IL-13-dependent autocrine signaling mediates altered responsiveness of IgE-sensitized airway smooth muscle

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Received 27 August 2001; accepted in final form 12 October 2001

Grunstein, M. M., H. Hakonarson, J. Leiter, M. Chen, R. Whelan, J. S. Grunstein, and S. Chuang. IL-13-dependent autocrine signaling mediates altered responsiveness of IgE-sensitized airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 282: L520–L528, 2002; 10.1152/ajplung.00343.2001.—In testing the hypothesis that interleukin-4 receptor α-subunit (IL-4Rα)-coupled signaling mediates altered airway smooth muscle (ASM) responsiveness in the atopic sensitized state, isolated rabbit tracheal ASM segments were passively sensitized with immunoglobulin E (IgE) immune complexes, both in the absence and presence of an IL-4Rα blocking antibody (anti-IL-4RαAb). Relative to control ASM, IgE-sensitized tissues exhibited enhanced isometric constrictor responses to administered ACh and attenuated relaxation responses to isoproterenol. These proasthmatic-like effects were prevented in IgE-sensitized ASM that were pretreated with anti-IL-4RαAb. In complementary experiments, IgE-sensitized cultured human ASM cells exhibited upregulated expression of IL-13 mRNA and protein, whereas IL-4 expression was undetected. Moreover, extended studies demonstrated that 1) exogenous IL-13 administration to naive ASM elicited augmented contractility to ACh and impaired relaxation to isoproterenol, 2) these effects of IL-13 were prevented by pretreating the tissues with an IL-5 receptor blocking antibody, and 3) IL-13 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 IgE-sensitized ASM is largely attributed to activation of an intrinsic Th2-type autocrine mechanism involving IL-13/IL-4Rα-coupled release and action of IL-5 in the sensitized ASM itself.

Th2 cytokines; immunoglobulin E; atopy; asthma; interleukin-13

THE AIRWAYS IN ALLERGIC ASTHMA are characterized by inflammation, mucus hypersecretion, obstruction, and constrictor hyperresponsiveness to spasmogenic stimuli. Although the mechanistic interplay between inflammation and the associated altered airway responsiveness remains to be elucidated, there exists ample evidence implicating the production of CD4+ Th2 lymphocyte-derived cytokines, including interleukin (IL)-4, IL-5, IL-10, and IL-13, in orchestrating the allergic pulmonary response and its associated changes in airway responsiveness. In this connection, IL-5 has been shown to regulate the growth, differentiation, and activation of eosinophils (2, 17, 29), whereas IL-4 and IL-13 were found to play both overlapping and independent roles in regulating IgE isotype switching in B cells and differentiation of naïve CD4+ T cells into the Th2 phenotype (1, 13, 23, 27). The overlapping biological functions of IL-4 and IL-13 are likely attributed to their commonly shared IL-4 receptor α-chain (IL-4Rα), which, when activated, dimerizes with other cytokine receptor moieties, subsequently leading to the activation of various signaling molecules, including the Th2 differentiating factor, STAT6 (1, 20, 27, 30).

When one considers the etiology of the characteristic changes in airway responsiveness in the atopic asthmatic state, it is of interest to note that, in light of emerging new evidence, the above paradigm involving proinflammatory Th2-dependent mechanisms has recently been somewhat redefined. In this regard, recent studies conducted in animal models of allergic asthma have demonstrated that phenotypic expression of airway constrictor hyperresponsiveness may be manifested independently of pulmonary inflammation (12, 18, 25). Moreover, recent reports have determined that under specific conditions, including atopic sensitization (6–9) and rhinovirus exposure (4, 11), the airway smooth muscle (ASM) itself has the capacity to autologously elicit proinflammatory-like changes in its constrictor and relaxant responsiveness secondary to the induced release and autocrine actions of various proinflammatory cytokines, including certain Th1- and Th2-type cytokines (4, 9, 10). In this context, in ultimately leading to altered ASM responsiveness under conditions of atopic sensitization, immunoglobulin E (IgE)-dependent autologous release of cytokines by ASM was found to display a temporal pattern of sequential autocrine action, as shown by an initial IL-5-mediated induction of the subsequent release of IL-1β in the atopic sensitized ASM (9). Given this evidence,
together with that establishing that IL-4Rα-dependent signaling is fundamentally important in eliciting the Th2 phenotype of cytokine expression (1, 13, 20, 23, 27, 30), the present study tested the hypothesis that atopic-dependent (i.e., IgE-mediated) changes in ASM responsiveness are attributed to IL-4Rα-coupled activation of an intrinsic Th2 mechanism in ASM. The results provide new evidence demonstrating that IgE sensitization of ASM activates an endogenously expressed Th2-type autocrine mechanism that involves induced upregulated expression of IL-13 and that the latter results in IL-4Rα-mediated release and action of IL-5 in the sensitized ASM to produce proinflammatory-like changes in ASM responsiveness.

MATERIALS AND METHODS

Animals. Twenty-one 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. The animals had no signs of respiratory disease for several weeks before the study.

Preparation and IgE sensitization of rabbit ASM tissue. After general anesthesia with xylazine (10 mg/kg) and ketamine (50 mg/kg), rabbits were killed with an overdose of pentobarbital sodium (130 mg/kg). As described previously (7, 10), the tracheae were removed via open thoracotomy, the loose connective tissue and epithelium were scraped and removed, and the tracheae were divided into eight ring segments of 6–8 mm in length. Each alternate ring was incubated for 24 h at room temperature in either vehicle alone (control) or IgE immune complexes, comprising 15 μg/ml human IgE and 5 μg/ml anti-IgE (goat polyclonal IgG), as previously described by our laboratory (5). In parallel experiments, 1 h before incubation in control or IgE-containing medium, ASM segments were treated with either an IgG2A-type anti-IL-4 receptor blocking antibody (anti-IL-4Rα) or an IgG2A-isotype control Ab (cAb). All the tissues studied were aerated with a continuous supplemental O2 mixture (5% CO2 in oxygen, a pH of 7.35–7.40, 1.46 MgSO4) and maintained, and the organ bath temperature was held at 37°C. Following primer sets: IL-4: 5′-GAGGATGCTTCTG-3′, 3′-primer: 5′-AACGTACTCTGTGTTGCTTA-3′, product is 321 bp; IL-13: 5′-TTTACAACTGGGCCACCTC-3′, 3′-primer: 5′-GACGTCATACCTCATTACAGG-3′, product is 419 bp. The cycling protocol used included human-5′-actin gene. cDNA was synthesized from total RNA isolated from ASM cells incubated for 0, 3, 6, 12, and 24 h in control or IgE-containing medium or exposed to IL-5 in the absence and presence of anti-IL-4Rα Ab. The cDNA was primed with oligo(dT)12–18, and extended with Superscript II RT (Gibco BRL). The PCR was used to amplify the specific products from each cDNA reaction, based on the published sequences of the human IL-4, IL-13, IL-5, and β-actin genes, and including the following primer sets: IL-4: 5′-primer: 5′-GTGCGGATATCCCTTACAGG-3′, 3′-primer: 5′-AACGTACTCTGTGTTGCTTA-3′, product is 321 bp; IL-13: 5′-primer: 5′-TGAGGAGTCTGTTGCAAACTCA-3′, 3′-primer: 5′-TTTACAACTGGGCCACCTC-3′, product is 419 bp; IL-5: 5′-primer: 5′-GACGTCATACCTCATTACAGG-3′, product is 419 bp. The cycling profile used was as follows: denaturation: 95°C for 1 min; annealing: 52–55°C for 1 min; and extension: 72°C for 1 min, with 35, 30, 25, and 25 cycles for the IL-4, IL-13, IL-5, and β-actin genes.
respectively. The number of cycles was determined to be in the linear range of the PCR products. The PCR reactions for the primers were performed using equivalent amounts of cDNA prepared from 2.5 μg of total RNA. Equal aliquots of each PCR reaction 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 immobilized by ultraviolet cross-linking using a Stratallinker UV Crosslinker 2400 at 120,000 μM/cm² (Stratagene). Prehybridization in a Techne hybridization oven was conducted for 2–3 h at 42°C in 50% formamide, 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-4, IL-13, IL-5, and β-actin DNA levels were assayed by Southern blot analysis using 32P-labeled probes, prepared by pooling several RT-PCR reactions for the individual PCR fragments and purifying them from a 1.2% agarose gel using the Qiagen II agarose gel extraction kit. The individual PCR products were subsequently sequenced for confirmation. 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 × 1 min at 50°C in 0.1× SSC, 0.1% SDS.

Determination of IL-13 and IL-5 intracellular proteins in ASM cells by flow cytometry. Intracellular protein expression of IL-13 and IL-5 was examined in the cultured human ASM cells with a Coulter EPICS Elite flow cytometer (Coulter EPICS Division, Hialeah, FL) equipped with a 5-W argon laser operated at 488 nM and 300-mW output. Fluorescence signals were accumulated as two-parameter fluorescence histograms, with both percent positive cells and mean channel fluorescence intensity (MFI) being recorded. Cells treated for 24 h with control or IgE-containing medium were carefully washed, scraped from the culture flasks, and then resuspended in PBS buffer. The cells were then dispensed by pipetting through a 23-gauge needle and orbital shaking, and subsequently fixed and permeabilized using reagents provided in a commercially available cell fixation/permeabilization kit (PharmMingen, San Diego, CA). The cells were then stained with mouse anti-human monoclonal antibodies to IL-13 and IL-5. To examine for nonspecific binding, the primary antibody was replaced by immunoglobulins of the same isotype following the manufacturer’s protocol, with mouse IgG1 serving as a negative control. After serial washing, the cells were stained with FITC-conjugated goat antimouse secondary antibody. The antibody-stained cells were then evaluated by flow cytometry and analyzed with the Elite Immuno 4 statistical software (Coulter EPICS Division). Fluorescence intensities are expressed as percent positive cells as well as MFI.

ELISA measurement of IL-5 protein release. IL-5 protein levels were also assayed in the culture media of ASM cells that were exposed for varying durations up to 24 h to vehicle alone, exogenous IL-13 (20 ng/ml), or IgE immune complexes. The IL-5 protein levels were quantitatively assessed using an enzyme-specific immunoassay, as previously described (9). The latter assay was performed using a double-antibody sandwich strategy in which an ACh esterase, Fab-conjugated IL-5-specific secondary antibody, is targeted to a first IL-5-captured antibody. The enzymatic activity of the ACh was measured spectrophotometrically, and, relative to a linear standard curve, the results were used to quantify the amount of the targeted IL-5 present in the culture media.

Statistical analysis. Unless otherwise indicated, the results are expressed as mean ± SE values. Statistical analysis of the ASM constrictor and relaxation dose-response relationships was performed using ANOVA with multiple comparisons of means, and analysis of the flow cytometric and ELISA data was conducted using the two-tailed Student’s t-test. P values < 0.05 were considered significant.

Reagents. The human ASM cells and SMBM were obtained from Clonetics (San Diego, CA). The IL-4, IL-13, IL-5, and β-actin primers were obtained from Integrated DNA Technologies (Coralville, IA). The IL-13 and IL-5 intracellular staining antibodies used in the flow cytometric studies were purchased from BioSource International (Camarillo, CA). The anti-IL-4Ra antibody, the IL-5 ELISA kit, and the mouse anti-human IL-5 primary antibody and the anti-mouse secondary antibody 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 both concentrations. Isoproterenol and ACh were freshly made for each experiment and were dissolved in normal saline to prepare 10–3 M stock solutions.

RESULTS

Role of IL-4Ra in regulating agonist responsiveness in IgE-sensitized ASM. To determine the role of IL-4Ra activation in regulating ASM responsiveness in the IgE-sensitized state, agonist-mediated constrictor and relaxation responses were compared in paired isolated rabbit ASM segments 24 h after exposure to IgE immune complexes (6), both in the absence and presence of an anti-IL-4RaAb or an isotype cAb. In agreement with our earlier findings (6), relative to control (vehicle exposed) tissues, the constrictor responses to exogenously administered ACh were significantly increased in IgE-treated ASM (Fig. 1). Accordingly, the mean ± SE Tmax values amounted to 97.8 ± 7.6 and 122.9 ± 9.0 g/g ASM wt in the control and IgE-sensitized tissues, respectively (P < 0.01). These increased constrictor

Fig. 1. Comparison of constrictor dose-response relationships to ACh in paired control (○) and immunoglobulin E (IgE)-sensitized airway smooth muscle (ASM) tissue segments in the absence (●) and presence of interleukin-4 receptor α-subunit blocking antibody (anti-IL-4RaAb, □) or an isotype control Ab (cAb, ■). Note: relative to control ASM, the heightened constrictor responses to ACh were largely ablated by cotreatment of the IgE-sensitized tissues with anti-IL-4RaAb, whereas cotreatment with cAb had no effect. Data represent mean ± SE values from 6 paired experiments.

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We previously reported that ASM cells and IL-13 appreciably affect the ASM relaxant responsiveness to isoproterenol (10). In light of this earlier evidence, together with the above results implicating a role for IL-4Rα in mediating the observed changes in agonist responsiveness in IgE-sensitized ASM, we next examined whether cultured human ASM cells express mRNAs for IL-4 and IL-13, the endogenous ligands for IL-4Rα, and whether mRNA expression of the latter cytokines is modulated in the IgE-sensitized state. For the mRNA analyses, Southern blots were prepared and probed with human cDNA probes specific for the human IL-4 and IL-13 genes, and a 419-bp β-actin probe was also prepared as a control for gel loading (see MATERIALS AND METHODS). There was no detectable IL-4 mRNA signal and an absent or only faintly detected mRNA signal for IL-13 in control (untreated) cells. Moreover, as shown by a representative experiment in Fig. 3, IL-4 mRNA expression was also undetected in IgE-sensitized cells. In contrast, relative to the unaltered constitutively expressed β-actin signal, IL-13 mRNA expression was progressively enhanced at all times for up to 24 h after exposure of the cells to IgE. Qualitatively, the temporal pattern of upregulated IL-13 mRNA expression in the IgE-treated cells appeared similar in three separate experiments, with distinctly increased IL-13 mRNA detected as early as 3 h after exposure of the cells to IgE and a somewhat reduced intensity of the mRNA signal detected at 24 h (i.e., as per Fig. 3). For all three experiments, the average maximal intensity of the IL-13 mRNA signals detected at 12 h, each expressed as a ratio of the respective intensity of the β-actin signal, amounted to 70.7 ± 9.1-fold above the corresponding mean intensity ratio detected at the 0-h time point.

In extending the above observations, we next examined whether human ASM cells express IL-4 and IL-13 proteins and also assessed whether expression of these cytokines is modulated in cells exposed for 24 h to IgE. Using flow cytometric analysis for intracellular detection of IL-4Rα membrane protein expression was upregulated after exposure of the cells to high IgE-containing atopic asthmatic serum (10). In light of this earlier evidence, together with the above results implicating a role for IL-4Rα in mediating the observed changes in agonist responsiveness in IgE-sensitized ASM, we next examined whether cultured human ASM cells express mRNAs for IL-4 and IL-13, the endogenous ligands for IL-4Rα, and whether mRNA expression of the latter cytokines is modulated in the IgE-sensitized state. For the mRNA analyses, Southern blots were prepared and probed with human cDNA probes specific for the human IL-4 and IL-13 genes, and a 419-bp β-actin probe was also prepared as a control for gel loading (see MATERIALS AND METHODS). There was no detectable IL-4 mRNA signal and an absent or only faintly detected mRNA signal for IL-13 in control (untreated) cells. Moreover, as shown by a representative experiment in Fig. 3, IL-4 mRNA expression was also undetected in IgE-sensitized cells. In contrast, relative to the unaltered constitutively expressed β-actin signal, IL-13 mRNA expression was progressively enhanced at all times for up to 24 h after exposure of the cells to IgE. Qualitatively, the temporal pattern of upregulated IL-13 mRNA expression in the IgE-treated cells appeared similar in three separate experiments, with distinctly increased IL-13 mRNA detected as early as 3 h after exposure of the cells to IgE and a somewhat reduced intensity of the mRNA signal detected at 24 h (i.e., as per Fig. 3). For all three experiments, the average maximal intensity of the IL-13 mRNA signals detected at 12 h, each expressed as a ratio of the respective intensity of the β-actin signal, amounted to 70.7 ± 9.1-fold above the corresponding mean intensity ratio detected at the 0-h time point.

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**Effects of IgE sensitization on ASM expression of IL-4 and IL-13.** We previously reported that ASM cells express IL-4Rα protein on their cell surface and that

**Fig. 3.** Southern blots depicting IL-4 and IL-13 mRNA expression in cultured human ASM cells after 0-, 3-, 6-, 12-, and 24-h exposure to IgE. Constitutive expression of β-actin mRNA was used to control for gel loading. RNA isolated from T lymphocytes was used as a positive control for detection of IL-4 cDNA. The blots were probed with human-specific IL-4, IL-13, and β-actin 32P-labeled cDNA probes (see MATERIALS AND METHODS). Note: relative to the undetectable IL-4 signal, the mRNA signal for IL-13 was progressively enhanced at the various times after exposure of cells to IgE, whereas the intensities of the mRNA signal for the constitutively expressed β-actin gene were essentially unaltered.

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**Fig. 2.** Comparison of relaxation dose-response relationships to isoproterenol in paired control (○) and IgE-sensitized ASM tissue segments in the absence (●) and presence of anti-IL-4Rα Ab (□) or cAb (■). Note: relative to control ASM, the attenuated relaxation responses to isoproterenol were prevented by cotreatment of the IgE-sensitized tissues with anti-IL-4Rα Ab (Fig. 1, filled squares). Moreover, in related experiments, neither anti-IL-4Rα Ab nor the isotype cAb had no effect (Fig. 1, filled squares). More generally, in related experiments, neither anti-IL-4Rα Ab nor the isotype cAb had no effect (Fig. 1, filled squares). More generally, in related experiments, neither anti-IL-4Rα Ab nor the isotype cAb had no effect (Fig. 1, filled squares).
tion of IL-4 and IL-13, we found no evidence for expression of IL-4 protein in either control (vehicle exposed) or IgE-treated cells. In contrast, as exemplified in Fig. 4A, relatively low levels of intracellular IL-13 protein were detected under control conditions, and the expression of IL-13 was markedly upregulated in ASM cells exposed to IgE, whereas corresponding nonspecific background staining using FITC-conjugated isotype cAb remained unaltered (Fig. 4B). Based on the results from four experiments, control cells displayed mean ± SE values for percent positive staining and MFI for IL-13 of 0.41 ± 0.09 and 6.7 ± 3.1%, respectively, compared with the corresponding increased values of 58.3 ± 7.9% (P < 0.001) and 22.6 ± 6.4% (P < 0.01), respectively, obtained in IgE-treated cells.

Role of IL-5 in mediating IL-13-induced changes in ASM responsiveness. Since, under experimental conditions comparable to those described herein, induced autocrine release and action of IL-5 were previously implicated in mediating similar observed changes in agonist responsiveness in atopic sensitized ASM (9), given the above present observations, a series of studies was conducted to further elucidate the role of IL-13 in regulating ASM responsiveness and investigate whether its action is mechanistically coupled to the previously reported contribution of IL-5. In addressing these issues, in our initial experiments we examined the effects of exogenous administration of human recombinant IL-13 to naive ASM tissues on their agonist constrictor and relaxant responsiveness, both in the absence and presence of pretreatment of the tissues with an anti-IL-5R-Ab. As shown in Fig. 5, exposure of tissues for 24 h to a maximally effective concentration of IL-13 (20 ng/ml) elicited significantly increased ASM constrictor responsiveness to ACh, wherein the T\text{max} values in the IL-13-treated averaged 129.7 ± 8.6 g/g ASM, compared with the mean T\text{max} value of 115.7 ± 8.7 g/g ASM obtained in control (vehicle treated) ASM (P < 0.05). Moreover, as demonstrated in Fig. 5, the heightened constrictor responses to ACh were completely abrogated in IL-13-treated tissues that were pretreated with anti-IL-5R-Ab (10 μg/ml), whereas an isotype cAb had no effect. Comparably, relative to their respective controls, ASM treated with IL-13 also exhibited significantly attenuated relaxation responses to isoproterenol (Fig. 6), with mean ± SE R\text{max} values amounting to 35.69 ± 4.97 vs. 53.70 ± 6.27% in the IL-13-treated vs. control ASM, respectively (P < 0.01). Furthermore, this impaired relaxation responsiveness to isoproterenol was also completely inhibited in IL-13-exposed ASM that were pretreated with anti-IL-5R-Ab (Fig. 6; open squares), whereas the isotype control Ab had no effect (Fig. 6; filled squares).

Effects of IL-13 on IL-5 mRNA and protein expression. Given the above observations, we next investigated whether the action of exogenously administered...
IL-13 is associated with an induced altered endogenous expression of IL-5 by the IL-13-exposed ASM. In these studies, cultured human ASM cells were exposed to IL-13, both in the absence and presence of IL-4R Ab, for varying durations up to 24 h. Thereafter, in one series of experiments, the cells were harvested for analysis of temporal changes in IL-5 mRNA expression. The IL-5 mRNA signal was only faintly detected in control (vehicle-exposed) ASM cells. In contrast, as depicted in Fig. 7, relative to the unaltered constitutively expressed β-actin mRNA signal, IL-5 mRNA expression was progressively enhanced in the IL-13-treated cells at all times for up to 24 h after IL-13 exposure. Moreover, as further shown in Fig. 7, the IL-13-induced upregulated expression of IL-5 mRNA was largely ablated in ASM cells that were concomitantly treated with IL-4R Ab.

In another series of experiments, the culture medium of ASM cells exposed to IL-13 was collected for measurements of IL-5 protein release by immunoassay (see MATERIALS AND METHODS). As shown in Fig. 8, relative to the low levels of expression of IL-5 protein from control cells, IL-13-exposed cells exhibited a progressively enhanced elaboration of IL-5 protein into the cell culture medium, with markedly increased (near maximal) levels of IL-5 detected as early as 6 h after IL-13 exposure and maximal levels attained at 24 h. For comparison, also shown in Fig. 8 are results obtained in ASM cells exposed to IgE. Relative to IL-13-exposed cells, IgE-treated cells released significantly less IL-5 protein at 6 h; however, similar levels of IL-5 concentration were attained by 24 h.

Finally, in light of our observed effects of IgE and IL-13 on IL-5 release, to further substantiate the above observations implicating IL-13/IL-5-coupled autocrine signaling in mediating IgE-induced changes in ASM responsiveness, we next examined the role of IL-4R activation in regulating IgE-induced expression of IL-5. In these experiments, using flow cytometry to detect intracellular IL-5 protein, we assayed the latter in cultured human ASM cells under control (vehicle exposed) conditions and, after exposure to IgE, in the absence and presence of pretreatment of the cells with anti-IL-4R Ab. In concert with the above results, relative to control cells, IgE-treated cells exhibited increased intracellular IL-5 protein expression, and this effect was inhibited in IgE-exposed cells that were pretreated with anti-IL-4R Ab (Fig. 9).
activated IL-4R/H9251 associated with binding of STAT6 protein to the activated constrictor responsiveness and impaired beta-adrenoceptor-mediated ASM relaxation (Figs. 1 and 2). In the evaluation of the collection of present findings, certain noteworthy considerations are raised. Among these, it is relevant to note that, despite the reported presence of IL-4Rα in ASM cells (10, 15) and its upregulated expression in the atopic sensitized state (10), we found no evidence for ASM cell expression of IL-4 mRNA or protein under control or IgE-sensitized conditions. In contrast, IL-13 mRNA expression was present and distinctly increased as early as 3 h after incubation of the cells with IgE (Fig. 3). Of interest, this temporal pattern of induced IL-13 mRNA expression closely paralleled the time course of induction of IL-5 mRNA by IL-13 administration (Fig. 7). To address the extent that, under experimental conditions comparable to those described herein, the induced mRNA expression and associated release of IL-5 protein by atopic asthmatic serum-sensitized ASM were previously shown to elicit the same observed changes in ASM responsiveness (9), our present results suggested that the temporal association between the induced changes in IL-13 and IL-5 expression may be mechanistically related. In addressing this possibility, our extended observations demonstrated that 1) exogenous administration of IL-13 induced both an increased expression of IL-5 mRNA (Fig. 7) and release of IL-5 protein (Fig. 8); and 2) comparable to the effect of IgE sensitization, exogenous IL-13 administration to naïve ASM tissues elicited proasthmatic-like changes in ASM constriction and relaxant responsiveness that were prevented by pretreating the tissues with an IL-5RαAb (Figs. 5 and 6). These findings, together with the observations that IgE sensitization induced the release of IL-5 protein and that this effect was inhibited in ASM cells pretreated with anti-IL-4RαAb (Figs. 8 and 9), support the concept of a causal association between induced IL-13 and IL-5 expression in the IgE-sensitized state. Accordingly, the results are consistent with the notion that IgE-induced IL-5 release by ASM is mechanistically dependent on the autocrine induction and action of IL-13 in the IgE-exposed ASM.

Our collection of findings is based on studies conducted using rabbit ASM tissues and cultured human ASM cells. Although the experiments using these different preparations provided results that were largely complementary in nature, the issue of potential species sensitization of ASM activates an endogenously expressed Th2-type autocrine mechanism that involves 1) IgE-induced upregulated expression of IL-13 by the sensitized ASM and 2) the latter cytokine acting in an autocrine fashion to mediate IL4Rα-coupled release and action of IL-5, which evokes proasthmatic-like changes in ASM responsiveness.

To our knowledge, the present observations are the first to demonstrate that IgE sensitization of ASM elicits the sequential autocrine release of IL-13 and IL-5 by the sensitized ASM itself and that this Th2-type autocrine response contributes to the changes in ASM responsiveness that characterize the atopic asthmatic phenotype, including heightened agonist-mediated constrictor responsiveness and impaired beta-adrenoceptor-mediated ASM relaxation (Figs. 1 and 2). In the evaluation of the collection of present findings, certain noteworthy considerations are raised. Among these, it is relevant to note that, despite the reported presence of IL-4Rα in ASM cells (10, 15) and its upregulated expression in the atopic sensitized state (10), we found no evidence for ASM cell expression of IL-4 mRNA or protein under control or IgE-sensitized conditions. In contrast, IL-13 mRNA expression was present and distinctly increased as early as 3 h after incubation of the cells with IgE (Fig. 3). Of interest, this temporal pattern of induced IL-13 mRNA expression closely paralleled the time course of induction of IL-5 mRNA by IL-13 administration (Fig. 7). To the extent that, under experimental conditions comparable to those described herein, the induced mRNA expression and associated release of IL-5 protein by atopic asthmatic serum-sensitized ASM were previously shown to elicit the same observed changes in ASM responsiveness (9), our present results suggested that the temporal association between the induced changes in IL-13 and IL-5 expression may be mechanistically related. In addressing this possibility, our extended observations demonstrated that 1) exogenous administration of IL-13 induced both an increased expression of IL-5 mRNA (Fig. 7) and release of IL-5 protein (Fig. 8); and 2) comparable to the effect of IgE sensitization, exogenous IL-13 administration to naïve ASM tissues elicited proasthmatic-like changes in ASM constriction and relaxant responsiveness that were prevented by pretreating the tissues with an IL-5RαAb (Figs. 5 and 6). These findings, together with the observations that IgE sensitization induced the release of IL-5 protein and that this effect was inhibited in ASM cells pretreated with anti-IL-4RαAb (Figs. 8 and 9), support the concept of a causal association between induced IL-13 and IL-5 expression in the IgE-sensitized state. Accordingly, the results are consistent with the notion that IgE-induced IL-5 release by ASM is mechanistically dependent on the autocrine induction and action of IL-13 in the IgE-exposed ASM.

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**DISCUSSION**

It is well established that IL-4R signaling is required for differentiation of naïve T lymphocytes into Th2 cytokine-producing cells. This phenomenon has been associated with binding of STAT6 protein to the activated IL-4Rα subunit, where it becomes tyrosyl phosphorylated, and migration of phosphorylated dimerized STAT6 to the nucleus where, together with other transcription factors, transcription of various IL-4R-inducible genes is activated (14, 16, 19, 20, 22, 30). Recently, IL-4Rα signaling has also been identified as a potent regulator of the characteristic airway constriction and hyperresponsiveness seen in murine models of allergic asthma (3, 24, 26, 28). This action of IL-4Rα activation was found to be largely mediated by IL-13 binding, as the airway constrictor hyperresponsiveness was prevented in IL-4Rα-deficient mice and in mice receiving a soluble IL-13α2-IgG Fc fusion protein to neutralize IL-13 (3, 28). Although these findings clearly emphasize a crucial role for IL-4Rα-mediated signaling in mediating the mechanistic action of IL-4R activation in regulating the induction of changes in airway responsiveness remains to be elucidated. In this context, it is relevant to note that IL-4Rα expression was recently identified in human ASM cells (10, 15) and that ASM cell expression of IL-4Rα was found to be upregulated in the atopic asthmatic sensitized state, in association with upregulated expression of other Th2-type cytokines, including IL-5 and granulocyte-monocyte colony-stimulating factor (10). In light of this evidence, the present study tested the hypothesis that atopic-dependent (i.e., IgE mediated) changes in ASM responsiveness are attributed to IL-4Rα-coupled activation of an intrinsic Th2 mechanism in ASM. The results demonstrate that IgE

![Graph](http://ajplung.physiology.org/)

**Fig. 9.** Comparison by flow cytometric analysis of intracellular IL-5 protein expression in representative control (untreated) and IgE-treated human ASM cells in the absence and presence of cotreatment with anti-IL-4RαAb. Cells were stained with mouse anti-human STAT6 protein expression, and this effect was prevented in IgE-treated cells that were concomitantly treated with anti-IL-4RαAb. In contrast, exposure of control cells to anti-IL-4RαAb had no effect on IL-5 protein expression (data not shown).
differences warrants consideration. In this regard, it is relevant to note that in earlier studies using atopic asthmatic serum sensitization of isolated rabbit ASM (4, 6, 7, 9), we found changes in ASM constrictor and relaxant responsiveness that, in general, were qualitatively similar to those reported in a number of other studies conducted on isolated human airways passively sensitized with atopic asthmatic serum or with exogenously administered IgE (see review Ref. 21). Moreover, we found that atopic asthmatic serum sensitization elicited qualitatively similar upregulated expression of the low affinity receptor for IgE, FcεRII (i.e., CD23), in both rabbit and human ASM cells (5, 6), as well as increased release of IL-1β protein from both cell types (4). Similarly, rhinovirus inoculation of rabbit and human ASM cells was also found to provoke the release of IL-1β from both cell types (4, 11). Finally, in concert with the present observations on IL-13-induced changes in agonist responsiveness in rabbit ASM tissues, Laporte et al. (15) recently reported a similar attenuated β-adrenergic responsiveness in cultured human ASM cells treated with IL-13. Thus the findings from these earlier reports, together with those of the present study, suggest that there exists a good concordance between rabbit and human ASM cells, at least with respect to the effects of atopic sensitization and IL-13 administration on ASM cell function. Although it remains to be established whether such an interspecies concordance also exists in vivo, it is noteworthy that, in agreement with the present in vitro observations, in vivo administration of an IL-4R antagonist in murine models of allergic asthma was shown to prevent the induction of changes in airway responsiveness (3, 24, 26, 28) and to inhibit allergen-induced release of certain Th2 cytokines (notably including IL-5) into the bronchoalveolar lavage fluid (24).

The central findings of this study lend an extended scope to the prevailing concept of a Th2 cytokine-dependent overall mechanism underlying the pathobiology of allergic asthma. In this regard, whereas 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 IgE (atopic)-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 (4, 9, 10), and this phenomenon was largely attributed to activation of FcεRII (CD23), expressed on the ASM cell surface, by the elevated IgE present in the atopic sensitizing serum (4, 6). 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-13/IL-4Rα-coupled induced release and action of IL-5 also exists in ASM that, when activated in the IgE-sensitized state, contributes to the proasthmatic changes in ASM responsiveness.

In conclusion, the present study investigated the role and mechanism of action of IL-4R signaling in regulating the altered agonist responsiveness of IgE-sensitized ASM. The results demonstrate that 1) the induced proasthmatic-like changes in agonist constrictor and relaxant responsiveness in IgE-sensitized ASM are prevented by blocking the IL-4αR in the sensitized ASM, 2) both IL-13 and IL-5 mRNA and protein expression are upregulated in IgE-sensitized ASM, 3) IL-13 elicits upregulated IL-5 mRNA expression and release of IL-5 protein from ASM cells, and 4) the latter IL-13/IL-4Rα-coupled induced autocrine release of IL-5 is responsible for mediating 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-sensitized state, elicits autologous proasthmatic perturbations in airway responsiveness.

The authors thank M. Brown for typing the manuscript. This work was supported in part by National Heart, Lung and Blood Institute Grants HL-31467, HL-58245, HL-61038, and HL-59906.

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