

The Journal of Immunology

This information is current as
of November 4, 2010

**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

J. Immunol. 2010;185;6105-6114; originally published
online Oct 15, 2010;
doi:10.4049/jimmunol.1000638
<http://www.jimmunol.org/cgi/content/full/185/10/6105>

References

This article **cites 40 articles**, 28 of which can be accessed free
at:
<http://www.jimmunol.org/cgi/content/full/185/10/6105#BIBL>

Subscriptions

Information about subscribing to *The Journal of Immunology* is
online at <http://www.jimmunol.org/subscriptions/>

Permissions

Submit copyright permission requests at
<http://www.aai.org/ji/copyright.html>

Email Alerts

Receive free email alerts when new articles cite this article. Sign
up at <http://www.jimmunol.org/subscriptions/etoc.shtml>

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 $\alpha 5\beta 1$ 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.

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 bronchoscopic examination. The study was approved by the Leicestershire Ethics Committees. All patients gave their written informed consent.

Institute for Lung Health, Department of Infection, Inflammation, and Immunity, University of Leicester, Leicester, United Kingdom

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.).

Address correspondence and reprint requests to Prof. Christopher E. Brightling, Institute for Lung Health, Department of Infection, Inflammation, and Immunity, University of Leicester, Glenfield Hospital, LE3 9QP, Leicester, United Kingdom. E-mail address: ceb17@le.ac.uk

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.

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10/\$16.00

Immunohistochemistry

Sequential 2- μ m sections were cut from glycomethacrylate-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, Carpinteria, 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 IB10 (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 (1×10^5 cells/well) were seeded onto six-well plates uncoated or coated with 5–40 μ g/ml fibronectin (Sigma-Aldrich, Poole, Dorset, U.K.) or 60 μ g/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 counted. The percentage of elongated adherent HMC-1 cells was calculated from five random high-powered fields. Adherent cells were then harvested using Accutase (Insight Biotechnology, Wembley, London, U.K.) and the percentage of adherence determined as the proportion of adherent cells/total cells.

Mast cell coculture with ASM. ASM cells (5×10^4 cells/60 mm dish for HLMC coculture; 2.5×10^5 cells/T75 flask for HMC-1 cocultures) were incubated at 37°C for 48 h, prior to incubation with ITS media at 37°C for 24 h. HLMCs or HMC-1 cells were then added in ITS media at a 1 (mast cell):4 (ASM cell) ratio (based on the mean mast cell density in

asthmatic ASM bundles in bronchial biopsies) and cultured over 15 d. Mast cell monoculture controls were established in parallel in ITS media plus SCF where appropriate.

To study the functional contribution of receptors for HMC-1/HLMC adhesion, cells were pretreated with anti- α 5 β 1 (6.5 μ g/ml, clone JBS5; Chemicon International, Hampshire, U.K.) and CADM1 [10 μ g/ml, clone 9D2, a generous gift from Dr. A. Ito (22)] or appropriate isotype controls (IgG1 [Dako], chicken IgY [R&D Systems, Abingdon, Oxfordshire, U.K.]), either for the duration of 1 h prior to seeding onto ASM or fibronectin (40 μ g/ml) or throughout the 7-d incubation for histamine release and PGD₂ release. After 6–7 d, the percentage of adherent cells was calculated.

HMC-1 cells were added in ITS media at a 1:4 ratio to Transwell membranes (0.4 μ m; Corning, Corning, NY) inserted into wells containing ASM cells (1×10^5 cells/well in six-well plates, incubated at 37°C for 24–48 h, prior to incubation with ITS media for 24 h) or supernatant from ASM incubated in ITS media for 24 h or ITS media alone. After 6 d, the percentage of adhesion was calculated.

Following ECM/coculture assays, cells were stained for mast cell/fibroblast markers, where appropriate, by flow cytometry. Elongation of mast cells in coculture with ASM was also visualized by immunofluorescence.

Flow cytometry

HMC-1 cells were stained, where appropriate, with Abs to Thy-1, IB10, 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 allophycocyanin (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, IB10, or appropriate isotype controls; indirectly labeled with FITC or allophycocyanin; 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

Table I. Clinical characteristics of subjects

	Normal	Mild–Moderate Asthma (Global Initiative for Asthma 1–3)	Severe Asthma (Global Initiative for Asthma 4–5)
Number	16	13	13
Age ^a	43 (4)	48 (5)	50 (3)
Male/Female	8/8	7/6	5/8
Never/current/ex-smokers	14/0/2	8/0/5	9/0/4
Pack-years ^a	0 (0)	4 (2)	2 (1)
Atopy (n)	8	9	9
PC ₂₀ FEV ₁ (mg/ml) ^b	>16	0.3 (0.1–0.9)	0.5 (0.2–1.7)
FEV ₁ (%) predicted ^a	99 (3)	88 (5)	84 (5)
Pre-BD FEV ₁ /FVC (%) ^a	81 (2)	74 (3)	75 (3)
BD response (%) ^a	1 (1)	8 (3)	11 (4)
ICS (n)	0	7	13
ICS (μ g/day BDP equivalent) ^c	0	0 (800)	1600 (940)
Long-acting β -agonist (n)	0	2	13
Oral corticosteroids (n)	0	0	3

^aMean (SE).

^bGeometric mean (95% CI).

^cMedian (IQR).

BD, bronchodilator; BDP, beclomethasone dipropionate; ICS, inhaled corticosteroids.

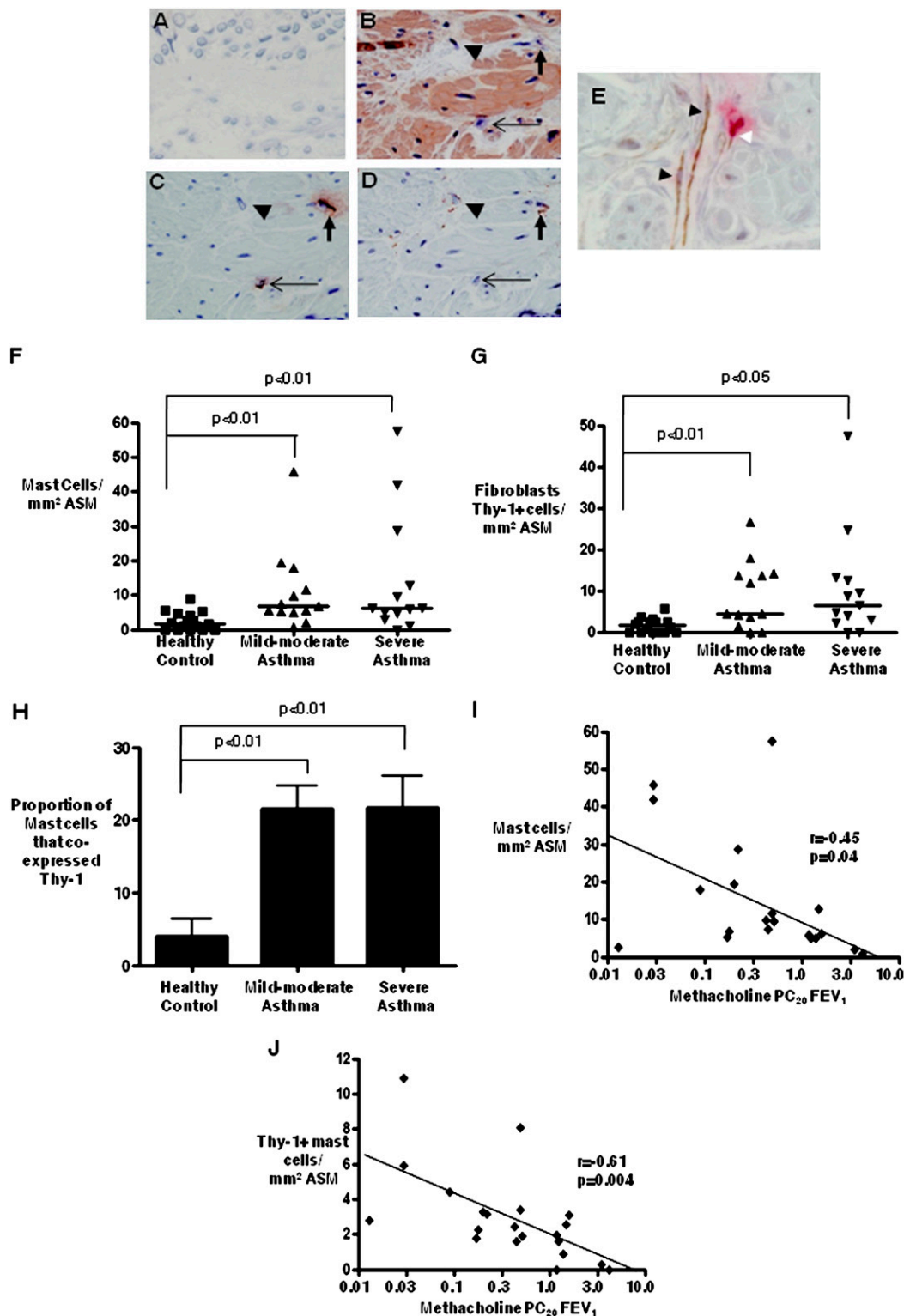


FIGURE 1. Mast cells in the ASM bundle express fibroblastic markers. Representative photomicrographs of a bronchial biopsy from an asthmatic (original magnification $\times 400$) stained for isotype control, illustrating the epithelium and lamina propria (A), and sequential sections of the ASM bundle (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 PC₂₀ FEV₁; correlation coefficient and p value are given on the figure.

1.25×10^5 HMC-1 cells were resuspended in 144 μ l ITS media $\pm$ stimulus, with 299 μ l collagen, 37 μ l $10\times$ DMEM (Invitrogen), and 20 μ l 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 μ l ITS media $\pm$ 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 $\pm$ 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).

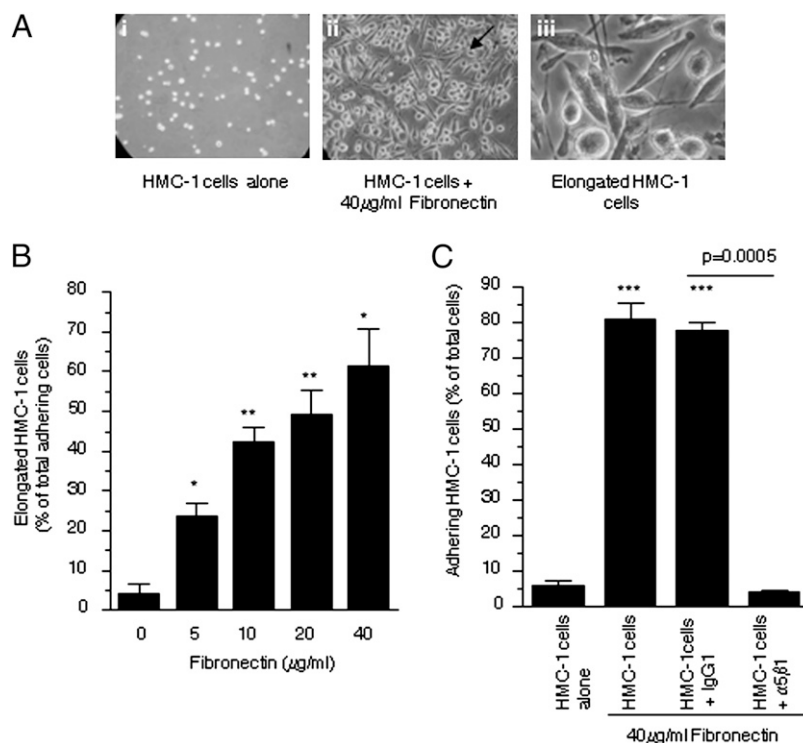
The number of fibroblasts was also increased in the ASM bundle in mild-moderate (4.4 [11.5] cells/mm² ASM) and severe asthma (6.5 [10.2] cells/mm² ASM), compared with controls (1.5 [2.5] cells/mm² ASM; $p = 0.003$ Kruskal–Wallis, $p < 0.05$ asthma groups versus controls) (Fig. 1G). The proportion of mast cells that coexpressed fibroblast markers (tryptase⁺/Thy-1⁺ cells) was increased in the ASM bundle ($18 \pm 3\%$) compared with the lamina propria ($2.9 \pm 0.6\%$; $p < 0.0001$), and in the ASM bundle the proportion was increased in mild-moderate ($22 \pm 3\%$) and severe asthma ($22 \pm 4\%$), compared with healthy controls ($4 \pm 2\%$; $p = 0.0002$ 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. 1I). 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. HMC-1 cells that adhere to fibronectin develop fibroblastic morphology. A, Representative images of HMC-1 cells cultured with serum-free media over 6 d without fibronectin (i) or cultured on fibronectin (40 μ g/ml) (ii); original magnification $\times 40$. The black arrow indicates the area of detailed morphology, of elongated HMC-1 cells shown in iii. B and C, HMC-1 cell adhesion on fibronectin, the percentage of elongated (B) ($n = 4$ or 5 experiments) and adhering (C) HMC-1 cells ($n = 3$ –6 experiments) on fibronectin over 6 d. The percentage of elongated/adhering cells was calculated as described in *Materials and Methods*. Data are presented as mean $\pm$ SEM. Comparisons for adhering cells were made against cells without fibronectin (0), using the paired *t* test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The *p* value for the paired *t* test of the effect of $\alpha 5\beta 1$ inhibition, compared with its isotype control, is given on the figure.



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 $\mu\text{g}/\text{ml}$. Therefore this concentration of fibronectin was used throughout the study. At this concentration, HMC-1 cells adhered avidly to fibronectin after 6 d ($81 \pm 5\%$, $n = 8$; $p < 0.001$), and adhesion was markedly inhibited by anti- $\alpha 5\beta 1$ blocking Ab ($4.3 \pm 0.4\%$ versus isotype control, $78 \pm 2\%$; $p = 0.0005$; $n = 3$; Fig. 2C).

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

Mast cells adherent to ECM express fibroblast markers

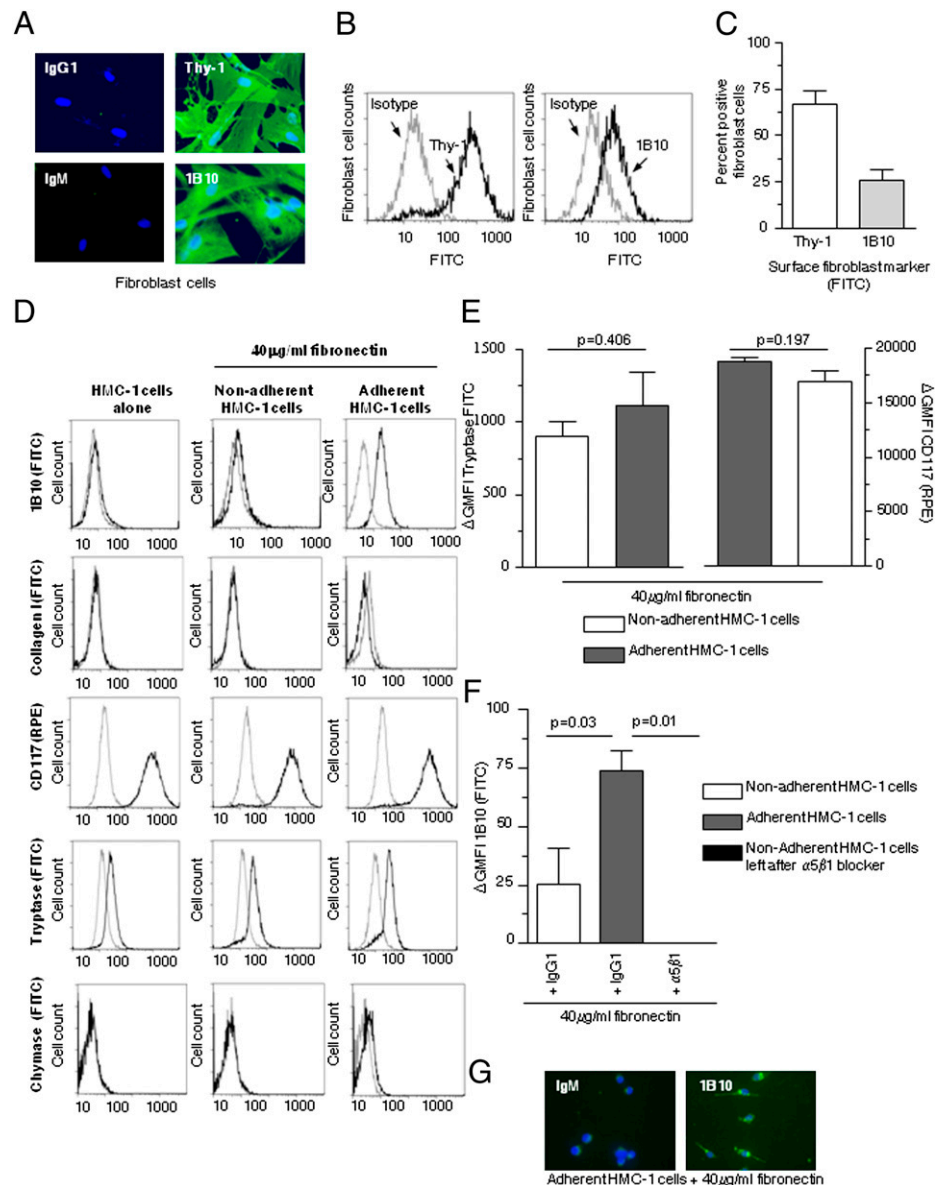
The fibroblast markers Thy-1 and 1B10 were highly expressed on cultured fibroblast cells, as evidenced by immunofluorescence (Fig. 3A) and by flow cytometry (Fig. 3B). The proportion of fibro-

blast cells that expressed Thy-1 was $67 \pm 7\%$ ($p = 0.001$; $n = 13$), and 1B10 was $26 \pm 5\%$ ($p = 0.003$; $n = 7$) (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, 1B10, CD117, and intracellular tryptase expression. 1B10 expression was significantly increased in the adherent HMC-1 cells (Δ geometric mean fluorescence intensity [GMFI] 137 ± 21 ; $n = 6$) compared with nonadherent cells (64 ± 14 ; $p = 0.001$) and controls (81 ± 23 ; $p = 0.002$). Example histograms are shown in Fig. 3D. No difference was seen in the expression of collagen I, CD117, or tryptase by the adherent or nonadherent HMC-1 cells (Fig. 3D, 3E). However, there was a significant increase in chymase expression in adherent HMC-1 cells (Δ GMFI 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- $\alpha 5\beta 1$ 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 Δ GMFI of CD117 ($n = 5$ experiments) and tryptase expression ($n = 5$ experiments) (E) and 1B10 expression with and without $\alpha 5\beta 1$ fibronectin blocking Ab ($n = 3$) (F) in nonadherent versus adherent HMC-1 cells cultured on fibronectin over 6 d. The Δ GMFI 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 $\mu\text{g}/\text{ml}$) over 6 d. Data presented as mean $\pm$ SEM. Statistical differences were assessed using the t tests and p values as shown. Original magnification $\times 400$.



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 $\mu\text{g}/\text{ml}$) 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 sug-

gests 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 $\pm$ SEM percentage size of gel compared with baseline was $66 \pm 4\%$ ($n = 7$) for nonadherent cells versus $82 \pm 4\%$ 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 ($27 \pm 5.8\%$; $p = 0.05$; $n = 13$; Fig. 5C, 5D) compared with cells in coculture ($9.85 \pm 5.3\%$; $n = 7$) and cells adherent to fibronectin ($10.1 \pm 3.3\%$; $n = 6$; $p = 0.02$) over 1 d.

In contrast to HMC-1 cells, HLMCs expressed 1B10 in the absence of fibronectin and/or ASM ($13 \pm 5\%$; $n = 3$), whereas the percentage of Thy-1-positive HLMCs in the absence of fibronectin and ASM was low ($4.7 \pm 1.7\%$). 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 ($22 \pm 5\%$; $p = 0.005$; $n = 6$) and 15 d ($45 \pm 8\%$; $p = 0.004$; $n = 6$) (Fig. 5F). A small, albeit significant, increase in Thy-1 expression was observed for HMC-1 cells ($9 \pm 2\%$; $p = 0.011$; $n = 7$) 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 TGF β -1 or 2 (0–10 ng/ml) (data not shown).

Mast cell fibroblastoid transition in coculture with ASM is mediated via $\alpha 5\beta 1$

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 $57 \pm 7\%$, $n = 4$. 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 ($58 \pm 9\%$ versus isotype control $57 \pm 9\%$; $n = 4$; $p = 0.67$) but was markedly inhibited by $\alpha 5\beta 1$ blockade ($26 \pm 4\%$ versus isotype control $68 \pm 6\%$; $p = 0.0008$; $n = 6$) (Fig. 6A). The blockade of CADM1 and $\alpha 5\beta 1$ in concert was not different to $\alpha 5\beta 1$ alone ($p = 0.49$) (Fig. 6A).

In coculture with ASM and in the presence of the $\alpha 5\beta 1$ blocking Ab, the proportion of Thy-1⁺ HMC-1 cells ($6.4 \pm 4.3\%$; $n = 4$) and HLMCs ($4.9 \pm 1.4\%$; $n = 5$) was significantly decreased

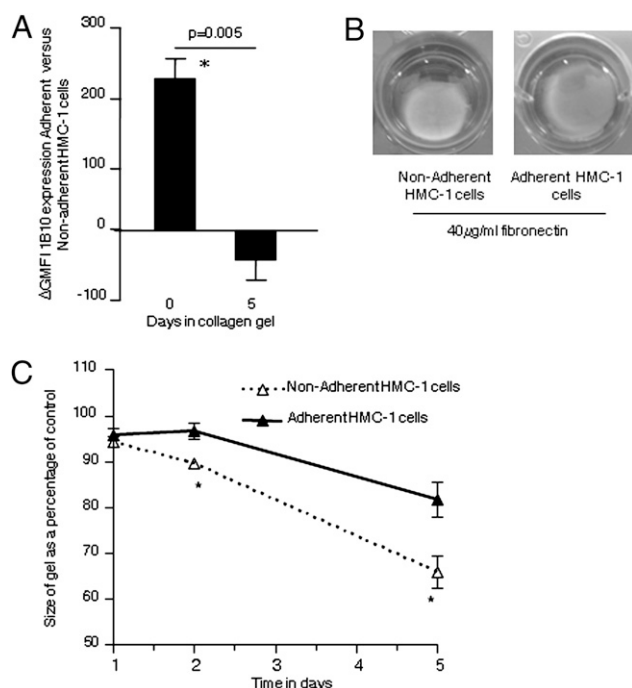


FIGURE 4. Mast cell fibroblastoid differentiation is associated with gel contraction. **A**, 1B10 expression was increased in the adherent versus nonadherent cells at day 0 prior to impregnating the collagen gels, but after 5 d in the gels the 1B10 expression was no longer different between the previously adherent and nonadherent cells. **B** and **C**, Example photographs (**B**) and time course (**C**) of contraction of collagen gels impregnated with nonadherent and adherent HMC-1 cells following culture in serum-free media over 6 d with fibronectin (40 $\mu\text{g}/\text{ml}$) cells. Cells were left in the collagen gels without stimulation over 5 d ($n = 7$). Comparisons for nonadhering cells were made against adherent cells, with data presented as mean $\pm$ SEM. Statistical differences were assessed using the *t* tests; * $p < 0.05$.

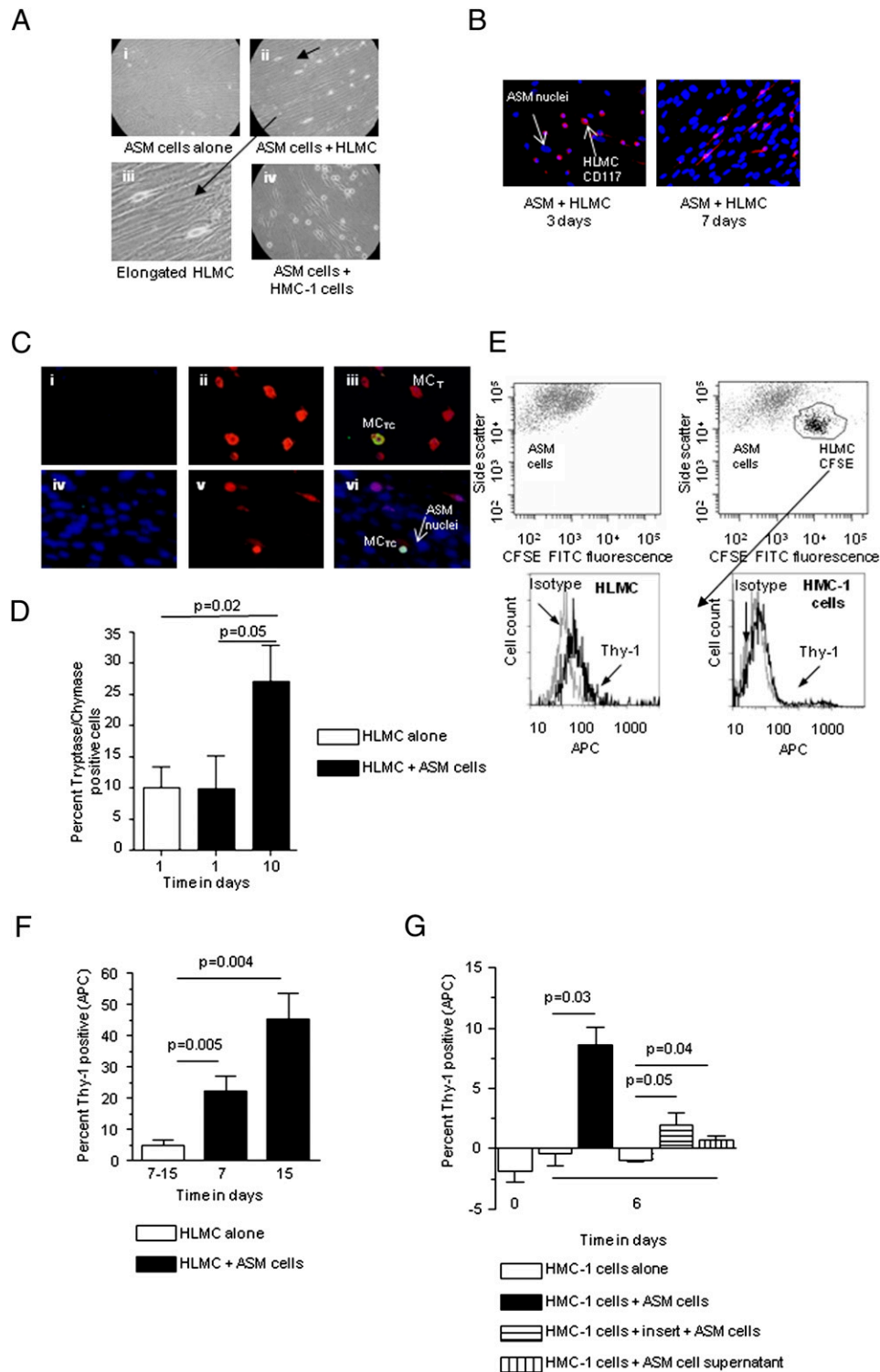
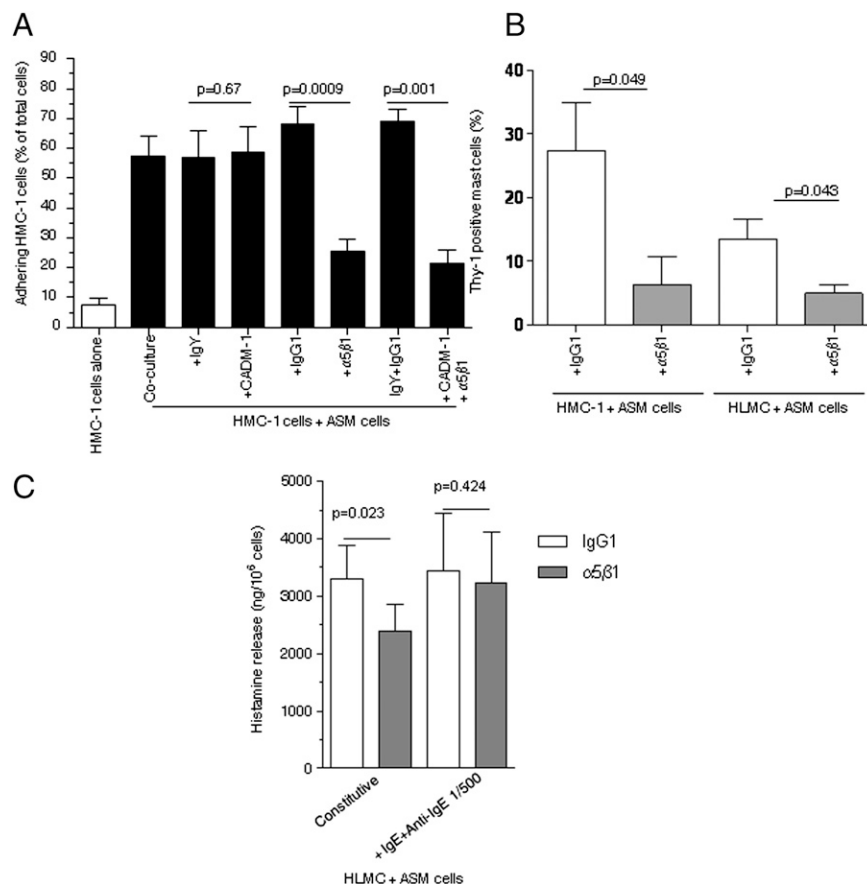


FIGURE 5. Mast cell-ASM coculture promotes fibroblastoid differentiation. *A*, Representative photomicrographs of ASM cells cultured alone with serum-free media (*i*), over 7 d with HLMCs (*ii*, *iii*), or HMC-1 cells over 6 d (*iv*) (original magnification $\times 40$). The black arrow indicates the 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 $\times 200$. *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 $\times 400$. *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 or ASM cells ($n = 4$), and with ASM cell supernatants ($n = 4$). Data are presented as mean $\pm$ SEM. Statistical differences were assessed using ANOVA and *t* tests as appropriate, and *p* values are as shown.

FIGURE 6. Mast cell fibroblastoid differentiation promoted by ASM-derived ECM proteins is $\alpha 5\beta 1$ 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 $\alpha 5\beta 1$ 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- $\alpha 5\beta 1$ 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- $\alpha 5\beta 1$ integrin or isotype control (IgG1) ($n = 2$ ASM cell donors and 4 HLMC donors). Data are presented as mean $\pm$ SEM. Statistical differences were assessed using the *t* tests and *p* values as shown.



compared with their isotype controls ($27.4 \pm 7.5\%$ and $13.4 \pm 3.1\%$, respectively; $p < 0.05$) (Fig. 6B). Blockade of CADM1 did not affect Thy-1 expression by HLMCs ($15.8 \pm 3.9\%$ versus $17.7 \pm 1.3\%$; $p = 0.59$).

The histamine concentration in the media corrected for cell number following coculture of HLMCs with ASM for 7 d was significantly reduced in the presence of the anti- $\alpha 5\beta 1$ blocking Ab compared with its isotype control ($2.4 \pm 0.5 \mu\text{g}/10^6$ cells versus $3.3 \pm 0.6 \mu\text{g}/10^6$ cells; $p = 0.02$; $n = 6$) (Fig. 6C). However, IgE-anti-IgE activation of HLMCs prior to incubating with ASM did not significantly reduce histamine release (Fig. 6C). PGD₂ release by HLMCs was not affected by anti- $\alpha 5\beta 1$ ($117.7 \pm 47.2 \text{ ng}/10^6$ cells; $p = 0.9$) compared with IgG1 control ($109.9 \pm 18.8 \text{ ng}/10^6$ cells).

Discussion

This study is the first to demonstrate that mast cells localized within the ASM bundle in asthma express fibroblast markers. In vitro we determined that this mast cell fibroblastoid differentiation is ASM-derived ECM protein dependent. These fibroblastoid mast cells have an increased expression of chymase and are in a heightened state of activation, with increased constitutive release of histamine. Taken together, our findings suggest that ASM-derived ECM-mediated mast cell fibroblastoid transition is important in the development of airway dysfunction in asthma.

Our study provides further evidence to support the view that, in asthma, mast cell localization to the ASM bundle is important in the pathogenesis of AHR. Several studies have consistently reported increased mast cell numbers in the ASM bundle in asthma (7, 8, 13, 16, 17, 24, 25). Using stepwise logistic regression to explore the association between AHR and immunopathological features of asthma, we observed that mast cell infiltration of the

ASM bundle was the strongest independent predictor of AHR (8). In this paper, we extend these earlier studies to demonstrate that the association with AHR is stronger for the number of “fibroblastic” mast cells than for the total mast cells within the ASM bundle, suggesting that this fibroblastoid-mast cell transition may play a pivotal role in mast cell-ASM interactions and the development of disordered airway function.

Consistent with our in vivo findings, we confirmed in vitro that mast cells have the capacity to undergo fibroblastoid differentiation. Primary HLMCs and human mast cell lines changed their morphology to spindle-shaped cells in the presence of the ECM proteins fibronectin or collagen I. These mast cells expressed proteins usually associated with fibroblasts, confirmed with two different Abs using a combination of techniques. Importantly, although the mast cells acquired fibroblast-like features and increased chymase expression, there was no downregulation in their CD117 or β -tryptase expression. This fibroblastoid transition was replicated in coculture with ASM and was associated with a change in cell size and increased constitutive histamine release, but not PGD₂. This fibroblastoid transition was associated with an increase in gel contraction, which may be a consequence of cell size and function. This finding was consistent with earlier observations determined by electron microscopy whereby mast cells within the ASM bundle were found to be smaller with reduced granule content, as a consequence of piecemeal degranulation (17).

We have reported previously that HLMCs and HMC-1 cells in vitro adhere to ASM, in part via CADM1 (14), and in coculture with ASM mast cells survive and proliferate (23). In contrast, we report in this paper that the mast cell transition toward a fibroblastoid phenotype was CADM1 independent. Indeed, this phenotypic transition was dependent on mast cell-ECM adhesion. One possible explanation for this apparent paradox is that CADM1 is

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 $\alpha 5\beta 1$ 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 TLR3 activation, which consequently abrogates mast cell attachment-dependent potentiation of IgE-mediated responses (28). Similarly, reduction in mast cell $\beta 1$ integrin surface expression as a consequence of diminished surface receptor stability in rabaptin-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 β -tryptase results in an upregulation of α -SMA intensity mediated via autocrine TGF- β release (35). Consequently, the ASM undergoes a phenotypic switch to a more contractile phenotype. This is supported by a very strong correlation in vivo between the number of mast cells within the ASM bundle and the ASM α -SMA intensity. We found that TGF- β did not influence the mast cell phenotype. However, TGF- β is an important inducer of ECM protein production by ASM, in particular, fibronectin (36, 37). This increased release of fibronectin will in turn promote ASM synthetic function (38, 39) and mast cell activation (26, 40), 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 $\alpha 5\beta 1$ 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.

References

- Braman, S. S. 2006. The global burden of asthma. *Chest* 130(1, Suppl)4S–12S.
- Chanez, P., S. E. Wenzel, G. P. Anderson, J. M. Anto, E. H. Bel, L. P. Boulet, C. E. Brightling, W. W. Busse, M. Castro, B. Dahlen, et al. 2007. Severe asthma in adults: what are the important questions? *J. Allergy Clin. Immunol.* 119: 1337–1348.
- Haldar, P., I. D. Pavord, D. E. Shaw, M. A. Berry, M. Thomas, C. E. Brightling, A. J. Wardlaw, and R. H. Green. 2008. Cluster analysis and clinical asthma phenotypes. *Am. J. Respir. Crit. Care Med.* 178: 218–224.
- Leckie, M. J., A. ten Brinke, J. Khan, Z. Z. Diamant, B. J. O'Connor, C. M. Walls, A. K. Mathur, H. C. Cowley, K. F. Chung, R. Djukanovic, et al. 2000. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356: 2144–2148.
- Haldar, P., C. E. Brightling, B. Hargadon, S. Gupta, W. Monteiro, A. Sousa, R. P. Marshall, P. Bradding, R. H. Green, A. J. Wardlaw, and I. D. Pavord. 2009. Mepolizumab and exacerbations of eosinophilic refractory asthma. *N. Engl. J. Med.* 360: 973–984.
- Brightling, C. E., R. Ward, K. L. Goh, A. J. Wardlaw, and I. D. Pavord. 1999. Eosinophilic bronchitis is an important cause of chronic cough. *Am. J. Respir. Crit. Care Med.* 160: 406–410.
- Brightling, C. E., P. Bradding, F. A. Symon, S. T. Holgate, A. J. Wardlaw, and I. D. Pavord. 2002. Mast-cell infiltration of airway smooth muscle in asthma. *N. Engl. J. Med.* 346: 1699–1705.
- Siddiqui, S., V. Mistry, C. Doe, K. Roach, A. Morgan, A. Wardlaw, I. D. Pavord, P. Bradding, and C. E. Brightling. 2008. Airway hyperresponsiveness is dissociated from airway wall structural remodeling. *J. Allergy Clin. Immunol.* 122: 335–341, 341, e1–e3.
- Weller, C. L., S. J. Collington, J. K. Brown, H. R. Miller, A. Al-Kashi, P. Clark, P. J. Jose, A. Hartnell, and T. J. Williams. 2005. Leukotriene B₄, an activation product of mast cells, is a chemoattractant for their progenitors. *J. Exp. Med.* 201: 1961–1971.
- Ochi, H., W. M. Hirani, Q. Yuan, D. S. Friend, K. F. Austen, and J. A. Boyce. 1999. T helper cell type 2 cytokine-mediated mitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. *J. Exp. Med.* 190: 267–280.
- Abonia, J. P., J. Hallgren, T. Jones, T. Shi, Y. Xu, P. Koni, R. A. Flavell, J. A. Boyce, K. F. Austen, and M. F. Gurish. 2006. Alpha-4 integrins and VCAM-1, but not MAdCAM1, are essential for recruitment of mast cell progenitors to the inflamed lung. *Blood* 108: 1588–1594.
- Siddiqui, S., F. Hollins, S. Saha, and C. E. Brightling. 2007. Inflammatory cell microlocalisation and airway dysfunction: cause and effect? *Eur. Respir. J.* 30: 1043–1056.
- Brightling, C. E., A. J. Ammit, D. Kaur, J. L. Black, A. J. Wardlaw, J. M. Hughes, and P. Bradding. 2005. The CXCL10/CXCR3 axis mediates human lung mast cell migration to asthmatic airway smooth muscle. *Am. J. Respir. Crit. Care Med.* 171: 1103–1108.
- Yang, W., D. Kaur, Y. Okayama, A. Ito, A. J. Wardlaw, C. E. Brightling, and P. Bradding. 2006. Human lung mast cells adhere to human airway smooth

- muscle, in part, via tumor suppressor in lung cancer-1. *J. Immunol.* 176: 1238–1243.
15. Brightling, C. E., F. A. Symon, S. T. Holgate, A. J. Wardlaw, I. D. Pavord, and P. Bradding. 2003. Interleukin-4 and -13 expression is co-localized to mast cells within the airway smooth muscle in asthma. *Clin. Exp. Allergy* 33: 1711–1716.
 16. Saha, S. K., M. A. Berry, D. Parker, S. Siddiqui, A. Morgan, R. May, P. Monk, P. Bradding, A. J. Wardlaw, I. D. Pavord, and C. E. Brightling. 2008. Increased sputum and bronchial biopsy IL-13 expression in severe asthma. *J. Allergy Clin. Immunol.* 121: 685–691.
 17. Begueret, H., P. Berger, J. M. Vernejoux, L. Dubuisson, R. Marthan, and J. M. Tunon-de-Lara. 2007. Inflammation of bronchial smooth muscle in allergic asthma. *Thorax* 62: 8–15.
 18. Siddiqui, S., F. Hollins, and C. E. Brightling. 2008. What can we learn about airway smooth muscle from the company it keeps? *Eur. Respir. J.* 32: 9–11.
 19. Araujo, B. B., M. Dolhnikoff, L. F. Silva, J. Elliot, J. H. Lindeman, D. S. Ferreira, A. Mulder, H. A. Gomes, S. M. Fernandez, A. James, and T. Mauad. 2008. Extracellular matrix components and regulators in the airway smooth muscle in asthma. *Eur. Respir. J.* 32: 61–69.
 20. Fouty, B., T. Moss, V. Solodushko, and M. Kraft. 2006. Dexamethasone can stimulate G1-S phase transition in human airway fibroblasts in asthma. *Eur. Respir. J.* 27: 1160–1167.
 21. Kaur, D., R. Saunders, P. Berger, S. Siddiqui, L. Woodman, A. Wardlaw, P. Bradding, and C. E. Brightling. 2006. Airway smooth muscle and mast cell-derived CC chemokine ligand 19 mediate airway smooth muscle migration in asthma. *Am. J. Respir. Crit. Care Med.* 174: 1179–1188.
 22. Furuno, T., A. A. Ito, Y. Koma, K. Wetabe, H. Yokozaki, J. Bienstock, M. Nakanishi, and Y. Kitamura. 2005. The spermatogenic Ig superfamily/synaptic cell adhesion molecule mast-cell adhesion molecule promotes interaction with nerves. *J. Immunol.* 174: 6934–6942.
 23. Hollins, F., D. Kaur, W. Yang, G. Cruse, R. Saunders, A. Sutcliffe, P. Berger, A. Ito, C. E. Brightling, and P. Bradding. 2008. Human airway smooth muscle promotes human lung mast cell survival, proliferation, and constitutive activation: cooperative roles for CADM1, stem cell factor, and IL-6. *J. Immunol.* 181: 2772–2780.
 24. Berger, P., P. O. Girodet, H. Begueret, O. Ousova, D. W. Perng, R. Marthan, A. F. Walls, and J. M. Tunon de Lara. 2003. Tryptase-stimulated human airway smooth muscle cells induce cytokine synthesis and mast cell chemotaxis. *FASEB J.* 17: 2139–2141.
 25. El-Shazly, A., P. Berger, P. O. Girodet, O. Ousova, M. Fayon, J. M. Vernejoux, R. Marthan, and J. M. Tunon-de-Lara. 2006. Fractalkine produced by airway smooth muscle cells contributes to mast cell recruitment in asthma. *J. Immunol.* 176: 1860–1868.
 26. Swieter, M., M. M. Hamawy, R. P. Siraganian, and S. E. Mergenhagen. 1993. Mast cells and their microenvironment: the influence of fibronectin and fibroblasts on the functional repertoire of rat basophilic leukemia cells. *J. Periodontol.* 64(5, Suppl):492–496.
 27. Küchler, J., A. Grützkau, B. M. Henz, and S. Krüger-Krasagakis. 2006. Morphological analysis of integrin-mediated adhesion of immature human mast cells to extracellular matrix proteins. *Arch. Dermatol. Res.* 298: 153–161.
 28. Kulka, M., and D. D. Metcalfe. 2006. TLR3 activation inhibits human mast cell attachment to fibronectin and vitronectin. *Mol. Immunol.* 43: 1579–1586.
 29. Dasty, J., and D. D. Metcalfe. 1994. Stem cell factor induces mast cell adhesion to fibronectin. *J. Immunol.* 152: 213–219.
 30. Dasty, J., J. Wyszokowska, and D. D. Metcalfe. 2001. Characterization of alpha 5-integrin-dependent mast cell adhesion following Fc epsilon RI aggregation. *Int. Arch. Allergy Immunol.* 125: 152–159.
 31. Levi-Schaffer, F., K. F. Austen, J. P. Caulfield, A. Hein, W. F. Bloes, and R. L. Stevens. 1985. Fibroblasts maintain the phenotype and viability of the rat heparin-containing mast cell in vitro. *J. Immunol.* 135: 3454–3462.
 32. Rios, E. J., A. M. Piliponsky, C. Ra, J. Kalesnikoff, and S. J. Galli. 2008. Rabaptin-5 regulates receptor expression and functional activation in mast cells. *Blood* 112: 4148–4157.
 33. Lavens, S. E., K. Goldring, L. H. Thomas, and J. A. Warner. 1996. Effects of integrin clustering on human lung mast cells and basophils. *Am. J. Respir. Cell Mol. Biol.* 14: 95–103.
 34. Hamawy, M. M., C. Oliver, S. E. Mergenhagen, and R. P. Siraganian. 1992. Adherence of rat basophilic leukemia (RBL-2H3) cells to fibronectin-coated surfaces enhances secretion. *J. Immunol.* 149: 615–621.
 35. Woodman, L., S. Siddiqui, G. Cruse, A. Sutcliffe, R. Saunders, D. Kaur, P. Bradding, and C. E. Brightling. 2008. Mast cells promote airway smooth muscle cell differentiation via autocrine up-regulation of TGF- β 1. *J. Immunol.* 181: 5001–5007.
 36. Johnson, P. R., J. K. Burgess, Q. Ge, M. Poniris, S. Boustany, S. M. Twigg, and J. L. Black. 2006. Connective tissue growth factor induces extracellular matrix in asthmatic airway smooth muscle. *Am. J. Respir. Crit. Care Med.* 173: 32–41.
 37. Moir, L. M., J. K. Burgess, and J. L. Black. 2008. Transforming growth factor beta 1 increases fibronectin deposition through integrin receptor alpha 5 beta 1 on human airway smooth muscle. *J. Allergy Clin. Immunol.* 121: 1034–1039, e4.
 38. Peng, Q., D. Lai, T. T. Nguyen, V. Chan, T. Matsuda, and S. J. Hirst. 2005. Multiple beta 1 integrins mediate enhancement of human airway smooth muscle cytokine secretion by fibronectin and type I collagen. *J. Immunol.* 174: 2258–2264.
 39. Chan, V., J. K. Burgess, J. C. Ratoff, B. J. O'Connor, A. Greenough, T. H. Lee, and S. J. Hirst. 2006. Extracellular matrix regulates enhanced eotaxin expression in asthmatic airway smooth muscle cells. *Am. J. Respir. Crit. Care Med.* 174: 379–385.
 40. Lam, V., J. Kalesnikoff, C. W. Lee, V. Hernandez-Hansen, B. S. Wilson, J. M. Oliver, and G. Krystal. 2003. IgE alone stimulates mast cell adhesion to fibronectin via pathways similar to those used by IgE + antigen but distinct from those used by Steel factor. *Blood* 102: 1405–1413.