Autocrine cytokine signaling mediates effects of rhinovirus on airway responsiveness

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Grunstein, Michael M., Hakon Hakonarson, Neil Maskeri, and Sing Chuang. Autocrine cytokine signaling mediates effects of rhinovirus on airway responsiveness. Am J Physiol Lung Cell Mol Physiol 278: L1146–L1153, 2000.—The airway responses to allergen exposure in allergic asthma are qualitatively similar to those elicited by specific viral respiratory pathogens, most notably rhinovirus (RV), suggesting that the altered airway responsiveness seen in allergic asthma and that elicited by viral respiratory tract infection may share a common underlying mechanism. To the extent that T helper cell type 2 (Th2) cytokines have been implicated in the pathogenesis of allergic asthma, this study examined the potential role(s) of Th2-type cytokines in mediating pro-asthmatic-like changes in airway smooth muscle (ASM) responsiveness after inoculation of naive ASM with human RV. Isolated rabbit ASM tissues and cultured human ASM cells were exposed to RV (serotype 16) for 24 h in the absence and presence of monoclonal blocking antibodies (MAbs) or antagonists directed against either the Th2-type cytokines interleukin (IL)-4 and IL-5, intercellular adhesion molecule (ICAM)-1 (the endogenous host receptor for most RVs), or the pleiotropic proinflammatory cytokine IL-1α. Relative to control (vehicle-treated) tissues, RV-exposed ASM exhibited significantly enhanced isometric contractility to acetylcholine and impaired relaxation to isoproterenol. These pro-asthmatic-like changes in ASM responsiveness were ablated by pretreating the RV-exposed tissues with either IL-5 receptor-α blocking antibody or human recombinant IL-1-β receptor antagonist, whereas IL-4 neutralizing antibody had no effect. Extended studies further demonstrated that inoculation of ASM cells with RV elicited 1) an increased mRNA expression and release of IL-5 protein, which was inhibited in the presence of anti-ICAM-1 MAb, and 2) an enhanced release of IL-1β protein, which was inhibited in the presence of IL-5 receptor-α antibody. Collectively, these observations provide new evidence demonstrating that RV-induced changes in ASM responsiveness are largely attributable to ICAM-1-dependent activation of a cooperative autocrine signaling mechanism involving upregulated IL-5-mediated release of IL-1β by the RV-exposed ASM itself.

T helper cell type 2 cytokines; airway smooth muscle; asthma

IN ASTHMATIC INDIVIDUALS, it is clinically well recognized that infection of the respiratory tract with certain viral pathogens often precipitates acute wheezing and symptom deterioration. Among these viral agents, rhinovirus (RV), the principal pathogen responsible for the common cold, represents the most frequent trigger of exacerbation of asthma symptoms (8, 12, 13, 27). Moreover, in this connection, a transient airway hyperreactivity has also been documented in normal (i.e., nonasthmatic) individuals after respiratory tract infection with RV (7, 12). Although the basic mechanism(s) mediating RV-induced exacerbation of asthma and airway hyperreactivity remains to be identified, a host of studies (25, 32, 34, 37) with various cell types (e.g., respiratory epithelium, fibroblasts, monocytes, and macrophages) have demonstrated that infection with RV elicits the expression and elaboration of various proinflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8, IL-9, IL-11, and tumor necrosis factor (TNF)α. It is generally believed that the actions of these cytokines, individually or in combination, likely underlie the clinical manifestation of symptomatic RV infection. In support of this concept, we recently identified that, notwithstanding the above-mentioned cell types, RV is also capable of directly infecting airway smooth muscle (ASM) tissue and cultured ASM cells and that this phenomenon is associated with the induction of “pro-asthmatic-like” changes in ASM responsiveness, including heightened constrictor agonist-mediated ASM contraction and impaired β-adrenergic receptor-mediated ASM relaxation (17, 23). Both these RV-induced changes in ASM responsiveness are qualitatively similar to those obtained in atopic asthmatic serum-sensitized ASM (19, 20), suggesting that the induced altered ASM responses in allergic asthma and those elicited after specific viral respiratory infections (e.g., with RV) share certain common mechanistic pathways. Indeed, in support of this concept, the collection of recent evidence from studies independently examining mechanisms of altered ASM responsiveness in the atopic asthmatic sensitized state (19) and after RV infection (17) of isolated ASM demonstrates that both of these conditions are associated with an induced endogenous release and autocrine action of the pleiotropic cytokine IL-1β. In light of this evidence, together with the wealth of information that implicates a crucial role of T helper cell type 2 (Th2)-type cytokines (e.g., IL-4 and IL-5) in mediating the pulmonary inflammatory response and airway hyperreactivity in allergic asthma (1, 11, 24, 29, 36), the present study examined whether RV-induced changes in ASM responsiveness are, at least in part, mechanistically coupled to activation of the Th2-type cytokine signaling pathways in the
RV-infected state. The results provide evidence demonstrating that 1) exposure of isolated ASM to RV elicits increased contractility of ASM to acetylcholine (ACh) and attenuated relaxation of the tissue to β-adrenoceptor stimulation with isoproterenol; 2) these changes in ASM responsiveness are associated with an induced upregulated mRNA expression and release of IL-5 protein by the RV-exposed ASM; and 3) the latter autologous release of IL-5 acts in an autocrine fashion to mediate the subsequent autologous release of IL-1β, which, in turn, is responsible for the RV-induced changes in ASM responsiveness. Collectively, these findings demonstrate that the effects of RV on airway responsiveness are largely attributed to the induction of a cooperative autocrine signaling mechanism that involves IL-5 and IL-1β release and action in the RV-exposed ASM.

METHODS

Animals. Twenty-one adult New Zealand White rabbits were used in this study, which was approved by the Institutional Animal Care and Use Committee of the Joseph Stokes Research Institute at Children's Hospital of Philadelphia (Philadelphia, PA). The animals had no signs of respiratory disease for several weeks before study.

Culture of human RV. A stock solution of human RV serotype 16 (RV16) was prepared by infecting monolayer cultures of human embryonic lung fibroblasts (MRC-5) with freshly isolated RV16 from the Clinical Virology Laboratory at the Children's Hospital of Philadelphia. As previously described (17, 23), the cultures were grown in modified MEM supplemented with Earle's balanced salt solution, 1% L-glutamine, 7.5% fetal bovine serum, HEPES buffer, and antimicrobial agents (5 µg/ml of gentamicin, 10 µg/ml of vancomycin, and 10 µg/ml of amphotericin B). When the infection was notably advanced, as evidenced by cytopathic effects, the cell supernatants were harvested and frozen in aliquots (–2–4 × 10^6 virus particles/aliquot) at –70°C for use in the experiments described in RV inoculation of ASM tissue.

RV inoculation of ASM tissue. Our method for preparing rabbit ASM tissue has been previously described (19, 20). In brief, after anesthesia (with ketamine [10 mg/kg] and xylazine [5 mg/kg]), the animals were killed with an intravenous overdose ofpentobarbital sodium (130 mg/kg). The tracheae were removed, scraped of loose connective tissue and epithelium, and then divided into 6- to 8-mm ring segments. Each alternate adjacent ASM ring segment was then incubated for 24 h at the optimal replication temperature for human RV in Dubbeco's modified Eagle's medium in the absence and presence of a maximal effective concentration of RV (1–10^6 viral particles/ml) as previously reported by our laboratory (17, 23). The inoculation experiments were conducted in the absence and presence of 1 h of pretreatment of the tissues with either an IL-4 neutralizing monoclonal antibody (NAb; 150 ng/ml), an IL-5 receptor antagonist (IL-5ra; blocking antibody (50 ng/ml), or the recombinant human IL-1 receptor antagonist (IL-1ra; 140 ng/ml). The tissues were aerated with a supplemental O2 mixture (95% O2–5% CO2) throughout the incubation period, and, thereafter, the responsiveness of the tissues to specific constrictor and relaxant agonists was compared as described in Pharmacodynamic studies of ASM responsiveness.

Pharmacodynamic studies of ASM responsiveness. After incubation of the tissue preparations, each ASM segment was suspended longituinally 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 continuously displayed on a multichannel recorder. Care was taken to place the membranous portion of the trachea between the supports to maximize the recorded tension generated by the contracting trachealis muscle. The tissues were bathed in modified Krebs-Ringer solution containing (in mM) 125 NaCl, 14 NaHCO3, 4 KCl, 2.25 CaCl2 · H2O, 1.46 MgSO4 ·H2O, 1.2 NaH2PO4·H2O, and 11 glucose. The baths were aerated with 5% CO2 in oxygen, 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 by our laboratory (20). 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 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, in separate studies, relaxation dose-response curves to isoproterenol (10^-10 to 10^-4 M) were conducted in tissues half-maximally contracted with ACh. The initial concentration of specific markers that confirmed their selective smooth muscle phenotype and excluded any contamination with other cell types. The standard experimental protocol involved growing the cells to confluence in the above medium and then starving the cells in unsupplemented SMBM for 24 h, at which time the cells were treated with 0, 3, 6, and 24 h with RV in the absence and presence of maximally effective concentrations of IL-4 NAb, IL-5ra blocking antibody, or a monoclonal blocking antibody (Mab) to intercellular adhesion molecule (ICAM)-1. The cells were then examined for mRNA and protein level of IL-4 and IL-5Ra expression by RT-PCR and ELISA measurements of IL-5 and IL-1β protein release.

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 previous described by our laboratory (17, 22). 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 IL-5, we used a RT-PCR protocol that included a human-specific primer for the cytokine as well as for the constitutively expressed ribosomal
protein (RP) L7. cDNA was synthesized from total RNA isolated from ASM cells grown under control (untreated) and RV-inoculated conditions. 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-5 and RPL7 genes (9, 30) and included the primer sets 5'-GTATGCAATCCACAGAAGA-3' (5'-primer; product is 433 bp) and 5'-TACAGAGTTCACACGACC-3' (3'-primer) for IL-5 and 5'-AAGAGGCTTCTTTTCTCAG-3' (5'-primer; product is 157 bp) and 5'-TCCGTCTCTC-CCTAATGTGCC-3' (3'-primer) for RPL7.

The culture procedure was used as follows: denaturation at 95°C for 1 min, annealing at 52–55°C for 1 min, and extension at 72°C for 1 min for 34 cycles for the IL-5 gene and 26 cycles for the RPL7 gene. The number of cycles was determined to be in the linear range of the PCR products. The PCRs for the human IL-5 and RPL7 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 immobilized by ultraviolet cross-linking with a Stratalinker UV Crosslinker 2400 at 120,000 µJ/cm² (Stratagene). Prehybridization in a 1:10 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-5 and RPL7 DNA levels were assayed by Southern blot analysis with 32P-labeled probes prepared by pooling several RT-PCRs for the individual IL-5 and RPL7 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: 1 × 15 min in 2× saline-sodium citrate (SSC)-0.1% SDS; 1 × 15 min in 0.1× SSC-0.1% SDS, both at room temperature; and 2 × 15 min at 50°C in 0.1× SSC-0.1% SDS. Southern blots were quantitated by direct measurement of the radioactivity in each band with a Pharmalog Magor (Molecular Dynamics).

ELISA measurements of IL-5 and IL-1β protein release. Both IL-5 and IL-1β protein levels were assessed in the culture medium of ASM cells that were exposed for 0, 3, 6, and 24 h to vehicle alone (control) or to RV in the absence and presence of anti-ICAM-1 MAb, IL-4 NAb, and IL-5Rα antibody. The IL-5 and IL-1β protein levels were quantitatively assessed with an enzyme-specific immunoassay as previously described by our laboratory (19, 22). The latter assay was performed with a double-antibody sandwich strategy in which an acetylcholinesterase-F-ab-conjugated IL-5 or IL-1β secondary antibody is targeted to a first cytokine-captured antibody. The enzymatic activity of acetylcholinesterase was measured spectrophotometrically, and relative to a linear standard curve (range 0–250 pg/ml), the results were used to quantify the amount of the targeted IL-5 and IL-1β present in the cell culture medium.

Reagents. The human ASM cells and SMBM were obtained from Clonetics (San Diego, CA). The IL-5 and RPL7 primers were obtained from Integrated DNA Technologies (Coralville, IA). IL-1ra, anti-human IL-5Rα antibody, IL-4 NAb, the IL-5 and IL-1β ELISA kits, the mouse anti-human IL-1β 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 bath concentrations. Isoproterenol and ACh were made fresh for each experiment and were dissolved in normal saline to prepare 10−4 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. P values < 0.05 were considered significant.

RESULTS

Role of IL-5 and IL-1β in mediating RV-induced changes in ASM responsiveness. In light of recent evidence demonstrating that an induced autocrine interaction between IL-5 and IL-1β mediates the altered responsiveness of ASM in the atopic asthmatic sensitized state (21), studies were conducted to initially evaluate the potential roles of the Th2-type cytokines IL-4 and IL-5 as well as the pleiotropic proinflammatory cytokine IL-1β in regulating ASM responsiveness in the presence of RV inoculation. Agonist constrictor and relaxation responses were separately examined in isolated rabbit ASM segments 24 h after inoculation of the tissues with vehicle alone (control) or with RV16 in the absence and presence of either IL-4 NAb, IL-5Rα antibody, or IL-1ra (see METHODS). As depicted in Fig. 1A, relative to control tissues, the constrictor responses and sensitivities (pD50; i.e., −log ED50 values) to exogenously administered ACh were significantly increased in ASM exposed to RV. Accordingly, the mean maximal constrictor response (Tmax) generated at the highest administered concentration of ACh (10−3 M) amounted to 101.9 ± 5.9 and 122.4 ± 8.5 g tracheal smooth muscle (TSM) in the control and RV-exposed tissues, respectively (P < 0.01), representing an RV-induced relative increase in Tmax of 20.1 ± 3.8% above the control value. Comparably, the corresponding mean pD50 values amounted to 4.99 ± 0.08 and 5.45 ± 0.09 −log M in the control and RV-exposed ASM, respectively (P < 0.01). These induced enhanced ASM constrictor responses to ACh were unaffected by pretreating RV-exposed tissues with IL-4 NAb (data not shown), wherein the mean Tmax values remained elevated at 128.3 ± 9.2 g TSM and 5.51 ± 0.09 −log M, respectively. In contrast, the augmented constrictor responses to ACh were prevented in RV-exposed tissues that were pretreated with a maximally effective concentration (i.e., 50 ng/ml) of IL-5Rα antibody (Fig. 1A). In these tissues, the mean Tmax and pD50 values remained elevated at 128.3 ± 9.2 g TSM and 5.51 ± 0.09 −log M, respectively, and both these values were not significantly different from those obtained in control ASM. Similarly, in comparable experiments, consistent with earlier observations by Hakonarson et al. (18), we found that pretreatment of RV-exposed ASM with human recombinant IL-1ra (140 ng/ml) also ablated the heightened constrictor responsiveness of the tissues to ACh (Fig. 1B). Accordingly, the mean T max values in the RV-exposed and control tissues amounted to 131.6 ± 10.7 and 104.3 ± 6.2 g TSM, respectively (P < 0.01), and the corresponding pD50 values averaged 5.17 ± 0.09 and 4.78 ± 0.09 −log M, respectively (P < 0.05). In the presence of IL-1ra, the Tmax and pD50 values in the RV-exposed ASM were similar to those obtained in
control tissues and averaged \(107.4 \pm 5.1\) g/g TSM and \(4.83 \pm 0.11\) \(-\log M\), respectively. In contrast to these observations obtained in RV-exposed ASM, neither IL-4 NAb, IL-5R\(\alpha\) antibody, nor IL-1ra had any appreciable effect on ASM contractility to ACh in control tissues (data not shown).

In separate studies during comparable levels of initial ACh-induced contractions in control and RV-exposed ASM segments averaging \(-45\% of T_{\text{max}}\), administration of the \(\beta\)-adrenoceptor agonist isoproterenol elicited cumulative dose-dependent relaxation of the precontracted tissues. As depicted in Fig. 2A, relative to control ASM, the maximal relaxation (R\(_{\text{max}}\)) responses and sensitivities (pD\(_{50}\) values) to isoproterenol were significantly reduced in the corresponding RV-exposed tissues. Accordingly, the mean R\(_{\text{max}}\) values in the RV-exposed and control ASM amounted to \(47.5 \pm 5.4\) and \(59.9 \pm 7.2\%\), respectively (\(P < 0.01\)), and the corresponding pD\(_{50}\) values for isoproterenol averaged \(5.81 \pm 0.11\) and \(6.19 \pm 0.12\) \(-\log M\), respectively (\(P < 0.05\)). These attenuated relaxation responses to isoproterenol were unaffected in RV-exposed tissues that were pretreated with IL-4 NAb where their R\(_{\text{max}}\) and pD\(_{50}\) values averaged \(49.2 \pm 7.1\%\) and \(5.83 \pm 0.08\) \(-\log M\), respectively (data not shown). In contrast, the impaired relaxation responses to isoproterenol were prevented in RV-exposed tissues that were pretreated with IL-5R\(\alpha\) antibody (Fig. 2A) where the mean R\(_{\text{max}}\) and pD\(_{50}\) values averaged \(56.2 \pm 5.9\%\) and \(6.11 \pm 0.11\) \(-\log M\), respectively. Similarly, in comparable experiments, the attenuated relaxation responses to isoproterenol were also ablated in RV-exposed ASM that was pretreated with IL-1ra (Fig. 2B). In contrast to the observations pertaining to RV-exposed ASM, we found that in control tissues, neither pretreatment with IL-4 NAb, IL-5R\(\alpha\) antibody, nor IL-1ra affected the subsequent relax-
Regulation of IL-5 expression in RV-inoculated ASM. The above observations are consistent with those in a recent study by Hakonarson et al. (17) demonstrating that exposure of isolated rabbit ASM tissue and cultured rabbit or human ASM cells to RV induces an upregulated mRNA expression and release of IL-1β protein into the ASM culture medium, an effect detectable after 6 h of exposure to the viral pathogen. Moreover, in the latter study, we found that the RV-induced upregulated release of IL-1β was responsible for the observed changes in ASM responsiveness in the RV-inoculated ASM (17). Given these previous findings, when evaluated in light of our above extended present observations implicating a role for IL-5, the consideration is raised that IL-5 and IL-1β may share a common or interactive mechanism in mediating the observed changes in ASM responsiveness in the RV-exposed state. In addressing this possibility, a series of experiments was conducted to initially examine whether cultured human ASM cells are induced to express altered mRNA and protein release of IL-5 in the RV-exposed state. For analysis of IL-5 mRNA, with the use of RT-PCR and a human IL-5-specific primer, cDNA was reverse transcribed from total isolated RNA primed with oligo(dT), and Southern blots were subsequently prepared and probed with the human cDNA probe specific for the human IL-5 gene (see METHODS). In addition, a 157-bp probe for the constitutively expressed RPL7 gene was also prepared and used to control for gel loading, and the signals for the individual PCR products were quantitated with a PhosphorImager. As shown by one of three representative experiments in Fig. 3, exposure of cultured human ASM cells to RV induced a marked upregulated expression of the IL-5 mRNA signal, distinctly evident at 3, 6, and 24 h after RV inoculation. In contrast, the intensity of the IL-5 mRNA signal obtained in control cells was relatively low and remained essentially unaltered during the 24-h period of exposure to vehicle alone. Of note, the corresponding constitutively expressed RPL7 signal also remained unaltered in both the control and RV-exposed cells.

In view of the above observations, complementary studies were conducted with a cytokine-specific radiomunoassay (see METHODS) to examine whether the RV-induced changes in IL-5 mRNA expression above are accompanied by corresponding changes in the elaboration of IL-5 protein into the cell culture medium by the RV-exposed human ASM cells. The initial basal concentrations of IL-5 protein in the culture medium of control and RV-exposed cells were similar and averaged 1.28 ± 0.32 and 1.59 ± 0.51 pg/ml, respectively. As demonstrated in Fig. 4, the levels of IL-5 protein did not systematically change in control cells during the 24-h study period. In contrast, the IL-5 protein levels in the culture medium of RV-exposed cells progressively increased, reaching maximal levels of 7.13 ± 0.82 and 6.89 ± 0.78 pg/ml at 6 and 24 h, respectively. Moreover, as further depicted in Fig. 4, the induced increases in IL-5 protein release were largely ablated in RV-exposed ASM cells that were pretreated with a MAb to ICAM-1 (Fig. 4). Insofar as ICAM-1 represents the endogenous host receptor for RV16 (15, 35), the observed inhibitory effect of anti-ICAM-1 MAb on RV-induced IL-5 release...
Role of IL-5 in mediating IL-1\(\beta\) release in RV-exposed ASM. In considering the above results together with recent findings by Hakonarson et al. (17) demonstrating that the changes in agonist responsiveness in RV-exposed ASM are mediated by an induced upregulated release and autocrine action of IL-1\(\beta\), we next examined whether the above observed effects of RV on IL-5 release are mechanistically coupled to the induction of IL-1\(\beta\) release from RV-exposed ASM cells. Accordingly, IL-1\(\beta\) protein levels were measured by radioimmunoassay of the culture medium of human ASM cells exposed for 24 h to vehicle alone and to RV16 in the absence and presence of IL-4 NAb and IL-5R\(\alpha\) antibody. As depicted in Fig. 5, relative to control cells, the elaboration of IL-1\(\beta\) protein was markedly increased in RV-exposed ASM cells, and although IL-4 NAb had no effect, pretreatment with IL-4 NAb had no effect, RV-induced enhanced release of IL-1\(\beta\) was largely responsible for the reported increase in IL-1\(\beta\) release from RV-exposed ASM (17). Interestingly, Hakonarson et al. (21) recently demonstrated that a similar sequential pattern of initial IL-5 release that, in turn, mediates a secondary release of IL-1\(\beta\) is responsible for the induction of comparable changes in agonist constrictor and relaxant responsiveness in isolated rabbit ASM passively sensitized with human atopic asthmatic serum.

**DISCUSSION**

The fundamental mechanism(s) underlying the well-known changes in airway reactivity that occur after infection of the respiratory tract with certain viral pathogens remains to be elucidated. In this context, it is relevant to note that current evidence supports the general concept that the airway responses in allergic asthma and those obtained after infection with specific viral respiratory pathogens may share certain common mechanistic pathways. Accordingly, it is well recognized that, as in allergic asthma, infection of the respiratory tract with specific viral pathogens can induce airway inflammation (5, 12), cytokine release (25, 32, 34, 37), and IgE production (2, 16). Insofar as these proinflammatory responses may be associated with altered airway reactivity that is similar to that seen in allergic asthma, it is likely that a mechanistic interplay between specific viral respiratory pathogens and activated inflammatory cells, airway epithelial cells, and other cell types importantly contributes to the viral-induced pro-asthmatic-like phenotype of altered airway responsiveness. In light of this consideration, together with the well-documented crucial role of Th2-type cytokines in the pathogenesis of allergic asthma (1, 11, 24, 29, 36), the present study examined the potential contributions of the Th2-type cytokines IL-4 and IL-5 in mediating RV-induced changes in ASM responsiveness. The results provided new evidence demonstrating that 1) exposure of naive ASM tissue to RV induced heightened ASM constrictor responsiveness and attenuated \(\beta\)-adrenoceptor-mediated ASM relaxation; 2) these RV-induced changes in ASM responsiveness were associated with an upregulated expression of IL-5 mRNA and increased IL-5 protein release in the RV-exposed state; and 3) the latter enhanced release of IL-5 was largely responsible for an associated increase in IL-1\(\beta\) release by ASM in the RV-exposed state.

To our knowledge, this study is the first to demonstrate that the induced endogenous release of IL-5 and IL-1\(\beta\) by RV-exposed ASM results in its autocrine manifestation of altered agonist responsiveness. In addressing the collection of evidence supporting this central finding, certain issues pertaining to the present observations are worthy of consideration. Among these, it is relevant to note that, in general, our observed changes in constrictor and relaxant responsiveness in isolated RV-exposed ASM (Figs. 1 and 2) mimicked the perturbations in airway function that characterize the asthmatic condition in vivo and isolated asthmatic airways in vitro, including enhanced bronchoconstrictor responsiveness and impaired \(\beta\)-adrenoceptor-mediated airway relaxation (3, 4, 10, 14, 33). Moreover, to the extent that these effects were found to be largely ablated in RV-exposed ASM that was pretreated with IL-5R\(\alpha\) antibody or IL-1ra (Figs. 1 and 2), as a first approximation, these results suggested that both IL-5 and IL-1\(\beta\) are key mediators of RV-induced changes in ASM responsiveness.

**Fig. 5.** Comparison of IL-1\(\beta\) protein elaboration into culture medium of human ASM cells after 24 h of treatment with vehicle alone (control) and exposure to RV in absence and presence of IL-4 neutralizing antibody (NAb) and IL-5R\(\alpha\) antibody. Note that relative to control cells, release of IL-1\(\beta\) protein was significantly increased in RV-exposed ASM cells, and although IL-4 NAb had no effect, RV-induced enhanced release of IL-1\(\beta\) was inhibited in cells pretreated with IL-5R\(\alpha\) antibody. **NS, not significant.**
and IL-1β were endogenously released by the RV-exposed ASM and that these cytokines acted similarly or interacted cooperatively in ultimately mediating the observed changes in ASM responsiveness. Both these considerations concur with the collection of related information based on other published reports. In this context, previous studies (6, 26, 28, 31) have demonstrated that apart from certain leukocytes, various non-bone marrow-derived resident tissue cells (e.g., epithelial cells, endothelial cells, keratinocytes) can be induced to elaborate specific cytokines, including Th2-type cytokines. Moreover, Hakonarson and colleagues (19, 22) recently demonstrated that atopic asthmatic serum-sensitized ASM cells can also be induced to sequentially release IL-5 and IL-1β in the atopic sensitized state. Our present observations extend this earlier evidence by demonstrating that ASM cells can additionally be induced to autologously express IL-5 and IL-1β and elaborate these cytokines in response to inoculation with RV (Figs. 4 and 5).

In addressing the potential mechanism by which IL-5 and IL-1β may interact in mediating the observed changes in ASM responsiveness in the RV-exposed state, the present study examined the possibility that the induced release of these cytokines represented a mechanistically related phenomenon. Indeed, as depicted in Fig. 5, when RV-inoculated ASM cells were pretreated with an IL-5-receptor blocking antibody, the increased elaboration of IL-1β was largely prevented. Thus, when taken together, the present findings support the notion that our observed changes in ASM responsiveness in the RV-exposed state largely reflected the behavior of a cooperative system of sequential autocrine signaling that involved an initial viral-induced release of IL-5 that, in turn, elicited the subsequent release of IL-1β by the ASM itself. Of note, this concept of sequential autocrine signaling involving IL-5 and IL-1β is consistent with the recent findings by Hakonarson et al. (21) demonstrating a similar pattern of sequential IL-5 and IL-1β release in mediating the changes in agonist responsiveness obtained in atopic asthmatic-sensitized ASM. Moreover, in this respect, it is relevant to note that, based on previous findings by Hakonarson and colleagues (18, 20), the mechanism by which IL-1β release ultimately leads to changes in ASM responsiveness is apparently due to its induction of enhanced expression and action of G protein, specifically Gαi2 and Gαi3, which inhibit intracellular cAMP accumulation.

An important consideration raised by the findings of the present study relates to the potential relevance of our in vitro observations to the in vivo condition. In this context, it is generally believed that the airway responses to respiratory tract infection with RV are largely mediated by cytokine release from infected and damaged airway epithelial cells and activated leukocytes infiltrating the affected airways (25, 32, 34, 37). Our present observations extend this contemporary view by identifying an important role for RV-induced cytokine release by the ASM itself in autologously mediating changes in ASM responsiveness. This evidence concurs with the emerging compelling concept that the ASM itself constitutes an autologously regulated system that when activated in the sensitized state (e.g., with RV infection or with atopic sensitization) elicits the expression of specific cell surface proteins (e.g., ICAM-1) and the release of specific cytokines (e.g., IL-1β) that lead to pro-asthmatic-like perturbations in ASM responsiveness (17, 19, 20, 22, 23). Moreover, in extending this concept, because ICAM-1 activation has been well established as a crucial mechanism for immune effector cell mobilization and action, our present findings raise the possibility that RV-induced activation of ICAM-1 in ASM may serve to mediate both the altered ASM responsiveness and the localized accumulation of inflammatory cells in the airways in the RV-infected state. This possibility remains to be systematically investigated.

In conclusion, the results of the present study provide new evidence demonstrating that both the Th2-type cytokine IL-5 and the pleiotropic proinflammatory cytokine IL-1β are endogenously released by ASM cells in response to inoculation with RV and that these cytokines act cooperatively in mediating the induced changes in ASM responsiveness in the RV-exposed state. In addition, the results demonstrate that the nature of the latter cytokine interaction is given by IL-5-mediated induction of the autocrine release and action of IL-1β in the RV-exposed ASM. Thus together with the conventional concepts related to the roles of various inflammatory cells in the pathogenesis of the airway response to RV infection, the present findings identify a potentially important mechanism by which the resident ASM itself may autologously regulate its own state of altered reactivity in response to RV infection. Moreover, in this connection, insofar as Th2-type cytokines are importantly implicated in the pathobiology of allergic asthma, the findings of the present study provide new evidence in support of the notion that the altered airway responses seen in allergic asthma and in the RV-infected state share a common mechanism that involves the autocrine release and actions of IL-5 and IL-1β in the affected ASM.

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REFERENCES


