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Interleukin-8 plays a significant role in IgE-mediated lung inflammation

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ABSTRACT: Interleukin (IL)-8 is a potentially important cytokine in allergic respiratory responses since it is released by many resident lung cells, and it is a potent granulocyte chemoattractant.

Therefore, we induced an immunoglobulin (Ig)E-mediated response in human lung samples and studied whether IL-8 was produced in sufficient quantities to promote human neutrophil and eosinophil migration across naked filters and endothelial and pulmonary epithelial monolayers cultured on these filters. Fresh human lung fragments from 16 thoracotomy specimens were treated with either a 1:100 dilution of anti-IgE or buffer (control) for 30 min.

All anti-IgE treated lung samples had significant release of histamine and neutrophil and eosinophil chemotactic activity. Fourteen of the 16 lung samples had a significant increase in IL-8 subsequent to anti-IgE treatment ($p<0.01$). Anti-IL-8 antibody (4 $\mu g \cdot mL^{-1}$) inhibited 42% and 53% of neutrophil and eosinophil chemotactic activity respectively, contained in supernatants from anti-IgE-treated lung samples. Finally, we found that IL-8 at a concentration near that measured after anti-IgE treatment of lung samples (2,000 $pg \cdot mL^{-1}$) induced neutrophil and eosinophil migration through naked filters and endothelial and pulmonary epithelial cell monolayers.

Thus, human lung IgE-mediated responses *in vitro* results in the rapid release of interleukin-8 in amounts sufficient to affect a biological response, granulocyte transcellular migration, indicating that interleukin-8 may play a significant role in allergic respiratory diseases.

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Allergic airway inflammation is characterized by an influx of inflammatory cells, especially neutrophils and eosinophils, subsequent to the release of mediators and cytokines. Several studies have shown increased levels of cytokines in bronchoalveolar lavage fluid (BALF) in symp-tomatic asthmatics *versus* asymptomatic and nonasthma-tics [1, 2]. In addition, the levels of cytokines in BALF correlated with the number of inflammatory cells and se-verity of disease [2–4].

Recent evidence suggests that immunoglobulin (Ig)E-dependent mast cell degranulation results in the release of an array of cytokines [5]. One such cytokine which could be important in the pathogenesis of lung allergic inflammatory responses is interleukin (IL)-8. We have shown IL-8 to be chemotactic for eosinophils and neutrophils *in vitro* [6–8]. Several investigators have shown that the levels of IL-8 in BALF were increased in asthmatics *versus* nonasthmatics [2, 9]. In patients with asthma, the levels of IL-8 correlated with the number of neutrophils present in BALF [2].

The presence of IL-8 messenger ribonucleic acid (mRNA) and product has been examined in numerous cells. YOUSEFI *et al*. [9] have shown that eosinophils have mRNA for IL-8 and constititively express IL-8 protein which is re-leased *in vitro* after various stimuli. IL-8 expression and release have been found to be greater by airway epithelial cells from symptomatic asthmatics *versus* nonasthmatics [4]. JOHNSTON [10] demonstrated increased levels of IL-8 protein released from both pulmonary epithelial cells and peripheral blood mononuclear cells in subjects with wild type rhinovirus infections. Human neutrophils produce and release IL-8 [11]. Human mast cells also express mRNA for IL-8 and release IL-8 [12, 13]. Thus, many cells in the lung are capable of producing IL-8 in response to a variety of stimuli.

Mast cells and other cells that produce chemotactic factors for granulocytes have been postulated to be key in inducing IgE-mediated inflammatory responses in the airways [14, 15]. In previous studies we directly examined whether anti-IgE treatment of human lung leads to the production of chemotactic activity [16]. We found that human lung released neutrophil and eosinophil chemotactic activity spontaneously, and to a much greater degree subsequent to anti-IgE treatment of the lung samples. The chemotactic activity generated was capable of inducing neutrophil and eosinophil migration through both endothelial and epithelial monolayers in dose-responsive fashion. Furthermore, some of the chemotactic activity produced was attributable to tumour necrosing factor (TNF-α). However, we have shown that the capacity of TNF-α to induce

neutrophil transcellular migration is largely dependent on the ability of the endothelial or epithelial barrier to produce IL-8 [17, 18]. Since IL-8 is a potent neutrophil and eosinophil chemoattractant released by key lung cells, it may be a putatively important cytokine in mediating allergic airway inflammation. We therefore extended our previous studies to examine whether anti-IgE treatment of human lung tissue resulted in the production of chemotactic activity for both neutrophils and eosinophils that was in part attributable to IL-8 release.

Materials and methods

Cell lines

Human umbilical vein endothelial cells (HUVEC) and A549 human type II-like lung epithelial (A549) cells were purchased from the American Type Culture Collection, Rockville, MD, USA.

Cell culture

HUVEC and A549 cells were grown as monolayers in tissue culture flasks or dishes incubated in 100% humidity and 5% CO_2 at 37°C. HUVEC were grown in Ham's F12K medium supplemented with foetal calf serum (FCS) (20%), heparin (100 μg·mL^{-1}), endothelial cell growth supplement (50 μg·mL^{-1}), penicillin (20 Units(u)·mL^{-1}) and strep-tomycin (20 μg·mL^{-1}). A549 cells were grown in Ham's F12K medium supplemented with FCS (10%) [6–8, 16–18].

The cells from the monolayers were harvested with trypsin (0.25%) and ethylenediaminetetra-acetic acid (EDTA) (0.1%) in phosphate-buffered saline, centrifuged at low speed (250 g, 5 min) and resuspended in fresh medium before growing on permeable filters in Transwell tissue culture plates (Costar, Cambridge, MA, USA). The cells were grown to confluence on these filters in Transwell plates over 2–3 days using the same conditions as outlined above for the tissue culture dishes or flasks. Monolayer integrity was ensured by phase-contrast microscopy and negative controls. Moreover, we have found that HWEC and A549 monolayers have negligible bovine serum albumin (BSA) permeability in the absence of granulocytes and chemoattractants. Transwell plates have 24 wells that are separated into upper and lower chambers by polycarbonate filters that have a diameter of 6.5 mm and a pore size of 3.0 μm. Chemotactic experiments were done using the Transwell plates. Culture flasks and filters for HWEC experiments were treated with collagen (50 μg·mL^{-1}) overnight followed by rinsing with Hanks' balanced salt solution (HBSS).

Isolation of neutrophils and chromium labelling

Neutrophils were isolated from 0.2% EDTA-anticoagulated whole blood collected by venipuncture from normal donors. Neutrophils were obtained using a modification of the density gradient technique (1.095 Hypaque-Ficoll) described by Ferrante and Thong [19]. The isolated neutrophils were washed once with HBSS without calcium (Ca^{2+}) or magnesium (Mg^{2+}). Neutrophils were then resus-pended in 4 mL of HBSS without Ca^{2+} or Mg^{2+}, and resi-dual red cells were lysed using 1% ammonium oxalate. The neutrophils were then washed again in HBSS with Ca^{2+} or Mg^{2+} before performing cell counts and viability determinations by the trypan blue exclusion method. The isolated neutrophils were at least 98% pure and 99% viable.

Isolated neutrophils were labelled with ^{51}Cr (Chromium-51, sodium chromate (Na_2CrO_4)) (New England Nuclear, Boston, MA, USA) prior to placing them in the chemotactic chambers by using a modification of the procedure described by Gallin *et al*. [20]. Neutrophils at concentrations of 15–45×10^6 cells·mL^{-1} were incubated in Ca^{2+}/Mg^{2+}-free HBSS with 0.5 mCi of ^{51}Cr (5 mCi·mL^{-1}) at 37°C for 1 h with vigorous mixing. The labelling was terminated at the end of the incubation period by diluting the cells to 15 mL with HBSS without Ca^{2+} or Mg^{2+}. The neutrophils were subsequently washed three times in HBSS before resuspension in HBSS with 0.2% BSA, Ca^{2+} and Mg^{2+}. Aliquots of the cell suspension were removed and assayed for total counts and unbound ^{51}Cr to determine the cell-associated counts per minute as described by Gallin *et al*. [20].

Isolation of eosinophils

Eosinophils were isolated from 0.1% EDTA-anticoagulated blood (110–180 mL) collected by venipuncture from atopic donors. Four parts of blood were mixed with one part of 4.5% dextran in 0.85% sodium chloride (NaCl), and the red cells were allowed to sediment over 45 min. The dextran supernatant (6 mL) was then layered over Ficoll-Hypaque 1.076 (3 mL) in 15 mL tubes and centrifuged at 400 × g for 15 min to further fractionate the leucocytes. The mononuclear cell band was removed, and the granulocyte pellet was washed and resuspended in Ca^{2+}/Mg^{2+}-free HBSS with 5% heat-inactivated neonatal calf serum to a concentration of 20×10^6 cells·mL^{-1}. Two millilitre aliquots of the granulocyte suspension were then layered over five-layer, discontinuous density gradients of Percoll (Sigma Chemical Co., St. Louis, MO, USA) (1.080, 1.085, 1.090, 1.095 and 1.100 g·mL^{-1}) prepared as previously described [7, 8]. The density gradients were centrifuged at 760 × g for 20 min, and the cell bands that formed at each density interface in the gradient were collected. After lysis of residual red cells with 1% ammonium oxalate, each band was analysed for eosinophil and neutrophil number and purity using a Neubauer haemocytometer and Wright-stained cytocentrifuge preparation. Only normodense eosinophil populations with purity greater than 75% recovered from band 5 (density Š1.095) were used in eosinophil experiments. Eosinophils were not ^{51}Cr-labelled because eosinophils were isolated in much smaller quantities. Thus, we could not afford to lose eosinophils during the ^{51}Cr-labelling and subsequent washes. Quantitation of eosinophils migrating through filters was done by haemocytometer counting. Both counting methods (^{51}Cr and haemocytometer) give equivalent results. We have previously shown that the degree of eosinophil migration was not dependent upon the concentration of contaminating eosinophils [7].

Chemotaxis, transepithelial and transendothelial migration

All neutrophil and eosinophil chemotaxis and transmigration studies were done using a standardized protocol [6–8, 16–18]. A total of 0.5–1.5×10^6 ^{51}Cr-labelled neutrophils or 1.5–5×10^5 unlabelled eosinophils in 100 μL of buffer (HBSS with Ca^{2+} and Mg^{2+} and 0.2% BSA) were placed in the upper chamber, above the filter or monolayer. The chemoattractant (lung supernatant or IL-8 (R & D Systems, Minneapolis, MN, USA)) in 500 μL of buffer was placed in the lower chamber (below the filter). Negative (buffer) and positive (formyl-methionyl-leucyl-phenylalanine) controls were included for each experiment. Each variable was tested in triplicate. Plates were incubated at 37°C in 5% CO_2 and 100% humidity for 3 h in the dose-response experiments. After incubation, the lower chamber contents were collected and counted in a gamma counter for ^{51}Cr-labelled neutrophils or with a haemocytometer for unlabelled eosinophils. The counts per minute of the neutrophil samples were calculated as counts per minute per 10^6 cells using the formula by GALLIN *et al*. [20]. Data are expres-sed as per cent net stimulated migration (NSM) using the formula:

$$\frac{\text{(cell counts in experimental sample) - (cell counts in negative control sample)}}{\text{total cell counts added to chamber}} \times 100$$

The cells counted in the negative control samples (buffer) were generally less than 5% of the total added to the top chambers.

Neutralization experiments

Anti-IL-8 (R & D System) at 4 μg·mL^{-1} was pre-incubated for 30 min at room temperature with a 1:16 dilution of supernatant from lung samples treated with buffer (control) or anti-IgE for 30 min. These lung samples were distinct from those used to measure IL-8. The methods used to induce IgE-mediated responses are listed below. The 1:16 dilution was determined previously to give optimal supernatant-induced chemotactic responses [16]. Chemotaxis experiments using naked filter barriers were then performed as outlined above.

Preparation of minced lung

Human peripheral lung tissue was obtained from surgical specimens of 16 patients undergoing resection of lung tumours. Prior approval from the University of Iowa Institutional Review Board for Human Studies was obtained. Macroscopically normal lung tissue was utilized within 2 h of resection. The tissue was dissected free of pleura, large blood vessels and bronchi and was cut into small pieces (2–3 mm in diameter).

Lung IgE-mediated responses

The minced lung pieces were incubated in human serum from an atopic donor at 37°C for 1 h and were subsequently washed with N-2-hydroxyethylpiperazine-N-2-ethansulphonic acid (HEPES) buffer. HEPES buffer, pH 7.4, contained (g·L^{-1}): HEPES, 4.76; sodium chloride (NaCl), 8.0; potassium chloride (KCl), 0.2; dextrose, 1.0; sodium bicarbonate ($NaHCO_3$), 2.0; magnesium chloride ($MgCl_2$), 0.1; and calcium chloride ($CaCl_2$), 0.2 (0.7 mM Ca^{2+}). Anti-human IgE (Sigma Chemical) was then added to induce histamine release in one set of duplicate tubes. Both control and anti-IgE treated samples contained the same amount of lung tissue in identical volumes. The lyophilized goat anti-human IgE was reconstituted in distilled water and diluted with HEPES buffer to achieve a 1:100 final dilution (0.45 mg protein·mL^{-1}), a concentration predetermined to be optimal for stimulating histamine release from human lung in our experiments [21]. HEPES buffer alone was added to another set of duplicate tubes incubated in the same manner as a control and for determination of spontaneous histamine release. The reaction was allowed to proceed for 30 min after addition of anti-IgE or buffer, and was then stopped by placing the tubes on ice. The minced lung pieces were then immediately separated from the supernatants. Both the supernatants and lung fragments from a second set of duplicate tubes were prepared for assay of their histamine content. Histamine was measured using a radioimmunoassay technique (AMAC, Westbrook ME, USA) [21]. The total histamine content of each sample was determined as the released histamine measured in supernatants plus residual histamine measured from corresponding minced lung pieces. The percentage of histamine released was determined as (amount of histamine in supernatant/amount of total histamine) × 100.

IL-8 analysis

IL-8 measurements were made using an enzyme-linked immunosorbent assay (ELISA) (R & D Systems) performed on control and experimental (anti-IgE treated) lung supernatants. Minimum detection level of IL-8 was 4.7 pg·mL^{-1}. IL-8 data are expressed as either: mean IL-8 concentration (pg·mL^{-1}); or difference in IL-8 level between matching anti-IgE and buffer (control)-treated samples.

Statistics

All statistical analyses were done using SigmaStat®, (Statistical Products and Service Solutions (SPSS), Inc., Chicago, Ill, USA). IL-8 measurements in control and anti-IgE treated lung samples were tested for normality using the Kolmogorov-Smirov test. Based on the results of this test, the data were analysed for differences using a paired-sample t-test.

The effects of anti-IL-8 on neutrophil and eosinophil chemotactic activity from buffer and anti-IgE-treated lung samples were analysed by paired sample t-test.

Results

Histamine release

Before performing chemotactic assays we first determined whether anti-IgE treatment of the human lung samples caused mast cell mediator release. We measured both

spontaneous and anti-IgE-stimulated histamine release from the lung samples at 30 min. This time period was chosen based on previous data showing that most (>80%) of the stimulated histamine release occurred within 15–30 min [21, 22]. As previously reported, the percentage histamine release values for control and anti-IgE-treated lung samples averaged <5% and >24%, respectively [16]. All lung samples analysed for chemotactic activity or IL-8 product had significant net histamine release after anti-IgE treatment.

IL-8 release

We first asked whether anti-IgE treatment of human lung lead to the release of IL-8. The levels of IL-8 varied widely between different human lung samples. For example, most control values were around 1,000 pg·mL^{-1}, whereas others were in excess of 2,000 pg·mL^{-1}. Thus, although the mean IL-8 levels shown on the left side of figure 1a indicate only a modest increase subsequent to anti-IgE treatment, the actual increase for the paired samples (control *versus* experimental) was significant, p<0.01. Of 16 human lung supernatants 14 contained increased levels of IL-8 subsequent to anti-IgE *versus* control treatment (fig. 1a). The changes in IL-8 subsequent to anti-IgE treatment for each of the 16 lung samples are shown in figure 1b.

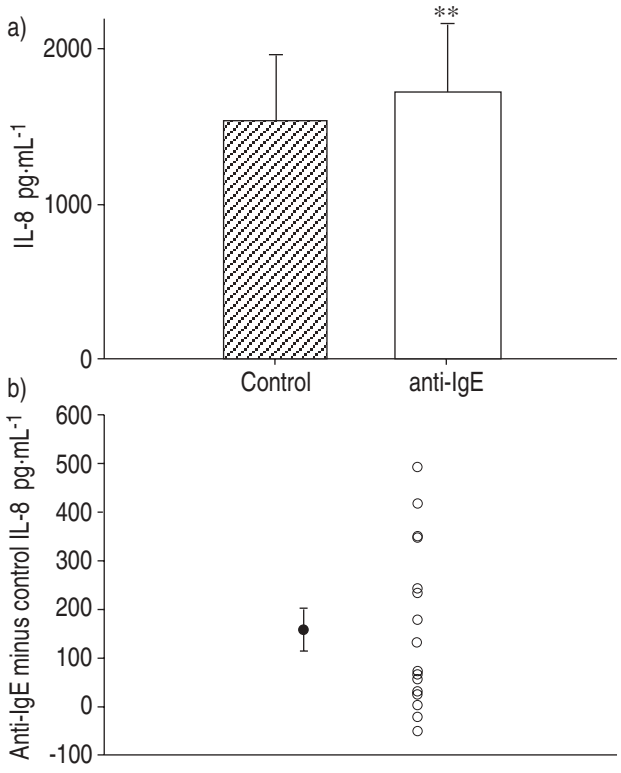

Fig. 1. – Interleukin (IL-8) concentration in supernatants from: a) anti-immunoglobulin (Ig)-E and buffer (control)-treated human lungs; and b) individual data points (○; n=16) and mean±SEM (●) expressed as the change in IL-8 concentration between paired anti-IgE and buffer-treated samples. IL-8 levels were measured after 30 min incubation with anti-IgE or buffer, respectively. Data are presented as mean±SEM cytokine values. **: p<0.01. anti-IgE treatment *versus* control for paired samples.

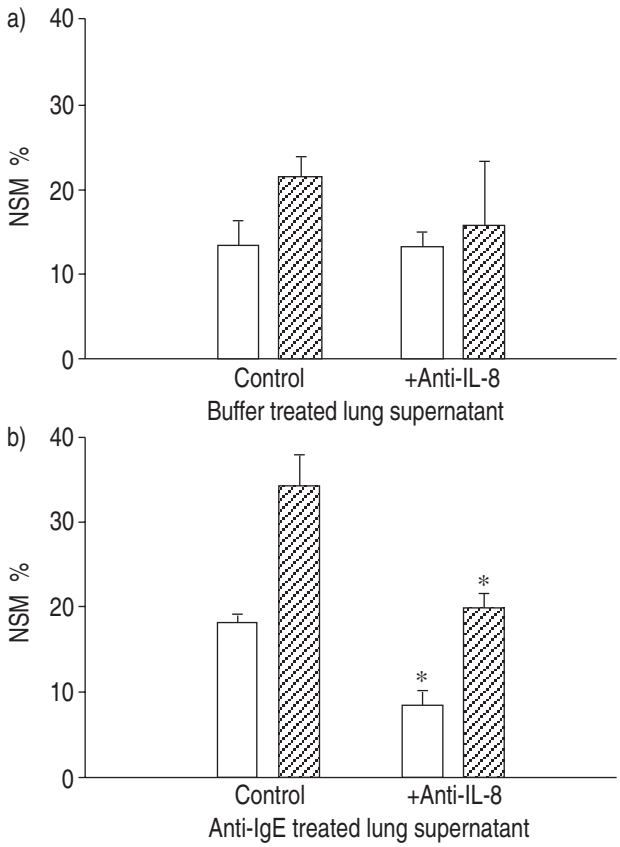

Fig. 2. – Effect of monoclonal anti-interleukin (IL)-8 (4 μg·mL^{-1}) on neutrophil and eosinophil chemotactic activity from: a) buffer; and b) anti-immunoglobulin (Ig)E-treated lung. A 1:16 (final) dilution of supernatants from buffer or anti-IgE-treated lungs were incubated without (control) or with (+) anti-IL-8. Granulocyte migration was measured at 3 h through naked filters. Data are presented as mean±SEM of net stimulated migration (NSM) of neutrophils and eosinophils. Data are from three observations. ▭: eosinophil; ▨: neutrophil. *: p<0.01, anti-IL8 treatment *versus* control.

Effects of anti-IL-8

As indicated, we previously reported that human lung spontaneously, and to a much greater degree after anti-IgE treatment, produced neutrophil and eosinophil chemotactic activity [16]. To determine whether the IL-8 produced subsequent to anti-IgE treatment of the lung, contributed to the chemotactic activity of lung supernatants, we examined the effects of a neutralizing antibody. Four micrograms per millilitre of monoclonal anti-IL-8 were incubated with a 1:16 dilution of supernatant from three lung samples treated with buffer or anti-IgE for 30 min. We chose 4 μg·mL^{-1} anti-IL-8 because we have found that dosages varying from 2–8 μg·mL^{-1} generally inhibited >75% of neutrophil and eosinophil migration due to 3,000 pM IL-8, a value significantly higher than those measured in the present study [6, 7]. We first examined the inhibitory effects of anti-IL-8 on the buffer-treated lung supernatants. Figure 2a shows that there was slight, but not significant inhibition of neutrophil or eosinophil migration in response to this lung supernatant when it was co-in-cubated with anti-IL-8. However, figure 2b illustrates that anti-IL-8 was capable of significantly inhibiting neutro-phil and eosinophil chemotactic activity present in

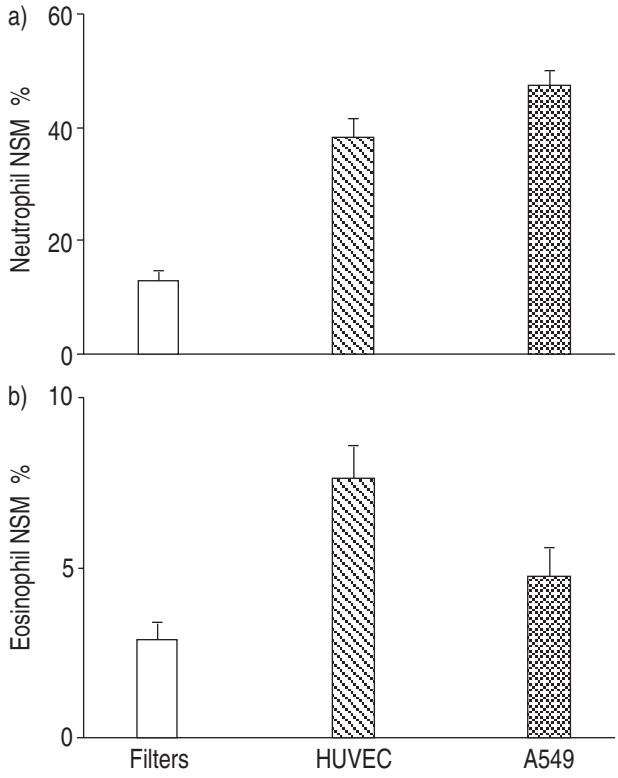

Fig. 3. – Physiological concentrations of interleukin (IL)-8 can induce neutrophil and eosinophil migration across naked filters, and human umbilical vein endothelial cell (HUVEC) and A549 cell monolayers grown on these filters. Data are presented as mean±SEM of net stimulated migration (NSM) of: a) neutrophils; and b) eosinophils, in res-ponse to 2,000 pg·mL^{-1} IL-8, a concentration measured in supernatants from anti-immunoglobulin (Ig)E-treated lung. Data are from three observations.

lung supernatants subsequent to anti-IgE treatment. Anti-IL-8 inhibited 42% and 53% of neutrophil and eosinophil che-motactic activity, respectively, contained in supernatants from anti-IgE treated lung samples.

IL-8-induced migration

Finally, we determined whether the concentration of IL-8 released after anti-IgE treatment of lung was capable of inducing neutrophil and eosinophil migration through HUVEC and A549 cells. At 2,000 pg·mL^{-1}, a level close to the mean concentration measured in supernatants from anti-IgE stimulated lung, IL-8 was capable of inducing significant neutrophil (fig. 3a) and eosinophil (fig. 3b) migration through naked filters and cellular monolayers. Taken together, these data suggest that IL-8 is an important factor in the chemotactic activity generated subsequent to human lung IgE-mediated responses.

Discussion

Sensitized asthmatics exposed to allergen exhibit dramatic lung inflammatory responses [23, 24]. The stimuli that induce inflammatory responses likely involve a myriad of cells and mediators. To better understand this inflammatory process, we treated human lung with anti-IgE and found that this treatment resulted in the significant release of neutrophil and eosinophil chemotactic activity (fig. 2 and [16]). The chemotactic activity of anti-IgE-treated lung supernatants may be due to several mediators and cytokines such as TNF, colony stimulating factors, ILs, chemokines, platelet activating factor, leukotrienes, and mono- and di-hydroxyeicosatetraenoic acids [15]. Indeed, we have demonstrated that a variety of mediators and cytokines can induce neutrophil and eosinophil transendothelial and transepithelial migration [6–8, 16–18].

We have previously documented the contribution of TNF-α to the chemotactic activity produced subsequent to lung IgE-mediated responses [16]. However, the role of TNF-α in stimulating neutrophils and eosinophils to migrate across cellular barriers is likely to be more indirect than direct. The capacity of TNF-α to induce neutrophil transcellular migration is largely due to IL-8 production [17, 18]. Indeed, IL-8 has been shown to be released from numerous cells [4, 9–13, 25, 26], be increased in the airways of asthmatics [2, 9] and be a chemoattractant for both eosinophils [7, 8] and neutrophils [6, 8]. Thus, we examined directly the contribution of IL-8 to the chemotactic activity produced by IgE-mediated human lung responses. Both control (buffer-treated) and anti-IgE-treated lung supernatants contained measurable quantities of IL-8 (fig. 1). However, greater than 85% of the supernatants from anti-IgE-treated lungs had significantly more IL-8 than corresponding controls.

To examine whether IL-8 was a contributing factor to neutrophil and eosinophil chemotactic activity released subsequent to the treatment of human lung with anti-IgE, we studied the inhibitable capacity of antibodies to IL-8 (figs. 2a and b). Anti-IL-8 slightly inhibited the migration induced by the control (buffer treated) supernatants. However, anti-IL-8 was capable of inhibiting 53 and 42% of the eosinophil and neutrophil chemotactic capacity found in anti-IgE-treated lung supernatants, respectively. Although we did not specifically measure the IL-8 levels in the samples used in these antibody studies, our data suggest that the levels of IL-8 necessary to affect a chemotactic response are more likely to occur subsequent to an IgE-mediated event. It is not surprising that anti-IL-8 did not completely inhibit the chemotactic activity since other mediators such as leukotrienes, platelet activating factor and other cytokines may be contributing factors.

The source of the released IL-8 is not known with certainty. As indicated, IL-8 has been shown to be released from many unstimulated and stimulated cells [4, 9–13, 25, 26]. Mast cells, monocytes, macrophages, eosinophils and epithelial cells are among the cells which constitutively express IL-8. Moreover, these cells significantly increase the production of IL-8 after anti-IgE, lipopolysaccharide or cytokine stimulation. However, the rapidity of the release of IL-8 (30 min) suggests that cells with preformed stores are the most likely sources. Mast cells, eosinophils and monocytes have been reported to have high affinity IgE receptors [27–29] and produce IL-8 [9, 12, 13, 25, 26]. Because the mast cell has a substantial number of high affinity IgE receptors it is likely that it is a major source of released IL-8 in the human lung subsequent to IgE-mediated activation.

We have previously shown that IL-8 is capable of inducing neutrophil and eosinophil transendothelial and transepithelial migration [6, 7]. We have now demonstrated that the quantity of IL-8 measured in anti-IgE-stimulated lung supernatants is capable of induc-

ing neutrophil and eosinophil migration through naked filters and end- othelial and epithelial cell monolayers (figs. 3a and b). However, this concentration of IL-8 induced significantly less eosinophil *versus* neutrophil migration. Nonetheless, the *in vivo* administration of IL-8 has been shown to in-duce a neutrophilic [30, 31] and eosinophilic [32] res- ponse in experimental animal models of inflammation.

Thus, the release of IL-8 subsequent to IgE-mediated responses in the respiratory tract could be a key factor in the inflammatory responses noted in diseases such as aller-gic asthma. Inhalation of antigen could lead to the release of chemotactic activity including IL-8 by numerous cells. This could lead to the chemotaxis of neutrophils and eosinophils, key effector cells in the pathogenesis of allergic inflammation. The influx of these inflammatory cells could then trigger a cascade of events perpetuating the production of IL-8 and the continued promotion of lung inflammation.

Several lines of investigation support a prominent role for IL-8 in the pathogenesis of asthma. Firstly, IL-8 message and product are increased in lung samples from asthmatics *versus* nonasthmatics. Furthermore, the symptomatic asthmatic state has been shown to result in greater IL-8 expression [2, 4, 9]. Once released, IL-8 can have a variety of important biological effects including the promotion of lung inflammation *via* chemotaxis of neutrophils, eosinophils and lymphocytes [6, 7, 33], and the induction of plasma leakage [31]. IL-8 has also been shown to induce airway contraction and hyperresponsiveness [34, 35]. Moreover, IL-8 has been shown to induce the release of histamine from basophils [36], and histamine has been shown to cause IL-8 secretion [37]. Our data further support an important role for IL-8 by demonstrating that IgE receptor triggering leads to the release of sufficient quantities of IL-8 from human lung to affect neutrophil and eosinophil migration across endothelium and pulmonary epithelium. Thus, IL-8 may indeed play an important role in IgE-mediated lung inflammation in asthma.

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References

1. Broide DH, Lotz M, Cuomo AJ, Coburn DA, Federman EC, Wasserman SI. Cytokines in symptomatic asthma airways. *J Allergy Clin Immunol* 1992; 89: 958–967.
2. Nocker RE, Schoonbrood DF, van de Graaf EA, *et al*. Interleukin-8 in airway inflammation in patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* 1996; 109: 183–191.
3. Woolley KL, Adelroth E, Wooley MJ, Ellis R, Jordana M, O'Byrne PM. Granulocyte-macrophage colony-stimulating factor, eosinophils and eosinophil cationic protein in subjects with and without mild, stable, atopic asthma. *Eur Respir J* 1994; 7: 1576–1584.
4. Marini M, Vittori E, Hollemborg J, Mattoli S. Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol* 1992; 89: 1001–1009.
5. Borish L, Joseph BZ. Inflammation and the allergic response. *Med Clin North Am* 1992; 76: 765–787.
6. Smart SJ, Casale TB. Interleukin-8-induced transcellular neutrophil migration is facilitated by endothelial and pulmonary epithelial cells. *Am J Respir Cell Mol Biol* 1993; 9: 489–495.
7. Erger RA, Casale TB. Interleukin-8 is a potent mediator of eosinophil chemotaxis through endothelium and epithelium. *Am J Physiol* 1995; 268 (Lung Cell Mol Physiol 12): L117–L122.
8. Bittleman DB, Erger RA, Casale TB. Cytokines induce selective granulocyte chemotactic responses. *Inflamm Res* 1996; 45: 89–95.
9. Yousefi S, Hemmann S, Weber M, *et al.* IL-8 is expressed by human peripheral blood eosinophils. Evidence for increased secretion in asthma. *J Immunol* 1995; 154: 5481–5490.
10. Johnston SL. Natural and experimental rhinovirus infections of the lower respiratory tract. *Am J Respir Crit Care Med* 1995; 152: S46–S52.
11. Cassatella MA, Bazzoni F, Ceska M, Ferro I, Baggiolini M, Berton G. IL-8 production by human polymorphonuclear leukocytes: the chemoattractant formyl-methionyl-leucyl-phenylalanine induces the gene expression and release of IL-8 through a pertussis toxin-sensitive pathway. *J Immunol* 1992; 148: 3216–3220.
12. Buckley MG, Williams WM, Thompson J, *et al.* IL-4 enhances IL-3 and IL-8 gene expression in human leukemic mast cell line. *Immunology* 1995; 84: 410–415.
13. Möeller A, Lippert U, Lessmann D, *et al*. Human mast cells produce IL-8. *J Immunol* 1993; 151: 3261–3266.
14. Bittleman DB, Casale TB. Allergic models and cytokines. *Am J Respir Crit Care Med* 1994; 150: S72–S76.
15. Smart SJ, Casale TB. Pathogenesis of asthma: mediators and mechanisms. *In*: Gershwin ME, Halpern G, eds. Bronchial Asthma: Principles of Diagnosis and Treatment, New Jersey , The Humana Press, Inc., 1994; pp. 23–55.
16. Casale TB, Costa JJ, Galli SJ. TNFα is important in human lung allergic reactions. *Am J Respir Cell Mol Biol* 1996; 15: 35–44.
17. Smart SJ, Casale TB. TNF-α-induced transendothelial neutrophil migration is IL-8 dependent. *Am J Physiol* 1994; 266: L238–L245.
18. Smart SJ, Casale TB. Pulmonary epithelial cells facilitate TNF-α-induced neutrophil chemotaxis. A role for cytokine networking. *J Immunol* 1994; 152: 4087–4094.
19. Ferrante A, Thong YH. A rapid one-step procedure for purification of mononuclear and polymorphonuclear leukocytes from human blood using a modification of the Hypaque-Ficoll technique. *J Immunol Meth* 1978; 24: 389–393.
20. Gallin JI, Clark RA, Kimball HR. Granulocyte chemotaxis: an improved *in vitro* assay employing ^{51}Cr-labeled granulocytes. *J Immunol* 1973; 110: 233–240.
21. Erger RA, Casale TB. Comparison of methodologies to measure human lung histamine. *J Immunol Meth* 1992; 152: 115–121.
22. Casale TB. Acute effects of *in vitro* mast cell degranulation on human lung muscarinic receptors. *Am Rev Respir Dis* 1993; 147: 940–945.
23. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989; 139: 806–817.
24. Holgate ST, Twentyman OP, Rafferty P, *et al*. Primary and secondary effector cells in the pathogenesis of bronchial asthma. *Int Arch Allergy Appl Immunol* 1987; 82: 498–506.
25. Hallsworth MP, Soh CPC, Lane SJ, Arm JP, Lee TH. Selective enhancement of GM-CSF, TNF-α, IL-1β, and

IL-8 production by monocytes and macrophages of asthmatic subjects. *Eur Respir J* 1994; 7: 1096–1102.
26. Grabbe J, Welker P, Moller A, Dippel E, Ashman LK, Czarnetzki BM. Comparative cytokine release from human monocytes, monocyte-derived immature mast cells, and human mast cell line (HMC-1). *J Invest Dermatol* 1994; 103: 504–508.
27. Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993; 366: 421–428.
28. Gounni, AS, Lamkhioued B, Ochiai K, *et al*. High-affinity IgE receptor on eosinophils is involved in defense against parasites. *Nature* 1994; 367: 183–186.
29. Maurer D, Fiebiger E, Reininger B, *et al*. Expression of functional high affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. *J Exp Med* 1994; 179: 745–750.
30. Van Zee KJ, Fischer E, Hawes AS, *et al.* Effects of intravenous IL-8 administration in nonhuman primates. *J Immunol* 1992; 148: 1746–1752.
31. Colditz IG, Zwahlen RD, Baggiolini M. Neutrophil accumulation and plasma leakage induced *in vivo* by neutrophil-activating peptide-1. *J Leukocyte Biol* 1990; 48: 129–137.
32. Collins PD, Weg VB, Faccioli LH, Watson ML, Moqbel R, Williams TJ. Eosinophil accumulation induced by human interleukin-8 in the guinea-pig *in vivo*. *Immunology* 1993; 79: 312–318.
33. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science* 1989; 243: 1464–1466.
34. Burrows LJ, Piper PJ. Human recombinant neutrophil activating factor/interleukin-8 is a spasmogen of airway smooth muscle. *Cytokine* 1989; 1: 100 (Abstract).
35. Xiu Q, Fujimura M, Nomura M, *et al*. Bronchial hyperresponsiveness and airway neutrophil accumulation induced by interleukin-8 and the effect of thromboxane A_2 antagonist S-1452 in guinea-pigs. *Clin Exp Allergy* 1995; 25: 51–59.
36. Bischoff SC, Baggiolini M, de Weck AL, Dahinden CA. Interleukin 8-inhibitor and inducer of histamine and leukotriene release in human basophils. *Biochem Biophys Res Commun* 1991; 179: 628–633.
37. Jeannin P, Delneste Y, Gosset P, *et al*. Histamine induces interleukin-8 secretion by endothelial cells. *Blood* 1994; 84: 2229–2233.