Human Lung Mast Cells Adhere to Human Airway Smooth Muscle, in Part, via Tumor Suppressor in Lung Cancer-1[1]

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Mast cells infiltrate the airway smooth muscle (ASM) of patients with asthma, an event which is likely to be a key factor in the development of this disease. Adhesion is a fundamental mechanism facilitating cellular cross-talk. We have examined whether human lung mast cells (HLMC) and ASM adhere, and have also examined the mechanism involved. Primary cultures of HLMC and confluent human ASM were cocultured for 30 min, then nonadherent HLMC were removed by centrifugation. HLMC adhered avidly to ASM monolayers (mean ± SEM adhesion 43.2 ± 1.2%, $n = 41$). Adhesion was increased to 58.8 ± 2.7% by 1 mM Mn^{2+} ($p = 0.015$), and was reduced by EDTA and EGTA to 20.5 ± 1.5% and 21.0 ± 1.3%, respectively ($p < 0.0001$). Adhesion-blocking Abs for ICAM-1, VCAM-1, CD18, and the α_4 and β_1 integrins had no effect on HLMC adhesion. HLMC expressed tumor suppressor in lung cancer-1 (TSLC-1) and blocking this reduced adhesion from 38.5 ± 4.8% to 28.3 ± 3.7% ($p = 0.004$, $n = 7$). ASM did not express TSLC-1, indicating that TSLC-1 acts as a heterophilic adhesion molecule. In summary, HLMC adhere avidly to ASM in part via TSLC-1 and in part via an as-yet-undefined Ca^{2+}-dependent pathway. This supports the hypothesis that adhesion is important in the recruitment and retention of HLMC by the ASM in asthma, and for the functional interaction of these cells. *The Journal of Immunology,* **2006, 176: 1238–1243.**

Compelling evidence implicates mast cells as key effector cells in the pathophysiology of asthma (reviewed in Refs. 1 and 2). Mast cells infiltrate three key structures in asthmatic airways: the bronchial epithelium (3), mucous glands (4), and airway smooth muscle (ASM)[3] (5). This tissue microlocalization, coupled with the knowledge that these mast cells are in an activated state, supports the notion that they play an important role in disease pathogenesis, in terms of both symptomatology and airway wall remodeling. The demonstration that mast cells (but not T cells or eosinophils) infiltrate the ASM in patients with asthma but not normal subjects or patients with eosinophilic bronchitis is particularly interesting (5). This is because patients with eosinophilic bronchitis have high levels of mast cell mediators in their sputum and airway eosinophilia, but no bronchial hyperresponsiveness or symptoms of airflow obstruction. Furthermore, the mucosal immunopathology of eosinophilic bronchitis and asthma are virtually identical in terms of inflammatory cell infiltration, Th2 cytokine expression, and subbasement membrane thickening. Taken together, these observations suggest that mast cell infiltration of the ASM is a critical determinant of the asthmatic phenotype.

The microlocalization of inflammatory cells within specific airway structures is likely to be critical in determining the downstream consequences of their activation. Adhesion between cell types is a fundamental part of this interactive process, leading to specifically directed signals through both cell-cell attachment and soluble mediators with implications for cell maturation, differentiation, activation, and survival. Because mast cells infiltrate the ASM in asthma, we hypothesized that human lung mast cell (HLMC) adhere to ASM through a specific interaction involving adhesion molecules expressed by the two cell types. To test this hypothesis, we have studied the adhesion of purified HLMC and the human mast cell line HMC-1 to primary cultures of human ASM.

Materials and Methods

Antibodies

The following mouse IgG mAbs (IgG1 isotype unless otherwise stated) were purchased: the Ags they are directed against are shown in parentheses, i.e., clone (Ag). Mouse IgG controls and MHM23 (CD18) (Dako-Cytomation); BBIG-I1 (ICAM-1), BBIG-V1 (VCAM-1), P4C10 (β_1 chain, CD29) (R&D Systems); YB5B8 (CD117) (LCG Bioscience); LM609 (CD51/61) (Chemicon International); HP2/1 (α_4 chain, CD49d) (Serotec). Chicken anti-tumor suppressor in lung cancer-1 (TSLC-1) mAbs 3E1 and 9D2 were generated as described previously (6). Although these Abs were raised against the mouse protein, they recognize the human protein with equal specificity and affinity.

Cytokines and reagents

Stem cell factor (SCF), IL-6, and IL-10 were purchased from R&D Systems; β-galactosidase (from bovine testis), EDTA, EGTA, Mn^{2+}, and paraformaldehyde were purchased from Sigma-Aldrich. Histamine and *S*-adenosyl-L-[*methyl*-^{3}H]methionine were purchased from Amersham Life Science; rat kidney histamine methyltransferase was a gift from Dr. S. Harper (AstraZeneca R&D, Charnwood, U.K.).

HLMC purification

The study was approved by the Leicestershire Research Ethics Committee, and all tissue donors gave written informed consent. HLMC were dispersed from macroscopically normal lung ($n = 16$ donors) obtained within 1 h of resection for lung cancer. The enzymatic dispersal procedure and cell purification using immunomagnetic affinity selection were as previously described (7). Final mast cell purity was >99% and viability was >98%.

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[3] Abbreviations used in this paper: ASM, airway smooth muscle; HLMC, human lung mast cell; SCF, stem cell factor; TSLC-1, tumor suppressor in lung cancer-1.

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Mast cell culture

After purification, HLMC were cultured overnight in DMEM/Glutamax/HEPES containing antibiotic/antimycotic solution, nonessential amino acids, 10% FCS, and cytokines (100 ng/ml SCF, 50 ng/ml IL-6, and 10 ng/ml IL-10) as described previously (8). HMC-1 cells were cultured in Iscove's medium containing 10% iron-supplemented FCS and 1.2 mM monothioglycerol as previously described (7).

ASM culture

Pure ASM bundles in airways isolated from lung resection ($n = 14$ donors) were dissected free of surrounding tissue. The small muscle bundles were cultured in DMEM supplemented with 10% FBS, 4 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, and 0.25 μg/ml amphotericin. ASM characteristics were determined by immunofluorescence and light microscopy with α-smooth muscle actin-FITC direct conjugate and myosin indirectly conjugated with FITC (Sigma-Aldrich).

Mast cell-ASM cell adhesion assay

The adhesion assay used was the same as that described previously for HLMC adhesion to bronchial epithelium (7). Briefly, ASM cells (passage 3 or 4) were grown to confluence in 96-well tissue culture plates and then starved of serum in medium containing insulin, transferrin, and selenium for 3 days. A total of 1×10^4 HLMC or 3×10^4 HMC-1, suspended in 100 μl of mast cell medium, were added to each well. Mast cells were allowed to adhere for 30 min at 37°C. After 30 min, 10 μl of supernatant was gently removed and stored for later histamine analysis to monitor release during the assay. The wells were then filled completely with medium, sealed with Mylar sealing tape (Sigma-Aldrich), inverted, then subjected to centrifugation at $15 \times g$ for 5 min to remove nonadherent cells. All medium was then removed, and the remaining adherent cells lysed in 100 μl of sterile water. Plates were frozen at −80°C until later histamine analysis. A total of 10^4 HLMC or 3×10^4 HMC-1 were lysed in sterile water and stored for measurement of total histamine content. All experiments were performed in quadruplicate. The percentage of adhesion was calculated as the amount of histamine remaining in a well divided by the total amount of histamine added originally.

Immunofluorescence and confocal microscopy

ASM cells were grown to confluence in chamber slides and starved of serum in medium for 3 days, and then 5×10^4 HLMC were added to the wells for 30 min at 37°C. After 30 min, the cells were washed, fixed with methanol, and labeled with mouse anti-human α-smooth muscle actin-FITC (Sigma-Aldrich) and either mouse anti-human tryptase-biotin (Chemicon International) indirectly labeled with Texas Red streptavidin (Vector Laboratories) or chicken anti-human TSLC-1 IgY mAb (3E1) indirectly demonstrated with rhodamine-labeled goat anti IgY polyclonal Ab (Abcam). Cells were counterstained with 4′,6-diamidino-2-phenylindole (Sigma-Aldrich) and mounted with photo bleach-retardant mounting medium (DakoCytomation Fluorescent mounting medium; DakoCytomation). Appropriate isotype controls were performed. The cells were then either studied by a standard fluorescent microscope or studied using an MRC 600 confocal laser scanning attachment (Bio-Rad Microsciences) linked to a Zeiss Axiovert epifluorescence microscope. Digital images and data files of immunofluorescence intensity transecting specimens in the x,y plane were recorded using COMOS software (Bio-Rad). Images and intensity scan graphs were rendered using Microsoft Excel and Adobe Photoshop.

Histamine assay

Histamine was measured by sensitive radioenzymatic assay based on the conversion of histamine to [^{3}H]methylhistamine in the presence of the enzyme histamine-*N*-methyltransferase as previously described (7).

Adhesion modulation assays

Adhesion-blocking mAb. The following well-characterized adhesion-blocking Abs were incubated with the ASM monolayers at room temperature for 15 min before the adhesion assay: 10 μg/ml BBIG-I1 (ICAM-1) (9); 30 μg/ml BBIG-V1 (VCAM-1) (10). The following adhesion-blocking mAb were incubated with HLMC for 15 min at room temperature before the adhesion assay: 10 μg/ml BBIG-I1 (ICAM-1); 20 μg/ml MHM23 (CD18) (11); 25 μg/ml HP2/1 (α_4 chain) (11, 12); 10 μg/ml LM609 (CD51/61, vitronectin receptor) (13); 20 μg/ml P4C10 (β_1 chain) (11); 10 μg/ml 9D2 (TSLC-1) (6). Controls were performed with IgG control at 20 μg/ml and chicken IgY control at 10 μg/ml. All of the aforementioned Abs remained present during the adhesion assay.

Requirement for divalent cations. The Ca^{2+} and Mg^{2+} chelating agents EDTA (5 mM) and EGTA (5 mM) were added to HLMC at 37°C for 10 min before the assay. Because they cause detachment of the ASM monolayer, the ASM cells were fixed in 0.4% paraformaldehyde for 5 min at room temperature, then washed twice before use. Mast cells were also incubated with Mn^{2+} (1 mM) for 10 min at 37°C to assess the effect of integrin activation on adhesion.

To allow for histamine release from mast cells in response to the aforementioned experimental procedures, appropriate aliquots of treated cells were kept for measurement of total histamine content.

Cell viability

Mast cell viability was monitored before and after the aforementioned experiments by exclusion of trypan blue. No treatment was cytotoxic and mast cell viability was >97% in all experiments.

GeneChip expression analysis

Expression of TSLC-1 in human cord blood-derived, peripheral blood-derived, lung and skin mast cells was examined in a gene expression database generated previously using the Human Genome U133A probe array (GeneChip; Affymetrix), which contains the oligonucleotide probe set for ~22,000 full-length genes. This was performed in accordance with the manufacturer's protocol (Expression Analysis Technical Manual). Other results from this database have been published previously (14, 15).

Flow cytometry

The surface expression of TSLC-1 on HLMC, HMC-1, and ASM was examined using flow cytometry as described previously (7). Pure populations of HLMC, HMC-1, and ASM were resuspended in PBS containing 0.5% BSA at a concentration of 1×10^6 cells/ml. Cells were incubated with the 3E1 chicken anti-TSLC-1 IgY mAb 10 μg/ml, then indirectly stained with FITC-labeled rabbit anti-IgY polyclonal Ab (Abcam). Appropriate chicken isotype control was also used (Santa Cruz Biotechnology). Cells were analyzed by flow cytometry on a FACScan (BD Biosciences).

Data presentation and analysis

The percentage of cells adhering is expressed as mean ± SEM. Data comparing maneuvers aimed at modulating mast cell adhesion were analyzed using Student's paired *t* test (two-tailed). TSLC-1 expression determined from the gene chip database is presented as the percentage of GAPDH control.

Results

Mast cell-ASM adhesion assay

The histamine-based adhesion assay was highly reproducible with an intraassay coefficient of variation of 7.1%. This is similar to our previous experience with bronchial epithelium where the intraassay coefficient of variation was 12.5% (7). There was no net release of histamine during the assay compared with control cells, and none of the experimental procedures released histamine from mast cells. Spiked histamine was not taken up or destroyed by ASM cultures (97.0 ± 0.03% recovery over the dose range 10–1000 μM after 1 h in culture at 37°C), and was not detectable in lysed ASM cells after histamine spiking.

HLMC adherence to ASM

A high proportion of both HLMC and HMC-1 adhered to primary cultures of human ASM (Fig. 1*A*). Thus a mean 43.2 ± 1.2% HLMC ($n = 41$ experiments using 16 HLMC donors and 14 ASM donors) and 38.6 ± 4.9% HMC-1 ($n = 8$ experiments using 8 ASM donors) adhered to ASM (Fig. 1*B*). Activating ASM with a mixture of IFN-γ, TNF-α, and IL-1β (50 ng/ml each) did not influence mast cell adhesion ($p = 0.99$, $n = 6$ experiments) (Fig. 1*C*).

Baseline mast cell adhesion to ASM is partially Ca^{2+}-dependent

Integrin- and C-type lectin-mediated adhesion generally requires the presence of Ca^{2+} (16). Because ASM monolayers detach in the presence of EDTA and EGTA, they were fixed in 0.4% paraformaldehyde before addition of EDTA or EGTA. With 0.4% paraformaldehyde fixation there was no significant change in adhesion

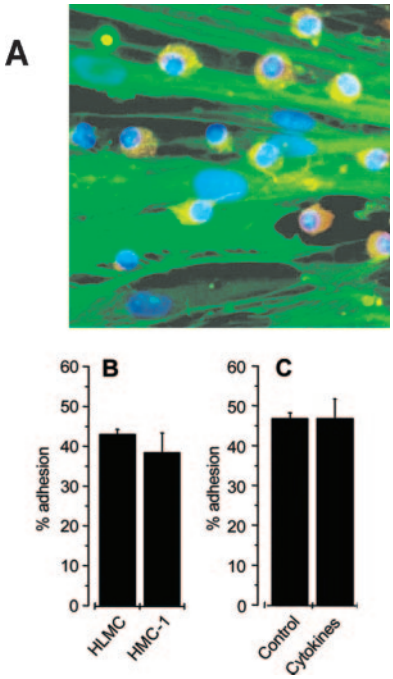

FIGURE 1. Adhesion of HLMC to ASM. *A*, Immunofluorescent staining demonstrating ASM cells stained for α-smooth muscle actin (green) and HLMC stained for tryptase (red). Nuclei stained blue with 4′,6-diamidino-2-phenylindole. *B*, Baseline adhesion of HLMC ($n = 41$) and HMC-1 ($n = 8$) to ASM expressed as mean ± SEM percentage of cells adhering. *C*, Adhesion of HLMC to unstimulated ASM (control) and ASM activated with IFN-γ, IL-1β, and TNF-α (cytokines) ($n = 6$). Expressed as mean ± SEM percentage of cells adhering.

(Fig. 2). Adhesion was significantly but partially attenuated after chelation of cations with EDTA or EGTA (5 mM) ($n = 30$) (Fig. 2). In contrast, HLMC adhesion to fibronectin-coated wells (38.5 ± 2.5%) was almost completely abrogated in the presence of EDTA (3.1 ± 1.6%, $n = 3$, $p = 0.012$).

There are several potential mast cell-ASM integrin-Ig superfamily interactions that could mediate the adhesive process. Mast cell adhesion was not modulated by preincubation of mast cells with adhesion-blocking mAb to CD18, CD51/61 (vitronectin receptor), α_4, β_1, or ICAM-1, or preincubation of ASM cells with adhesion-blocking mAb to ICAM-1 or VCAM-1 (Fig. 2). In contrast, in our hands, the β_1-blocking mAb (P4C10) completely inhibits adhesion of HLMC cells to plasma fibronectin (7) and attenuates eosinophil adhesion to nasal polyp endothelium (11), the anti-CD18 mAb (MHM23) attenuates neutrophil adhesion to nasal polyp endothelium (11), and the anti-α_4 mAb (HP2/1) inhibits eosinophil adhesion to nasal polyp endothelium (11). Integrin activation on mast cells with 1 mM Mn^{2+} increased adhesion from 50.7 ± 1.4% to 58.8 ± 2.7% ($n = 6$, $p = 0.015$), suggesting the potential for an integrin-like dependent mechanism to contribute to adhesion under certain conditions.

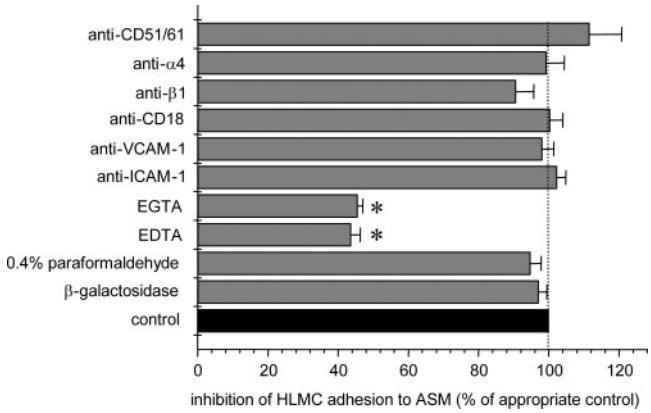

FIGURE 2. Effects of cation chelation, blocking Abs, and β-galactosidase on the adhesion of HLMC to ASM. Adhesion assays were performed in the presence of EGTA and EDTA, in the presence of adhesion blocking Abs, and after pretreatment of HLMC with β-galactosidase. Experiments were performed at least 10 times, except for CD51/61 where $n = 4$. Data are expressed as mean ± SEM adhesion relative to the appropriate control. *, $p < 0.0001$ compared with control.

Similar results were obtained with the HMC-1 human mast cell line (data not shown). β-Galactosidase partially inhibits HLMC adhesion to bronchial epithelium (7) but had no effect on adhesion to ASM (Fig. 2).

HLMC adhesion to ASM is mediated in part via TSLC-1

TSLC-1 is involved in the adhesion of mouse mast cells to mouse fibroblasts (17). Interrogation of our Affymetrix high density oligonucleotide probe array database (14, 15) revealed that HLMC, human cord blood-derived mast cells, human peripheral blood-derived mast cells, and human skin mast cells each express mRNA for TSLC-1. The respective mRNA expression levels as a percentage of GAPDH control were 12.9, 2.8, 16.4, and 40.7%. Both HLMC and HMC-1 showed strong expression of TSLC-1 when analyzed by both confocal immunofluorescence and flow cytometry using the 3E1 anti-TSLC-1 Ab (Fig. 3). The mean number of cells analyzed by flow cytometry that expressed this molecule was 78.5 ± 1.5% for HLMC ($n = 3$, $p = 0.0007$) and 96.7 ± 0.2% for HMC-1 ($n = 3$, $p < 0.0001$).

The adhesion-blocking anti-TSLC-1 Ab 9D2 inhibited the adhesion of both HLMC and HMC-1 to human ASM (Fig. 4*A*). HLMC was reduced by 22.3 ± 3.0% ($p = 0.004$, $n = 7$ experiments using four HLMC donors and seven ASM donors), while HMC-1 adhesion was reduced by 21.5 ± 2.6% ($p = 0.0027$, $n = 4$ experiments using four ASM donors). Additional experiments demonstrated that the effects of inhibiting adhesion with EDTA and anti-TSLC-1 were additive, indicating that TSLC-1 contributes to the Ca^{2+}-independent adhesion pathway (Fig. 4*B*). TSLC-1 was not expressed by ASM as assessed by both flow cytometry (data not shown) and immunofluorescence (Fig. 3*A*), indicating that TSLC-1 expressed by HLMC mediates heterotypic adhesion with an unknown ASM ligand.

In mice, the adhesion of mast cells to fibroblasts mediated by TSLC-1 is dependent on an interaction between membrane-bound SCF and CD117 (18). However, function-blocking Abs to CD117 or SCF did not alter baseline adhesion or the response to TSLC-1 blockade (data not shown).

Discussion

This is the first study to demonstrate that both HLMC and the human mast cell line HMC-1 adhere to cultured human ASM cells. The degree of adhesion demonstrated under basal conditions, ~45% of mast cells, was high compared with that reported for T cells and eosinophils (19, 20), and similar to that described previously for HLMC adhesion to human bronchial epithelium (7) and human skin mast cell adhesion to human skin fibroblasts (21).

ASM expresses several molecules with potential adhesive capability including Ig superfamily members (VCAM-1, ICAM-1) and membrane-bound SCF (19, 20, 22), with complimentary ligands expressed on HLMC and HMC-1 (CD11a/CD18, $\alpha_4\beta_1$,

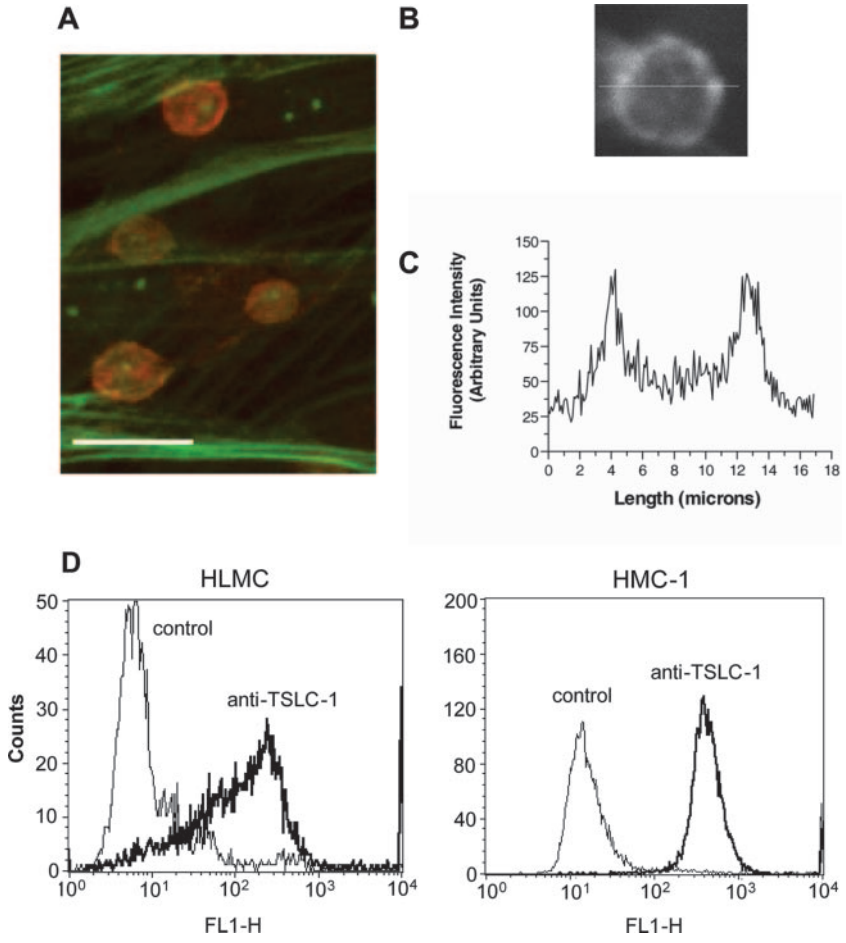

FIGURE 3. Surface expression of TSLC-1 by HLMC. *A*, Confocal dual channel immunofluorescence demonstrating the adherence of TSLC-1$^+$ HLMC (red) to α-smooth muscle actin$^+$ ASM cells (green). Note that ASM is negative for TSLC-1. Scale bar, 25 μm. *B*, Fluorescence was scanned across the cells as indicated by the white line. *C*, The plot shows the variation in fluorescence intensity along the line. Notably, the cell surface is the most intensely fluorescent. *D*, Flow cytometric analysis of TSLC-1 expression by HLMC and HMC-1. Staining was performed with the 3E1 anti-TSLC-1 Ab and analyzed by flow cytometry. Representative examples of three experiments performed on each cell type.

CD117) (7). However, these molecules are also expressed by human bronchial epithelium and fibroblasts but do not mediate human mast cell adhesion to these cells (7, 21). We previously have undertaken an extensive investigation into the adhesion of HLMC to airway epithelial cells (7). Numerous potential adhesive interactions were examined, including integrins expressed by HLMC, but none were involved in the adhesion mechanism. This HLMC-epithelial adhesion pathway did not depend at all on Ca^{2+}, but was inhibited in part by the glycosidases β-galactosidase and endo-α*N*-acetylgalactosaminidase, suggesting the involvement of *O*-linked galactose residues. Trautmann et al. (21) described the adhesion of HMC-1 cells and human skin mast cells to human fibroblasts. Again, high baseline adhesion was observed, in the order of 95% for HMC-1 cells and 60% for skin mast cells, but a detailed extensive investigation of potential adhesion pathways failed to define a mechanism. Adhesion was not mediated by a number of known adhesion receptors or sugars, and was only partially sensitive to calcium depletion (21). With respect to our current study, adhesion to ASM was not inhibited by β-galactosidase, or by adhesion-blocking mAb to integrins, VCAM-1, or ICAM-1. However, the ability of Mn^{2+} to enhance HLMC adhesion to ASM suggests that integrin pathways might be activated under certain conditions. Because adhesion was partially Ca^{2+} dependent, it most closely resembles skin mast cell adhesion to cutaneous fibroblasts, although adhesion to epithelium could involve common elements. Taken together, these three studies provide firm evidence that the bulk of human mast cell adhesion to airway epithelium, ASM, and skin fibroblasts involves novel pathways that remain to be defined.

Adhesion was consistently attenuated by a blocking Ab to TSLC-1. TSLC-1 is a 75 kDa *N*-linked glycoprotein that functions as a tumor suppressor in epithelial cells (23). It is also known by a variety of other names depending on where and by whom it was discovered. These include spermatogenic Ig superfamily (SgIGSF) (24), Ig superfamily member 4 (IGSF-4) (25), RA175 (26), synaptic cell adhesion molecule-1 (SynCAM-1) (27), and nectin-like molecule-2 (Necl-2) (28). In keeping with other Ig-like cell adhesion molecules, TSLC-1 consists of three extracellular Ig-like loops, a transmembrane domain, and a cytoplasmic domain linked to the cytoskeleton. The human and mouse proteins are 98% identical at the amino acid level (29). The molecule is widely expressed, particularly in epithelium, but not in mouse fibroblasts (28). TSLC-1 mediates both homophilic and heterophilic cell-cell adhesion, both of which are Ca^{2+} independent (28, 30). Interestingly, TSLC-1 is also expressed by mouse mast cells, and contributes to their adhesion to mouse fibroblasts and nerves (6, 31). Therefore, it is of great interest that we have confirmed for the first time that human mast cells also express TSLC-1 at both the mRNA and protein level, and that blocking this attenuates mast cell adhesion to ASM. This adhesive interaction appears to be heterophilic in that ASM did not express TSLC-1. The ASM counter ligands await identification, but TSLC-1 is known to bind to other nectins and nectin-like molecules including nectin-3 and necl-1 (28). The observation that the effects of Ca^{2+} chelation and TSLC-1 blockade on HMC-1 and HLMC adhesion to ASM were additive further suggests that the TSLC-1-dependent adhesion mechanism is involved in the Ca^{2+}-independent adhesion observed. In the mouse, TSLC-1-dependent adhesion to fibroblasts,

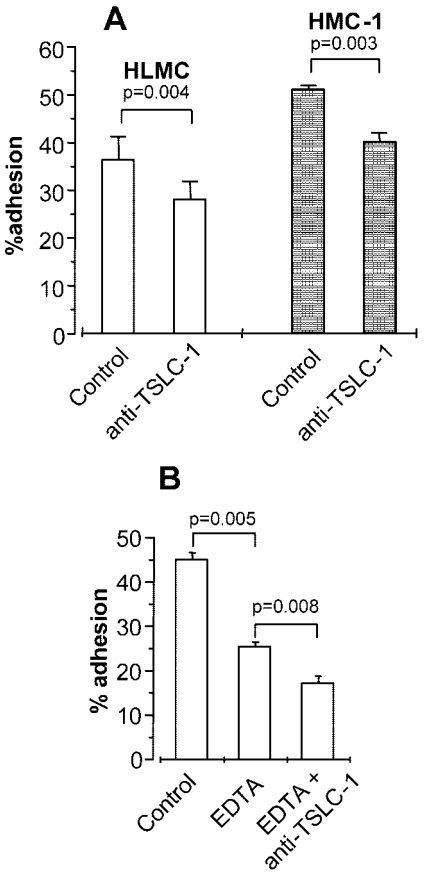

FIGURE 4. Attenuation of HLMC-ASM adhesion by an anti-TSLC-1 Ab. *A*, Attenuation of HLMC ($n = 7$) and HMC-1 ($n = 4$) adhesion to ASM by the 9D2 adhesion-blocking anti-TSLC-1 Ab. *B*, Attenuation of HMC-1 adhesion to ASM in the presence of EDTA and in the presence of EDTA plus anti-TSLC-1 blocking Ab ($n = 3$).

but not nerves, also requires signaling via CD117 (32). We could not demonstrate any dependence of either baseline or TSLC-1-mediated HLMC-ASM adhesion on CD117, which is consistent with previous observations relating human mast cell adhesion to fibroblasts and epithelium (7, 21). Whether TSLC-1 contributes to the adhesion of human mast cells to fibroblasts and airway epithelium needs addressing in future work.

The concentration of the 9D2 anti-TSLC-1 Ab we used (10 μg/ml) was just below that which is optimal for inhibition of the homotypic aggregation of TSLC-1 transfectants (14 mg/ml) (6). At this concentration, this Ab reduces TSLC-1 homotypic adhesion by ~60%. The effects of 9D2 on the Ca^{2+}-independent adhesion of HLMC to ASM is close to this (~50%), suggesting that TSLC-1 probably accounts for all the Ca^{2+}-independent adhesion observed. At present 9D2 is the only adhesion-blocking mAb available, so it is not possible at this stage to examine this further. The Ca^{2+}-dependent HLMC-ASM adhesion pathway awaits further characterization. For the reasons indicated above this is likely to be novel, so rather than undertaking a further extensive investigation of all known potential adhesion pathways between HLMC and ASM, which may prove fruitless, work is in progress to generate short-chain Fv adhesion-blocking Abs and to characterize the ligands to which they bind.

The high baseline adhesion of HLMC to ASM is very interesting. HLMC infiltrate the ASM in asthma, numbers of which correlate inversely with the degree of bronchial hyperresponsiveness (5). In contrast, T cells and eosinophils are not evident in the ASM in asthma (5). The observation that, in contrast to HLMC, resting T cells do not adhere to ASM (19), and eosinophils adhere relatively poorly to ASM (M. Hughes, personal communication) (19, 20) suggests that the adhesion mechanism for HLMC might play an important role in the recruitment and retention of HLMC by the ASM in asthma. Furthermore, it is likely that the ability of the cells to adhere will have important implications for the function of both cell types which is the subject of on-going studies.

In summary, HLMC adhere avidly to ASM, in part via TSLC-1 and in part via an as-yet-undefined Ca^{2+}-dependent pathway. This may have important implications for the pathogenesis of asthma, and for the design of novel treatments.

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Disclosures

The authors have no financial conflict of interest.

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