Human airway epithelial cell determinants of survival and functional phenotype for primary human mast cells

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Mast cells (MCs) are found in increased numbers at airway mucosal surfaces in asthmatic patients. Because human airway epithelial cells (HAECs) actively participate in airway inflammatory responses and are in direct contact with MCs in the mucosa, we hypothesized that HAEC–MC interactions may contribute to the differentiation and survival of MCs in the airway mucosa. Here, we show that HAECs express mRNA and protein for soluble and membrane-bound stem cell factor, releasing soluble stem cell factor into the cell culture supernatant at a concentration of 5.9 ± 0.1 ng per 10^6 HAEC. HAECs were able to support MC survival in coculture in the absence of any exogenous cytokines for at least 4 d. Before the initiation of coculture, MCs were uniformly tryptase and chymase (MC_{TC}) double positive, but by 2 d of coculture the majority of MCs expressed tryptase (MC_T) alone. MCs supported in coculture generated low amounts of cysteinyl-leukotrienes (cys-LT) after FcεRI-dependent activation (0.2 ± 0.1 ng of cys-LT per 10^6 cells) and required priming with IL-4 and IL-3 during coculture to achieve a quantity of cys-LT generation within the range expected for human lung mucosal MC (26.5 ± 16 ng of cys-LT per 10^6 cells). In these culture conditions, HAECs were able to direct mucosal MC protease phenotype, but T cell-derived Th2 cytokines were required for the expression of a functional airway MC eicosanoid phenotype. Thus, distinct cell types may direct unique aspects of reactive mucosal MC phenotype in the airways.

airway inflammation | stem cell factor

Airway epithelial cells actively participate in the inflammatory response in asthma by secreting cytokines, reactive oxygen metabolites, and other mediators that regulate infiltrating inflammatory cells such as lymphocytes, eosinophils, and mast cells (MCs) (1). MCs are dispersed widely throughout the body, and their numbers are highest at mucosal surfaces (2). As primary effector cells in immediate-type hypersensitivity reactions, MCs have a critical role in anaphylaxis, allergic rhinitis, and asthma (3). Tissue MC development in rodents and humans requires the interaction between the receptor tyrosine kinase *c-kit* (CD117) and its ligand stem cell factor (SCF) and is further modulated by additional tissue factors that account for MC heterogeneity (4). In the lung, a constitutive baseline MC subpopulation resides in the perivascular connective tissue in the bronchial submucosa, whereas an immunohistochemically and functionally distinctive reactive MC subpopulation develops at mucosal surfaces in response to inflammation (5). In asthmatic airways, MCs are found in the airway mucosa adjacent to the airway epithelium, with MC survival presumably supported by SCF expression by lung epithelial cells (6–8). SCF expression is increased in the airways of asthmatics as compared with controls (9).

MC tissue heterogeneity has historically been understood in terms of protease content. MCs expressing only tryptase, a serine endopeptidase with trypsin-like substrate specificity, designated by MC_T or reactive MC phenotype, predominate at mucosal surfaces of the intestine and lung. MCs containing both tryptase and chymase (TC), a chymotryptic-like serine endopeptidase (and designated by MC_{TC} or constitutive phenotype), predominate in the skin and submucosa of the lung. More than 90% of the MCs in the epithelium and lumen of bronchioles and bronchi as well as in the alveolar walls are of MC_T phenotype, whereas MC_{TC} are found in the subepithelial regions of the bronchi and in the connective tissue (6). MCs also differ in their profiles of eicosanoid biosynthesis in response to FcεRI-dependent activation after isolation from various dispersed tissue sources, with lung MCs capable of generating from 22–50 ng of cysteinyl-leukotriene (cys-LT) per 10^6 MC (10, 11). Both total cys-LT generation and cys-LT generation relative to prostaglandin (PG)D_2 generation are marked by quantitative and relative tissue-specific differences. Because MCs derive from a single lineage of circulating committed progenitors under the influence of SCF, their heterogeneous profiles of eicosanoid generation are determined by additional local factors associated at mucosal surfaces (11, 12).

A number of model systems involving coculture of human MCs with human intestinal fibroblasts, keratinocyte epithelium, umbilical vein endothelial cells, and lung fibroblasts have been developed to study the interaction of MCs with cells in their resident microenvironment (13–16). Because human airway epithelial cells (HAECs) regulate airway inflammatory responses and directly contact MCs in the airway mucosa, we hypothesized that HAEC:MC interactions may contribute to MC function and survival at the epithelial surface. To test this hypothesis, we developed a model system to study the functional interactions between cord blood-derived primary human MCs and primary HAECs in coculture. We demonstrate that MCs grown in coculture adopt a reactive MC_T protease phenotype but retain a constitutive eicosanoid phenotype; only in the presence of additional exogenous Th2 cytokines do MCs developed in coculture adopt an eicosanoid phenotype similar to that of human airway MCs *in vivo*. These data suggest that distinct pathways may control specific aspects of MC phenotype and that the functional identity of a mucosal MC may not be controlled by anatomical location alone.

Materials and Methods

Isolation, Purification, and Culture of Human MCs. Mature human MCs were developed from umbilical cord blood as described in refs. 17 and 18 in compliance with institutional review board guidelines (see *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site). Cells were seeded at 10^6 per ml and cultured in the presence of 100 ng/ml SCF, 50 ng/ml IL-6, and 10 ng/ml IL-10 (R & D Systems) in RPM1 1640-based media (Invitrogen). Nonadherent cells were passaged every week into media containing fresh cytokines. Once cells

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Abbreviations: cys-LT, cysteinyl-leukotriene; HAEC, human airway epithelial cell; MC, mast cell; MITF, microphthalmia transcription factor; PG, prostaglandin; SCF, stem cell factor; STAT, signal transducer and activator of transcription; TC, tryptase and chymase.

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reached maturity, defined by >95% toluidine-blue positivity and positive immunostaining for both tryptase and chymase, they were used for experiments.

HAEC Isolation and Culture. HAECs were isolated as described in ref. 19. Normal lung tissue was obtained from donor lungs not used for transplantation or surgical specimens after lobectomy for lung cancer according to institutional guidelines (see *Supporting Materials and Methods*). Cells were seeded on precoated tissue culture plates in serum-free bronchial epithelial basal medium (Clonetics, San Diego) with 1% penicillin-streptomycin-fungizone. The purified HAECs were frozen and individual vials thawed and seeded for experiments to reduce interdonor variability. Only passage 1–3 HAECs were used for experiments.

MC–HAEC Coculture Assay. Mature MCs (1×10^6) were washed with PBS and seeded onto washed subconfluent HAEC monolayers in 12-well plates (Costar) in media containing 1% heat-inactivated FBS and penicillin, streptomycin, and gentamicin in the presence or absence of Transwell inserts, 0.4-μm pore size (Costar), with MCs in the upper chamber and HAECs in the lower chamber. Cell number and viability were assessed on a daily basis by trypan-blue dye exclusion and purity by toluidine-blue staining (Sigma). MCs grown in monoculture with recombinant SCF were resuspended into the same media as that used for coculture. A blocking anti-human SCF Ab (PeproTech) was used in the indicated experiments.

Immunocytochemistry. Cytospin preparations (6×10^4 cells per condition) were fixed for 10 min in Carnoy's fixative (60% ethanol/30% chloroform/10% glacial acetic acid) and blocked with VECTASTAIN blocking reagent for 30 min (Vector Laboratories). Immunocytochemistry was performed by using Abs directed against human SCF (1:500, R & D Systems), human tryptase (1:1,000, Chemicon), human chymase (1:250, Novus Biologicals, Littleton, CO), or isotype control (Zymed) as primary Abs at 4°C overnight. Detection of the primary Ab was by a streptavidin-biotin-vector red detection system (Vector Laboratories).

RNA Isolation and RT-PCR. Total RNA was extracted from HAECs with TRIzol (Invitrogen), and two-step RT-PCR was performed using standard protocols. Primers for PCR were as follows: SCF, 5′-GAAGGGATCTGCAGGAATCGTGTG-3′ and 5′-GCCCAGTGTAGGCTGGAGTCTCCA-3′; β-actin, 5′-CCCTGGAGAAGAGCTACGA-3′ and 5′-TAAAGCCATGCCAATCTCAT-3′ (Invitrogen). One-fifth volume of the PCR reaction mix was resolved on 1% agarose gel and documented on a Gel Print 2000i (Genomic Solutions, Ann Arbor, MI).

Analysis of SCF Protein Expression. Analysis of SCF protein expression from HAEC supernatants was performed by using a sandwich ELISA specific for human SCF (Chemicon).

Analysis of MC Mediator Release. IgE-dependent histamine release was assessed by overnight treatment with 1 μg/ml human purified IgE (Chemicon) before stimulation with goat anti-human IgE Ab (Chemicon) at a concentration of 1 μg/ml for 30 min at 37°C. This dose was determined to be optimal for degranulation without toxicity when testing (anti-IgE) from 0.1 to 10 μg/ml, with no differences noted at varying (anti-IgE) between MCs in monoculture or coculture. Recombinant human IL-4 and IL-3 were used for priming as indicated (PeproTech). Cell supernatants were harvested and stored at −70°C before assay. Cell pellet fractions were resuspended and lysed by rapid freeze/thaw. Histamine in the supernatant and pellet fractions was quantitated by histamine ELISA (Beckman Coulter). Percentage of histamine release was calculated by the following: histamine in supernatant/(histamine in supernatant + histamine in pellet) × 100.

Fig. 1. Expression of SCF mRNA by HAECs. Soluble (572 bp) and membrane-bound (488 bp) SCF expression by HAECs was identified by RT-PCR analysis. SCF cDNA was amplified by 35 cycles of PCR. Lanes 1 and 2, HAEC donor 1; lanes 3 and 4, primary human MC; lanes 5 and 6, HAEC donor 2; lanes 7 and 8, HAEC donor 3. Expected size of β-actin: 508 bp.

Eicosanoid biosynthesis was quantitated after IgE-dependent activation from cell supernatants stored at −70°C before assay. Cys-LT generation in the supernatant was measured by ELISA for $LTC_4/D_4/E_4$ (Amersham Pharmacia). PGD_2 generation in the supernatant was measured with a PGD_2 ELISA (Cayman Chemical, Ann Arbor, MI).

Statistical Analysis. Unless otherwise indicated, results are reported as the mean ± SEM from at least three independent experiments with cells from different donors. Statistical differences were determined with the independent group Student's *t* test. Comparison of MC survival in coculture was performed by using single factor ANOVA.

Results

SCF Production by HAECs Supports the Survival of MCs in Coculture. SCF signaling is recognized to be essential for MC development and survival, although recent coculture experiments suggest that additional factors may be involved. SCF is expressed in soluble and membrane-bound forms with the soluble form generated by proteolytic cleavage within exon 6 (20–22). Membrane-bound SCF is generated by excising exon 6 by alternative RNA splicing. We first confirmed that HAECs expressed both soluble and membrane-bound forms of SCF by RT-PCR analysis by using PCR primers located in exons 2 and 7, respectively, designed to span exon 6 (Fig. 1). Although the predominant mRNA species identified was for soluble SCF, the band for membrane-bound SCF could always be detected. Soluble SCF protein was released into the cell culture supernatant in a time-dependent manner (Fig. 2). Maximal expression of soluble SCF protein was observed at 3–4 d followed by a plateau, with a maximal concentration of 3.8 ± 0.7 ng/ml (or 5.9 ± 0.1 ng per 10^6 cells; $n = 6$). Membrane-bound SCF protein was detected uniformly on HAECs by immunohistochemistry (Fig. 2).

MCs were then cultured with HAECs directly on HAEC monolayers or separated by transwell membranes. HAECs were able to support the survival of MCs for at least 4 d in coculture with no statistically significant differences in survival seen between direct coculture and cells cultured by separation with a transwell insert. MC survival in coculture with direct cell contact was equivalent to survival in suspension monoculture in SCF 100 ng/ml. Although there was a trend for less survival in transwell coculture and in HAEC-conditioned media alone, only the differences between suspension monoculture with SCF 100 ng/ml vs. suspension monoculture in media without cytokines was statistically significant ($P < 0.002$) by single-factor ANOVA (Fig. 3*A*). Suspension monoculture with 4 ng/ml SCF, studied based on the peak SCF concentration identified in Fig. 2, was not as effective as monoculture in SCF 100 ng/ml in supporting MC survival. Addition of an anti-SCF blocking Ab during SCF-dependent monoculture or during coculture with HAECs was able to completely inhibit MC survival (Fig. 3*B*).

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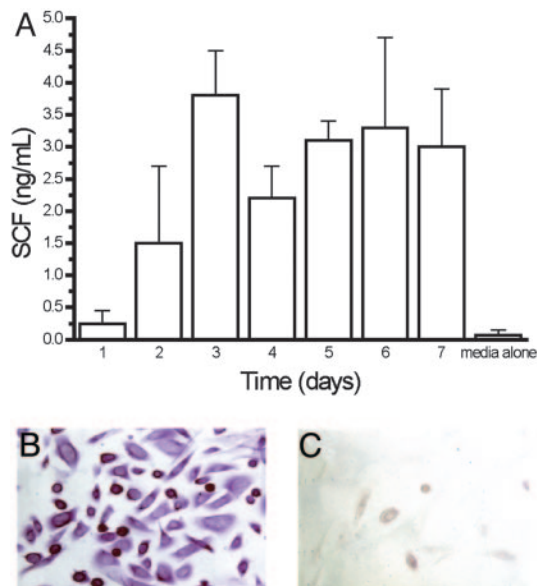

Fig. 2. SCF protein expression by HAECs. Release of soluble SCF protein into HAEC culture media was detected by SCF-ELISA. (*A*) All data are mean ± SEM ($n = 4$). Detection of membrane-bound SCF expression by immunohistochemical staining of HAECs by using goat-anti-human SCF Ab (*B*) or irrelevant IgG (*C*) demonstrates membrane-bound expression of SCF protein ($n = 2$). (Magnification: ×200.)

Effect of HAECs on MC Protease Phenotype. As MCs located at mucosal surfaces in contact with HAECs predominantly express MC_T phenotype, we examined protease expression by immunocytochemistry to characterize MC phenotype in coculture. MCs cultured in recombinant human SCF, IL-6, and IL-10 were nearly 100% MC_{TC} before the initiation of coculture. At 48 h, MCs cultured in SCF alone were 99 ± 1% positive for tryptase and 90 ± 3% positive for chymase. In contrast, the majority of MCs grown in HAEC coculture or HAEC-conditioned media expressed MC_T phenotype (99 ± 1% positive for tryptase in all conditions and 25 ± 2% positive for chymase for MC with direct coculture, 30 ± 3% with transwell coculture, and 31 ± 4% with HAEC-conditioned media). MCs grown without SCF lost both tryptase and chymase expression. At 4 d, the majority of MCs were of MC_T phenotype, with MC 99 ± 1% positive for tryptase in all conditions and 11 ± 3% positive for chymase in direct coculture, 16 ± 4% positive in coculture separated by a transwell membrane, and 25 ± 7% positive with HAEC-conditioned media (data not shown). The intensity of tryptase and chymase immunostaining did not appreciably change with progressive coculture (Fig. 4 and Fig. 8, which is published as supporting information on the PNAS web site). Thus, membrane-bound or soluble factors elaborated by HAECs are able to direct MC protease phenotype consistent with that observed with mucosal or reactive airway MCs *in vivo* (6).

Effect of HAECs on MC Eicosanoid Phenotype in Coculture. Because lung MC_T cells have an enhanced capacity for cys-LT generation upon IgE-dependent activation, we examined the ability of MC_T developed in coculture to generate cys-LT. When compared with MCs grown in SCF monoculture alone, MCs in coculture with HAECs released comparable amounts of histamine (17 ± 3% vs. 11 ± 4%, $n = 4$) and increased amounts of PGD_2 (16.2 ± 10.3 vs. 35 ± 11 ng per 10^6 cells, $P = 0.05$, $n = 4$) after sensitization with IgE (Fig. 5). However, MCs grown in coculture had little increase in cys-LT production when compared with SCF-monoculture alone (0.2 ± 0.1 vs. 0.2 ± 0.3 ng per 10^6 cells, $n = 4$). This result was unexpected, for after 4 d of coculture, MCs were predominantly

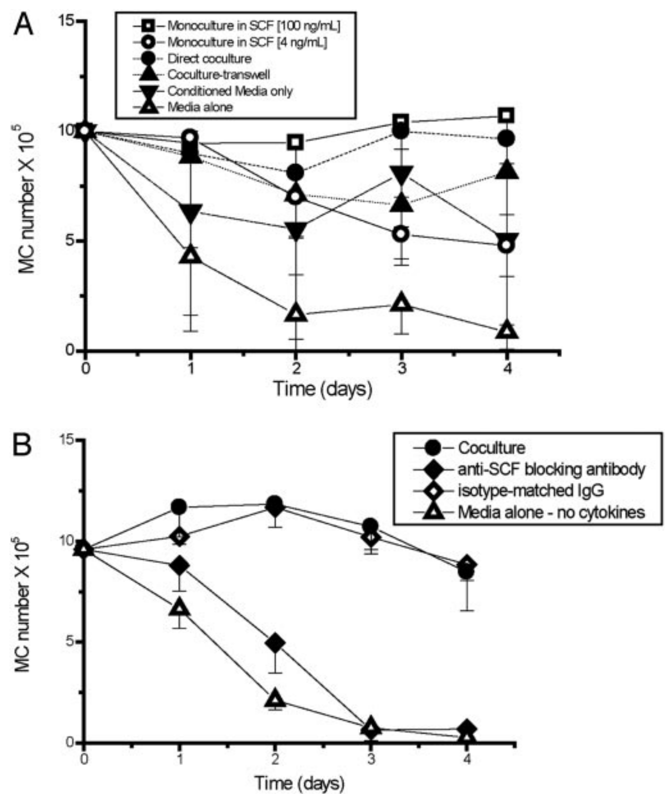

Fig. 3. MC survival in coculture. (*A*) MCs were grown in monoculture with recombinant human SCF (100 ng/ml or 4 ng/ml), in coculture with HAECs by seeding MCs directly onto subconfluent HAECs (direct coculture), in coculture with HAECs by using a transwell insert (coculture-transwell), with HAEC-conditioned media in the absence of any HAECs (conditioned media alone), or with media alone (media). All data are shown as mean ± SEM. When compared with monoculture in SCF, only media alone was unable to support MC survival ($n = 4$; comparison made by one-way ANOVA; *, $P < 0.002$). All other conditions were not statistically different from culture with SCF 100 ng/ml. (*B*) Inhibitory effect of anti-SCF blocking Ab (5 μg/ml) on MC survival in coculture compared with coculture without blocking Ab, isotype-matched IgG (5 μg/ml), or media without SCF. Data presented are mean ± SEM ($n = 3$).

MC_T and might have been expected to adopt a mucosal lung eicosanoid phenotype.

Effect of HAEC Coculture and Th2 Cytokine Priming on MC Eicosanoid Phenotype. Because coculture supported a reactive protease phenotype (MC_T) but not a reported lung MC eicosanoid phenotype, we investigated whether priming MCs in coculture with additional Th2 cytokines could augment MC cys-LT-biosynthetic capacity. MC_{TC} primed with IL-4 and IL-3 or IL-5 in monoculture were previously shown to have augmented cys-LT-generating capacity with IgE-dependent stimulation (17). With IL-4 priming, MCs in monoculture or coculture both had increased histamine release (77 ± 3% and 49 ± 10%, $n = 4$; Fig. 6) when compared with histamine release in the absence of IL-4 (17 ± 3% vs. 11 ± 4%, $n = 4$; Fig. 5). IL-4 priming also increased both cys-LT generation by MCs in monoculture (by 40-fold) and coculture (by 20-fold) and PGD_2 production by MCs in monoculture (by 2.8-fold) when compared with MCs grown in the absence of IL-4 (after IL-4 priming, for cys-LT generation: 8 ± 2.4 ng per 10^6 cells for monoculture and 4 ± 1.9 ng per 10^6 cells for coculture, $P < 0.05$ for both conditions when compared with unprimed MCs, $n = 4$; for PGD_2 production, 46 ± 12.8 ng per 10^6 cells for monoculture and 30 ± 14.2 ng per 10^6 cells for coculture, $n = 4$, $P < 0.05$ for PGD_2 production compared with unprimed MCs grown in SCF monocul-

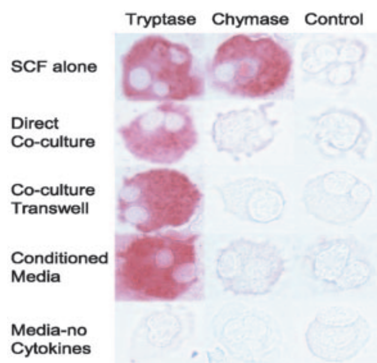

Fig. 4. MC protease expression identified by immunostaining over time. MCs were recovered after 2 d of culture in liquid suspension culture with SCF alone, in direct coculture with HAECs, in HAEC coculture separated by a transwell membrane, with HAEC-conditioned media, or in suspension culture lacking any cytokines. MCs were fixed with Carnoy's fixative, and expression of tryptase and chymase granule proteases was detected by immunocytochemistry. Representative cells for each condition are shown. (Magnification: ×400.) Cells (100) were counted per condition for each replicate (n = 4).

ture; Fig. 6). Priming with IL-3 alone in monoculture or coculture produced only a small increase in IgE-dependent cys-LT generation (1.2 ± 0.9 and 1.1 ± 0.7 ng per 10^6 cells, n = 3; data not shown). Priming MCs in mono- or coculture with IL-4 and IL-3 was able to selectively increase the amount of cys-LT generated upon IgE-dependent activation (when compared with IL-4 priming alone, an additional 2.8-fold increase for monoculture and 6.5-fold increase for coculture), whereas the amount of PGD_2 released remained relatively constant (for cys-LT generation: 23.7 ± 12.5 ng per 10^6 cells for monoculture and 26.5 ± 16 ng per 10^6 cells for coculture, $P < 0.05$ for both conditions when compared with MCs primed with IL-4 alone, n = 4; for PGD_2 production, 52 ± 8.9 ng per 10^6 cells for monoculture and 43 ± 16.2 ng per 10^6 cells for coculture, n = 4; Fig. 7). No difference in cell-surface FcεRI expression was noted by FACS analysis between MCs grown in coculture or monoculture (data not shown).

Discussion

The positioning of MCs at airway mucosal surfaces is one of the hallmarks of bronchial asthma, where the presence of Th2 cytokines released by Th2-polarized lymphocytes may influence the ability of MCs to release eicosanoid metabolites and other mediators implicated in the pathogenesis of asthma (23–25). SCF plays a crucial role in MC biology, acting as a critical survival factor, a chemotactic factor, an adhesion molecule, and an activation factor (26–32). In the lung, many different cell types potentially produce SCF, including fibroblasts, airway smooth muscle cells, endothelial cells, and airway epithelial cells (8, 13, 21, 33, 34). SCF expression is increased in human asthmatic airway epithelium as determined by quantitation of SCF mRNA-positive cells and SCF immunoreactivity by immunocytochemistry (9). We identified constitutive expression of SCF mRNA and protein for both the soluble and membrane-bound forms by HAECs from different donors. HAECs *ex vivo* secrete SCF within the reported range for serum SCF of 1.3–8.0 ng/ml (35). Expression of membrane-bound SCF by HAECs also provides a mechanism by which HAECs may attract MCs to the epithelial surface by SCF-mediated chemotaxis and provide adhesion and pro-survival signals to maintain MCs at the mucosal surface.

Here, cord blood-derived MCs survive in direct or separated coculture with HAECs in the absence of any exogenous cytokines, whereas MCs in suspension culture without cytokines die within a few days (Fig. 3). In contrast, studies of MC coculture with human umbilical vein endothelial cells (HUVECs) suggest that membrane-bound SCF mediates HUVEC-dependent MC survival as the

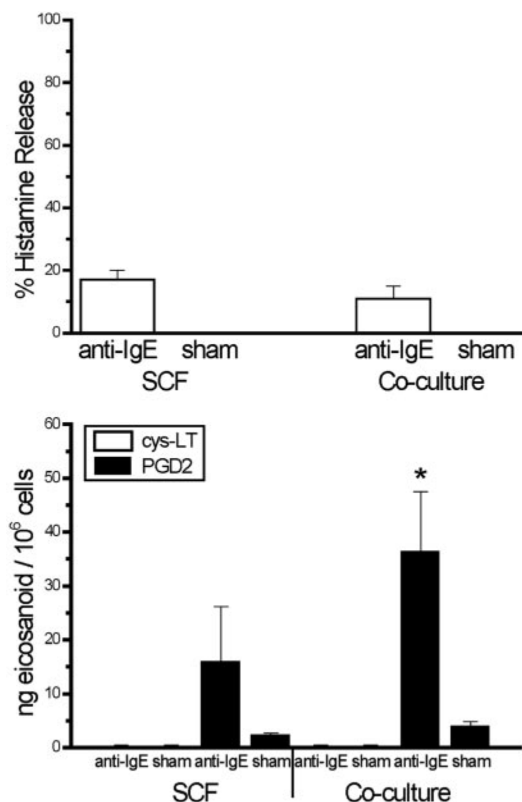

Fig. 5. Effect of coculture of MCs with HAECs on IgE-dependent histamine release and eicosanoid generation as measured by ELISA. MCs were maintained in monoculture with SCF or in coculture with HAECs for 4 d. Results depict percentage histamine release, cys-LT generation, and PGD_2 production after 1 d of passive sensitization with IgE followed by activation with anti-IgE. Results are the mean ± SEM of four experiments. *, P = 0.05 when compared with PGD_2 generation of MCs grown in SCF monoculture.

pro-survival effect of HUVECs was abolished by separation with a 0.4-μm transwell membrane (13). In this system, HUVECs produced little soluble SCF (<9 pg per 10^6 cells). In coculture experiments of intestinal MCs with intestinal fibroblasts, there was no difference in survival noted between MCs grown in direct or transwell coculture (14). In this system, blocking Abs to SCF or *c-kit* did not abrogate survival after 6 d. In our HAEC coculture experiments, blocking anti-SCF Abs did block SCF-dependent MC survival.

Under these culture conditions, MC survival in monoculture with 4 ng/ml SCF was not as robust as in 100 ng/ml SCF, and coculture with direct cell contact or in transwells more closely approximated survival in 100 ng/ml SCF. Membrane-bound SCF induces more persistent *c-kit* activation than soluble SCF (36), an effect that is approximated by using high-concentrations of soluble SCF. *In vivo* HAECs are likely in direct contact with MCs. In monoculture with lower concentrations of SCF, the amount of available SCF may be limiting given the number of cells in culture, whereas in the coculture with live HAECs, SCF is continuously being produced. Thus, the survival curve in monoculture with 4 ng/ml SCF is similar to that of HAEC-conditioned media, where the available SCF is not being renewed. Also, recombinant SCF produced in *Escherichia coli* lacks glycosylation, whereas glycosylated SCF is produced by human cells. It is also possible that other HAEC-derived mediators contribute to MC survival; although in this system, MC survival was SCF-dependent, as demonstrated by the anti-SCF blocking experiments (Fig. 3). Previous studies have demonstrated that SCF is required for the comitogenic effect of a number of cytokines (such as IL-6) on MC proliferation (18).

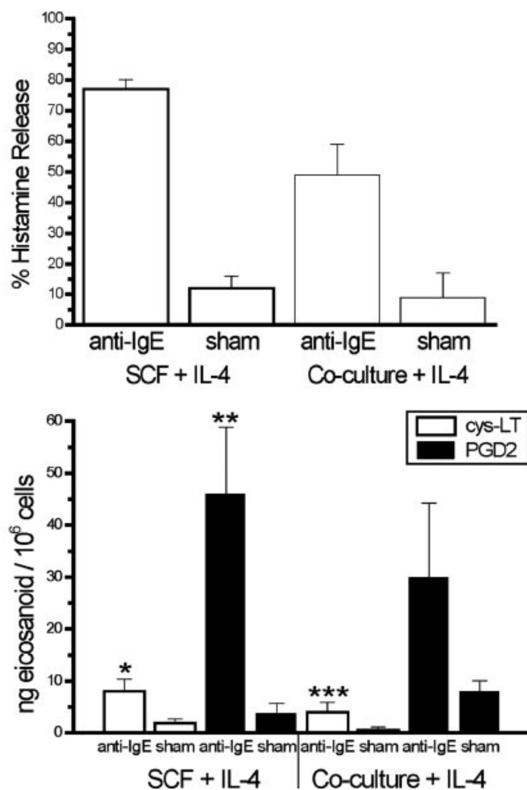

Fig. 6. Effect of coculture with IL-4 priming of MCs with HAECs on IgE-dependent histamine release and eicosanoid generation. MCs were maintained in monoculture with SCF or in coculture with HAECs for 4 d in the presence of recombinant human IL-4 (10 ng/ml). Results depict percentage histamine release, cys-LT generation, and PGD_2 production after 1 d of passive sensitization with IgE followed by activation with anti-IgE. Results are the mean ± SEM of four experiments. *, $P < 0.05$ when compared with cys-LT generation of unprimed MCs grown in SCF monoculture; **, $P < 0.05$ when compared with PGD_2 generation of unprimed MCs grown in SCF monoculture; ***, $P < 0.05$ when compared with cys-LT generation of unprimed MCs grown with HAEC coculture.

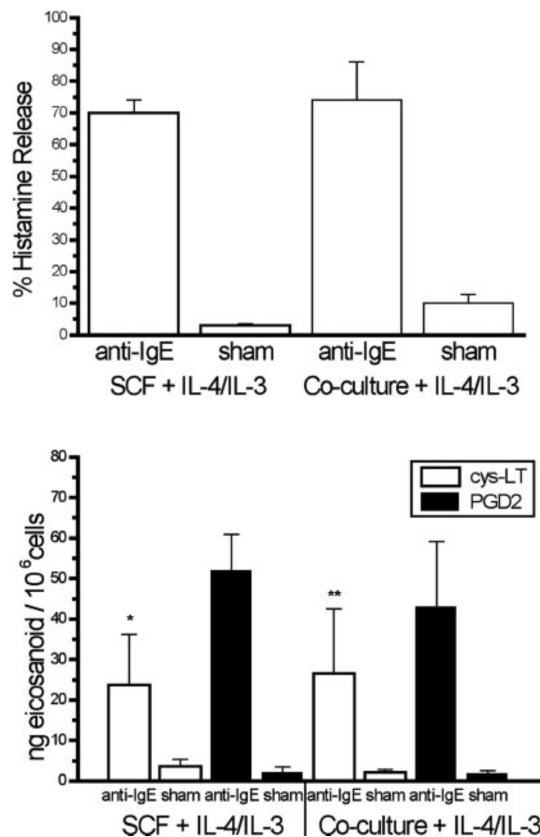

Fig. 7. Effect of coculture with IL-4 and IL-3 priming of MCs with HAECs on IgE-dependent histamine release and eicosanoid generation. MCs were maintained in monoculture with SCF or in coculture with HAECs for 4 d in the presence of recombinant human IL-4 (10 ng/ml) and IL-3 (5 ng/ml). Results depict percentage histamine release, cys-LT generation, and PGD_2 production after 1 d of passive sensitization with IgE followed by activation with anti-IgE. Results are the mean ± SEM of four experiments. *, $P < 0.05$ when compared with cys-LT generation of IL-4 primed MCs grown with SCF in monoculture; **, $P < 0.05$ when compared with cys-LT generation of IL-4 primed MCs grown in HAEC coculture.

In the human lung, the majority of MCs in the epithelium of the bronchioles and bronchi are MC_T, whereas the majority of MCs in the subepithelial regions of the bronchi and in the connective tissue are MC_{TC} (6). Cord blood-derived MC developed in SCF, IL-6, and IL-10 are uniformly MC_{TC}-positive, mirroring the phenotype of the constitutive, T cell-independent connective tissue MCs that expand at sites of inflammation under the influence of T cell-derived cytokines (5, 18). When these uniformly MC_{TC} cells were grown in coculture with HAEC, within 2–4 d a majority of the MC were MC_T phenotype. In contrast, MC_{TC} maintained for the same period in suspension culture with SCF alone or with SCF, IL-6, and IL-10 remained predominantly MC_{TC}. Thus, the MC mucosal protease phenotype was influenced by HAECs in a T cell-independent manner. In MC:HUVEC coculture, a starting population of 20–40% MC_{TC} adopted an 85% MC_{TC} phenotype at 14 d, suggesting that HUVECs were able to influence the expected constitutive MC_{TC} protease phenotype (13). A similar trend was observed in intestinal MC:fibroblast coculture (14). It is less likely that the MC_T cells recovered in HAECs coculture represent expansion of a latent predetermined mucosal MC progenitor, because the starting MC population was nearly uniformly MC_{TC}. Under these conditions, the transition from MC_{TC} to MC_T may represent a phenotypic change in protease expression, possibly mirroring the *in vivo* transition of constitutive to reactive mucosal MCs in the presence of inflammatory signals.

Metabolically active HAECs express a diverse array of mediators that may influence MC function. Airway epithelial cells can elaborate PGE_2 under certain conditions, and cord blood-derived MCs developed in SCF, IL-6, and PGE_2 express a predominantly MC_T phenotype (37, 38). Cord blood-derived MCs cultured in the presence of 200 ng/ml SCF have increased chymase expression compared with those cultured in the 50 ng/ml, suggesting that higher concentrations of SCF may promote chymase expression (39). Activated signal transducer and activator of transcription (STAT) 4 has been associated in murine MC with a connective-tissue MC_{TC} phenotype, so negative regulators of STAT4 activation may be expected to influence MC protease expression (40). Microphthalmia transcription factor (MITF) *mi/mi* mutant mice express little mouse MC protease-5, a human α-chymase homolog (41). As MITF phosphorylation leads to protein inhibitor of activated STAT (PIAS)3 dissociation from MITF and association with STAT3 (42), negative regulation of MITF expression and STAT3 activation may regulate MC chymase expression. The relative contribution of these candidates is currently under investigation.

Despite the adoption of a mucosal protease phenotype, MCs in coculture had low levels of IgE-dependent cys-LT generation, lower than that reported for human lung MCs (11). MC eicosanoid biosynthesis after IgE-dependent activation is tissue-specific and recent studies have identified potential mechanisms by which

specific Th2 cytokines may influence IgE-dependent MC cys-LT generation. IL-4 up-regulates the expression of FcεRI on the surface of the MCs (43), which may account for the increased histamine release seen after IL-4 priming of MCs in mono- or coculture. IL-4 also up-regulates the expression of LTC_4 synthase, an integral membrane protein that conjugates reduced glutathione to leukotriene A4 in the cys-LT biosynthetic pathway (17), and may account for the modest up-regulation in the total quantitative production of cys-LT with IL-4 priming alone of MCs grown in mono- or coculture. Only in the presence of IL-4 and additional Th2 cytokines (in this case, IL-3 used as a prototypic cytokine) was there an up-regulation of the total amount of cys-LT elaborated to an amount consistent with that expected for lung MC *in vivo*. A diverse array of Th2 cytokines, including IL-4 and IL-3, are found in bronchial biopsy specimens from asthmatic patients, and IL-3 protein can be detected in the bronchial epithelium of patients with or without asthma; so it is possible that these specific cytokines, which modulate IgE-dependent cys-LT generation *in vitro*, may also have a similar role *in vivo* (44, 45).

MCs in coculture under these experimental conditions adopted a predominantly mucosal protease phenotype yet retained an eicosanoid phenotype more consistent with a constitutive MC. This observation suggests that MC functional phenotype may be adaptable to a specific biological context, and that individual aspects of MC phenotype may be controlled by distinct mechanisms and cell types. Although T cell-derived cytokines may be required for the expansion of constitutive MC_{TC} into MC_T cells at the intestinal mucosal surface (46), it may be that other cell types such as epithelial cells are able to direct other aspects of MC phenotype, such as protease expression. However, these observations are in contrast to data suggesting that human lung-derived MC_{TC} cells generate significant amounts of cys-LT after IgE-dependent activation (although less than lung-derived MC_T cells) and that lung-derived MC_T cells treated for 1 wk in the presence of IL-6 do not increase in percentage of MC_{TC}s (47). The conversion of MC_{TC} to MC_T was not specifically addressed, although the implication is that MC protease development is parallel rather than sequential. The MCs used in our study were derived in culture from cord blood in recombinant human cytokines in serum-containing media, which may not reproduce the microenvironmental context of lung MC development *in vivo*, and the priming of MCs with as yet undefined tissue-derived factors in the human lung may account for these differences. In addition, IL-6 may not be the only factor that may influence chymase expression in the lung.

HAECs play a central role in directing inflammatory responses at the airway mucosal surface and HAEC-derived mediators such as nitric oxide, cytokines, and adhesion molecules may affect MC function (48–50). Here, we suggest that regulation of HAEC expression of SCF may serve as a primary mechanism by which MCs are positioned and maintained at the mucosal surface in allergic inflammation.

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1. Davies, D. E. (2001) *Curr. Allergy Asthma Rep.* **1,** 127–133.
2. Schulman, E. S., MacGlashan, D. W., Jr., Peters, S. P., Schleimer, R. P., Newball, H. H. & Lichtenstein, L. M. (1982) *J. Immunol.* **129,** 2662–2667.
3. Bingham, C. O., III, & Austen, K. F. (2000) *J. Allergy Clin. Immunol.* **105,** S527–S534.
4. Galli, S. J. (1990) *Lab. Invest.* **62,** 5–33.
5. Austen, K. F. & Boyce, J. A. (2001) *Leuk. Res.* **25,** 511–518.
6. Schwartz, L. B., Bradford, T. R., Irani, A. M., DeBlois, G. & Craig, S. S. (1987) *Am. Rev. Respir. Dis.* **135,** 1186–1189.
7. Pompen, M., Smids, B. S., Dingemans, K. P., Jansen, H. M., Out, T. A. & Lutter, R. (2000) *Clin. Exp. Allergy* **30,** 1104–1112.
8. Wen, L. P., Fahrni, J. A., Matsui, S. & Rosen, G. D. (1996) *Biochim. Biophys. Acta* **1314,** 183–186.
9. Al Muhsen, S. Z., Shablovsky, G., Olivenstein, R., Mazer, B. & Hamid, Q. (2004) *Clin. Exp. Allergy* **34,** 911–916.
10. Fox, C. C., Kagey-Sobotka, A., Schleimer, R. P., Peters, S. P., MacGlashan, D. W., Jr., & Lichtenstein, L. M. (1985) *Int. Arch. Allergy Appl. Immunol.* **77,** 130–136.
11. Peters, S. P., MacGlashan, D. W., Jr., Schulman, E. S., Schleimer, R. P., Hayes, E. C., Rokach, J., Adkinson, N. F., Jr., & Lichtenstein, L. M. (1984) *J. Immunol.* **132,** 1972–1979.
12. Nakano, T., Sonoda, T., Hayashi, C., Yamatodani, A., Kanayama, Y., Yamamura, T., Asai, H., Yonezawa, T., Kitamura, Y. & Galli, S. J. (1985) *J. Exp. Med.* **162,** 1025–1043.
13. Mierke, C. T., Ballmaier, M., Werner, U., Manns, M. P., Welte, K. & Bischoff, S. C. (2000) *J. Exp. Med.* **192,** 801–811.
14. Sellge, G., Lorentz, A., Gebhardt, T., Levi-Schaffer, F., Bektas, H., Manns, M. P., Schuppan, D. & Bischoff, S. C. (2004) *J. Immunol.* **172,** 260–267.
15. Artuc, M., Steckelings, U. M. & Henz, B. M. (2002) *J. Invest. Dermatol.* **118,** 391–395.
16. Artuc, M., Steckelings, U. M., Grutzkau, A., Smorodchenko, A. & Henz, B. M. (2002) *J. Invest. Dermatol.* **119,** 411–415.
17. Hsieh, F. H., Lam, B. K., Penrose, J. F., Austen, K. F. & Boyce, J. A. (2001) *J. Exp. Med.* **193,** 123–133.
18. Ochi, H., Hirani, W. M., Yuan, Q., Friend, D. S., Austen, K. F. & Boyce, J. A. (1999) *J. Exp. Med.* **190,** 267–280.
19. Zheng, S., Xu, W., Bose, S., Banerjee, A. K., Haque, S. J. & Erzurum, S. C. (2004) *Am. J. Physiol.* **287,** L374–L381.
20. Hogaboam, C., Kunkel, S. L., Strieter, R. M., Taub, D. D., Lincoln, P., Standiford, T. J. & Lukacs, N. W. (1998) *J. Immunol.* **160,** 6166–6171.
21. Kassel, O., Schmidlin, F., Duvernelle, C., Gasser, B., Massard, G. & Frossard, N. (1999) *Eur. Respir. J.* **13,** 951–954.
22. Slanicka, K. M., Nissen, C., Manz, C. Y., Toksoz, D., Lyman, S. D. & Wodnar-Filipowicz, A. (1998) *Exp. Hematol.* **26,** 365–373.
23. Laitinen, L. A., Laitinen, A. & Haahtela, T. (1993) *Am. Rev. Respir. Dis.* **147,** 697–704.
24. Wenzel, S. E., Westcott, J. Y., Smith, H. R. & Larsen, G. L. (1989) *Am. Rev. Respir. Dis.* **139,** 450–457.
25. Wenzel, S. E., Larsen, G. L., Johnston, K., Voelkel, N. F. & Westcott, J. Y. (1990) *Am. Rev. Respir. Dis.* **142,** 112–119.
26. Galli, S. J., Zsebo, K. M. & Geissler, E. N. (1994) *Adv. Immunol.* **55,** 1–96.
27. Meininger, C. J., Yano, H., Rottapel, R., Bernstein, A., Zsebo, K. M. & Zetter, B. R. (1992) *Blood* **79,** 958–963.
28. Coleman, J. W., Holliday, M. R., Kimber, I., Zsebo, K. M. & Galli, S. J. (1993) *J. Immunol.* **150,** 556–562.
29. Columbo, M., Horowitz, E. M., Botana, L. M., MacGlashan, D. W., Jr., Bochner, B. S., Gillis, S., Zsebo, K. M., Galli, S. J. & Lichtenstein, L. M. (1992) *J. Immunol.* **149,** 599–608.
30. Okayama, Y., Kobayashi, H., Ashman, L. K., Dobashi, K., Nakazawa, T., Holgate, S. T., Church, M. K. & Mori, M. (1998) *Eur. J. Immunol.* **28,** 708–715.
31. Gibbs, B. F., Arm, J. P., Gibson, K., Lee, T. H. & Pearce, F. L. (1997) *Eur. J. Pharmacol.* **327,** 73–78.
32. Koma, Y., Ito, A., Watabe, K., Hirata, T., Mizuki, M., Yokozaki, H., Kitamura, T., Kanakura, Y. & Kitamura, Y. (2005) *Lab. Invest.* **85,** 426–435.
33. Huang, E. J., Nocka, K. H., Buck, J. & Besmer, P. (1992) *Mol. Biol. Cell* **3,** 349–362.
34. Flanagan, J. G., Chan, D. C. & Leder, P. (1991) *Cell* **64,** 1025–1035.
35. Bowen, D., Yancik, S., Bennett, L., Culligan, D. & Resser, K. (1993) *Br. J. Haematol.* **85,** 63–66.
36. Miyazawa, K., Williams, D. A., Gotoh, A., Nishimaki, J., Broxmeyer, H. E. & Toyama, K. (1995) *Blood* **85,** 641–649.
37. Wu, T., Rieves, R. D., Larivee, P., Logun, C., Lawrence, M. G. & Shelhamer, J. H. (1993) *Am. J. Respir. Cell Mol. Biol.* **8,** 282–290.
38. Saito, H., Ebisawa, M., Tachimoto, H., Shichijo, M., Fukagawa, K., Matsumoto, K., Iikura, Y., Awaji, T., Tsujimoto, G., Yanagida, M., *et al.* (1996) *J. Immunol.* **157,** 343–350.
39. Ahn, K., Takai, S., Pawankar, R., Kuramasu, A., Ohtsu, H., Kempuraj, D., Tomita, H., Iida, M., Matsumoto, K., Akasawa, A., *et al.* (2000) *J. Allergy Clin. Immunol.* **106,** 321–328.
40. Kataoka, T. R., Komazawa, N., Morii, E., Oboki, K. & Nakano, T. (2005) *Blood* **105,** 1016–1020.
41. Morii, E., Jippo, T., Tsujimura, T., Hashimoto, K., Kim, D. K., Lee, Y. M., Ogihara, H., Tsujino, K., Kim, H. M. & Kitamura, Y. (1997) *Blood* **90,** 3057–3066.
42. Sonnenblick, A., Levy, C. & Razin, E. (2004) *Mol. Cell. Biol.* **24,** 10584–10592.
43. Toru, H., Ra, C., Nonoyama, S., Suzuki, K., Yata, J. & Nakahata, T. (1996) *Int. Immunol.* **8,** 1367–1373.
44. Humbert, M., Durham, S. R., Ying, S., Kimmitt, P., Barkans, J., Assoufi, B., Pfister, R., Menz, G., Robinson, D. S., Kay, A. B., *et al.* (1996) *Am. J. Respir. Crit. Care Med.* **154,** 1497–1504.
45. Woolley, K. L., Adelroth, E., Woolley, M. J., Ramis, I., Abrams, J. S., Jordana, M. & O'Byrne, P. M. (1996) *Am. J. Respir. Crit. Care Med.* **153,** 350–355.
46. Irani, A. M., Craig, S. S., DeBlois, G., Elson, C. O., Schechter, N. M. & Schwartz, L. B. (1987) *J. Immunol.* **138,** 4381–4386.
47. Oskeritzian, C. A., Zhao, W., Min, H. K., Xia, H. Z., Pozez, A., Kiev, J. & Schwartz, L. B. (2005) *J. Allergy Clin. Immunol.* **115,** 1162–1168.
48. Guo, F. H., Uetani, K., Haque, S. J., Williams, B. R., Dweik, R. A., Thunnissen, F. B., Calhoun, W. & Erzurum, S. C. (1997) *J. Clin. Invest.* **100,** 829–838.
49. Davis, B. J., Flanagan, B. F., Gilfillan, A. M., Metcalfe, D. D. & Coleman, J. W. (2004) *J. Immunol.* **173,** 6914–6920.
50. Propst, S. M., Denson, R., Rothstein, E., Estell, K. & Schwiebert, L. M. (2000) *J. Immunol.* **165,** 2214–2221.