 may play a part in amplifying the inflammatory response through the release of proinflammatory mediators. It is proposed that IL-17 may play an important role in neutrophil recruitment via stimulating the release of IL-8, Groα, and G-CSF from airway epithelial cells. IL-17 therefore may represent a novel target for respiratory diseases that are associated with elevated levels of neutrophils.

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