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*J Immunol* 2001; 166:293-303; doi: 10.4049/jimmunol.166.1.293

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Bi-Directional Activation Between Human Airway Smooth Muscle Cells and T Lymphocytes: Role in Induction of Altered Airway Responsiveness

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Because both T lymphocyte and airway smooth muscle (ASM) cell activation are events fundamentally implicated in the pathobiology of asthma, this study tested the hypothesis that cooperative intercellular signaling between activated T cells and ASM cells mediates proasthmatic changes in ASM responsiveness. Contrasting the lack of effect of resting human T cells, anti-CD3-activated T cells were found to adhere to the surface of naive human ASM cells, increase ASM CD25 cell surface expression, and induce increased constrictor responsiveness to acetylcholine and impaired relaxation responsiveness to isoproterenol in isolated rabbit ASM tissues. Comparably, exposure of resting T cells to ASM cells prestimulated with IgE immune complexes reciprocally elicited T cell adhesion to ASM cells and up-regulated T cell expression of CD25. Extended studies demonstrated that: 1) ASM cells express mRNAs and proteins for the cell adhesion molecules (CAMs)/costimulatory molecules, CD40, CD40L, CD80, CD86, ICAM-1 (CD54), and LFA-1 (CD11a/CD18); 2) apart from LFA-1, ASM cell surface expression of the latter molecules is up-regulated in the presence of activated T cells; and 3) pretreatment of ASM cells and tissues with mAbs directed either against CD11a or the combination of CD40 and CD86 completely abrogated both the activated T cell-induced changes in expression of the above CAMs/costimulatory molecules in ASM cells and altered ASM tissue responsiveness. Collectively, these observations identify the presence of bi-directional cross-talk between activated T cells and ASM cells that involves coligation of specific CAMs/costimulatory molecules, and this cooperative intercellular signaling mediates the induction of proasthmatic-like changes in ASM responsiveness. The Journal of Immunology, 2001, 166: 293–303.

A wealth of information gathered over the past decade has established a crucial role for CD4+ Th lymphocytes, most notably of the Th2 phenotype, in the pathogenesis of allergic asthma (1–4). Specifically, Th2 cell-derived cytokines, such as IL-4, IL-13, and IL-5, orchestrate various critical humoral and cellular immune functions including IgE synthesis by B cells, as well as eosinophil proliferation, recruitment, and activation, all of which serve to facilitate expression of the airway inflammatory response that characterizes the atopic asthmatic phenotype (5–8). Notwithstanding this fundamental role played by T lymphocytes, together with the actions attributed to other leukocytes (e.g., mast cells, macrophages, eosinophils, etc.), certain nonbone marrow-derived cell types in the lung (e.g., airway epithelial, neurovascular, endothelial cells, fibroblasts, etc.) have also been importantly implicated in the overall pathobiology of allergic asthma (9–12). Among the latter cell types, the resident airway smooth muscle (ASM)3 itself has been recently found to display a variety of proinflammatory actions in the atopic asthmatic sensitized state, including the release of different cytokines such as Th1- and Th2-type cytokines, IL-1β, and others (13–17). Moreover, the autologous release by the ASM of these cytokines, individually or in combination, was found to act in an autocrine manner on the ASM itself to induce proasthmatic-like changes in its constrictor and relaxant responsiveness (18–20). Thus, apart from its obvious functional role as a regulator of airway caliber, given its intrinsic capability to elaborate a variety of proinflammatory cytokines, it is reasonable to hypothesize that the ASM may also serve as a potential regulator of the local airway immune response in the atopic asthmatic sensitized state. In light of the latter hypothesis, the present study examined whether, under specific conditions of cellular activation: 1) isolated ASM tissues and cultured ASM cells can directly communicate with isolated T lymphocytes; and 2) induced stimulatory reciprocal “cross-talk” between these cell types can elicit proasthmatic-like changes in ASM constrictor and relaxant responsiveness. The results provide new evidence demonstrating that reciprocal cross-talk involving ligation of specific costimulatory/cell adhesion molecules exists between activated T cells and ASM cells and that the cooperative signaling established by this intercellular communication induces proasthmatic-like changes in ASM constrictor and relaxant responsiveness.

Materials and Methods

Preparation of ASM tissue and coinoculation 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...
To examine whether activated T lymphocytes have the capacity to induce proinflammatory changes in ASM responsiveness, anti-CD3-activated T cells (see below) were coincubated with isolated rabbit ASM tissue and media were aspirated off the cells at the 24-h time point, thereby separating the T cells from the ASM cells (see below). The T cells were prepared for flow cytometric studies, or further incubated with HASM cells for 0–24 h and, in separate experiments, inactive T cells were coincubated with isolated rabbit ASM tissue and media were aspirated off the cells at the 24-h time point, thereby separating the T cells from the ASM cells (see below). T cell purity was confirmed by staining the cells with an anti-CD3 PE-conjugated Ab, which demonstrated >90% T cell purity and <2% of the isolated cells stained positive with anti-CD19- and anti-CD14-labeled Abs, as assessed by flow cytometry. T cell growth was measured every 24 h using a hemocytometer (Reichert, Buffalo, NY). The cells were counted by taking 10 μl of the culture, pipetting them under the coverslip, and averaging the cell counts within the two 20 × 20-μm areas.

**HASM cell/T cell interaction**

IL-2- and anti-CD3-activated T lymphocytes were incubated with naive HASM cells for 0–24 h and, in separate experiments, inactive T cells were incubated with HASM cells that were activated with IgE-immune complex (see below). The T lymphocytes were subsequently collected and the cells washed three times with PBS. Photographs were obtained with a digital camera under a microscope to determine the level of T cell adherence. Upon removal of the T lymphocytes and media, the HASM cells were scraped off the flask with a rubber spatula. Both sets of cells were then separately prepared and stained for flow cytometric studies, as described below.

**Flow cytometric analysis**

Expression of CAMs/costimulatory cell surface molecules was examined in both HASM cells and T lymphocytes using a Coulter EPICS Elite flow cytometer (Coulter Electronics, 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 positive cells and mean channel fluorescence being recorded. As described above, the culture medium was aspirated off the cells at the 24-h time point, and the cells were prepared for flow cytometric analysis. Five milliliters of Versene (PBS lacking Ca2+ and Mg2+), with 0.2 g/l EDTA and 0.5 M KCl was added to the flask containing the tissue culture dish, which was then incubated for 15 min with 0.1% trypsin to remove the T lymphocytes and media, the HASM cells were scraped off the tissue culture dish. The cells were之力 centrifuged at 1500 rpm for 2 min, resuspended in minimum PBS lacking Ca2+ and Mg2+, and counted by taking 10 μl of the culture, pipetting them under the coverslip, and averaging the cell counts within the two 20 × 20-μm areas.

**Preparation and treatment of cultured ASM cells**

Human airway smooth muscle (HASM) cells were derived from a 16-year-old and a 21-year-old male donor (Clonetics, San Diego, CA) who had no signs of pulmonary disease, and the cells were cultured at 37°C in a humidified atmosphere of 5% CO2/95% air. The cells were examined for changes in the tissues’ agonist-mediated constrictor and relaxation responsiveness. In brief, after anesthesia with xylazine (10 mg/kg) and ketamine (50 mg/kg), rabbits were sacrificed with an overdose of pentobarbital (130 mg/kg). The tracheae were removed via open thoracotomy, cleared of loose connective tissue and epithelium, divided into eight ring segments of 6–8 mm length, and each alternative smooth muscle ring was incubated for 24 h at room temperature in either: 1) DMEM alone, or 2) DMEM in the presence of naïve (resting) vs anti-CD3-activated T lymphocytes (1 × 10⁵/ml), with and without 1 h of pretreatment with various combinations of mouse anti-human CD40 (4 μg/ml), CD40L (4 μg/ml), -CD80 (2 μg/ml), -CD86 (1.5 μg/ml) mAbs, or with anti-CD11a (3 μg/ml) or anti-CD54 (4 μg/ml) mAbs, in separate experiments. The incubation media were aerated with a continuous supplemental O₂ mixture (95% O₂/5% CO₂) during the incubation phase.

**Pharmacodynamic studies**

After incubation of the ASM tissue samples, each segment was suspended longitudinally between stainless steel triangular supports in siliconized Harvard 20-ml organ baths (Harvard Apparatus, South Natick, MA). 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 FT03C force transducer (Validyne Engineering, Northridge, CA) from which isometric tension was continuously displayed on a multichannel recorder, as previously described (21, 22). 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 125 mM NaCl, 14 mM NaHCO₃, 4 mM KCl, 2.25 mM CaCl₂, 1.16 mM MgSO₄·7H₂O, 1.2 mM NaH₂PO₄·H₂O, and 11 mM glucose. The baths were aerated with 5% CO₂ in oxygen; a pH of 7.35–7.40 was maintained, and the organ bath was washed with SFM before the incubations.

**Preparation of human T lymphocytes**

IL-2- and anti-CD3-activated T lymphocytes were isolated from whole blood of nonatopic/nonasthmatic healthy donors (n = 4), mixed with PrepaCyte-SC medium (Bioergonomics), thereby separating the T cells from the erythrocytes, mature granulocytes, and monocytes. The T cells were then centrifuged, washed with PBS, and resuspended in either basal medium alone, activation medium (igen International, Gaithersburg, MD) containing IL-2, or in basal medium containing maximum effective concentrations of anti-CD3 ligand (determined by dose-dependent effects of anti Ab), at a cell concentration of ~1 × 10⁶ cells/ml. The T lymphocytes were then incubated in 24-well tissue culture plates (Costar) in the presence of naive (resting) vs anti-CD3 activated T cells, because these tissues typically exhibited enhanced constrictor sensitivity to ACh (see Results). The relaxant responses to isoproterenol were analyzed in terms 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 (pD₂ₐ₀) value.

**Preparation of human T lymphocytes**

**Preparation and treatment of cultured ASM cells**

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of 15–25 µg of intact RNA from each T-75 flask of HASM cells and per each tissue specimen under study. To analyze for mRNA expression of CD40, CD40L, CD80, CD86, CD54, and CD11a, we used RT-PCR and human-specific primers for these molecules. cDNA was synthesized from total RNA isolated from untreated cells and from human ASM cells passively sensitized with IgE immune complexes and anti-CD3-activated T lymphocytes. The cDNAs were primed with oligo(dt)12–18 and extended with Superscript II reverse transcriptase (Life Technologies, Rockville, MD). The PCR was used to amplify the specific products from each cDNA reaction, based on the published sequences of the human CD40, CD40L, CD80, CD86, CD54, and CD11a genes, and included the following primer sets: CD40: 5′-primer 5′-CTGGGCTAGCGATACAGGAG-3′, 3′-primer 5′-TGAGTTTGGCCAA CAGAAAT-3′, 256-bp product; CD40L: 5′-primer 5′-AAATTGGCAG CATTGCTCATA-3′, 3′-primer 5′-ATCTACGGGGGACTTATTAGG-3′, 240-bp product; CD80: 5′-primer 5′-CATCACCATCAAGTGTCACA-3′, 3′-primer 5′-CAAAAGTGCTGCGTGTGCTG-3′, 255-bp product; CD86: 5′-primer 5′-CTCCTCTGCCTCCTCCTCCT-3′, 3′-primer 5′-ATCTGCTGTAAGTC GAAAT-3′, 319-bp product; CD54: 5′-primer 5′-GAGCTTGTTGAGA CACCTCT-3′, 3′-primer 5′-TCACACTTCTCTCTGACACC-3′, 367-bp product; CD11a: 5′-primer 5′-GTCTCTGCTGAGCCTTACA-3′, 3′-primer 5′-ATGCTGTCGTGAC-3′, 377-bp product; and ribosomal protein L7 (RPL7): 3′-primer 5′-GAAGAGCTCTCATTCTGCCCC-3′, 3′- primer 5′-TCCCTCTGCTGAC-3′, 157-bp product.

The cycling profile used was as follows: denaturation, 95°C for 1 min; annealing, 52–56°C for 1.0–1.5 min; and extension, 72°C for 1.0–1.5 min and 34–40 cycles for the CD40, CD40L, CD80, CD86, CD54, and CD11a genes, and 30 cycles for the RPL7 gene. The number of cycles was determined to be in the linear range of the individual PCR products. The PCR for the individual products were performed using equivalent amounts of cDNA (averaged from 2.5 µg of total RNA, and equal aliquots of each PCR mixture were then run on a 1.2% agarose gel and subsequently transferred to a ζ-probe membrane overnight in 0.4 N NaOH. After capillary transfer, the DNA was immobilized by UV-cross-linking using a Stratallinker UV Cross-linker 2400 at 120,000 mJ/cm² (Stratagene, La Jolla, CA). Hybridization in a Techne (Princeton, NJ) hybridization oven was conducted for 2–3 h at 42°C in 50% formaldehyde, 7% (v/v) SDS, 0.25 M NaCl, 0.12 M NaHPO₄ (pH 7.2), and 1 mM EDTA. Hybridization was for 20 h at 42°C in the same solution. The CD40, CD40L, CD80, CD86, CD54, and CD11a and RPL7 DNA levels were assayed by Southern blot analysis using 32P-labeled probes, prepared by separately pooling several RT-PCRs for the individual PCR fragments and purifying them from a 1.2% agarose gel using the Qiaex II agarose gel extraction kit (Qiagen, Chatsworth, CA).

Statistical analyses
Statistical analyses was performed using the two-tailed paired Student’s t test and ANOVA with multiple comparison of means, where appropriate. Values of p < 0.05 were considered significant.

Reagents
The HASM cells and SmBM were obtained from Clonetics (Walkersville, MD). The human CD40, CD40L, CD80, CD86, CD54, CD11a, and RPL7 primers were purchased from the Integrated DNA Technologies (Corvallis, IA). The mouse anti-human CD40, CD40L, CD80, CD86, and MHC-IIDR mAbs were obtained from Serotec (Raleigh, NC). The CD11a, CD11b, and CD54 mAbs and the anti-mouse secondary Ab and IL-2 were purchased from R&D Systems (Minneapolis, MN). The anti-CD3 activation Ab was prepared in our laboratory from a NK3 hybridoma cell line purchased from American Type Culture Collection (Manassas, VA). ACh and isoprotenerol were purchased from Sigma (St. Louis, MO). All drug concentrations are expressed as final bath concentrations. Isoprotenerol and ACh were made fresh for each experiment and were dissolved in normal saline to prepare 10⁻⁴ M and 10⁻³ M solutions, respectively.

Results
Effects of activated T cells on ASM responsiveness
To assess whether activated T lymphocytes have the capacity to directly provoke changes in ASM tissue responsiveness, agonist constrictor and relaxation responses were compared in isolated rabbit ASM segments that were incubated for 24 h with vehicle alone (control) and with either resting (inactivated) T cells or T cells activated by cross-linking the TCR with anti-CD3 Ab (see Materials and Methods). As shown in Fig. 1, the maximal constrictor responses (Tₘₐₓ) and sensitivities (pD₅₀ values, i.e., −log ED₅₀ values) to exogenously administered ACh obtained in control (○) and resting T cell-exposed (■) ASM tissues were similar. In contrast, relative to controls, the constrictor responses to ACh were significantly enhanced in ASM that were exposed to anti-CD3-activated T cells (●), wherein the mean ± SE Tₘₐₓ values in the control vs activated T cell-exposed tissues, amounted to 76.92 ± 3.4 vs 111.0 ± 7.45 g/g ASM weight, respectively (p < 0.01), and the corresponding pD₅₀ values averaged 5.17 ± 0.09 vs 5.28 ± 0.14 −log M, respectively (p < 0.05).

In separate studies conducted under the same experimental conditions, during comparable levels of initial sustained ACh-induced contractions (averaging ~40% of Tₘₐₓ), administration of the β-adrenoceptor agonist, isoprotenerol, elicited cumulative dose-dependent relaxation of the precontracted ASM segments. As shown in Fig. 2, whereas the Rₘₐₓ responses and sensitivities (pD₅₀ values) to isoprotenerol were similar in control and resting T cell-exposed ASM tissues, the relaxation responses to isoprotenerol were significantly attenuated in ASM exposed to anti-CD3-activated T cells. Accordingly, the mean ± SE Rₘₐₓ values to isoprotenerol amounted to 49.44 ± 8.02 vs 61.5 ± 9.5% in the activated T cell-exposed vs control ASM (p < 0.05), and the corresponding pD₅₀ values averaged 6.14 ± 0.12 vs 6.30 ± 0.06 −log M, respectively (p < 0.05).

Expression and regulation of cell adhesion molecules (CAMs)/costimulatory molecules
Given the above evidence implicating a role for anti-CD3-activated T cells in directly inducing changes in ASM responsiveness, a series of studies were pursued to further elucidate the nature of this T cell/ASM cell interaction and to assess whether reciprocal costimulatory “cross-talk” exists among these cell types. In addressing these issues, initial experiments examined whether

FIGURE 1. Comparison of ASM constrictor responses to ACh in rabbit tissues after 24 h of exposure to vehicle alone (○), resting T cells (○), or anti-CD3-activated T cells (●). Note: Relative to tissues incubated with vehicle alone (n = 6) or with resting T cells (n = 6), both Tₘₐₓ and ED₅₀ responses to ACh were significantly enhanced (p < 0.01 and p < 0.05, respectively) in ASM samples that were coincubated with activated T lymphocytes (n = 6). In contrast, no difference was observed in ACh responses between ASMs that were incubated with inactive T cells and with vehicle alone.
intercellular adhesion between T cells and cultured (near-confluent) human ASM cells is elicited after selective preactivation of either the T cells with anti-CD3 or the ASM cells with IgE immune complexes, as previously described (17, 19). Consistent with previous reports (23), when compared with inactivated T cells (Fig. 3a), anti-CD3-activated T cells inoculated into resting ASM cell cultures displayed (after 24 h) distinctive adhesive clustering on the surface of the resting ASM cells (Fig. 3b). Comparably, similar adhesive clustering was also observed after resting T cells were inoculated into cultures of ASM cells that were preactivated with IgE immune complexes (Fig. 3c).

Because, apart from T cells and other leukocytes, ASM cells were recently shown to also express certain CAMs on their cell surface (22–24), in light of the above observations, we next examined whether specific CAMs and other costimulatory molecules implicated in intercellular immune responses are expressed in ASM and modulated in the presence of ASM cell activation under the above experimental conditions. mRNA and cell surface protein expression of ICAM-1 (CD54), its endogenous counterreceptor ligand, LFA-1 (CD11a/CD18), as well as the “accessory” costimulatory molecules, CD40, CD40L, CD80, and CD86, was first examined in cultured HASM cells in the absence and presence of activation with exogenously administered IgE immune complexes. Using RT-PCR for mRNA analysis, ASM cells were found to constitutively express mRNAs for CD40, CD40L, CD80, and CD86, as well as for CD54 and CD11a, under control conditions (Fig. 4, left). When activated by the administration of IgE immune complexes, with exception to unaltered CD11a expression, these mRNA signals were markedly up-regulated at 3 h and remained enhanced at 24 h (Fig. 4, right). In contrast, mRNA expression of the constitutively expressed RPL7 was unaffected, and the signal was similar in intensity under both treatment conditions. In separate studies, qualitatively similar up-regulation of mRNA expression of the above CAMs/costimulatory molecules was also detected in T cells that were activated by CD3 engagement (data not shown).

In accordance with the above results, using flow cytometric analysis, we found that cell surface expression of the above CAMs/costimulatory molecules was also detected in ASM cells under resting conditions (Fig. 5, left) and that, with the exception of CD11a, expression of these molecules was distinctly enhanced at

![FIGURE 2](http://www.jimmunol.org/)  Comparison of ASM relaxant responses to isoproterenol in rabbit tissues after 24 h of exposure to vehicle alone (○), resting T cells (□) or anti-CD3-activated T cells (■). Note: Relative to tissues incubated with vehicle alone (n = 6) or with resting T cells (n = 6), both R_{max} and pD_{50} responses to isoproterenol were significantly attenuated (p < 0.05 and p < 0.05, respectively) in ASM samples that were coincubated with activated T lymphocytes (n = 6). In contrast, no differences were observed in isoproterenol responses between ASM that were incubated with inactive T cells and with vehicle alone.

![FIGURE 3](http://www.jimmunol.org/)  Representative experiment demonstrating cell-to-cell surface adhesion between cultured HASM cells and isolated peripheral blood T lymphocytes. Note: As shown in b, relative to resting T cells (a), 24 h of exposure of naive human ASM cells to anti-CD3-activated T cells produced notable adhesion between the activated T cells and ASM cells. Comparable adhesion formation was also observed when resting T cells were exposed to IgE-immune complex-activated human ASM cells (c). Experiments were performed in triplicate.

![FIGURE 4](http://www.jimmunol.org/)  mRNAs expression of the costimulatory molecules, CD40, CD40L, CD80, and CD86; the ICAM, CD54, and its natural counterligand, CD11a, in human ASM cells in the absence (left) and presence (right) of 0, 3, and 24 h of exposure to IgE immune complexes. Note: Human ASM cells express mRNAs for the CD40, CD40L, CD80, and CD86, and CD54, as well as for CD11a under control conditions (Fig. 4, left). When activated by the administration of IgE immune complexes, with exception to unaltered CD11a expression, these mRNA signals were markedly up-regulated at 3 h and remained enhanced at 24 h (Fig. 4, right). In contrast, mRNA expression of the constitutively expressed RPL7 was unaffected, and the signal was similar in intensity under both treatment conditions. In separate studies, qualitatively similar up-regulation of mRNA expression of the above CAMs/costimulatory molecules was also detected in T cells that were activated by CD3 engagement (data not shown).

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24 h after activation of the cells with IgE immune complexes (Fig. 5, right). Accordingly, as displayed in Fig. 5, increases in both the percentage of cells positively stained for these proteins and their respective mean fluorescence intensity (MFI) values were observed. Relative to the resting condition, the increases in the percentage of positive staining for CD40, CD40L, CD80, and CD86 amounted to 2.1-, 9.2-, 5.6-, and 4.4-fold, respectively, and the corresponding changes in MFI amounted to 1.3-, 0.95-, 2.1-, and 2.2-fold, respectively. As further depicted in Fig. 5, >95% of ASM cells also stained positively for the MHC class II Ag, HLA-DR, under resting conditions, and the MFI for HLA-DR expression was enhanced by 33% in the presence of ASM cell activation.
Bi-directional activation by stimulated ASM and T cells

In view of the above results, we next examined for related evidence of reciprocal activation between ASM cells and T lymphocytes. In these flow cytometric experiments, using cell surface expression of CD25 (IL-2R protein) as a marker for cell “activation,” we found that CD25 was markedly enhanced both in anti-CD3-activated T cells (Fig. 6A) and in ASM cells that were stimulated with IgE immune complexes (Fig. 6B), with increases in the percentage of positive staining for CD25 in the activated T cells and ASM cells amounting to ~4-fold and 3-fold, respectively. Moreover, as demonstrated by the representative experiments in Fig. 7, when resting T cells were exposed to ASM cells preactivated with IgE immune complexes, their CD25 protein expression was also markedly increased by ~4-fold in the percentage of positive staining (Fig. 7A) and, comparably, when naive ASM cells were exposed to anti-CD3-activated T cells, the ASM cells also displayed up-regulated expression of CD25 (~4-fold increase in the percentage of positive staining), whereas exposure to resting T cells had no effect (Fig. 7B). Finally, in association with these observed changes in CD25 expression, exposure of resting ASM cells to anti-CD3-activated T cells was also found to up-regulate the expression of CD40, CD86, and CD54 on the surface of the naive ASM cells (Fig. 8).

In addressing the potential mechanism(s) underlying the above evidence of T cell/ASM cell reciprocal activation, extended studies demonstrated that this phenomenon is primarily attributed to an induced coligation between specific cell surface CAMs/costimulatory molecules expressed in these cell types. Accordingly, as shown by representative experiments in Fig. 9, anti-CD3-activated T cell-induced up-regulation of CD25, CD40, CD86, and CD54 expression in naive ASM cells was found to be completely inhibited by pretreating the resting ASM cells with either the combination of neutralizing mAbs directed against CD40 and CD86 (i.e., anti-CD40/CD86 mAbs) (Fig. 9, middle) or anti-CD11a mAb

FIGURE 6. Cell surface expression of the “activation marker,” CD25, in isolated peripheral blood T lymphocytes and cultured HASM cells in the absence (left) and presence (right) of cell activation (see Materials and Methods). Data show the percentage of positive cells and MFI values relative to isotype control Abs (left histogram). Note: Expression of CD25 was markedly enhanced (range: 3- to 5-fold) in the anti-CD3-activated T lymphocytes, and comparable induction in CD25 expression was also observed in HASM cells that were exposed to IgE immune complexes. Experiments were performed in triplicate.

FIGURE 7. A, Cell surface expression of the activation marker, CD25, in isolated peripheral blood T lymphocytes after exposure of the cells to resting (left) and IgE immune complex-activated (right) human ASM cells. B, Cell surface expression of CD25 in human ASM cells after exposure of the cells to resting (left) and anti-CD3-activated (right) T cells. Data show the percentage of positive cells and MFI values relative to isotype control Abs (left histogram). Note: CD25 expression in T lymphocytes that were exposed to activated human ASM cells was markedly enhanced (range: 3- to 4-fold), and comparable induction in CD25 expression was also observed in human ASM cells that were exposed to activated T lymphocytes. Experiments were performed in triplicate.
anti-CD40/CD86 mAbs or anti-CD11a mAb. As shown in Fig. 11, absence and presence of pretreatment of the tissues with either posed for 24 h to resting and anti-CD3-activated T cells in the responses to isoproterenol were separately examined in tissues ex-
possibility, ASM constrictor responses to ACh and relaxation re-
naive ASM tissues to activated T cells (Fig. 1). In addressing this
served changes in ASM responsiveness induced by exposure of naive ASM to anti-CD3-activated T cells (data not shown).
In contrast, pretreatment of naive ASM with a mAb directed
against the related β2 integrin, Mac-1 (i.e., anti-CD11b mAb), had no preventative effect on activated T cell-induced changes in ASM responsiveness (data not shown).

Discussion
Bronchial asthma is characterized by exaggerated agonist-induced bronchoconstriction, attenuated β-adrenergic receptor-mediated airway relaxation and airway inflammation, the latter principally involving infiltration of the airways with T lymphocytes, mast cells, and eosinophils (1–8, 25–28). Although the mechanistic interplay between airway inflammation and the associated changes in ASM responsiveness remains to be fully characterized, the wealth of evidence accumulated to date suggests that the altered airway responsiveness in asthma is primarily attributed to the actions of various cytokines, which are primarily produced by infiltrating CD4+ T lymphocytes expressing the Th2 profile of cytokine release (1–8, 27, 28). In keeping with this notion, the observed changes in ASM responsiveness in asthmatic individuals and in animal models of allergic asthma have been associated with elevated bronchoalveolar lavage fluid and serum levels of the Th2-type cytokines, notably IL-4, IL-5, IL-13, and GM-CSF (15, 16, 27–30). Moreover, relative to nonatopic, nonasthmatic subjects, mononuclear cells isolated from peripheral blood and bronchoalveolar lavage fluid samples obtained from atopic asthmatic patients have also been found to display enhanced production of these Th2-type cytokines when these cells are activated in vitro, whereas their profile of Th1-type cytokine expression is suppressed (30, 31). Thus, although multiple cell types are known to actively participate in the proinflammatory reaction in allergic asthma, it is the general consensus that CD4+ T lymphocytes play a critical role in regulating both the initiation and perpetuation of the net airway inflammatory response in this disease (1–3, 31, 32).

Notwithstanding the crucial role played by T lymphocyte and other airway infiltrating leukocytes in atopic asthma, emerging new evidence demonstrates that various cytokines, including those of the Th1 and Th2 phenotypes, are also expressed by certain nonbone marrow-derived airway structural cells, such as bronchial epithelial cells and microvascular endothelial cells (9–12), as well as by the ASM cell itself (13–15, 18). In this regard, apart from its intrinsic role as a regulator of airway caliber, given its extended capacity to elaborate a variety of cytokines, it is reasonable to speculate that the ASM may also serve as a regulator of the local airway immune response in atopic asthma. In light of this consideration, and given the well-established importance of T lymphocyte activation in the overall pathobiology of atopic asthma, this study examined the potential mechanistic interplay between activated T cells and ASM cells in the regulation of changes in ASM responsiveness. The results provide new evidence demonstrating that reciprocal cross-talk involving ligation of specific CAMs/costimulatory molecules exists between activated T cells and ASM alone (Fig. 9, right). Comparably, pretreatment of resting T cells with either anti-CD40/CD86 mAbs or anti-CD11a mAb alone was also found to completely prevent their up-regulated expression of CD25 and the above CAMs/costimulatory molecules in response to inoculation into ASM cell cultures preactivated with IgE immune complexes (data not shown). Finally, in concert with these results, pretreatment of naive ASM cells with either anti-CD40/CD86 mAbs or anti-CD11a mAb was further found to abrogate the adhesion of anti-CD3-activated T cells after their inoculation into the ASM cell cultures (Fig. 10).

Role of CAMs/costimulatory molecules in mediating T cell-induced changes in ASM responsiveness
The above results implicating CAM/costimulatory molecules in ASM cell/T cell reciprocal activation raised the consideration that coligation of these molecules with their endogenous counter-receptor ligands may be responsible for eliciting the observed changes in ASM responsiveness induced by exposure of naive ASM tissues to activated T cells (Fig. 1). In addressing this possibility, ASM constrictor responses to ACh and relaxation responses to isoproterenol were separately examined in tissues exposed for 24 h to resting and anti-CD3-activated T cells in the absence and presence of pretreatment of the tissues with either anti-CD40/CD86 mAbs or anti-CD11a mAb. As shown in Fig. 11, pretreatment with either anti-CD40/CD86 mAbs (Fig. 11a) or anti-
CD11a mAb (Fig. 11b) prevented the induction of increased constrictor responsiveness of ASM tissues exposed to anti-CD3-activated T cells. Similarly, pretreatment with either anti-CD40/CD86 mAbs (Fig. 12A) or anti-CD11a mAb (Fig. 12B) also largely abrogated the induction of impaired relaxation responsiveness to isoproterenol in ASM exposed to the activated T cells. In accordance with these observations, extended experiments further demonstrated that pretreatment of ASM tissues with a neutralizing mAb directed against ICAM-1, the endogenous counterreceptor ligand for CD11a/CD18, also completely prevented the changes in agonist constrictor and relaxant responsiveness induced by exposure of naive ASM to anti-CD3-activated T cells (data not shown).

![ASM cells exposed to activated T cells](image_url)
cells and that the cooperative signaling established by this intercellular communication induces proasthmatic-like phenotypic changes in ASM constrictor and relaxant responsiveness.

To our knowledge, this study is the first to demonstrate that anti-CD3-activated T lymphocytes have the capacity to directly elicit proasthmatic-like changes in agonist-induced ASM responsiveness, including increased ASM contractility to ACh (Fig. 1) and impaired β-adrenoceptor-mediated ASM relaxation (Fig. 2). To address the mechanism underlying this phenomenon, a series of studies were conducted to examine for evidence of direct T cell/ASM cell interaction and to further assess whether reciprocal costimulatory “cross-talk” exists among these cell types. As shown in Fig. 3, intercellular adhesion was elicited between anti-CD3-activated T cells and HASM cells. The finding of induced adhesion between activated T cells and naive ASM cells in culture (Fig. 3b) is consistent with previous reports (23, 33), and the present results further demonstrate that such intercellular adhesion is similarly elicited by exposure of resting T cells to cultured ASM cells preactivated with IgE immune complexes (Fig. 3c). In this context, it should be noted that our rationale for using IgE immune complexes to initially selectively activate the ASM cells was based on our previous studies demonstrating that ASM cells express the low affinity receptor to IgE, FcεRII (CD23), and that exposure of the ASM cells to IgE (and most notably to IgE immune complexes) effectively stimulates the cells via binding and activation of FcεRII on the ASM cell surface (17, 19). The efficacy of this approach to activate the ASM cells was further evidenced in the present study, wherein the results demonstrated that treatment of the cells with IgE immune complexes evoked up-regulation of their cell surface expression of various CAMs/costimulatory molecules (Figs. 4 and 5).

Constitutive expression of specific CAMs on the surface of ASM cells has been previously reported (6, 22, 23), including ICAM-1 (CD54), LFA-1 (CD11a/CD18), and VCAM-1 (6, 22–24). Moreover, we recently identified that ICAM-1 expression in ASM cells and tissue is up-regulated after inoculation of the ASM with rhinovirus-16 (22) or exposure of the ASM to atopic asthmatic serum (24). In contrast, neither experimental condition was found to be associated with altered expression of LFA-1 (22, 24). Our present results largely concur with these recent findings and demonstrate that, whereas CD54 mRNA and surface protein are up-regulated in ASM cells treated with IgE immune complexes, expression of CD11a is essentially unaltered (Figs. 4 and 5). The present observations provide additional new evidence demonstrating that the intercellular adhesion/costimulatory molecules, CD40, CD40L, CD80, and CD86, as well as the MHC class II Ag, HLA-DR, are also constitutively expressed by ASM cells and that the expression of these molecules is up-regulated after exposure of the ASM cells to IgE immune complexes (Figs. 4 and 5).
Although the latter molecules are known to be characteristically expressed on the surface of various inflammatory cells and professional APCs, the observations herein support the concept that ASM cells possess the cell surface molecular machinery for potential coligation with inflammatory cells involved in local (peripheral tissue) immune responses. Indeed, in this context, identification of the above CAMs/costimulatory molecules on the surface of ASM cells, together with their observed up-regulated expression in the ASM activated state, suggested a potential mechanistic role for these molecules in ASM cell/T cell coactivation, as discussed below.

Up-regulated expression of the cell activation marker, CD25, was independently elicited in anti-CD3-activated T cells and in ASM cells stimulated by IgE immune complexes (Fig. 6). Moreover, the results demonstrated that CD25 expression (Fig. 7), together with cell surface expression of CD40, CD86, and CD54, was also up-regulated in resting T cells and ASM cells when either cell type was co-incubated with its preactivated counterpart (Fig. 8). Thus, both cell types exhibited evidence of reciprocal activation, supporting the concept that there exists a dynamic interaction involving bi-directional signaling between these cell types. Additionally, because the observed stimulatory effects of this reciprocal activation on CD25 expression in both T cells and ASM cells (Fig. 7) were roughly comparable in magnitude to those elicited by pre-activation of either cell type independently (Fig. 6), the results suggest that the phenomenon of T cell/ASM cell reciprocal activation represents a potent mechanism of intercellular communication. In considering these observations, it is relevant to note that previous studies have reported that vascular smooth muscle cells can also activate both allogeneic and HLA-matched T cells, as reflected by induced expression of CD25 and release of IL-2 by the T cells (34, 35). Although, in the latter studies, the induced T cell activation was not accompanied by enhanced T cell proliferation (detected by [3H]thymidine incorporation), due to arrest of the T cells in the G1 phase of the cell cycle, these data suggested that the vascular smooth muscle cells were capable of processing and presenting Ag to T cells (34, 35). Our results herein fundamentally concur with this concept in demonstrating that ASM cells express the above collection of CAMs/costimulatory molecules and, by virtue of their high constitutive expression of the MHC class II protein, HLA-DR, the possibility is raised that ASM cells also have the capacity to act as APCs. In this respect, Ag presentation to both autologous and allogeneic T cells by nonprofessional APCs has been clearly demonstrated in other parenchymal cell types, including keratinocytes, fibroblasts, epithelial cells, and endothelial cells (36–38). However, with respect to smooth muscle cells, although several studies have implicated such cells as potential APCs (39, 40), other reports have suggested that smooth muscle cells, including ASM cells (23), may inhibit the process of T cell activation via the release of a putative inhibitory factor (e.g., cytokine), resulting in inhibition of the progression of activated T cell clones through the cell cycle, despite their increased expression of CD25 and release of IL-2 (34, 35). Thus, evidence defining the role of smooth muscle cells as APCs is, at present, somewhat inconclusive, and this issue remains to be systematically investigated.

In addressing the potential mechanism underlying T cell/ASM cell reciprocal activation, our extended present observations demonstrated that responses related to this phenomenon were completely abrogated by pretreating either cell type with anti-CD40/CD86 mAbs or with anti-CD11a mAb alone (Figs. 9 and 10). Thus, the results suggest that induced molecular interactions involving coligation of CD40, CD86, and LFA-1 with their respective CAMs/counterreceptor ligands are events that fundamentally underlie T cell/T cell reciprocal activation.
cell/ASM cell adhesion and reciprocal activation. Moreover, in this respect, to the extent that anti-CD40/CD86 mAbs and anti-CD11a mAb also abrogated the proasthmatic-like changes in ASM tissue responsiveness elicited by exposure of the tissues to activated T cells (Figs. 11 and 12), the results further imply that the mechanism of T cell-induced changes in ASM responsiveness is also fundamentally dependent on intercellular coligation involving these CAMs/costimulatory molecules. In this connection, it should be noted that molecular coligation involving T cells and other cell types is a process typically characterized by the evoked release of various cytokines (2–5, 30–32, 41–43). In this regard, with respect to ASM activation, we recently identified that the elicitation of proasthmatic-like changes in agonist responsiveness (i.e., as depicted herein) in ASM tissues exposed to atopic asthmatic serum is primarily prevented by pretreating the ASM tissues with either the combination of anti-CD40 and CD86 mAbs combined (A), or with anti-CD11a mAb alone (B). Note: Relative to tissues incubated with resting T cells, both the R_max and pD_20 responses to isoproterenol were significantly attenuated (p < 0.05 and p < 0.05, respectively) in ASM segments that were exposed to activated T cells, whereas the latter effects were primarily prevented by pretreating the ASM tissues with either the combination of anti-CD40 and CD86 mAbs (A) or anti-CD11a mAb alone (B).

FIGURE 12. Comparison of ASM relaxant responses to isoproterenol in rabbit tracheal tissues after 24 h of exposure to resting T cells (○) or to anti-CD3-activated T cells in the absence (■) and presence (□) of pretreatment with anti-CD40 and anti-CD86 mAbs combined (A), or with anti-CD11a mAb alone (B).


