

T lymphocyte-mediated changes in airway smooth muscle responsiveness are attributed to induced autocrine release and actions of IL-5 and IL-1 β

Hakon Hakonarson, MD, PhD,^a Russell Whelan, MS,^a Jennifer Leiter, MSc,^a Cecilia Kim, MS,^a Mei Chen, BS,^a Donald Campbell, PhD,^b and Michael M. Grunstein, MD, PhD^a Philadelphia, Pa

Background: Bidirectional stimulatory cross-talk was recently found to exist between activated T cells and airway smooth muscle (ASM) cells, a process that involves coligation of specific cellular adhesion–costimulatory molecules that results in the induction of proasthmatic-like changes in ASM responsiveness. **Objective:** The present study examined whether the cooperative intercellular signaling between activated T cells and ASM cells is coupled to the induced expression and actions of IL-5 and IL-1 β .

Methods: Agonist-induced constrictor and relaxant responses were examined in rabbit ASM segments exposed to resting and anti-CD3-activated T cells in the absence and presence of either an anti-IL-5 receptor mAb or the recombinant human IL-1 receptor antagonist. In addition, mRNA and protein expression of IL-5 and IL-1 β were assayed under control and anti-CD3-stimulated conditions.

Results: Relative to inactive T cells, incubation of ASM tissues with anti-CD3-activated T cells induced proasthmatic-like changes in agonist-mediated ASM responsiveness. This T cell-induced perturbation in ASM responsiveness was ablated by pretreating the tissues with either an anti-IL-5 receptor mAb or IL-1 receptor antagonist. Moreover, exposure of ASM cells to anti-CD3-activated T cells elicited an initial increased mRNA expression and release of IL-5, followed by an enhanced expression and release of IL-1 β , and the induced release of these cytokines was prevented in ASM cells that were pretreated with an anti-IL-5 receptor mAb.

Conclusion: Collectively, these observations provide new evidence demonstrating that exposure of naive ASM cells to activated T cells induces the sequential release of IL-5 and IL-1 β from the ASM cells and that the latter cytokines act in an autocrine manner to elicit the proasthmatic phenotype of altered ASM responsiveness. (*J Allergy Clin Immunol* 2002;110:624-33.)

Key words: IL-5, IL-1 β , airway hyperresponsiveness, airway smooth muscle cells, T lymphocytes, cell-to-cell interactions

Specific CD4⁺ T_H2 cytokines have been implicated in the pathogenesis of the pulmonary inflammatory response and altered bronchial responsiveness in allergic asthma.¹⁻⁵ Apart from orchestrating various critical humoral and cellular immune functions, the T_H2 cell-derived cytokines, notably including IL-4, IL-5, and IL-13, also regulate IgE synthesis by B lymphocytes and promote eosinophil proliferation, recruitment, and activation, all of which reportedly serve to facilitate expression of the airway inflammatory response and airway constrictor hyperresponsiveness in asthma.⁶⁻¹⁰ In this regard it has been demonstrated that, relative to nonallergic and nonasthmatic subjects, children and adults with atopic asthma show increased levels of the T_H2 cytokines IL-4, IL-13, and IL-5 in their serum, as well as in bronchoalveolar lavage (BAL) fluid samples.¹¹⁻¹⁷ Moreover, it has been reported that mononuclear cells isolated from serum or BAL fluid samples from atopic asthmatic individuals demonstrate a relatively enhanced expression of the T_H2 phenotype when the cells are stimulated with antigen.^{12,14,15,18} Finally, studies on the basis of genetic analyses of families with allergic asthma have demonstrated a significant direct correlation between altered airway constrictor responsiveness and IL-4 and IL-5 cytokine levels in serum or BAL fluid samples.^{16,19}

Notwithstanding the fundamental role played by T lymphocytes, together with the actions attributed to other leukocytes (eg, mast cells, macrophages, and eosinophils), certain non–bone marrow–derived cell types in the lung (eg, airway epithelial, neurovascular cells, endothelial cells, and fibroblasts) have also been implicated in the overall pathobiology of allergic asthma.²⁰⁻²² Among the latter cell types, the resident airway smooth muscle (ASM) itself has more recently been found to display a variety of proinflammatory actions in the atopic asthmatic sensitized state, including the autologous expression and release of various proinflammatory and T_H1- and T_H2-type cytokines.^{2,10,23-28} Moreover, under simulated conditions of atopic sensitization involving IgE-mediated stimulation of human ASM cells, the latter were found to activate naive resting T cells, and conversely, anti-CD3-activated T cells were shown to reciprocally activate naive resting ASM cells.²⁹ This phenomenon of bidirectional stimulatory cross-talk between T

From the Divisions of ^aPulmonary Medicine and ^bAllergy, Immunologic and Infectious Diseases, The Joseph Stokes Jr Research Institute, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia.

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Reprint requests: Hakon Hakonarson, MD, PhD, Division of Pulmonary Medicine, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, 34th St and Civic Center Blvd, Philadelphia, PA 19104.

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Abbreviations used

ACh: Acetylcholine
ASM: Airway smooth muscle
BAL: Bronchoalveolar lavage
ED50: Median effective dose
IL-1ra: IL-1 receptor antagonist
IL-5r: IL-5 receptor
MFI: Mean fluorescence intensity
Rmax: Maximal relaxation
SmBM: Smooth muscle basal medium

lymphocytes and ASM cells was attributed to intercellular coligation of specific cellular adhesion molecules/costimulatory molecules and was found to elicit proasthmatic-like changes in ASM tissue constrictor and relaxant responsiveness.²⁹ In light of these findings, the present study was designed to elucidate the mechanism underlying activated T cell-induced changes in ASM responsiveness. The results provide new evidence demonstrating that the interaction between anti-CD3-activated T cells and ASM cells evokes a sequential upregulated expression and release of the T_H2 -type cytokine IL-5 and the pleiotropic proinflammatory cytokine IL-1 β and that the cooperative actions of these cytokines are responsible for inducing proasthmatic-like changes in ASM responsiveness.

METHODS

Preparation of ASM tissue and coincubation with T lymphocytes

Fourteen adult New Zealand white rabbits were used in this study, which was approved by the Biosafety, Animal Research, and Institutional Review Board Committees of the Joseph Stokes Research Institute at Children's Hospital of Philadelphia. The animals had no signs of respiratory disease before the study. Anti-CD3-activated T cells (see below) were coincubated with isolated rabbit ASM tissue and examined for changes in the tissues' agonist-mediated constrictor and relaxation responsiveness, as previously described, to determine whether activated T lymphocytes have the capacity to induce proasthmatic changes in ASM responsiveness.²⁹ The tracheal rings were incubated for 24 hours at room temperature in either Dulbecco's modified Eagle's medium alone or Dulbecco's modified Eagle's medium in the presence of naive (resting) versus anti-CD3-activated T lymphocytes (1×10^6 /mL) with and without 1 hour of pretreatment with an anti-IL-5 receptor (IL-5r) mAb (2 μ g/mL), an IL-4 neutralizing antibody (1 μ g/mL), or the IL-1 receptor antagonist (IL-1ra; 140 ng/mL). The incubation media were aerated with a continuous supplemental O_2 mixture (95% O_2 /5% CO_2) during the incubation phase.

Pharmacodynamic studies

After incubation of the tissue samples, each ASM segment was suspended longitudinally between stainless-steel triangular supports in siliconized, Harvard, 20-mL organ baths, as previously described.^{30,31} Cholinergic contractility was assessed in the ASM segments by means of cumulative administration of acetylcholine (ACh) in final bath concentrations ranging from 10^{-10} to 10^{-3} mol/L. Thereafter, in separate studies relaxation dose-response curves to isoproterenol (10^{-10} - 10^{-4} mol/L) were generated in tissues half-maximally contracted with ACh. The relaxant responses to isoproterenol were analyzed in terms of the percentage of maximal

relaxation (Rmax) from the active cholinergic contraction, and sensitivity to the relaxing agent was determined as the negative logarithm of the dose of the relaxing agent producing 50% of Rmax (PD50; ie, geometric mean median effective dose [ED50] value).

Preparation and treatment of cultured ASM cells

Human ASM cells were derived from a 16- and a 21-year-old male donor (Clonetics) who had no evidence of pulmonary disease. The cells were cultured at 37°C in a mixture of 5% smooth muscle basal medium (SmBM), as previously described.^{23,31} Before their selective treatments (see below), the ASM cells were grown to approximately 95% confluency, at which time the cells were starved for 24 hours in 10 mL of unsupplemented SmBM.

T-lymphocyte isolation and activation

T lymphocytes were isolated from whole blood by using the WBP1010 Acticyte-TC Kit (Bioergonomics), as previously described.²⁹ T-cell purity was confirmed by staining the cells with an anti-CD3 phycoerythrin-conjugated antibody, which demonstrated greater than 96% T-cell purity. Less than 2% of the isolated cells stained positive with anti-CD19- and anti-CD14-labeled antibodies, as assessed by means of flow cytometry. T-cell proliferation was examined every 24 hours with a hemocytometer (Reichert).

Human ASM cell/T-cell interaction

Anti-CD3-activated T lymphocytes were incubated with naive ASM cells for various time points (up to 24 hours) in the absence and presence of 1 hour of pretreatment with various combinations of an anti-IL-5r mAb (2 μ g/mL), IL-4 neutralizing antibody (1 μ g/mL), or IL-1ra (140 ng/mL), each in separate experiments. The media containing the ASM cells and T lymphocytes were subsequently collected, and the cells were washed 3 times with PBS. After removal of the T lymphocytes and media, the ASM cells were scraped off the flask with a rubber spatula. We verified that the T cells were effectively removed by demonstrating their absence when staining the cell preparation with a CD3 antibody. Both sets of cells were then separately prepared for either mRNA extraction or flow cytometric studies, as described below.

Determination of IL-5 and IL-1 β mRNA expression

Total RNA was isolated from the human T lymphocytes and cultured HASM cell preparations by using 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.^{23,31} To analyze for mRNA expression of IL-5 and IL-1 β , we used RT-PCR and human-specific primers for these molecules. RNA was isolated from untreated cells and human ASM cells that were exposed to inactive versus anti-CD3-activated T lymphocytes. The cDNAs were prepared and primed with oligo(dT). PCR was used to amplify the specific products from each cDNA reaction by using primers based on the published sequences of the human *IL5* and *IL1 β* genes, as previously described.²⁴ The human ASM cells were also examined for CD3 expression to exclude contamination from T cells. The IL-5, IL-1 β , CD3, and β -globulin DNA levels were assayed by means of Southern blot analysis with product-specific phosphorous 32-labeled probes. Southern blots were quantitated by means of direct measurements of radioactivity in each band with a PhosphorImager (Molecular Dynamics).

Flow cytometric analysis

Expression of intracellular IL-5 and IL-1 β was examined in both ASM cells and T lymphocytes in the absence and presence of IL-5r

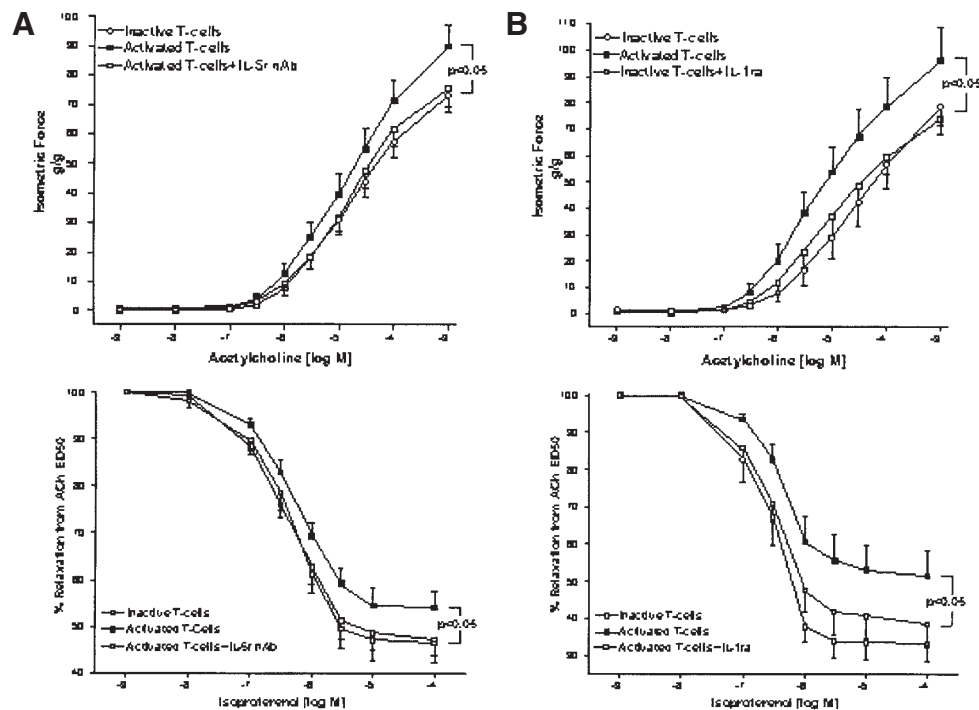


FIG 1. Comparison of ASM constrictor responses to ACh (upper panels) and ASM relaxant responses to isoproterenol (lower panels) in rabbit ASM tissues after 24 hours' exposure to resting T cells (open circles) or to anti-CD3-activated T cells in the absence (filled squares) and presence (open squares) of pretreatment with an anti-IL-5r mAb (A) or with IL-1ra (B). Data represent mean $\pm$ SE values from 6 experiments.

mAb with a Coulter EPICS Elite flow cytometer, as previously described.²⁹ The cells were stained with a direct phycoerythrin-conjugated mouse anti-human mAb to IL-5 and intracellular IL-1 β by using a direct FITC-conjugated mouse anti-human IL-1 β mAb. In addition, the cells were stained with mouse IgG antibodies of the identical isotypes as the anti-IL-5 and anti-IL-1 β mAbs to assess for background fluorescence (ie, IgG1 negative controls). The cells were also stained with smooth muscle actin and anti-CD3 antibodies to ensure that the ASM cell sample was not contaminated with T cells. Once stained, the cells were examined by means of flow cytometry and analyzed with the Elite Immuno 4 statistical software (Coulter EPICS Division).

ELISA measurements of IL-5 and IL-1 β proteins

IL-5 and IL-1 β protein levels were measured initially in the cell culture media at 0.5 hours. Thereafter, the protein levels were assayed in the culture media of ASM cells that were exposed for 3, 6, and 24 hours to either naive T cells (inactive) or to anti-CD3-activated T cells. The IL-5 and IL-1 β protein levels were quantitatively assessed with an enzyme-specific immunoassay, as previously described.²⁵

Statistical analyses

Statistical analyses were performed by using the 2-tailed paired Student *t* test and ANOVA with multiple comparison of means, where appropriate. *P* values of less than .05 were considered significant.

Reagents

The human ASM cells and SmBM were obtained from Clonetics. IL-5; IL-1 β ; all the mouse anti-human mAbs to IL-5, IL-1 β , IL-

5r, IL-4, and IL-1ra; and IL-5 and IL-1 β ELISA kits were purchased from R&D Systems Inc. The anti-CD3 activation antibody was prepared from an NK3 hybridoma cell line purchased from ATCC.

RESULTS

Roles of IL-5 and IL-1 β in regulating agonist responsiveness in T cell-activated ASM

We previously determined that the proinflammatory cytokines IL-5 and IL-1 β are critically involved in mediating proasthmatic-like changes in agonist responsiveness in ASM sensitized under atopic and nonatopic conditions, including passive sensitization with atopic asthmatic serum and inoculation with rhinovirus, respectively.^{23,24,26,28,32} Isolated rabbit ASM tissue segments were exposed to inactive (resting) or anti-CD3-activated T cells, both in the absence and presence of pretreatment of the tissues, either with a blocking mAb directed against the IL-5 receptor (anti-IL-5r mAb) or with the endogenous IL-1ra (see the "Methods" section) to address the potential roles of these cytokines in regulating T cell-mediated modulation of ASM function. As shown in Fig 1, A (top panel), relative to ASM tissues exposed to vehicle alone or to inactive T cells, tissues exposed to anti-CD3-activated T cells exhibited increased constrictor responses to exogenously administered ACh. Accordingly, the mean $\pm$ SE maximal constrictor responses and sensitivities (PD50; ie, $-\log$ ED50 values) to ACh

Human Airway Smooth Muscle Cells

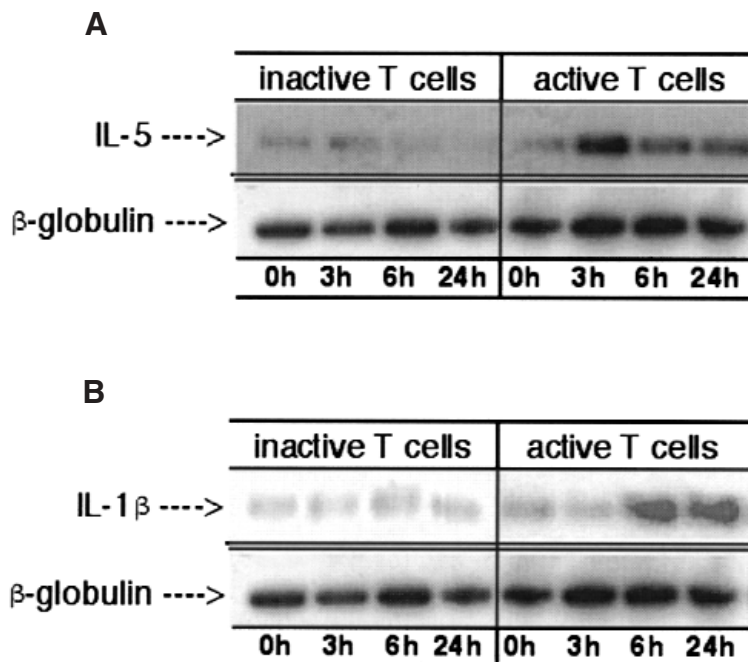


FIG 2. Comparison of IL-5 (**A**) and IL-1 β (**B**) mRNAs expressed by cultured human ASM cells exposed for 0, 3, 6, or 24 hours to resting T cells (*left panels*) and to anti-CD3-activated T cells (*right panels*). Note the mRNA expression signals for both IL-5 and IL-1 β were notably enhanced (3- to 5-fold) in the anti-CD3-activated, T cell-exposed ASM cells at 3 (IL-5) and 6 (IL-1 β) hours, and the increased IL-1 β mRNA signal was sustained for up to 24 hours.

amounted to 89.78 ± 7.28 g/g ASM weight and 5.00 ± 0.10 $-\log$ mol/L, respectively, in the tissues exposed to activated T cells, relative to the corresponding values of 73.20 ± 5.70 g/g ASM weight and 4.80 ± 0.09 $-\log$ mol/L obtained in control ASM ($P < .05$). These augmented constrictor responses to ACh were prevented, however, when ASM tissues exposed to activated T cells were pretreated with a maximally effective concentration (2 μ g/mL) of anti-IL-5r mAb, and hence both the maximal constrictor responses and PD50 values to ACh in these tissues were similar to those obtained in control ASM. Comparably, in another group of experiments (Fig 1, *B*), we found that the enhanced constrictor responses to ACh (Fig 1, *B*, top panel) were also completely abrogated when ASM tissues exposed to activated T cells were pretreated with IL-1ra. In contrast to these findings, it should be noted that neither anti-IL-5r mAb nor IL-1ra was found to have any effect on the constrictor responses to ACh in control (vehicle-exposed) ASM (data not shown). Moreover, contrasting with the protective effects of the anti-IL-5r mAb and IL-1ra, pretreatment of ASM exposed to activated T cells with either an IgG1 isotype control mAb or an IL-4 protein neutralizing antibody had no effect on the tissues' heightened constrictor responses to ACh (data not shown).

In separate studies we also compared the ASM relaxation responses to the β -adrenoceptor agonist isoproterenol, which was cumulatively administered to tissues initially half-maximally contracted with their respective ED50 doses of ACh under the same treatment conditions described above. As shown in Fig 1 (lower panels), relative to tissues exposed to vehicle alone or to inactive T cells, the relaxation responses to isoproterenol were significantly attenuated in ASM exposed to anti-CD3-activated T cells. Accordingly, as exemplified by the data displayed in Fig 2, *A*, the mean $\pm$ SE R_{max} and sensitivities (PD50 values) to isoproterenol amounted to 45.88% and 6.29 $-\log$ mol/L, respectively, in the tissues exposed to activated T cells relative to the corresponding values of 53.70% and 6.36 $-\log$ mol/L obtained in control ASM ($P < .05$). In comparable experiments these impaired relaxation responses to isoproterenol were prevented when ASM tissues exposed to activated T cells were pretreated with either anti-IL-5r mAb (Fig 1, *A*, lower panel) or IL-1ra (Fig 1, *B*, lower panel).

Expression of IL-5 and IL-1 β in T cell-activated ASM cells

In light of the above observations implicating roles for IL-5 and IL-1 β in mediating the observed changes in ASM

constrictor and relaxant responsiveness, we examined whether the expression of mRNAs and proteins for these cytokines are modulated in cultured human ASM cells exposed to the activated T cells. As depicted in Fig 2, exposure of cultured ASM cells to activated T cells induced upregulated expression of both IL-5 (Fig 2, A) and IL-1 β (Fig 2, B) mRNA signals in the ASM cells. Accordingly, compared with cells exposed to inactive T cells, wherein the intensities of the IL-5 and IL-1 β mRNA signals were low or barely detectable, the mRNAs for both these cytokines were markedly upregulated in the T cell-activated human ASM cells, whereas the corresponding mRNA signal for the constitutively expressed gene encoding β -globulin remained essentially unaltered. In further examining Fig 2, it can be seen that although both the IL-5 and IL-1 β mRNA signals were upregulated in the activated ASM cells, the temporal patterns of enhanced mRNA expression of these cytokines were notably different. Thus, whereas the IL-5 mRNA signal was distinctly increased as early as 3 hours in the T cell-activated ASM cells, the signal was subsequently attenuated by 6 and 24 hours. In contrast, upregulated expression of the corresponding IL-1 β mRNA signal was detected at 6 hours and was sustained for up to 24 hours in ASM cells that were exposed to the activated T cells. To ensure that the T cells were effectively removed from the ASM cells, we stained the cell preparation with a CD3 antibody specific for detecting the presence of T cells and demonstrated their absence by negative CD3 expression (data not shown).

In complementary studies with cytokine-specific immunoassays (see the "Methods" section), we next examined whether the above temporal patterns of changes in IL-5 and IL-1 β mRNA expression were also reflected by corresponding changes in the release of these cytokines into the culture media of human ASM cells that were exposed to resting versus anti-CD3-activated T cells. The basal protein concentrations of IL-5 and IL-1 β detected in the culture media of ASM cells that were subsequently exposed to resting and anti-CD3-activated T cells (ie, at 30 minutes) averaged 0.70 ± 0.40 and 1.10 ± 0.60 pg/mL for IL-5 (Fig 3) and 1.40 ± 1.10 and 1.30 ± 1.20 pg/mL for IL-1 β (Fig 4), respectively. In contrast to media from ASM cells exposed to resting T cells, wherein the cytokine levels were minimally affected for up to 24 hours, the IL-5 (Fig 3) and IL-1 β (Fig 4) protein levels progressively increased in the culture media of ASM cells that were coincubated with activated T cells, attaining greater than 20- and 80-fold increases above their corresponding basal levels, respectively. Moreover, in accordance with the above different temporal patterns of mRNA expression (Fig 2), the temporal patterns of IL-5 and IL-1 β release also differed, with an earlier initial increase in IL-5 protein (ie, at 3 hours) detected relative to the release of IL-1 β (ie, at 6 hours). Given these results, we next examined whether the induced release of IL-5 and IL-1 β were causally related. As also shown in Fig 4, pretreatment of ASM cells with anti-IL-5r mAb largely prevented their release of IL-1 β into the cell culture medium on subsequent exposure to anti-CD3-acti-

vated T cells. The latter finding is consistent with those in our previous studies demonstrating that IL-5 induces the release of IL-1 β from atopic asthmatic serum-sensitized²⁴ and rhinovirus-inoculated ASM.³²

Intracellular detection of IL-5 and IL-1 β proteins

Extended studies were conducted wherein both cell types were stained with antibodies specific for intracellular detection of human IL-5 and IL-1 β proteins to elucidate the cellular origin of IL-5 and IL-1 β protein release under the above conditions of T-cell/ASM-cell coinubation. In examining the IL-5 protein levels, flow cytometric analysis demonstrated that, relative to the resting state, the detection signals for IL-5 were distinctly increased in both the coinubated ASM cells and the anti-CD3-activated T cells, as depicted in Fig 5. Accordingly, as exemplified, relative to the values of the percentage of positive cell staining and mean fluorescence intensity (MFI) obtained in ASM cells under resting conditions, which amounted to 0.46% and 2.03, respectively (Fig 5, A, upper panel), increased corresponding values of 94.29% and 34.52, respectively, were obtained in the coinubated T cell-activated ASM cells (Fig 5, B, upper panel). Comparably, the values of the percentage of positive cell staining and MFI for IL-5 were also similarly increased in the anti-CD3-activated T cells, amounting to 86.04% and 15.56, respectively (Fig 5, B, lower panel), versus the corresponding values of 3.61% and 2.36, respectively, obtained in the resting T cells (Fig 5, A, lower panel). Enhanced intracellular expression of IL-1 β protein was also detected in the activated ASM cells, wherein the values for the percentage of positive staining and MFI amounted to 21.0% and 10.86, respectively (Fig 5, D, upper panel), versus 1.83% and 3.17, respectively, obtained under resting conditions (Fig 5, C, upper panel). In contrast, the percentage of positive intracellular IL-1 β staining was only minimally increased in the activated (Fig 5, D, lower panel) compared with the resting (Fig 5, C, lower panel) T cells (ie, 2.51% vs 3.43%, respectively), although MFI for IL-1 β was notably increased in the activated versus resting T cells (ie, 12.55 vs 2.29, respectively). Thus although the T lymphocytes might have contributed to the release of IL-5 and IL-1 β in the experiments shown in Figs 3 and 4, the intracellular antibody experiments show that human ASM cells also contributed to the release of these cytokines (Fig 5).

Given the above results, we further investigated whether the observed changes in intracellular IL-5 and IL-1 β protein expression in the T cell-activated ASM cells were causally related. In these studies intracellular staining of IL-1 β was assessed in ASM cells exposed to either resting T cells or anti-CD3-activated T cells, both in the absence and presence of pretreatment of the ASM cells with anti-IL-5r mAb. As shown in Fig 6, relative to the low levels detected under resting conditions, the percentage of positive cell staining for IL-1 β was markedly enhanced by greater than 50-fold in ASM cells coincubated with activated T cells. Furthermore, in accordance with the above observations on extracellular release of IL-

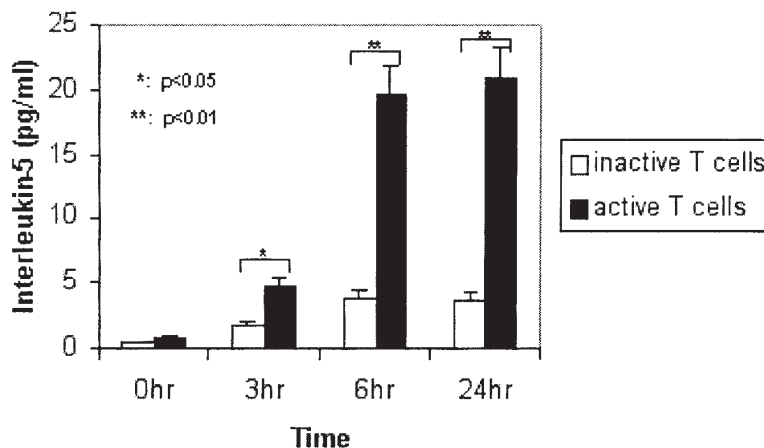


FIG 3. Comparison of the release of IL-5 protein into the culture media of human ASM cells after 0-, 3-, 6-, and 24-hour exposure to resting T cells (*open bars*) and anti-CD3-activated T cells (*filled bars*). Note ASM cells that were exposed to anti-CD3-activated T cells showed significantly increased levels of IL-5 release into the cell culture medium. Data represent mean $\pm$ SE values.

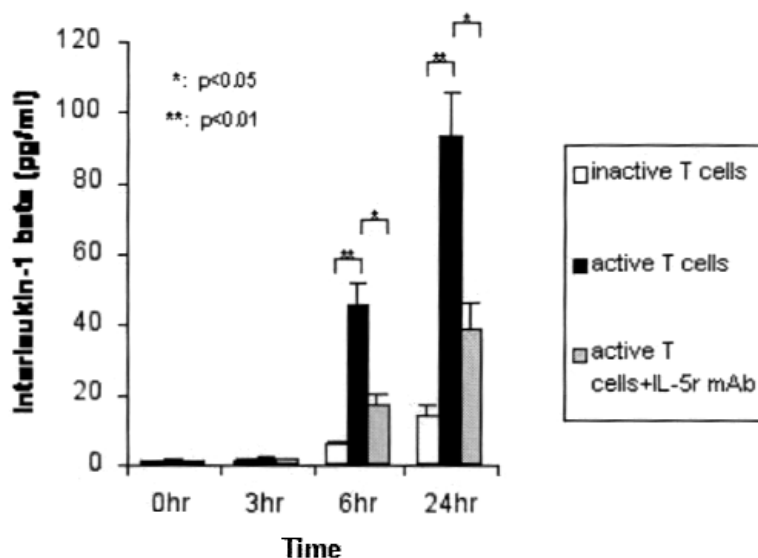


FIG 4. Comparison of the release of IL-1 β protein into the culture media of human ASM cells after 0-, 3-, 6-, and 24-hour exposure to resting T cells (*open bars*) and to anti-CD3-activated T cells in the absence (*filled bars*) and presence (*shaded bars*) of an anti-IL-5r mAb. Note whereas ASM cells that were exposed to anti-CD3-activated T cells showed significantly increased levels of IL-1 β release into the cell culture medium, it was largely prevented by pretreatment of the activated cells with anti-IL-5r mAb. Data represent mean $\pm$ SE values.

1 β protein into the cell culture medium (Fig 4), the increase in intracellular IL-1 β detected in ASM cells co-incubated with activated T cells was largely ablated when the ASM cells were pretreated with anti-IL-5r mAb.

DISCUSSION

Although multiple cell types are known to actively participate in the airway proinflammatory process in allergic asthma, the contemporary view is that CD4⁺ T lymphocytes play a particularly crucial role in both initi-

ating and regulating the perpetuation of the net airway inflammatory response.^{1-10,33} Notwithstanding the important role played by T lymphocytes and other airway infiltrating leukocytes in atopic asthma, however, there is compelling new evidence demonstrating that various proinflammatory cytokines are also expressed by certain non-bone marrow-derived airway structural cells, including the ASM cell,^{20-23,26} and that these cytokines might importantly contribute to the overall pathobiology of allergic asthma.²⁰⁻²² Thus apart from its intrinsic role as a regulator of airway caliber, the ASM itself was also

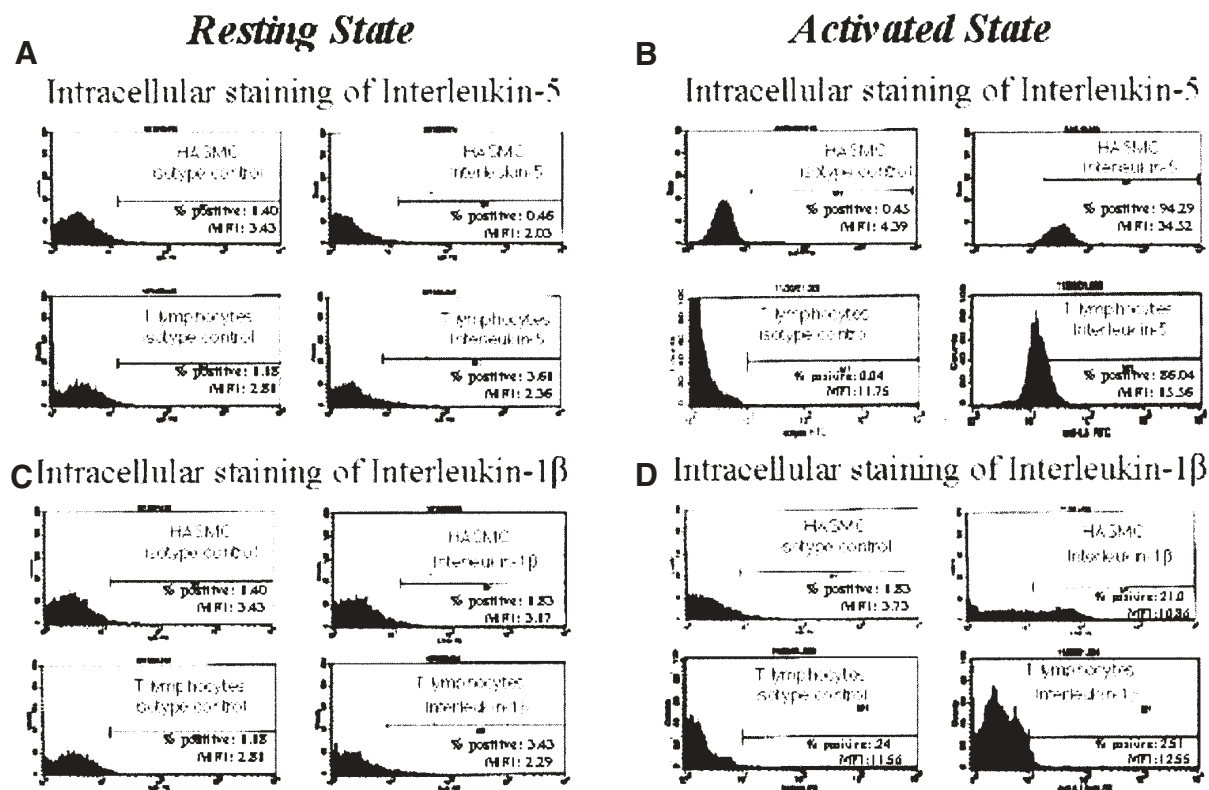


FIG 5. Flow cytometric analysis (percentage of positive cells and MFI) of intracellular staining of IL-5 (A and B) and IL-1β (C and D) proteins in cultured human ASM cells (upper panels, Fig 5, A-D) in the presence of 24 hours' exposure to resting (Fig 5, A and C) versus anti-CD3-activated T cells (Fig 5, B and D) and in T cells (lower panels, Fig 5, A-D) in the absence (Fig 5, A and C) and presence (Fig 5, B and D) of anti-CD3-mediated activation. Note intracellular staining of IL-5 was markedly increased in both ASM and T cells in the activated state. In contrast, whereas intracellular staining of IL-1β was only minimally enhanced in activated T cells, it was markedly increased in ASM cells that were exposed to activated T cells. Data are representative of 2 separate experiments.

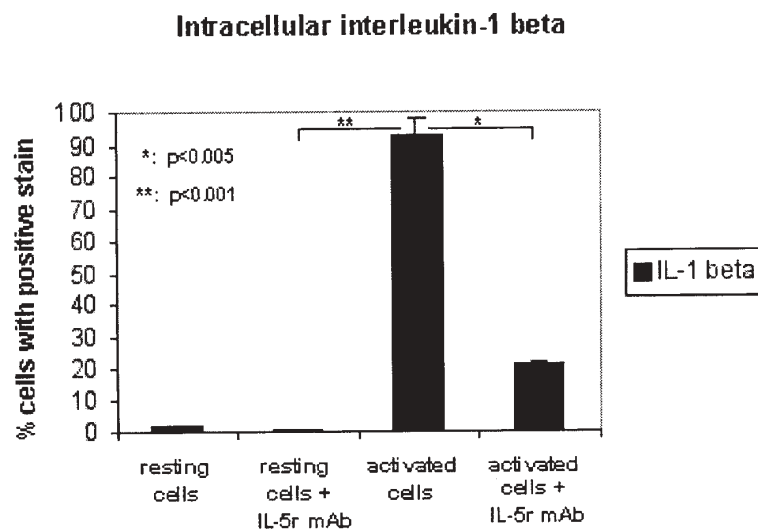


FIG 6. Comparison of intracellular IL-1β protein expression detected by means of flow cytometry in ASM cells coincubated for 24 hours with either resting or anti-CD3-activated T cells in the absence and presence of anti-IL-5r mAb. Note whereas intracellular staining of IL-1β was increased by greater than 50-fold in ASM cells exposed to anti-CD3-activated T cells, it was largely inhibited in the presence of anti-IL-5r mAb. Experiments were performed in triplicate.

identified as a potentially important regulator of the local airway immune response in atopic asthma.^{23,24} In this regard we recently demonstrated that reciprocal cross-talk involving coligation of specific cellular adhesion molecules/costimulatory molecules exists between activated T cells and ASM cells and that the cooperative signaling established by this intercellular communication induces proasthmatic-like phenotypic changes in ASM constrictor and relaxant responsiveness.²⁹ In light of this evidence, together with the crucial role attributed to T-lymphocyte activation in atopic asthma, the present study examined whether the reported cooperative intercellular signaling between activated T cells and ASM cells is coupled to the induced release and actions of IL-5 and IL-1 β , since these cytokines were previously shown to mediate proasthmatic changes in ASM responsiveness both in atopic sensitized and rhinovirus-inoculated ASM.^{24,32} The results provide new evidence demonstrating that exposure of naive ASM cells to anti-CD3-activated T cells elicits their sequential release of the T_H2-type cytokine IL-5 and the pleiotropic proinflammatory cytokine IL-1 β and that both of these cytokines act in a mutually inclusive autocrine fashion to mediate proasthmatic perturbations in ASM responsiveness.

In addressing the collection of evidence supporting the above central findings, certain issues pertaining to the present observations are worthy of consideration. Among these, it is relevant to note that our observed changes in constrictor (Fig 1, top panels) and relaxant (Fig 1, bottom panels) responsiveness of T cell-activated ASM tissues closely mimicked the perturbations in airway function that characterize the *in vivo* asthmatic condition, including enhanced muscarinic cholinergic-mediated bronchoconstriction and impaired β -adrenoceptor-mediated airway relaxation.^{25,27} The present results further demonstrated that these proasthmatic-like changes in agonist responsiveness were prevented in activated T cell-exposed ASM tissues that were pretreated with either anti-IL-5r mAb (Fig 1, A) or IL-1ra (Fig 1, B). These observations are consistent with the substantial collection of previous evidence implicating crucial roles for IL-5 and IL-1 β in mediating the changes in airway responsiveness observed in various *in vivo* animal^{34,35} and *in vitro* tissue^{23-26,36} models of allergic asthma. In this regard it should be noted that although the source of these cytokines is commonly attributed to various activated leukocytes, other non-bone marrow-derived resident tissue cells (eg, epithelial cells and keratinocytes) have been found to also elaborate these and other cytokines under certain proinflammatory conditions.²⁰⁻²² Indeed, among such resident tissue cell types, the ASM cell itself has been shown to release IL-1 β and IL-5, as well as other T_H2-type cytokines, under different proasthmatic experimental conditions, including passive sensitization of ASM with atopic asthmatic serum and rhinovirus inoculation of isolated ASM.^{24,32} Moreover, exogenously administered IL-5 and IL-1 β were each found to elicit comparable proasthmatic-like changes in ASM responsiveness.^{24,32,36} Our present observations

lend extended support to this earlier evidence and further demonstrate that, when exposed to activated T cells, naive ASM cells are induced to autologously express mRNAs (Fig 2) and proteins (Figs 3-5) for both IL-5 and IL-1 β . Furthermore, when examined by means of intracellular staining, enhanced IL-5 protein levels were detected in both the coincubated anti-CD3-activated T cells and ASM cells, and interestingly, although both cell types displayed similarly increased IL-5 protein levels, enhanced expression of IL-1 β was selectively exhibited by the ASM cells (Fig 5).

In further considering the present observations, it is important to note that the temporal patterns of upregulated mRNA expression and release of IL-5 and IL-1 β by the ASM cells were found to be distinctly different, as evidenced by a relatively earlier induced upregulated expression of IL-5 mRNA (Fig 2) and release of IL-5 protein (Figs 3 and 4). These findings raised the consideration that the temporal differences in induced IL-5 and IL-1 β expression were causally related. In addressing this possibility, our extended observations demonstrated that both the enhanced intracellular detection (Fig 6) and release of IL-1 β protein (Fig 4) by T cell-activated ASM cells were largely inhibited when the cells were pretreated with anti-IL-5r mAb. Thus the results support the notion that the induced release of IL-1 β was attributed, at least in part, to an autocrine action elicited by the initial elaboration of IL-5. The latter concept is consistent with our recent findings demonstrating a similar pattern of sequential cytokine signaling in atopic sensitized ASM, wherein an initial induced release of IL-5 protein elicits, in an autocrine manner, the subsequent elaboration and autocrine action of IL-1 β in the atopic sensitized ASM.²⁴ Given this evidence, when considered in light of our present experimental conditions and observations, it is reasonable to speculate that stimulatory cross-talk between activated T lymphocytes and ASM cells is characterized by sequential IL-5/IL-1 β -coupled autocrine signaling that ultimately results in the induction of proasthmatic changes in ASM responsiveness and that this mechanism parallels that which is manifested in the atopic asthmatic sensitized state. To the extent that the present observations pertain to *in vitro* experimental conditions, however, the potential role of the above mechanism of sequential cytokine-driven autocrine signaling in inducing comparable changes in airway responsiveness *in vivo* remains to be established.

The central findings of the present study lend an extended scope to the prevailing concept of a T_H2 cytokine-dependent mechanism underlying the pathobiology of allergic asthma. In this regard, whereas the contemporary T_H2 paradigm related to allergic asthma largely reflects the role played by CD4⁺ T cells expressing the T_H2 phenotype of cytokine release, the present findings expand this model to include a cooperative T_H2-type autocrine role intrinsically expressed by the ASM itself that contributes to proasthmatic changes in ASM responsiveness. In this regard, recent reports have demonstrated the ability of atopic asthmatic serum-sensitized ASM to autologously express both T_H1- and T_H2-type cytokines, as well as the

pleiotropic proinflammatory cytokine IL-1 β ,^{23,24,26} and this phenomenon was largely attributed to activation of the low-affinity receptor for IgE, Fc ϵ RII (CD23), which is endogenously expressed on the ASM cell surface and activated by the increased IgE present in the atopic sensitizing serum.^{25,27} In light of this recent information, together with the observations presented in this study, there is ample evidence to support the notion that, notwithstanding the crucial role independently played by CD4⁺ T_H2 lymphocytes, an extended autocrine T_H2-type cytokine network involving induced IL-5-mediated release and autocrine action of IL-1 β also exists in ASM, which, when stimulated in the presence of activated T cells, cooperatively contributes to overall expression of the proasthmatic phenotype of altered ASM responsiveness.

In conclusion, the present study examined the role and mechanism of T-cell interaction with ASM in eliciting changes in ASM responsiveness. The results provide new evidence demonstrating the following: (1) relative to inactive T cells, incubation of ASM tissues with anti-CD3-activated T cells induces proasthmatic-like changes in agonist-mediated ASM responsiveness; (2) this T cell-induced perturbation in ASM responsiveness is ablated by pretreating the ASM tissues with either anti-IL-5r mAb or IL-1ra, whereas an IL-4 protein neutralizing antibody has no effect; (3) exposure of human ASM cells to anti-CD3-activated T cells elicits an initial increased mRNA expression and release of IL-5 protein from both cell types, followed by an enhanced expression and release of IL-1 β from the ASM cells; and (4) the induced secondary release of IL-1 β is prevented in ASM cells that are pretreated with an anti-IL-5r mAb. Collectively, these observations provide new evidence demonstrating that exposure of naive ASM cells to activated T cells induces the sequential release of IL-5 and IL-1 β from the ASM cells and that the latter cytokines act in an interdependent autocrine manner to elicit proasthmatic changes in ASM responsiveness.

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