Autocrine signaling by IL-10 mediates altered responsiveness of atopic sensitized airway smooth muscle

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Grunstein, M. M., H. Hakonarson, J. Leiter, M. Chen, R. Whelan, J. S. Grunstein, and S. Chuang. Autocrine signaling by IL-10 mediates altered responsiveness of atopic sensitized airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 281: L1130–L1137, 2001.—To elucidate the role and mechanism of action of interleukin (IL)-10 in regulating airway smooth muscle (ASM) responsiveness in the atopic asthmatic state, isolated rabbit tracheal ASM segments were passively sensitized with serum from atopic asthmatic patients or nonatopic nonasthmatic (control) subjects in both the absence and presence of an anti-IL-10 receptor blocking antibody (Ab). Relative to control ASM, atopic asthmatic serum-sensitized tissues exhibited enhanced isometric contractile responses to administered acetylcholine and attenuated the relaxation responses to isoproterenol. These proasthmatic effects were prevented in atopic asthmatic serum-sensitized ASM that was pretreated with anti-IL-10 receptor Ab. In complementary experiments, exposure of cultured human ASM cells to atopic asthmatic serum induced upregulated expression of IL-10 mRNA. Moreover, extended studies demonstrated that 1) exogenous IL-10 administration to naive ASM elicited augmented contractility to acetylcholine and impaired relaxation to isoproterenol, 2) these effects of IL-10 were prevented by pretreating the tissues with an IL-5 receptor Ab, and 3) IL-10 administration induced upregulated mRNA expression and release of IL-5 protein from cultured ASM cells. Collectively, these findings provide new evidence demonstrating that the altered responsiveness of atopic asthmatic serum-sensitized ASM is largely attributed to activation of an intrinsic T helper type 2 autocrine mechanism involving IL-10-mediated release and the action of IL-5 in the sensitized ASM itself.

interleukin; T helper type 2 cytokines; signal transduction; asthma

BRONCHIAL ASThma is characterized by enhanced agonist-mediated bronchoconstriction, impaired β-adrenoceptor-mediated airway relaxation, and airway inflammation. Although the mechanism(s) underlying these inflammation-associated changes in airway responsiveness remain to be elucidated, substantial evidence accumulated in recent years has implicated a crucial role for CD4+ T helper (Th) type 2-type cytokines in the pathophysiology of the airway inflammatory response in allergic asthma and its accompanying changes in airway responsiveness. Notably, among the Th2 cytokines, interleukin (IL)-4, IL-13, and IL-5 are known to orchestrate various humoral and cellular immune responses that are characteristic of allergic asthma, including IgE synthesis and eosinophil recruitment and activation (3, 14, 16, 21). Accordingly, it is generally believed that these cytokines modulate airway responsiveness either indirectly through extended activation of an inflammatory cascade or directly by acting on the atopic asthmatic serum-sensitized airway smooth muscle (ASM) itself (4, 5, 12, 15, 26).

Of interest, the above paradigm involving Th2-dependent proinflammatory mechanisms in atopic asthma is currently being somewhat redefined in light of emerging new evidence in animal models of allergic asthma that phenotypic expression of airway constrictor hyperresponsiveness may be manifested independent of pulmonary inflammation (14a, 18, 25). In this regard, recent in vivo studies (14a, 18, 25) in allergen-sensitized mice have demonstrated that the expression and action of the Th2-type cytokine IL-10 is required for the development of airway constrictor hyperresponsiveness but not for pulmonary inflammation or eosinophilia after allergen challenge in the sensitized state. Because IL-10 is generally recognized for its immunosuppressive properties in animal models (1, 19, 28), this recent evidence suggests a potentially important role for IL-10 downstream from the pulmonary inflammatory cascade in regulating airway responsiveness in the allergic asthmatic state. In light of this consideration, the present study was designed to elucidate the role and mechanism of action of IL-10 in regulating agonist-mediated airway constrictor and relaxant responsiveness in atopic asthmatic serum-sensitized ASM. The results provide new evidence demonstrating that the altered agonist responsiveness of atopic asthmatic serum-sensitized ASM is largely attributed to activation of an endogenously expressed Th2-type autocrine mechanism involving sequentially induced IL-
10-mediated release and the action of IL-5 in the atopic sensitized ASM itself.

MATERIALS AND METHODS

Animals. Seventeen adult 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 (Philadelphia, PA). The animals had no signs of respiratory disease for several weeks before the study.

Preparation and sensitization of rabbit ASM tissue. After general anesthesia with xylazine (10 mg/kg) and ketamine (50 mg/kg), the rabbits were killed with an overdose of pentobarbital sodium (130 mg/kg). As previously described (10), the tracheae were removed via an open thoracotomy, the loose connective tissue and epithelium were scraped and removed, and the tracheae were divided into eight ring segments 6–8 mm in length. Each alternate ring was incubated for 24 h at room temperature in either 1) human serum containing >800 IU/ml of IgE obtained from allergic patients with moderate to severe asthma who demonstrated 4–5 or 6+ radioallergosorbent test (RAST)-positive specific IgE concentrations of >17.5 Phadebas RAST units/ml to Dermatophagoides pteronyssimus, D. farinae, or ragweed, and who had positive skin tests to these antigens or 2) human serum from nonatopic nonasthmatic (control) individuals with normal serum IgE levels (<70 IU/ml) and negative skin test reactivity to D. pteronyssimus, D. farinae, and ragweed. In parallel experiments, 1 h before incubation in control or atopic asthmatic serum, ASM segments were treated with either an IgG1-type anti-IL-10 receptor (anti-IL-10R) blocking antibody (Ab), an IgG2-type anti-IL-10 protein neutralizing monoclonal Ab (MAb), or an IgG1-type anti-vascular cell adhesion molecule (VCAM)-1 MAb. All the tissues studied were aerated with a continuous supplemental O2 mixture (95% O2:5% CO2) during the incubation phase.

Pharmacodynamic studies of ASM responsiveness. After incubation of the tissue preparations, each ASM segment was suspended longitudinally between stainless steel triangular supports in siliconized Harvard 20-ml organ baths. The lower support was secured to the base of the organ bath, and the upper support was attached via a gold chain to a Grass FT.01C force transducer from which isometric tension was continuously displayed on a multichannel recorder. Care was taken to place the membranous portion of each tracheal segment between the supports to maximize the recorded tension generated by the contracting tracheal muscle. The tissues were bathed in modified Krebs-Ringer solution containing (in mM) 125 NaCl, 14 NaHCO3, 4 KCl, 2.25 CaCl2·H2O, 1.46 MgSO4·H2O, 1.2 NaH2PO4, and 11 glucose. The baths were aerated with 5% CO2 in O2, a pH of 7.35–7.40 was maintained, and the organ bath temperature was held at 37°C. Passive resting tension of each ASM segment was set at 1.5–2.0 g after the tissue had been passively stretched to a tension of 8 g to optimize its resting length for contraction, as previously described (10, 13). The tissues were allowed to equilibrate in the organ 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 subsequently assessed in the ASM segments by cumulative administration of ACh in final bath concentrations ranging from 10–10 to 10–3 M. Thereafter, the tissues were repeatedly rinsed with fresh buffer, and, subsequently, relaxation dose-response curves to isoproterenol (10–10 to 10–4 M) were conducted after the tissues were half-maximally contracted with their respective doses (ED50) of ACh. The initial constrictor dose-response curves to ACh were analyzed in terms of the maximal isometric contractile force (Tmax) of the tissues to the agonist. The subsequent relaxation responses to isoproterenol were analyzed in terms of percent maximal relaxation (Rmax) from the initial level of active cholinergic contraction, and sensitivity to the relaxing agonist was determined as the corresponding pD50 value (i.e., geometric mean ED50 value) associated with 50% of Rmax.

Preparation and sensitization of cultured human ASM cells. Cultured human ASM cells were obtained from Clonetics (San Diego, CA). The ASM cells were derived from two male donors, 16 and 21 yr of age, who had no evidence of lung disease. The cells were characterized by the manufacturer with specific markers to confirm their selective smooth muscle phenotype and to exclude contamination with other cell types. The cells were grown in smooth muscle basal medium (SMBM) supplemented with 5% fetal bovine serum, 5 ng/ml of insulin, 10 ng/ml of epidermal growth factor (human recombinant), 2 ng/ml of fibroblast growth factor (human recombinant), 50 ng/ml of gentamicin, and 50 ng/ml of amphotericin B. The experimental protocol involved growing the cells to confluence in the above medium. Thereafter, in separate experiments, the cells were starved in unsupplemented SMBM for 24 h, at which time the cells were treated for 0, 3, 6, 12, and 24 h with either 10% human control serum, 10% human atopic asthmatic serum, or serum-free medium in the presence and absence of exogenously administered IL-10. The cells were then examined for the expression of IL-10 and IL-5 mRNAs as well as for the elaboration of IL-5 protein into the cell culture medium as described in Determination of IL-10 and IL-5 mRNA expression in human ASM cells.

Determination of IL-10 and IL-5 mRNA expression in human ASM cells. Total RNA was isolated from the ASM cell preparations with the modified guanidinium thiocyanate-phenol-chloroform extraction method to include proteinase K (in 5% SDS) for digestion of protein in the initial RNA pellet as previously described by our laboratory (12, 13). The concentration of each RNA sample was determined spectrophotometrically. This procedure consistently produced yields of 15–25 μg of intact RNA from each T-75 flask of cultured human ASM cells. To analyze for mRNA expression of the IL-10 and IL-5 genes, we used a RT-PCR protocol that included human-specific primers for these genes as well as for the constitutively expressed ribosomal protein (RP) L7 gene. cDNA was synthesized from the total RNA isolated from ASM cells incubated for 0, 3, 6, 12, and 24 h with control or atopic asthmatic serum or exposed to serum-free medium in the absence and presence of IL-10. The cDNA was primed with oligo(dT)12-18 and extended with SuperScript II reverse transcriptase (GIBCO BRL). The PCR was used to amplify the specific products from each cDNA reaction based on the published sequences of the human IL-10, IL-5, and RPL7 genes and including the following primer sets: 5′-primer-5′-TATTTTGTTCCGCTGTGCT-3′ and 3′-primer-5′-TTTGGTGCAAGGGAGGCC-3′ (211-bp product) for IL-10, 5′-prime5′-TATTTTGTTCCGCTGTGCT-3′ and 3′-primer-5′-TATTTTGTTCCGCTGTGCT-3′ and 3′-primer-5′-TTTGGTGCAAGGGAGGCC-3′ (157-bp product) for RPL7. The cycling profile used was as follows: denaturation at 95°C for 1 min, annealing at 52–55°C for 1.0 min, and extension at 72°C for 1.0 min, with 35, 30, and 25 cycles for the IL-10, IL-5, and RPL7 genes, respectively. The number of cycles was determined to be in the linear range of the PCR products. The PCR products for the primers were performed with equivalent amounts of cDNA prepared from 2.5 μg of total RNA. Equal aliquots of each PCR were then run on a 1.2% agarose gel and
subsequently transferred to a Zeta-probe membrane overnight in 0.4 N NaOH. After capillary transfer, the DNA was immobi-
lized by ultraviolet cross-linking with a Stratalinker UV
Crosslinker 2400 at 120,000 μJ/cm² (Stratagene). Prehybrid-
ization in a Techne 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 Na2HPO4 (pH 7.2), and 1 mM EDTA. Hybridization was for
20 h at 42°C in the same solution. The IL-10, IL-5, and RPL7
DNA levels were assayed by Southern blot analysis with 32P-
labelled probes that were prepared by pooling several RT-PCRs
for the individual PCR fragments and purifying them from a
1.2% agarose gel with the QIAEX II agarose gel extraction kit.
The individual PCR products were subsequently sequenced for
confirmation. Washes were as follows: one time for 15 min in 2×
SSC-0.1% SDS, one time for 15 min in 0.1× SSC-0.1% SDS
(both at room temperature), and two times for 1 min each at
50°C in 0.1× SSC-0.1% SDS.

ELISA measurement of IL-5 protein release. IL-5 protein
levels were assayed in the culture medium of ASM cells that
were exposed for varying durations up to 24 h to either 10%
control or atopic asthmatic serum. The IL-5 protein levels
were quantitatively assessed with an enzyme-specific immu-
noassay as previously described (11). The latter assay was
performed with a double-antibody sandwich strategy in
which an acetylcholinesterase-F(ab)-conjugated IL-5-specific
secondary antibody was targeted first to an IL-5-captured
antibody. The enzymatic activity of the acetylcholinesterase
was measured spectrophotometrically, and the results were
used to quantify, relative to a linear standard curve, the
amount of the targeted IL-5 present in the culture medium.

Reagents. The human ASM cells and SMBM were obtained
from Clonetics. The IL-10, IL-5, and RPL7 primers were
obtained from Integrated DNA Technologies (Coralville, IA).
The anti-IL-10R, anti-IL-10, and anti-VCAM-1 neutralizing
Abs, the IL-5 ELISA kit, the mouse anti-human IL-5 primary
Abs, the IL-5 ELISA kit, the mouse anti-human IL-5 primary
Ab, and the anti-mouse secondary Ab used in the protein
neutralizing MAb or an anti-VCAM-1 MAb. As
described in related experiments, neither the anti-IL-10R Ab nor
the anti-IL-10 MAb had no effect. In comparable experi-
ments, we found that the heightened constrictor responses to ACh were also ablated in atopic asthmatic
serum-sensitized tissues that were pretreated with 0.5
μg/ml of anti-IL-10 MAb (data not shown). Moreover,
in related experiments, neither the anti-IL-10R Ab nor
the anti-IL-10 MAb was found to appreciably affect the
ASM constrictor responses to ACh in control se-
rum-exposed tissues (data not shown).

RESULTS

Role of IL-10 in regulating agonist responsiveness in
atopic asthmatic serum-sensitized ASM. To determine
the role of IL-10 in regulating ASM responsiveness in
the atopic asthmatic serum-sensitized state, agonist-
mediated constrictor and relaxation responses were
separately compared in paired isolated rabbit ASM
segments 24 h after exposure to serum from either
atopic asthmatic individuals or nonatopic nonasth-
matic (control) subjects in both the absence and pres-
ence of an anti-IL-10R blocking Ab, an anti-IL-10 pro-
tein neutralizing MAb or an anti-VCAM-1 MAb. As
shown in Fig. 1, relative to control serum-exposed
tissues, the constrictor responses to exogenously ad-
ministered ACh were significantly increased in atopic
asthmatic serum-sensitized ASM. Accordingly, the
Tmax values amounted to 109.9 ± 7.9 (SE) and 131.2 ±
9.7 g/g ASM weight in the control and atopic asthmatic
serum-sensitized tissues, respectively (P < 0.01), rep-
resenting an average increase in Tmax of ~ 20% above
the control value in the atopic asthmatic serum-sensi-
tized ASM. These increased constrictor responses to ACh were abrogated in atopic asthmatic-sensitized tis-
sues that were pretreated with a maximally effective
concentration (1.0 μg/ml) of anti-IL-10R Ab (Fig. 1),
whereas pretreatment with the isotype control anti-
VCAM-1 MAb had no effect. In comparable experi-
ments, we found that the heightened constrictor re-
sponses to ACh were also ablated in atopic asthmatic
serum-sensitized tissues that were pretreated with 0.5
μg/ml of anti-IL-10 MAb (data not shown). Moreover,
in related experiments, neither the anti-IL-10R Ab nor
the anti-IL-10 MAb was found to appreciably affect the
ASM constrictor responsiveness to ACh in control se-
rum-exposed tissues (data not shown).

In further studies, during comparable levels of initial
sustained ACh-induced contractions in control and
atopic asthmatic serum-sensitized ASM segments,
averaging ~ 50% of Tmax, administration of the β-adre-
neceptor agonist isoproterenol produced cumulative
dose-dependent relaxation of the precontracted tis-
ues. As depicted in Fig. 2, relative to control serum-
exposed ASM, the Rmax responses and pD50 values to
isoproterenol were significantly attenuated in the cor-
responding atopic asthmatic serum-sensitized tissues.
Accordingly, the Rmax values in the atopic asthmatic
serum-sensitized and control ASM amounted to 41.5 ±
7.8 and 67.3 ± 6.4%, respectively (P < 0.01), and the

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exposed ASM, the Rmax responses and pD50 values to
isoproterenol were significantly attenuated in the cor-
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Accordingly, the Rmax values in the atopic asthmatic
serum-sensitized and control ASM amounted to 41.5 ±
7.8 and 67.3 ± 6.4%, respectively (P < 0.01), and the
corresponding pD$_{50}$ values for isoproterenol averaged 6.01 ± 0.05 and 6.50 ± 0.06 $\log$ M, respectively ($P < 0.01$). This impaired relaxation responsiveness to isoproterenol was largely prevented in atopic asthmatic serum-sensitized tissues that were pretreated with an anti-IL-10R Ab (Fig. 2), whereas pretreatment with the isotype control anti-VCAM-1 MAb had no effect. The impaired relaxation responses to isoproterenol were also ablated in atopic asthmatic serum-sensitized tissues that were pretreated with anti-IL-10 MAb (data not shown). Moreover, in comparable experiments, neither pretreatment with anti-IL-10R Ab nor anti-IL-10 MAb was found to significantly affect the ASM relaxant responsiveness to isoproterenol in control serum-exposed tissues (data not shown).

**Induced mRNA expression of IL-10 and IL-5 in atopic asthmatic-sensitized ASM.** Grunstein et al. (6) and Hakonarson et al. (11) previously reported that exposure of naive ASM to atopic asthmatic serum induces upregulated mRNA expression and release of the Th2-type cytokine IL-5 by the atopic asthmatic serum-sensitized tissue. In light of this earlier evidence, together with the above results implying a role for IL-10 in mediating the observed changes in agonist responsiveness in atopic asthmatic serum-sensitized state (6, 11), given the above present observations, a series of studies was conducted to further elucidate the role of IL-10 in regulating ASM responsiveness and investigate whether its action is mechanistically associated with the previously reported contribution of IL-5. In addressing this issue, initial experiments examined the effects of the administration of exogenous IL-10 to naive ASM tissues on their agonist constrictor and relaxant responsiveness in both the absence and presence of pretreatment of the tissues with an IL-5R a-chain blocking Ab. As shown in Fig. 4, exposure of tissues for 24 h to a maximally effective concentration of IL-10 (25 ng/ml) elicited significantly increased ASM constriction responsiveness to ACh, where the $T_{\text{max}}$ values in the IL-10-treated tissues averaged 141.1 ± 12.6 g/g ASM compared with the mean $T_{\text{max}}$ value of 116.9 ± 11.4 g/g ASM obtained in control (vehicle-treated) ASM ($P < 0.05$). Moreover, as further demonstrated in Fig. 4, the heightened constrictor responses to ACh were completely abrogated in IL-10-exposed ASM that was pretreated with anti-IL-5R Ab (10 g/ml).
Comparably, relative to the respective control tissue, ASM treated with IL-10 also exhibited significantly attenuated relaxation responsiveness to isoproterenol (Fig. 5), with $R_{\text{max}}$ values amounting to 54.9 ± 5.0 and 65.1 ± 4.1% in the IL-10-treated and control ASM, respectively ($P < 0.05$). Furthermore, these impaired relaxation responses to isoproterenol were also completely inhibited in IL-10-exposed ASM that was concomitantly treated with an anti-IL-5R Ab (Fig. 5).

**Effect of IL-10 on IL-5 mRNA and protein expression.** In view of the above results, we next examined whether the action of exogenously administered IL-10 is mechanistically associated with an induced altered endogenous expression of IL-5 by the IL-10-exposed ASM. In these studies, cultured human ASM cells were exposed to vehicle alone or to IL-10 for various durations up to 24 h. Thereafter, in separate experiments, the cells were harvested for analysis of temporal changes in IL-5 mRNA expression, and the cell culture medium was extracted for measurement of IL-5 protein release by immunoassay (see MATERIALS AND METHODS). IL-5 mRNA was essentially undetectable in control (vehicle-exposed) ASM cells. In contrast, as depicted in Fig. 6, relative to the unaltered constitutively expressed RPL7 mRNA signal, IL-5 mRNA expression was progressively enhanced in the IL-10-treated cells at all times for up to 24 h after IL-10 exposure. Comparably, the IL-10-treated ASM cells also exhibited an enhanced elaboration of IL-5 protein into the cell culture medium, with increased levels of IL-5 protein detected as early as 3 h after IL-10 exposure and maximal levels attained at 12 h (Fig. 7).

**DISCUSSION**

Although generally recognized for its anti-inflammatory properties, the role of IL-10 in bronchial asthma is controversial. In this regard, several studies (1, 2) have reported that cellular expression of IL-10 is reduced in allergic and nonatopic asthmatic individuals, whereas other reports have demonstrated increased levels of IL-10 expression in the lungs of asthmatic subjects (17, 22, 24) as well as in the skin and peripheral blood cells of allergic individuals after allergen challenge (22). Although these apparently disparate findings regarding the expression of IL-10 in asthma remain to be explained, emerging evidence based on in vivo studies with animal models of allergic asthma suggests that in contrast to other Th2-type cytokines that are proinflammatory in nature, IL-10 may regulate the induction of airway constrictor hyperresponsiveness independent of pulmonary inflammation (18, 25). This recent evidence raises the speculation that, notwith-
sented in ASM responsiveness that characterize the atopic asthmatic phenotype, including heightened agonist-mediated constrictor responsiveness and impaired β-adrenoceptor-mediated ASM relaxation (Figs. 1 and 2). In evaluating the collection of present findings, certain issues are worthy of consideration. Among these, it is relevant to note that IL-10 mRNA expression in ASM cells was essentially undetectable under control conditions but was distinctly increased as early as 3 h after incubation of the cells with atopic asthmatic serum (Fig. 3). Of interest, this temporal pattern of IL-10 mRNA induction closely paralleled the time course of induction of mRNA expression of the Th2-type cytokine IL-5 by the atopic asthmatic serum-sensitized ASM cells (Fig. 3). To the extent that, under the same experimental conditions described here, the induced mRNA expression and associated release of IL-5 protein by atopic sensitized ASM were previously shown to elicit the same observed changes in ASM responsiveness as in the atopic asthmatic serum-sensitized state (6, 13), our present results suggested that the temporal association between the induced changes in IL-10 and IL-5 expression may be mechanistically related. In addressing this possibility, our extended observations demonstrated that 1) exogenous administration of IL-10 to naive ASM cells induced their up-regulated expression of IL-5 mRNA (Fig. 6) and release of IL-5 protein (Fig. 7) and 2) comparable to atopic asthmatic serum, exogenous IL-10 administration to naive ASM tissue elicited proasthmatic-like changes in ASM constrictor and relaxant responsiveness that were prevented by pretreating the tissues with an IL-5R blocking Ab (Figs. 4 and 5). Taken together, these results support the concept of a causal association between induced upregulated IL-10 and IL-5 expression in the atopic sensitized state, where the release of IL-5 is mechanistically dependent on the autocrine induction and action of IL-10 in the sensitized ASM.

In a recent in vivo study (25), intratracheal instillation of recombinant murine IL-10 to antigen-challenged mice was found to produce increased bronchoalveolar lavage (BAL) fluid levels of IL-10, whereas BAL fluid levels of IL-5 as well as of IL-4 and interferon-γ were found to be reduced. In contrast, in another in vivo study with antigen-sensitized mice, similarly elevated BAL fluid levels of IL-5 and IL-4 were detected in both wild-type and IL-10-deficient mice after allergen challenge, and reconstitution of the IL-10 gene in the IL-10-deficient mice failed to induce any changes in the BAL fluid levels of these cytokines (18). Moreover, in other recent investigations with IL-10 gene knockout murine models of allergic asthma, one study (14a) reported that BAL fluid levels of IL-4, IL-5, and IL-13 were upregulated in the lungs of the IL-10 gene knockout mice, whereas another study (27) reported that IL-5 production and lung eosinophilic infiltration were attenuated in the IL-10-deficient mice. Although the apparent discrepancy between the findings of some of these in vivo studies is not readily explained, given our present in vitro observations, the general consideration is raised that determination of BAL fluid levels of certain cytokines may not be reflective of potential autocrine-induced changes in the expression of such cytokines in the microenvironment of the ASM itself. Furthermore, in this context, the possibility also exists that IL-10 exerts its cytokine-modulatory effects in a cell type-specific manner, with potential immunosuppressive actions occurring in certain cells such as peripheral blood mononuclear cells (23) and, as demonstrated herein, cytokine-stimulatory actions manifested in other cell types (e.g., ASM).

The central findings of this study provide an extended scope to the prevailing concept of a Th2 cytokine-dependent overall mechanism underlying the pathobiology of allergic asthma. In this regard, although the contemporary Th2 paradigm related to allergic asthma largely reflects the role played by CD4+ T cells expressing the Th2 phenotype of cytokine release, the present findings expand this model to include an apparent Th2-type autocrine role intrinsically
expressed by the ASM itself in the atopic asthmatic serum-sensitized state. The ability of atopic asthmatic serum-sensitized ASM to autologously express both Th1- and Th2-type cytokines as well as the pleiotropic proinflammatory cytokine IL-1β has been previously demonstrated (6, 9, 11, 12); and this phenomenon was largely attributed to activation of the low-affinity receptor for IgE, FcεRII (CD23), expressed on the ASM cell, by the elevated IgE present in the atopic sensitizing serum (7, 8). In light of this previous information, together with the observations presented in the present study, there is ample evidence to support the notion that, notwithstanding the crucial role played by CD4+ Th2 lymphocytes, an extended autocrine Th2-type cytokine network involving IL-10-mediated release and action of IL-5 also exists in ASM that, when activated in the atopic asthmatic serum-sensitized state, contributes to the proasthmatic changes in ASM responsiveness.

In conclusion, the present study investigated the role and mechanism of action of IL-10 in regulating the altered agonist responsiveness of atopic asthmatic serum-sensitized ASM. The results demonstrate that 1) the induced proasthmatic-like changes in agonist constrictor and relaxant responsiveness in atopic asthmatic serum-sensitized ASM are prevented by blocking the IL-10R in the sensitized ASM; 2) both IL-10 and IL-5 mRNA expression are upregulated in atopic asthmatic serum-sensitized ASM; 3) IL-10 elicits up-regulated IL-5 mRNA expression and release of IL-5 protein from ASM cells; and 4) the latter IL-10-induced autocrine release of IL-5 is responsible for IL-10-mediated proasthmatic changes in ASM responsiveness. Collectively, these findings lend extended support to the concept that, apart from the important role played by inflammatory cells, the ASM itself constitutes a Th2-type cytokine autocrine system that when activated in the atopic asthmatic serum-sensitized state, elicits autologous proasthmatic perturbations in airway responsiveness.

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