

Stimulation of human bronchial epithelial cells by IgE-dependent histamine-releasing factor

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Yoneda, Kazuo, Kazuhito Rokutan, Yoichi Nakamura, Hiroaki Yanagawa, Shigetada Kondo-Teshima, and Saburo Sone. Stimulation of human bronchial epithelial cells by IgE-dependent histamine-releasing factor. *Am J Physiol Lung Cell Mol Physiol* 286: L174–L181, 2004. First published August 29, 2003; 10.1152/ajplung.00118.2003.—An IgE-dependent histamine-releasing factor (HRF p23; also known as translationally controlled tumor protein or p23) stimulates the release of histamine, IL-4, and IL-13 from a subpopulation of highly allergic donor basophils. It has also been shown to act as a chemoattractant for eosinophils. To elucidate novel functions of HRF p23 in airway inflammation, we examined the effects of human recombinant HRF p23 (hrHRF) on bronchial epithelium and found that hrHRF stimulated the secretions of IL-8 and granulocyte/macrophage colony-stimulating factor by both primary cultures of human bronchial epithelial cells and BEAS-2B cells. In response to hrHRF, these cells induced IL-8 mRNA expression within 4 h. H₂O₂, but not IL-1 β or tumor necrosis factor- α , stimulated secretion of HRF p23 by BEAS-2B cells, suggesting that oxidative stress may trigger the release of HRF p23 from bronchial epithelial cells. Bronchoalveolar lavage (BAL) from healthy volunteers contained only trivial or undetectable amounts of HRF p23. Significantly higher amounts of HRF p23 were recovered from BAL fluid taken from asthmatic patients, and the amounts of HRF p23 were further elevated in patients with idiopathic eosinophilic pneumonia. Our results demonstrate for the first time that HRF p23 can stimulate nonimmune epithelium. HRF p23 derived from bronchial epithelial cells may regulate complex cytokine networks in eosinophil-dependent inflammation of the human airway.

interleukin-8; granulocyte/macrophage colony-stimulating factor; airway inflammation

BRONCHIAL ASTHMA is a chronic inflammatory disease characterized by an increase in airway responsiveness to a variety of specific and nonspecific stimuli. This hyperresponsiveness is characterized by tissue infiltration of lymphocytes, eosinophils, and mast cells, among which eosinophils play a prominent role (2, 5, 8). Eosinophils cause tissue damage through the release of toxic proteases, lipid mediators, cytokines, and reactive oxygen intermediates, leading to desquamation of the bronchial epithelium, enhanced bronchial responsiveness, airway obstruction, and remodeling of the airway wall. Th2 cells play a central role in the regulation of eosinophilic inflammation (2, 19, 27). In addition to these inflammatory cells, it has been suggested that airway epithelial cells themselves participate in inflammation and remodeling processes, by producing proinflammatory mediators [platelet-activating factor (PAF) and

prostaglandins], cytokines [granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-6], chemokines [IL-8, monocyte chemoattractant peptide-1 and -4, regulated on activation, normal T cell expressed secreted (RANTES), eotaxins, monocyte-derived chemokine, and thymus- and activation-regulated chemokines], and other factors (11). Many of these, especially CCL chemokines, are known to target eosinophils (23). IL-8 is an important bronchial epithelial product with concentrations in bronchoalveolar lavage (BAL) from patients with asthma that are known to be several times higher than in BAL from healthy individuals (17).

A translationally controlled tumor protein, p23 (TCTP), was originally identified as a growth-related tumor protein whose synthesis is controlled mainly at the translational level (4, 30). This protein has been recognized as a cell cycle-dependent, tubulin-binding protein having calcium-binding sites (9). In addition to this growth-related function as a cytosolic protein, TCTP is now known to act as a secretory protein. It has been uniquely characterized as an IgE-dependent histamine-releasing factor (HRF p23) (14). HRF p23 mRNA is ubiquitously expressed, including in B cells, T cells, monocyte-macrophages, and fibroblasts (14). In addition to nasal lavages (13) and skin blister fluids (29) from patients with late-phase allergic inflammation, BAL fluid from bronchial asthma was reported to contain an activity that stimulates release of histamine from basophils in an IgE-dependent fashion (12). Human recombinant HRF p23 (hrHRF) was shown to be able to prime basophils, triggering secretion of IL-4 and IL-13 as well as histamine (20, 21). In these earlier papers of MacDonald and colleagues, it was shown that the histamine-releasing capacity of hrHRF was dependent on the type of IgE (termed IgE⁺) but not on Fc ϵ RI. However, they recently showed (28) that the histamine-releasing activity of hrHRF did not appear to involve the IgE molecule. It discriminates between basophiles, not between different IgE molecules (IgE or IgE⁺). We reported (24) that mouse recombinant HRF p23 specifically triggers eosinophil recruitment in ovalbumin-sensitized mice but not in normal mice. Subsequently, Bheekha-Escura et al. (3) confirmed that hrHRF had a chemoattractant activity with regard to human eosinophils and could stimulate IL-8 secretion by these cells, which do not normally possess cell surface Fc ϵ RI. This suggested that hrHRF may stimulate distinct types of cells other than basophils, thus participating in the late phase of allergic inflammation. We recently showed (18) that dioxin, the most toxic man-made pollutant, stimulates synthesis and se-

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cretion of HRF p23 from mouse embryonic stem cells, suggesting a potential role for environmental pollutant-induced inflammation.

At present, HRF p23 is recognized as a cytokine that stimulates inflammatory cells such as basophils and eosinophils. To elucidate potential roles of HRF p23 in airway inflammation, this study tested whether hrHRF can stimulate human bronchial epithelial cells.

MATERIALS AND METHODS

Preparation and culture of bronchial epithelial cells. The present experiments were approved by the Ethics Committee at the University of Tokushima. Human bronchial epithelial cells were obtained by brushing subsegmental bronchi of the left B⁵ under bronchoscopy from healthy volunteers (16). Each volunteer gave written consent after receiving an explanation of the entire experimental procedures. After isolated cells were washed by centrifugation at 400 *g* at 4°C, they were suspended in LHC-9-RPMI 1640 medium and cultured for 7–10 days in type 1 collagen-coated wells of 24-well culture plates at 37°C under 5% CO₂ in air. The medium was changed at 24 h and then

every other day thereafter. When cells became confluent, they were passaged in 48-well culture plates. Immunocytochemical analysis with a monoclonal antibody against human cytokeratin or against vimentin showed that the growing cells were bronchial epithelial cells and that vimentin-positive fibroblasts were not contaminated, as previously documented (16).

A human bronchial epithelial cell line transformed with SV40 (BEAS-2B; American Type Culture Collection, Rockville, MD) was cultured in LHC-9-RPMI 1640 medium, and cells at passages 43–45 were used for experiments.

Preparation of hrHRF p23. The open reading frame of human HRF p23 cDNA was obtained by RT-PCR with the following primers: sense primer, 5'-AAAAGGATCCATGATTATCTACCGGGAC-3'; antisense primer, 5'-AAAAGAATTCTTAACATTTTTCATTCTTAA-3' (10). A resultant PCR product was confirmed to be the HRF p23 cDNA by cycle sequencing with a DNA sequencer (model ABI 373; Applied Biosystems). The RT-PCR product was restriction digested with *Bam*HI and *Eco*RI, ligated into the pGEX-4T-2 plasmid, and transfected into JM109-competent cells. The glutathione S-transferase fusion protein hrHRF was prepared, and glutathione S-transferase was then enzymatically cleaved by treatment with

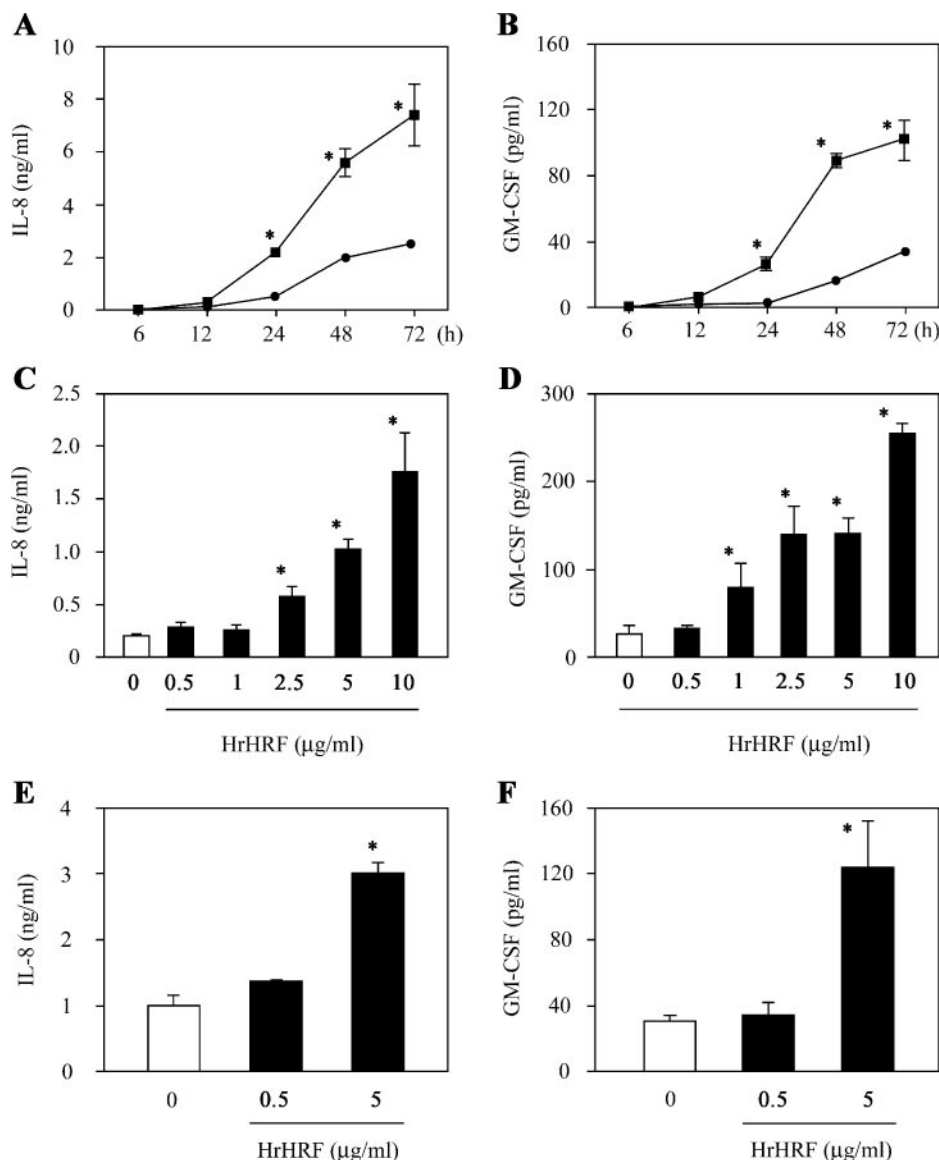


Fig. 1. Effects of human recombinant histamine-releasing factor (hrHRF) on production of IL-8 or granulocyte-macrophage colony-stimulating factor (GM-CSF) by human bronchial epithelial cells. BEAS-2B cells were cultured in LHC-9-RPMI 1640 medium in 24-well plates. Cells at confluence were washed and incubated for the indicated times with 5 μ g/ml hrHRF in serum-free RPMI 1640 medium (A and B) or were treated with different concentrations of hrHRF for 48 h (C and D). Freshly isolated human bronchial cells growing at confluence in 24-well culture plates were treated with 1 or 10 μ g/ml hrHRF for 48 h in serum-free RPMI 1640 medium (E and F). The amounts of IL-8 and GM-CSF secreted into the medium were measured by ELISA as described in MATERIALS AND METHODS. Values are expressed as means $\pm$ SE in 4 independent experiments. *Significant differences from the values of untreated control cells ($P < 0.05$).

thrombin, as previously described (24). Before the treatment with thrombin, contaminated LPS was removed by sedimentation with β -(1,4)-2-amino-2-deoxy-D-glucan (Kurita Water Industry, Tokyo, Japan). The *Limulus* ameocyte lysate assay (Endospecy; Seikagaku Kogyo, Tokyo, Japan) showed that LPS contaminated in hrHRF preparations was consistently <10 pg/ μ g hrHRF.

Immunohistochemistry. Transverse sections (4 μ m) were prepared from surgically resected lung tissues that had been fixed with 3% formaldehyde in PBS and embedded in paraffin. After nonspecific binding sites were blocked with 1% goat serum in PBS for 20 min, the sections were incubated at room temperature for 1 h with a rabbit polyclonal antibody directed against residues 91–107 of human HRF p23 (24). After washing with PBS, sections were incubated with biotinylated goat anti-rabbit IgG for 30 min. Bound antibodies were visualized with an alkaline phosphatase-conjugated anti-rabbit IgG (Sigma) and Vector red alkaline phosphatase substrate kit I (Vector, Burlingame, CA). Preimmune rabbit serum was used to assess non-specific reactions.

Northern blot analysis. Total RNA was extracted from cells with an acid guanidinium-isothiocyanate-phenol-chloroform mixture (Nippon Gene, Toyama, Japan). Ten micrograms of total RNA was subjected to electrophoresis in a 1% agarose gel containing 0.6 M formaldehyde. The RNA was then transferred to a nylon membrane (Hybond N-plus; Amersham Pharmacia). After prehybridization, the membrane was subjected to Northern blot hybridization with an *Eco*RI-digested fragment of the human IL-8 cDNA (Teijin, Tokyo, Japan) or a cDNA probe for β -actin (Wako Chemical, Osaka, Japan). These probes were pre-labeled by the multiprime DNA-labeling method. After hybridization, the membrane was washed four times for 15 min each with $2\times$ SSPE (0.15 M NaCl, 10 mM NaH_2PO_4 , 1 mM EDTA, pH 7.4) containing 0.1% SDS at 42°C and then with $1\times$ SSPE containing 0.1% SDS for 30 min at 42°C. The membrane was autoradiographed

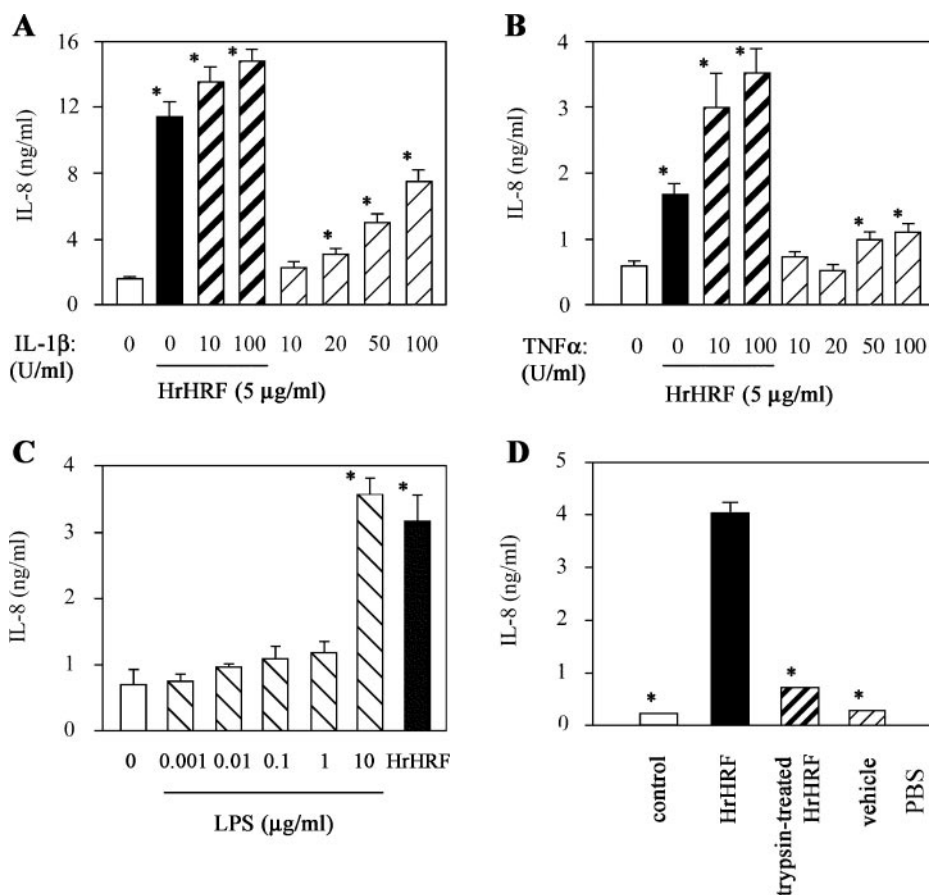
by exposure to Kodak XAR-5 film at -80°C for an appropriate time. Bound probes were quantified with a FUJIX Bio-Image Analyzer (BAS2000; Fuji Film, Tokyo, Japan).

Measurement of HRF p23. Whole-cell proteins were extracted from freshly isolated, cultured bronchial epithelial cells and BEAS-2B cells, and intracellular HRF p23 levels were measured by immunoblot analysis as described previously (24). For measurement of HRF p23 release from BEAS-2B cells into culture media, the cells were stimulated with 20 U/ml IL-1 β , 100 U/ml tumor necrosis factor (TNF)- α , or 50–250 μ M H_2O_2 in serum-free RPMI 1640. The medium was collected into a microcentrifuge tube and centrifuged at 900 g for 5 min. The supernatants were concentrated 50 times by centrifugal filtration with a 10,000 nominal molecular weight limit filter tube (Millipore, Bedford, MA) and stored at -80°C until further examination. BAL was collected as described previously (1). The lavage fluid was passed through sterile gauze and centrifuged at 250 g for 10 min at 4°C. The supernatants were concentrated 100 times with an ultrafiltration membrane in a stirred cell (Amicon, Beverly, MA) and stored at -80°C until they could be examined. The number of precipitated cells was counted in a hemocytometer, and cell populations were determined after staining with May-Giemsa stain. The amounts of HRF p23 in the culture supernatants and BAL fluids were measured by Western blot analysis with the anti-HRF p23 antibody (24), and the levels were quantified by laser densitometry, with reference to simultaneously electrophoresed hrHRF standards (10–200 ng).

Measurements of cytokines. GM-CSF and IL-8 were measured with ELISA kits (R & D Systems, Minneapolis, MN). Minimum detectable concentrations of human GM-CSF and IL-8 were 1.5 and 20 pg/ml, respectively.

Statistical analysis. All results are expressed as means $\pm$ SE. Statistical analysis was performed with the Student's two-tailed un-

Fig. 2. Stimulation of IL-8 production from BEAS-2B cells by IL-1 β , tumor necrosis factor (TNF)- α , or LPS. BEAS-2B cells were stimulated by the indicated concentrations of IL-1 β (A) or TNF- α (B) in the presence or absence of 5 μ g/ml hrHRF. They were also treated with different concentrations of LPS (C). The amounts of IL-8 secreted into the medium were measured by ELISA. *Significant differences from the values measured for untreated control cells ($P < 0.05$). hrHRF was treated with trypsin in PBS. D: BEAS-2B cells were incubated with 5 μ g/ml hrHRF, trypsin-treated HrHRF, or vehicle (PBS) for 48 h, and the release of IL-8 was measured by ELISA. *Significant differences from the values of hrHRF-treated cells ($P < 0.05$).



paired *t*-test for comparisons between two groups. Correlations between two parameters were evaluated with Pearson's test. Differences were considered significant if *P* values were <0.05.

RESULTS

Secretion of IL-8 and GM-CSF by bronchial epithelial cells. Although hrHrF has been shown to target basophils and eosinophils, we tested whether hrHrF could act also on bronchial epithelial cells to trigger the secretion of proinflammatory cytokines. The human bronchial epithelial cell line BEAS-2B secretes inflammatory cytokines in response to LPS, TNF- α , or IL-1 β (16). When BEAS-2B cells were treated with different concentrations of hrHrF, release of IL-8 (Fig. 1, A and C) and GM-CSF (Fig. 1, B and D) were significantly stimulated in a dose- and time-dependent fashion, compared with the values for the control medium. Human bronchial epithelial cells freshly prepared from healthy volunteers also responded to hrHrF, leading to upregulated secretion of IL-8 and GM-CSF (Fig. 1, E and F). This suggested that bronchial epithelium might be a target for hrHrF.

Under our experimental conditions, the stimulatory action of hrHrF at 5 $\mu\text{g/ml}$ (217 nM) on IL-8 secretion was more potent than that of IL-1 β or TNF- α alone (Fig. 2, A and B) and was comparable to that of 10 $\mu\text{g/ml}$ LPS (Fig. 2C). Furthermore, hrHrF additively enhanced IL-8 secretion from BEAS-2 cells that were stimulated by IL-1 β or TNF- α (Fig. 2, A and B). Low-endotoxin hrHrF (<10 pg/ μg hrHrF) was used in this study. Bronchial epithelial cells were relatively insensitive to LPS, and the contaminated LPS at lower concentrations in hrHrF did not stimulate BEAS-2B cells (Fig. 2C). Furthermore, treatment with trypsin did not decompose LPS, but the protease digestion of hrHrF almost completely cancelled the stimulatory actions of HrHrF (Fig. 2D), thus excluding potential LPS contamination.

Stimulation of IL-8 mRNA expression by hrHrF. Bronchial epithelial cell lines HS-24, BET-1A, and BEAS-2B express IL-8 mRNA and upregulate its expression in response to TNF- α or IL-1 (15, 22). As shown in Fig. 3, BEAS-2B cells rapidly increased expression of IL-8 mRNA, with a peak at 1 h after stimulation (Fig. 3A). Furthermore, 1 $\mu\text{g/ml}$ (43 nM) or

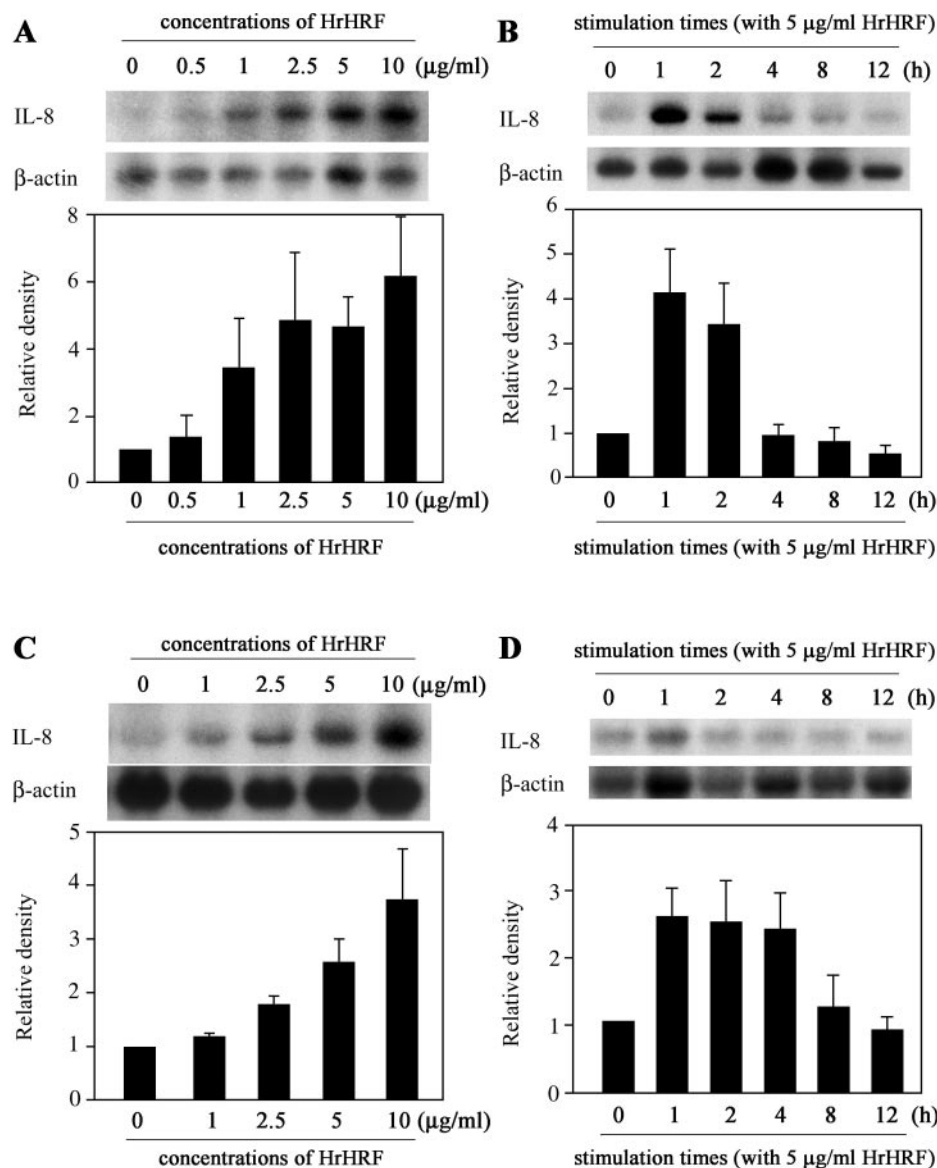


Fig. 3. Stimulation of IL-8 mRNA expression in bronchial epithelium by hrHrF. BEAS-2B cells (A and B) or primary cultured bronchial epithelial cells (C and D) were incubated with different concentrations of hrHrF for 48 h or stimulated with 5 $\mu\text{g/ml}$ hrHrF for the indicated times. Total RNA was extracted from these cells and subjected to Northern blot hybridization as described in MATERIALS AND METHODS. The amounts of IL-8 and β -actin mRNAs were quantified with a FUJIX Bio-Image Analyzer, and the results are shown in A–D, bottom. Values are means $\pm$ SE for 3 separate experiments.

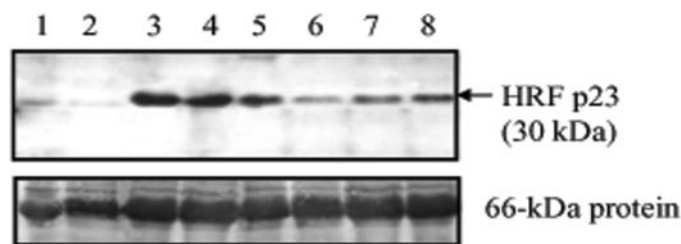


Fig. 4. Detection of HRF p23 in bronchoalveolar lavage (BAL). BAL was collected from healthy volunteers (lanes 1 and 2), eosinophilic pneumonia patients (lanes 3–5), and asthmatic patients (lanes 6–8). The fluids were concentrated and subjected to Western blot analysis as described in MATERIALS AND METHODS. The amount of sample applied to each lane was roughly determined by comparing the amounts of a 66-kDa protein dominantly stained with Coomassie blue R-250 as shown at bottom.

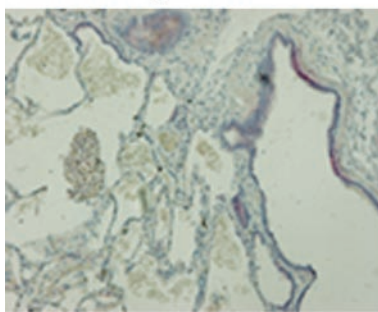
higher concentrations of hrHRF significantly stimulated IL-8 transcript expression in these cells (Fig. 3B). Freshly isolated, cultured human bronchial epithelial cells also responded to hrHRF, and IL-8 mRNA expression increased 1–4 h after treatment with hrHRF. This upregulation occurred in response to 2.5 $\mu\text{g/ml}$ or higher concentrations of hrHRF (Fig. 3C).

Detection of HRF p23 in BAL fluid. Next, we examined whether BAL fluid actually contains HRF p23 protein. Collected BAL fluid was concentrated 100 times and subjected to immunoblot analysis (Fig. 4). BAL fluids from healthy volunteers contained undetectable or small amounts of HRF p23. Although we examined limited numbers of patients, BALs from asthmatic patients were seen to contain significantly higher concentrations of HRF p23 than BALs from normal

subjects (Fig. 4). It should be noted that BAL fluids from patients with idiopathic eosinophilic pneumonia appeared to contain higher levels of HRF than those from asthmatic patients (Fig. 4).

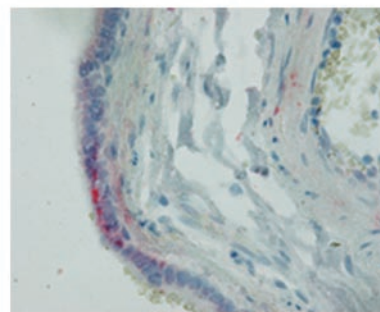
Source of HRF p23 in human airway. The expression of HRF p23 was examined in surgically resected lung tissues. The tissues were subjected to immunohistochemical detection of HRF p23-containing cells. As shown in Fig. 5, human bronchial cells were positive for HRF p23. We next examined potential sources of hrHRF in BAL fluid. To address the possibility that bronchial epithelial cells themselves may secrete HRF p23 into bronchial lumen, we examined possible stimulants that may trigger HRF p23 release from BEAS-2B cells. Induction of HRF p23 is mainly regulated at the translational level; therefore, intra- and extracellular levels of HRF p23 were assessed by Western blot analysis. First, we tested whether IL-1 β or TNF- α triggers release of HRF p23 from BEAS-2B cells. As shown in Fig. 6, BEAS-2B cells in serum-free culture spontaneously released small amounts of HRF p23; however, neither IL-1 β nor TNF- α enhanced this secretion, and intracellular levels of HRF p23 were constant in these cells. On the other hand, exposure to 50–250 μM H₂O₂ significantly stimulated HRF p23 secretion from BEAS-2B cells. Figure 6 shows data with 50 mM H₂O₂. However, in doses up to 250 mM (data not shown) there was significant stimulation. We also confirmed that H₂O₂ up to 250 μM did not cause cell damage throughout the experiments, which were based on continued Trypan blue exclusion, adherence to the culture plates, and no significant increase in lactate dehydrogenase release (data not shown).

anti-HRF p23 Ab



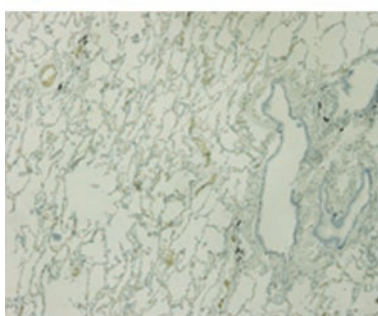
x 40

anti-HRF p23 Ab



x 100

normal rabbit serum



x 40

Fig. 5. Immunohistochemical detection of HRF p23-possessing cells. Surgically resected lung tissues were subjected to immunohistochemical detection of HRF p23-containing cells as described in MATERIALS AND METHODS. Preimmune rabbit serum was used to assess nonspecific reactions. Original magnifications are shown below each panel.

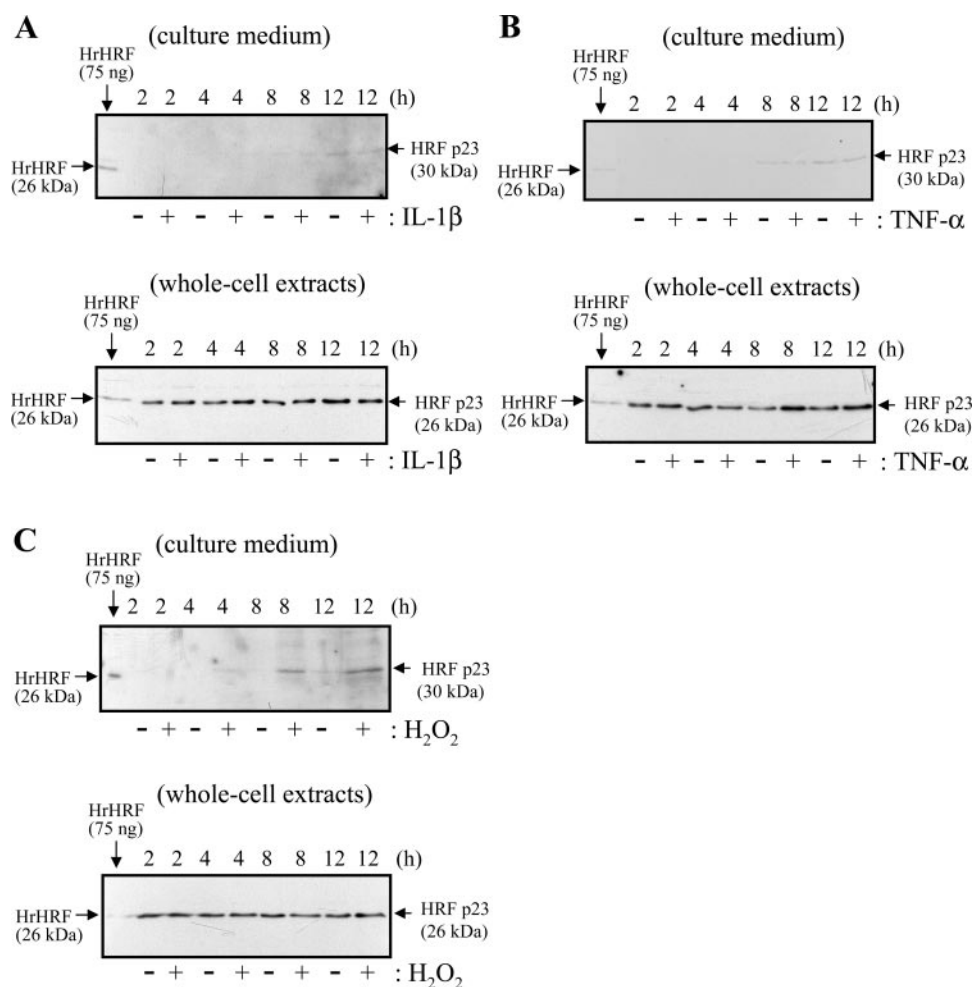


Fig. 6. Secretion of HRF p23 by BEAS-2B cells. BEAS-2B cells were treated with 100 U/ml IL-1 β (A), TNF- α (B), or 50 μ M H₂O₂ (C) for the indicated numbers of hours. Whole cell proteins were extracted as previously described (15). Reaction media were collected and concentrated 50 times as described in MATERIALS AND METHODS. The amounts of HRF p23 in the whole cell proteins and concentrated media were measured by Western blot analysis with an anti-HRF p23 antibody, with 75 ng of hrHRF as a standard.

DISCUSSION

Bronchial epithelial cells not only form a physical barrier to the outside environment but also serve important biochemical and immunologic roles in the initiation of inflammatory and immune responses against various types of environmental stimuli. We previously showed (16) that the Th2 cell-derived cytokines IL-4 and IL-13 stimulate GM-CSF secretion by bronchial epithelial cells and suggested that these cells may be actively involved in Th2-associated airway inflammation. To the best of our knowledge, we have demonstrated for the first time here that hrHRF can stimulate human bronchial epithelial cells and cause secretion of proinflammatory cytokines, at least in vitro. We also confirmed that these cells are one of the potential sources of this factor in the airway. Thus our results suggest, but do not prove, the hypothesis that HRF p23 may be involved in the regulation of complex cytokine networks in airway inflammation.

Compared with IL-1 β and TNF- α , higher concentrations (micrograms/milliliter) of hrHRF were required for the stimulation of IL-8 and GM-CSF secretion by bronchial epithelial cells. It is difficult to correctly measure the concentration of HRF p23 in the airway; however, immunoblot analysis showed that patients with asthma and eosinophilic pneumonia had increased levels of HRF p23 in BAL. If bronchial epithelial cells could secrete HRF p23 into the lumen, it might be

concentrated enough to stimulate bronchial epithelial cells themselves by autocrine/paracrine mechanisms. BAL from asthmatic patients contains concentrations of a neutrophil chemoattractant IL-8 that are several times higher than those measured in healthy subjects (17). GM-CSF stimulates the proliferation, differentiation, and survival of eosinophils. Furthermore, it was found that GM-CSF primed eosinophils for enhanced IL-8 secretion that was stimulated by RANTES or PAF, as well as by hrHRF (3). It is possible that HRF p23 may directly or indirectly participate in the progression of eosinophilic inflammation in the airway. However, further clinical studies are required to prove this hypothesis.

In a previous report (24), we showed that an intraperitoneal injection of recombinant mouse HRF p23 could trigger infiltration of eosinophils within 4 h in antigen-sensitized mice but not in normal mice. Using human eosinophils prepared from patients with mild asthma, Bheekha-Escura et al. (3) demonstrated that hrHRF was chemotactic for eosinophils, although its potency was much lower than that of PAF or RANTES. They also showed that hrHRF stimulated IL-8 mRNA expression within 1 h, and this was followed by an increase in the secretion of IL-8 by eosinophils. The concentration of hrHRF (700 nM) used to stimulate human eosinophils or the eosinophilic cell line AML 14-3D10 (3) was higher than the concentrations used in our experiments (~40–400 nM). IL-8

production from eosinophils stimulated by hrHRF required prepriming with GM-CSF (3), but hrHRF alone effectively stimulated IL-8 and GM-CSF production by bronchial epithelial cells, implying that bronchial epithelial cells might be more sensitive to hrHRF than eosinophils. We also documented that hrHRF augmented IL-8 secretion from bronchial epithelial cells in the presence of IL-1 β or TNF- α . This was similar to results reported for eosinophils stimulated by hrHRF combined with IL-5 or TNF- α (3). Neither IL-1 β nor TNF- α upregulated synthesis or secretion of HRF p23 in bronchial epithelial cells. Thus hrHRF appeared to stimulate IL-8 production independently of IL-1 β and TNF- α .

Finally, we found that low concentrations of H₂O₂ trigger HRF p23 secretion by BEAS-2B cells. Cell viability was carefully monitored, and the culture medium contained only a secretory form of the 30-kDa HRF p23 but not an intracellular form of the 26-kDa HRF p23. This was similar to observations reported for macrophages stimulated by macrophage colony-stimulating factor (24). Human HRF p23 has an ER-targeting signal and an N-glycosylation site, whereas it does not have an obvious signal sequence at the NH₂ termini (10). The secretory mechanism is still unknown; however, BEAS-2B cells appeared to actively secrete HRF p23 under oxidative stress, and this was supported by our recent report (18) that dioxin also stimulates secretion of HRF p23 from mouse embryonic stem cells. These findings imply that air pollutants or reactive oxygen species generated during inflammation may trigger HRF p23 secretion from bronchial epithelial cells, thus aggravating airway inflammation.

Campbell et al. reported that bronchial epithelial cells expressed a low-affinity (7) or high-affinity (6) receptor for IgE on bronchial cells of asthmatic patients, and it had been suggested that HRF p23 might interact with IgE⁺ or Fc ϵ RI. However, several lines of evidence have revealed that HRF is able to stimulate mediator releases independently of these two molecules (3, 21, 28).

A recent structural analysis of *Schizosaccharomyces pombe* HRF p23 has revealed that this protein forms a structural superfamily with the Mss4/Dss4 family of proteins (members of the Ras superfamily) (25), which have been termed guanine nucleotide-free chaperones. By comparing the differences in histamine-releasing capability of basophils to hrHRF between IgE⁺ and IgE⁻ donors, Vonakis et al. (26) showed that the release rates were negatively associated with the levels of Src homology 2 domain-containing inositol 5'-phosphatase. This suggests a possible involvement of phosphatidylinositol triphosphate as a second messenger for hrHRF-induced histamine release in a distinct population of basophils (2, 19, 27). At present, no specific receptor for HRF p23 has been identified and HRF p23-specific activation pathways in bronchial epithelia are unknown. To develop novel therapeutic strategies for allergic airway diseases, further studies are necessary to clarify the pathophysiology of HRF p23 in inflammatory responses of the airway.

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