Autocrine Role of Interleukin 1β in Altered Responsiveness of Atopic Asthmatic Sensitized Airway Smooth Muscle

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Abstract

The role of IL-1β in regulating altered airway responsiveness in the atopic/asthmatic sensitized state was examined in isolated rabbit tracheal smooth muscle (TSM) tissue and cultured cells passively sensitized with sera from atopic asthmatic patients or nonatopic/nonasthmatic (control) subjects. During half-maximal isometric contraction of the tissues with acetylcholine, relative to control TSM, the atopic sensitized TSM exhibited significant attenuation of both their maximal relaxation ($P < 0.001$) and sensitivity (i.e., −log dose producing 50% maximal relaxation) to isoproterenol and PGE_2 ($P < 0.05$), whereas the relaxation responses to direct stimulation of adenylate cyclase with forskolin were similar in both tissue groups. The impaired relaxation responses to isoproterenol and PGE_2 were ablated in sensitized TSM that were pretreated with either the IL-1 recombinant human receptor antagonist or an IL-1β–neutralizing antibody. Moreover, extended studies demonstrated that, in contrast to their respective controls, both passively sensitized rabbit TSM tissue and cultured cells exhibited markedly induced expression of IL-1β mRNA at 6 h after exposure to the sensitizing serum, a finding similar to that also obtained in passively sensitized human bronchial smooth muscle tissue. Finally, unlike their respective controls, passively sensitized TSM tissue and cultured cells also displayed progressively enhanced release of IL-1β protein into the culture media for up to 24 h after exposure to atopic/asthmatic serum. Collectively, these observations provide new evidence demonstrating that the altered responsiveness of atopic/asthmatic sensitized airway smooth muscle is largely attributed to its autologously induced expression and autocrine action of IL-1β. (*J. Clin. Invest.* 1997. 99:117-124.) Key words: interleukin 1 • cytokines • β-adrenergic receptors • airway relaxation • asthma

Introduction

Substantial evidence accumulated in recent years has implicated various cytokines in orchestrating and perpetuating the airway inflammatory response in asthma (1–5). Accordingly, studies have reported increased concentrations of different cytokines in the bronchoalveolar lavage fluid isolated from allergic asthmatic individuals (6, 7) and, while the exact role of each cytokine remains to be identified, these agents are known to collectively exert a host of the proinflammatory actions. These include induction of IgE production (8, 9), antigen presentation by macrophages (10), activation of structural cells (3, 11, 12), altered expression of adhesion molecules (13, 14), induced secretion of various growth factors (15), recruitment of inflammatory cells from the circulation (16, 17), and other proinflammatory events.

Apart from potentially producing the above proinflammatory effects in atopic asthmatic airways, specific cytokines, notably IL-1β and, to a lesser extent, TNF-α, may also contribute to the altered airway responsiveness in asthma. In this regard, treatment of antigen-sensitized animals with an IL-1 receptor antagonist has been shown to inhibit their in vivo bronchial hyperreactivity to histamine (1) or substance P (18), as well as the accompanying pulmonary inflammation with leukocytes, including eosinophils and neutrophils (1, 18, 19). Moreover, exogenous administration of IL-1β to isolated guinea pig (20) and rabbit (21) airways has also been shown to impair isoproterenol-mediated relaxation in vitro, a phenomenon that appears to be mechanistically explained by enhanced induction of G_i protein expression, resulting in reduced receptor-coupled adenylate cyclase activation and cAMP accumulation (21). Finally, we recently reported that these IL-1β–induced changes in airway responsiveness are analogous to those elicited by incubating naive airway smooth muscle (ASM)[1] with human atopic/asthmatic serum (21, 22).

Collectively, the above findings support the concept that certain cytokines, particularly IL-1β, may be significantly involved in mediating both the proinflammatory processes and changes in airway responsiveness that characterize the atopic/asthmatic state. In further addressing this issue, this study examined the specific etiologic role of IL-1β in inducing altered responsiveness in airway smooth muscle that has been passively sensitized with human atopic/asthmatic serum. The results demonstrate that (*a*) the enhanced constrictor and attenuated relaxation responsiveness induced in sensitized airways are prevented by the blockade of IL-1β action; and (*b*) exposure of ASM tissue or cultured cells to atopic/asthmatic serum induces the autologous mRNA expression and elaboration of IL-1β by the ASM. Taken together, these observations pro-

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1. *Abbreviations used in this paper:* ACh, acetylcholine; ASM, airway smooth muscle; GAPDH, glyceraldehyde phosphate dehydrogenase; IL-1βab, IL-1β–neutralizing antibody; IL-1ra, IL-1 receptor agonist; pD_{50}, log dose producing 50% maximal relaxation; R_{max}, maximal relaxation response; RPL7, ribosomal protein L7; RT, reverse transcription; T_{max}, maximal contractile response; TSM, tracheal smooth muscle.

vide new evidence that the altered responsiveness of atopic/asthmatic sensitized airways is largely attributed to the induced autocrine expression and autologous action of IL-1β in ASM.

Methods

Preparation and sensitization of airway tissues. Healthy New Zealand White rabbits were used in this study, which was approved by the Biosafety and Animal Research Committee of the Joseph Stokes, Jr. Research Institute at Children's Hospital of Philadelphia. After anesthesia with xylazine (10 mg/kg) and ketamine (50 mg/kg), the animals were killed with an overdose of pentobarbital (130 mg/kg). The tracheae were removed, cleared of loose connective tissue, and each trachea was divided into ring segments of 6–8 mm in length. The sensitization protocol was conducted as described previously (22). Briefly, adjacent ring segments were alternately incubated for 24 h at room temperature in either (*a*) human serum containing > 700 IU/ml IgE, obtained from allergic patients with moderate to severe asthma ($n = 4$) and 4–5/6+ radioallergosorbent test (RAST)-positive (specific IgE concentration > 17.5 Phadebas RAST units [PRU]/ml) to *Dermatophagoides pteronyssinus*, *D. farinae*, *and ragweed*, and with positive skin tests to these antigens; or (*b*) human serum from nonatopic, nonasthmatic individuals ($n = 3$) with normal serum IgE levels (i.e., < 70 IU/ml) and negative skin test reactivity, including to *D. pteronyssinus*, *D. farinae*, and ragweed. In parallel studies, these incubations were also conducted in the presence of a maximal effective concentration of the recombinant human IL-1 receptor antagonist (IL-1ra; 140 ng/ml) or IL-1β monoclonal blocking antibody (IL-1βab; 280 ng/ml). The incubating sera were aerated with a continuous supplemental O_2 mixture (95% O_2/5% CO_2) and maintained at a glucose concentration of 150–200 mg/dl.

In two comparable experiments, the passive sensitization protocol described above was also conducted on human bronchial smooth muscle segments isolated from the surgically resected lung of a 60-yr-old man with peripheral lung carcinoma and no evidence of obstructive lung disease, as assessed by routine preoperative pulmonary function testing. From microscopically tumor-free parts of the specimen, third and fourth generation bronchi were carefully cleaned of loose connective tissue and epithelium, and then cut into ring segments. The latter were cleared of cartilage, and each alternate adjacent segment was incubated in either control or atopic/asthmatic serum, as described above, for subsequent determination of IL-1β mRNA expression (see below).

Pharmacodynamic studies. After incubation, each airway tissue segment was suspended between stainless steel triangular supports in siliconized 20-ml organ baths (Harvard Apparatus, Inc., South Natick, MA). The lower support was secured to the base of the organ bath, and the upper support was attached via a gold chain to a force transducer (FT.03C; Grass Instrument Co., Quincy, MA), from which isometric tension was continuously displayed on a multichannel recorder. Care was taken not to injure the epithelia and to place the membranous portion of the trachea between the supports to maximize the recorded tension generated by the contracting trachealis muscle. The tissues were bathed in modified Krebs-Ringer solution containing (in mM) 125 NaCl, 14 $NaHCO_3$, 4 KCl, 2.25 $CaCl_2 \cdot 2H_2O$, 1.46 $MgSO_4 \cdot 7H_2O$, 1.2 $NaH_2PO_4 \cdot H_2O$, and 11 glucose. The baths were aerated with 5% CO_2 in oxygen, a pH of 7.35–7.40 was maintained, and the temperature was held at 37°C. Passive resting tension of each tracheal smooth muscle (TSM) segment was set at 2.0 *g* after each tissue had been passively stretched to a tension of 8 *g* to optimize the resting length of each segment (23). After a thorough rinsing, the tissues equilibrated in the baths for 45 min, at which time each tissue was primed with a 1-min exposure to 10^{-4} M acetylcholine (ACh). Cholinergic contractility was initially assessed in the TSM by cumulative administration of ACh in final bath concentrations ranging from 10^{-9} to 10^{-3} M, both in the absence and presence of IL-1ra or IL-1βab. After a thorough rinsing, each tissue segment was then half-maximally contracted with ACh and, in separate studies, the tissues were treated with cumulative administration of isoproterenol (10^{-9}–10^{-4} M), PGE_2 (10^{-9}–10^{-4} M), or forskolin (10^{-9}–10^{-4} M). The relaxant responses to isoproterenol, PGE_2, and forskolin were analyzed in terms of percent maximal relaxation (R_{max}) from the active cholinergic contraction, and sensitivity to the relaxing agent was determined as the negative logarithm of the dose of the relaxing agent producing 50% of R_{max} (pD_{50}; i.e., geometric mean ED_{50} value).

Reverse transcription PCR (RT-PCR) determination of IL-1β mRNA levels. Total RNA was isolated from the sensitized and control rabbit TSM and human bronchial smooth muscle segments using a modified acid guanidinium thiocyanate phenol-chloroform extraction method (24) that included proteinase K (in 0.5% SDS) digestion of protein in the initial RNA pellet. The concentration of each RNA sample was then determined spectrophotometrically, and the procedure consistently yielded 20–25 μg of intact RNA per tissue sample. To examine for IL-β mRNA expression, we used a RT-PCR protocol wherein cDNA was reverse transcribed from total RNA primed with oligo (dT). The IL-1β primers used for PCR included (*a*) rabbit-specific 5′-GCACCTCTCAGACAGAGTAC-3′ and 5′-GTGGTTGCTGATAGAAGCTG-3′ (20 mer) (25); and (*b*) human-specific 5′-AGATGAAGTGCTCCTTCCAG-3′ (20 mer) and 5-CAACACGCAGGACAGGTACAG-′3 (21-mer) (26). To control for the transcription levels of the samples, we used (*a*) the rabbit-specific α-actin primers 5′-CGACATCAAGGAGAAGCTG-′3 and 5′-CTAGAAGCATTTGCGGTGC-′3 (19 mer), and the glyceraldehyde phospate dehydrogenase (GAPDH) primers 5′-GATCCATTCATTGACCTCC-′3 (19 mer) and 5′-GATCTCGCTCCTGGAAGATG-′3 (20 mer) (27); and (*b*) the human-specific ribosomal protein L7 (RPL7) primers 5′-AAGAGGCTCTCATTTTCCTGGCTG-′3 (24 mer) and 5′-TCCGTTCCTCCCCATAATGTTCC-′3 (23 mer) (28). The cycling profile used was as follows: denaturation, 95°C × 1 min; annealling, 52°C × 1 min; extension, 72°C × 1 min for 35 cycles for IL-1β and RPL7 and 22 cycles for α-actin and GAPDH. The number of cycles were determined to be in the linear range of the PCR product. The PCR reactions with the rabbit IL-1β, α-actin and GAPDH, and the human IL-1β and RPL7 primers were performed with equivalent amounts of cDNA prepared from 2.5 μg of total RNA from either the rabbit TSM or human bronchial smooth muscle incubated for 6 h with human control or atopic/asthmatic serum. Equal aliquots of each PCR reaction were run on a 1.2% agarose gel and then transferred to a Zeta-Probe (Bio Rad Laboratories, Hercules, CA) membrane overnight in 0.4 N NaOH. After capillary transfer, the DNA was immobilized by UV cross-linking at 120,000 μJ/cm^2 (Stratalinker UV Crosslinker 2400; Stratagene, La Jolla, CA). Prehybridization in a hybridization oven was conducted for 2–3 h at 42°C in 50% formaldehyde, 7% (wt/vol) SDS, 0.25 M NaCl, 0.12 M Na_2HPO_4 (pH 7.2), and 1 mM EDTA. Hybridization was for 20 h at 42°C in the same solution. The IL-1β, α-actin, GAPDH, and RPL7 DNA levels were measured by Southern blot analysis using ^{32}P-labeled cDNA probes prepared by random priming. The human IL-1β probe used was the 1,047-bp cDNA fragment cloned from blood-derived macrophages (26). The other probes were generated by pooling several RT-PCR reactions of individual PCR fragments, followed by gel purification from 1.2% agarose gel using a Qiaex II agarose gel extraction kit (Qiagen, Chatsworth, CA). Washes were as follows: 1 × 15 min in 2 × SSC, 0.1% SDS; 1 × 15 min in 0.1 × SSC, 0.1% SDS both at room temperature, and 2 × 15 min at 50°C in 0.1 × SSC, 0.1% SDS. The specific activities of the above radiolabeled probes were in the range of 5×10^8–1×10^9 cpm/μg DNA. The Southern blots were quantitated by direct measurement of radioactivity in each band using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Preparation of cultured airway smooth muscle cells. Separate sensitization studies were conducted using cultured rabbit ASM cells, previously characterized in detail with respect to their distinguishing morphological, histological, and immunological features (29). The cell isolation and subcultivation procedures were described previ-

ously (29). Briefly, ASM cells were isolated from epithelium-denuded trachealis muscle from adult New Zealand white rabbits. After digestion in Ham's F-12 culture medium (F-12) containing 30 mg/ml protease, 55 mg/ml type IV collagenase, and 100 mg/ml trypsin inhibitor, the dissociated cells were centrifuged and resuspended in Ham's F-12 containing 10% FBS and 100 mg/ml of gentamicin sulfate. The cells were then inoculated in 100-mm tissue culture dishes and, after 4 wk, the cells had sufficiently proliferated to permit routine subcultivations. At weekly intervals, the subcultivated cells were suspended, inoculated at a density of 10^4 cells/cm^2 in 75-cm^2 tissue culture flasks containing Ham's F-12 with 20% FBS, and incubated at 37°C in a humidified atmosphere of 5% CO_2–95% air. For the sensitization protocol, cells initially seeded at 10^4 cells/cm^2 in 75-cm^2 tissue culture flasks were grown to confluence in Ham's F-12 medium containing 20% FBS. The original culture medium was then replaced with serum-free Ham's F-12 containing 5% control or atopic/asthmatic serum for varying durations.

Northern blot analysis. Total RNA was isolated from the cultured rabbit ASM cells, as described above. 20 μg of total RNA was fractionated in 1% agarose, 2.2 M formaldehyde denaturing gels for each time point (0, 6, and 24 h) after exposure of the cells to atopic/asthmatic or control serum. After capillary transfer to Zeta-Probe membranes (Bio Rad) in 10 × SSC (1 × SSC = 0.015 M sodium citrate), RNA was immobilized by UV cross-linking using a Stratalinker UV Crosslinker 2400 (Strategene) at 120,000 μJ/cm^2. Prehybridization in a hybridization oven was conducted for 2–3 h at 42°C in 50% formaldehyde, 7% (wt/vol) SDS, 0.25 M NaCl, 0.12 M Na_2HPO_4 (pH 7.2), and 1 mM EDTA. Hybridization was for 20–22 h at 42°C in the same solution. The IL-1β mRNA level was examined by Northern blot analysis using an IL-1β probe ^{32}P labeled in random primer reactions as described above, and a rabbit-specific α-actin probe was used as a control for RNA loading.

Plasma IL-1β immunoassay. Plasma levels of IL-1β were measured in the blood samples drawn from the asthmatic and control donors using an enzyme-specific immunoassay. The immunometric assay was performed using a double-antibody sandwich strategy in which an ACh esterase–, Fab-conjugated IL-1β–specific secondary antibody is targeted to a first cytokine-captured antibody. The enzymatic activity of the ACh esterase was measured spectrophotometrically and, relative to a linear standard curve (range = 0–250 pg/ml), the results were used to quantify the amount of the targeted IL-1β present in the plasma samples.

IL-1β radioimmunoassay. IL-1β protein levels were measured in the culture media from TSM tissues and ASM cells at 0, 3, 6, and 24 h using a commercially available radioimmunoassay kit that specifically quantitates rabbit IL-1β (Endogen, Cambridge, MA). The assay is based on the competition between unlabeled rabbit IL-1β in the test sample and ^{125}I-radiolabeled recombinant rabbit IL-1β (tracer) for binding sites on a specific IL-1β primary antibody. A second antibody is then used to immunoprecipitate primary antibody bound with either tracer or unlabeled IL-1β. The immunoprecipitate is then counted with a gamma counter, and the values are read from a standard curve.

Statistical analysis. Unless otherwise indicated, results are expressed as mean±SE. Statistical analysis was performed by means of a two-tailed paired Student's t test. $P < 0.05$ was considered significant.

Reagents. IL-1ra and IL-1βab were obtained from R&D Systems (Minneapolis, MN). ACh, isoproterenol hydrochloride, PGE_2, and forskolin were obtained from Sigma Chemical Co. (St. Louis, MO). The IL-1β, α-actin, GAPDH, and RPL7 primers were from Cruachem Inc. (Dulles, VA). The rabbit IL-1β radioimmunoassay kit was from Endogen, (Cambridge, MA). All drug concentrations are expressed as final bath concentrations. Forskolin was dissolved in DMSO to prepare a 10^{-4} M solution; dilutions of 10^{-5} M and lower were prepared in demineralized H_2O. Isoproterenol and ACh were made fresh for each experiment, and were dissolved in normal saline to prepare 10^{-4} M and 10^{-3} M solutions, respectively. PGE_2 was dissolved in demineralized H_2O to prepare a 10^{-4} M solution. Addition of these diluents to the organ baths at the appropriate concentrations had no effect on either the resting nor half-maximal cholinergic tone of the TSM tissues.

Results

Role of IL-1 in altered constriction of sensitized airways. Contractile Shultz-Dale responses to the administration of *D. pteronyssinus*, *D. farinae*, and ragweed antigens were compared in paired TSM exposed to control vs. atopic/asthmatic serum. As previously described using this sensitization protocol (22), contractile responses to antigen were observed only in the atopic sensitized tissues, indicating that the control airway segments were not sensitized to the antigens.

To assess whether IL-1 plays a role in regulating TSM constrictor responsiveness in passively sensitized tissues, contractile dose–response relationships to ACh were compared in sensitized and control TSM in the absence and presence of IL-1ra. As shown in Fig. 1 *A*, maximal constrictor responsiveness to ACh was significantly increased in sensitized vs. control TSM, which provided mean±SE maximal isometric force (T_{max}) values of 131.76±10.87 vs. 104.70±5.90 g/g TSM wt, re-

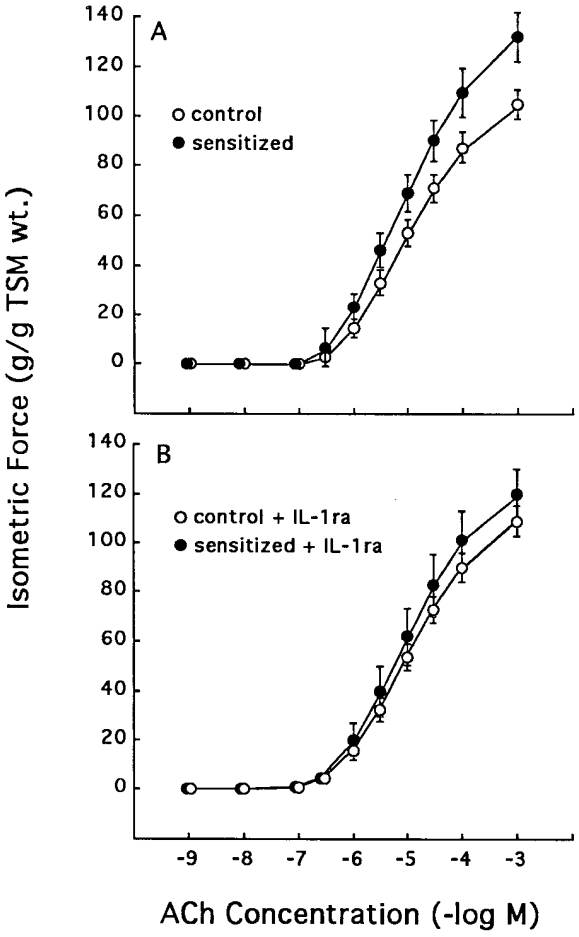

Figure 1. Comparison of contractile dose–response curves to ACh in paired control (*open symbols*) and passively sensitized (*closed symbols*) TSM segments in the absence (*A*) and presence (*B*) of IL-1ra. Data represent means±SE from eight experiments.

spectively ($P < 0.01$). Similarly, constrictor sensitivity to ACh was also significantly enhanced in sensitized vs. control tissues, wherein the mean pD_{50} values amounted to 5.11±0.11 vs. 5.03±0.09 −log M, respectively ($P < 0.05$). As depicted in Fig. 1 *B*, pretreatment of the tissues with a maximally effective concentration of IL-1ra (140 ng/ml) ablated the differences between sensitized and control TSM. Specifically, IL-1ra selectively attenuated the T_{max} responses to ACh in the sensitized tissues, and there were no differences between either the T_{max} and pD_{50} values in sensitized vs. control tissues in the presence of IL-1ra, wherein the mean T_{max} values amounted to 119.69±10.13 vs. 108.62±6.13 g/g TSM wt ($P = 0.14$), respectively, and the corresponding pD_{50} values averaged 4.99±0.19 vs. 5.00±0.92 −log M, respectively ($P = 0.84$).

Role of IL-1 in altered relaxation of sensitized airways. In separate studies, we also compared agonist-mediated relaxation responses in control vs. sensitized TSM. During comparable levels of initial sustained ACh-induced contractions in the sensitized and control tissues, averaging 41.25±1.47 and 39.52±1.46% of T_{max}, respectively, administration of the β-adrenergic agonist, isoproterenol, PGE_2, or the direct adenylate cyclase activator, forskolin, elicited cumulative dose-dependent relaxation of the precontracted TSM segments. Relative to their respective controls, the mean R_{max} and sensitivities (pD_{50}) to isoproterenol and PGE_2 were significantly attenuated in the sensitized tissues. As exemplified for the isoproterenol-treated tissues in Fig. 2 *A*, the mean R_{max} values to isoproterenol amounted to 28.7±4.5 and 68.9±5.5% in the sensitized and control TSM, respectively ($P < 0.001$), with corresponding pD_{50} values of 6.17±0.11 and 6.29±0.12 −log M, respectively ($P < 0.05$). Pretreatment of the TSM with IL-1ra inhibited the attenuated relaxation responses to isoproterenol in the sensitized tissues, while relaxation responsiveness was unaffected by IL-1ra in control TSM (Fig 2 *B*). Similarly, the reduced relaxation responses to PGE_2 were also ablated by pretreatment of the sensitized tissues with IL-1ra. Accordingly, the mean R_{max} and pD_{50} values to PGE_2 in the sensitized tissues averaged 22.5±5.2 and 49.9±5.2% ($P < 0.001$) and 6.00±0.02 and 6.20±0.04 −log M ($P < 0.01$) in the absence and presence of IL-1ra, respectively; and the latter values in the presence of IL-1ra were not significantly different from those obtained in corresponding control tissues (i.e., R_{max} = 52.4±8.3% and pD_{50} = 6.24±0.06 −log M). Thus, in the presence of IL-1ra, there were no differences in the relaxation responses to isoproterenol or PGE_2 between sensitized and control TSM.

In comparable experiments, we found that the above changes in relaxation responsiveness to isoproterenol and PGE_2 in sensitized TSM were also prevented by coincubation of the tissues with IL-1βab (280 ng/ml). Accordingly, in the absence of IL-1βab, the R_{max} values to isoproterenol and PGE_2 obtained in sensitized TSM amounted to 31.2±7.8 and 23.8±4.2%, respectively; whereas the corresponding values obtained in sensitized TSM treated with IL-1βab averaged 45.2±3.0 ($P < 0.01$) and 43.3±1.1% ($P < 0.05$), respectively. The latter R_{max} values obtained in the presence of IL-1βab were similar to those generated in control tissues (i.e., 59.4±6.8 and 45.0±1.0% for isoproterenal and PGE_2, respectively), wherein IL-1βab had no effect on the relaxation responses to either isoproterenol or PGE_2.

In contrast to the described observations above, there were no differences in the relaxation responses to forskolin between the tissue groups, wherein the mean R_{max} values to forskolin

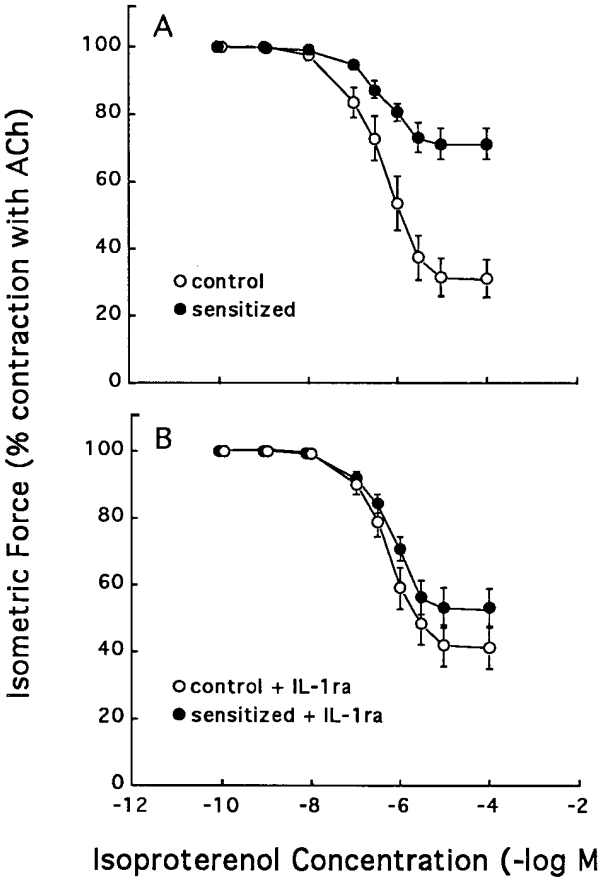

Figure 2. Comparison of airway relaxation responses to isoproterenol in paired control (*open symbols*) and atopic sensitized (*closed symbols*) TSM segments that were initially half-maximally contracted with respective ED_{50} doses of acetylcholine in the absence (*A*) and presence (*B*) of IL-1ra. Each data point represents the mean±SE from 8–10 paired tissue samples.

amounted to 88.8±2.8 and 92.0±5.8% in the sensitized and control TSM, respectively ($P = 0.78$), and the corresponding pD_{50} values averaged 5.96±0.07 and 6.00±0.05 −log M, respectively ($P = 0.52$). Furthermore, IL-1ra had no effect on the relaxation responses to forskolin in either of the tissue groups. Thus, taken together, the above observations demonstrate that (*a*) reversal of ACh-induced airway constriction with isoproterenol or PGE_2, both of which are associated with receptor/G protein–coupled activation of adenylate cyclase, is attenuated in sensitized tissues; (*b*) the latter effect is prevented by IL-1ra or IL-1βab; and (*c*) in contrast, non–receptor-coupled TSM relaxation to forskolin, a diterpene that directly activates the catalytic unit of adenylate cyclase, is unaltered in the sensitized state and unaffected by the blockade of IL-1β action.

IL-1β expression in sensitized airway smooth muscle. In light of the above findings, we tested for the presence of IL-1β by enzyme immunoassay in the plasma from the atopic/asthmatic and control blood samples used for incubation of the airway tissues. There were no detectable levels of IL-1β in the plasma samples from either the asthmatic or control subjects. Accordingly, we examined whether the above changes in relaxant responsiveness in the sensitized tissues were related to altered

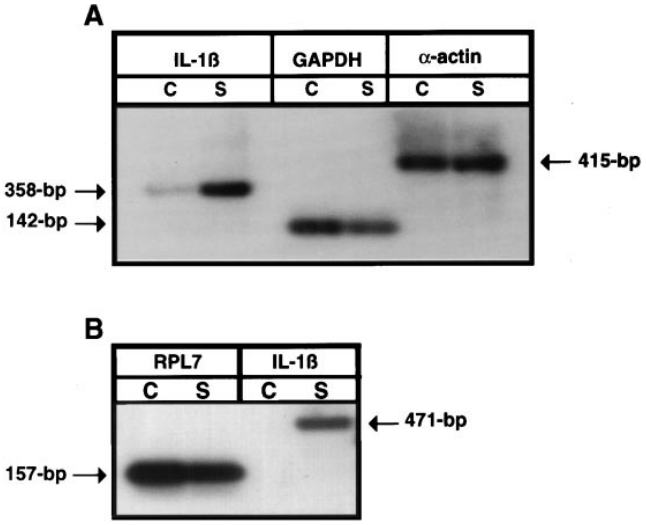

Figure 3. Representative Southern blots probed with 358-bp IL-1β, 142-bp GAPDH, and 415-bp α-actin rabbit cDNA fragments (*A*), and 471-bp IL-1β and 157-bp RPL7 human cDNA fragments (*B*). Paired rabbit TSM and human bronchial smooth muscle segments were incubated with atopic/asthmatic or control serum for 6 h. cDNA was transcribed from total RNA primed with oligo (dT). PCR products were amplified using rabbit-specific IL-1β, GAPDH and α-actin primers, and human-specific IL-1β and RPL7 primers run on 1.2% agarose gels and then probed with the various cDNA probes (see Methods). Note the marked induction of IL-1β expression in both the atopic/asthmatic serum–sensitized (*S*) rabbit (*A*) and human (*B*) tissues as compared to their respective controls (*C*), whereas expression of GAPDH, α-actin, and RPL7 were similar in both tissue samples.

endogenous expression of IL-1β by sensitized TSM. A rabbit cDNA was synthesized to compare the expression of IL-1β mRNA in control and sensitized TSM by Southern blot analysis using a RT-PCR protocol (see Methods). cDNA was reverse transcribed from total RNA primed with oligo (dT), and 10 μl of each PCR reaction was used to load each lane of a 1.2% agarose gel. The Southern blot was analyzed with rabbit-specific probes, including a 358-bp IL-1β probe, a 415-bp α-actin probe, and a 142-pb GAPDH probe, all of which were ^{32}P labeled by random priming. In this semiquantitative RT-PCR assay, the signals for the IL-1β, α-actin, and GAPDH PCR products were quantitated with a Molecular Dynamics PhosphorImager. After 6 h of treatment with control or atopic/asthmatic serum, the signals for α-actin and GAPDH were not significantly different between the control and sensitized samples, as exemplified in Fig. 3 *A*. In contrast, it will be noted that while the signal for IL-1β was faintly detectable in tissue treated with control serum, IL-1β expression was markedly enhanced (> 10-fold) after exposure to the atopic/asthmatic serum.

Similar results were obtained in two comparable experiments conducted on passively sensitized human bronchial smooth muscle tissues, wherein PCR products generated using oligo (dT)-primed human cDNA and human-specific IL-1β and RPL7 primer sets were loaded in separate lanes on a 1.2% agarose gel. The Southern blot was analyzed with human-specific IL-1β (1,047-bp) and RPL7 (157-bp) probes that were ^{32}P labeled by random priming. As depicted in Fig. 3 *B*, after 6 h of treatment with control or atopic/asthmatic serum, the signals for constitutively expressed RPL7 were similar in the control and atopic sensitized samples. In contrast, IL-1β expression was markedly induced in the atopic/asthmatic serum–treated tissue, whereas the IL-1β signal was undetectable in the corresponding control serum–treated sample.

Since the above observations pertain to TSM tissue preparations that intrinsically contain various cell types (i.e., in addition to ASM per se), the above studies were extended to include analysis of IL-1β mRNA expression specifically in cultured rabbit ASM cells. As depicted by the Northern blot in Fig. 4, relative to constitutively expressed α-actin mRNA, ASM cells exposed to control serum showed virtually nondetectable levels of IL-1β mRNA. In contrast, cells treated with atopic/asthmatic serum demonstrated a transient induced expression of IL-1β mRNA at 6 h, with significant reversal of the increased IL-1β mRNA expression at 24 h.

Given the above observations, additional experiments were conducted to evaluate the effects of exposure of TSM tissue and cultured ASM cells to control or atopic/asthmatic serum on the release of IL-1β into the culture medium, which was examined by radioimmunoassay. There were no detectable levels of IL-1β in the culture medium of either the TSM tissue or cultured ASM cell preparations that were exposed to control serum. In contrast, as exemplified in Fig. 5, both the tissues (Fig. 5 *A*) and cultured cells (Fig. 5 *B*) exposed to atopic/asthmatic serum depicted progressively enhanced elaboration of IL-1β into the culture media for up to 24 h. Thus, taken together, the above observations demonstrate that, whereas control serum had no effect, exposure of TSM tissue or cultured ASM cells to atopic/asthmatic serum induced the mRNA expression and elaboration of IL-1β in the sensitized state.

Discussion

Bronchial asthma is characterized by inflammation of the airways associated with exaggerated bronchoconstrictor responsiveness to contractile agonists and impaired airway relaxation to β-adrenoceptor stimulation (30–32). The etiology of these changes in airway function remains to be identified, although some recent studies have provided certain valuable insights into potential mechanisms. Accordingly, it has been demon-

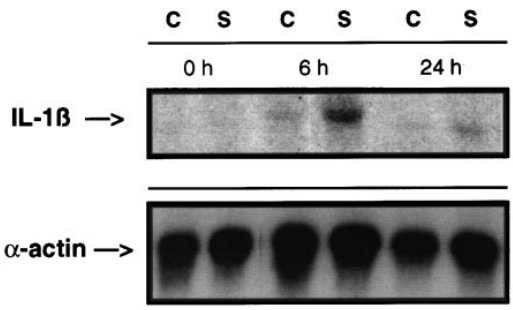

Figure 4. Representative Northern blot probed with rabbit-specific IL-1β and α-actin probes. Rabbit ASM cells were incubated with 5% control or atopic/asthmatic serum for 0, 6, and 24 h. 20 μg of RNA was loaded in each lane and run on a 1% agarose, 2.2 M formaldehyde denaturing gel. Note the induced enhanced expression of IL-1β mRNA (*top*) in the atopic/asthmatic serum–treated samples at 6 and 24 h, whereas corresponding α-actin mRNA expression (*bottom*) was not significantly affected.

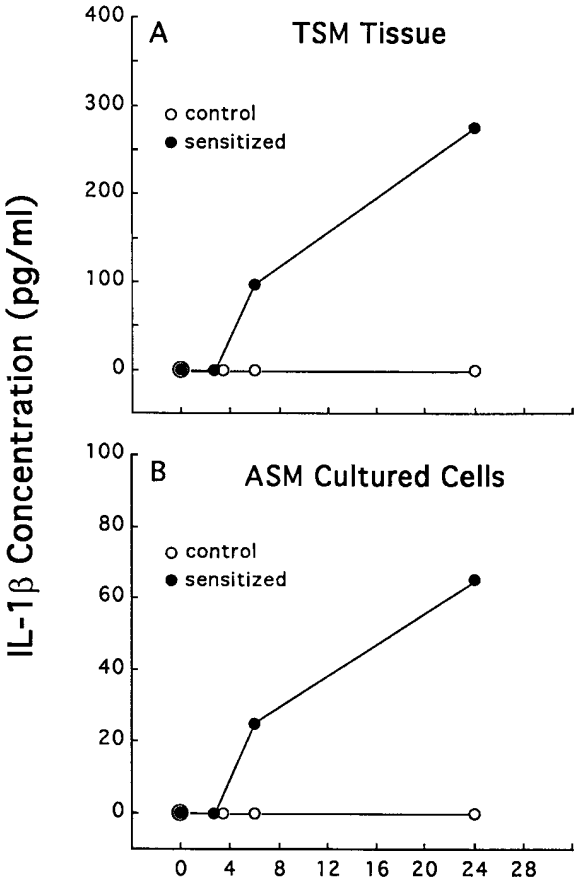

Figure 5. Comparison of levels of rabbit IL-1β protein released into the culture media of TSM tissues (*A*) and cultured ASM cells (*B*) at various times after exposure to control (*open symbols*) or atopic/asthmatic serum (*filled symbols*). Data respresent the mean values from three separate experiments.

strated that the attenuated β-adrenoceptor responsiveness in asthmatic airways could not be accounted for by a decrease in β-adrenergic receptor density or affinity (33–35), but rather, likely reflects a disruption of the β-adrenoceptor/adenylate cyclase–coupled transmembrane signaling mechanism. Indeed, in support of this concept, we recently reported that the induced impairment of β-adrenoceptor–mediated relaxation in rabbit ASM passively sensitized with human atopic/asthmatic serum is attributed to enhanced muscarinic M_2 receptor/G_i protein–coupled expression and interaction, leading to attenuated receptor-coupled cAMP accumulation (22). Moreover, in an extended study, we found that administration of IL-1β to rabbit ASM also produced impaired β-adrenoceptor–mediated relaxation associated with induced G_i protein expression (21), and that these changes were analogous to those elicited by incubating the tissue with human atopic/asthmatic serum (22).

In light of the above findings, and given emerging evidence implicating certain cytokines, most notably IL-1β, in producing airway dysfunction and pulmonary inflammation in antigen-sensitized animals (1, 18–20), this study examined the specific etiological role of IL-1β in inducing altered responsiveness in airways passively sensitized with human atopic/asthmatic serum. The results obtained support the concept that IL-1β plays an important role in mediating changes in airway responsiveness that characterize the atopic/asthmatic phenotype. Moreover, new evidence is provided that demonstrates induced expression of IL-1β by the ASM itself, and strongly implicates the autocrine release and action of IL-1β by the ASM in autologously inducing its altered responsiveness in the sensitized state.

Our use of passive sensitization of rabbit airways with human atopic/asthmatic serum provided a practical in vitro experimental approach to examine the regulation of airway responsiveness in the atopic/asthmatic sensitized state (22). In this regard, the observed changes in responsiveness in the sensitized tissues mimick the pertubations in airway function that characterize the in vivo asthmatic condition, and are similar to those obtained in passively sensitized human ASM in vitro (36–38). The mechanistic link, however, between exposure of the airway tissue to atopic/asthmatic serum and its altered responsiveness remains to be identified, although it appears to be likely attributed to the action of serum IgE bound to Fc receptors on airway-infiltrating cells (37–39) or to the activation of Fc receptors present on the ASM cell itself. Indeed, in preliminary studies (40, 41), we found upregulated expression and activation of the low affinity FcεRII receptor for IgE in both rabbit TSM and cultured human ASM cells passively sensitized with human atopic/asthmatic serum, as performed herein. Notwithstanding the potential triggering mechanism involved, the present results demonstrate that the elaboration and action of IL-1β plays a fundamental etiologic role in mediating the observed changes in airway responsiveness in the sensitized state.

The observations that both the β-adrenoceptor– and PGE_2-mediated relaxation responses, but not those elicited by forskolin, were attenuated in sensitized TSM suggest that the impairment in airway relaxation involved a non–receptor-specific signaling mechanism upstream to adenylate cyclase activation (22). Moreover, to the extent that pretreatment of the sensitized tissues with IL-1ra or IL-1βab was found to inhibit their impaired responsiveness to β-adrenoceptor stimulation (Fig. 2), while having no effect on the tissues' responses to forskolin, the results further support the notion that IL-1β does not modulate the signal transduction mechanism distal to adenylate cyclase activation (21). Thus, taken together, these observations are consistent with our recent findings that the attenuating action of IL-1β on receptor-coupled airway relaxation is caused by induced upregulated expression and inhibitory action of G_i protein (i.e., specifically $G_i\alpha_2$ and $G_i\alpha_3$ subunits) on cAMP accumulation (21).

In further examining the role of IL-1β in the sensitized airway tissues, our finding of a lack of any detectable levels of IL-1β in either the control or atopic/asthmatic plasma samples suggested that the source of IL-1β likely resides within the airway tissue. Indeed, using RT-PCR and Southern blot analysis with rabbit- and human-specific IL-1β cDNA probes, we found markedly induced expression of IL-1β mRNA in the sensitized tissues (Fig. 3). Moreover, Northern blot analysis of IL-1β mRNA expression in cultured rabbit ASM cells demonstrated induced expression of IL-1β mRNA in cells exposed to atopic/asthmatic serum (Fig. 4). Finally, in extending the above findings, we found that the transiently induced IL-1β mRNA expression in sensitized TSM and cultured ASM cells was associated with an induced elaboration of IL-1β into the tissue and cell culture media for up to 24 h after exposure to the atopic/asthmatic serum (Fig. 5). Taken together, these ob-

servations demonstrate that passive sensitization of ASM induces its autologous expression of IL-1β mRNA, and that this involves an autocrine action of the ASM cell that is associated with its extracellular release of IL-1β protein. The relative contributions of IL-1β gene transcription vs. posttranscriptional regulation of the altered IL-1β mRNA expression in ASM in the sensitized state remain to be determined.

Collectively, the above observations support the novel concept of an autocrine role for the ASM cell wherein, via its induced expression of IL-1β, the ASM autologously mediates its altered responsiveness in the atopic/asthmatic sensitized state. While comparable autocrine mechanisms in structural cells have recently been implicated in other cell types and may be involved in certain disease states (11, 15, 42, 43), the findings in this report have potentially significant implications regarding the pathobiology of asthma. Given the diverse proinflammatory actions of IL-1β and its direct effects on ASM function, it is conceivable that both the changes in bronchial responsiveness and associated airways inflammation in atopic asthma may be largely initiated by the autocrine role of the sensitized ASM cell itself, with possible amplification and perpetuation of proinflammatory processes occurring secondary to the paracrine recruitment and activation of other cell types. This compelling consideration offers some new directives for further investigation into potentially important mechanisms that underlie airway structural and functional abnormalities in asthma.

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