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Mastocytosis associated with a rare germline *KIT* K509I mutation displays a well-differentiated mast cell phenotype

Eunice Ching Chan, PhD^{a,*}, Yun Bai, MSc^{a,*}, Arnold. S. Kirshenbaum, MD^a, Elizabeth R. Fischer, MA^b, Olga Simakova, PhD^c, Geethani Bandara, PhD^a, Linda M. Scott, CRNP^a, Laura B. Wisch, MSN^a, Daly Cantave, MSN^a, Melody C. Carter, MD^a, John C. Lewis, MD^d, Pierre Noel, MD^e, Irina Maric, MD^c, Alasdair M. Gilfillan, PhD^a, Dean D. Metcalfe, MD^a, and Todd M. Wilson, DO^a

^aMast Cell Biology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

^bResearch Technologies Section, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT

^cDepartment of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, MD

^dDivision of Allergy, Mayo Clinic Arizona, Scottsdale, Arizona

^eDivision of Hematology/Oncology, Mayo Clinic Arizona, Scottsdale, Arizona

Abstract

Background—Mastocytosis associated with germline *KIT* activating mutations is exceedingly rare. We report the unique clinicopathologic features of a patient with systemic mastocytosis caused by a *de novo* germline *KIT* K509I mutation.

Objectives—To investigate the impact of the germline *KIT* K509I mutation on human mast cell development and function.

Methods—Primary human mast cells derived from CD34⁺ peripheral blood progenitors were examined for growth, development, survival and IgE-mediated activation. In addition, a mast cell transduction system which stably expressed the *KIT* K509I mutation was established.

Results—*KIT* K509I biopsied mast cells were round, CD25(–) and well differentiated. *KIT* K509I progenitors, cultured in SCF, demonstrated a ten-fold expansion compared to progenitors from healthy subjects and developed into mature, hypergranular mast cells with enhanced antigen-mediated degranulation. *KIT* K509I progenitors cultured in the absence of SCF survived, however

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Correspondence: Todd M. Wilson, D.O., 6701 Democracy Blvd., Rm. 914 National Institutes of Health, Bethesda, MD 20892-1881, twilson@mail.nih.gov, Tel: (301) 435-0768, FAX: (301) 480-3661.

*These authors contributed equally to this work.

Conflict of Interest

The authors declare no conflict of interest.

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lacked expansion and developed into hypogranular mast cells. A *KIT* K509I mast cell transduction system revealed the SCF-independent survival to be reliant on the preferential splicing of *KIT* at the adjacent exonic junction.

Conclusion—Germline *KIT* mutations associated with mastocytosis drive a well-differentiated mast cell phenotype, distinct to that of somatic *KIT* D816V disease, whose oncogenic potential may be influenced by SCF and selective *KIT* splicing.

Clinical Implications—Mastocytosis associated with reported germline *KIT* activating mutations, in this case *KIT* K509I, display a mature, well-differentiated mast cell phenotype distinct to that of somatic *KIT* D816V disease.

Keywords

KIT; K509I; mastocytosis; germline; mast cells; well-differentiated

INTRODUCTION

Systemic mastocytosis is a myeloproliferative neoplasm characterized by the pathologic expansion and infiltration of mast cells within tissues.(1–3) Affecting mainly adults, the onset is sporadic and often associated with acquired gain-of-function mutations in the receptor tyrosine kinase, *KIT*. *KIT* signaling through its ligand, stem cell factor (SCF), influences mast cell proliferation, activation and differentiation. The most common somatic mutation, *KIT* D816V, is located in the second intracellular tyrosine kinase domain, induces SCF-independent activation and is observed in greater than 90% of adult patients with systemic mastocytosis.(4)

Rarely, mastocytosis may be associated with germline *KIT* mutations, as underscored by seven reports in the literature.(5–11) The inheritance pattern is generally autosomal dominant and a consequence of non-synonymous point mutations involving either the extracellular, transmembrane or juxtamembrane regions of *KIT*. These mutations are thought to enhance *KIT* dimerization and/or impair kinase regulation; while generally maintaining sensitivity to the tyrosine kinase inhibitor, imatinib. An exception is the recent report of a family with cutaneous mastocytosis accompanied by a germline *KIT* N822I mutation.(10) This mutation is located within the kinase domain and was found to be resistant to imatinib. A germline *KIT* D816V mutation to date has not been reported.

Cell culture systems to effectively study the primary mast cells of patients with mastocytosis are lacking; mainly due to the limited recovery of neoplastic mast cells from tissues and a lack of significant clonal expansion *ex vivo*. Therefore, studies to understand the effects of *KIT* activating mutations *in vitro* have relied primarily on mast cell lines or transduction experiments, often in non-mast cell lineages. Although much has been learned utilizing these alternative approaches, the capacity to expand and study primary mast cells from patients with mastocytosis is favored.

In this study, we report the unique clinicopathologic features of well-differentiated systemic mastocytosis (WDSM) driven by a *de novo* germline *KIT* K509I mutation. WDSM is a rare variant of systemic mastocytosis characterized by compact aggregates of mature, round, fully

granulated mast cells in the bone marrow; lacking the *KIT* D816V mutation, as well as the aberrant expression of CD25/CD2 markers.(12–15) The germline nature of this presentation permitted the growth of *KIT* K509I CD34⁺ derived human mast cells (HuMCs) from the patient. The HuMCs displayed a mature phenotype with enhanced proliferation, granulation and activation. Moreover, SCF-independent growth and development was determined to be dependent on the preferential splicing of *KIT*. We propose that the activating potential of germline *KIT* mutations may retain significant ligand and molecular regulation, thus resulting in a well-differentiated HuMC phenotype.

MATERIALS

Patient

The patient is a white female who, at the age of 6 weeks, was reportedly diagnosed with cutaneous mastocytosis after developing “blisters” on her skin. Throughout childhood, she reported sporadic flushing, pruritus and urticaria (Figure 1A). In addition, she reported recurrent episodes of abdominal discomfort requiring hospitalization on three occasions. By the age of 19, her gastrointestinal symptoms regressed and skin symptoms stabilized to the point of requiring no antihistamines. At 22 years old, she developed significant morning stiffness and arthralgia involving her hands, shoulders and knees; she was subsequently diagnosed with seronegative rheumatoid arthritis.

At the age of 24, the patient’s mastocytosis-related symptoms flared after moving to Arizona. Symptoms included diarrhea, abdominal pain, musculoskeletal pains, flushing and headaches. Her skin displayed a diffuse pattern of involvement, erythrodermic in nature, accompanied by scattered nodules (subcutaneous benign lipoma) and significant pruritus. This diffuse cutaneous presentation is in contrast to the urticaria pigmentosa classically observed in adult onset *KIT* D816V systemic mastocytosis (Figure 1B). A bone marrow exam revealed 90% cellularity (almost entirely mast cells) and the aspirate was 75% mast cells with round nuclei and variable granularity. The bone marrow mast cells displayed no evidence of “spindling” or CD25 expression (Figure 1C). A serum total tryptase level was 189 ng/ml. A diagnosis of indolent systemic mastocytosis was established according WHO criteria(2, 3) and the disease was further defined as WDSM. Sanger sequencing of *KIT* was performed after initial *KIT* D816V mutation testing was negative. A heterozygous *KIT* K509I mutation was identified in the cDNA of the bone marrow mononuclear cells (Figure 1D), as well as the gDNA of peripheral blood mononuclear cells, buccal mucosa and hair samples (Supplemental Figure 1). This mutation was not detected in either parent and suggests the *KIT* K509I mutation was a *de novo* germline event.

The patient’s significant symptoms, bone marrow mast cell involvement, and known sensitivity of the *KIT* K509I mutation to imatinib(9) prompted a trial of imatinib 300 mg daily. Although an encouraging decrease in her serum tryptase was noted (57 ng/ml), she temporarily discontinued imatinib secondary to an exacerbation of her headaches. Imatinib was restarted at 100 mg daily and upon increasing to 200 mg daily, her headaches and skin erythema worsened (Figure 2A) requiring imatinib to be held. To control the reactions, prednisone 40 mg daily was started one week prior to the re-initiation of imatinib 100 mg every other day. Prednisone was tapered weekly by 10 mg increments until reaching a stable

regimen of prednisone 10 mg daily and imatinib 100 mg every other day. At this time, mast cells involved approximately 25% of the bone marrow, 10% of the aspirate and her serum total tryptase level was 37.2 ng/ml. Despite these measures, imatinib was ultimately discontinued by the patient due to intolerance and a desire to possibly conceive.

The patient received symptoms based treatment under guarded observation for approximately 3 years. Following an initial increase, her tryptase level plateaued at approximately 100 ng/ml (Figure 2B). However, her bone marrow mast cell involvement progressively increased to 50%, accompanied by increased daily flushing, pruritus and severe bone pain, limiting her daily activity. Aspirin, Gastrocrom and UVA/UVB phototherapy were added to her antihistamine regimen with minimal relief. At 28 years old, imatinib was restarted at 50 mg daily and was gradually increased over 4 months. She ultimately achieved 100 mg daily which resulted in a normal tryptase level (Figure 2B); clearance of the bone marrow mast cells (Figure 2C), and a modest reduction in symptoms.

All patients and healthy donors provided informed consent on NIH IRB-approved protocols. (NCT00044122, NCT00050193 and NCT00001756)

Mutational analysis

Total RNA, cDNA and gDNA was prepared as described.(16) Overlapping *KIT* PCR amplification products were purified and directly sequenced by Macrogen USA, Rockville MD. Sequencing data was analyzed using Sequencher (Version 4.5, Softgenetics). Detection of the *KIT* D816V mutation was assessed by PCR/RFLP as described.(17)

CD34⁺ derived human mast cell (HuMC) cultures

Leukapheresis and enrichment of peripheral CD34⁺ cells was performed as described (18) with the exception that a mobilizing agent (G-CSF) was not administered to the patient. The percent of enriched CD34⁺ cells obtained by leukapheresis was determined using a FITC-conjugated anti-CD34⁺ antibody (BD Biosciences, San Jose, CA) on a FACSCalibur (BD Biosciences) with CellQuest 3.3 software (BD Biosciences). HuMCs were cultured in StemPro complete media including L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 mg/ml), IL-3 (30 ng/ml, first week only) and IL-6 (100 ng/ml) in the presence or absence of SCF (100 ng/ml) (Pepro Tech, Rocky Hill, NJ) using an equal starting number of CD34⁺ progenitor cells. (19) For all HuMC studies, different healthy donors were used as controls for each set of experiments. HMC 1.1, HMC1.2 and LAD2 mast cell lines were cultured as described. (20, 21)

Light and electron microscopy (EM)

Cytospin preparations followed by toluidine blue staining were performed using standard protocols.(18) For EM, HuMCs grown on 13 mm thermanox coverslips (Nunc, Naperville, IL) were fixed overnight at 4° C with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7. Samples were post-fixed 30 min with 0.5% osmium tetroxide/0.8% potassium ferricyanide in 0.1 M sodium cacodylate, 1 h with 1% tannic acid and overnight with 1% uranyl acetate at 4° C. Samples were dehydrated with a graded ethanol series, and embedded in Spurr's resin. Thin sections were cut with a Leica EM UC6 ultramicrotome (Leica,

Vienna, Austria), and stained with 1% uranyl acetate and Reynold's lead citrate prior to viewing at 120 kV on a Tecnai BT Spirit transmission electron microscope (FEI, Hillsboro, OR). Digital images were acquired with a Hamamatsu XR-100 side mount digital camera system (Advanced Microscopy Techniques, Danvers, MA) and processed using Adobe Photoshop (Adobe Systems Inc., San Jose, CA).

Multiparameter flow cytometry

Bone marrow mast cells and HuMCs were analyzed as described(17) using the following antibodies: CD45-PerCP, CD2-PE, CD25-FITC, CD117-APC, CD69-FITC, CD63-FITC, CD203-PE (BD Biosciences) and/or FcεRI-PE (eBioscience, San Diego, CA) with a FACSCanto II flow cytometer (BD Biosciences).

MTT and apoptosis assays

HuMCs were plated at 2×10^5 cells/ml (MTT assay) or 1×10^5 cells/ml (apoptosis assay) with different concentrations of SCF for 72 h. The MTT assay (Cell Growth Determination Kit, Sigma, St. Louis, MO) was performed at the final 3 h of incubation according to the manufacturer's instructions. Apoptosis was evaluated using the cellular Annexin V-FITC Apoptosis Kit (Biovision, Mountain View, CA) according to the manufacturer's instructions.

HuMC antigen-mediated activation assays

HuMCs were sensitized overnight with biotinylated-human IgE (100 ng/ml) in the absence of SCF and subsequently stimulated with various concentrations of streptavidin (referred to as Ag), in the presence or absence of SCF (100 ng/ml) at 37°C for 30 min.(22) β-hexosaminidase (β-hex) release was determined as described.(23) Prostaglandin D2 (PGD2) release was determined using the PGD2-MOX ELISA kit (Cayman Chemical Company, Ann Arbor, MI) according to the manufacturer's instructions. Ca^{2+} flux was measured in sensitized and activated Fura 2-AM (2 μM) (Molecular Probes)-loaded HuMCs as described. (24)

IC2 mast cell transduction system

The IL-3 dependent immature murine mast cell line, IC2, expresses FcεRI, however lacks surface KIT expression and exhibits minimal granule formation.(25) *KIT* WT and D816V associated with GNNK +/- variants were cloned into the pMX-puro retroviral expression vector (Cell BioLabs, San Diego, CA) as described.(26) *KIT* K509I was subsequently generated using the QuickChange II XL site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. Transduction of IC2 cells (including empty vector control) was performed as described.(26) For metabolism experiments, transduced IC2 cells were washed free of IL-3 and cultured in the presence or absence of SCF (100 ng/ml) for one week. 2×10^5 cells/ml were subsequently incubated in a 96 well-plate for 24 h and the MTT assay performed as above. To assess proliferation, the transduced IC2 cells maintained in SCF were subsequently seeded at 2×10^5 cells/ml in a 96 well plate in the presence or absence of SCF. Viable cells were counted on day 3 using the Cellometer Auto T4 cell counter (Nexcelom Bioscience, Lawrence, MA).

Immunoblotting

For KIT activation experiments, HuMCs sensitized in the absence of SCF overnight were stimulated with SCF (10 ng/ml) at 37°C for 2 min. Cell lysates were prepared and loaded on to 4 – 12% NuPAGE Bis-Tris gels (Invitrogen) for electrophoretic separation and immunoblotting as described.(27) Anti-human c-Kit mAb (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-human phospho-c-Kit (Tyr703) mAb (Cell signaling technology, Danvers, MA), anti-human CD226 mAb (Santa Cruz Biotechnology) and anti-β-actin mAb (Sigma) were used for immunoblotting. Immunoreactive proteins were visualized with enhanced chemiluminescence ECL (Perkin Elmer Life Sciences, San Jose, CA).

Statistical analysis

Data are presented as the mean ± standard error of mean (SEM). Comparison between two groups was analyzed by unpaired Student t test. P-values <0.05 were considered statistically significant. Analysis was performed using PRISM software, version 5 (GraphPad Software, San Diego, CA). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0005$, **** $p < 0.0001$

RESULTS

***KIT* K509I CD34⁺ progenitors display enhanced proliferation and develop into hypergranular HuMCs**

To investigate the impact of the *KIT* K509I mutation on primary mast cell development, CD34⁺ progenitors were cultured *ex vivo*. At 8 weeks, the *KIT* K509I progenitors cultured in SCF demonstrated a ten-fold expansion compared to progenitors from healthy donors ($p < 0.05$; Figure 3A). *KIT* K509I HuMC expansion continued for up to 20 weeks, exceeding normal survival (10–12 weeks). Preservation of the *KIT* K509I mutation at 8 weeks was confirmed by Sanger sequencing (Supplemental Figure 1). The morphology of the *KIT* K509I HuMCs was examined by light and electron microscopy (EM). Similar to our bone marrow observations, the *KIT* K509I HuMCs grown in SCF appeared round and mature as indicated by the well-condensed nuclei, with an apparent increase in granulation by EM (Figure 3B). This hypergranular phenotype was supported by an increase in side scatter when the cells were analyzed by flow cytometry (Figure 3C).

SCF-depletion impacts *KIT* K509I HuMC proliferation, development and survival

As HuMCs are dependent on SCF for survival, proliferation, activation and differentiation, we next investigated the oncogenic potential of the *KIT* K509I HuMCs in the absence of SCF. After 3 days of SCF depletion, a significant decline in the survival of both the control HuMCs ($p < 0.0001$) and *KIT* K509I HuMC's ($p < 0.0001$) was observed by MTT assay (Figure 4A). However, the *KIT* K509I HuMCs were less sensitive to SCF depletion and demonstrated a survival advantage over the control HuMCs (85% vs. 26% survival, $p < 0.0001$). An apoptosis assay, performed in parallel, revealed that SCF withdrawal resulted in minimal apoptosis in the *KIT* K509I HuMCs ($p = 0.055$) and a significant induction of apoptosis ($p < 0.05$) in the control HuMCs (Figure 4B). This observation is further illustrated when the degree of apoptosis in the *KIT* K509I HuMCs is directly compared to controls (15% vs. 67% apoptosis, $p < 0.01$). Consistent with these observations, the *KIT*

K509I HuMCs displayed minimal autophosphorylation of KIT in the absence of SCF (Figure 4C).

With the observation that the *KIT* K509I HuMCs retained a marginal survival dependence on SCF, we sought to determine if the *KIT* K509I mutation may support SCF-independent HuMC growth and development. *KIT* K509I and control CD34⁺ progenitors were cultured in the absence of SCF for 8 weeks. Only the *KIT* K509I CD34⁺ progenitors survived and developed into HuMCs, however these cells displayed minimal expansion by week 8 (Figure 4D). HuMCs accounted for nearly 100% of the culture and appeared mature, although hypogranular (Figure 4E).

***KIT* K509I transduced IC2 cells are dependent on the *KIT* GNNK⁻ isoform for SCF independent survival**

As a result of alternative mRNA splicing, two major isoforms of KIT are co-expressed in mast cells, characterized by the presence or absence of four amino acids (GNNK) in the juxta-membrane region of the extracellular domain. (26, 28–30) The GNNK⁻ variant is the predominant transcript; as was observed in the patient's bone marrow mononuclear cells (Figure 1D). The *KIT* K509I mutation is located adjacent to this GNNK splice region; therefore we hypothesized that the activating potential of the mutation may be influenced by GNNK splicing. We expressed the *KIT* K509I GNNK⁻ or GNNK⁺ isoforms in the IL-3 dependent immature mast cell line, IC2, to assess the potential impact on SCF independent growth and survival. As reported, in the presence of SCF, WT KIT metabolism (Figure 5A) and growth (Figure 5C) was enhanced when associated with the GNNK⁻ variant as compared to the GNNK⁺ variant ($p < 0.0005$). However, this difference was negated by the presence of either *KIT* K509I or *KIT* D816V (Figures 5A, 5C). With SCF absent, WT KIT transduced IC2 cells succumbed, while *KIT* D816V supported ligand independent metabolism (Figure 5B) and growth (Figure 5D). SCF independent survival of *KIT* K509I transduced IC2 cells was only observed when associated with the GNNK⁻ isoform (Figure 5B, 5D) and displayed a far weaker oncogenic potential as compared to the *KIT* D816V mutation. Of note, the GNNK⁻ variant appears to influence *KIT* D816V ligand independent metabolism (Figure 5B), but not cell proliferation (Figure 5D) as reported.(26)

***KIT* K509I HuMCs display enhanced antigen-mediated degranulation, PGD2 release and intracellular calcium flux**

Enhanced HuMC degranulation via KIT activation is well recognized.(24, 31) In the presence (Figure 6A) or absence of SCF (Figure 6B), the *KIT* K509I HuMCs displayed enhanced FcεR1 mediated degranulation with increasing antigen concentrations as compared to controls. No increase in baseline degranulation was observed in the *KIT* K509I HuMCs in the absence of antigen, irrespective of the presence of SCF. In contrast, SCF alone produced a baseline PGD2 release in both the *KIT* K509I and control HuMCs (Figure 6C); a reported effect.(32) However, in the absence of SCF, the *KIT* K509I HuMCs displayed enhanced PGD2 release with increasing antigen concentrations (Figure 6D). Antigen-mediated intracellular calcium flux was also examined in the presence (Figure 6E) or absence of SCF (Figure 6F), with an enhanced signal observed in the *KIT* K509I HuMCs.

***KIT* K509I HuMCs exhibit increased expression of FcεRI and CD226**

With enhanced antigen-mediated activation confirmed in the *KIT* K509I HuMCs, we sought to identify potential contributing features. FACS analysis revealed no difference in CD117, CD25, CD2, CD203c, CD63 or CD69 expression. However, surface expression of the IgE receptor, FcεRI, was markedly increased (Figure 6E). To identify alterations in gene regulation, a limited gene expression array was performed with the *KIT* K509I HuMCs (not shown). As compared to a control, the gene with the most profound increase in expression (10 fold) was the immunomodulating transmembrane glycoprotein, CD226. CD226 immunoblotting with *KIT* K509I HuMC lysates confirmed this preliminary finding and minimal CD226 expression was observed in the control HuMCs (Figure 6F). We expanded this analysis and observed moderate CD226 expression in HMC1.1 cells (*KIT* D816V negative) and a lack of CD226 expression in HMC1.2 cells (*KIT* D816V positive). LAD2 cells (*KIT* D816V negative) were found to overexpress CD226, in excess of the positive control, Jurkat cells.

DISCUSSION

In this study, we demonstrate that a germline juxtamembrane *KIT* K509I activating mutation enhances proliferation and development of mature, round, hypergranular HuMCs which lack the CD2/CD25 activation markers characteristically observed in *KIT* D816V driven disease. This well-differentiated HuMC phenotype(12, 14, 15) appears common to mastocytosis associated with germline *KIT* mutations as summarized in Table 1. Interestingly, this shared morphology does not appear entirely dependent on the location of the activating mutation within *KIT*. Although the mutations tend to cluster in the *KIT* juxta and transmembrane regions, mutations in the extracellular and tyrosine kinase domains are also reported and suggest other factors may be involved in the tolerance of these oncogenic events.

We have herein demonstrated that *KIT* K509I HuMCs retain a modest dependency on SCF for growth, development and survival. Perhaps germline *KIT* mutations share a common reliance on SCF and as a result, their oncogenic potential is limited and compatible with a mature HuMC phenotype and long term survival. Consistent with this hypothesis, the *KIT* K509I HuMCs displayed negligible ligand independent autophosphorylation of *KIT* and an enhanced response to SCF, as evidenced by increased expansion and activation. It is plausible this hyper-responsiveness coupled with the potential for autocrine SCF production by the *KIT* K509I HuMCs(33) may account for the survival and protection from apoptosis observed in the “SCF-free” culture conditions. Future studies with the *KIT* K509I HuMCs, as well as other germline *KIT* mutations, may define the critical signaling pathways contributing to the enhanced responsiveness to SCF and their association with a well-differentiated phenotype.

Alternative splicing has a recognized role in oncogenesis(34, 35) and the proximity of the K509I mutation to the *KIT* GNNK splicing region has prompted interest. A normal GNNK⁻ predominance in the patient’s bone marrow mononuclear cells was in agreement with Zhang et al (9), who observed that the K509I mutation did not alter the GNNK splicing profile in a family with mastocytosis. Nonetheless, selective expression of the GNNK isoforms had a significant impact on the *KIT* K509I oncogenic potential, with SCF-independent survival

only achieved when the *KIT* K509I mutation was associated with the GNNK⁻ isoform in the transduced IC2 mast cells. In contrast, the GNNK isoforms have relatively little influence on the robust oncogenic potential of the *KIT* D816V mutation.(26, 36) Our observation suggests that the survival, growth and development of the *KIT* K509I HuMCs in absence of SCF were supported solely by the GNNK⁻ isoform. It is provocative to consider that HuMCs *in vivo* could regulate the activating potential of the *KIT* K509I mutation through selective GNNK splicing; however no evidence presently exists to support this conclusion.

In our attempt to identify additional factors contributing to the enhanced degranulation of the *KIT* K509I HuMCs, we observed increased expression of FcεRI and CD226. Aggregation of surface FcεRI, the high-affinity IgE receptor, is central to antigen mediated HuMC degranulation and it is plausible that upregulation of FcεRI may enhance this activation. CD226 is a transmembrane glycoprotein which mediates cell adhesion, cytokine secretion and cytotoxicity.(37) Moreover, co-engagement of CD226 with FcεRI on mast cells synergistically augments degranulation.(38) Interestingly, CD226 appeared overexpressed in neoplastic mast cell lines lacking the *KIT* D816V mutation, most profoundly the LAD2 cell line.(23) Taken together, these observations suggest the existence of interplay between CD226, FcεRI and KIT signaling which enhances mast cell activation.

To our knowledge, this study is the first to evaluate IgE-mediated degranulation and activation of primary mast cells from a patient with mastocytosis. The *KIT* K509I HuMCs displayed heightened antigen-dependent activation. This data supports the clinical observation that IgE mediated systemic reactions (anaphylaxis) are more prevalent in adult patients with systemic mastocytosis.(39, 40) Indeed, enhanced degranulation was observed at lower antigen concentrations in the *KIT* K509I HuMCs (most notably in the presence of SCF), however IgE sensitization alone (no antigen) was insufficient to induce basal degranulation in our assays.

In summary, our study contributes to a larger body of evidence suggesting that germline *KIT* mutations associated with mastocytosis drive a well differentiated mast cell phenotype, markedly different to that of somatic *KIT* D816V driven disease, whose activating potential may be influenced by SCF and selective *KIT* splicing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

HuMC	CD34 ⁺ derived human mast cell
SCF	stem cell factor

EM	electron microscopy
WDSM	well-differentiated systemic mastocytosis
PGD2	prostaglandin D2
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
IRB	Institutional Review Board

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FIG. 1.

Clinicopathological features of germline *KIT* K509I well-differentiated systemic mastocytosis. **A**, Cutaneous presentation as an infant. **B**, Diffuse cutaneous presentation as an adult with comparison to typical *KIT* D816V urticarial pigmentosa. **C**, KIT and CD25 staining of bone marrow mast cells with comparison to typical *KIT* D816V morphology. **D**, Diagram of KIT and location of the heterozygous K509I mutation in relationship to GNNK splice site.

FIG. 2. Clinical reaction and response to imatinib. **A**, Erythematous rash exaggerated by the initiation of imatinib. **B**, Serum total tryptase levels upon discontinuation and reinitiation of imatinib. **C**, Tryptase staining of bone marrow biopsy demonstrating mast cell involvement pre and post imatinib.

FIG. 3.

KIT K509I CD34⁺ progenitors display enhanced proliferation and develop into hypergranular HuMCs in the presence of SCF. **A**, Growth of K509I HuMCs in the presence of SCF. Data are the mean $\pm$ SEM of 3 independent experiments performed in duplicate. **B**, Light microscopy with toluidine blue staining (20x magnification) and electron microscopy. **C**, Side scatter (SSC) histogram. Representative of 3 experiments.

FIG. 4. SCF-depletion impacts *KIT* K509I HuMC proliferation, development and survival. *KIT* K509I HuMC survival (**A**) and apoptosis (**B**) with various SCF concentrations. Data are the mean $\pm$ SEM of 3 independent experiments performed in triplicate. **C**, Immunoblot of KIT phosphorylation pre and post SCF stimulation. Representative of 3 experiments. **D**, SCF independent *KIT* K509I HuMC growth. Data are the mean $\pm$ SEM of 3 independent experiments performed in duplicate. **E**, Light microscopy with toluidine blue staining (20x magnification).

FIG. 5.

Activating potential of *KIT* K509I is dependent on the GNNK⁻ isoform. MTT assay of transduced IC2 cells cultured in the (A) presence or (B) absence of SCF. Data are the mean $\pm$ SEM of 3 independent experiments performed in triplicate. Growth and survival of transduced IC2 cells cultured in the (C) presence or (D) absence of SCF. Data are the mean $\pm$ SEM of 2 independent experiments performed in duplicate. Control: empty vector.

FIG. 6.

KIT K509I HuMCs display enhanced antigen-mediated activation. β -hexosaminidase release (**A** and **B**), PGD₂ release (**C** and **D**) and intracellular Ca²⁺ flux (**E** and **F**) in the presence or absence of SCF. Data are the mean $\pm$ SEM of 3 independent experiments performed in triplicate. Ca²⁺ flux represents an experiment performed in triplicate. **G**, Fc ϵ R1 surface expression by flow cytometry. Representative of 3 experiments. **H**, CD226 immunoblotting of whole cell lysates. Representative of 2 experiments.

Table 1

Mastocytosis associated with germline *KIT* mutations

Reference	KIT mutation	Exon	Location	Genotype	Inheritance pattern	Mast cell morphology	CD25 expression	Cutaneous involvement	Systemic progression	Imatinib sensitive
Beghini, A. <i>Cancer</i> . 2001;92(3):657–662	A559V	10	JMI	Het	AD	Round, mature	*	Urticaria Pigmentosa	*	*
Tang, X. <i>J Med Genet</i> . 2004;41e88	A533D	10	TM	Het	AD	Round, mature	*	Diffuse Cutaneous	yes	*
Akin C. <i>Blood</i> . 2004;103(8):3222–3225	F522C	10	TM	Het	De novo	Round, mature, hypergranular	Negative	Urticaria Pigmentosa	yes	yes
Hartmann K. <i>Gastroenterology</i> . 2005;129:1042–1046	Del 419 Asp	8	EX	Het	AD	Round, mature	*	Diffuse Cutaneous	yes	*
Zhang, LY. <i>Leukemia. Research</i> . 2006;30:373–378	K509I	9	JME	Het	AD	Round, granular	*	Diffuse Cutaneous	yes	yes
Wasag B. <i>Exp Hematology</i> . 2011;39:859–865	N822I	17	TK	Het	AD	Round, mature	Negative	Urticaria Pigmentosa	no	no
Speight SA. <i>J of Clinical Oncology</i> . 2013; 31:16:e245–e247	K509I	9	JME	Het	De novo	Round, mature	*	Diffuse Cutaneous	yes	no

* Not reported,

Personal communication, JMI= juxtamembrane intracellular, TM=transmembrane, EX=extracellular, JME= juxtamembrane extracellular, TK=tyrosine kinase domain, AD=autosomal dominant