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 constrictor 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-type 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 (i.e., <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.03C force transducer from which isometric tension was measured (in mN) for digestion of protein in the initial RNA pellet. The organ bath temperature was held at 37°C. Passive resting tension of each ASM segment was set at a tension of 8 g to optimize its resting length for contraction. 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 (Tₘₕₜ) of the tissues to the agonist. The subsequent relaxation responses to isoprep-renol were analyzed in terms of percent maximal relaxation (Rₘₕₜ) from the initial level of active cholinergic contraction, and sensitivity to the relaxing agent was determined as the corresponding pD₅₀ value (i.e., geometric mean ED₅₀ value) associated with 50% of Rₘₕₜ.

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 mRNA 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)₁₂–₁₅ 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'-TATTTGTCCTGGCTGTGCCT-3' and 3'-primer 5'-TTTGGCTACAAAGGAGCC-3' (215-bp product) for IL-5, 5'-primer 5'-TATTTTGGTCTGGCTTGTC-3' and 3'-primer 5'-CTTTCTTGGGCCTCATATTCTC-3' (215-bp product) for IL-5, and 5'-primer 5'-AAGAGGCTCTATTCTTCTTGGTGTG-3' and 3'-primer 5'-TCCGGTCTCCTCCCATATTTGTACC-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 1.5 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 PCRs 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
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subsequently transferred to a Zeta-probe membrane overnight in 0.4 N NaOH. After capillary transfer, the DNA was immobilized by ultraviolet cross-linking with a Stratalinker UV Crosslinker 2400 at 120,000 μl/cm² (Stratagene). Prehybridization 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-labeled 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 immunoassay 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 Ab, and the anti-mouse secondary Ab used in the protein assay studies were purchased from R&D Systems (Minneapolis, MN). ACh and isoproterenol were purchased from Sigma (St. Louis, MO). All drug concentrations are expressed as final bath concentrations. Isoproterenol and ACh were made fresh for each experiment and were dissolved in normal saline to prepare 10−3 M stock solutions.

Statistical analysis. Unless otherwise indicated, the results are expressed as means ± SE. Statistical analysis was performed with two-tailed Student’s t-test or ANOVA with multiple comparison of means where appropriate. P values < 0.05 were considered significant.

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 nonasthmatic (control) subjects in both the absence and presence of an anti-IL-10 blocking Ab, an anti-IL-10 protein neutralizing MAb or an anti-VCAM-1 MAb. As shown in Fig. 1, relative to control serum-exposed tissues, the constrictor responses to exogenously administered 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), representing an average increase in Tmax of ~ 20% above the control value in the atopic asthmatic serum-sensitized ASM. These increased constrictor responses to ACh were ablatated in atopic asthmatic serum-sensitized tissues 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 experiments, 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 responsiveness to ACh in control serum-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-adrenoceptor agonist isoproterenol produced cumulative dose-dependent relaxation of the precontracted tissues. As depicted in Fig. 2, relative to control serum-exposed ASM, the Rmax responses and pD50 values to isoproterenol were significantly attenuated in the corresponding 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
Role of IL-5 in mediating IL-10-induced changes in ASM responsiveness. Because, under the same experimental conditions described here, an induced autocrine release and action of IL-5 were previously implicated in mediating the observed changes in ASM responsiveness in the 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 α-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 constrictor responsiveness to ACh, where the T_{max} values in the IL-10-treated tissues averaged 141.1 ± 12.6 g/g ASM compared with the mean T_{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).

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 ASM, we next examined whether cultured human ASM cells endogenously express mRNA for IL-10 and whether expression of the latter is modulated in the atopic asthmatic serum-sensitized state in temporal association with the induced changes in IL-5 mRNA expression. For the mRNA analyses, Southern blots were prepared and probed with human cDNA probes specific for the human IL-10 and IL-5 genes, and a 157-bp RPL7 probe was also prepared as a control for gel loading (see MATERIALS AND METHODS). As shown by a representative experiment in Fig. 3, the mRNA signals for IL-10 and IL-5 were only faintly detected relative to the unaltered constitutively expressed RPL7 signal, at all times after exposure of the ASM cells to control serum. In contrast, cells exposed to atopic asthmatic serum displayed notably increased expression of both IL-10 and IL-5 mRNAs at all times for up to 24 h after exposure to the atopic asthmatic serum-sensitizing serum. Qualitatively, the temporal changes in IL-10 and IL-5 mRNA expression in the atopic asthmatic serum-sensitized cells appeared generally similar, with distinctly increased expression of these mRNAs detected as early as 3 h after exposure of the cells to the sensitizing serum.

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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-10 R 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-10 R 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).

### Fig. 2. Comparison of relaxation dose-response relationships to isoproterenol in paired control serum-incubated and AS-sensitized ASM tissue segments in the absence and presence of anti-IL-10 R Ab. Values are means ± SE from 6 paired experiments. Relative to control serum-incubated ASM, the attenuated relaxation responses to isoproterenol were prevented by cotreatment of the AS-sensitized tissues with anti-IL-10 R Ab.
Comparably, relative to the respective control tissue, ASM treated with IL-10 also exhibited significantly attenuated relaxation responsiveness to isoproterenol (Fig. 5), with R_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 after exposure of cells to IL-10.

**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, notwth-
responsiveness as in the atopic asthmatic serum-sen-
senitized 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 adminis-
tration 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 associa-
tion between induced upregulated IL-10 and IL-5 ex-
pression in the atopic sensitized state, where the re-
lease of IL-5 is mechanistically dependent on the
autocrine induction and action of IL-10 in the sensi-
tized ASM.

In a recent in vivo study (25), intratracheal instilla-
tion of recombinant murine IL-10 to antigen-chal-
lenge mice was found to produce increased bronchoal-
veolar 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 ele-
vated BAL fluid levels of IL-5 and IL-4 were detected
in both wild-type and IL-10-deficient mice after aller-
gen 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 knock-
out 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 consider-
ation 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.
Furthemore, in this context, the possibility also exists
that IL-10 exerts its cytokine-modulatory effects in a
cell type-specific manner, with potential immunosup-
pressive actions occurring in certain cells such as periph-
eral 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 ex-
tended scope to the prevailing concept of a Th2 cyto-
kine-dependent overall mechanism underlying the
pathobiology of allergic asthma. In this regard, al-
though the contemporary Th2 paradigm related to al-
lergic asthma largely reflects the role played by CD4+
T cells expressing the Th2 phenotype of cytokine re-
lease, the present findings expand this model to in-
clude an apparent Th2-type autocrine role intrinsically

Fig. 7. Comparison of the release of IL-5 protein into the culture
medium of human ASM cells after exposure to vehicle alone (control)
and IL-10. In contrast to control cells where IL-5 protein release was
undetectable, ASM cells exposed to IL-10 showed induced release of
IL-5, with maximal levels attained 12 h after exposure to IL-10.

standing its reported anti-inflammatory properties,
IL-10 may play an important role in allergic asthma by
acting directly on the sensitized ASM itself. The
present study addressed this possibility, and the re-
results provide new evidence demonstrating that the
altered agonist responsiveness of atopic asthmatic
seum-sensitized ASM is largely attributed to activation
of an endogenously expressed autocrine mechanism
that involves the autologous induction of IL-10 medi-
at releases and the action of IL-5 in the sensitized
ASM itself.

To our knowledge, the present observations are the
first to demonstrate that IL-10 exerts significant ef-
fects on airway responsiveness by acting directly on the
ASM itself and, accordingly, contributes to the changes
in ASM responsiveness that characterize the atopic
asthmatic phenotype, including heightened agonist-
mated 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 expres-
sion 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 asth-
matic 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, un-
der 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 asthmatic serum-sen-
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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