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

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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: We sought to investigate the effect 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 that 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 stem cell factor (SCF) demonstrated a 10-fold expansion compared with 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 but lacked expansion and developed into hypogranular mast cells. A KIT K509I mast cell transduction system revealed 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, the oncogenic potential of which might be influenced by SCF and selective *KIT* splicing. (J Allergy Clin Immunol 2014;134:178-87.)

Key words: *KIT, K509I, mastocytosis, germline, mast cells, well differentiated*

Abbreviations used
EM: Electron microscopy
FITC: Fluorescein isothiocyanate
HuMC: CD34$^+$ derived human mast cell
PE: Phycoerythrin
PGD_2: Prostaglandin D_2
SCF: Stem cell factor
WDSM: Well-differentiated systemic mastocytosis
WT: Wild-type

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 7 reports in the literature.[5-11] The inheritance pattern is generally autosomal dominant and a consequence of nonsynonymous point mutations involving either the extracellular, transmembrane, or juxtamembrane regions of KIT. These mutations are thought to enhance KIT dimerization, impair kinase regulation, or both, 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. To date, a germline *KIT* D816V mutation has not been reported.

Cell-culture systems to effectively study the primary mast cells of patients with mastocytosis are lacking, mainly because of 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 by using 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 that lack the *KIT* D816V mutation, as well as the aberrant expression of CD25/CD2 markers.[12-15] The germline nature of

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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 were determined to be dependent on the preferential splicing of *KIT*. We propose that the activating potential of germline *KIT* mutations might retain significant ligand and molecular regulation, thus resulting in a well-differentiated HuMC phenotype.

METHODS

The patient

The patient is a white woman who, at the age of 6 weeks, was reportedly given a diagnosis of cutaneous mastocytosis after having "blisters" on her skin. Throughout childhood, she reported sporadic flushing, pruritus, and urticaria (Fig 1, *A*). In addition, she reported recurrent episodes of abdominal discomfort, requiring hospitalization on 3 occasions. By the age of 19 years, her gastrointestinal symptoms regressed and skin symptoms stabilized to the point of requiring no antihistamines. At 22 years old, she had significant morning stiffness and arthralgia involving her hands, shoulders, and knees; she was subsequently given a diagnosis of seronegative rheumatoid arthritis.

At the age of 24 years, the patient's mastocytosis-related symptoms flared after moving to Arizona. Symptoms included diarrhea, abdominal pain, musculoskeletal pain, flushing, and headaches. Her skin displayed a diffuse pattern of involvement that was erythrodermic in nature and accompanied by scattered nodules (subcutaneous benign lipoma) and significant pruritus. This diffuse cutaneous presentation is in contrast to the urticaria pigmentosa classically observed in patients with adult-onset *KIT* D816V systemic mastocytosis (Fig 1, *B*). A bone marrow examination 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 (Fig 1, *C*). The serum total tryptase level was 189 ng/mL. A diagnosis of indolent systemic mastocytosis was established according to World Health Organization criteria,[2,3] and the disease was further defined as WDSM. Sanger sequencing of *KIT* was performed after initial *KIT* D816V mutation test results were negative. A heterozygous *KIT* K509I mutation was identified in the cDNA of the bone marrow mononuclear cells (Fig 1, *D*), as well as the genomic DNA of PBMCs, buccal mucosa, and hair samples (see Fig E1 in this article's Online Repository at www.jacionline.org). This mutation was not detected in either parent, and this suggests the *KIT* K509I mutation was a *de novo* germline event.

The patient's significant symptoms and bone marrow mast cell involvement and the known sensitivity of the KIT K509I mutation to imatinib[9] prompted a trial of 300 mg/d imatinib. Although an encouraging decrease in her serum tryptase level was noted (57 ng/mL), she temporarily discontinued imatinib after an exacerbation of her headaches. Imatinib was restarted at 100 mg/d, and on increasing to 200 mg/d, her headaches and skin erythema worsened (Fig 2, *A*), requiring imatinib to be held. Prednisone, 40 mg/d, was started 1 week before reinitiation of 100 mg of imatinib every other day to control the reactions. Prednisone was tapered weekly by 10-mg increments until reaching a stable regimen of 10 mg/d prednisone and 100 mg of imatinib every other day. At this time, mast cells involved approximately 25% of the bone marrow and 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 because of intolerance and a desire to possibly conceive.

The patient received symptoms-based treatment under guarded observation for approximately 3 years. After an initial increase, her tryptase level reached a plateau at approximately 100 ng/mL (Fig 2, *B*). 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, cromolyn sodium (Gastrocrom; Celltech Pharmaceuticals, Rochester, NY), and UVA/UVB phototherapy were added to her antihistamine regimen, with minimal relief. At 28 years old, imatinib was restarted at 50 mg/d and gradually increased over 4 months. She ultimately achieved a dosage of 100 mg/d, which resulted in a normal tryptase level (Fig 2, *B*), clearance of the bone marrow mast cells (Fig 2, *C*), and a modest reduction in symptoms.

All patients and healthy donors provided informed consent on National Institutes of Health Institutional Review Board–approved protocols (NCT00044122, NCT00050193 and NCT00001756).

Mutational analysis

Total RNA, cDNA, and genomic DNA was prepared, as previously described.[16] Overlapping *KIT* PCR amplification products were purified and directly sequenced by Macrogen USA (Rockville Md). Sequencing data were analyzed with Sequencher (Version 4.5; SoftGenetics, State College, Pa). Detection of the *KIT* D816V mutation was assessed by using PCR/RFLP, as previously described.[17]

HuMC cultures

Leukapheresis and enrichment of peripheral $CD34^+$ cells was performed, as previously described,[18] with the exception that a mobilizing agent (granulocyte colony-stimulating factor) was not administered to the patient. The percentage of enriched $CD34^+$ cells obtained by means of leukapheresis was determined with a fluorescein isothiocyanate (FITC)–conjugated anti-$CD34^+$ antibody (BD Biosciences, San Jose, Calif) on a FACSCalibur (BD Biosciences) with CellQuest 3.3 software (BD Biosciences). HuMCs were cultured in StemPro complete media including L-glutamine (2 mmol/L), penicillin (100 U/mL), streptomycin (100 mg/mL), IL-3 (30 ng/mL for the first week only), and IL-6 (100 ng/mL) in the presence or absence of SCF (100 ng/mL; PeproTech, Rocky Hill, NJ) by using an equal starting number of $CD34^+$ progenitor cells.[19] For all HuMC studies, different healthy donors were used as control subjects for each set of experiments. HMC 1.1, HMC1.2, and LAD2 mast cell lines were cultured, as previously described.[20,21]

Light and electron microscopy

Cytospin preparations followed by toluidine blue staining were performed by using standard protocols.[18] For electron microscopy (EM), HuMCs grown on 13-mm Thermanox Coverslips (Nunc, Naperville, Ill) were fixed overnight at 4°C with 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7. Samples were postfixed for 30 minutes with 0.5% osmium tetroxide/0.8% potassium ferricyanide in 0.1 mol/L sodium cacodylate, for 1 hour 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 resin. Thin sections were cut with a Leica EM UC6 ultramicrotome (Leica, Vienna, Austria) and stained with 1% uranyl acetate and Reynold lead citrate before viewing at 120 kV on a Tecnai BT Spirit transmission electron microscope (FEI, Hillsboro, Ore). Digital images were acquired with a Hammamatsu XR-100 side mount digital camera system (Advanced Microscopy Techniques, Danvers, Mass) and processed with Adobe Photoshop (Adobe Systems, San Jose, Calif).

Multiparameter flow cytometry

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

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 hours. The MTT assay (Cell Growth Determination Kit; Sigma, St Louis, Mo) was performed at the final 3 hours of incubation, according to the manufacturer's instructions. Apoptosis was evaluated by using the cellular Annexin V–FITC