

EFFECTS OF RAGWEED AND Th-2 CYTOKINES ON THE SECRETION OF IL-8 IN HUMAN AIRWAY EPITHELIAL CELLS

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☐ *Ragweed-induced asthma is a very common pulmonary disease. An important part of asthma is a large increase in inflammatory cells, including neutrophils and eosinophils, in ragweed-sensitized subjects. In this study, we determined if ragweed treatment could induce the release of interleukin-8 (IL-8) by human airway epithelial cells. We found that ragweed induces a substantial increase of the secretion of IL-8 from A549 cells in a dose- and time-dependent manner. Th-2 cytokines, IL-4 and IL-13, partially inhibited the ragweed-induced secretion of IL-8. Our results suggest that airway epithelial cells may be one of the cell sources that provide IL-8 during ragweed-induced asthma. The results also indicate that IL-4 and IL-13 may exert inhibitory effects on IL-8 secretion by airway epithelium.*

Keywords *IL-8, ragweed, Th-2 cytokines*

Asthma is an inflammatory airway disease that can be caused by inhalation of various allergens like ragweed [1]. One feature of asthma is the inflammation characterized by an accumulation of various types of cells, including neutrophils and eosinophils [2–4]. Interleukin-8 (IL-8) is a chemokine that is known to mediate the migration of blood cells to the sites of inflammation in the airways [5–7]. IL-8 has been shown to have the chemotactic activity for both neutrophils and eosinophils [5, 6]. Airway epithelial cells can be a major source of IL-8, and they play an important role in mediating inflammatory processes in a variety of other disorders [8]. It is known that viruses and various macrophage-derived cytokines can trigger the release of IL-8 from airway epithelial cells [8, 9]. It is not known if allergens, like ragweed, can activate airway epithelial cells in a similar manner.

Asthma is characterized by increased amounts of Th-2 cytokines in the airways [10, 11]. Th-2 cytokines are produced by a subsets of T helper lymphocytes and they play an important role in regulating the inflammatory

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response in asthma [12–14]. Th-2 cytokines can mediate either anti-inflammatory [15–17] or proinflammatory [18, 19] effects on various types of inflammation.

Ragweed is a very common allergen that causes asthma [1]. It has been shown that inhalation of ragweed induces the accumulation of neutrophils and eosinophils in lung tissues of ragweed-sensitized subjects by 90-fold and 800-fold, respectively [20]. In this study, we investigated whether ragweed could induce the secretion of IL-8 by human airway epithelial cells. The effects of Th-2 cytokines on the secretion of IL-8 were also studied. We found that ragweed induced the secretion of IL-8 by the human airway epithelial cell line, A549 cells. IL-4 and IL-13 significantly inhibited the secretion of IL-8 induced by ragweed.

MATERIALS AND METHODS

Reagents

A549 cell, a human airway epithelial cell line, was purchased from American Tissue Culture Collection (ATCC) (Rockville, MD). Eagle's minimal essential media and fetal bovine serum were purchased from Life Technologies (Gaithersburg, MD). Trypsin/EDTA was purchased from Irvine Scientific (Santa Ana, CA). Penicillin and streptomycin were purchased from the University of Iowa Cancer Center. Gentamicin was purchased from Elkins-Sinn, Inc. (Cherry Hill, NJ). Short ragweed pollen extract was purchased from the repository of the National Institute of Allergy and Infectious Diseases (Rockville, MD). Bovine serum albumin was purchased from Sigma Chemical Co. (Saint Louis, MO). ELISA reagents and Th-2 cytokines were purchased from R & D systems (Rochester, MN). Horseradish peroxidase streptavidin was purchased from Vector Laboratories, Inc. (Burlingame, CA). The ELISA substrate reagents were purchased from Genzyme Diagnostics (San Carlos, CA).

Cell Culture

A549 cells were cultured in Eagle's minimal essential media supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 U/mL streptomycin, and 80 µg/mL gentamicin. The cells were maintained in a 37°C tissue culture incubator supplemented with 5% CO₂. They were subcultured by trypsinization twice a week. The cell passage numbers used in the experiments were from 77th passage to 95th passage, as recommended by ATCC.

ELISA

The enzyme-linked immunosorbent assay (ELISA) analysis procedure is based on the one provided by the manufacturer (R & D systems) and described briefly as follows. The 96-well Nunc-Immuno plates (Nalge Nunc International, Denmark) were coated with appropriately diluted capture antibodies at 25°C overnight. The coating antibodies were aspirated and the plates were washed with wash buffer (0.05% Tween 20 in phosphate-buffered saline, pH 7.4) and blocked by blocked solution (1% bovine serum albumin, 5% sucrose, 0.5% NaN₃ in phosphate-buffered saline, pH 7.4). After the plates were washed with the buffer, the cell supernatants were added into the plates and incubated at 25°C for 1 hour. The antigens were aspirated, the plates washed, and appropriately diluted biotinylated antibodies were added into the plates and incubated at 25°C for 1 hour. Then the plates were washed and horseradish peroxidase streptavidin was added for the 20-minute incubation at 25°C, followed by addition of the substrate solution. After the enzyme reactions had proceeded for 20 to 30 minutes, they were stopped by addition of 3 M sulfuric acid. The optical densities at 405 nm were then determined by using an ELISA reader (Bio-Tek Instruments, Inc, Winooski, VT).

Statistics

The data were expressed as mean $\pm$ SE. Student's *t* test was applied for the data analysis. *P* value < .05 was considered to be significant.

RESULTS

Ragweed Increases the Secretion of IL-8 From Human Airway Epithelial Cells

It has been shown that ragweed challenge in human lung results in increased accumulation of various kinds of inflammatory cells, including eosinophils and neutrophils [20]. Because IL-8 is a chemokine able to recruit both kinds of cells, we investigated whether ragweed could increase the secretion of IL-8 from human airway epithelial cells. We treated A549 cells with different amounts of ragweed antigen and incubated them for 48 hours. Then the cell supernatants containing the secreted IL-8 from control cells and ragweed-treated cells were collected and subjected to ELISA. Compared with the control cells, ragweed induced a 6-fold and 8-fold increase in the release of IL-8 from A549 cells at concentrations of 4 units/mL and 8 units/

IL-8 (ng/ml)

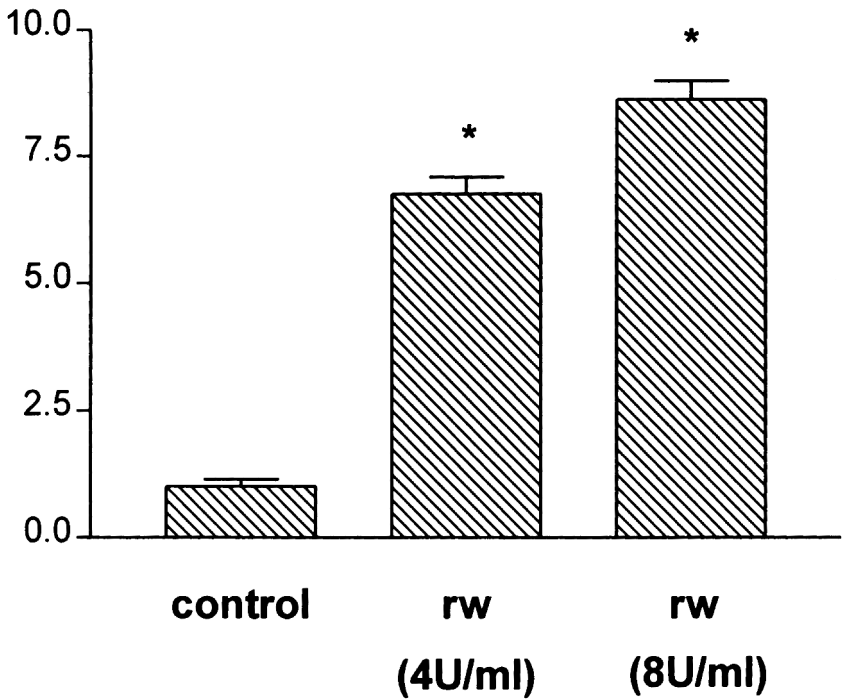


FIGURE 1 Ragweed increased the secretion of IL-8 in A549 cells in a dose-dependent manner. A549 cells were treated with either medium alone (control) or various concentrations of ragweed (rw) and maintained in culture for 48 hours. The cell supernatants containing the secreted cytokines were then harvested and subjected to ELISA for measurement of IL-8. At concentrations of 4 U/mL and 8 U/mL (the activity of ragweed was represented by antigen E units), ragweed increased the secretion of IL-8 by about 6- and 8-fold, respectively (each was $P < .05$, $n = 5$).

mL (the activity of ragweed was represented by antigen E units), respectively (Figure 1). The induction of IL-8 from A549 cells by ragweed at different time points was also tested. At a concentration of 8 units/mL, ragweed was added into the cell culture and incubated for 3, 24, and 48 hours. Figure 2 shows that ragweed induced the secretion of IL-8 from A549 cells in a time-dependent manner. In order to exclude a possible effect from lipopolysaccharide (LPS), we next tested the induction of IL-8 secretion from A549 cells by ragweed in the presence of polymyxin B, which blocks the effects of LPS [21]. After 48 hours of incubation, ragweed (at concentrations of 4 and 8 units/mL) induced the release of IL-8 from A549 cells to the similar level in the absence and presence of polymyxin B (100 μ g/mL) (Figure 3). Further, LPS at a concentration up to 20 ng/mL had little effect on the release of IL-8 from A549 cells (data not shown).

IL-8 (ng/ml)

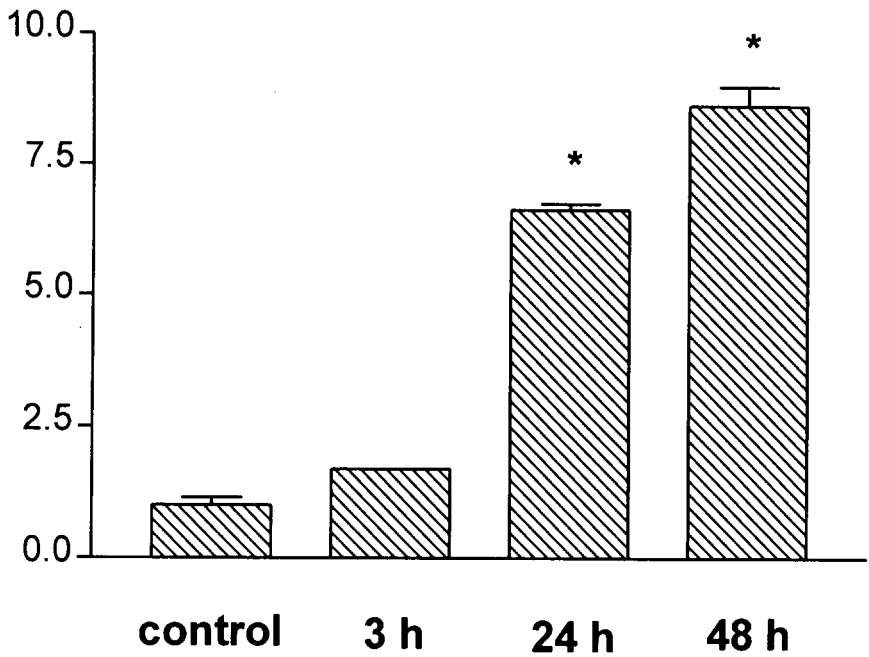


FIGURE 2 Ragweed increased the secretion of IL-8 in A549 cells in time-dependent manner. A549 cells were treated with either medium alone or ragweed at 8 U/mL for 3, 24, and 48 hours, respectively. The cell supernatants containing the secreted cytokines were then harvested and subjected to ELISA for measurement of IL-8. At 24 and 48 hours, ragweed increased the secretion of IL-8 by about 6- and 8-fold, respectively (each was $P < .05$, $n = 5$).

IL-4 and IL-3 Partially Inhibit Ragweed-Induced Secretion of IL-8 by A549 Cells

Th-2 cytokines have been shown to play important regulatory roles during various inflammatory processes [15–19]. We next tested whether Th-2 cytokines have any regulatory effects on the secretion of IL-8 from A549 cells induced by ragweed. A549 cells were pretreated for 1 hour with each of the following Th-2 cytokines, IL-4, IL-5, IL-10, and IL-13 (10 ng/mL). Ragweed (8 units/mL) was then added into the cell culture for a 48-hour incubation. Whereas IL-5 and IL-10 had no effects on the secretion of IL-8 induced by ragweed in A549 cells (Figures 4B, C), IL-4 and IL-13 significantly inhibited the secretion of IL-8 by 36% and 53%, respectively ($P < .05$) (Figures 4A, D).

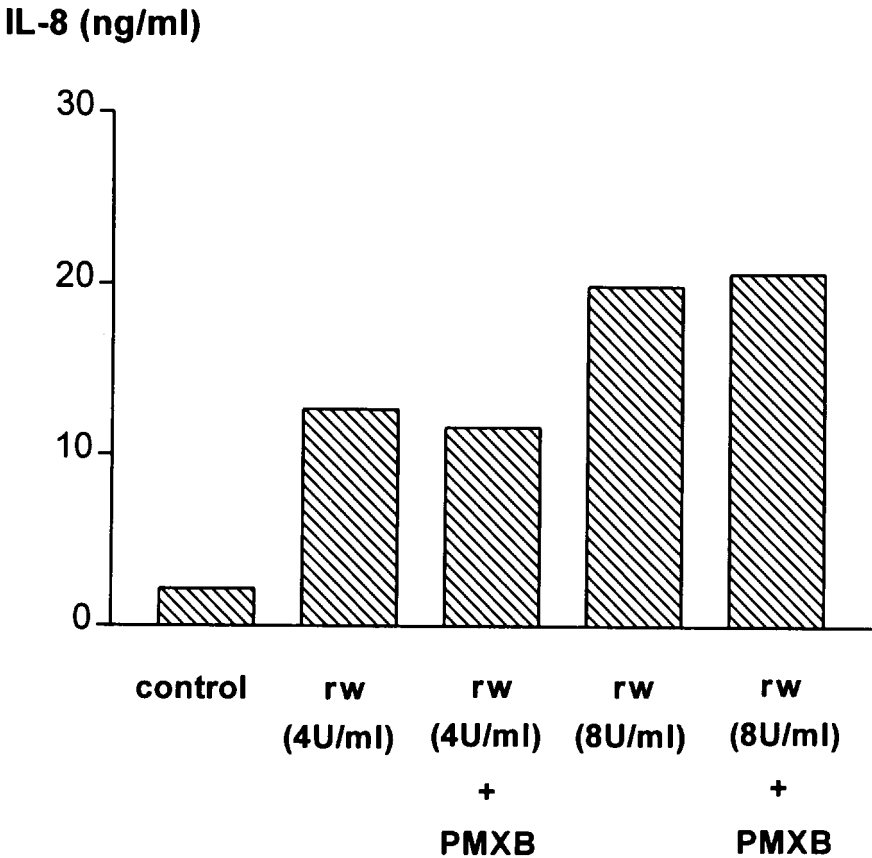


FIGURE 3 Blocking the effect of LPS did not affect the release of IL-8 induced by ragweed from A549 cells. A549 cells were treated with medium alone, ragweed (rw) alone (at 4 U/mL and 8 U/mL) or pretreated with polymyxin B (PMXB, 100 µg/mL) for 1 hour before the addition of ragweed. The cells were cultured for 48 hours and then the cell supernatants containing the secreted cytokines were harvested and subjected to ELISA for measurement of IL-8. Ragweed induced the release of IL-8 to the similar level in the absence and presence of polymyxin B.

DISCUSSION

It has been shown that ragweed antigen induces a marked increase in the numbers of neutrophils and eosinophils in asthmatic subjects [20]. Airway epithelial cells not only function as a protection barrier, but also play important regulatory roles during various inflammatory processes. For example, airway epithelial cells are known to be one of the major sources of IL-8 in inflammatory airways [8]. In this study, we investigated whether ragweed could increase the secretion of IL-8 by human airway epithelial cells. We found that ragweed induced a substantial increase in IL-8 secretion by A549 cells in a time- and dose-dependent manner. This result demonstrates that the airway epithelial cells may be one of the cell sources providing IL-8 fol-

IL-8 (ng/ml)

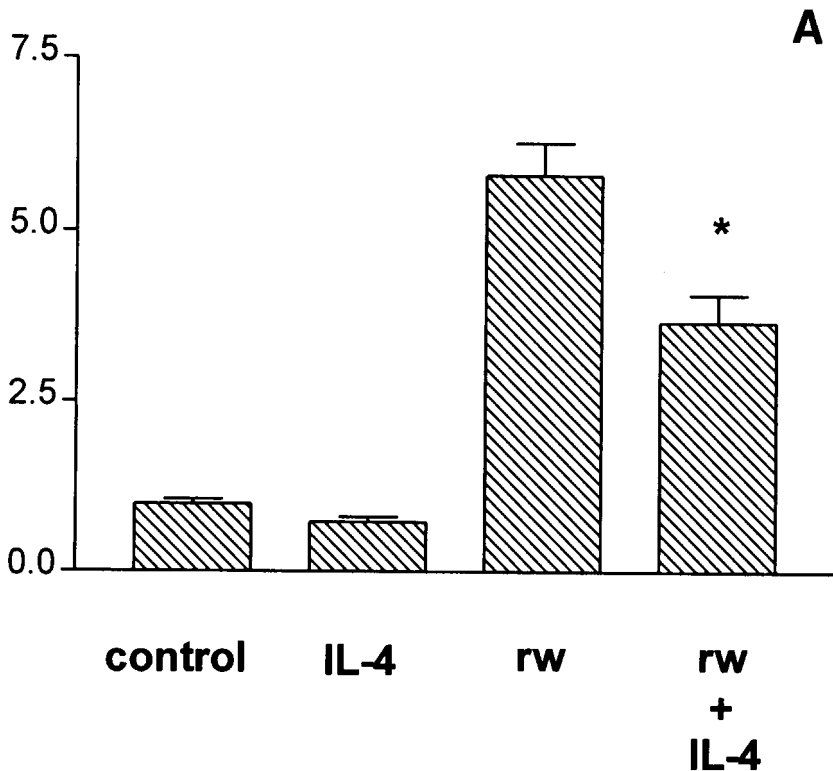


FIGURE 4 IL-4 and IL-13 inhibited ragweed-induced secretion of IL-8 in A549 cells. A549 cells were treated with medium alone, ragweed (rw) alone at 8 U/mL or pretreated with various Th-2 cytokines (10 ng/mL) for 1 hour before the addition of ragweed. The cells were cultured for 48 hours and then the cell supernatants containing the secreted cytokines were harvested and subjected to ELISA for measurement of IL-8. Although IL-5 (B) and IL-10 (C) had no effects, IL-4 (A) and IL-13 (D) inhibited ragweed-induced secretion of IL-8 by about 36% and 53%, respectively (each was $P < .05$, $n = 6$).

lowing inhalation of allergens. Interestingly, the Th-2 cytokines, IL-4 and IL-13, partially suppressed this release of IL-8. This observation suggests that IL-4 and IL-13 may have some anti-inflammatory effects in the airways in asthma. These studies do not exclude a role for other inflammatory cells, like macrophages or monocytes, which can also contribute to the production of IL-8.

The short ragweed is composed of at least 52 antigens, such as antigens E, K, and Ra [22–24]. Each antigen may have several types of isoforms [22]. At the present time, it is not known which of these antigens induced the release of IL-8 from A549 cells. The mechanism(s) by which ragweed stimulated epithelial cells is also not clear. Other investigators found that the short ragweed can attach to epithelial cells via binding to surfactant protein A (SP-

IL-8 (ng/ml)

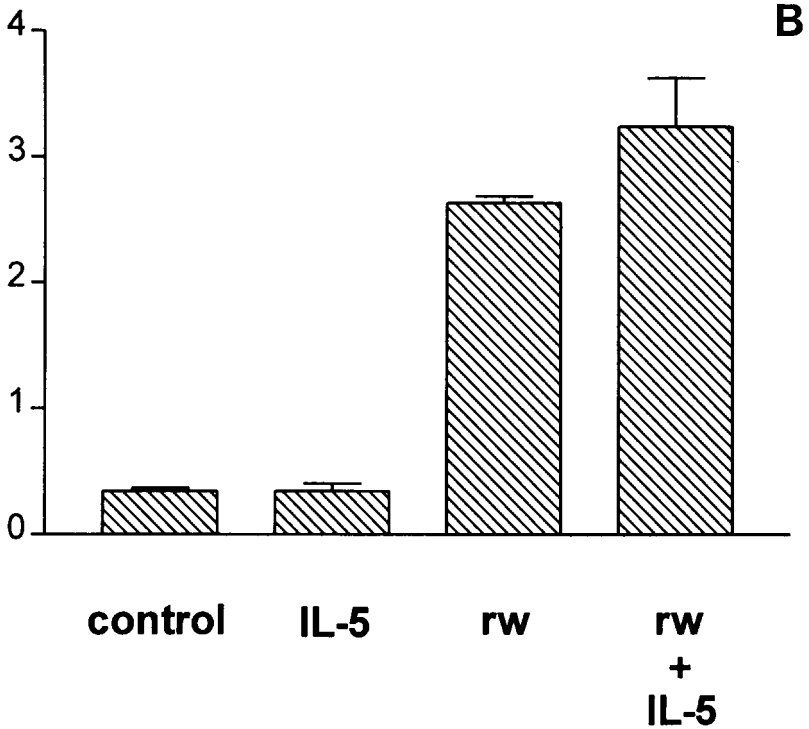
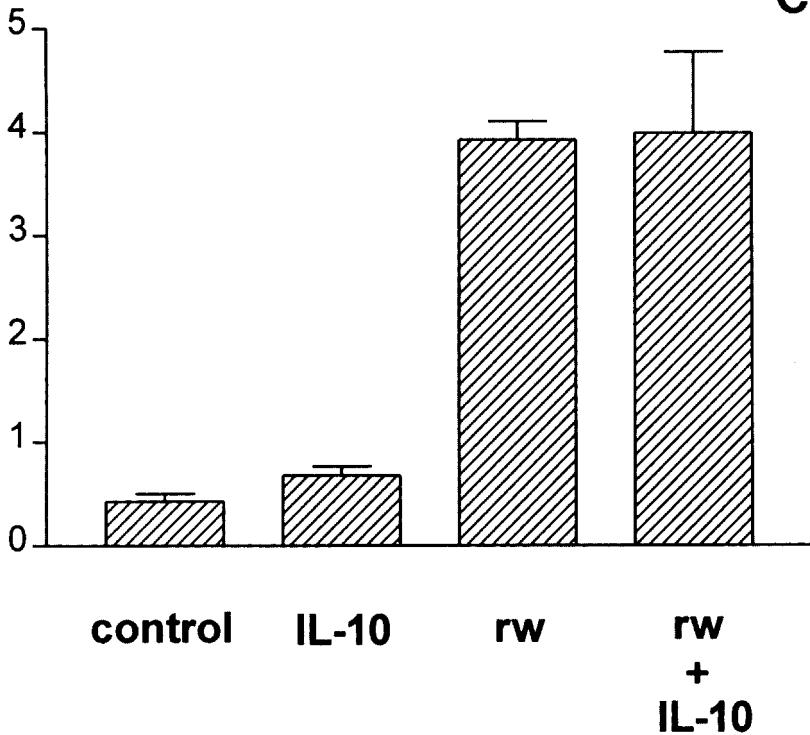


FIGURE 4—Continued

A) and the SP-A receptor on the cells [25, 26]. It is unlikely that SP-A mediated that attachment of ragweed to the epithelial cells in this study, because SP-A was not present in the culture. Further, it is likely that this mechanism is relevant in vivo only to the terminal airways where SP-A is present.

Th-2 cytokines play important regulatory roles during various inflammatory processes [12]. In our study, we found that IL-4 and IL-13 have inhibitory effects on ragweed-induced secretion of IL-8 by A549 cells. One of the possible mechanisms for this effect may be the involvement of nitric oxide (NO), a mediator in many inflammatory processes [27]. Other investigators have shown that the expression of NO synthase is increased in human airway epithelial cells during asthma process [28]. NO is closely related to the expression of proinflammatory cytokines, including IL-8 [29]. Inhibition of NO synthase can result in the inhibition of IL-8 production [29]. In human airway epithelial cells, IL-4 and IL-13 have been shown to inhibit the activity of NO synthase [30]. It may be possible that IL-4 and IL-13 inhibit the ragweed-induced secretion of IL-8 by blocking the activity of NO synthase.

IL-8 (ng/ml)**C****FIGURE 4**—Continued

Besides the possible role of NO synthase, other mechanisms that are involved in the inhibition of ragweed-induced IL-8 release from A549 cells also need to be considered. Cyclooxygenase (COX) is the enzyme involved in many inflammatory processes [31]. It was found that bradykinin induces the release of IL-8 from airway smooth muscle cells and COX is involved in this induction process [32]. Other investigators found that Th-2 cytokines (IL-4, IL-10, and IL-13) inhibit the expression of COX in alveolar monocytes [33]. Whether Th-2 cytokines inhibit the ragweed-induced release of IL-8 from A549 cells through COX-mediated mechanisms needs to be studied.

In summary, we found that ragweed induced a substantial increase of IL-8 secretion in A549 cells, suggesting that airway epithelial cells may be involved in the recruitment of neutrophils and eosinophils during the inflammatory process of asthma. IL-4 and IL-13 inhibited the ragweed-induced secretion of IL-8 from A549 cells, indicating possible regulatory roles for these cytokines in asthma.

IL-8 (ng/ml)

D

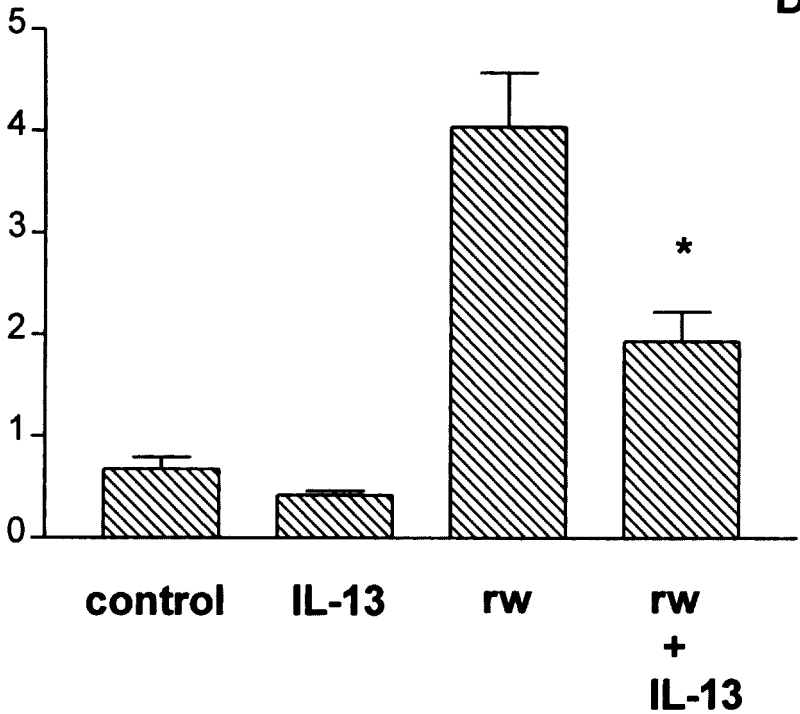


FIGURE 4—Continued

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