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*J Immunol* 2010; 185:6105-6114; Prepublished online 15 October 2010;
doi: 10.4049/jimmunol.1000638
http://www.jimmunol.org/content/185/10/6105

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*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Mast Cell Fibroblastoid Differentiation Mediated by Airway Smooth Muscle in Asthma

Davinder Kaur, Ruth Saunders, Fay Hollins, Lucy Woodman, Camille Doe, Salman Siddiqui, Peter Bradding, and Christopher Brightling

Mast cell microlocalization to the airway smooth muscle (ASM) bundle is a key feature of asthma, but whether these mast cells have an altered phenotype is uncertain. In this paper, we report that in vivo, mast cells within the ASM bundle, in contrast to mast cells in the bronchial submucosa, commonly expressed fibroblast markers and the number of these cells was closely related to the degree of airway hyperresponsiveness. In vitro human lung mast cells and mast cell lines cultured with fibronectin or with primary human ASM cells acquired typical fibroblastic markers and morphology. This differentiation toward a fibroblastoid phenotype was mediated by ASM-derived extracellular matrix proteins, independent of cell adhesion molecule-1, and was attenuated by α5β1 blockade. Fibroblastoid mast cells demonstrated increased chymase expression and activation with exaggerated spontaneous histamine release. Together these data indicate that in asthma, ASM-derived extracellular matrix proteins mediate human mast cell transition to a fibroblastoid phenotype, suggesting that this may be pivotal in the development of airway dysfunction in asthma. The Journal of Immunology, 2010, 185: 6105–6114.

Abbreviations used in this paper: AHR, airway hyperresponsiveness; ASM, airway smooth muscle; BD, bronchodilator; BDP, beclomethasone dipropionate; CADM1, cell adhesion molecule-1; ECM, extracellular matrix; GMFI, geometric mean fluorescence intensity; HLMC, human lung mast cell; HMC-1, human mastocytoma cell line; ICS, inhaled corticosteroid; IQR, interquartile range; α-SMA, α-smooth muscle actin.

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Received for publication February 24, 2010. Accepted for publication September 3, 2010.

This work was supported by Asthma UK, Department of Health Clinician Scientist Award (to C.B.), Wellcome Value in People Award (to R.S.), and Wellcome Senior Clinical Fellowship (to C.B.).

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1000638

Asthma is a major cause of morbidity and mortality (1). It is characterized by variable airflow obstruction and airway hyperresponsiveness (AHR) in association with Th2 eosinophilic airway inflammation and remodeling (2). Asthma is a heterogeneous condition, and the relationship between airway inflammation and dysfunction is complex (3). Indeed, anti–IL-5 therapy has resulted in successful reduction in eosinophilic inflammation without changes in AHR (4, 5), illustrating that eosinophilic inflammation and AHR are dissociated. Comparative studies between asthma and eosinophilic bronchitis, a common cause of cough (6), have been very informative about the cause of disordered airway physiology in asthma and suggest that the microlocalization of mast cells within the airway smooth muscle (ASM) bundle is critical for the development of AHR (7, 8).

Mast cell progenitor recruitment to the lung is in part mediated by leukotriene B4 (9) and chemokines (10) and is integrin dependent (11). In the airway, mast cell trafficking to different compartments is under the influence of several chemotactic factors (reviewed in Ref. 12). Recruitment to the ASM bundle in asthma is predominantly via activation of the CXCR3/CXCL10 axis (13). Within the ASM bundle, mast cells adhere to ASM cells, in part via heterophilic adhesion mediated by cell adhesion molecule-1 (CADM1) expressed by mast cells (14). However, the CADM1-independent adhesion mechanism remains unexplained. Mast cells within the ASM bundle are in an activated state with increased IL-4 and IL-13 expression (15, 16), are smaller than mast cells observed in the lamina propria, and undergo piecemeal degranulation as evidenced by electron microscopy (17). Whether these changes simply represent mast cell activation or a transition to an altered phenotype is unknown. ASM-bundle–resident mast cells are intimately associated with both the ASM cells and the local matrix environment. Therefore, the potential for mast cells to undergo phenotypic transition as a consequence of interactions with ASM and extracellular matrix (ECM) proteins needs to be explored further.

The ECM composition varies between different compartments in the airway wall, between asthma versus a healthy state, and across disease severity. This matrix is not a benign bystander that simply constitutes the scaffold to build airway structures and allow inflammatory cell trafficking, but can alter the biophysical properties of the airway wall and modulate cell function (18). In those with fatal asthma, compared with nonasthmatics and asthmatics who died of other causes, elastin fibers and fibronectin are increased in the ASM bundle (19). We therefore hypothesized that ASM-derived ECM proteins exert important effects on mast cells localized within the ASM bundle.

In this study, we have demonstrated that mast cells localized within the ASM bundle express fibroblast markers and their number in the ASM bundle is closely related to AHR. We have identified in vitro that this fibroblastoid mast cell transition is mediated by ASM-derived ECM proteins. These fibroblastoid mast cells are activated and release increased concentrations of histamine, and their number in the ASM bundle is closely related to AHR.

Materials and Methods

Subjects

Asthmatic subjects and nonasthmatic controls were recruited from Leicester, U.K. Asthmatic subjects had a consistent history and objective evidence of asthma, as described previously (7, 13). Subjects underwent extensive clinical characterization including video-assisted fiberoptic bronchoscopy examination. The study was approved by the Leicestershire Ethics Committees. All patients gave their written informed consent.
**Immunohistochemistry**

Sequential 2-μm sections were cut from glycolmethacrylate-embedded bronchial biopsies and stained using mAbs against α-smooth muscle actin (α-SMA; Dako, Ely, U.K.), mast cell tryptase (Dako), the fibroblast marker Thy-1 (Ab1) (Calbiochem, San Diego, CA) (20), and appropriate isotype controls (Dako). Thy-1/′tryptase′ (fibroblast), Thy-1′tryptase′ (mast cell) and Thy-1′tryptase+ cells (fibroblastoid mast cell) were enumerated per square millimeter ASM by colocalization (7, 13, 15). The whole biopsy was assessed, and a minimum area of 0.1 mm² was considered assessable, as described previously (7). Double immunostaining for tryptase and Thy-1 fibroblast marker was performed using the EnVision Doublestain Kit (DakoCytomation, Carpinet, CA) according to the manufacturer’s instructions. Thy-1 expression was developed with peroxidase and 3,3′-diaminobenzidine tetrahydrochloride (brown reaction product) and for tryptase alkaline phosphatase and fast red (red reaction product). Sections were then counterstained with hematoxylin and mounted in an aqueous mounting medium (BDH Chemicals, Poole, U.K.). Appropriate isotype controls were performed in which the primary Abs were replaced by irrelevant mouse mAb of the same isotype and at the same concentration as the specific primary mAb.

**Cell isolation and culture**

Pure ASM bundles were isolated from bronchoscopic samples (n = 12; 10 asthmatic subjects, 2 nonasthmatic) and from lung resection (n = 10). Primary ASM was cultured and characterized as previously described (21). Cells used were passage 2–5. Primary fibroblasts were isolated from bronchial biopsies, characterized for the fibroblast markers Thy-1 and 1B10 (AbCam, Cambridge, U.K.), and used between passage 6 and 8 (n = 13). The human lung mast cells (HLMCs) were isolated and cultured from nonasthmatic lung (n = 19), as previously described (21). The human mastocytoma cell line (HMC-1) was a generous gift from Dr. J. Butterfield (Mayo Clinic, Rochester, MN), and was cultured as described previously (21).

**Adhesion, elongation, and differentiation assays**

**Mast cells and ECM proteins.** HMC-1 cells (1 x 10⁶ cells/well) were seeded onto six-well plates uncoated or coated with 5–40 μg/ml fibronectin (Sigma-Aldrich, Poole, Dorset, U.K.) or 60 μg/ml collagen (Inamed Biomaterials, Fremont, CA) in ITS Media (ASM media with 1% ITS+3 supplement (Sigma-Aldrich) in place of FBS). After 6 d, nonadherent cells were collected and grown further. Adherent cells were then harvested using Accutase (Invitrogen Molecular Probes, Paisley, U.K.).

**Flow cytometry**

HMC-1 cells were stained, where appropriate, with Abs to Thy-1, 1B10, Collagen 1 (Chemicon, Temecula, CA), CD117 (BD Pharmingen, San Diego, CA), chymase (Chemicon, Hampshire, U.K.), and tryptase (Dako), or their appropriate isotype controls indirectly labeled with FITC (Dako) or allylpropocyanin (R&D Systems) and analyzed by one-color or two-color flow cytometry (in coculture samples) on a FACSCanto (BD Biosciences, Oxford, U.K.). Mast cells were distinguished from ASM by prelabeling with CellTrace CFSE prior to coculture (according to manufacturer’s instructions, Invitrogen Molecular Probes, Paisley, Scotland, U.K.) or by staining with CD117-RPE (Dako).

**Immunofluorescence**

Cells were stained, where appropriate, for surface Thy-1, 1B10, or appropriate isotype controls; indirectly labeled with FITC or allylpropocyanin; and counterstained with DAPI (Sigma). Mast cells were distinguished from ASM cells by prelabeling with CellTrace CFSE or by staining with CD117-RPE and indirect labeling with NorthernLights-RPE (R&D Systems). Double immunofluorescence was performed on cocultured ASM and mast cells and mast cells adherent to fibronectin using biotinylated tryptase mAb (Promega, Madison, WI) and chymase mAb (Chemicon, Temecula, CA). The biotinylated Ab binding was detected using Streptavidin-Texas Red, and chymase Ab binding was detected using secondary FITC-labeled Ab. Sections were counterstained with DAPI. The slide was then mounted with fluorescent mounting medium and analyzed.

**Assessment of cell contraction by collagen gel analysis**

Following culture in fibronectin-coated six-well plates over 6 d, the extent of collagen gel contraction by HMC-1 cells was assessed. A total of

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*Mean (SE).
*Median (IQR).
BD, bronchodilator; BDP, beclomethasone dipropionate; ICS, inhaled corticosteroids.
FIGURE 1. Mast cells in the ASM bundle express fibroblastic markers. Representative photomicrographs of a bronchial biopsy from an asthmatic (original magnification ×400) stained for isotype control, illustrating the epithelium and lamina propria (A), and sequential sections of the ASM bundle (B–D), with arrows highlighting the same cells across the three sections stained for α-SMA (B), mast cell tryptase (C), and the fibroblast marker Thy-1 (Ab1) (D). The arrowhead and the long and short arrows illustrate cells that are Thy-1−tryptase− (fibroblast), Thy-1−tryptase+ (mast cell), and Thy-1−tryptase+ (fibroblastic mast cell), respectively. Single staining of Thy-1+ (brown, black arrowhead) and double staining of Thy-1+ (brown) and tryptase+ of mast cells (red staining, white arrowhead, E). Dot plot of the number of mast cells per square millimeter of ASM (F) and Thy-1+ cells per square millimeter of ASM (G) in subjects with or without asthma. Horizontal bars represent the median, p < 0.05, Kruskal–Wallis test, for all across-group comparisons; the p value for Dunn’s posttest is given on the figure. H, Percentage of mast cells colocalizing with Thy-1+ cells in subjects with or without asthma. Mean (SEM), p < 0.05 ANOVA; p value for Tukey’s post hoc test is given on the figure. Correlation of mast cells per square millimeter of ASM (I) and Thy-1+ mast cells per square millimeter of ASM, with methacholine PC_{20} FEV_{1}; correlation coefficient and p value are given on the figure.
1.25 × 10^5 HMC-1 cells were resuspended in 144 μl ITS media ± stimulus, with 299 μl collagen, 37 μl 10× DMEM (Invitrogen), and 20 μl sodium bicarbonate (Invitrogen), and added to 24-well plates. The mixture was left to polymerize into gels at 37°C for 90 min prior to detachment from the well; 500 μl ITS media ± stimulus was added and incubated over 5 d. Gel surface area was measured using ImageJ (http://rsb.info.nih.gov/ij) by a blinded observer. Collagenase (1.9 mg/ml; Sigma-Aldrich) for 20 min at 37°C was used to extract HMC-1 cells from the gels, to further characterize mast cell differentiation.

Quantification of histamine release by mast cells

Histamine was measured by a sensitive radioenzymatic assay, as described previously, and corrected for cell number (23).

ELISA

PGD2 expression in cocultured cell supernatants was measured by ELISA (Cayman Chemical, Ann Arbor, MI).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 4 (GraphPad, San Diego, CA). Parametric data are presented as mean ± SEM; log normally distributed data were log transformed and expressed as geometric mean (95% confidence interval), and nonparametric data are presented as median (interquartile range [IQR]). Comparisons across groups was analyzed by ANOVA and post hoc Tukey’s test or by Kruskal–Wallis testing with post hoc Dunn’s pairwise comparisons for nonparametric data. Between-group comparisons were made by paired and unpaired t tests, as appropriate. Differences were considered significant when p < 0.05.

Results

Mast cells express fibroblast markers in the ASM bundle in asthma

To determine the localization of mast cells, fibroblasts, and cells coexpressing markers for both cell types in the airway wall, bronchial biopsies from 16 healthy controls and 26 subjects with asthma were studied. The clinical characteristics of these subjects are shown in Table I. Representative photomicrographs of mast cells (tryptase⁺ cells) and fibroblasts (Thy-1⁺ [Ab1]⁺ cells) within the ASM bundle are shown in Fig. 1A–D. Mast cells were observed within the ASM bundles typically surrounded by matrix, as illustrated (Fig. 1C). Double immunostaining for Thy-1 and tryptase on the same section are shown in Fig. 1E, where the Thy-1 cells are stained individually and double stained with tryptase⁺ mast cells within the ASM bundles (n = 3 severe asthmatics). The median (IQR) mast cell number within the ASM bundle was increased in mild-moderate (6.8 [9.8] cells/mm² ASM) and severe asthma (6.2 [16.4] cells/mm² ASM) compared with healthy controls (1.7 [4.4] cells/mm² ASM; p = 0.001 Kruskal–Wallis; p < 0.01 asthma groups versus controls, Dunn’s pairwise comparison) (Fig. 1F).

The number of fibroblasts was also increased in the ASM bundle in mild-moderate (4.4 [11.5] cells/mm² ASM) and severe asthma (6.5 [10.2] cells/mm² ASM), compared with controls (1.5 [2.5] cells/mm² ASM; p = 0.003 Kruskal–Wallis; p < 0.05 asthma groups versus controls) (Fig. 1G). The proportion of mast cells that coexpressed fibroblast markers (tryptase⁺/Thy-1⁺) cells) was increased in the ASM bundle (18 ± 3%) compared with the lamina propria (2.9 ± 0.6%; p < 0.0001), and in the ASM bundle the proportion was increased in mild-moderate (22 ± 3%) and severe asthma (22 ± 4%), compared with healthy controls (4 ± 2%; p = 0.0002 ANOVA, p < 0.01 asthma groups versus controls, Tukey’s post hoc comparison) (Fig. 1H).

In the 21 asthmatic subjects who underwent bronchial challenge, the number of mast cells within the ASM bundle was significantly inversely correlated with the degree of AHR (r = −0.45; p = 0.04; Fig. 1J). There was no significant correlation between the number of fibroblasts in the ASM bundle and AHR (data not shown). The number of mast cells that coexpressed fibroblast markers was strongly correlated with the degree of AHR (r = −0.61; p = 0.004; Fig. 1J). There was no significant correlation between FEV₁ percentage predicted and the number of mast cells, fibroblasts, or fibroblastoid mast cells in the ASM bundle (data not shown).

Mast cells adhere to ECM and undergo morphological transition

We therefore considered whether mast cells differentiate to a fibroblastoid phenotype in the presence of ECM proteins. Indeed,

![FIGURE 2](http://www.jimmunol.org/)
we found that in vitro the morphology of the HMC-1 changed over 6 d in the presence of plasma fibronectin, with cells becoming elongated (Fig. 2A). The percentage of elongated cells was related to the concentration of fibronectin in a dose-dependent manner (Fig. 2B). The number of elongated cells was greatest in the presence of fibronectin 40 μg/ml. Therefore this concentration of fibronectin was used throughout the study. At this concentration, HMC-1 cells adhered avidly to fibronectin after 6 d (81 ± 5%, n = 8; p < 0.001), and adhesion was markedly inhibited by anti-α5β1 blocking Ab (4.3 ± 0.4% versus isotype control, 78 ± 2%; p = 0.0005; n = 3; Fig. 2C).

Previous reports have shown HMC-1 cells to adhere to collagen at a maximum concentration of 60 μg/ml (6). At this concentration we found that the proportion of HMC-1 cells that adhered to collagen after 6 d was 59 ± 2% (n = 5; p < 0.001), and the proportion of elongated cells was increased (44 ± 2.4%; n = 4; p < 0.001).

Mast cells adherent to ECM express fibroblast markers

The fibroblast markers Thy-1 and 1B10 were highly expressed on cultured fibroblast cells, as evidenced by immunofluorescence (Fig. 3A) and by flow cytometry (Fig. 3B). The proportion of fibroblast cells that expressed Thy-1 was 67 ± 7% (p = 0.001; n = 13), and 1B10 was 26 ± 5 (p = 0.003; n = 7) (Fig. 3C).

HMC-1 cells cultured for 6 d without fibronectin (control), HMC-1 cells cultured with fibronectin that did not adhere (non-adherent cells) and those cells that adhered (adherent cells) were analyzed by flow cytometry for surface Thy-1, IB10, CD117, and intracellular tryptase expression. 1B10 expression was significantly increased in the adherent HMC-1 cells (ΔGMFI 21 ± 2.3; n = 3) compared with control (6.7 ± 1.76; p = 0.002). HMC-1 cells cultured with or without fibronectin did not significantly express Thy1 (n = 5; data not shown).

In the presence of the anti-α5β1 blocking Ab, HMC-1 cells did not express 1B10 (Fig. 3F). We were unable to compare 1B10 expression in adherent versus nonadherent HMC-1 cells in the presence of the blocking Ab, as it markedly inhibited adhesion and

**FIGURE 3.** HMC-1 cells adherent to fibronectin develop fibroblastic markers Thy-1 and 1B10; fibroblast marker expression was confirmed by cultured fibroblast cells by immunofluorescence (A) and flow cytometry (B). The example fluorescent histograms represent populations of Thy-1 and 1B10 positive cells (black line) plotted with the corresponding isotype control (gray line). C. Percent positive analysis of fibroblast cells demonstrates surface Thy-1 (n = 13 experiments) and 1B10 (n = 7 experiments) expression. D. Representative histograms of surface 1B10, CD117, intracellular collagen I, tryptase, and chymase expression on HMC-1 cells cultured with serum-free media over 6 d without fibronectin, versus nonadherent cells and adherent cells with fibronectin. E and F. The ΔGMFI of CD117 (n = 5 experiments) and tryptase expression (n = 5 experiments) (E) and 1B10 expression with and without α5β1 fibronectin blocking Ab (n = 3) (F) in nonadherent versus adherent HMC-1 cells cultured on fibronectin over 6 d. The ΔGMFI was determined by the difference between the total binding expression of total GMFI minus the total binding of matched isotype control for 1B10, CD117, and tryptase. G. 1B10 expression was also confirmed by immunofluorescence on adherent HMC-1 cells on fibronectin (40 μg/ml) over 6 d. Data presented as mean ± SEM. Statistical differences were assessed using the t tests and p values as shown. Original magnification ×400.
therefore resulted in insufficient adherent cells for flow cytometry. Expression of 1B10 by adherent HMC-1 cells was confirmed by immunofluorescence (Fig. 3G).

In the presence of collagen (60 μg/ml) for 6 d, adherent HMC-1 cells demonstrated increased 1B10 expression (ΔGMFI 79 ± 5; n = 3) compared with nonadherent cells (33 ± 9; p = 0.025) and HMC-1 cells alone (28 ± 6; n = 3).

Mast cell fibroblastoid transition causes collagen gel contraction

We considered whether during fibroblastoid transition in a three-dimensional matrix mast cells undergo shape change that induces contraction. Therefore, to test whether the HMC-1 cells that were nonadherent to fibronectin in two-dimensional culture after 6 d have the capacity to undergo fibroblastic transition in a three-dimensional matrix, we cultured adherent and nonadherent HMC-1 cells in collagen gels for 5 d.

We confirmed that after 6 d culture with fibronectin, the 1B10 expression was increased in the adherent versus nonadherent HMC-1 cells (ΔGMFI [95% confidence interval] 233 [140–325]; p = 0.003; n = 3) (Fig. 4A). Following culture in collagen gels for a further 5 d, there was no difference in 1B10 expression between the adherent and the nonadherent HMC-1 cells (ΔGMFI [95% confidence interval] 43 [−26–112]; p = 0.18), but the change from baseline in 1B10 expression in the nonadherent cells was significantly increased compared with that in the adherent cells (280; 95% CI [142–418]; p = 0.005) (Fig. 4A). This observation suggests that the nonadherent mast cells in the two-dimensional culture had indeed undergone fibroblastoid transition in collagen gels.

This HMC-1 fibroblastoid transition was associated with a significant reduction in the size of the collagen gel. Representative gels are as shown (Fig. 4B). After 5 d the mean ± SEM percentage size of gel compared with baseline was 66 ± 4% (n = 7) for nonadherent cells versus 82 ± 4% for adherent cells (p = 0.025) (Fig. 4C). This finding illustrates that the mast cells had undergone concomitant changes in cell shape and expression of fibroblast markers in the collagen gels.

Mast cell fibroblastoid transition in coculture with ASM

To investigate the effects of mast cell-ASM interactions on mast cell phenotype, we extended our model to a coculture system. Primary HLMCs and HMC-1 cells cocultured with primary ASM cells over 7 d changed their cell morphology to become more elongated (Fig. 5A). This change in cell morphology was also confirmed by immunofluorescence (Fig. 5B). The percentage of tryptase/chymase-positive cells increased significantly after 10 d (27 ± 5.8%; p = 0.05; n = 13; Fig. 5C, 5D) compared with cells in coculture (9.85 ± 5.3%; n = 7) and cells adherent to fibronectin (10.1 ± 3.3%; n = 6; p = 0.02) over 1 d.

In contrast to HMC-1 cells, HLMCs expressed 1B10 in the absence of fibronectin and/or ASM (13 ± 5%; n = 3), whereas the percentage of Thy-1–positive HLMCs in the absence of fibronectin and ASM was low (4.7 ± 1.7%). Therefore, Thy-1 expression by mast cells was studied in coculture with ASM cells.

To track mast cells in coculture with ASM over 6–15 d, mast cells were labeled with the fluorescent marker CFSE, a stable dye that is not passed between cells upon adhesion. With use of flow cytometry, CFSE-labeled mast cells were gated and analyzed for Thy-1 expression. Example flow cytometry dot plots and histograms are as shown, illustrating Thy-1 expression by HLMCs, HMC-1 cells, and in cells cocultured with ASM (Fig. 5E). Following coculture with ASM, HLMC Thy-1 expression was increased compared with control after 7 d (22 ± 5%; p = 0.005; n = 6) and 15 d (45 ± 8%; p = 0.004; n = 6) (Fig. 5F). A small, albeit significant, increase in Thy-1 expression was observed for HMC-1 cells (9 ± 2%; p = 0.011; n = 7) cocultured with ASM for 6 d, compared with cells alone (Fig. 5G). Similarly, Thy-1 expression by HMC-1 cells cultured with ASM separated by a Transwell insert or with ASM-conditioned media was increased, compared with that in controls. This increased expression was reduced in comparison with that of HMC-1 cells in coculture with ASM (Fig. 5G; p < 0.05).

HMC-1 cell Thy-1 expression was not modulated by TGFβ-1 or 2 (0–10 ng/ml) (data not shown).

Mast cell fibroblastoid transition in coculture with ASM is mediated via α5β1

We considered whether this mast cell fibroblastoid transition was dependent upon mast cell-ASM adhesion or mast cell ECM-ASM interactions. The percentage adhesion of HMC-1 cells to ASM after 6 d coculture was 57 ± 7%, n = 4. In contrast to short-term culture (14), this HMC-1 adhesion to ASM after long-term culture was not inhibited by preincubation with the CADM1 blocking Ab (58 ± 9% versus isotype control 57 ± 9%; n = 4; p = 0.67) but was markedly inhibited by α5β1 blockade (26 ± 4% versus isotype control 68 ± 6%; p = 0.0008; n = 6) (Fig. 6A). The blockade of CADM1 and α5β1 in concert was not different to α5β1 alone (p = 0.49) (Fig. 6A).

In coculture with ASM and in the presence of the α5β1 blocking Ab, the proportion of Thy-1+h HMC-1 cells (6.4 ± 4.3%; n = 4) and HLMCs (4.9 ± 1.4%; n = 5) was significantly decreased...
FIGURE 5. Mast cell-ASM coculture promotes fibroblastoid differentiation. A, Representative photomicrographs of ASM cells cultured alone with serum-free media (i), over 7 d with HLMCs (ii, iii), or HMC-1 cells over 6 d (iv) (original magnification ×40). The black arrow indicates the area in ii that is shown in detail in iii. B, CD117 expression of HLMCs cultured on ASM cells over 3 and 7 d, showing elongated cells at day 7 (nuclei stained blue). Original magnification ×200. C, Immunofluorescence staining demonstrating HLMCs cultured on fibronectin for 1 d (i–iii) and cocultured on ASM cells over 10 d (iv–vi); i and iv, double isotype control; ii and v, red tryptase+ve HLMCs; iii and vi, double stained HLMCs for tryptase (red) and chymase (green) (n = 6–13). Original magnification ×400. D, Percentage of tryptase/chymase-positive HLMCs cultured alone (1 d; n = 6) and with ASM (1–10 d; n = 6–13). E, Representative dot plots for ASM alone and ASM cultured with HLMCs (prelabeled with CFSE FITC). Labeled CFSE HLMCs were gated and analyzed for Thy-1 expression, using the allophycocyanin secondary Ab. Shown are representative flow cytometric histograms for HLMCs and HMC-1 cells prelabeled with CFSE and stained for Thy-1–allophycocyanin (black line) plotted with the corresponding isotype control (gray line) over 6 d culture. F, Percentage of Thy-1–positive HLMCs cultured alone (7–15 d; n = 10 donors) and with ASM for 7 d (n = 6) and 15 d (n = 6). G, Percentage of Thy-1–positive HMC-1 cells cultured alone (n = 3–5), with ASM (n = 7), on inserts over ASM cells (n = 4), and with ASM cell supernatants (n = 4). Data are presented as mean ± SEM. Statistical differences were assessed using ANOVA and t tests as appropriate, and p values are as shown.
A, Percentage inhibition of HMC-1 cell adherence to ASM cells by adhesion blocking Abs directed against CADM1 (n = 4) and α5β1 integrin (n = 6) alone and in combination (n = 4), compared with appropriate isotype controls (IgY and IgG1). B, Percentage of Thy-1-positive HMC-1 cells (n = 4) and HLMCs (n = 3–5) cocultured with ASM for 6 d (HMC-1) and 7 d (HLMC) in the presence of anti-α5β1 mAb determined by flow cytometry. C, Constitutive and net IgE-dependent histamine release from HLMC was measured after 7 d coculture with ASM cells in the presence of the anti-α5β1 integrin or isotype control (IgG1) (n = 2 ASM cell donors and 4 HLMC donors). Data are presented as mean ± SEM. Statistical differences were assessed using the t tests and p values as shown.

FIGURE 6. Mast cell fibroblastoid differentiation promoted by ASM-derived ECM proteins is α5β1 dependent and is associated with mediator release. A, Percentage inhibition of HMC-1 cell adherence to ASM cells by adhesion blocking Abs directed against CADM1 (n = 4) and α5β1 integrin (n = 6) alone and in combination (n = 4), compared with appropriate isotype controls (IgY and IgG1). B, Percentage of Thy-1-positive HMC-1 cells (n = 4) and HLMCs (n = 3–5) cocultured with ASM for 6 d (HMC-1) and 7 d (HLMC) in the presence of anti-α5β1 mAb determined by flow cytometry. C, Constitutive and net IgE-dependent histamine release from HLMC was measured after 7 d coculture with ASM cells in the presence of the anti-α5β1 integrin or isotype control (IgG1) (n = 2 ASM cell donors and 4 HLMC donors). Data are presented as mean ± SEM. Statistical differences were assessed using the t tests and p values as shown.
important for the initial adhesion of mast cells to ASM and may initiate important cell signaling events, but that sustained adhesion of mast cells within the ASM bundle is maintained by mast cell interactions within a local ECM protein environment. This observation provides an explanation for the lack of effect we reported for blocking β-integrins on short-term adhesion for 30 min between ASM and mast cells, as well as providing a rationale for our inability to inhibit mast cell adhesion to ASM by CADM1 blockade after coculture for several days. Mast cell adhesion to fibronectin has been consistently reported (26–28), and in some circumstances, stem cell factor and IgE cross-linking promote mast cell adhesion to fibronectin (29, 30). We report in this paper that the development of fibroblastoid mast cells cultured with ECM proteins increased in a dose-dependent manner. This also occurred in coculture with ASM and was present, albeit to a substantially reduced extent, in the absence of direct contact with ASM when using Transwells and ASM supernatants and may have been mediated by soluble fibronectin. All of these effects were inhibited by αβ1 integrin blockade, implicating mast cell-fibronectin interactions. Importantly, fibronectin is increased in the ASM bundle in asthma deaths (19) suggesting that this ECM protein is important in asthma, particularly in severe disease, and therefore has the capacity to mediate important mast cell-ECM interactions. Consistent with this view, previous reports have shown that rat mast cells cocultured with fibroblasts (31) or fibronectin result in increased histamine release (26). Similarly, human mast cells that adhere to fibronectin become more oblong, with pronounced formation of filopodia (27). This adhesion is attenuated by TRAIL activation, which consequently abrogates mast cell attachment-dependent potentiation of IgE-mediated responses (28). Similarly, reduction in mast cell β1 integrin surface expression as a consequence of diminished surface receptor stability in rapabiptin-5 knockdown also leads to inhibition of mast cell adhesion, migration, and activation (32). Perhaps of most importance, adhesion to fibronectin does not increase IgE-dependent histamine release from HLMCs (33), supported by data reported in this paper, in contrast to findings in rodent mast cells (34). This suggests that ASM-driven HLMC mediator release, which is in part dependent on this matrix protein, drives the chronic mast cell activation evident within the ASM bundle in asthma in vivo (17). Taken together, these data suggest that ASM-derived ECM-mast cell interactions are critical for the transition of mast cells to a fibroblastoid phenotype and are likely to be central for the functional effects of this altered mast cell phenotype.

Our findings lead us to propose a new paradigm for the development of disordered airway physiology in asthma whereby the location of the mast cell within the ASM bundle leads to important reciprocal phenotypic changes in both cell types, which are perpetuated via positive feedback. We have reported that in coculture IgE-independent mast cell release of histamine, as well as transition to fibroblastoid phenotype. This altered phenotype has the capacity to further drive the ASM to a more contractile phenotype, therefore propagating the cycle to promote AHR (35).

One potential criticism of our findings is the cross-sectional design of the bronchoscopy study, which does not allow us to determine whether the strong relationship between the number of activated fibroblastoid mast cells in the ASM bundle and AHR is causal or an epiphenomenon. To confirm that fibroblastoid mast cells and their products promote airway dysfunction, bronchoscopic evaluation is required as part of interventional studies of therapies that attenuate AHR. In addition, to date there is paucity of robust animal models of asthma in which mast cell infiltration of the ASM bundle is a feature. Thus there is a need for improved models and new therapies to further study the importance of this mast cell fibroblastoid transition in asthma. Whether this transition is reversible and the fibroblastoid mast cell phenotype is plastic is also important and warrants further study.

In conclusion, we report in this paper the first evidence that mast cells develop a fibroblastoid phenotype mediated via ASM-derived ECM proteins, dependent on the αβ1 integrin. This novel mast cell phenotype demonstrates increased mediator release of histamine, and its presence in the ASM bundle is closely related to AHR. This presents a new paradigm for asthma and underlines the importance of mast cell-ASM interactions as a therapeutic target.

Acknowledgments
We thank B. Hargadon and S. McKenna for assistance in clinical characterization of subjects who underwent bronchoscopy.

Disclosures
The authors have no financial conflicts of interest.

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