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Up-Regulation of Functional Kinin B1 Receptors in Allergic Airway Inflammation

Sandra C. Christiansen,* Jane Eddleston,* Katharine M. Woessner,* Sandra S. Chambers,* Richard Ye,† Zhixing K. Pan,* and Bruce L. Zuraw**

B1 receptors are known to be induced during allergic airway inflammation in animal models. However, little is known regarding in vivo B1 receptor expression in humans. We examined B1 receptor mRNA expression in nasal tissue samples from allergic rhinitis and normal subjects. Allergic rhinitis subjects displayed significantly higher expression of B1 receptor mRNA than did the normal subjects, and nasal allergen challenge increased B1 receptor mRNA expression at 8 to 24 h time points in allergic rhinitis subjects. No significant difference was found in B2 receptor expression. To confirm B2 and B1 receptor functional activity, subjects were challenged with kinin agonists. Nasal challenge with the B1 receptor ligand, Lys-des-Arg-bradykinin (BK), activated extracellular signal-regulated kinase in allergic rhinitis, but not normal, subjects. Nasal challenge with the B2 receptor ligand, BK, activated extracellular signal-regulated kinase in both allergic rhinitis and normal subjects. The consequences of B1 receptor activation were investigated using the human airway epithelial cell lines A549 and BEAS-2B. We demonstrated that Lys-des-Arg-BK activates the transcription factor AP-1. Taken together, these results show that functional B1 receptors are induced in the airway during allergic inflammation and suggest that they participate in the regulation of gene expression.

Several observations support a participatory role for kinins in the pathogenesis of allergic airway disease. Symptomatic and physiologic changes which mimic naturally occurring rhinitis and asthma are provoked by inhaled challenge with bradykinin (BK)3 (1, 2). Increased levels of kinines have been detected in secretions from individuals with allergic rhinitis as well as following nasal allergen challenge in atopic subjects (3–5). In asthmatics, increased levels of kinines are present in the bronchoalveolar lavage fluid of patients with active disease (6). Similar to findings in the nasal model, local challenge with relevant allergen in allergic asthmatics increase kinin levels in conjunction with histamine release at both immediate and late-phase time points (7, 8).

Two subtypes of kinin receptors, the B2 receptor and the B1 receptor, were originally distinguished based upon functional data in animal tissues (9). In humans, cloned gene sequences of the receptors have confirmed distinct B2 and B1 receptors, each belonging to the seven transmembrane G protein-coupled superfamily (10, 11). BK and Lys-BK (kallidin) are equally effective agonists for the B2 receptor; however, the carboxypeptidase metabolite of Lys-BK, Lys-des-Arg-BK, is the only known potent agonist for the human B1 receptor (11, 12). B2 receptors are constitutively expressed on many cell types (13) including human airway epithelial cells (14), whereas B1 receptors are expressed at low levels in normal tissues but can be induced in response to pathophysiological stimuli (15–17).

In the human airway, the majority of evidence has supported mediation of kinins’ effects occurring through the B2 receptor. Inhalation of BK or Lys-BK provoke acute bronchoconstriction (18). Icatibant (HOE-140), a B2 receptor antagonist, abolished hyperresponsiveness to histamine and reduced nasal eosinophilia induced by Ag in subjects with allergic rhinitis (19). In asthmatic subjects treated with icatibant, pulmonary function gradually improves over a 4-wk treatment phase, suggesting that blockade of the B2 receptor also had an anti-inflammatory role (20). In contrast, human airway provocation with Lys-des-Arg-BK failed to cause either bronchoconstriction in asthmatic subjects or increased glandular secretion and vascular permeability in the upper airways of subjects with allergic rhinitis (21). Participation of the B1 receptor in human airway disease is thus uncertain. However, in an allergen rat model a B1 receptor antagonist inhibited allergen-induced bronchial hyperresponsiveness (22).

In the current investigation, we sought to better define the potential role of B1 receptors in human airway inflammation. We examined the expression of B1 receptor mRNA in nasal tissue from subjects with allergic rhinitis compared with normal controls, and measured the effects of nasal allergen challenge on B1 receptor mRNA expression. Finally, we assessed the ability of Lys-des-Arg-BK to transduce in vivo activation of a mitogen-activated protein kinase (MAPK) in nasal tissue, and investigated the effect of this ligand on transcription factor activation in cultured airway epithelial cells.

Materials and Methods

Subject characteristics

Non-smoking adults with allergic rhinitis (diagnosis based on a consistent history and confirmatory aeroallergen skin tests) and normal controls were recruited to participate. Informed consent was obtained in accordance with the Human Subjects Committee at The Scripps Research Institute (La Jolla, CA 92037) and the Rose Stein Charitable Trust Fund.
CA). Allergic rhinitis subjects undergoing nasal challenges were excluded if they had received prior immunotherapy, and all allergy and asthma medications were held for at least 2 wk before challenge. Participants were screened with prick skin tests for common aeroallergens (Greer Laboratories, Lenoir, NC). A positive reaction was defined as a wheal diameter at least 3 mm greater than the saline control. Screening panels included grass mixture, Bermuda grass, tree mixture, weed mixture, dust mite (Dermatophagoides pteronyssinus and Dermatophagoides farinae), cat, and dog.

Nasal challenge protocols

**Allergen challenge.** Subjects with mild or quiescent allergic rhinitis underwent graded nasal allergen challenge to establish the provoking dose for symptomatic rhinitis. Six subjects were challenged with mite (D. farinae and D. pteronyssinus), two with cat, and seven with grass allergen. Subjects were asked to record their global severity score using a visual analog scale with 0 for none and 10 for severe. Subjects were questioned for the appearance of typical symptoms of allergic rhinitis including sneezing, itching, burning, congestion, rhinorrhea, throat irritation, and ocular symptoms of itching, tearing, or burning. Allergen was delivered by gently instilling 100 μl aliquots into the nasal passage using a tuberculin syringe that was placed in the nostril. The dose of allergen was increased at 10-fold increments every 5 min from an initial 1/100,000 dilution of concentrated extract until symptoms were elicited of 5 or greater on the visual analog scale. The cumulative dose of allergen delivered into the nare eliciting the symptomatic response was then designated as the provoking dose. For pollen sensitive subjects, challenges were conducted out of season.

One month after the provoking dose was determined, subjects returned for a baseline nasal sample. Subsequent nasal samples were obtained using one of two protocols. Subjects studied using the first protocol had a baseline nasal sample obtained, then returned for allergen challenge 1 wk later. At that time, each subject underwent bilateral nasal allergen challenge, increasing the provoking dose delivered in each nare. Two timed nasal samples were collected following allergen challenge, one from each nare. Allergic rhinitis subjects studied using the second protocol underwent both relevant and irrelevant nasal allergen challenges at least 4 wk apart. Irrelevant allergens were selected to be allergens to which the subjects were skin test negative and had no history of clinical symptoms with natural exposure. The relevant allergen dose was the previously established provoking dose while the irrelevant allergen dose was selected at the average provoking dose for the relevant allergen challenges (1/100 dilution of the allergen concentrate). To be included, relevant allergen challenges needed to cause an increase in nasal symptoms while irrelevant challenges needed not to cause increased nasal symptoms. For each of the challenges, subjects had a baseline nasal sample obtained from one nare then immediately underwent nasal allergen challenge in the contralateral nare. Four to 8 h after challenge, subjects had an additional nasal sample obtained from the challenged nare.

**Kinin challenges.** Baseline nasal sample was obtained from one nare, immediately followed by instillation of the appropriate concentration of either BK or Lys-des-Arg-BK in a 100 μl sprayer onto the inferior turbinate of the contralateral nare. The kinin was instilled by gentle insufflation in the region of the inferior turbinate using a tuberculin syringe placed in the nare under direct visualization. A nasal speculum. A subsequent nasal sample was collected from the challenged nare 20 min after kinin was instilled. Kinin challenges were conducted at least 1 mo after allergen challenge and at least 2 wk apart. BK was obtained from PolyPeptide Laboratories (Wolfenbuttel, Germany) at >98% purity as a trifluoroacetate salt reconstituted in sterile water. Lys-des-Arg-BK was obtained from Bachem (Torrance, CA) at >99% purity as a trifluoroacetate salt reconstituted in sterile water.

**Sample collection**

**Nasal sample collection.** Nasal samples were collected by gently scraping the inferior turbinate with a Rhinoprobe curette (Arlington Scientific, Springville, UT). The sample was then placed into RLT lysis buffer (Qiagen, Valencia, CA) and snap frozen. Total RNA was extracted from the samples using the RNeasy mini kit (Qiagen) according to the manufacturer’s instructions. Protein was isolated from the RNeasy using the Mini Spin column flow-through by TCA precipitation. An equal volume of cold 10% TCA solution was added to the flow-through and kept on ice for 20 min, followed by a 15-min microcentrifugation. Pellets were washed with 100% ice-cold ethanol and resuspended in sample buffer for immunoblotting.

**Isolation of nasal epithelial cells.** Nasal samples were immediately placed in 1 ml of disaggregation buffer (0.1% collagenase XI, 0.1 mg/ml DNAse, 0.1 mg/ml FCS), and gently rotated for 20 min at room temperature. The nasal cells were then washed three times in cold sterile PBS containing 0.1% BSA and then resuspended in 1 ml PBS containing 20% FCS. Epithelial cells were isolated from the cell suspension using the CELlection Epithelial Enrich system (Dynal Biotech, Lake Success, NY), according to the manufacturer’s instructions.

**Ribonuclease protection assay (RPA)**

Specific mRNA levels for the B1 receptor, B2 receptor, IL-8 gene, and L-32 housekeeping gene in the nasal samples were measured by multiprobe RPA as previously described (14). PCR products for each of these genes were cloned into the pGEM3Z plasmid (Promega, Madison, WI) using BamHI and HindIII restriction sites, and the correct sequence was confirmed on a DNA sequencer (373A Sequencer; Applied Biosystems, Foster City, CA). Twenty micrograms of each plasmid were linearized with the appropriate restriction enzyme and antisense RNA probes were prepared by in vitro transcription with either T7 (B2 receptor and L32) or SP6 (B1 receptor and IL-8) RNA polymerases (Promega) with the incorporation of [α-32P]UTP (800 Ci/mmol; Amer sham, Arlington Heights, IL). Plasmid DNA was digested with RNase-free DNase I (10 U; Stratagene, La Jolla, CA). The labeled riboprobes were then gel-purified on a 6% polyacrylamide gel. Riboprobes were diluted in hybridization buffer (Torrey Pines Biolog, Houston, TX) to 1 × 104 cpm/μl. Total RNA was quantitated fluorometrically using SYBR green II (Molecular Probes, Eugene, OR) (23). Ten microliters of RNA sample (1 μg total RNA in diethyl pyrocarbonate-treated H2O) was heated at 95°C for 5 min then hybridized with 1 μl of labeled riboprobe mix for 16 h at 37°C in a total volume of 20 μl hybridization buffer. Unhybridized single-stranded RNA was digested with 100 μl of RNase digestion mixture for 1 h at 30°C. After the addition of 100 μl stop buffer (Torrey Pines Biologs), the tube was vortexed and incubated at 23°C for 10 min. The undigested RNA was then purified by ethanol precipitation, and separated on a 6% acrylamide/urea sequencing gel. The protected bands were visualized and quantified by scanning the gels using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**Extracellular signal-regulated kinase (ERK) immunoblotting**

Ten microliters of extracted nasal protein (0.5–1.0 μg/μl) per lane was separated by 12% SDS-PAGE then electrophoretically transferred to nitrocellulose membranes. Phosphorylated ERK was detected by immunoblotting the membrane with a mouse derived mAb specific for the phosphorylated tyrosine motif of 1/2000 dilution (Phospho-P44/42 MAP Kinase E10 Monoclonal Ab; Cell Signaling Technology, Beverly, MA) followed by HRP-conjugated goat anti-mouse IgG (Cal tag Laboratories, Burlingame, CA). Protein bands were detected by ECL (SuperSignal West Pico Chemiluminescent Substrate; Pierce, Rockford, IL). The membranes were then stripped and total ERK levels were measured by immunoblotting with a rabbit p44/42 MAPK Ab at 1/10000 dilution (Cell Signaling Technology), followed by HRP-conjugated anti-rabbit Ab (Cell Signaling Technology) and ECL (Pierce).

**Cell culture**

The transformed human bronchial epithelial cell line, BEAS-2B (CRL- 9609; American Type Culture Collection, Manassas, VA) was grown as monolayers in 100% humidity and 5% CO2 at 37°C in the serum-free defined growth media (Clonetics, San Diego, CA). The type II pneumocyte-like human lung adenocarcinoma cell line, A549, was grown as monolayers in 100% humidity and 5% CO2 at 37°C in Ham’s F12K medium containing 2 mM l-glutamine, penicillin (100 IU/ml), streptomycin (50 μg/ml), and 10% FBS. Normal primary human bronchial epithelial cells were purchased from Clonetics, grown in serum-free defined growth media, and used on passages 2 to 4.

**EMSA**

Nuclear extracts were prepared by a modified method of Dignam et al. (24) and EMSA were performed as previously described (25). Oligonucleotides and their complementary strands for EMSAs were from Promega. The sequence of the AP-1 oligonucleotide was 5′-CGCCCTGAGTATGTCACG CGGAGA-3′ (E3202). Double-stranded oligonucleotide (5 pmol) was 32P-labeled with T4 polynucleotide kinase. [γ-32P]ATP (5000 Ci/mmol) was from Amersham.

**Real-time quantitative RT-PCR**

Total RNA was extracted from nasal scrape samples as described above and contaminating DNA digested using a DNase-treatment kit (Qiagen). Total nasal RNA (250–500 ng) was reverse-transcribed using the Omni- script RT kit (Qiagen), and one-twentieth of the cDNA was used for subsequent PCR.

The Bio-Rad iCycler (Hercules, CA) was used for real-time quantitative PCR. The primers used were as follows: B1 receptor forward primer, 5′-CA ACGGACCTGCCGAGAATTCAC; B1 receptor reverse primer, 5′-CAA
The reaction conditions were 95 °C and 1 U of AmpliTaq Gold (Applied Biosystems) in a total volume of 50 µl.

FIGURE 1. B1 receptor mRNA is expressed in higher levels in nasal samples from allergic rhinitis subjects than in normal subjects. Nasal samples from two normal subjects (lanes 1 and 2) and four allergic rhinitis subjects (lanes 3–6) were analyzed by multiprobe RPA as described in Materials and Methods. The position of protected bands for the B1 receptor (B1 BKR), IL-8, B2 receptor (B2 BKR), and L32 are indicated. The autoradiograph is shown.

Statistics
All analyses were performed using nonparametric techniques. Non-paired comparisons were analyzed using the Mann-Whitney U test and paired comparisons were analyzed using the Wilcoxon signed rank test. Correlation between two variables was assessed using the Spearman rank correlation test.

Results
Expression of B1 receptor mRNA is increased in subjects with allergic rhinitis compared with normal subjects
Nasal samples were obtained using the Rhinprobe curette from 34 subjects with active allergic rhinitis and 17 normal subjects. Steady-state mRNA levels of the B1 receptor as well as the B2 receptor, IL-8 gene, and L32 ribosomal housekeeping gene were measured by multiprobe RPA. In all cases, BK receptor and IL-8 mRNA levels were normalized to the level of L32 mRNA expression based on the density of the respective bands as determined by phosphorimaging. Fig. 1 shows a representative pattern from four allergic rhinitis subjects and two normal controls. Strong protected B1 receptor mRNA bands were detected in two of the allergic rhinitis samples with a third allergic rhinitis subject showing a weak but clear band. B1 receptor protected bands were not seen in the normal subject samples. In contrast, all six samples (allergic rhinitis and normal) showed clear B2 receptor bands.

The mean level of B1 receptor mRNA expression in the 34 allergic rhinitis subjects was significantly greater than that in the 17 normal subjects (p < 0.05, Mann-Whitney). IL-8 mRNA expression was also significantly increased in the allergic rhinitis subjects, however its expression did not significantly correlate with B1 receptor expression (p > 0.05, Spearman rank correlation). B2 receptor mRNA was greater in the allergic rhinitis subjects than in the normal controls, but the difference was not statistically significant (p > 0.05, Mann-Whitney). Fig. 2 summarizes the B1 receptor mRNA expression in all 51 subjects. Interestingly 14 of 17 (82.4%) normal subjects did not express detectable B1 receptor mRNA; however, even among the allergic rhinitis subjects, 13 of 34 (38.2%) also did not express detectable B1 receptor mRNA. To assess the repeatability of the multiprobe RPA, several nasal scrape samples from allergic rhinitis subjects were pooled, then analyzed, in five replicates. The mean B1 receptor/L32 mRNA ratio for the five replicates was 33.04% with a standard error of 2.32%.

In vivo nasal allergen challenge increases B1 receptor expression
To assess whether allergic inflammation could directly increase B1 receptor expression, we measured B1 receptor mRNA in nasal tissue samples before and after in vivo nasal allergen challenge. Seven allergic rhinitis subjects with mild or quiescent symptoms at baseline were challenged with relevant allergen and experienced a spectrum of typical symptoms including itch, congestion, sneeze, rhinorrhea, sore throat, and ocular watering and itch. Nasal samples were obtained 1 wk before allergen challenge (baseline) then 8 and 24 h after allergen challenge in six subjects or 4 and 8 h after allergen challenge in one subject. B1 receptor and L32 mRNA levels were measured by RPA, and the B1 receptor expression was normalized to L32.

Six of seven allergen-challenged allergic rhinitis subjects demonstrated increased B1 receptor mRNA following allergen challenge. Fig. 3 shows the changes in B1 receptor mRNA expression at the times following challenge compared with the baseline values. The peak increase in B1 receptor mRNA occurred at 8 h in four of the seven subjects. Two subjects showed peak B1 receptor mRNA at 24 h, and one subject did not show any change. Comparing each subjects’ maximal postchallenge level to the baseline level, allergen challenge resulted in a statistically significant increase in B1 receptor mRNA expression (p < 0.05; Wilcoxon signed rank test). As an additional control, a single normal subject was challenged with grass allergen and measurements of B1 receptor message analyzed at 4 and 8 h time points by RPA. No detectable message was found in the baseline or postchallenge samples (data not shown).

B1 receptor mRNA levels were also measured in six allergic rhinitis subjects by quantitative real-time RT-PCR before and after...
nasal challenge with relevant and irrelevant allergen. Relevant allergen challenge resulted in a mean 2.37 ± 0.64-fold increase in the B1 receptor-GAPDH mRNA ratio compared with baseline. In contrast, irrelevant allergen challenge resulted in a small decrease (0.63 ± 0.19-fold) in the B1 receptor/GAPDH mRNA ratio compared with baseline. Comparing the matched challenges, relevant challenge increased B1 receptor mRNA expression to a greater degree than the irrelevant allergen challenge in all six of the subjects and this was a statistically significant effect (p = 0.0273, Wilcoxon signed rank test).

To determine whether nasal epithelial cells expressed B1 receptor mRNA, we isolated nasal epithelial cells from the nasal scrape samples in two normal and two allergic rhinitis subjects. Total RNA was isolated from the epithelial cells and the ratio of B1 receptor to GAPDH mRNA was measured by real-time quantitative RT-PCR. Detectable B1 receptor was found in all four subjects, and the mean B1 receptor/GAPDH mRNA ratio in the two normal subjects was virtually identical to the ratio found in total nasal scrape samples (0.0040 and 0.0057). However, one of the allergic rhinitis subjects showed a >10-fold higher level of B1 receptor compared with the normal subjects.

Activation of ERK in cultured airway epithelial cells following kinin challenge

BK has been previously reported to activate the MAPK ERK in a variety of cell types (26, 27). Therefore, we examined the ability of BK and Lys-des-Arg to activate ERK-1 and ERK-2 in human airway epithelial cells. The transformed human bronchial epithelial cell line, BEAS-2B, constitutively expresses both B2 and B1 receptors (data not shown). Following stimulation with BK, an increase in phosphorylated ERK-1 and ERK-2 was seen beginning by 7.5 min (the earliest time point sampled) and continuing for at least 22.5 min (Fig. 4). Stimulation of BEAS-2B cells with Lys-des-Arg-BK also increased phospho-ERK1/2, with the increase first seen at 15 min and continuing for at least 22.5 min (Fig. 4).

Unlike the BEAS-2B cells, primary human bronchial epithelial cells constitutively express B2, but not B1, receptors (data not shown). BK stimulated increases in phospho-ERK are seen within 10 min in primary human bronchial epithelial cells, peak at 15 min, and begin to decrease by 20 min (Fig. 5). However, in marked contrast to the BEAS-2B cells, primary human bronchial epithelial cells do not activate ERK in response to Lys-des-Arg-BK (Fig. 5). These results show that the B1 receptor is required in order for Lys-des-Arg-BK to activate ERK.

Activation of ERK in nasal samples following kinin challenge

To assess whether the increased B1 receptor mRNA found in nasal samples from allergic rhinitis subjects was associated with expression of functional receptors, we performed nasal challenges with BK or Lys-des-Arg-BK in allergic rhinitis and normal subjects then measured in vivo activation of ERK in baseline and 20 min postchallenge nasal samples. Both normal and allergic subjects reported transient nasal burning, sore throat, and congestion in response to the BK challenge, usually maximal at 1–5 min with resolution by 20 min. No subjects experienced any symptoms in response to Lys-des-Arg-BK challenge.

As would be expected for the constitutively expressed B2 receptor, instillation of 200 μg BK resulted in activation of ERK in both normal and allergic rhinitis subjects (Fig. 6A). In contrast,
Subjects were instilled with 500 or 200 μg of Lys-des-Arg-BK activated ERK in allergic rhinitis subjects while instillation of 500 μg Lys-des-Arg-BK did not activate ERK in a normal subject (Fig. 6B). Summary data from in vivo kinin challenges are summarized in Fig. 7. Values are expressed as the fold change in the ratio of phospho-ERK to total ERK in the postchallenge sample vs the matched baseline sample. All seven of the allergic rhinitis subjects challenged with Lys-des-Arg-BK demonstrated an increase in phospho-ERK (four challenged with 500 μg and three challenged with 200 μg). In contrast, six of seven normal controls challenged with Lys-des-Arg-BK (five challenged with 500 μg and one challenged with 200 μg) showed no increase in phospho-ERK. A single normal subject, challenged with 500 μg Lys-des-Arg-BK, showed a small increase in phospho-ERK. The difference in response to Lys-des-Arg-BK challenge between the allergic rhinitis and normal subjects was statistically significant (p < 0.005; Mann-Whitney U test). Fig. 7 also shows that all eight allergic rhinitis and four normal subjects challenged with BK showed increased activation of ERK in the 20-min sample compared with the baseline sample. These results are compatible with constitutive nasal expression of B2 receptors in both subject groups with expression of functional B1 receptors only in the allergic rhinitis subjects.

Effects of Lys-des-Arg-BK on transcription factor activation in human airway epithelial cells

BK-mediated activation of ERK through the Ras-Raf pathway has been associated with activation of the transcription factor AP-1 (27). Therefore, we assessed the effects of BK and Lys-des-Arg-BK on transcription factor activation in A549 cells, a transformed type II pneumocyte cell line (Fig. 8). As we previously reported, BK-stimulated activation of transcription factor NF-κB (Fig. 8A, lane 4), and this could be blocked by preincubation with a B2 receptor antagonist (Fig. 8A, lane 10). In contrast, Lys-des-Arg-BK did not stimulate NF-κB activation (Fig. 8A, lane 3). However, both BK and Lys-des-Arg-BK stimulated AP-1 activation (Fig. 8B, lanes 3 and 4). BK-stimulated AP-1 activation was blocked by a B2 receptor antagonist (Fig. 8B, lane 10), but not a B1 receptor antagonist (Fig. 8B, lane 7); while Lys-des-Arg-BK-stimulated AP-1 activation was blocked by a B1 receptor antagonist (Fig. 8B, lane 6), but not a B2 receptor antagonist (Fig. 8B, lane 9). Lys-des-Arg-BK also activated AP-1 in BEAS-2B bronchial epithelial cells (data not shown).

Discussion

Although kinin B2 receptors are well-recognized to play an important role in allergic airway inflammation (1–8, 18–20), the role of kinin B1 receptors in humans is controversial (21, 22). A substantive body of evidence from studies involving intact animals as well as in vitro tissue supports the concept that expression of B1 receptors is tightly regulated (17), being absent or expressed at low levels in normal tissue and induced during inflammation (28–30). Therefore, we sought to determine whether B1 receptors are expressed in the airway during allergic inflammation and, if so, whether they were functional and transduced signals that could be relevant to inflammation.

We report the novel observation that kinin B1 receptors are induced during allergic airway inflammation in human subjects. Using multiprobe RPA to measure expression in nasal samples, we found a statistically significant increased level of B1 receptor mRNA in 34 allergic rhinitis subjects compared with 17 normal
controls. B1 receptor mRNA was not detectable in 82% of normal subjects (14 or 17), in keeping with animal and human cell line data indicating that the B1 receptor is usually absent in normal tissue and only expressed in response to pathophysiologic stimuli (17). Within the group of allergic subjects, there was significant variability in the level of message expression, with 38% of allergic rhinitis subjects having no detectable receptor. B1 receptor mRNA expression was also measured by quantitative real-time RT-PCR. Using this more sensitive technique, we could consistently detect low level expression of B1 receptor mRNA in the normal subjects, however the allergic rhinitis subjects showed significantly higher levels of expression. Using multiprobe RPA and real-time quantitative RT-PCR, we also demonstrated that relevant allergen challenge significantly increases nasal B1 receptor mRNA expression in subjects with mild or quiescent allergic rhinitis. Although a certain amount of variability was evident with respect to B1 receptor expression, there was clear evidence of elevated B1 receptor mRNA expression in allergic rhinitis subjects which was further increased in the majority of subjects by allergen challenge.

Little is known regarding the potential consequences of agonist binding to the expressed B1 receptors in the airway. The lack of a phosphorylation motif in its C-terminal intracellular domain has been reported to cause the B1 receptor to be only minimally internalized and desensitized, thus allowing for protracted activity (31). Studies in transiently transfected human embryonic kidney 293 cells have also shown that the B1 receptor exhibits a high degree of constitutive activity even in the absence of ligand (32). BK has been reported to activate the MAPK ERK in a variety of cells (27, 33). Although des-Arg-BK has also been reported to activate ERK in human embryonic kidney 293 cells overexpressing B1 receptors (34), the significance of this observation is uncertain because des-Arg-BK is not an effective agonist for the human B1 receptor (11, 12).

Using cultured human airway epithelial cells, we demonstrated that engagement of either the B1 or B2 receptor by its appropriate agonist resulted in rapid phosphorylation of ERK-1/ERK-2. BK was used as the B2 receptor agonist and Lys-des-Arg-BK was used as the B1 agonist. The specificity of their agonist activity at the concentrations used have been carefully documented. BK and Lys-BK (kallidin) are equally effective agonists for the B2 receptor; however, Lys-BK, but not BK, shows some agonist activity for the B1 receptor (11, 12). Their carboxypeptidase metabolites (des-Arg-BK and Lys-des-Arg-BK) have very different B1 receptor agonist activities; Lys-des-Arg-BK is a potent agonist for the human B1 receptor, while des-Arg-BK is at least 1000-fold less potent (11, 12).

We then used nasal kinin challenges to examine in vivo activation of ERK in nasal cells. Seven allergic rhinitis subjects and seven normal controls were challenged with Lys-des-Arg-BK. Postchallenge samples were collected at 20 min based on the in vitro response to agonists in BEAS-2B cells. All seven allergic rhinitis subjects demonstrated clear activation of ERK following challenge with either 500 or 200 μg of Lys-des-Arg-BK. The seven normal subjects were all challenged with 500 μg of Lys-des-Arg-BK, however only a single normal subject showed minimal activation of ERK. In contrast, BK nasal challenge activated ERK in both allergic rhinitis and normal subjects. These results both confirm the expression results discussed above and demonstrate that the nasal B1 receptor mRNA found in allergic rhinitis subjects is associated with expression of functional B1 receptors.

Activation of ERK has been linked to activation of the transcription factor AP-1 (27, 35), and previous studies have suggested that signaling through the B1 receptor may contribute to the regulation of gene expression (36–38). We demonstrated that Lys-des-Arg-BK could activate AP-1 in transformed human respiratory epithelial cells (A549 and BEAS-2B). AP-1 has an array of associations with potentially important regulatory pathways involved in allergic inflammation of the airways, including stimulation of cytokine and chemokine transcription and functioning as an effector of growth factor signaling required for normal cell cycle progression and in the apoptotic response (39–41). Preliminary experiments in our laboratory suggest, moreover, that Lys-des-Arg-BK stimulates increased expression of chemokine receptors in cultured airway epithelial cells (data not shown). The timing of B1 receptor expression following allergen challenge suggests the possibility that B1 receptors may contribute to the phenomena of the late-phase response, which has been clearly linked to chronic airway inflammation (42). Additional studies will be required to define the potential effects and role of the B1 receptor in airway gene expression during allergic inflammation.

In summary, we have demonstrated the up-regulation of B1 receptors in naturally occurring allergic rhinitis and in response to challenge with relevant allergen. Evidence is also presented confirming the functional signaling of the B1 receptors in response to ligand exposure in allergic, but not normal, subjects. Finally, we have provided evidence that engagement of the B1 receptor with its ligand leads to activation of AP-1, a transcription factor that regulates both basal and inducible transcription of many genes involved in cellular proliferation and differentiation. The ability of the B1 receptor to activate AP-1 represents a novel and potentially important functional consequence of receptor up-regulation. We hypothesize that the kinin system contributes to the development of airway inflammation initially through the B2 receptor, with subsequent up-regulation of the B1 receptor. Therefore, generation of kinins in the airway will activate both NF-κB and AP-1 which act together to stimulate transcription of many genes involved in the inflammatory response. The B1 receptor is postulated through these avenues to play a role in sustaining and amplifying chronic inflammation.

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References


