

Bradykinin increases the in vivo expression of the CXC chemokine receptors CXCR1 and CXCR2 in patients with allergic rhinitis

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Background: Increased levels of bradykinin and IL-8 have been detected within the airways of individuals with active symptoms of allergic rhinitis and asthma.

Objective: We sought to investigate the in vivo effect of bradykinin on the expression of the IL-8 receptors CXCR1 and CXCR2 in nasal cells.

Methods: Nasal samples were obtained from patients with active allergic rhinitis; patients with mild, quiescent allergic rhinitis; and healthy control subjects. CXCR1 and CXCR2 mRNA expression in the nasal cells was measured by means of quantitative real-time RT-PCR in baseline samples from all subjects, as well as in samples obtained after in vivo bradykinin challenge in healthy control subjects and patients with mild allergic rhinitis. CXCR1 and CXCR2 cell-surface expression was also assessed by means of flow cytometry in nasal epithelial cells at baseline and after ex vivo bradykinin challenge.

Results: No difference was seen in CXCR1 or CXCR2 mRNA expression between healthy control subjects and patients with quiescent allergic rhinitis at baseline; however, patients with active allergic rhinitis had increased baseline expression of both CXCR1 and CXCR2 mRNA. In vivo nasal bradykinin challenge significantly increased CXCR1 and CXCR2 mRNA expression in patients with quiescent allergic rhinitis but had no effect in healthy control subjects. Low levels of CXCR1 but not CXCR2 cell-surface expression was detected in nasal epithelial cells at baseline, and ex vivo bradykinin challenge induced CXCR2 cell-surface expression in nasal epithelial cells from patients with mild allergic rhinitis.

Conclusion: This study demonstrates the in vivo regulation of chemokine receptors by means of bradykinin in human airway tissue in patients with allergic rhinitis. (J Allergy Clin Immunol 2003;111:106-12.)

Key words: Bradykinin, IL-8, allergic rhinitis, CXCR1, CXCR2

The CXC chemokine IL-8 is regarded as one of the major chemokines involved in airway inflammation.^{1,2} IL-8 plays a crucial role in inflammation, recruiting neutrophils and potentially eosinophils to the site of inflam-

Abbreviations used

ENA: Epithelial neutrophil-activating protein
FAM: 6-Carboxy-fluorescein
GAPDH: Reduced glyceraldehyde-phosphate dehydrogenase
GRO: Growth-related oncogene
TAMRA: 6' Carboxy-tetramethyl-rhodamine

mation.³⁻⁵ A variety of airway resident cells, as well as cells infiltrating the airways during inflammation, are known to have the capacity to produce IL-8, and elevated levels of IL-8 have been detected within the airways of patients with allergic rhinitis.⁶⁻¹⁰ Furthermore, in vivo expression of IL-8 by normal human nasal epithelium has been demonstrated.¹¹ Multiple inflammatory mediators, including bradykinin, can induce the production of IL-8 by airway cells.¹² Increased levels of bradykinin have been found within the airways of allergic individuals with active symptoms of rhinitis and asthma, as well as following allergen challenge.¹³⁻¹⁸ A recent in vivo study demonstrated an association between bradykinin and IL-8 in the development of allergic rhinitis, whereby a bradykinin antagonist reduced the antigen-induced increase of IL-8 in nasal lavage fluid.¹⁹

Inappropriate activation of chemokine networks is associated with numerous human diseases.²⁰ There is increasing evidence that the levels of the chemokine receptors, as well as the chemokines themselves, potentially play an important role in the progression and attenuation of airway inflammation. For example, CCR3, along with its ligand, eotaxin, have been colocalized to the airway epithelium of asthmatic patients.²¹⁻²³ In addition, there is evidence that chemokines, including eotaxin and IL-8, might be regulated in an autocrine fashion.^{25,25} Such an autoregulatory pathway for chemokines within the airway could contribute to the amplification of the allergic inflammatory response.

Little is known regarding the expression of the IL-8 receptors. Bradykinin is known to be involved in the upregulation of IL-8 during allergic rhinitis; therefore it is of interest to ascertain the effect of bradykinin on IL-8 receptor expression. An increase in IL-8 receptor expression could render the airway more responsive to constitutive and induced IL-8. This investigation assessed the in vivo effect of bradykinin on the levels of the IL-8 recep-

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tors CXCR1 and CXCR2 in the nasal tissue of patients with allergic rhinitis and healthy control subjects.

METHODS

Subject characteristics, nasal challenge protocols, and nasal sample collection

Nonsmoking adults with mild, quiescent allergic rhinitis (diagnosis on the basis of a consistent history and confirmatory skin tests) and healthy control subjects were recruited to participate in the study. Informed consent was obtained in accordance with the Human Subjects Committee at The Scripps Research Institute. Patients with allergic rhinitis were excluded if they had received prior immunotherapy. All allergy medications, asthma medications, or both were withheld for at least 2 weeks before challenge. Baseline nasal samples were obtained from the left naris, followed by instillation of 200 μ g of bradykinin (reconstituted in sterile water) in a 100- μ L spray onto the inferior turbinate of the contralateral naris. A subsequent nasal sample was collected from the challenged naris after 4 hours. After a period of at least 2 weeks, the subject returned for a repeat challenge. A baseline nasal sample was collected from the left naris, and the subject was challenged with bradykinin as previously described, except that the challenged nasal sample was taken 8 hours after bradykinin was instilled. Nasal samples were collected by means of gentle scraping of the inferior turbinate with a Rhinoprobe curette (Arlington Scientific Inc). The sample was placed directly into RLT buffer (Qiagen Inc) and placed immediately at -70°C .

A separate group of subjects with active symptoms of allergic rhinitis but who were not taking medications for their symptoms were recruited from the allergy clinic. Baseline nasal samples were collected as described above.

Isolation of nasal epithelial cells

Nasal epithelial cells were isolated from nasal tissue samples by using the CELLection Epithelial Enrich kit (DynaL A.S.). Before epithelial cell isolation, the nasal tissue sample was disaggregated in 1 mL of PBS containing 0.1% collagenase XI (Sigma), 0.1 mg/mL DNase I (Sigma), and 3 mmol/L dithiothreitol and gently shaken for 20 minutes at room temperature. The cells were pelleted and washed 3 times in PBS. After the final wash, the sample was gently suspended in 1 mL of PBS/20% FBS. Nasal epithelial cells were then isolated from the nasal sample, according to the manufacturer's instructions.

RNA isolation and reverse transcription

Total RNA was extracted from nasal tissue samples and nasal epithelial cells by using the RNeasy mini kit (Qiagen Inc). DNA was digested during the RNA isolation procedure by using the RNase-free DNase 1 treatment kit (Qiagen Inc). A third of the nasal RNA was reverse transcribed with the Omniscript RT kit (Qiagen Inc). A one-twentieth aliquot of the RT product was used for subsequent PCR.

Real-time quantitative PCR

The iCycler iQ (Bio-Rad) was used for real-time quantitative PCR. The primers used were as follows: CXCR1 forward primer, 5'-gcagctctactgttgacaca-3'; CXCR1 reverse primer, 5'-catgtcctcttcagttcagca-3'; CXCR2 forward primer, 5'-tggtgatcagcaagactc-3'; CXCR2 reverse primer, 5'-gccctgaagaagagccaaca-3'; reduced glyceraldehyde-phosphate dehydrogenase (GAPDH) forward primer, 5'-gggaaggtgaagtcggagt-3'; and GAPDH reverse primer, 5'-tccactttaccagagttaaagcag-3'. The following dual-labeled probes were obtained from BioSearch Technologies: GAPDH, 5' FAM 6-carboxy-fluorescein (FAM)-ttgtctgtattggcgctgt-3' 6' carboxy-tetramethyl-rhodamine (TAMRA); CXCR1, 5' FAM-ctg-

gcggtgtcttcagttatgacaaacc-3'TAMRA; and CXCR2, 5' FAM-ctgccaagacagcagcgtctct-3'TAMRA. Standards, from 10 to 0.0001 attomoles of the PCR product cloned into pGEMTeasy (Promega Life Sciences), were run alongside the samples to generate a standard curve. All samples and standards were analyzed in triplicate. The PCR reaction consisted of 1.5 mmol/L Tris-HCl; 5 mmol/L KCl; 2 mmol/L deoxyribonucleoside triphosphates; 200 ng of sense and antisense primers; either 10 pmol of CXCR1 probe, 5 pmol of CXCR2 probe, or 12 pmol of GAPDH probe; either 3 mmol/L Mg^{2+} for CXCR1 and CXCR2 amplification or 4 mmol/L Mg^{2+} for GAPDH amplification; and 1 U of AmpliTaq gold (Applied Biosystems) in a total volume of 50 μ L. The reaction conditions were 95°C for 10 minutes, followed by 50 cycles of 30 seconds at 94°C , 30 seconds at 60°C , and 30 seconds at 72°C . The starting amount of cDNA in each sample was calculated by using the iCycler iQ software package (Bio-Rad).

Ex vivo stimulation of nasal samples with bradykinin

Nasal tissue was gently scraped from one naris and placed into 1 mL of basal keratinocyte serum-free medium (Gibco Invitrogen Corp). A nasal sample from the contralateral naris was placed into 1 mL of basal keratinocyte medium containing bradykinin (10^{-6} mol/L). The cells were incubated at 37°C in 100% humidity and 5% CO_2 for 8 hours before flow cytometry.

Flow cytometry

Nasal tissue samples from participating subjects were disaggregated in 1 mL of PBS containing 0.1% collagenase XI (Sigma), 0.1 mg/mL DNase I (Sigma), and 3 mmol/L dithiothreitol; gently shaken for 20 minutes at room temperature; washed in FACS buffer (PBS containing 0.2% BSA and 0.09% sodium azide); and resuspended in 100 μ L of FACS buffer. Cells from 3 donors of the same subject group were pooled and then split equally into 4 aliquots. Nasal cells were dual labeled with FITC-conjugated mouse anti-human IG1 Ber-EP4 epithelial marker (DAKO Corp) and a phycoerythrin-conjugated mouse anti-human IG2A antibody specific for either CXCR1 or CXCR2 (R&D Systems Inc). The isotype control antibodies used were FITC-conjugated goat anti-mouse IG1 and phycoerythrin-conjugated goat anti-mouse IG2A (R&D Systems Inc). Cells were incubated with the appropriate antibodies for 45 minutes at 4°C , washed twice in FACS buffer, and suspended in 400 μ L of FACS buffer containing 0.2% paraformaldehyde to fix the cells for analysis. Cells were analyzed on a FACScaliber (BD Biosciences). CXC receptor staining was determined on cells gated on forward- and side-scatter characteristics that stained positive for the Ber-EP4 epithelial marker.

Statistics

Statistics were performed with the software program Statview 5.0 (SAS Institute). Data were analyzed by using the Mann-Whitney U test, the unpaired t test, or the Wilcoxon signed-rank test.

RESULTS

In vivo expression of CXCR1 and CXCR2 mRNA in human nasal tissue

Initially, we assessed the expression of CXCR1 and CXCR2 mRNA in nasal tissue samples from patients with mild, quiescent allergic rhinitis and healthy control subjects by means of real-time quantitative RT-PCR. Levels of CXCR1 and CXCR2 mRNA were normalized to the housekeeping gene *GAPDH*. CXCR1 and CXCR2 mRNA were detected in nasal tissue samples from all 7

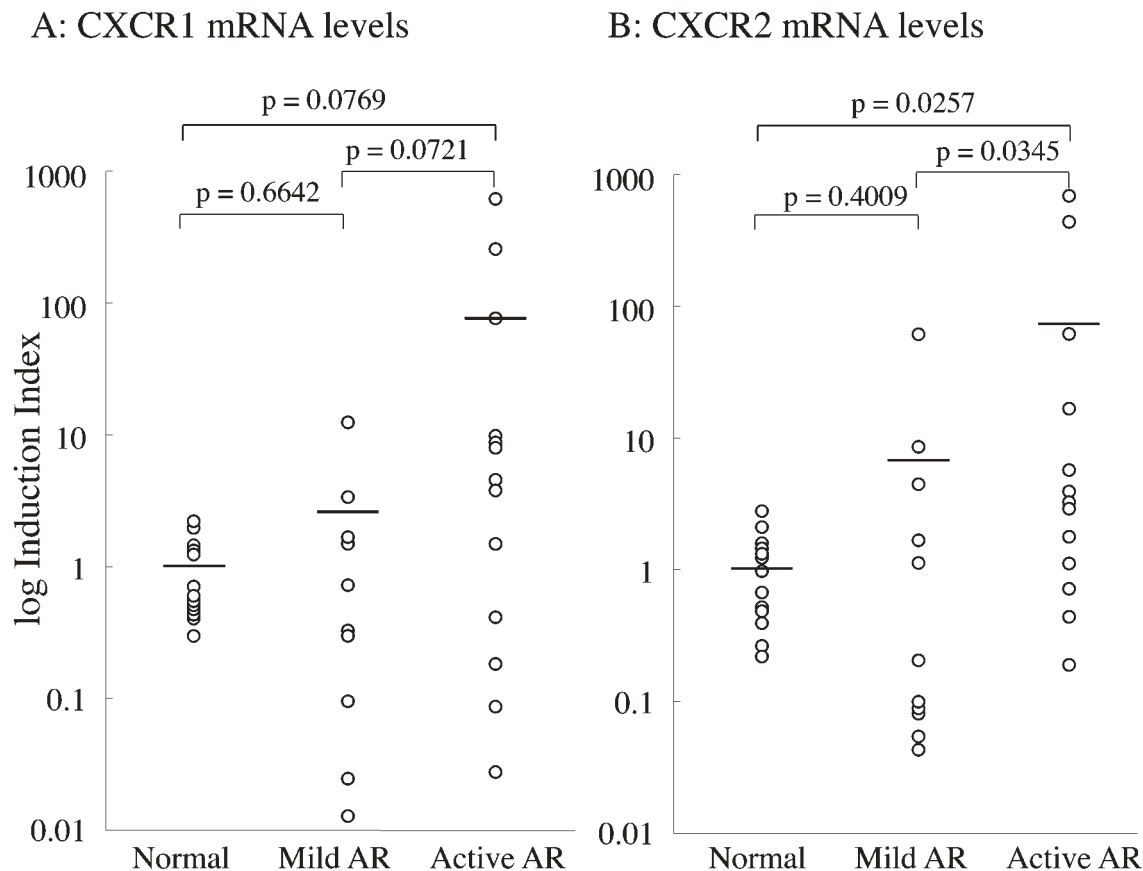


FIG 1. Increased levels of CXCR1 and CXCR2 mRNA in the nasal tissue of patients with active allergic rhinitis. Nasal tissue was obtained from 13 patients with active allergic rhinitis (*active AR*); 11 patients with mild, quiescent allergic rhinitis (*mild AR*); and 13 healthy control subjects. Total RNA was isolated, and each sample was analyzed in triplicate for CXCR1, CXCR2, and GAPDH by means of real-time quantitative RT-PCR. Levels of CXC chemokine receptor mRNA were normalized to GAPDH. An induction index was calculated for each sample. The induction index is defined as the ratio of the chemokine mRNA expression in a given sample divided by the mean chemokine mRNA expression of the matched healthy control subjects. The levels of CXCR1 (**A**) and CXCR2 (**B**) mRNA levels were compared between the subjects groups.

control subjects and 7 patients with allergic rhinitis. There was no difference in the level of either CXCR1 or CXCR2 mRNA expression in the nasal tissue of patients with mild, quiescent allergic rhinitis compared with that seen in healthy control subjects (Mann-Whitney *U* test).

Increased levels of CXCR1 and CXCR2 mRNA in nasal tissue from patients with active symptoms of allergic rhinitis

There was no difference in the levels of CXCR1 or CXCR2 mRNA between control subjects and patients with mild, quiescent allergic rhinitis; therefore we expanded our study to investigate the levels of CXCR1 and CXCR2 mRNA in the nasal tissue of patients with active symptoms of allergic rhinitis. For this purpose, we enrolled 13 patients with active symptoms of allergic rhinitis who were not receiving treatment for their allergic

rhinitis. An equal number of healthy subjects were recruited as control subjects. Nasal tissue samples were obtained from the subjects, RNA was isolated, and the levels of CXCR1, CXCR2, and GAPDH mRNA were assessed by means of real-time quantitative PCR. We normalized the chemokine receptor mRNA data to allow comparison of results obtained from experiments performed at widely separate times. Since groups of samples from patients with allergic rhinitis were always analyzed with a matching group of samples from healthy control subjects, we calculated the induction index (defined as the ratio of the chemokine mRNA expression in a given sample divided by the mean chemokine mRNA expression of the matched healthy control subjects).

The levels of CXCR1 mRNA in nasal tissue samples from 9 of the 13 subjects with active symptoms of allergic rhinitis were increased compared with the mean level of CXCR1 mRNA in the nasal tissue from healthy con-

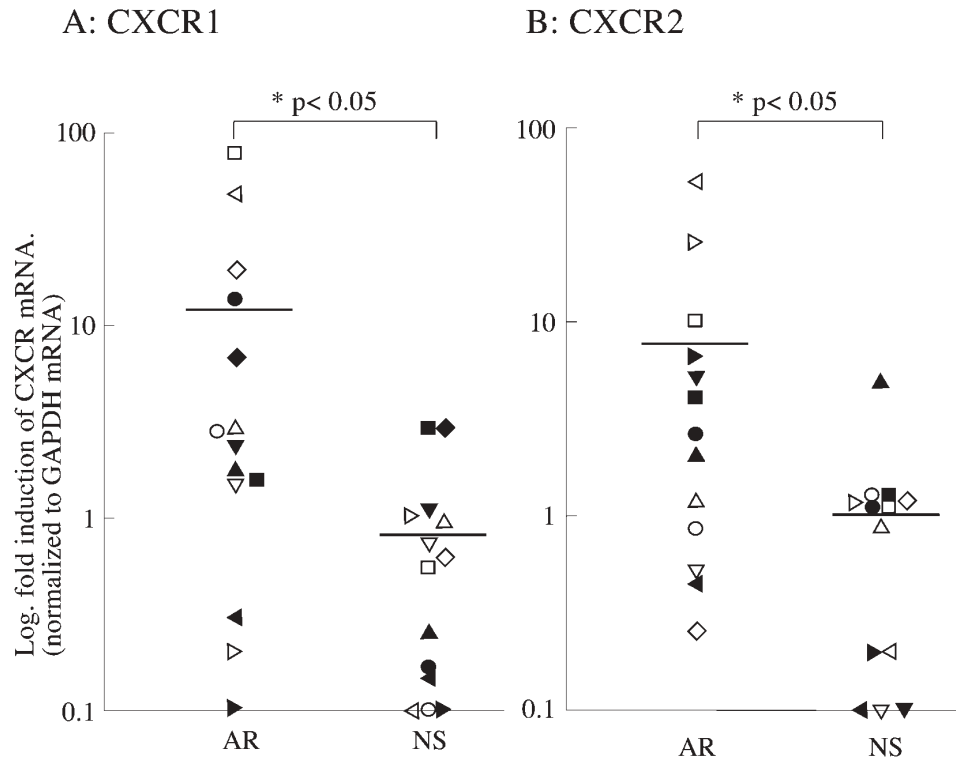


FIG 2. Effect of nasal bradykinin on the in vivo level of CXCR1 and CXCR2 mRNA in human nasal tissue. Patients with allergic rhinitis (AR) and healthy control subjects (NS) were challenged with intranasal bradykinin (200 μ g), and nasal tissue was obtained 4 or 8 hours later. Total RNA was isolated, and each sample was analyzed in triplicate for CXCR1, CXCR2, and GAPDH expression by means of real-time quantitative RT-PCR. Levels of CXCR1 and CXCR2 mRNA were normalized to GAPDH. Each subject is represented by a different shape, with open shapes representing the level of induction of either CXCR1 (A) or CXCR2 (B) mRNA at 4 hours, and filled shapes representing the level of induction of CXCR mRNA after 8 hours compared with the matched baseline level of CXCR mRNA.

trol subjects, whereas 8 were higher than the mean level of the mild, quiescent allergic rhinitis group (Fig 1, A). These differences were not significant ($P = .0769$ and $P = .0721$, respectively; Mann-Whitney U test) but showed a strong trend toward an increase in CXCR1 mRNA levels in the active allergic rhinitis group. CXCR2 mRNA levels in patients with active allergic rhinitis were statistically significantly higher than levels observed in both healthy control subjects and patients with mild allergic rhinitis ($P = .0257$ and $P = .0345$, respectively; Fig 1, B).

Bradykinin increases CXCR1 and CXCR2 mRNA expression in vivo in human nasal tissue

As increased levels of CXCR1 and CXCR2 mRNA were observed in subjects with active symptoms of allergic rhinitis, we investigated whether mediators that are increased following allergen challenge could affect the expression of these CXC receptors. To assess whether exposure to bradykinin could regulate airway expression

of CXCR1 and CXCR2 in vivo, we measured CXCR1 and CXCR2 mRNA levels in nasal tissue samples before and after nasal bradykinin challenge. CXCR1 and CXCR2 mRNA levels were measured by means of real-time quantitative RT-PCR at baseline as well as at 4 and 8 hours after bradykinin challenge in 7 patients with mild, quiescent allergic rhinitis and 7 healthy control subjects. Levels of CXCR1 and CXCR2 mRNA were normalized to the housekeeping gene *GAPDH*. The effect of bradykinin on CXCR1 and CXCR2 mRNA levels at both 4 and 8 hours after bradykinin challenge was compared between the 2 subject groups (Fig 2). Bradykinin stimulated a significantly greater increase in CXCR1 and CXCR2 mRNA levels in patients with allergic rhinitis than it did in healthy control subjects ($P < .05$, Mann-Whitney U test; $P < .05$, log-transformed t test).

To further analyze the response to bradykinin, we compared the effect of bradykinin challenge on CXCR1 and CXCR2 mRNA levels within each subject group. The maximum level of induction of CXCR1 or CXCR2 mRNA for each subject was used for this statistical

TABLE I. CXCR1 and CXCR2 cell-surface expression on nasal epithelial cells before and after bradykinin challenge

Cell treatment	Mean fluorescence intensity		
	Isotype control	CXCR1	CXCR2
Medium alone	15.06	36.41	10.76
Bradykinin stimulated	15.06	33.13	21.43

analysis. Subgroup analysis revealed that bradykinin challenge significantly increased the level of CXCR1 mRNA in the patients with mild, quiescent allergic rhinitis ($P = .028$, Wilcoxon signed-rank test) but not in the control group ($P =$ not significant, Wilcoxon signed-rank test). Similarly, bradykinin challenge significantly increased the level of CXCR2 mRNA in the patients with mild, quiescent allergic rhinitis ($P = .028$, Wilcoxon signed-rank test) but not in healthy control subjects ($P =$ not significant, Wilcoxon signed-rank test).

To control for the mechanical effect of the nasal challenge, we assessed the effect of nasal saline challenge on CXCR1 and CXCR2 mRNA levels. A 4-hour saline challenge was performed on 5 patients with allergic rhinitis and 4 healthy control subjects by using the same procedure as described for the nasal bradykinin challenge. The saline challenge had no significant effect on CXCR1 or CXCR2 mRNA levels in either subject group. However, a trend toward decreased CXCR1 and CXCR2 mRNA levels after saline challenge in both groups was observed (data not shown).

Expression of CXCR1 and CXCR2 mRNA by nasal epithelial cells

We have consistently observed that nasal tissue samples are composed primarily of epithelial cells. Epithelial cells were isolated from nasal tissue samples from 2 patients with mild, quiescent allergic rhinitis and 2 healthy control subjects to confirm that nasal epithelial cells expressed CXCR1 and CXCR2 mRNA. Isolated mRNA from the nasal epithelial cells was assessed for CXCR1, CXCR2, and GAPDH mRNA by means of real-time quantitative RT-PCR. CXCR1 and CXCR2 mRNA was detected in all 4 nasal epithelial samples.

Cell-surface expression of CXCR1 on nasal epithelial cells

To further investigate the expression of CXCR1 and CXCR2 in allergic rhinitis, we assessed the cell-surface expression of these CXC chemokine receptors in nasal epithelial cells. Nasal epithelial cells from patients with mild allergic rhinitis and healthy control subjects were assessed for CXCR1 and CXCR2 cell-surface receptor expression by means of FACS analysis. CXC receptor expression was determined on nasal cells gated by physical characteristics that also stained positive for the epithelial marker Ber-EP4. A small but consistent increase in the geometric mean fluorescence intensity of staining to greater than that of the isotype control was

seen for CXCR1, but not for CXCR2, in nasal epithelial cells of patients with mild allergic rhinitis and healthy control subjects (Table I).

Bradykinin induces CXCR2 cell-surface expression on the nasal epithelial cells of patients with mild allergic rhinitis

Since we showed that bradykinin induced an increase in the levels of CXCR1 and CXCR2 mRNA in nasal epithelial cells of patients with mild allergic rhinitis, we next investigated whether bradykinin also induced an increase in chemokine receptor cell-surface expression. Three patients with mild, quiescent allergic rhinitis were recruited for each experiment. One naris was scraped, and the sample was placed into medium alone, whereas the nasal tissue from the contralateral naris was placed into medium containing bradykinin (10^{-6} mol/L). After 8 hours, the nasal cells were disaggregated, and the expression of CXCR1 and CXCR2 cell-surface expression was determined by means of flow cytometry. No difference was seen in CXCR1 staining at the single 8-hour time point studied. Bradykinin stimulation, however, induced a consistent increase in the mean fluorescence intensity of CXCR2 staining compared with that of the matched nasal cells exposed to medium alone (Table I). Thus bradykinin induced cell-surface expression of CXCR2 on nasal epithelial cells.

DISCUSSION

In this investigation we demonstrated for the first time the *in vivo* expression of the IL-8 receptors CXCR1 and CXCR2 in human nasal tissue. CXCR1 and CXCR2 mRNA was detected in nasal tissue samples from patients with mild, quiescent allergic rhinitis, as well as in samples from healthy control subjects, and there was no difference in the baseline mRNA levels of either receptor. Patients with active allergic rhinitis, however, showed increased levels of CXCR1 and CXCR2 mRNA in the nasal samples compared with those of either healthy control subjects or patients with mild, quiescent allergic rhinitis.

To further explore this observation, we examined whether the inflammatory mediator bradykinin, which is increased during allergic airway inflammation, could contribute to the increased levels of nasal tissue CXCR1 and CXCR2 mRNA seen in patients with active allergic rhinitis. Here we report the novel observation that bradykinin is able to modulate *in vivo* the expression of CXCR1 and CXCR2 mRNA in human airway tissue. Nasal bradykinin challenge significantly increased the levels of both CXCR1 and CXCR2 mRNA in patients with mild, quiescent allergic rhinitis. Reflecting the heterogeneity of the human population, the kinetics of the bradykinin-induced increase in the levels of CXCR1 and CXCR2 mRNA differed between subjects, with peak levels of CXCR1 or CXCR2 mRNA reached at either 4 or 8 hours after nasal challenge.

These data also demonstrate an intrinsic physiologic difference between normal nasal tissue and the nasal tissue of patients with mild allergic rhinitis, as bradykinin had no effect on the mRNA levels of either receptor in the nasal tissue of healthy control subjects. These differences could potentially be due to an upregulation of bradykinin cell-surface receptor expression or possibly an augmentation in functional activity of bradykinin receptor signaling in patients with allergic rhinitis. Recent work in our laboratory was unable to identify any difference in the level of bradykinin B2 receptor mRNA in the nasal tissue of patients with mild allergic rhinitis and healthy control subjects.²⁶ Further in-depth studies will be required to ascertain whether the bradykinin B2 receptor is upregulated on the cell surface of nasal epithelial cells or whether other inflammatory mediators generated in patients with allergic rhinitis might prime the nasal epithelial cell response to bradykinin.

Nasal scrapes are composed of a variety of cell types, including epithelial cells, mast cells, basophils, and eosinophils. The dominant cell type is the epithelial cell, which we postulate to be the most likely source of the CXCR1 and CXCR2 mRNA detected in the nasal tissue samples. To address this issue, we investigated the expression of CXCR1 and CXCR2 in RNA from epithelial cells isolated from nasal tissue samples. CXCR1 and CXCR2 mRNA were detected in nasal epithelial cells from both patients with allergic rhinitis and healthy control subjects. We cannot, however, exclude the possibility that the bradykinin-induced increases in CXCR1 and CXCR2 mRNA levels were due in part to inflammatory cell influx rather than the regulation of nasal epithelium CXCR1 and CXCR2 mRNA levels.

We next examined whether the bradykinin-induced increase in the levels of CXCR1 and CXCR2 mRNA was accompanied by an increase in the chemokine cell-surface receptor expression by means of flow cytometry. Initially, we assessed whether cell-surface CXCR1 and CXCR2 receptors were present on nasal epithelial cells of patients with mild allergic rhinitis and healthy control subjects. CXCR1 cell-surface receptor was expressed by nasal epithelial cells from both groups; however, CXCR2 cell-surface receptor was not detected on the nasal epithelial cells from either subject group. Interestingly, we found that ex vivo stimulation with bradykinin for 8 hours induced the cell-surface expression of CXCR2 on nasal epithelial cells from patients with mild allergic rhinitis, correlating with the increased levels of CXCR2 mRNA after nasal bradykinin challenge. In conjunction with the other data presented in this study, this evidence strongly suggests that bradykinin regulates the expression of CXCR2 at both the mRNA and cell-surface expression level in nasal epithelial cells in vivo. In contrast, CXCR1 cell-surface expression remained unchanged after ex vivo bradykinin stimulation, although it is possible that upregulation of cell-surface expression would be seen if other time points had been examined.

A large number of human chemokines and chemokine receptors have been discovered in recent years.²⁷ CXCR1 is specific for IL-8, whereas CXCR2 has a variety of ligands, including IL-8, growth-related oncogene (GRO), and epithelial neutrophil-activating protein 78 (ENA-78). IL-8 is produced by a variety of human resident airway cells and inflammatory cells, including airway smooth muscle, airway epithelium, and eosinophils.⁶⁻¹⁰ Human airway epithelial cells and bronchoalveolar lavage macrophages constitutively express GRO, whereas ENA-78 has been associated with allergic airway disease.^{28,29} In a model of allergic airway disease, allergen challenge resulted in a significant increase in ENA-78 within the airways, with mast cells observed as the major source of this chemokine. IL-8 has been shown to be regulated in an autocrine fashion; therefore increased levels of IL-8 receptor expression by airway cells could contribute to the increased levels of IL-8 and potentially other CXC chemokines observed during allergic airway inflammation. Elevated levels of CXC chemokines might prolong the allergic response, in part by encouraging the migration of neutrophils, mast cells, and possibly eosinophils, themselves sources of inflammatory mediators and destructive proteases.

Although bradykinin has been demonstrated to have the ability to orchestrate the symptomatic and physiologic changes associated with rhinitis and asthma,^{30,31} including inducing airway permeability and bronchoconstriction, the mechanisms by which bradykinin potentially influences the development of airway inflammation have remained obscure. This study demonstrates a novel in vivo effect of bradykinin on human airway tissue, whereby bradykinin is potentially involved in controlling the sensitivity of airway tissue to chemokines through the regulation of chemokine receptor expression.

In conclusion, this investigation clearly demonstrates that the levels of CXCR1 and CXCR2 mRNA are increased in the nasal cells of subjects with active symptoms of allergic rhinitis. Moreover, nasal bradykinin selectively upregulates in vivo CXCR1 and CXCR2 mRNA in airway tissue of patients with mild allergic rhinitis, and furthermore, bradykinin induces the cell-surface expression of CXCR2 on nasal epithelial cells ex vivo. Therefore we speculate that as clinical symptoms of allergic rhinitis increase, there is an increase in the levels of inflammatory mediators, such as bradykinin, which is accompanied by an increase in CXCR1 and CXCR2 receptor levels. Since bradykinin has previously been shown to regulate airway lumen IL-8 expression after allergen challenge, bradykinin might act to specifically increase the sensitivity of the airway tissue to the CXC chemokines, including IL-8, ENA-78, and GRO. Thus our study further demonstrates the potentially significant role of kinins in the progression and amplification of allergic airway inflammation.

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