T Lymphocytes Adhere to Airway Smooth Muscle Cells Via Integrins and CD44 and Induce Smooth Muscle Cell DNA Synthesis

By Aili L. Lazaar,*‡ Steven M. Albelda,*‡ Joseph M. Pilewski,* Brian Brennan,* Ellen Puré,‡ and Reynold A. Panettieri, Jr.*

*From the *Pulmonary and Critical Care Division, Department of Medicine, University of Pennsylvania Medical Center, and ‡The Wistar Institute, Philadelphia, PA 19104*

Summary

Asthma is a disease of airway inflammation and hyperreactivity that is associated with a lymphocytic infiltrate in the bronchial submucosa. The interactions between infiltrating T lymphocytes with cellular and extracellular matrix components of the airway and the consequences of these interactions have not been defined. We demonstrate the constitutive expression of CD44 on human airway smooth muscle (ASM) cells in culture as well as in human bronchial tissue transplanted into severe combined immunodeficient mice. In contrast, basal levels of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression are minimal but are induced on ASM by inflammatory mediators such as tumor necrosis factor α (TNF-α). Activated, but not resting T cells, adhere to cultured ASM; stimulation of the ASM with TNF-α enhanced this adhesion. Adhesion was partially blocked by monoclonal antibodies (mAb) specific for lymphocyte function-associated antigen 1 (LFA-1) and very late antigen 4 (VLA-4) on T cells and ICAM-1 and VCAM-1 on ASM cells. The observed integrin-independent adhesion was mediated by CD44/hyaluronate interactions as it was inhibited by anti-CD44 mAb 5F12 and by hyaluronidase. Furthermore, the adhesion of activated T lymphocytes induced DNA synthesis in growth-arrested ASM cells. Thus, the interaction between T cells and ASM may provide insight into the mechanisms that induce bronchial inflammation and possibly ASM cell hyperplasia seen in asthma.

Asthma, a disease of reversible bronchial obstruction, is characterized by airway inflammation, epithelial damage, airway smooth muscle hyperplasia, and airway hyperresponsiveness. Although the eosinophil is one important effector cell in asthma, other inflammatory cells, particularly lymphocytes, are postulated to be critical in the initiation and perpetuation of airway inflammation. Bronchial biopsies from asthmatics exhibit increased numbers of lymphocytes in the submucosa (1). In addition, an increase in the number of $CD4^+$ T cells with a phenotype associated with T cell activation is found in bronchoalveolar lavage of asthma patients (2–4). Activated T lymphocytes secrete a pattern of cytokines, including IL-4 and IL-5, characteristic of a Th2 phenotype (4). In turn, these cytokines promote allergic type inflammatory responses such as eosinophil recruitment and isotype switching of B cells to production of IgE (5).

In addition to secreting cytokines, T lymphocytes interact with other cell types by direct cell–cell contact. Recent attention has been directed toward the role of cell adhesion molecules in mediating airway inflammation (6). Previous studies showed increased expression of the β_1 and β_2 integrins very late antigen 4 (VLA-4)[1] and LFA-1, as well as of CD44, a receptor for hyaluronate (HA), on leukocytes recovered from the airways of patients with asthma (7, 8). Furthermore, Wegner et al. (9) demonstrated that antibodies against intercellular adhesion molecule 1 (ICAM-1), a counter-receptor for LFA-1, decreased eosinophil infiltration, and attenuated bronchial hyperresponsiveness in a primate model of asthma. Adhesion molecules also mediate lymphocyte–endothelial cell interactions during the process of cell recruitment and homing. Although extravasation of lymphocytes from the circulation is a relatively well defined early step in establishing a local inflammatory response, the subsequent interactions of the infiltrating lymphocytes with other cell types in the bronchial submucosa or extracellular matrix, a step that may be

[1] *Abbreviations used in this paper:* ASM, airway smooth muscle; BrdU, bromodeoxyuridine; HA, hyaluronate; ICAM-1, intercellular adhesion molecule 1; PDBU, phorbol 12,13-dibutyrate; PECAM-1, platelet endothelial cell adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; VLA-4, very late antigen 4; VSM, vascular smooth muscle.

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important for sustaining the inflammatory response, remain largely unstudied.

Infiltrating lymphocytes are in close proximity with airway smooth muscle (ASM) cells, a cell type that is important in the pathogenesis of asthma. Interestingly, virtually nothing is known about the expression or function of adhesion molecules on ASM cells. We postulated that ASM, like vascular smooth muscle (VSM), expresses cell adhesion molecules that may be upregulated during inflammation and that lymphocyte–ASM interactions could have important consequences for both cell types. We therefore investigated the adhesion molecule repertoire of ASM, the potential for and mechanisms of lymphocyte–ASM interactions to occur, and the consequences of such cell–cell interactions.

Materials and Methods

mAbs. The following murine mAbs specific for human antigens were used: RR6.5 (anti-ICAM-1, gift of Dr. R. Rothlein [Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT] [10]), 4B9 (anti-vascular cell adhesion molecule 1 [VCAM-1] gift of Dr. T. Carlos [University of Pittsburgh, Pittsburgh, PA] [11]), 5F12 (blocking anti-CD44, gift of Dr. B. Haynes [Duke University Medical Center, Durham, NC] [12]), Hermes III [nonblocking anti-CD44 [Stanford University, Palo Alto, CA] [13]), 4G6 (anti-platelet endothelial cell adhesion molecule 1 [PECAM-1] [14]), TS1/22 (anti-LFA-1 α chain [American Type Culture Collection, Rockville, MD] [15]), 163H (anti-VLA-4, gift of Dr. W. Gallatin [ICOS Corp., Bothell, WA]), DREG 56 (anti-L-selectin, gift of Dr. T. K. Kishimoto [Boehringer Ingelheim Pharmaceuticals, Inc.] [16]). RR6.5, 4B9, Hermes III, 4G6, and DREG 56 were used at a concentration of 10 μg/ml of purified antibody. TS1/22 was purified from an ammonium sulfate cut of serum-free supernatant and 5F12 was provided as a hybridoma supernatant. KM201 (rat antimurine CD44 [Dr. P. Kincade, Oklahoma Medical Research Foundation, Oklahoma City, OK] [17]) was purified from an ammonium sulfate cut of serum-free supernatant.

T Cells. Peripheral blood was obtained by venipuncture from healthy volunteers and the T cells purified (>95% $CD3^+$ by flow cytometry) by Ficoll (Pharmacia LKB, Piscataway, NJ) density gradient centrifugation and rosetting with neuraminidase-treated (Sigma Chemical Co., St. Louis, MO) sheep erythrocytes (Rockland Inc., Gilbertsville, PA) (18). Cells were maintained in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 100 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml fungizone. Where indicated, T cells were activated with phorbol 12,13-dibutyrate (PDBU, 5 ng/ml; Sigma Chemical Co.) and ionomycin (250 nM; Sigma Chemical Co.) for 42 h at 37°C. T cells used for adhesion assays were pulsed with [^{3}H]thymidine (1 μCi/10^6 cells; New England Nuclear, Boston, MA) for the final 16 h of incubation.

ASM. Human ASM cells were isolated from the trachealis muscle of lung transplant donors and purified as described by Panettieri et al. (19). These cells retain smooth muscle–specific actin expression as well as responsiveness to contractile agonists (19). Cells were maintained in Ham's F12 media supplemented with 10% FCS, 2 mM glutamine, 25 mM Hepes, 12 mM NaOH, 1.5 mM $CaCl_2$, 100 U/ml penicillin, and 100 μg/ml streptomycin. For the DNA synthesis experiments, cells were quiesced in serum-free Ham's F12 media supplemented with 5.7 μg/ml insulin, 5 μg/ml transferrin, 2 mM glutamine, 1.5 mM $CaCl_2$, 25 mM Hepes, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Flow Cytometry. ASM cells were recovered by digestion with trypsin/EDTA and reacted with mAb specific for PECAM-1, ICAM-1, or VCAM-1 for 45 min at 4°C, followed by FITC goat anti–mouse IgG (Organon Teknika Corp., Durham, NC). Samples were analyzed using an Ortho Cytofluorograf System 50, connected to a 2150 Data Handling System (Ortho Diagnostic Systems, Inc., Raritan, NJ).

Immunoprecipitation. Confluent ASM cells grown in a T25 flask were depleted in methionine-free media for 1 h and incubated overnight with [^{35}S]methionine (200 μCi; Amersham Corp., Arlington Heights, IL). After labeling, cells were washed and lysed in 10 mM Tris, 0.5% NP-40, 0.5 mM $CaCl_2$ and 1 mM phenyl methyl sulfonyl fluoride. Lysates were fractionated by centrifugation at 10,000 *g* and the NP-40–soluble extract precleared with Sepharose–protein A, 150 mM NaCl, 4% BSA. Lysates were reacted with murine mAb specific for ICAM-1, VCAM-1, CD44 (Hermes III), or PECAM-1, and the immune complex precipitated with Sepharose–protein A. Precipitates were washed with 0.5% deoxycholic acid, 50 mM Tris, 150 mM NaCl, 1% Triton X-100, 0.1% SDS. The immune complexes were then eluted by boiling in 2× Laemmli buffer and resolved by electrophoresis on a 6% nonreduced polyacrylamide gel.

Quantitative Adhesion Assay. Confluent ASM monolayers were treated with control media or 1000 U/ml TNF-α for 24 h. [^{3}H]thymidine-labeled activated T cells were washed and pretreated with murine mAbs specific for anti-LFA-1, anti-VLA-4, anti-CD44 (5F12), or normal mouse IgG (Fisher Scientific, Pittsburgh, PA) for 45 min at 4°C. Where stated, ASM cells were pretreated with mAbs specific for anti-ICAM-1 or anti-VCAM-1 for 45 min at 4°C. Cells (6 × 10^5/well) were added to resting or TNF-α–stimulated ASM monolayers in 24-well tissue culture plates and allowed to adhere for 1 h at 37°C. Nonadherent cells were removed by washing. Adherent cells were lysed with 1% Triton X-100 and quantitated by scintillation spectroscopy. Percent binding were calculated as counts recovered from adherent cells/total input counts ×100. Each condition was performed in triplicate and data were expressed as mean percent binding ± SD. Statistical significance was analyzed with a one way analysis of variance using the Bonferonni-Dunn test.

Detection of DNA Synthesis. ASM cells were grown to subconfluence in 2-well slide culture flasks and serum-deprived for 48 h to establish quiescence. Resting or activated T cells were irradiated with 3,500 rad using a ^{137}Cs source. T cells (5 × 10^5/well) were then added to the ASM and allowed to adhere for 42 h. During the final 24 h, cultures were incubated with 20 μM bromodeoxyuridine (BrdU; Sigma Chemical Co.). The slides were washed once with PBS, fixed in 70% ethanol for 30 min at room temperature, and allowed to air dry. Slides were exposed to 1 N HCl for 5 min and washed with PBS. Primary antibody staining was performed with the hybridoma culture supernatant containing anti-BrdU mAb BU-1 (20) and detected with 10 μg/ml of FITC-sheep anti–mouse IgG (Boehringer Mannheim, Indianapolis, IN). The cells were then counterstained with Evan's blue. Nuclei were visualized using a Leitz fluorescent microscope equipped with standard fluorescein and rhodamine filters. Random high power fields were chosen and photographed or the nuclei counted. The mitotic index was calculated as the ratio of BrdU positive nuclei/total nuclei ± SEM from 50 high power fields for each condition from three separate experiments. Statistical significance was determined using one way analysis of variance.

CD44 Transfectants. The stable AKR1.G10 transfectant ex-

pressing the hematopoietic form of murine CD44 was obtained from Dr. J. Lesley and Dr. R. Hyman (The Salk Institute, La Jolla, CA) (21).

SCID Model. Human bronchial xenografts were established as previously described (22). Briefly, segments of second to fifth generation human airway were dissected from surrounding adventitial tissue and transplanted subcutaneously into both flanks of SCID mice (23) obtained from a colony maintained in pathogen-free housing at the Wistar Institute. In this model, xenografts become revascularized within 1 wk of transplant and have a normal histologic appearance with little evidence of tissue rejection. A xenograft in one flank was injected with 50 μl of endotoxin-free saline containing 2 mg/ml BSA (Sigma Chemical Co.) as a protein carrier plus 1% colloidal carbon to identify the injection site. The xenograft on the contralateral side was injected with 6,000 U of recombinant human TNF-α (provided by Genentech Inc., S. San Francisco, CA) diluted in 50 μl endotoxin-free saline containing 2 mg/ml BSA. 6 or 18 h after the injection the xenografts were removed and flash frozen at −70°C. Frozen sections were stained as previously described (22). Primary antibody was detected using the Vectastain ABC Elite Kit (Vector Laboratories, Inc., Burlingame, CA).

Results

Constitutive and TNF-α–inducible Expression of Adhesion Molecules on ASM Cells In Vitro. Analysis of immunoprecipitates of extracts of [^{35}S]methionine-labeled ASM cells demonstrated constitutive expression of the ~90-kD hematopoietic form of CD44 that was not changed by treating the ASM with TNF-α (Fig. 1). In contrast, ICAM-1 was expressed at low levels and VCAM-1 was not detectable on untreated ASM cells; both were markedly upregulated after treatment with TNF-α for 24 h. Binding of radiolabeled anti-ICAM-1 antibody (data not shown) and immunofluorescent flow cytometry of single cell suspensions of ASM cells (Fig. 2) confirmed the cytokine-inducible expression of ICAM-1 and VCAM-1 on the cell surface. There was no expression of E-selectin on either resting or TNF-α–stimulated ASM (data not shown).

TNF-α induction of ICAM-1 expression on ASM was dose and time dependent. Confluent ASM cells were stimulated with varying concentrations of TNF-α for 24 h and analyzed by flow cytometry. TNF-α–induced expression of ICAM-1 was detectable with 10 U/ml TNF and linear up to 1,000 U/ml TNF (Fig. 3 *A*). To determine the kinetics of ICAM-1 expression, confluent ASM cells were stimulated with 100 U/ml of TNF-α and changes in surface expression of ICAM-1 were determined between 0 and 48 h by flow cytometry. ICAM-1 expression was detectable by 4 h and maximal at 36 h (Fig. 3 *B*).

Flow cytometry was also used to compare the effect of several other inflammatory mediators on ICAM-1 and VCAM-1 expression on ASM in vitro. Treatment with IL-1, LPS, and IFN-γ for 24 h increased ICAM-1 expression, whereas VCAM-1 expression was most notably increased by IL-1 (Fig. 2).

Adhesion Molecule Expression on ASM In Vivo. We extended the in vitro findings using an in vivo model of human bronchial tissue transplanted onto the flank of SCID mice (22).

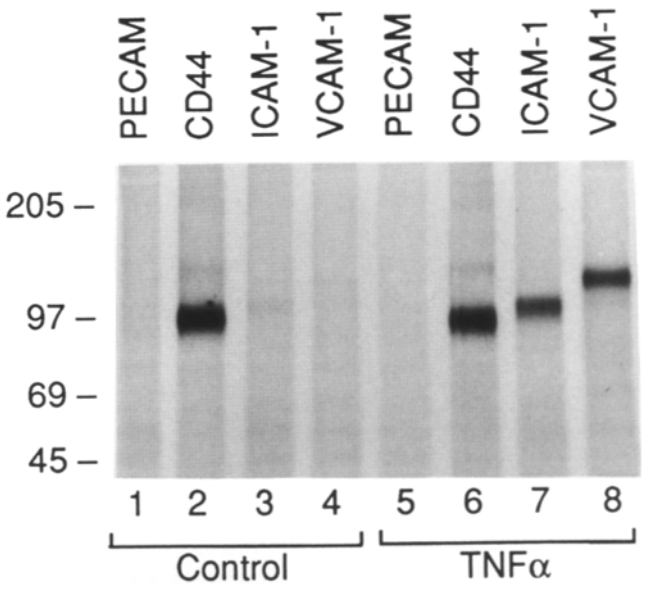

Figure 1. Constitutive and TNF-α–inducible expression of cell adhesion molecules on ASM. Immunoprecipitation of lysates from resting and TNF-α–stimulated ASM. [^{35}S]methionine-labeled protein from resting and TNF-α–stimulated (100 U/ml) ASM was precipitated with mAbs as indicated and resolved on a 6% SDS-PAGE gel.

Immunocytochemistry of frozen sections of the bronchial xenograft (Fig. 4) demonstrated that minimal amounts of ICAM-1 and VCAM-1 were expressed on the ASM cells as identified by staining for smooth muscle actin (Fig. 4, *C* and *F*) in untreated grafts (Fig. 4, *A* and *B*). However, injection of TNF-α into the bronchial lumen of the graft induced a

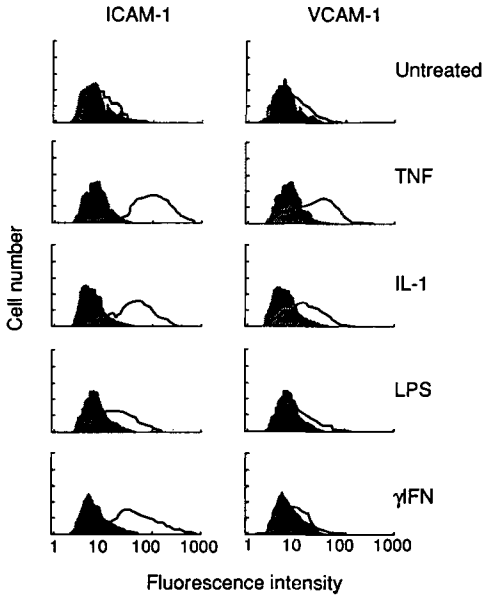

Figure 2. Induction of ICAM-1 and VCAM-1 by cytokines and LPS. Confluent ASM was treated with 100 U/ml TNF-α, 10 U/ml IL-1, 100 U/ml IFN-γ, or 1 μg/ml LPS for 24 h. Shaded areas represent profiles of a negative control antibody (anti-PECAM-1). Open curves represent staining with anti-ICAM-1 (*left*) or anti-VCAM-1 (*right*) mAbs.

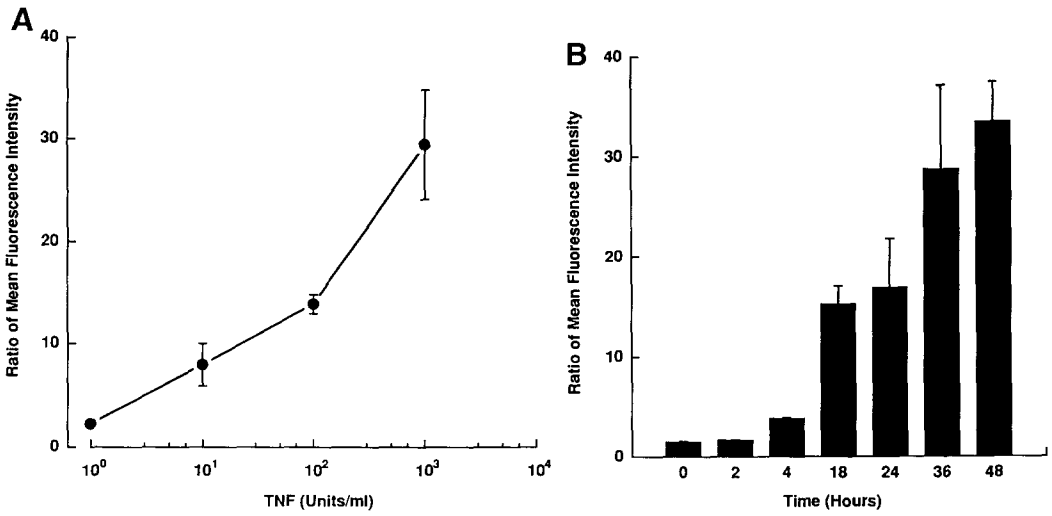

Figure 3. TNF-α–induced ICAM-1 expression is dose and time dependent. (*A*) Dose-dependent induction of ICAM-1 expression by TNF-α. Confluent ASM was stimulated with the indicated doses of TNF-α for 24 h. (*B*) Kinetics of TNF-α–induced ICAM-1 expression. Confluent ASM was stimulated with 100 U/ml TNF-α for the times indicated. Data are expressed as the ratio of mean fluorescent intensity of ICAM-1 mAb to an isotype matched negative control mAb ± SD. Data are representative of four separate experiments.

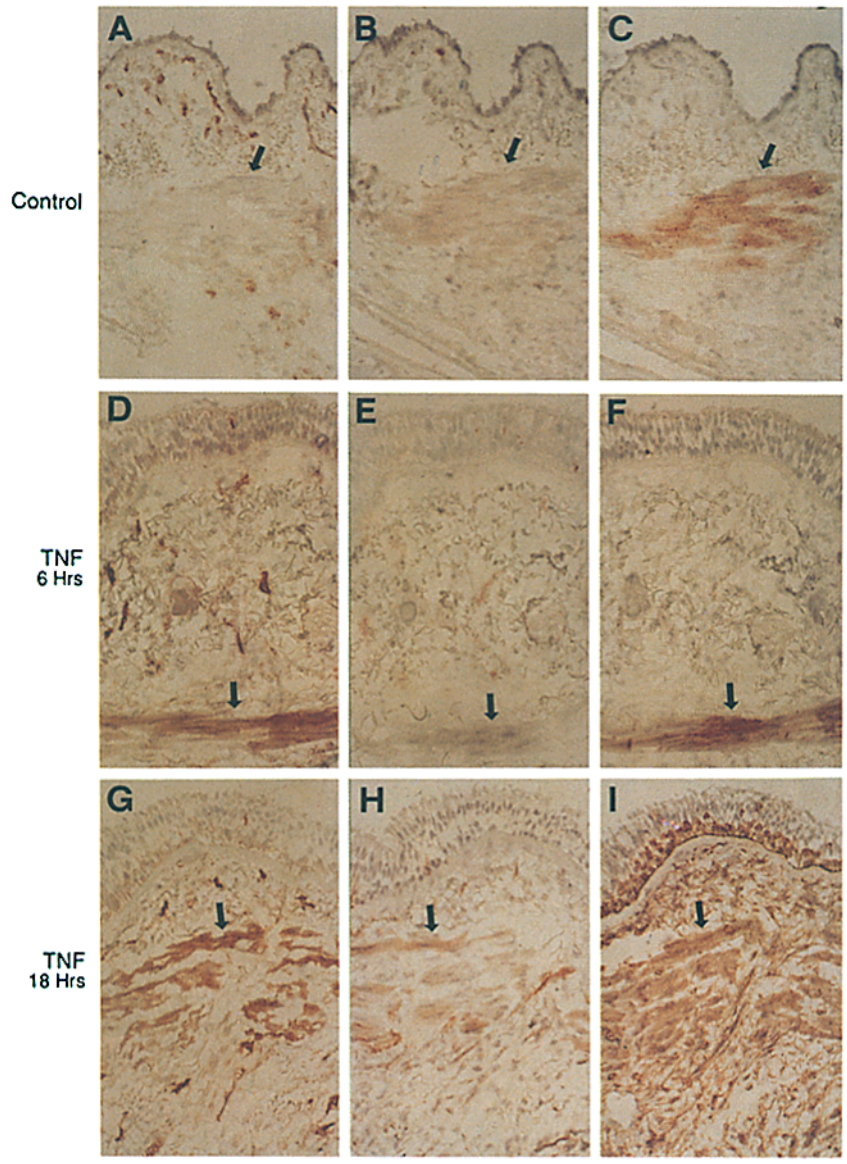

Figure 4. Adhesion molecule expression on human bronchial xenografts in SCID mice. Frozen sections of human bronchial xenografts were analyzed by immunohistochemical staining for expression of (*A*, *D*, and *G*) ICAM-1; (*B*, *E*, and *H*) VCAM-1; (*C* and *F*) myocyte actin; and (*I*) CD44. The bronchial xenografts were from SCID mice treated with vehicle alone (*A–C*), 6 (*D–F*) or 18 h (*G–I*) after TNF-α injection (6,000 U) directly into the graft. Human bronchial tissue was grafted onto the flank of a SCID mouse as previously described (22). Arrows indicate ASM tissue.

marked upregulation of ICAM-1 after 6 h (Fig. 4 *D*) and ICAM-1 and VCAM-1 after 18 h (Fig. 4, *G* and *H*) on the submucosal smooth muscle tissue. In addition, there was marked expression of CD44 on both the ASM and the epithelium in both the TNF-α–treated (Fig. 4 *I*) and untreated (data not shown) xenografts. In addition, the expected increases in endothelial expression of ICAM-1 and VCAM-1 in response to TNF-α stimulation were seen (Fig. 4, *D*, *G*, and *H*).

Activated T Lymphocytes Adhere to ASM. To examine the capacity of T cells to adhere to ASM, we performed a quantitative adhesion assay. Between 5 and 10% of unstimulated T cells bound to resting ASM cells (Fig. 5). In contrast, up to 30% of activated T cells bound to untreated ASM cells. Treatment of the ASM cells with TNF-α for 24 h did not significantly increase adhesion of resting T cells, but enhanced adhesion of activated T cells up to 50%. This indicates that activation of T lymphocytes is required for their adhesion to ASM cells. Although upregulated expression of cell adhesion receptors by the ASM cells was not sufficient to mediate resting T lymphocyte adhesion, it enhanced adhesion of activated T cells.

T Cell Adhesion to ASM Is Mediated by Lymphocyte Integrins and CD44. The role of specific adhesion receptors in mediating lymphocyte-ASM binding was determined using blocking mAbs (Fig. 6). We observed partial inhibition of binding to TNF-α–stimulated ASM when activated T cells were pretreated with mAbs against LFA-1 (Fig. 6, *A* and *B*) or when the TNF-α–stimulated ASM cells were pretreated with an antibody to ICAM-1 (Fig. 6 *A*). When used alone, antibodies specific for VLA-4 (Fig. 6, *A* and *B*) or one of its ligands, VCAM-1 (Fig. 6 *A*), had little inhibitory effect on adhesion of activated T cells to stimulated ASM. However, mAbs against LFA-1 and VLA-4 used together, or ICAM-1 and VCAM-1 in combination, inhibited binding

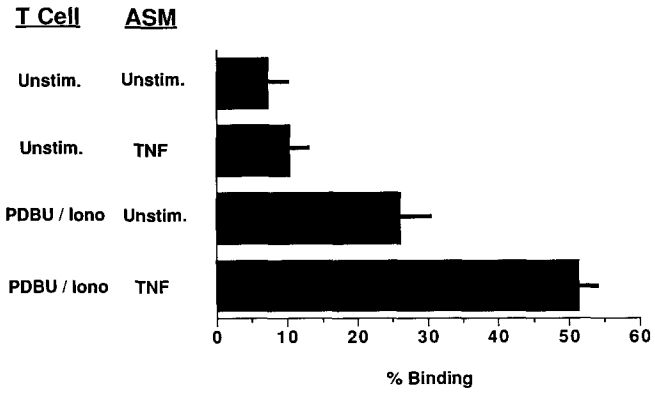

Figure 5. Activated T lymphocytes adhere to ASM cells. T cells were cultured without (unstim) or with PDBU and ionomycin (PDBU/Iono) and pulsed with [^{3}H]thymidine (1 μCi/10^6 cells) for the final 16 h of a 42-h culture period. ASM was stimulated with control media or TNF-α (1,000 U/ml) for 24 h. Percent adhesion was quantitated as described in Materials and Methods. Each condition was performed in triplicate and data are representative of five separate experiments.

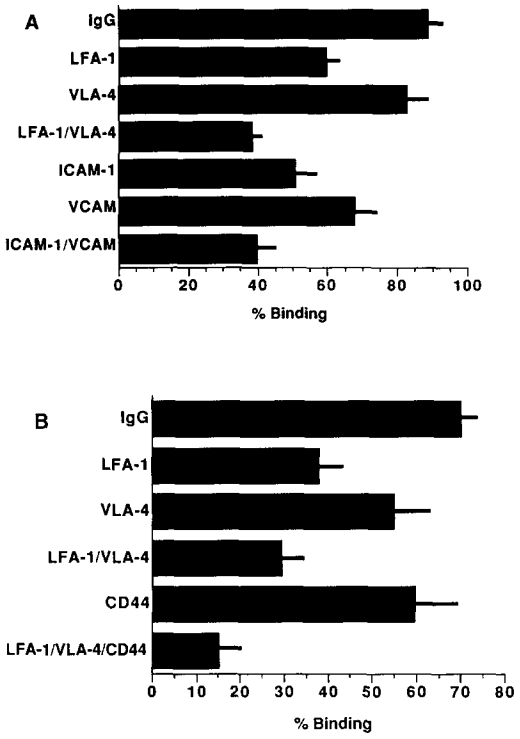

Figure 6. T cell adhesion to ASM is mediated by LFA-1, VLA-4, and CD44. Activated T cells were pretreated with mAbs specific as indicated. ASM was stimulated with TNF-α (1,000 U/ml) for 24 h; cells were also pretreated with mAbs specific for ICAM-1 and VCAM-1 where indicated. Percent adhesion was quantitated as described in Materials and Methods. Data from two (total n = 5) different experiments (*A* and *B*) are shown.

to a greater extent than anti-LFA-1 or anti-ICAM-1 alone. Adhesion of activated T cells to resting ASM, although less than adhesion to TNF-α–treated ASM, was inhibited proportionally to the same extent by these antibodies (data not shown).

Since we observed significant adhesion even in the presence of these combinations of mAbs, we investigated the potential role for a third adhesion pathway mediated by CD44-HA interactions. Pretreatment of T cells with a mAb specific for the HA binding site of CD44 (5F12) did not cause a decrease in activated T lymphocyte adhesion to TNF-α–stimulated ASM (Fig. 6 *B*). Pretreatment of the ASM monolayer with hyaluronidase also failed to decrease adhesion of activated T cells (data not shown). However, the combination of mAbs against LFA-1, VLA-4, and CD44 acted synergistically, reducing the binding of activated T cells to the level observed for resting T cells (Fig. 6 *B*). LFA-1/VLA-4–independent adhesion was also sensitive to hyaluronidase treatment (data not shown). These data indicate that CD44 also plays a role in mediating T cell adhesion to ASM and accounts for the integrin-independent adhesion. Pretreatment of activated T cells with mAbs against L-selectin or against an epitope of CD44 not involved in HA binding (Hermes III) had no effect on adhesion (data not shown).

CD44 Is Sufficient to Mediate T Cell Adhesion to ASM. We

observed that mAb against CD44 decreased T cell adhesion only in the absence of integrin-mediated adhesion. We therefore determined whether CD44 was sufficient to mediate adhesion or whether it was dependent on LFA-1 and/or VLA-4. For this purpose, we exploited the fact that murine LFA-1 does not bind to human ICAM-1 (24), whereas CD44-mediated binding is not species specific (25). AKR1.G10 is a murine lymphoma line that expresses LFA-1, but not VLA-4 or CD44. This line does not bind appreciably to HA unless transfected with CD44 (21). As expected, we also found that the LFA-1 expressed by this murine line could not mediate binding to human ASM cells. Thus, by comparing adhesion of the CD44$^-$ AKR1.G10 cells and AKR1.G10 transfected with murine CD44, we could test for integrin-independent, CD44-mediated adhesion. Staining with fluorescein-labeled Hermes III or HA indicated that the AKR1.G10 transfectants expressed similar levels of active CD44 compared with activated T lymphocytes (data not shown). AKR1.G10 cells transfected with vector alone did not bind significantly to human ASM, whereas cells transfected with murine CD44 demonstrated ~50% binding (Fig. 7). This interaction was specific, as it was inhibited by the mAb KM201 that blocks the HA binding site of murine CD44 (Fig. 7) as well as by soluble HA (data not shown). Furthermore, adhesion was reversible by digestion with hyaluronidase (Fig. 7). These studies indicate that CD44 is sufficient to mediate T cell adhesion to ASM under certain conditions.

T Cell Adhesion Induces ASM DNA Synthesis. Smooth muscle cell hypertrophy and hyperplasia are hallmarks of asthma (26), however, the mechanisms that induce this response in ASM cells remain unknown. We studied the effect of T lymphocyte adherence on DNA synthesis in ASM as measured by uptake of BrdU (27). We chose to use BrdU incorporation as a measure of DNA synthesis since this technique can distinguish DNA synthesis in individual cells. This method also allowed us to characterize the relationship between adherent T cells to ASM cells that had entered the cell cycle. Using fluorescence microscopy, we observed that irradiated activated T cells adhered and induced a significant increase in the number of ASM cells incorporating BrdU, compared with no effect in ASM cells cocultured with resting T cells (Fig. 8, *A* and *B* vs. *C* and *D*). Immunohistochemical analysis with an antibody against BrdU revealed fluorescence in less than 0.3 ± 0.2% of ASM maintained in serum-free media compared with 30 ± 2.4% of ASM treated with 10% FCS, a potent ASM mitogen (27) (Fig. 9). BrdU incorporation was detected in 8.8 ± 1.1% of growth-arrested ASM cocultured with activated T lymphocytes, whereas neither unstimulated T cells nor the conditioned media from activated T cells (data not shown) induced DNA synthesis. In addition, the supernatant from ASM-T cell cocultures did not induce DNA synthesis in ASM (data not shown). Similar results were obtained when uptake of [^{3}H]thymidine was measured (data not shown).

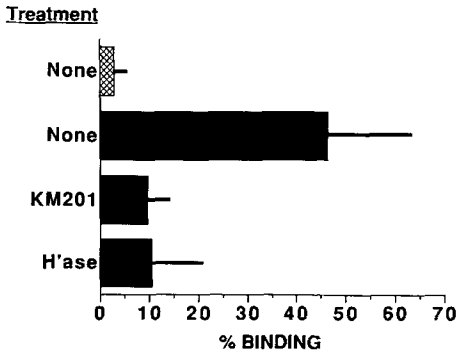

Figure 7. CD44 is sufficient for T cell adhesion to ASM. AKR1.G10 cells stably transfected with vector alone (*hatched bars*) or murine CD44 (*solid bars*) were pulsed with [^{3}H]thymidine for 16 h. ASM cells were stimulated with TNF-α (1,000 U/ml) for 24 h. AKR1.G10 cells were pretreated with control antibody or KM201 (50 μg/ml) as indicated. Hyaluronidase (200 μg/ml) was added 5 min before completion of the adhesion assay. Each condition was performed in triplicate; data are representative of two separate experiments.

Discussion

Adhesion of lymphocytes to endothelium is necessary for their migration to areas of tissue injury. What are less well understood are the effector functions of these cells and the signals that contribute to ongoing immune stimulation once cells are recruited from the circulation. As lymphocytes migrate from the vascular space to the airway, it is likely that they interact with both extracellular matrix components and with the cellular components of the airway such as fibroblasts, dendritic cells and smooth muscle cells (28). We show that ASM, both in vitro and in vivo, constitutively expresses high levels of CD44, and can be stimulated to express ICAM-1 and VCAM-1 in response to inflammatory mediators such as TNF-α. It is noteworthy, therefore, that TNF-α levels are significantly increased in the bronchoalveolar lavage fluid from asthmatics compared with normal patients, with levels ranging from 600 to 10,000 pg/ml (29, 30). Lymphocytes can interact with ASM cells in a regulated manner via specific adhesion receptors. Furthermore, the data illustrate that such interactions can stimulate DNA synthesis in ASM, which may be relevant to mechanisms that induce ASM cell hyperplasia.

The few studies that have examined the expression or function of adhesion molecules on smooth muscle cells have focused on vascular tissue. Li et al. (31) described an increase in VSM expression of VCAM-1 in response to IFN-γ and LPS, but not to IL-1 or TNF-α. Couffinhal et al. (32) found that VSM treated with TNF-α expressed functionally active ICAM-1 that mediated monocyte binding. In addition, Stemme et al. (33) demonstrated that ICAM-1 expression on VSM could be upregulated by IL-1, IFN-γ, and LPS and was accompanied by a modest increase in T lymphoblast binding. In turn, binding could be partially inhibited by mAbs specific for ICAM-1, LFA-1, and CD29, implicating these receptors in the adhesion of T cells to VSM. However, the fact that blocking by these mAbs was incomplete suggests that additional adhesion pathways might be involved with adhesion to VSM as well.

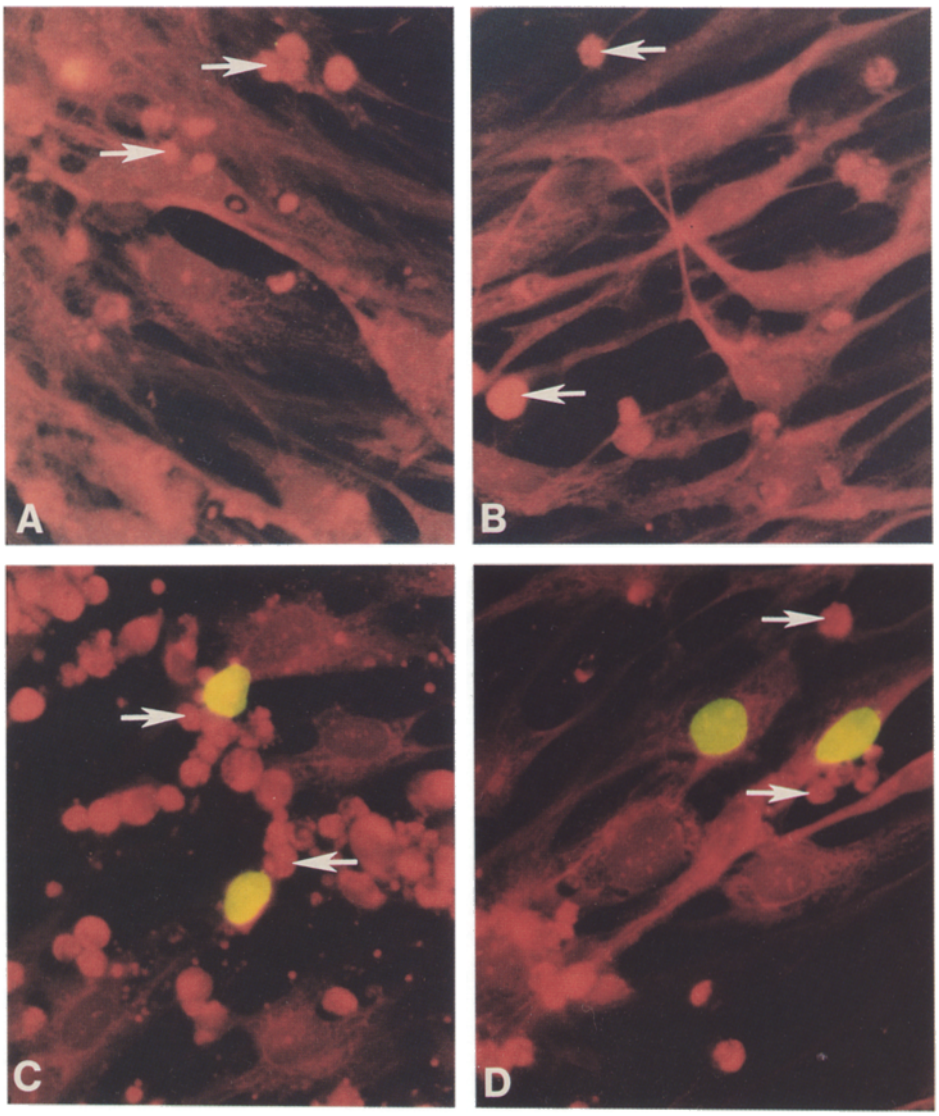

Figure 8. Activated T cells induce DNA synthesis in ASM. Representative (n = 4) fluorescent photomicrograph comparing BrdU incorporation by ASM cells cultured with resting (*A* and *B*) or stimulated (*C* and *D*) T cells. Arrows indicate T cells; ASM cell nuclei that stain positive for BrdU are yellow. Original magnification 800×.

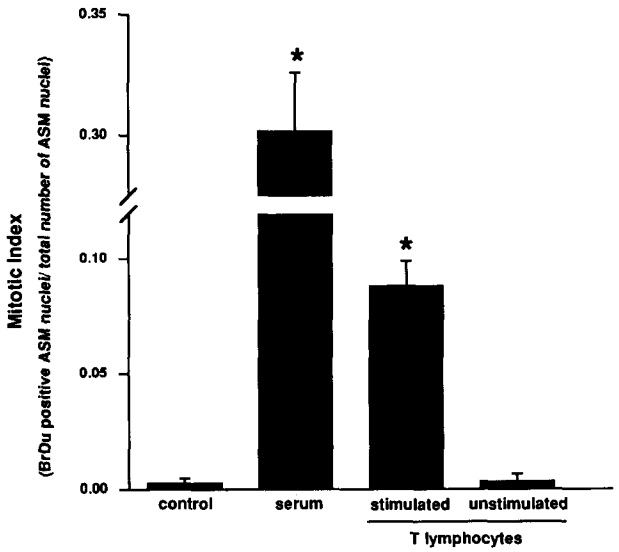

Figure 9. Quantitation of ASM cell DNA synthesis. BrdU incorporation by ASM from a representative experiment (n = 4) expressed as the ratio of positively staining nuclei/total nuclei ± SEM was determined under the indicated conditions. $*p < .001$ compared with control.

We observed that adhesion of activated T lymphocytes to ASM was also only partially mediated by an LFA-1/ICAM-1 interaction, and a role for VLA-4/VCAM-1 was only apparent in the absence of LFA-1–mediated adhesion. Similar findings suggesting the preferential use of LFA-1 over VLA-4 have also been reported for adhesion of activated T cells to endothelium (34–36). We obtained no evidence to implicate L-selectin in adhesion of T cells to ASM. This is in contrast to recent work suggesting that L-selectin is important in T cell adhesion to resting and activated endothelium (34).

One of the most important findings of this study was the definition of the integrin-independent adhesion of T cells to ASM as mediated by CD44. CD44, a glycoprotein with homology to core and link proteins, has been shown to serve as a homing molecule and as a receptor for HA (25, 37–39). CD44 is expressed on a wide variety of cells and is present in an inactive form on resting lymphocytes. The physiologic stimuli that induce adhesion function of CD44 remain unknown. Engagement of other adhesion receptors may activate CD44, either by increasing expression and/or by increasing

its affinity for HA. Studies by Lesley et al. (21, 40) indicate that cross-linking of CD44 by antibody can increase the affinity of CD44 for HA. In addition, there is evidence that T cell adhesion to endothelium is accompanied by the clustering of CD44 molecules on the surface of the lymphocyte oriented toward the endothelium (41). This receptor clustering may lead to intracellular signaling, effecting a further increase in adhesiveness of the integrin receptors such as LFA-1 (42–44). Similar examples of cross talk between receptors leading to cellular activation and adhesion have become increasingly recognized (45).

The expression of CD44 on ASM cells as well as on T lymphocytes, combined with the ability of hyaluronidase to disrupt the T cell–ASM interaction, suggests that T cell CD44 binds to ASM-associated HA and that HA possibly provides a bridge between these two CD44-positive populations. The demonstration that CD44 can act as a primary mediator of lymphocyte adhesion to smooth muscle cells underscores the potential importance of substrates such as hyaluronate in leukocyte trafficking, particularly in the context of inflammation. These results are consistent with our previous studies that indicated that CD44 expression is required for the generation of an optimal contact allergic response (46).

There are a number of potentially important implications for lymphocyte–ASM cell interactions in the pathogenesis of asthma. For example, smooth muscle cells are not classically considered antigen presenting cells. Several investigators have demonstrated, however, that VSM can express MHC class II antigens in response to IFN-γ stimulation (47, 48). We have found that MHC class II expression by ASM cells also is induced by IFN-γ (our unpublished observation). It is therefore possible that class II–expressing smooth muscle cells could serve an antigen presenting cells for preactivated T cells in the asthmatic airway.

Another role of this interaction may be in regulating ASM cell growth. We have demonstrated the ability of activated T lymphocytes to induce DNA synthesis in growth-arrested ASM. In addition, we found that maximal BrdU incorporation and uptake of [^{3}H]thymidine induced by T cell adhesion to ASM coincided with the time necessary for serum-stimulated ASM cells to cycle through S phase as determined by propidium iodide staining (data not shown). If T cell induced BrdU incorporation in ASM cells was due to DNA injury and repair, then we would not have expected such agreement in the time courses between these techniques. Whether this phenomenon occurs via direct cell–cell contact and/or local production of mediators needs to be determined. However, the ability of T lymphocyte adhesion to induce ASM proliferation in vitro suggests that this interaction may contribute to the airway remodeling that occurs in these patients.

In summary, this study has raised the possibility that the airway smooth muscle cell may be an important participant in the airway inflammatory response. ASM-lymphocyte interactions may have effects on both lymphocyte activation and function as well as on ASM cell growth and reactivity.

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Address correspondence to Dr. Ellen Puré, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104-4268.

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