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Characterization of a novel human mast cell line that responds to stem cell factor and expresses functional FcεRI

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Abstract

Background—Studies of human mast cells are constrained by the paucity of functional cell lines, the expense of maintaining mast cells in culture, and technical complexities.

Objective—We derived and characterized a human mast cell line that arose spontaneously from a culture of non-transformed hematopoietic progenitor cells.

Methods—CD34⁺-enriched mononuclear cells derived from a donor with aspirin exacerbated respiratory disease were cultured for 8 weeks with stem cell factor and interleukin-6 and with interleukin-3 for the first week only. The cells (termed LUVA cells) survived and proliferated without further addition of any growth factors and have been maintained in culture for ~2 years.

Results—LUVA cells possess metachromatic cytoplasmic granules that are immunoreactive for tryptase, cathepsin G, and carboxypeptidase A3. They express transcripts encoding genes for FcεRI, c-kit, chymase, tryptase, histidine decarboxylase, carboxypeptidase A3, and the type 1 receptor for cysteinyl leukotrienes. Flow cytometry confirmed uniform expression of FcεRI, c-kit and FcγRII. FcεRI cross-linkage induced the release of β-hexosaminidase, prostaglandin D₂, thromboxane A₂, and macrophage inflammatory protein-1β. Immortalization was not associated with either a known genomic mutation of c-kit in the donor or a somatic mutation of c-kit within the cells, and it was not associated with c-kit autophosphorylation.

CONCLUSIONS—LUVA cells are an immortalized human mast cell line that can be maintained without stem cell factor and display high levels of normally signaling c-kit and FcεRI. These cells will prove valuable for functional human mast cell studies.

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Keywords

Mast cell; SCF; FcεRI; c-Kit; Tryptase; CPA3

INTRODUCTION

Mast cells (MCs) are sentinels of innate and acquired immunity and allergic disorders. The clonal expansion of MCs results in mastocytosis and MC leukemias. MCs do not circulate in peripheral blood and are difficult to culture from tissue in large numbers. Thus, studies of human MC biology have primarily used MCs derived *in vitro* from cord blood^{1,2} or from peripheral blood hematopoietic progenitor cells.³ The maintenance of these cultures is technically difficult and requires large quantities of recombinant cytokines, usually stem cell factor (SCF) and interleukin (IL)-6. The two known human MC lines, HMC-1⁴ and LAD2,⁵ were derived from patients with MC leukemias. The HMC-1 cell line represents very immature transformed cells and they contain no chymase, very little tryptase,⁶ and do not express functional IgE receptors. LAD2 cells are functional MCs but have a widely variable doubling time, an unstable phenotype in culture, and can be difficult to freeze and recover.

We identified and characterized a new human MC line (termed LUVA cells), which arose spontaneously during the culture of peripheral blood CD34⁺ cells. LUVA cells respond to IgE receptor cross-linkage and resemble mature human MCs functionally and morphologically. They do not exhibit autophosphorylation of the c-kit receptor and respond to recombinant human c-kit ligand, SCF, with accelerated proliferation. However, neither SCF nor any other exogenous growth factor is necessary to maintain the survival or proliferation of LUVA cells. LUVA cells are the first MC line derived from a patient with no clinical MC disorder and no mutation in the c-kit receptor. They should be valuable for studies of human MC function.

METHODS

Development and maintenance of LUVA cells

MCs were grown from CD34⁺-enriched mononuclear cells derived from the peripheral blood of a donor with aspirin exacerbated respiratory disease according to the methodology of Kirshenbaum et al.³ The donor has no clinical symptoms consistent with mastocytosis or leukemia. Serum tryptase (Mayo Medical Laboratories, Rochester, MN) was within normal limits (1.38 ng/mL). Cells were cultured for 1 week with recombinant human SCF (100 ng/mL; generated in *E. coli* in our laboratory), IL-6 (100 ng/mL; generated in High FiveTM cells in our laboratory), and IL-3 (30 ng/mL; R&D Systems) in StemPro[®]-34 SFM medium (Invitrogen) that was half replaced every week and provided with fresh IL-6 and SCF. After 8 weeks the cells continued to increase in number and their dependency on exogenous cytokines for survival and proliferation was assessed. LUVA cells are maintained in our laboratory in StemPro[®]-34 SFM at a concentration of 5×10^5 cells/mL and are provided with 50% fresh medium weekly without exogenous cytokines.

Staining

Cells (1×10^4 /slide) were immobilized onto glass slides by centrifugation (Shandon Cytospin[®] 4 Cyto centrifuge) and air dried (Fig 1, A and F-J), or a cell suspension was fixed and embedded in HistoGelTM (Richard-Allan Scientific) and then in paraffin (Fig 1, B-E). 6-week-old MCs grown from CD34⁺-enriched mononuclear cells from the peripheral blood of a healthy donor were used for comparison, as were LAD2 cells.³ Cells were stained with toluidine blue for 1 minute and washed with water. For immunocytochemistry, cells were

fixed in Carnoy's fluid (60% ethanol, 30% chloroform, and 10% acetic acid) for 10 minutes at room temperature and washed with phosphate buffered saline. Samples were then incubated with a mouse anti-human tryptase antibody (clone AA1; Dako), chymase antibody (clone CC1; AbD Serotec), cathepsin G antibody (clone 19C3; Abcam), CPA3 antibody (Prestige Antibodies®) or with an irrelevant isotype-matched antibody (BD Biosciences).

Flow cytometric analysis of surface markers

Cells were incubated with monoclonal mouse anti-human antibodies specific for FcεRIα (eBioscience), c-kit, CD13, FcγRIII, CD25, FcγRII, FcγRI (all from BD Biosciences), IL-3 receptor (eBioscience), IL-4 receptor (eBioscience), IL-9 receptor (R&D Systems), thymic stromal lymphopoietin receptor (TSLP-R) (BioLegend), IL-33 receptor (IL-33R) (MBL International Corporation), or IL-25 receptor (IL-25R) (R&D Systems). The expression of FcεRIα was also examined after a 3 day incubation of the cells with IgE or IL-4 (PeproTech), and the expression of both FcγRIII and FcγRI was examined after an overnight incubation in the absence or presence of interferon gamma (IFN-γ) (PeproTech). Isotype controls were used for each experiment and at least 20 000 events were recorded for each analysis on a BD FACS Aria™ Flow Cytometer.

Mediator generation and release

Cells were incubated overnight with IgE, washed, and stimulated with a cross-linking rabbit anti-human polyclonal anti-IgE antibody. Supernatants and pellets were harvested at 30 minutes ± 6 μM calcium ionophore for quantification of β-hexosaminidase and at 6 hours for quantification of prostaglandin D₂ (PGD₂), thromboxane A₂ (TXA₂) (Cayman Chemical) and macrophage inflammatory protein (MIP-1β) (eBioscience) by enzyme-linked immunosorbent assay (ELISA). SCF (R&D Systems) and IFN-γ (eBioscience) were also measured by ELISA. Cysteinyl leukotriene (cys-LT) measurement was performed on supernatants from cells stimulated for 15 minutes with FcεRI cross-linkage or calcium ionophore (2 μM) using high-performance liquid chromatography (RP-HPLC) (Beckman Coulter) as described.⁷ The resolved products were quantified from the ratio of the peak areas to the area of the internal standard prostaglandin B₂.

The specific activity of the terminal cys-LT-generating enzyme leukotriene C₄ synthase (LTC₄S) was determined by measuring the conversion of leukotriene A₄ methyl ester (20 mM; Enzo Life Sciences International) to LTC₄ methyl ester in the presence of glutathione using RP-HPLC.^{8,9}

Proliferation and apoptosis

Triplicate samples of 10⁵ cells/mL were maintained in medium ± SCF at 1, 10, and 100 ng/mL for 3 days. In selected experiments the cells were cultured with varying doses of IL-3, IL-4, IL-5, IL-6, IL-9, IL-33, granulocyte-macrophage colony-stimulating factor (GM-CSF), or β-nerve growth factor, alone and in the presence of 10 ng/mL SCF. On day 3, 1 μCi of thymidine labeled with tritium (³H) was added to each well, and 18 hours later the [³H]thymidine incorporation was counted for each sample. The percentage of cells undergoing apoptosis was determined by culturing 5 × 10⁵ cells/mL in medium for 3 days in the presence or absence of SCF and in the presence or absence of the tyrosine kinase inhibitor imatinib mesilate (Novartis). Apoptotic cells were detected with fluorescein isothiocyanate (FITC)-labeled Annexin V (BD Biosciences). Fluorescence signals of FITC were detected on a BD FACS Aria™ Flow Cytometer. For each analysis at least 20 000 events were recorded. Growth curves and doubling times were established by plating LUVa cells at a density of 2 × 10⁵ cells/mL in StemPro®-34 ± SCF 100 ng/mL in triplicate wells; cells were harvested and counted at days 1, 3, and 4. LAD2 cells (a gift from Dr. A.

Kirshenbaum at the National Institutes of Health) and HMC-1.1 cells (a gift from Dr. C. Akin, Brigham and Women's Hospital) were used for comparison.

Quantitative PCR (qPCR)

RNA extracted with TRI Reagent® (Sigma-Aldrich) from LUVA cells and LAD2 cells was converted into cDNA templates with the RT² First Strand Kit (SABiosciences). The expression of transcripts encoding FcεRIα (*FCER1A*), c-kit (*KIT*), tryptase (*TPSAB1*), chymase (*CMA1*), carboxypeptidase A3 (*CPA3*), histidine decarboxylase (*HDC*), *LTC₄S*, PGD₂ synthase (*HPGDS*), microphthalmia transcription factor (*MITF*), and the type 1 and 2 receptors for cys-LTs (*CYSLTR1* and *CYSLTR2*) was examined using RT² SYBR® Green/ROX qPCR Master Mix (SABiosciences) and normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (all primers from SABiosciences).

Western blotting for c-kit signaling

LUVA, LAD2, and HMC-1 cells were washed and grown in fresh medium without SCF for 24 hours and then were stimulated in the presence or absence of SCF (100 ng/mL) for 10 minutes. Western blots were performed with antibodies against total c-kit, phosphorylated c-kit, total Akt, phosphorylated Akt, total mitogen-activated protein kinase-kinase (MEK), phosphorylated MEK, total extracellular signal regulated kinase (ERK), phosphorylated ERK, total cyclic AMP response element binding protein (CREB) and phosphorylated CREB (all antibodies from Cell Signaling).

Sequencing

Genomic DNA was purified from the peripheral blood of the donor with a Wizard® Genomic DNA Purification Kit (Promega), and RNA was extracted from LUVA cells with TRI Reagent® and converted to cDNA templates with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The *KIT* gene from LUVA cells was sequenced through the entire coding region and the receptor from the donor's peripheral blood was sequenced through the 6 known mutations in human MCs, at sites 419¹⁰, 522, 560¹¹, 550–580¹², 816¹⁴, and 839/840¹⁵. Primers spanning the coding region or each specific mutation were used to generate a PCR product that was subsequently electrophoresed and isolated from an agarose gel. The PCR products were sequenced at the University of Virginia sequencing facility (see Methods section of Online Repository for primer sequences).

Cryopreservation

The cells were frozen and stored according to the methodology of Kirshenbaum et al.,⁵ except that no SCF was used.

Statistical Analysis

Values are expressed as mean ± SD or mean ± SEM, as indicated in the text. All reported *P* values were 2-sided and were declared significant at levels <.05.

RESULTS

Morphologic characterization of LUVA cells

To establish that LUVA cells were similar to MCs, slides generated by cytocentrifugation or from sections of paraffin-embedded cell pellets were stained for markers characteristic of MCs. Primary MCs grown from peripheral blood progenitors were used for comparison. LUVA cells uniformly contained granules that stained metachromatically with toluidine blue (Fig 1, A), all of the cells were strongly positive for tryptase and cathepsin G, and

>80% were positive for CPA3 (Fig 1, *B, D, E*). Roughly half of the LUVA cells reacted strongly with chymase (Fig 1, *C*). LUVA cells were varied in size and most had round to oval nuclei with a large nuclear:cytoplasmic ratio compared to the primary cells. Approximately 2% of the LUVA cells were multinucleated. In contrast to LUVA cells, primary MCs were largely non-reactive for chymase and CPA3 (Fig. 1, *H and J*), and LAD2 cells were largely negative for toluidine blue, tryptase, and chymase, having apparently lost their original phenotype during the two-year period of culture in our laboratory (data not shown). The IgG control was negative in all cases.

Cytofluorographic characterization of LUVA cells

We performed cytofluorographic analyses to determine whether LUVA cells expressed surface markers characteristic of MCs. LUVA cells stained strongly and uniformly for c-kit (Fig 2, *A*), FcγRII (Fig 2, *B*), and FcεRIα (Fig 2, *D and E*). Slightly more than half of the cells stained positively FcγRI (Fig 2, *C*). Between 10 and 25% of the cells weakly expressed IL-4Rα, IL-25R, IL-33R, CD13, and CD25 (IL-2Rα), shown by a unimodal peak shift in the fluorescence intensity. No baseline surface expression of CD16, TSLP-R, IL-3Rα, or IL-9Rα was observed. Each marker was examined twice with similar results (data from one experiment are shown in Fig E1 in the Online Repository). As previously described for primary MCs, treatment of LUVA cells with either IL-4 (10 ng/mL)¹⁶ or varying concentrations of IgE (100–1000 ng/mL)^{17,18} increased the surface levels of FcεRIα (Fig 2, *D and E*). Treatment of the cells overnight with IFN-γ 15 ng/mL did not further increase the expression of Fcγ RI, nor did it change the expression of the other Fc receptors (data not shown). Supernatants from unstimulated and FcεRI cross-linked LUVA cells did not contain detectable IFN-γ as measured by ELISA (sensitivity limit of 4 pg/mL).

Release of mediators

LUVA cells and LAD2 cells were sensitized overnight with monoclonal IgE and challenged with polyclonal anti-IgE to cross-link FcεRI. The release of β-hexosaminidase was monitored as a measure of exocytosis; calcium ionophore stimulation (6 μM) was used as a positive control. Both LUVA and LAD2 cell lines released β-hexosaminidase upon FcεRI cross-linkage ($18.4 \pm 0.1\%$ net release from LUVA cells, mean $\pm$ SD, $n=3$ experiments) (Fig 3, *A*) and on stimulation with calcium ionophore. To evaluate arachidonic acid metabolism, we measured the generation of PGD₂, cys-LTs, and TXA₂ and we measured MIP-1β as a representative MC-derived chemokine.^{19, 20} In these experiments we also tested the effect of IL-4 priming because IL-4 increases the IgE-dependent production of both lipid mediators⁷ and cytokines¹⁹ in primary human MCs. LUVA cells released large quantities of both PGD₂ and TXA₂ (Fig 3, *B and C*), although no cys-LTs were detected in the supernatants from cells activated by FcεRI cross-linkage as determined by HPLC (sensitivity limit of ~2 ng) (data not shown). Stimulation of LUVA cells with 2 μM calcium ionophore induced the production of 50.9 pmol of cys-LTs/10⁶ cells, of which ~60% had been converted to LTE₄. LUVA cells secreted small amounts of MIP-1β spontaneously; this secretion increased from 0.58 ± 0.20 ng/10⁶ cells to 4.44 ± 0.30 ng/10⁶ cells (mean $\pm$ SD, $n=3$ experiments) after FcεRI -cross-linkage. Following 3 days of priming with IL-4 (10 ng/mL), FcεRI cross-linkage increased the productions of both PGD₂ and MIP-1β by ~2-fold compared to the unprimed replicates (Fig 3, *B and D*), though the production of TXA₂ was unaffected. LAD2 cells released slightly more β-hexosaminidase than LUVA cells, but much less PGD₂, TXA₂, and MIP-1β, and no priming effect of IL-4 was noted (Fig 3). LUVA cells had abundant LTC₄S activity (138.2 pmol of LTC₄-methyl ester/10⁶ cells were detected from unstimulated cells), which remained unchanged after a 3 day incubation with IL-4 (138.5 pmol LTC₄-methyl ester/10⁶ cells). Accordingly, the generation of cys-LTs in response to FcεRI cross-linkage or stimulation with ionophore was not enhanced by IL-4 priming (data not shown).

Expression analysis of MC-associated mRNA transcripts

We characterized the steady-state expression of MC-associated mRNAs in LUVA cells by qPCR. LUVA cells expressed slightly lower levels of tryptase than LAD2 cells and comparable levels of chymase, but expressed ~40-fold higher levels of CPA3 than LAD2 cells (Fig 4). LUVA cells also had detectable levels of transcript for *HDC*, *LTC4S*, *HPGDS*, *MITF*, *CYSLTR1*, *CYSLTR2*, the IgE high-affinity receptor *FCER1A*, and the SCF receptor *KIT* (Fig E2 in the Online Repository).

Measurement of proliferation and apoptosis

We monitored the proliferation rates of LUVA cells (by incorporation of [³H]thymidine) and their entry into apoptosis (by cytofluorographic detection of Annexin V) in the presence or absence of recombinant SCF. We used LAD2 cells as a control for these experiments, since they can survive and proliferate without SCF. Both LUVA cells and LAD2 cells exhibited substantial [³H]thymidine incorporation in the absence of SCF. LUVA cells showed a dose-dependent increase in proliferation in response to SCF (Fig 5). Various concentrations (1–10 ng/mL) of IL-3, IL-4, IL-5, IL-6, IL-9, IL-33, GM-CSF, β -nerve growth factor, LTD₄, or LTE₄ failed to elicit incremental proliferation by LUVA cells, either alone or in combination with SCF (data not shown).

Because primary human MCs depend on SCF for their survival, these cells were used as additional controls for the apoptosis experiments. In the absence of SCF, most of the LUVA cells remained viable for 3 days (Fig 6, A), whereas most of the primary human MCs became positive for surface annexin V (Fig 6, D). SCF did not change the percentages of LUVA cells that were Annexin V positive after 3 days of culture (Fig 6, B) but was strongly cytoprotective for the primary human MCs (Fig 6, E). Results from a parallel experiment with LAD2 cells yielded results similar to those for LUVA cells (data not shown). The addition of imatinib mesilate for 3 days did not affect the apoptosis of LUVA cells (Fig 6, C), but dramatically increased the apoptosis of primary human MCs (Fig 6, F). The doubling time of LUVA cells was 54.0 ± 0.5 hours in the absence of SCF and 43.3 ± 2.9 hours in the presence of SCF (100 ng/mL). This was faster than the growth of LAD2 cells, but slower than that of HMC-1 cells. (Table E1 and Fig E3).

c-Kit-dependent signaling

To determine whether LUVA cells exhibited constitutive activation of c-kit, we examined the phosphorylation of c-kit and its downstream signaling effectors in the presence or absence of SCF. We compared the responses of LUVA cells to those of LAD2 cells and HMC-1 cells. In the absence of stimulation both LUVA and LAD2 cell lines exhibited low levels of c-kit phosphorylation and stimulation with SCF (100 ng/mL) for 10 minutes resulted in robust phosphorylation in both of these cell lines (Fig 7, A), along with incremental phosphorylation of Akt, MEK, ERK, and CREB (Fig 7, B). In contrast, HMC-1 cells exhibited constitutive c-kit phosphorylation that did not change after stimulation with SCF; none of the above signaling proteins showed increased phosphorylation upon stimulation with SCF (data not shown). Supernatants from LUVA cells did not contain detectable SCF as measured by ELISA (sensitivity limit of 9.0 pg/mL). Sequencing of the entire *KIT* coding region revealed no mutations in the LUVA cell DNA, and no known mutation was found in the DNA from the donor's leukocytes.

DISCUSSION

We describe a human MC line that arose spontaneously during the *in vitro* derivation of MCs from CD34⁺ peripheral blood precursors from a patient with aspirin-exacerbated respiratory disease. To our knowledge, LUVA cells are the first human MC line derived

from an individual without a clonal MC disorder, based on a normal serum tryptase and the lack of a mutation in *KIT*. LUVA cells possess metachromatic granules (Fig 1, A) and have high expression of IgE receptors that increases further with IL-4 or exogenous IgE (Fig 2). As with primary cultured human MCs^{7, 19} (but not LAD2 or HMC-121), LUVA cells exhibit priming responses to IL-4 for IgE-dependent chemokine and PGD₂ production (Fig 3). Moreover, LUVA cells have remained phenotypically stable over 2 years of propagation in culture, even in the absence of SCF. Although they exhibit normal c-kit signaling, their continued growth and survival are mediated by an as yet-to-be determined c-kit-independent mechanism.

The characteristic metachromatic staining of MC granules is based on their content of sulfated proteoglycans bound to a peptide core.²² These proteoglycans bind the serine proteases tryptase- β , chymase, and cathepsin G, and the metalloexopeptidase CPA3. All human MCs express tryptase- β .²³ Two subsets of tissue MCs in humans are distinguished based on the presence or absence of chymase, cathepsin G, and carboxypeptidase, which are typically expressed as a cassette.^{24,25} In addition to uniformly strong immunoreactivity for tryptase (Fig 1, B), LUVA cells were also positive for cathepsin G (Fig 1, D) and CPA3 (Fig 1, E), the latter of which was absent in the primary MCs used as controls (Fig 1, J). Only a subset of LUVA cells strongly expressed chymase (Fig 1, C), similar to the characteristics reported for LAD2 cells. The strong expression of CPA3 was also evident in the qPCR analysis (Fig 4). A recent report demonstrated that CPA3 is expressed by MCs infiltrating the bronchial mucosa of individuals with severe asthma,²⁶ and is among the most strongly expressed mRNA transcripts in lung biopsy specimens from patients with asthma.²⁷ Interestingly, intraepithelial mast cells in bronchial biopsies from individuals with severe asthma lack chymase despite their strong CPA3 expression.²⁸ Thus LUVA cells (but not primary peripheral blood MCs) may recapitulate certain phenotypic features of intraepithelial MCs present in asthmatic patients.

In allergic responses, MC activation results from cross-linkage of the heterotetrameric form of Fc ϵ RI. In tissues, MCs also express Fc γ RII and Fc γ RI, but the latter is only expressed inducibly on primary peripheral blood-derived human MCs cultured *in vitro* with IFN- γ .²⁹ LUVA cells expressed all 3 of these Fc receptors constitutively (Fig 2). Whereas Fc ϵ RI could be upregulated by IL-4 and IgE, consistent with prior reports for primary human MCs, Fc γ RI was not further upregulated by IFN- γ , nor was IFN- γ detected in the media of LUVA cells.²⁹ Although Fc ϵ RI cross-linkage induced only modest release of β -hexosaminidase, indicative of preformed granule mediator release, it caused substantial production of both PGD₂ and TXA₂, indicating intact cytosolic phospholipase A₂-mediated arachidonic acid release and cyclooxygenase function (Fig 3). As was the case for the prostanoids, LUVA cells generated MIP-1 β at levels that were several-fold higher than those generated by LAD2 cells, indicating that systems for *de novo* cytokine transcription and translation are intact in LUVA cells (Fig 3). IL-4 primed the cells and augmented Fc ϵ RI function, reflected by increases in PGD₂ and MIP-1 β generation (Fig 3), which was consistent with previous reports on studies with primary human MCs.^{7,19} The absence of cys-LTs found in the supernatants of the cells stimulated by Fc ϵ RI cross-linkage may reflect the limits of detection (~ 2 ng of LTC₄) of the HPLC assay, as the strong stimulation provided by ionophore resulted in abundant release of cys-LTs (>50 pmol/10⁶ cells). Although the activity of LTC₄S, the terminal enzyme required to convert LTA₄ to LTC₄, was robust, it was not further upregulated by IL-4. This represents a marked difference between LUVA cells and cord-blood derived human mast cells, in which constitutive LTC₄S expression is low and profoundly increased by IL-4.⁷

SCF and its receptor, c-kit, are essential for normal development of MCs *in vivo*. In humans, clonal proliferation of MCs results from gain-of-function mutations in the *KIT* activation

domain. A point mutation producing a change from aspartic acid to valine at residue 816 (Asp816Val) results in SCF-independent phosphorylation of c-kit.¹⁵ *In vivo*, this mutation results in systemic mastocytosis and in a syndrome of idiopathic anaphylaxis associated with clonal MCs in the bone marrow.³⁰ The cytokine-independent human MC line HMC-1 also bears gain-of-function *KIT* mutations (Asp816Val and Val560Gly) and consistent with our findings, displays constitutive phosphorylation of the c-kit protein.³¹ Although LUVA cells do respond to SCF with a dose-dependent increase in proliferation (Fig 5), they show considerable SCF-independent proliferation and do not require the presence of SCF to sustain their viability (Fig 6). Consistent with this observation is the finding that LUVA cells did not undergo apoptosis when treated for 3 days with imatinib mesilate at doses that completely inhibit the function of c-kit *in vitro*.³² Additionally, LUVA cells did not exhibit significant constitutive c-kit receptor phosphorylation in the absence of SCF, but they did demonstrate phosphorylation in response to stimulation with exogenous SCF (Fig 7). Although MCs are absent in most organs of c-kit and SCF-mutant mice, both strains have MCs in the skin at 20–30% of normal levels. Combined with the lack of a *KIT* mutation in LUVA cells, our findings indicate that LUVA cells survive and proliferate by a c-kit-independent mechanism, a subject of ongoing analysis in our lab. Interestingly, LUVA cells express some CD25 (Fig E1), the alpha chain of the IL-2 receptor,³³ and a surface marker expressed by bone marrow MCs in patients with systemic mastocytosis bearing mutant c-kit. CD25 is not expressed by cultured human mast cells derived from CD34⁺ progenitor cells³⁴ or by MCs in normal tissues. Whether CD25 expression is a reflection of transformation or a marker of activation is unclear, and it is not known whether it is expressed by MCs in asthmatic bronchi or other areas where MC phenotype is altered by the inflammatory milieu.

The importance of MCs in allergic disease, host defense, and inflammation is broadly accepted. Mouse MC biology is greatly facilitated by the relative ease with which MCs can be cultured from bone marrow, as well as the development of multiple strains with targeted deletions in MC-associated genes. The unanticipated development of this fully functional MC line spontaneously arising from a patient without a clonal MC disorder should prove valuable for investigations focused on understanding human MC function.

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ABBREVIATIONS

CPA3	Carboxypeptidase A3
CREB	Cyclic AMP response element binding protein
cys-LT	Cysteinyl leukotriene
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal regulated kinase
FITC	Fluorescein isothiocyanate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
RP-HPLC	High-performance liquid chromatography
IL	Interleukin

IL-25R	Interleukin-25 receptor
IL-33R	Interleukin-33 receptor
LTC₄	Leukotriene C ₄
LTC₄S	Leukotriene C ₄ synthase
MIP-1β	Macrophage inflammatory protein
MC	Mast cell
MEK	Mitogen-activated protein kinase-kinase
PGD₂	Prostaglandin D ₂
qPCR	Quantitative PCR
SCF	Stem cell factor
TXA₂	Thromboxane A ₂
TSLP-R	Thymic stromal lymphopoietin receptor
³H	Tritium

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Figure 1. Morphological characterization of LUVA cells

400× magnifications of LUVA cells are in the left column and of CD34⁺-derived primary MCs are in the right column. Cells stained with toluidine blue (**A** and **F**), tryptase (**B** and **G**), chymase (**C** and **H**), cathepsin G (**D** and **I**), and CPA3 (**E** and **J**) are shown.

Figure 2. Cell surface expression of MC markers

Representative flow cytometric analyses are of the expression of c-kit (A), Fc γ RII/CD32 (B), and Fc γ RI/CD64 (C). Basal expression of Fc ϵ RI α and upregulation of Fc ϵ RI α after incubation with IgE (D) or IL-4 (E) are shown. Each analysis was carried out at least 3 times. Isotype controls are shown in gray.

Figure 3. Mediator release

Quantification of β -hexosaminidase (A), PGD₂ (B), TXA₂ (C), and MIP-1 β (D) levels in cell supernatants are shown. In some experiments the cells were primed for 3 days with IL-4 (10 ng/mL) before activation. Data are mean $\pm$ SD, n= 2 or 3 experiments for LUVA cells, and n=1 or 2 experiments for LAD2 cells.

Figure 4. Mast cell-specific transcript expression

Baseline mRNA transcript expressions of tryptase (*TPSAB1*), chymase (*CMA1*), and *CPA3* are shown. Each LUVA transcript is expressed as mean $\pm$ SEM, n=4 experiments, and in parallel are the baseline levels of the same transcripts by LAD2 cells (n=1).

Figure 5. Cellular proliferation of MCs in the presence or absence of SCF

Cells were stimulated with the indicated doses of SCF for 72 hours. Proliferation rates were determined by [^3H]thymidine incorporation. Data are mean $\pm$ SD, n=3 experiments.

Figure 6. Effect of exogenous SCF and imatinib mesilate on apoptosis

LUVAs cells were cultured in medium alone (A), with SCF (B), or with SCF and imatinib mesilate (C). Six week-old primary human cord blood-derived MCs (cbMCs) were cultured with the same conditions (D-F). The percentage of apoptotic cells was measured by surface expression of Annexin V, compared to isotype control in shaded gray. Each analysis was carried out at least 3 times with similar results.

Figure 7. Activation of c-kit and the c-kit signaling pathway in MCs

Cells were starved of SCF for 3 hours and then were stimulated for 10 minutes with SCF (100 ng/mL) or medium alone. SDS-PAGE immunoblotting comparing phosphorylated c-kit and total c-kit (A). Phosphorylation of Akt, MEK, p42/44 ERK and CREB in the same experiment (B). The data in a second experiment were similar.