Intrinsic ICAM-1/LFA-1 activation mediates altered responsiveness of atopic asthmatic airway smooth muscle

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Grunstein, Michael M., Hakon Hakonarson, Neil Maskeri, Cecilia Kim, and Sing Chuang. Intrinsic ICAM-1/LFA-1 activation mediates altered responsiveness of atopic asthmatic airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 278: L1154–L1163, 2000.—Cell adhesion molecules (CAMs) have been importantly implicated in the pathobiology of the airway responses in allergic asthma, including inflammatory cell recruitment into the lungs and altered bronchial responsiveness. To elucidate the mechanism of CAM-related mediation of altered airway responsiveness in the asthmatic state, the expressions and actions of intercellular adhesion molecule-1 (ICAM-1) and its counterreceptor ligand lymphocyte function-associated antigen-1 (LFA-1; i.e., CD11a/CD18) were examined in isolated rabbit airway smooth muscle (ASM) tissues and cultured human ASM cells passively sensitized with sera from atopic asthmatic patients or nonatopic nonasthmatic (control) subjects. Relative to control tissues, the atopic asthmatic sensitized ASM exhibited significantly enhanced maximal contractility to acetylcholine and attenuated relaxation responses to isoproterenol. These proasthmatic changes in agonist responsiveness were ablated by pretreating the atopic sensitized tissues with a monoclonal blocking antibody (MAb) to either ICAM-1 or CD11a, whereas a MAB directed against the related β2-integrin Mac-1 had no effect. Moreover, relative to control tissues, atopic asthmatic sensitized ASM cells displayed an autologously upregulated mRNA and cell surface expression of ICAM-1, whereas constitutive expression of CD11a was unaltered. Extended studies further demonstrated that 1) the enhanced expression and release of soluble ICAM-1 by atopic sensitized ASM cells was prevented when cells were pretreated with an interleukin (IL)-5-receptor-α blocking antibody and 2) administration of exogenous IL-5 to naïve (nonsensitized) ASM cells induced a pronounced soluble ICAM-1 release from the cells. Collectively, these observations provide new evidence demonstrating that activation of the CAM counterreceptor ligands ICAM-1 and LFA-1, both of which are endogenously expressed in ASM cells, elicits autologously upregulated IL-5 release and associated changes in ICAM-1 expression and agonist responsiveness in atopic asthmatic sensitized ASM.

The characteristic features of the airways in bronchial asthma include enhanced agonist-mediated bronchoconstriction, impaired β-adrenoceptor-mediated airway relaxation, and bronchial inflammation, the latter primarily involving infiltration of the airways with eosinophils, lymphocytes, and mast cells. In concert with the contemporary overall view of the development of bronchial inflammation and its association with changes in airway responsiveness, a number of studies (6, 13, 32, 39, 43) have identified important roles for various cell adhesion molecules (CAMs) in orchestrating the net process of inflammatory cell activation and recruitment into the affected airways. In this regard, among other relevant cell adhesion interactions, a critical role for the binding of intercellular adhesion molecule (ICAM)-1 (CD54) to its β2-integrin counterreceptor ligand lymphocyte function-associated antigen-1 (LFA-1; i.e., CD11a/CD18) has been demonstrated in a wide variety of cellular interactions including T-lymphocyte antigen-specific responses, leukocyte binding to vascular endothelium, and emigration of leukocytes into inflammatory foci (1, 8, 36). Moreover, in relation to allergic asthma, ICAM-1 activation has been proposed as mediating the airway eosinophilia and hyperresponsiveness found in a primate model of allergic asthma after chronic in vivo antigen challenge (43). Similarly, a crucial role for ICAM-1 activation in mediating antigen-induced airway responses was also demonstrated in studies with other experimental models (4, 5, 26, 33, 37, 44). The collection of evidence from these studies implicates ICAM-1 activation that is coupled to leukocyte (principally, eosinophil) influx in mediating the observed changes in airway responsiveness. However, the mechanistic interplay between ICAM-1 activation, airway leukocyte infiltration, and altered airway responsiveness remains largely unidentified. In this connection, in a recent study (38) conducted on antigen-sensitized Brown-Norway rats, administration of an anti-ICAM-1 antibody was found to attenuate the animals’ airway constrictor hyperresponsiveness without producing a concomitant decrease in airway inflammation. Thus although ICAM-1 activation may importantly contribute to the development of airway hyperresponsiveness, the mechanism underlying this phenomenon may not be completely dependent on inflammatory cell infiltration of the airways. Indeed, in considering the mechanism of action of ICAM-1, it is relevant to note that, apart from leukocytes per se, cell surface expression of...
ICAM-1 has also been identified in a variety of non-
bone marrow-derived cell types including certain air-
way structural cells and smooth muscle cells (21, 27,
34, 42). Moreover, expression of ICAM-1 on lung stro-
mal cells and epithelial cells was found to be upregu-
lated after exposure of asthmatic subjects to allergen
(15, 29), and ICAM-1 expression was also reportedly
increased in bronchial microvascular endothelial cells
isolated from asthmatic individuals (3). Taken to-
gether, these findings raise the notion that ICAM-1
activation on resident airway tissue cells may, at least
in part, contribute to the changes in airway responsive-
ness that characterize the proasthmatic phenotype.

In light of the above considerations, together with
more recent evidence demonstrating that the airway
smooth muscle (ASM) itself can be induced to autol-
gously express and respond to its autocrine release of
certain bronchoactive cytokines in the atopic asthmatic
sensitized state (17, 19, 20), the present study exam-
ined whether ASM endogenously expresses the CAM
counterreceptor ligands ICAM-1 and LFA-1 and whether
altered ICAM-1/LFA-1 expression and activation leads
to cytokine-coupled phenotypic changes in ASM respon-
siveness in the atopic asthmatic sensitized state. The
results provide new evidence demonstrating that 1)
pro-asthmatic-like changes in ASM responsiveness ob-
tained in atopic asthmatic serum-sensitized ASM are
mediated by ICAM-1/LFA-1 activation; 2) this process
is associated with an autologously upregulated cell
surface expression of ICAM-1 by the atopic sensitized
ASM, whereas LFA-1 expression is unaltered; and 3)
the enhanced expression of ICAM-1 is attributed to an
induced autocrine release and action of the T helper cell
type 2 (Th2)-type cytokine interleukin (IL)-5 in the
sensitized ASM itself. Collectively, these findings sup-
port the novel concept that activation of the CAM
counterreceptor ligands ICAM-1 and LFA-1, both of
which are endogenously expressed in ASM cells, leads
to cytokine-mediated autocrine changes in ASM respon-
siveness in the atopic asthmatic sensitized state.

RESULTS

Preparation and sensitization of rabbit ASM tissue.
Preparation of cultured human ASM cells.
Pharmacodynamic studies.

METHODS

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

Preparation and sensitization of rabbit ASM tissue. After
general anesthesia with xylazine (10 mg/kg) and ketamine
(50 mg/kg), the rabbits were killed with an overdose of
pentobarbital sodium (130 mg/kg). As previously described
(18), the trachea were removed via an open thoracotomy, the
loose connective tissue and epithelium were scraped and
removed, and the trachea were divided into eight ring
segments 6–8 mm long. Each alternate ring was incubated
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Preparation of cultured human ASM cells. Cultured hu-
man ASM cells were obtained from Clonetics (San Diego, CA). The ASM cells were derived from two male donors 16 and 21
yr of age who had no evidence of lung disease. The cells were

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cholinergic contraction, and sensitivity to the relaxing agent was determined as the negative logarithm of the dose of the relaxing agent producing 50% of $R_{\text{max}}$ (pD$_{50}$, i.e., geometric mean E$_{D_{50}}$).

Determination of ICAM-1 and CD11a mRNA expression in human ASM cells. Total RNA was isolated from the ASM cell preparations with the modified guanidinium thiocyanate-phenol-chloroform extraction method to include proteinase K (in 5% SDS) for digestion of protein in the initial RNA pellet as previously described by our laboratory (16, 17). 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 ICAM-1 and CD11a, we used a RT-PCR protocol that included human-specific primers for these genes as well as for the constitutively expressed ribosomal protein (RP) L7. cDNA was synthesized from total RNA isolated from ASM cells passively sensitized with human control or atopic asthmatic serum. The cDNA was primed with oligo(dT)$_{22-18}$ and extended with Superscript II RT (GIBCO BRL, Life Technologies). PCR was used to amplify the specific products from each cDNA reaction based on the published sequences of the human ICAM-1, CD11a, and RPL7 genes and including the following primer sets: 5'-GTCTCTCTCTGTAAGCTTACA-3' (5'-primer) and 5'-ATCTTTCTACCTTCCAGCAC-3' (3'-primer) for CD11a (product is 337 bp); 5'-GAGCTTGTGAAACACTCT-3' (5'-primer) and 5'-TCACACATTCTGACACTCT-3' (3'-primer) for ICAM-1 (product is 367 bp); and 5'-AAGAGGCTTCTATTGTCGTTG-3' (5'-primer) and 5'-TCCGTTCCTCCCATATAAC-3' (3'-primer) for RPL7 (product is 157 bp). The cycling profile used was 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, with 35 cycles for ICAM-1 and CD11a and 26 cycles for the RPL7 genes. The number of cycles was determined to be in the linear range of the PCR products. The PCRs for the human ICAM-1, CD11a, 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 subsequentially transferred to a Zeta-probe membrane overnight in 0.4 M NaOH. After capillary transfer, DNA was immobilized by ultraviolet cross-linking with a Stratalinker UV Crosslinker 2400 at 120,000 µJ/cm$^2$ (Stratagene). Prehybridization in a Techne hybridization oven was conducted for 2–3 h at 42°C in 50% formaldehyde, 7% (wt/vol) SDS, 0.25 M NaCl, 0.12 M Na$_2$HPO$_4$ (pH 7.2), and 1 mM EDTA. Hybridization was for 20 h at 42°C in the same solution. The ICAM-1, CD11, and RPL7 DNA levels were assayed by Southern blot analysis with $^{32}$P-labeled probes prepared by pooling several RT-PCRs for the individual CAM 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 0.1x SSC-0.1% SDS (both at room temperature), and 2 × 1 min at 50°C in 0.1x SSC-0.1% SDS. Southern blots were quantitated by direct measurements of radioactivity in each band with a PhosphorImager (Molecular Dynamics).

Determination of ICAM-1 and CD11a protein expression in ASM cells by flow cytometry. Cell surface protein expression of ICAM-1 and CD11a was examined in the cultured human ASM cells with a Coulter EPICS Elite flow cytometer (Coulter EPICS Division, Hialeah, FL) equipped with a 5-W argon laser operated at 488 nM and 300-mW output. Fluorescence signals were accumulated as two-parameter fluorescence histograms, with both percent positive cells and mean channel fluorescence intensity (MFI) being recorded. Cells treated for 24 h with 10% control or 10% atopic asthmatic serum were carefully washed, scraped from the culture flasks, and then resuspended in PBS buffer. The cells were then dispersed by pipetting through a 23-gauge needle and orbital shaking and then stained with mouse anti-human monoclonal antibodies to ICAM-1 and CD11a. To examine for nonspecific binding, the primary antibody was replaced by immunoglobulins of the same isotype following the manufacturer’s protocol, with mouse IgG1 as a negative control. After serial washing, the cells were stained with FITC-conjugated goat anti-mouse secondary antibody. The antibody-stained cells were then evaluated by flow cytometry and analyzed with the Elite Immuno 4 statistical software (Coulter EPICS Division). Fluorescence intensities are expressed as percent positive cells as well as MFI.

ELISA measurements of IL-5 and sICAM-1 protein release. IL-5 and sICAM-1 protein levels were initially measured in the control and atopic asthmatic sera. Thereafter, the protein levels were assayed in the culture medium of ASM cells that were exposed for 24 h to either control or atopic asthmatic sera. Both the absence and presence of specific MAb, including anti-ICAM-1 MAb, anti-CD11a MAb, or IL-5Ra antibody, sICAM-1 protein levels were also assayed in the culture medium of ASM cells at 0, 3, 6, 12, and 24 h after treatment with SFM alone or with SFM containing a maximal effective concentration of IL-5. The IL-5 and sICAM-1 protein levels were quantitatively assessed with an enzyme-specific immunoassay as previously described by our laboratory (20). The latter assay was performed with a double-antibody sandwich strategy in which an acetylcholinesterase-Fab-conjugated IL-5- or sICAM-1-specific secondary antibody is first targeted to an IL-5- or sICAM-1-captured antibody. The enzymatic activity of acetylcholinesterase was measured spectrophotometrically, and relative to a linear standard curve, the results were used to quantify the amounts of targeted IL-5 and sICAM-1 present in the cell culture medium.

Reagents. The human ASM cells and SMBM were obtained from Clonetics (San Diego, CA). The ICAM-1, CD11a, and RPL7 primers were obtained from Integrated DNA Technologies, (Coralville, IA). Anti-ICAM-1 MAb, anti-CD11a MAb, anti-CD11b MAb, anti-IL-5Ra antibody, the IL-5 and sICAM-1 ELISA kits, the mouse anti-human ICAM-1 and CD11a primary antibodies, and the anti-mouse secondary antibody used in the protein assay studies were purchased from R&D Systems (Minneapolis, MN). The FITC-mouse antibodies to human ICAM-1 and CD11a used in the flow cytometric studies were purchased from Caltag. 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 or ANOVA with multiple comparison of means where appropriate. P values < 0.05 were considered significant.

RESULTS

Role of ICAM-1/LFA-1 activation in regulating ASM responsiveness in the atopic asthmatic sensitized state. Agonist constriction and relaxation responses were separately examined in isolated rabbit tracheal ASM segments that were incubated for 24 h in either human...
atopic asthmatic serum or serum from nonatopic non-
asthmatic (i.e., control) individuals in both the absence
and presence of an anti-ICAM-1 MAb or blocking
antibodies to the natural counterreceptor ligands for
ICAM, including LFA-1 (i.e., anti-CD11a MAb) and
Mac-1 (i.e., anti-CD11b MAb; see METHODS). As depicted
in Fig. 1A, relative to control serum-exposed tissues,
the maximal constrictor responses (\(T_{\text{max}}\)) and sensitivi-
ties (\(pD_{50}\), i.e., \(-\log \text{ED}_{50}\) values) to exogenously admin-
istered ACh were significantly enhanced in ASM pas-
sively sensitized with atopic asthmatic serum.
Accordingly, the mean \(T_{\text{max}}\) values amounted to 110.0 \(\pm\) 13.4 and 138.7 \(\pm\) 15.1 g/g ASM weight in the control
and atopic sensitized tissues, respectively (\(P < 0.01\)),
and the corresponding mean \(pD_{50}\) values amounted to
5.28 \(\pm\) 0.05 and 5.74 \(\pm\) 0.07 \(-\log \text{M}\), respectively (\(P < 0.05\)). These induced, heightened constrictor responses to
ACh were abrogated in atopic sensitized ASM that
was pretreated with a maximally effective concentra-
tion (i.e., 350 ng/ml) of anti-ICAM-1 MAb (Fig. 1A). In
these tissues, the mean \(T_{\text{max}}\) and \(pD_{50}\) values amounted to
108.3 \(\pm\) 15.0 g/g ASM and 5.22 \(\pm\) 0.06 \(-\log \text{M}\),
respectively, and the latter determinations were simi-
lar to those obtained in control serum-treated ASM.
Similarly, in comparable experiments, we found that
pretreatment of atopic asthmatic serum-sensitized ASM
with anti-CD11a MAb (12.5 µg/ml) also ablated the
heightened constrictor responsiveness of the tissues to
ACh (Fig. 1B). Accordingly, in these studies, the \(T_{\text{max}}\)
values obtained in the atopic asthmatic serum-sensi-
tized and control serum-sensitized tissues averaged
129.5 \(\pm\) 7.9 and 104.1 \(\pm\) 13.2 g/g ASM, respectively (\(P < 0.01\)),
and the corresponding \(pD_{50}\) values amounted to
5.03 \(\pm\) 0.05 and 4.82 \(\pm\) 0.06 \(-\log \text{M}\), respectively (\(P <
0.01\)). In the presence of anti-CD11a MAb, the \(T_{\text{max}}\)
and \(pD_{50}\) values in the sensitized ASM were similar to those
obtained in the control serum-treated tissues and aver-
aged 101.9 \(\pm\) 11.2 g/g ASM and 4.79 \(\pm\) 0.06 \(-\log \text{M}\),
respectively. In contrast to these observations, in sepa-
rate studies, we found that the augmented constrictor
responses to ACh in atopic sensitized ASM were una-
fected by pretreatment of the sensitized tissues with
the anti-Mac-1-specific antibody (anti-CD11b MAb). In
these studies, the mean \(T_{\text{max}}\) and \(pD_{50}\) values averaged
109 \(\pm\) 8.9 g/g ASM and 5.01 \(\pm\) 0.05 \(-\log \text{M}\), respec-
tively, and both values were not significantly different
from the corresponding measurements obtained in
sensitized ASM in the absence of anti-CD11b MAb,
which amounted to 113 \(\pm\) 10.9 g/g ASM and 5.04 \(\pm\) 0.06
\(-\log \text{M}\), respectively. Moreover, in parallel experi-
ments, neither anti-ICAM-1 MAb, anti-CD11a MAb,
nor anti-CD11b MAb was found to appreciably affect
the ASM constrictor responsiveness to ACh in control
serum-exposed tissues (data not shown).

In further studies during comparable levels of ini-
tial sustained ACh-induced contractions in control
and atopic asthmatic sensitized ASM segments, averag-
ing \(\sim 50\%\) of \(T_{\text{max}}\) administration of the \(\beta\)-adrenoceptor
agonist isoproterenol elicited cumulative dose-depen-
dent relaxation of the precontracted tissues. As de-
picted in Fig. 2A, relative to control serum-treated
ASM, the \(R_{\text{max}}\) and \(pD_{50}\) values to isoproterenol were
significantly attenuated in the corresponding atopic
asthmatic sensitized tissues. Accordingly, the mean
\(R_{\text{max}}\) value for isoproterenol amounted to 22.2 \(\pm\) 6.1%
(SE) in the atopic sensitized ASM compared with
47.7 \(\pm\) 4.8% in the control serum-exposed ASM (\(P <
0.005\)), and the corresponding \(pD_{50}\) values averaged
6.15 \(\pm\) 0.05 and 6.36 \(\pm\) 0.04 \(-\log \text{M}\), respectively (\(P <
0.05\)). These attenuated relaxation responses to isopro-
terенол were unaffected in sensitized ASM segments
pretreated with anti-CD11b MAb where their \(R_{\text{max}}\)
and \(pD_{50}\) values averaged 27.3 \(\pm\) 6.1% and 6.07 \(\pm\) 0.07
\(-\log \text{M}\), respectively. In contrast, the impaired relaxation
responses to isoproterenol were prevented in atopic
asthmatic sensitized tissues that were pretreated with
anti-ICAM-1 MAb (Fig. 2A) where the mean \(R_{\text{max}}\)
and \(pD_{50}\) values averaged 45.2 \(\pm\) 4.9% and 6.35 \(\pm\) 0.05
\(-\log \text{M}\), respectively. Similarly, in comparable experiments,
the impaired isoproterenol-mediated relaxation re-

![Fig. 1. Comparison of constrictor dose-response relationships to ACh in paired human control serum-treated and atopic asthmatic serum-
treated rabbit airway smooth muscle (ASM) segments in absence
(sensitized) and presence [sensitized + anti-ICAM-1 MAb] of anti-ICAM-1 (A)
and anti-CD11a (B) MAbs. Values are means ± SE from 6 paired
experiments. Note that relative to control cells, heightened constrict-
or responses to ACh in atopic asthmatic serum-sensitized tissues were prevented by cotreatment of tissues with either anti-ICAM-1 or
anti-CD11a MAb.](image-url)
sponses were also ablated in atopic sensitized tissues that were pretreated with anti-CD11a MAb (Fig. 2B). In contrast to these findings obtained in atopic asthmatic serum-sensitized ASM, in tissues incubated with control serum, pretreatment with either anti-ICAM-1 MAb, anti-CD11a MAb, or anti-CD11b MAb had no effect on the subsequent relaxation responsiveness of the tissues to isoproterenol (data not shown).

Expression of ICAM-1 and LFA-1 in atopic asthmatic serum-sensitized ASM cells. Taken together, the above observations suggested a role for induced ICAM-1/LFA-1 activation in mediating the observed changes in ASM responsiveness in the atopic asthmatic sensitized state. To elucidate whether this role of ICAM-1/LFA-1 activation represents a phenomenon that is intrinsic to the atopic sensitized ASM itself, a series of experiments were conducted to examine whether cultured human ASM cells autologously express the mRNAs and proteins for the above cell adhesion molecules and to assess whether the expression of ICAM-1 and LFA-1 in ASM cells is altered in the atopic asthmatic sensitized state. For the mRNA analyses, Southern blots were prepared and probed with the human cDNA probes specific for the human CD11a and ICAM-1 genes (see METHODS). In addition, a 157-bp RPL7 probe was also used as a control for gel loading. As depicted by one of three representative experiments in Fig. 3, relative to the corresponding constitutively expressed RPL7 signal, mRNA expression of CD11a was modestly detected at various times after exposure of the cultured ASM cells to control or atopic asthmatic serum. Moreover, there were no pronounced differences in the intensities of the CD11a mRNA signals between the control and atopic asthmatic serum-sensitized cells. In contrast to these observations, as further shown in Fig. 3, relative to control serum-treated ASM cells where ICAM-1 mRNA expression was barely detectable after 3, 6, and 24 h of serum exposure, the intensity of the ICAM-1 mRNA signal was markedly upregulated at all these times after exposure of the ASM cells to atopic asthmatic sensitized serum.

In extending the above observations, we next examined whether human ASM cells express CD11a and ICAM-1 proteins on their cell surface and also assessed whether expression of these proteins is modulated in the atopic asthmatic sensitized state. With the use of flow cytometric analysis of human ASM cells exposed for 24 h to control and atopic asthmatic serum (see METHODS), as exemplified in Fig. 4, both the control serum-treated (Fig. 4A) and atopic asthmatic serum-sensitized (Fig. 4B) ASM cells were found to express CD11a protein on their cell surface. Of note, there were no distinct differences in CD11a expression between the control and atopic sensitized ASM cells; however, in contrast with the relatively unaltered CD11a expression, the atopic asthmatic serum-sensitized cells (Fig.

![Graph](image1.png)

**Fig. 2.** Comparison of relaxation dose-response relationships to isoproterenol in paired human control serum-treated and atopic asthmatic serum-treated rabbit ASM segments half-maximally contracted with ACh in absence and presence of anti-ICAM-1 (A) and anti-CD11a (B) MAbs. Values are means ± SE from 6 paired experiments. Note that relative to control cells, attenuated relaxation responses to isoproterenol in atopic asthmatic serum-sensitized tissues were prevented by cotreatment of tissues with either anti-ICAM-1 or anti-CD11a MAb.

![Graph](image2.png)

**Fig. 3.** Comparison of expression of CD11a and ICAM-1 mRNAs with RT-PCR and Southern blot analysis in human ASM cells after 0-, 3-, 6-, and 24-h exposures to 10% human control and 10% atopic asthmatic sera. Expression of ribosomal protein (RP) L7 mRNA was used to control for gel loading. Blots were probed with human-specific CD11a, ICAM-1, and RPL7 32P-labeled cDNA probes (see METHODS). Note that mRNA expression was faintly detected in control and atopic serum-sensitized cells, and there were no notable differences in mRNA expression between treatment conditions. In contrast, relative to control serum-treated cells, mRNA signals for ICAM-1 were markedly enhanced at all times after exposure of cells to atopic asthmatic serum. mRNA expression of constitutively expressed RPL7 gene was unaltered under both treatment conditions.
4D) exhibited a significantly enhanced expression of ICAM-1 relative to the control serum-exposed cells (Fig. 4C). Accordingly, cells positively stained for ICAM-1 averaged 9.1 and 23.4% in the control and atopic serum-exposed conditions, respectively, and the corresponding MFI value was also relatively enhanced in the atopic serum-sensitized cells by 2.3-fold. Qualitatively similar results were obtained in other comparable experiments where, relative to control serum-treated ASM cells, the percent positive staining for ICAM-1 and MFI was increased by an average of 1.8- and 2.5-fold, respectively, in atopic asthmatic serum-sensitized cells.

Role of IL-5 in regulating ICAM-1 expression in atopic asthmatic serum-sensitized ASM cells. Previous studies (23, 28, 32, 39) have demonstrated increased ICAM-1 expression and release of sICAM-1 into the lungs of asthmatic individuals and in animal models of allergic asthma. Moreover, in a recent study (35), inhalation of recombinant human IL-5 was found to elicit increases in the sputum concentration of sICAM-1 in atopic asthmatic subjects. This evidence, when coupled to recent observations by Hakonarson and colleagues (19, 20) demonstrating that human ASM cells are autologously induced to release IL-5 protein in the atopic asthmatic sensitized state raises the consideration that IL-5 may play a role in regulating the above observed changes in ICAM-1 expression in the atopic asthmatic serum-sensitized ASM cells. In addressing this possibility, studies were conducted to initially examine whether the reported elaboration of IL-5 protein by atopic asthmatic serum-sensitized human ASM cells is mechanistically coupled to ICAM-1/LFA-1 activation. With the use of an IL-5-specific immunoassay (see METHODS), IL-5 protein levels were measured in the culture medium of human ASM cells after 24 h of exposure of the cells to control serum or to atopic asthmatic serum in the absence and presence of anti-ICAM-1 MAb and anti-CD11a MAb. As shown in Fig. 5, relative to control serum-treated cells, IL-5 protein levels in the culture medium of atopic serum-sensitized cells were significantly increased by an average of approximately fourfold. Moreover, it will be noted that this enhanced elaboration of IL-5 protein...
was ablated in atopic asthmatic serum-sensitized ASM cells that were pretreated with either anti-ICAM-1 MAb or anti-CD11a MAb (Fig. 5).

In light of the above results, we next examined whether the ICAM-1/LFA-1-dependent release of IL-5 by atopic asthmatic serum-sensitized ASM cells is responsible for the observed induced upregulation of ICAM-1 expression in the atopic sensitized state (i.e., Figs. 3 and 4). Accordingly, levels of sICAM-1 were measured in the ASM culture medium with an sICAM-1-specific immunoassay (see Methods) after 24 h of exposure of the cells to control and atopic asthmatic sera in both the absence and presence of IL-5Rα antibody (150 ng/ml). As shown in Fig. 6, the sICAM-1 concentrations in the culture medium of control serum-treated ASM cells averaged 1.70 ± 0.78 ng/ml, and this value was similar to that obtained in control cells pretreated with IL-5Rα antibody. In contrast, the sICAM-1 level in the culture medium of atopic asthmatic serum-treated cells was significantly enhanced, averaging 24.28 ± 3.86 ng/ml. Of significance, this augmented elaboration of sICAM-1 was inhibited in atopic serum-sensitized cells that were pretreated with IL-5Rα antibody.

Given the above observations, to further substantiate the role of IL-5 in regulating sICAM-1 release from ASM cells, we next examined the time-dependent effect of exogenously administered IL-5 on sICAM-1 accumulation in the culture medium of naive (i.e., non-serum-exposed) ASM cells. As depicted in Fig. 7, relative to vehicle-treated (control) cells where the sICAM-1 level remained essentially unaltered, IL-5-treated cells displayed a progressive time-dependent increase in sICAM-1 release, and the average level of sICAM-1 accumulation in the culture medium at 24 h amounted to ~30-fold above the control level.

**DISCUSSION**

Substantial evidence accumulated in recent years has implicated a crucial role for CAMs in the overall pathobiology of the airway responses in allergic asthma, including the development of airway inflammation and the associated changes in airway responsiveness. In this regard, a number of studies (4, 5, 24, 26, 33, 38, 43, 44) have demonstrated that administration of blocking antibodies to specific CAMs including ICAM-1, LFA-1, and very late activating antigen-4 attenuates or abolishes the airway constrictor hyperresponsiveness and/or airway inflammation seen in different in vivo animal experimental models of asthma. For the most part, these studies have largely suggested that the specific CAM-dependent changes in airway responsiveness and airway inflammatory cell infiltration are casually related. More recent reports (24, 26, 31, 38), however, have provided compelling evidence that certain CAM-dependent effects on airway responsiveness may occur independent of leukocyte migration into the lungs. In light of this emerging new information, the consideration is raised that the reported protective effects of specific anti-CAM antibodies on airway hyperresponsiveness may be attributed to a more direct action(s) of CAM activation on resident airway tissue cells. In addressing this issue, the present study examined the role and mechanism of action of ICAM-1-coupled LFA-1 activation in regulating agonist responsiveness in atopic asthmatic serum-sensitized isolated ASM. Collectively, the results herein provide new evidence demonstrating that 1) the proasthmatic perturbations in ASM responsiveness seen in atopic asthmatic sensitized tissues are

![Fig. 6. Comparison of soluble ICAM-1 (sICAM-1) release into culture medium of human ASM cells after 24-h treatment of cells with 10% control and 10% atopic asthmatic sera in both absence and presence of IL-5-receptor-α (IL-5Rα) blocking antibody. Values are means ± SE from 4 experiments. Note that relative to control serum-exposed cells where IL-5Rα antibody had no effect, atopic serum-sensitized cells depicted a significantly increased elaboration of sICAM-1, and this effect was inhibited in atopic sensitized cells that were pretreated with IL-5Rα antibody.](http://ajplung.physiology.org/)
mediated by ICAM-1/LFA-1 activation; 2) atopic asthmatic serum-sensitized ASM cells also display an autologously upregulated mRNA and protein expression of ICAM-1, whereas CD11a expression is constitutively present on the surface of ASM cells but is unaltered in the sensitized state; and 3) the enhanced elaboration of sICAM-1 by atopic sensitized ASM cells is attributed to an ICAM-1/LFA-1-dependent autocrine release and action of the Th2-type cytokine IL-5 in the ASM itself.

To our knowledge, this study is the first to demonstrate that both ICAM-1 and its endogenous β2-integrin counterreceptor CD11a are constitutively expressed in ASM and that activation of these CAM counterreceptors in the atopic sensitized state mediated the observed changes in ASM responsiveness. In evaluating the collection of evidence supporting these 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 and relaxant agonist responsiveness in atopic asthmatic serum-sensitized ASM tissues (Figs. 1 and 2) mimicked the perturbations in airway function that characterize the in vivo asthmatic condition, including augmented bronchoconstrictor responsiveness and impaired β-adrenoceptor-mediated airway relaxation (2, 14, 18). Moreover, to the extent that our observed changes in ASM responsiveness were ablated in atopic sensitized tissues that were pretreated with blocking antibodies to either ICAM-1 or CD11a, these results suggested that both ICAM-1 and LFA-1 were expressed by ASM cells and that activation of these CAM counterreceptors in the atopic sensitized state mediated the observed changes in ASM responsiveness. In general, these considerations are consistent with a collection of related information based on other published reports. In this regard, previous studies (21, 27, 34, 42) have demonstrated that, apart from leukocytes, ICAM-1 is also expressed in various non-bone marrow-derived resident tissue cells (e.g., epithelial cells, endothelial cells) including ASM cells. Furthermore, as noted above, a host of studies (4, 5, 26, 33, 38, 43, 44) have demonstrated that activation of ICAM-1 and LFA-1 is crucial for the induction of airway hyperreactivity in various animal models of allergic asthma. Our present findings extend this earlier evidence by demonstrating that both ICAM-1 and LFA-1 are constitutively expressed in ASM cells (Fig. 4) and that their activation in the atopic sensitized state leads to altered ASM responsiveness in association with an induction of IL-5 release (Fig. 5) and enhanced ICAM-1 expression and ICAM-1 protein release (Fig. 6).

Our rationale for investigating the role of IL-5 in atopic asthmatic sensitized ASM is based on considerable evidence implicating an important contribution of this Th2-type cytokine in regulating the induction of airway constrictor hyperresponsiveness in various in vivo animal models of allergic asthma (7, 9, 22, 41). Moreover, in this connection, Hakonarson and colleagues (19, 20) have recently demonstrated that independent of the presence of inflammatory cells, IL-5 is endogenously released by atopic asthmatic serum-sensitized ASM cells and serves to mediate the altered constrictor and relaxant responsiveness in isolated sensitized ASM tissue. In extending these recent findings, our present observations demonstrated that the induced release of IL-5 by atopic asthmatic sensitized ASM cells was inhibited in the presence of either anti-ICAM-1 MAb or anti-CD11a MAb (Fig. 5), implicating ICAM-1/LFA-1 activation in the induction of IL-5 release in the sensitized state. These observations fundamentally concur with those of previous studies (12, 25, 30, 40) that also demonstrated that ICAM-1/LFA-1 activation elicits the release of proinflammatory cytokines (including IL-5) in other cell types.

Although the previous findings by Hakonarson et al. (19) demonstrated that the autocrine release and action of IL-5 in ASM mediate its altered responsiveness in the atopic asthmatic sensitized state, the present results provide additional evidence that ICAM-1/LFA-1-dependent IL-5 release is also responsible for inducing an enhanced elaboration of sICAM-1 from atopic asthmatic serum-sensitized ASM cells (Fig. 6). This finding is in agreement with those of a recent study (35) demonstrating that inhalation of recombinant human IL-5 produces increases in the sputum concentration of sICAM-1 in atopic asthmatic individuals. Moreover, in the latter study, because the sputum concentrations of sICAM-1 obtained after IL-5 challenge were found to exceed levels that could be accounted for by passive transudation of sICAM-1 from the circulation, the authors (35) suggested that the stimulatory action of IL-5 on sICAM release was attributed to a localized effect in the lungs. Our present extended observations provide evidence in support of the latter notion by demonstrating that exogenously administered IL-5 elicits a significant time-dependent increase in sICAM-1 release from cultured human ASM cells (Fig. 7). Thus when evaluated in light of our present results, it is conceivable that the reported IL-5-induced increase in the release of sICAM-1 in asthmatic lungs may, at least in part, be related to a stimulatory effect of IL-5 on ASM cells. Clearly, to the extent that our present observations pertain to in vitro experimental conditions, the involvement of other cell types in the lung in contributing to IL-5-induced increases in sICAM-1 release in vivo remains to be determined.

A fundamental issue pertaining to the collection of findings in the present study relates to the potential mechanism by which passive sensitization of isolated ASM tissue and cultured ASM cells with human atopic asthmatic serum induces ICAM-1/LFA-1 activation. Although this mechanism remains to be elucidated, it is relevant to note that under the same experimental conditions described herein, we previously identified that the induced changes in ASM responsiveness obtained after passive sensitization of rabbit ASM with atopic asthmatic serum were initiated by the binding of IgE (present in the sensitizing serum) to its low-affinity IgE receptor, FcεRII (i.e., CD23), on the surface of ASM cells (16). Furthermore, under these conditions, the changes in ASM responsiveness in the atopic sensitized state were found to be mediated by the induced sequen-
tial autocrine release and action of IL-5 and IL-1β (19), an effect resulting in altered ASM responsiveness secondary to IL-1β-mediated upregulated expression and action of Gαi, proteins, specifically Gαi1,2 and Gαi3, which inhibit intracellular cAMP accumulation (17, 18). Given this earlier evidence, when examined in light of the present observations, the consideration is raised that activation of ICAM-1/LFA-1 coupling in atopic asthmatic serum sensitized ASM represents a transmembrane signaling event that is triggered by CD23 activation in the ASM. Support for this speculated mechanism of action is, in part, provided by the findings of recent studies demonstrating that CD23 activation induces enhanced LFA-1-mediated adhesiveness in CD4+ T cells and increased CAM expression (10) as well as a Th2-type profile of cytokine release (11). The potential role and mechanism of CD23-dependent activation of CAM expression and action in airway smooth muscle remains to be systematically investigated.

In conclusion, the results of the present study provide new evidence demonstrating that pro-asthmatic-like changes in agonist constrictor and relaxant responsiveness in atopic asthmatic serum-sensitized ASM are attributed to ICAM-1/LFA-1 activation in the sensitized ASM itself. Moreover, the results demonstrate that ICAM-1/LFA-1 activation also elicits upregulated IL-5 protein release from sensitized ASM cells and that the latter phenomenon appears responsible for the associated increased expression and release of sICAM-1 by ASM cells in the atopic asthmatic sensitized state. Thus together with the conventional concept related to the interactive roles of specific CAMs and inflammatory cells in the pathobiology of asthma, the present findings identify a potentially important mechanism by which the resident ASM itself may, via autologous ICAM-1/LFA-1 activation, regulate its own state of altered responsiveness in the atopic asthmatic condition.

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