

Bradykinin stimulates eotaxin production by a human lung fibroblast cell line

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Background: Bradykinin, a potent inflammatory peptide, is increased in the airways of allergic patients. Accompanying the elevated bradykinin levels are increases in both eosinophils and fibroblasts. Eotaxin, a potent eosinophil-specific chemotactic factor, is released by fibroblasts and increased in the lower respiratory tract of allergic patients.

Objective: We sought to test the hypothesis that lung fibroblasts release eotaxin in response to bradykinin.

Methods: The potential of bradykinin to induce the release of eotaxin from the human lung fibroblast cell line HFL-1 was tested by cell culture and evaluation of the culture supernatant fluids and RNA for immunoreactive eotaxin and eotaxin messenger RNA.

Results: HFL-1 cells released eotaxin constitutively without stimulation, but bradykinin stimulated eotaxin release in a dose- and time-dependent manner and resulted in augmented expression of eotaxin messenger RNA. The release of eotaxin was sensitive to the action of glucocorticoids. Eosinophil chemotactic activity by HFL-1 supernatant fluids was inhibited by anti-human eotaxin-neutralizing antibody. Consistent with these results, inhibitors of bradykinin B2 receptors, but not bradykinin B1 receptors, inhibited bradykinin-induced eotaxin release.

Conclusion: These data demonstrate that bradykinin may stimulate lung fibroblasts to release eotaxin and suggest the potential for this mechanism to be important in modulation of lung inflammation. (*J Allergy Clin Immunol* 2000;106:117-23.)

Key words: Eosinophil, chemotaxis, corticosteroids

Bradykinin is a 9-amino-acid peptide that is generated from its plasma protein precursor, kininogen, by the action of plasma and tissue kallikreins. Bradykinin has a number of properties appropriate to a mediator of asthma.¹ Increased levels of kinins are evident in the upper airways of allergic patients after allergen challenge.² Similarly, in allergic subjects with asthma, bronchoalveolar lavage fluid levels of immunoreactive kinins, as well as kinin-generating enzymes, are significantly elevated after inhalation of antigen.³ Furthermore, the administra-

Abbreviations used

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

PIPES: Piperazine-N, N'-bis[2-ethanesulfonic acid]

tion of bradykinin receptor antagonists are reported to inhibit the increase of several inflammatory mediators in bronchoalveolar lavage fluid, airway hyperresponsiveness, and inflammation.^{4,5}

The airways of asthmatic patients are infiltrated by a large number of eosinophils. Several studies have suggested a strong association between eosinophil numbers and the severity of asthma.^{6,7} The chemokines are a family of potent protein chemotactic factors from inflammatory cells.⁸ Although several members of the chemokine family can induce eosinophil migration, eotaxin appears to be the most selective for eosinophil recruitment in vivo.⁹⁻¹² Eotaxin production is increased in some human disorders characterized by eosinophilic inflammation, including asthma.^{13,14} It is also increased in response to respiratory allergen challenge in animals^{9,15} and human subjects.^{16,17}

Previous reports have demonstrated the capacity of fibroblasts for eotaxin production and messenger (m)RNA expression.^{18,19} We postulated that bradykinin would stimulate eotaxin expression by lung fibroblasts. The results demonstrate bradykinin stimulation of lung fibroblasts to release eotaxin secretion and suggest a role for fibroblasts and eotaxin in eosinophil recruitment at sites of tissue injury and inflammation.

METHODS

Cell culture

Human fetal lung fibroblasts (HFL-1, lung, diploid, human, passage 14 cell line) were purchased from the American Type Tissue Culture Collection (Rockville, Md). These cells retain features of human lung fibroblasts, including collagen and fibronectin production.^{20,21} The HFL-1 cells were suspended at 1.0×10^6 cells/mL in Ham's F-12 supplemented penicillin (50 µg/mL, Gibco), streptomycin (50 µg/mL, Gibco), and 10% heat-inactivated FBS. Cell suspensions (3 mL) were added to 30-mm diameter tissue-culture dishes (Corning) and were cultured at 37°C in a 5% CO₂ atmosphere. After 2 to 3 days in culture, the cells had reached confluence, and the culture medium was replaced with 2 mL of medium supplemented as above and incubated for 1 additional day.

Exposure of HFL-1 cells to bradykinin

The culture medium was removed from the cells by washing twice with serum-free medium, and cells were incubated in the pres-

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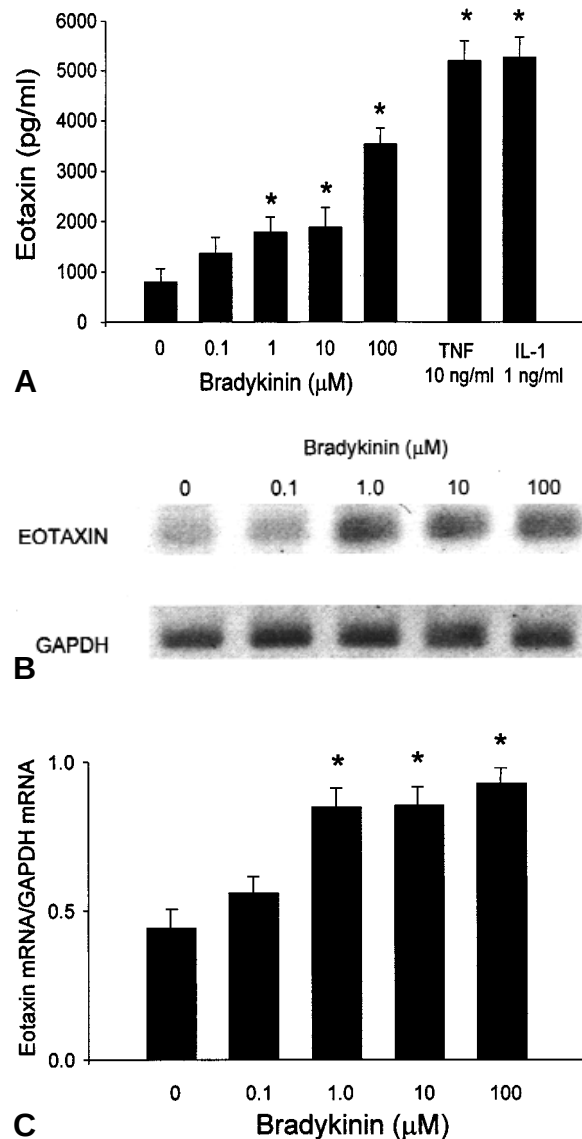


FIG 1. **A**, The dose-dependent release of eotaxin from HFL-1 cells in response to bradykinin after 48 hours' incubation ($n = 5$). TNF- α (10 ng/mL) and IL-1 β (1 ng/mL) were incubated with HFL-1 cells to be compared with bradykinin's effect. Eotaxin concentration is on the ordinate, and bradykinin concentration is on the abscissa. Values are expressed as means $\pm$ SEM. * $P < .05$ compared with supernatant fluids without bradykinin. **B**, The dose-dependent effect of bradykinin on eotaxin mRNA expression. Representative results with RT-PCR for eotaxin and GAPDH are shown. **C**, Densitometry data with eotaxin expressed as ratio of eotaxin mRNA to GAPDH mRNA (*eotaxin mRNA/GAPDH mRNA*). The ratio of eotaxin mRNA to GAPDH mRNA is on the ordinate, and bradykinin concentration is on the abscissa ($n = 3$). * $P < .05$ compared with cells without bradykinin.

ence and absence of bradykinin. To determine the dose-dependent release of eotaxin, the cultures were incubated with various concentrations of bradykinin (0, 0.1, 1, 10, and 100 μ M, Sigma) at 37°C in a humidified 5% CO₂ atmosphere for 48 hours. TNF- α (10 ng/mL, Sigma) or IL-1 β (1 ng/mL, Sigma) were used as positive controls.^{22,23} The time-dependent release of chemotactic activity was

evaluated by incubating HFL-1 cells for 6 to 48 hours in the presence and absence of bradykinin (100 μ M/L) because this concentration produced the maximal eotaxin stimulation of the doses tested. To test the capacity of glucocorticoids to inhibit bradykinin-induced eotaxin release, the cell cultures were incubated with bradykinin (100 μ M/L) and dexamethasone (0.1–1 μ M/L). Cell injury was evaluated by means of microscopy (cell shape and detachment from tissue culture dish) and trypan blue exclusion. The supernatant fluids were then harvested and stored at –80°C until assayed.

Measurement of eotaxin in the supernatant fluids

The concentrations of eotaxin were measured in the cell supernatant fluids from HFL-1 cells by using a commercially available ELISA (R&D Systems) according to the manufacturer's instructions. The minimum concentration detected by this method was 15.6 pg/mL.

Evaluation of eotaxin mRNA expression

Eotaxin mRNA was analyzed by using RT-PCR. Total cellular RNA was extracted from adherent cells by using a modification of the methods of Chomczynski and Sacchi.²⁴ The RNA was reverse transcribed by using a commercially available kit (Promega). One microgram of the reverse-transcribed DNA was then mixed with Ready to Go PCR Beads (Pharmacia), and the front and back primers were added at a final concentration of 0.2 μ M/L. PCR was performed in a Perkin-Elmer model 480 thermal cycler at 94°C for 2 minutes and 32 cycles consisting of 94°C for 45 seconds, 61°C for 45 seconds, 72°C for 2 minutes, and 72°C for an additional 7 minutes. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a *housekeeping gene* with PCR. The DNA was subjected to agarose gel, and the intensity of the bands was quantitated by using densitometry. The results were expressed as the ratio of intensity to the GAPDH. Sequences of the primers were as follows: eotaxin (F): 5'-ACATGAAGGTCTCCGACACTTC-3'; eotaxin (R): 5'-TTGGCCAGGTAAAGCAGVAGGTG-3'; GAPDH (F): 5'-ACCACAGTCCATGCCATCA-3'; and GAPDH (R): 5'-TCCAC-CACCCTGTTGCTGTA-3'.

Measurement of IL-1 β and effect of IL-1 β antibody on the release of eotaxin

It was reported that bradykinin stimulates lung fibroblasts to release IL-1 β ,²⁵ which then might stimulate the fibroblasts to release eotaxin. To test for this possibility, the monoclonal mouse anti-human IL-1 β antibody (10, 100, and 1000 ng/mL; Gnezyme) was used. Thirty minutes before the addition of bradykinin (100 μ M/L), anti-human IL-1 β antibody was added to the cultures. We measured the concentrations of IL-1 β in the cell supernatant fluids from HFL-1 cells by using a commercially available ELISA (R&D Systems) according to the manufacturer's instructions.

Effect of eotaxin antibody on eosinophil chemotactic activity by HFL-1 supernatant fluids

Eosinophils were isolated by using a modification of the method of Hansel et al²⁶ with a magnetic cell-separation system (Becton Dickinson). Briefly, venous blood anticoagulated with 130 mmol/L trisodium citrate was obtained from normal human volunteers and diluted with PBS in a 1:1 ratio. Diluted blood was overlaid on an isotonic Percoll solution (density, 1.082 g/mL; Sigma) and then centrifuged at 1000g for 30 minutes at 4°C. The supernatant and mononuclear cells at the interface were carefully removed, and red blood cells in the sediment were lysed with two cycles of hypotonic lysis (0.1% KHCO₃ and 0.83% NH₄Cl). Isolated granulocytes were washed two times with piperazine-N, N'-bis[2-ethanesulfonic

acid] (PIPES) buffer (25 mmol/L PIPES, 50 mmol/L NaCl, 5 mmol/L KCl, 25 mmol/L NaOH, and 5.4 mmol/L glucose, pH 7.4) containing 1% defined calf serum (Hyclone Laboratories), and an approximately equal volume of anti-CD16 antibody conjugated with magnetic particles (Miltenyi Biotec) was added to the cell pellet. After 60 minutes on ice, 5 mL of PIPES buffer with 1% defined calf serum (DCS) was added to the cell-antibody mixture. The resuspended cells were loaded onto the separation column positioned in the magnetic cell separation system with a strong magnetic field. The cells were eluted 3 times with 5 mL of PIPES buffer with 1% DCS. Purity of the eosinophils counted by using Randolph's stain was greater than 94%, and viability was greater than 98%. The eosinophils were resuspended in Gey's solution at 2.0×10^6 cells/mL and used for the chemotaxis assay.

Eosinophil chemotactic activity was assayed in 48-well microchemotaxis chambers (Neuroprobe, Inc), as previously described.²⁷ The bottom wells of the chamber were filled with 25 μ L of the bradykinin-stimulated HFL-1 supernatant fluids, which were incubated with anti-human eotaxin-neutralizing antibody (R&D systems, 0.1–10 μ g/mL) or normal goat IgG (R&D systems) for 1 hour before chemotactic assay. A 10- μ m thick polyvinylpyrrolidone-free polycarbonate filter with a pore size of 5 μ m was placed over the samples. The silicon gasket and the upper pieces of the chamber were applied, and 50 μ L of the cell suspension was placed into the upper wells. The chambers were incubated in humidified air in 5% CO₂ at 37°C for 90 minutes. Nonmigrated cells were wiped away from the filter. The filter was immersed in methanol for 5 minutes, stained with a modified Wright's stain, and mounted on a glass slide. Cells that had completely migrated through the filter were counted by means of light microscopy. Eosinophil chemotactic activity was expressed as the mean number of migrated cells per high-powered field from duplicate wells.

Effect of receptor determinants on the release of eotaxin

To determine the receptor responsible for the release of eotaxin, the bradykinin B1 receptor antagonist Des Arg⁹-[Leu⁸]-bradykinin (Sigma) and the bradykinin B2 receptor antagonist D-Arg-[Hyp³Thi^{5,8}, D-Phe⁷]-bradykinin (Sigma) were evaluated to determine the responsible bradykinin receptor.²⁸ Thirty minutes before the addition of bradykinin (10 μ mol/L), HFL-1 cells were treated with the bradykinin B1 or bradykinin B2 receptor antagonist (100 μ g/mL).

Statistical analysis

In experiments in which multiple experiments were made, differences between groups were tested for significance by using one-way analysis of variance with the Fisher multiple range test applied to data at specific time and dose points. In experiments in which a single measurement was made, the differences between groups were tested for significance by using the Student paired *t* test. In all cases a *P* value of less than .05 was considered significant. Data in figures and tables were expressed as mean values $\pm$ SEM.

RESULTS

Expression of eotaxin mRNA and release of eotaxin from the HFL-1 cell line

HFL-1 cells released eotaxin spontaneously without stimulation. However, HFL-1 cells released eotaxin in a dose-dependent manner in response to bradykinin (*P* < .05; Fig 1, A). The lowest dose of bradykinin to stimulate eotaxin release was 1.0 μ mol/L. Increasing concentrations of bradykinin progressively increased the

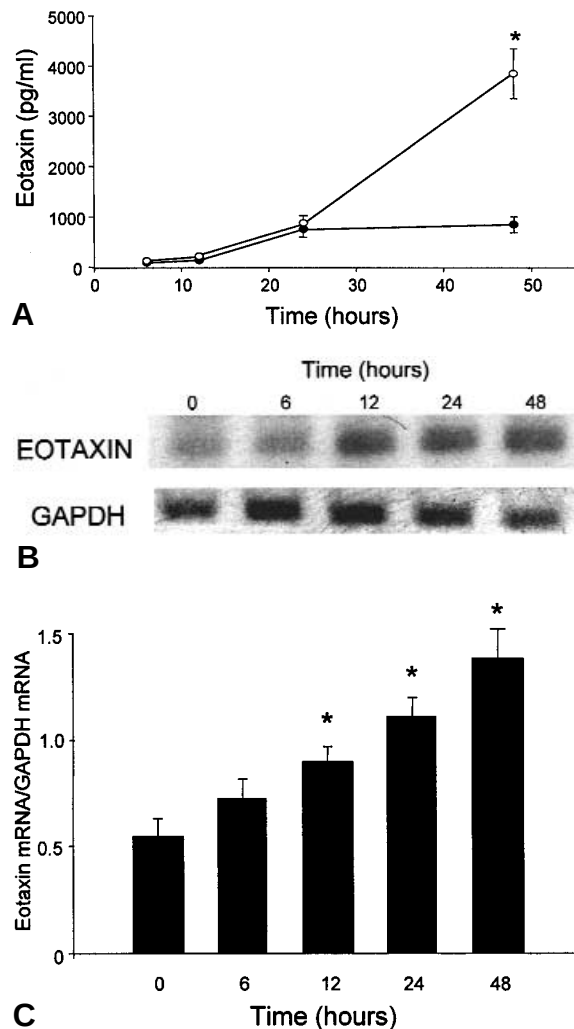


FIG 2. **A**, The time-related release of eotaxin in response to 100 μ mol/L bradykinin from HFL-1 cells (*n* = 4). Open circles indicate eotaxin release with bradykinin incubation, and closed circles indicate eotaxin release without bradykinin from HFL-1 cells. The amount of eotaxin released is on the ordinate, and time is shown on the abscissa. Values are expressed as means $\pm$ SEM. **P* < .05 compared with supernatant fluids without bradykinin. **B**, The time-dependent effect of bradykinin on eotaxin mRNA expression. Representative results with RT-PCR for eotaxin and GAPDH are shown. **C**, Densitometry data with eotaxin expressed as ratio of eotaxin mRNA to GAPDH mRNA (eotaxin mRNA/GAPDH mRNA). Eotaxin concentration is on the ordinate, and time is shown on the abscissa (*n* = 3). **P* < .05 compared with cells without bradykinin.

release of eotaxin up to 100 μ mol/L. Both TNF- α and IL-1 β also stimulated the release of eotaxin (Fig 1, A). Bradykinin also increased eotaxin in a dose-dependent manner, as demonstrated with RT-PCR (Fig 1, B and C). Bradykinin treatment of HFL-1 cells also resulted in the augmented release of eotaxin and expression of eotaxin mRNA in a time-dependent manner (Fig 2). HFL-1 release of eotaxin in response to bradykinin in a time-dependent manner was not significant compared with that elicited by media alone until 48 hours (*P* < .05; Fig

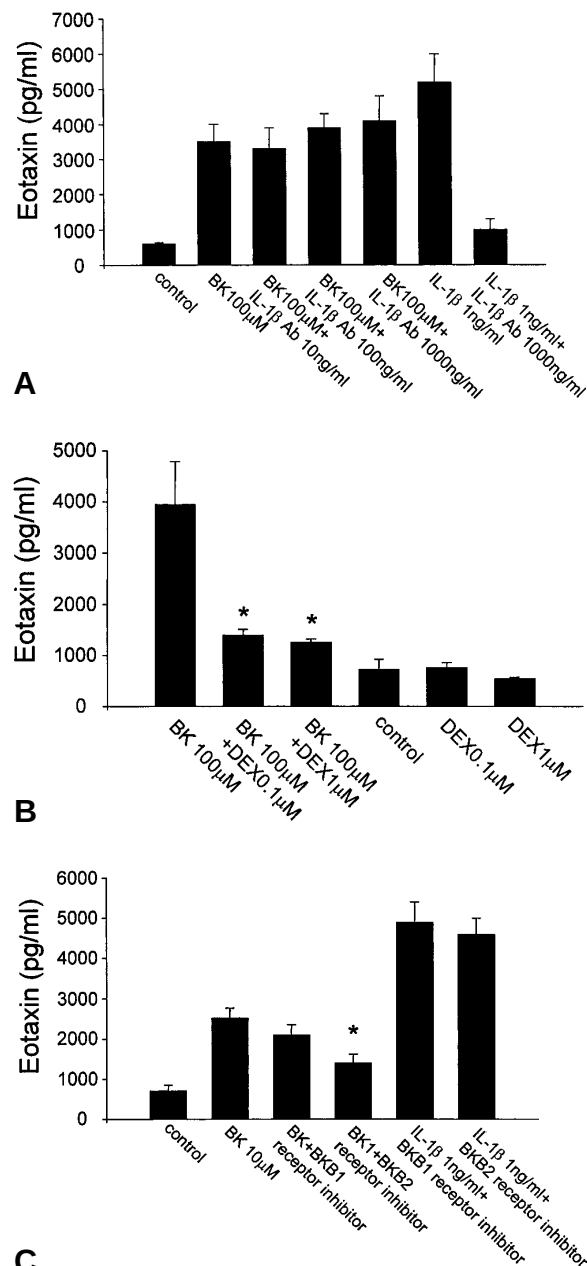


FIG 3. **A**, The effect of IL-1 β antibody on eotaxin release from HFL-1 cells stimulated by bradykinin (BK; $n = 4$). **B**, The effect of dexamethasone (DEX) on eotaxin release from HFL-1 cells ($n = 4$). **C**, The effect of bradykinin receptor antagonists Des Arg⁹-[Leu⁸]-bradykinin or D-Arg-[Hyp³Thi^{5,8}, D-Phe⁷]-bradykinin (100 μ M/L) on eotaxin release from HFL-1 cells in response to bradykinin (10 μ M/L, $n = 4$). In the panels eotaxin concentration is shown on the ordinate, and the experimental groups are listed on the abscissa. Values are expressed as means $\pm$ SEM. * $P < .05$ compared with bradykinin-treated supernatant fluids.

2, A). However, treatment of HFL-1 cells with bradykinin (100 μ M/L) increased eotaxin mRNA expression after 12 hours (Fig 2, B and C), with levels of eotaxin mRNA remaining elevated for the duration of the experiment (up to 48 hours). Increasing PCR cycles

gave increasing amounts of DNA through a fairly broad range, with linearity obtained from approximately cycles 24 to 32, beginning with 1 μ g of RNA ($r = 0.99$ for eotaxin and GAPDH; $P < .01$ for both comparisons). Similarly, different doses of starting material (10-1000 ng of RNA) produced different band intensities of DNA ($r = 0.99$ for eotaxin and GAPDH; $P = .011$ for eotaxin and $P < .01$ for GAPDH).

Consistent with these results, we also stimulated the lung epithelial cell lines BEAS 2B and A549 and another lung fibroblast cell line, CCL-202, with bradykinin. The eotaxin concentration by the epithelial cell lines was below the lower detection limit of the assay (15.6 pg/mL). In contrast, CCL-202 released eotaxin in response to bradykinin after 48 hours (media, 32.1 ± 4.1 pg/mL; 10 μ M/L bradykinin, 43.2 ± 5.3 pg/mL; 100 μ M/L bradykinin, 65.4 ± 7.3 pg/mL; $n = 4$; $P < .05$ for 10 and 100 μ M/L bradykinin compared with media alone).

Bradykinin did not cause HFL-1 injury (no deformity of cell shape, no detachment from tissue culture dish, and greater than 95% of cells viable by means of trypan blue exclusion) after 48 hours' incubation with 100 μ M/L bradykinin.

IL-1 β release and effect of IL-1 β antibody on the release of eotaxin by bradykinin stimulation

IL-1 β was not detected in HFL-1 supernatant fluids after bradykinin stimulation. IL-1 β antibody (10, 100, and 1000 ng/mL) also had no effect on eotaxin release caused by bradykinin stimulation ($P > .05$; Fig 3, A).

Effect of dexamethasone on eotaxin secretion

In cell cultures stimulated with bradykinin, dexamethasone pretreatment significantly inhibited eotaxin release ($P < .05$; Fig 3, B). Dexamethasone had no significant effect on eotaxin release from unstimulated cells.

Effect of bradykinin receptor antagonists on the release of eotaxin from HFL-1 cells

Bradykinin-induced eotaxin release was suppressed by pretreatment with D-Arg-[Hyp³Thi^{5,8}, D-Phe⁷]-bradykinin, a selective bradykinin B2 receptor antagonist (Fig 3, C). Des Arg⁹-[Leu⁸]-bradykinin, a bradykinin B1 receptor antagonist had no significant effect on eotaxin release. Higher concentrations of the bradykinin receptor antagonist (>100 μ M/L) had an apparent direct effect on eosinophil chemotactic activity.

Inhibition of eosinophil chemotactic activity by polyclonal antibody to eotaxin

HFL-1 cells released eotaxin spontaneously, and bradykinin stimulated the release of eotaxin. Therefore we evaluated the effect of anti-eotaxin antibody on eosinophil chemotactic activity released into the supernatant fluids by bradykinin-stimulated HFL-1 cells. The

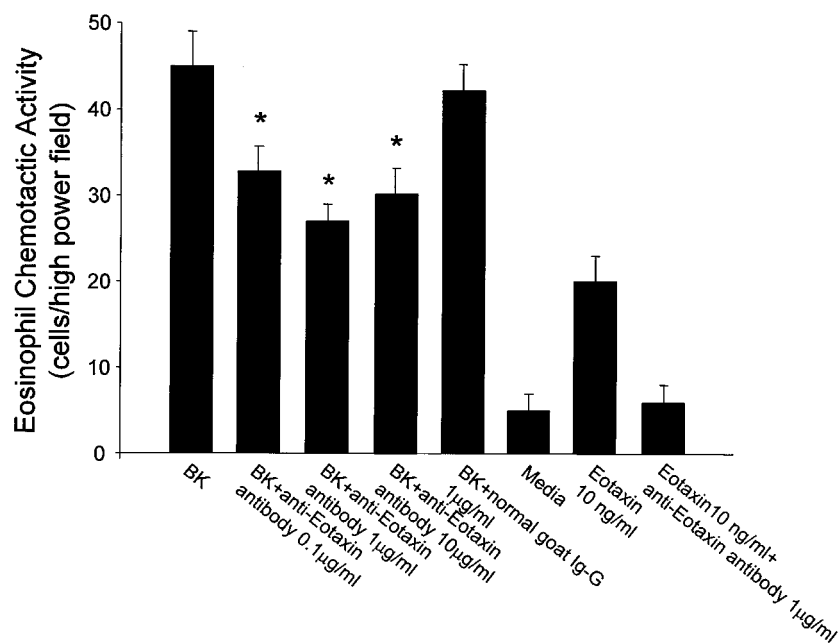


FIG 4. The inhibition of eosinophil chemotactic activity by anti-eotaxin antibody (1 µg/mL, $n = 4$). Eosinophil chemotactic activity is shown on the ordinate, and the experimental groups are listed on the abscissa. Values are expressed as means $\pm$ SEM. Media, Ham's F12 medium alone; BK, bradykinin. * $P < .05$ compared with bradykinin-treated supernatant fluids.

anti-eotaxin antibody inhibited eosinophil chemotactic activity induced by the release of bradykinin (100 µmol/L) by about 40% ($P < .05$, Fig 4), but a control antibody did not ($P > .05$, Fig 4). The anti-eotaxin antibody also reduced the eosinophil chemotactic activity of recombinant eotaxin (10 ng/mL, R&D Systems) to baseline values ($P > .05$ compared with media alone, Fig 4).

DISCUSSION

The present study demonstrated the spontaneous release of eotaxin by HFL-1 cells. However, eotaxin release was significantly augmented in response to bradykinin in a dose- and time-dependent manner. Consistent with these results, bradykinin treatment of HFL-1 cells also resulted in the augmented expression of eotaxin mRNA. Eotaxin release by bradykinin-stimulated HFL-1 cells was sensitive to the action of glucocorticoids, and eosinophil chemotactic activity by HFL-1 supernatant fluids was inhibited by an anti-eotaxin antibody. The receptor responsible for the release of eotaxin appears to be the bradykinin B2 receptor because the bradykinin B2 receptor antagonist, D-Arg-[Hyp³Thi^{5,8}, D-Phe⁷]-bradykinin, inhibited the release of eotaxin. These data suggest that bradykinin may play a role in eosinophil recruitment to the airways by stimulating eotaxin release from lung fibroblasts.

In clinical studies elevated levels of kinin and tissue kallikreins have been found in the bronchoalveolar lavage fluid of asthmatic subjects after antigen challenge.³ Consistent with the concept of bradykinin stimulating an influx of eosinophils into the airways, a signif-

icant correlation has been found between airway responsiveness to bradykinin and eosinophil count in bronchoalveolar lavage fluid, bronchial biopsy specimens, and sputum.^{29,30}

Previous reports have shown that various cytokines modulate eotaxin mRNA expression and release in several cell types, including lung epithelial cells and fibroblasts.²³ Recently, Teran et al¹⁸ reported that the amount of immunoreactive eotaxin induced by IL-4 and TNF- α from lung fibroblasts was 3800 to 20,000 times greater than that released by cytokine-stimulated A549 epithelial cells. Consistent with these results, we also stimulated the lung epithelial cell lines BEAS 2B and A549 and another lung fibroblast cell line, CCL-202, with bradykinin. The eotaxin concentrations in the supernatant fluid of epithelial cell lines were below the lower detection limit of the assay (15.6 pg/mL, data not shown), but the CCL-202 human fibroblast cell line could also be stimulated to release eotaxin in response to bradykinin. These observations are consistent with the concept that fibroblasts may be a major source of eotaxin in the lungs and a major source of eosinophil chemotactic activity in allergic airway disorders by releasing eotaxin in response to bradykinin.

IL-1 β is found at increased levels in lung lavage fluid from patients with asthma, and its spontaneous release is augmented in alveolar macrophages from adult patients with asthma and wheezy infants.^{31,32} Recently, bradykinin has been reported to stimulate IL-1 β release from the human lung fibroblast cell line WI-38.²⁵ Therefore one explanation for our findings is that bradykinin stimulation of fibroblasts releases IL-1 β , which in turn

stimulates the fibroblasts to release eotaxin. However, IL-1 β antibodies had no effect on bradykinin-induced eotaxin release, and IL-1 β was not detected in the supernatant fluids after bradykinin stimulation. These data suggest that bradykinin stimulation is not related to an IL-1 β effect in our experiments.

Eosinophil chemotactic activity by the HFL-1 cell line is probably not entirely caused by eotaxin because an anti-eotaxin antibody inhibited only about 40% of the eosinophil chemotactic activity released by bradykinin-stimulated HFL-1 cells. Nevertheless, eotaxin is still likely an important eosinophil chemotactic factor. Recent studies have demonstrated that fibroblasts are important participants in the orchestration of inflammation by virtue of their release various chemoattractants. We have previously reported that HFL-1 cells constitutively release GM-CSF and leukotriene B₄³³ as monocyte chemoattractants, and their concentrations are sufficient to function as chemotactic factors. These cytokines can also serve as eosinophil chemotactic factors and may explain some of the eosinophil chemotactic activity not inhibited by eotaxin antibodies.

The use of glucocorticoids is now a well-established treatment for asthma. Because glucocorticoids downregulate the production of numerous cytokines and are potent inhibitors of the eosinophil infiltrate seen in chronic allergic diseases,³⁴ we investigated the effect of dexamethasone on the secretion of eotaxin. Our results indicate that the release of eotaxin from lung fibroblasts is sensitive to the action of glucocorticoids. The inhibitory effect of glucocorticoids on eotaxin release may be relevant to their anti-inflammatory properties and their clinical efficacy in controlling airway inflammation.

Although it is reported that bradykinin B1 receptors have been shown to be induced in inflammation or after tissue injury,²⁸ the presence of bradykinin B1 receptor-binding sites in guinea pig trachea and lung tissue has not been observed in healthy animals.³⁵ However, the bradykinin B2 receptor is normally expressed in a large number of tissues, including lung tissue.³⁶ In this study bradykinin-induced eotaxin release was suppressed by pretreatment with D-Arg-[Hyp³Thi^{5,8}, D-Phe⁷]-bradykinin, a selective bradykinin B2 receptor antagonist, but not with Des Arg⁹-[Leu⁸]-bradykinin, a bradykinin B1 receptor antagonist.²⁸ These results suggest that bradykinin induces eotaxin release from lung fibroblasts through stimulation of bradykinin B2 receptors. Consistent with these observations, previous studies have reported that bradykinin B2 receptors are expressed on lung fibroblasts.^{37,38}

In conclusion, bradykinin stimulated HFL-1 cells to release eotaxin, and the secretion is inhibited by dexamethasone. These findings lead to the hypothesis that lung fibroblast-derived eotaxin may in part be responsible for the eosinophil infiltration observed in allergic disease of the airways.

REFERENCES

1. Proud D, Kaplan AP. Kinin formation: mechanisms and role in inflammatory disorders. *Annu Rev Immunol* 1988;6:49-83.

2. Proud D, Togias A, Naclerio RM, Crush SA, Norman PS, Lichtenstein LM. Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. *J Clin Invest* 1983;72:1678-85.
3. Christiansen SC, Proud D, Cochrane CG. Detection of tissue kallikrein in the bronchoalveolar lavage fluid of asthmatic subjects. *J Clin Invest* 1987;79:188-97.
4. Abraham WM, Burch RM, Farmer SG, Sienkiewicz MW, Ahmed A, Cortes A. A bradykinin antagonist modifies allergen-induced mediator release and late bronchial responses in sheep. *Am Rev Respir Dis* 1991;143:787-96.
5. Soler M, Sienkiewicz M, Abraham WM. A bradykinin-antagonist blocks antigen-induced airway hyperresponsiveness and inflammation in sheep. *Pulm Pharmacol* 1990;3:9-15.
6. Bousquet J, Chané P, Lacoste JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
7. Smith H. Asthma, inflammation, eosinophils and bronchial hyperresponsiveness. *Clin Exp Allergy* 1992;22:187-97.
8. Schall TJ, Bacon KB. Chemokines, leukocyte trafficking, and inflammation. *Curr Opin Immunol* 1994;6:865-73.
9. Jose PJ, Griffiths-Johnson DA, Collins PD, et al. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J Exp Med* 1994;179:881-7.
10. Ponath PD, Qin S, Ringler DJ, et al. Cloning of the human eosinophil chemoattractant, eotaxin. Expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J Clin Invest* 1996;97:604-12.
11. Rothenberg ME, Luster AD, Leder P. Murine eotaxin: an eosinophil chemoattractant inducible in endothelial cells and in interleukin 4-induced tumor suppression. *Proc Natl Acad Sci USA* 1995;92:8960-4.
12. Gonzalo JA, Lloyd CM, Kremer L, et al. Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. *J Clin Invest* 1996;98:2332-45.
13. Lamkhouié B, Renzi PM, Abi-Younes S, et al. Increased expression of eotaxin in bronchoalveolar lavage and airways of asthmatics contributes to the chemotaxis of eosinophils to the site of inflammation. *J Immunol* 1997;159:4593-601.
14. Ying S, Robinson DS, Meng Q, et al. Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. *Eur J Immunol* 1997;27:3507-16.
15. Luster AD, Rothenberg ME. Role of the monocyte chemoattractant protein and eotaxin subfamily of chemokines in allergic inflammation. *J Leukoc Biol* 1997;62:620-33.
16. Minshall EM, Cameron L, Lavigne F, et al. Eotaxin mRNA and protein expression in chronic sinusitis and allergen-induced nasal responses in seasonal allergic rhinitis. *Am J Respir Cell Mol Biol* 1997;17:683-90.
17. Brown JR, Kleimberg J, Marini M, Sun G, Bellini A, Mattoli S. Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients after allergen inhalation. *Clin Exp Immunol* 1998;114:137-46.
18. Teran LM, Mochizuki M, Bartels J, et al. Th1- and Th2-type cytokines regulate the expression and production of eotaxin and RANTES by human lung fibroblasts. *Am J Respir Cell Mol Biol* 1999;20:777-86.
19. Miyamasu M, Nakajima T, Misaki Y, et al. Dermal fibroblasts represent a potent major source of human eotaxin: In vitro production and cytokine-mediated regulation. *Cytokine* 1999;11:751-8.
20. Breul SD, Bradley KH, Hance AJ, Schafer MP, Berg RA, Crystal RG. Control of collagen production by human diploid lung fibroblasts. *J Biol Chem* 1980;255:5250-60.
21. Katayama K, Seyer JM, Raghoebar R, Kang AH. Regulation of extracellular matrix production by chemically synthesized subfragments of type I collagen carboxy propeptide. *Biochemistry* 1991;30:7097-104.
22. Chung KF, Patel HJ, Fadlon EJ, et al. Induction of eotaxin expression and release from human airway smooth muscle cells by IL-1 β and TNF α effects of IL-10 and corticosteroids. *Br J Pharmacol* 1999;127:1145-50.
23. Lilly CM, Nakamura H, Kesselman H, et al. Expression of eotaxin by human lung epithelial cells: induction by cytokines and inhibition by glucocorticoids. *J Clin Invest* 1997;99:1767-73.
24. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.

25. Pan ZK, Zuraw BL, Lung CC, Prossnitz ER, Browning DD, Ye RD. Bradykinin stimulates NF- κ B activation and interleukin 1 β gene expression in cultured human fibroblasts. *J Clin Invest* 1996;98:2042-9.
26. Hansel TT, De Vries IJ, Iff T, et al. An improved immunomagnetic procedure for the isolation of highly purified human blood eosinophils. *J Immunol Methods* 1991;145:105-10.
27. Harvath L, Falk W, Leonard EJ. Rapid quantification of neutrophil chemotaxis: use of polyvinylpyrrolidone-free polycarbonate membrane in a multiwell assembly. *J Immunol Methods* 1980;37:39-45.
28. Marceau F. Kinin B1 receptors: a review. *Immunopharmacology* 1995;30:1-26.
29. Roisman GL, Lacronique JG, Desmazes-Dufeu N, Carre C, Le Cae A, Dusser DJ. Airway responsiveness to bradykinin is related to eosinophilic inflammation in asthma. *Am J Respir Crit Care Med* 1996;153:381-90.
30. Polosa R, Renaud L, Cacciola R, Prosperini G, Crimi N, Djukanovic R. Sputum eosinophilia is more closely associated with airway responsiveness to bradykinin than methacholine in asthma. *Eur Respir J* 1998;12:551-6.
31. Virchow JC Jr, Walker C, Hafner D, et al. T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. *Am J Respir Crit Care Med* 1995;151:960-8.
32. Azevedo I, de Blic J, Dumarey CH, Scheinmann P, Vargaftig BB, Bachelet M. Increased spontaneous release of tumour necrosis factor- α by alveolar macrophages from wheezy infants. *Eur Respir J* 1997;10:1767-73.
33. Koyama S, Sato E, Masubuchi T, et al. Human lung fibroblasts release chemokinetic activity for monocytes constitutively. *Am J Physiol* 1998;275:L223-30.
34. Wang JH, Devalia JL, Xia C, Sapsford RJ, Davies RJ. Expression of RANTES by human bronchial epithelial cells in vitro and in vivo and the effect of corticosteroids. *Am J Respir Cell Mol Biol* 1996;14:27-35.
35. Farmer SG, Burch RM, Meeker SA, Wilkins DE. Evidence for a pulmonary B3 bradykinin receptor. *Mol Pharmacol* 1989;36:1-8.
36. Farmer SG, Burch RM. Biochemical and molecular pharmacology of kinin receptors. *Annu Rev Pharmacol Toxicol* 1992;32:511-36.
37. Baenziger NL, Jong YJ, Yocum SA, et al. Diversity of B2 bradykinin receptors with nanomolar affinity expressed in passaged IMR90 human lung fibroblasts. *Eur J Cell Biol* 1992;58:71-80.
38. Sawutz DG, Faunce DM, Houck WT, Haycock D. Characterization of bradykinin B2 receptors on human IMR-90 lung fibroblasts: stimulation of 45Ca²⁺ efflux by D-Phe substituted bradykinin analogues. *Eur J Pharmacol* 1992;227:309-15.

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