

Analysis of the lineage relationship between mast cells and basophils using the *c-kit* D816V mutation as a biologic signature

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Background: Mast cells and basophils share similar morphologic and functional properties; however, it is not known whether they are derived from a bilineage (basophil/mast cell)-restricted progenitor.

Objective: To assess whether basophils and mast cells are derived from common committed progenitors using the *c-kit* D816V mutation as a biologic signature.

Methods: The D816V *c-kit* mutation found in mast cells of patients with systemic mastocytosis is used as a trackable genetic marker to assess the lineage relationship between mast cells and basophils. Blood and bone marrow aspirates were collected from 33 consecutive patients with mastocytosis with different disease severity. Peripheral blood basophils, monocytes and neutrophils were sorted by immunomagnetic beads. Presence of the D816V *c-kit* mutation was analyzed by restriction fragment length polymorphism in the genomic DNA and mRNA from sorted cells in all patients and in the genomic DNA of individual basophils of 1 patient.

Results: The *c-kit* D816V mutation was detectable in basophils of 5 patients (15%). All 5 patients had the *c-kit* mutation also detectable in monocytes and thus had multilineage involvement. Single cell analysis of the genomic DNA in 1 patient showed a similar degree of clonal expansion in basophils, monocytes, and neutrophils. Mutated *c-kit* was expressed at the mRNA level in all 5 patients. There was no difference in surface Kit expression levels in basophils.

Conclusion: Basophils carrying the D816V *c-kit* mutation in mastocytosis were detected only in the context of a multilineage involvement. These results argue against the presence of a bilineage-restricted committed progenitor for mast cells and basophils. (J Allergy Clin Immunol 2005;115:1155-61.)

Key words: Mast cells, basophils, *c-kit*, mastocytosis, hematopoiesis

Basophils and mast cells are major effector cells in allergic reactions and share similar morphological and functional characteristics. Basophils and mast cells, as with other hematopoietic lineages, are derived from CD34⁺ progenitor cells.¹ However, although individual growth factor requirements and growth kinetics of basophils and mast cells have been studied extensively,^{2,3} the lineage relationship of basophils with mast cells still remains controversial.⁴

The existence of a multipotential basophil hematopoietic progenitor capable of giving rise to other lineages has been supported by colony assays in which basophils and eosinophils were observed in the same colony-forming unit.^{5,6} Similarly, granulocytes with a hybrid eosinophil/basophil phenotype have been reported in the peripheral blood of patients with chronic granulocytic and myelogenous leukemias.^{7,8} Further, the UT-7 cell line derived from a patient with megakaryoblastic leukemia has the capacity to differentiate into basophils, eosinophils, erythrocytes, and megakaryocytes under the influence of erythropoietin, GM-CSF, and IL-3.⁹ However, *in vitro* studies have largely failed to demonstrate a common committed progenitor for mast cells and basophils. To that end, mast cells and monocytes, but not basophils, were derived *in vitro* from a CD34⁺/Kit⁺/CD13⁺ cell population, whereas a CD34⁺/CD38⁺/HLA DR⁺ progenitor population gave rise only to mast cells.^{10,11} In other studies, Agis et al¹² have reported that mast cells differentiate from circulating CD34⁺/CD117⁺/Ly⁺/CD14⁺/CD17⁺ progenitor cells but not from monocytes and basophils. These studies as well as comparative analyses of the phenotypic properties of mast cells and basophils led to the suggestion that mast cells and basophils are derived from unique hematopoietic progenitors and are not closely related.¹³

On the other hand, a common bilineage precursor for basophils and mast cells has been suggested in other studies. The mAb 97A6 (CD203c) has been demonstrated to define specifically basophils, mast cells, and their progenitor cells.¹⁴ Metachromatic cells with morphologic and immunophenotypic features of both basophils and mast cells were reported in peripheral blood of patients with asthma, atopy, and allergic drug reactions.¹⁵ Although these cells displayed basophilic features such as a multilobulated nucleus, they also were Bsp1⁺ and expressed mast cell proteases as well as Kit.

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Abbreviation used

HMC-1: Human mast cell leukemia cell line

Genetic alterations, either at the chromosomal or molecular level, may be used as trackable genetic markers to investigate lineage relationships of hematopoietic cells *in vivo*. Systemic mastocytosis is a neoplastic disease of the mast cell lineage that is associated with activating mutations in the proto-oncogene *c-kit*.^{16,17} The presence of the D816V *c-kit* point mutation in systemic mastocytosis presents a unique opportunity to use this mutation as a biologic signature to determine the lineage relationship between mast cells and other cells of hematopoietic origin.¹⁸ This approach has been used to demonstrate that mastocytosis is a disorder of the hematopoietic progenitor cell, much like other myeloproliferative disorders such as chronic myeloid leukemia.^{19,20} However, there has been no study to our knowledge examining the presence of the D816V *c-kit* mutation in basophils in this context.

Therefore, to determine whether mast cells and basophils are derived from a common bilineage-restricted progenitor cell in mastocytosis, we analyzed the presence and the frequency of the D816V *c-kit* mutation in basophils, mast cells, and other myeloid lineages. Our results do not support the presence of a bilineage-restricted mast cell/basophil precursor.

METHODS**Patients**

Thirty-three consecutive patients with different variants of systemic mastocytosis evaluated at the National Institutes of Health Clinical Center were studied. Diagnosis of systemic mastocytosis was made according to the World Health Organization criteria.²¹⁻²³ Twenty-seven patients had indolent systemic mastocytosis, 4 had smoldering systemic mastocytosis, and 2 had systemic mastocytosis with an associated clonal hematologic nonmast cell lineage disease. Peripheral blood and bone marrow aspirates were obtained from all patients after informed consent. The study was reviewed and approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases.

Cell isolation from peripheral blood and bone marrow

Peripheral blood was collected in a tube and was diluted in an equal volume of PBS supplemented with 2 mmol/L EDTA and 0.1% FBS. The cell suspension was then layered on histopaque (specific gravity, 1.077; Sigma, St Louis, Mo) and centrifuged for 20 minutes at 400g.²⁴ Mononuclear cells in the middle layer and granulocytes in the bottom layer were collected into separate tubes. After washing once with PBS, the cells were suspended in 1 mL PBS, and red cells were lysed by incubation with NH₄Cl (StemCell Technologies, Vancouver, British Columbia, Canada) for 10 minutes. The cell suspensions were filtered across 30-μm nylon mesh to remove clumps before cell separation.

Basophils were isolated by using negative immunomagnetic selection (Miltenyi Biotec, Auburn, Calif). Cells from some patients were further purified by fluorescence-activated cell sorting (FACStar;

Becton Dickinson, San Jose, Calif) after staining with anti-IgE–fluorescein isothiocyanate (Biosource, Camarillo, Calif) and anti-CD123–phycoerythrin (BD-Pharmingen, San Diego, Calif). Monocytes and neutrophils were isolated by positive selection using ClinMACS CD14 MicroBeads and CD15 MicroBeads, respectively (Miltenyi Biotec). The isolation procedure was repeated twice to achieve higher purity. Cell purity was confirmed by staining cytospin preparations of isolated cells and/or flow-cytometric analysis and was always greater than 96%. Lesional bone marrow mast cells were enriched by immunomagnetic beads coated with antihuman CD25 antibody (Miltenyi Biotec) for 30 minutes at 4°C as described.²⁰ The amount of the available bone marrow aspirate and the recovered CD25⁺ cell fraction was sufficient to perform analysis of CD25⁺ cells in 12 patients.

Mutational analysis of the genomic DNA

Genomic DNA was extracted by using Qiagen DNeasy Tissue Kit (Qiagen Inc, Valencia, Calif). A 174-bp gene fragment of *c-kit* gene was amplified by PCR using the primers 5'-TCCTCCAACCTAAT-AGTGTATTACAG-3' and 5'-TTTGCAGGACTGTCAAGCAG-AGAATG-3'. The PCR was performed in a final volume of 50 μL containing 1 mmol/L Ampliqa Gold DNA polymerase buffer (Applied Biosystems, Foster City, Calif), 3 mmol/L MgCl₂, 0.4 μmol/L of each primer, 200 μmol/L of each deoxynucleotide triphosphate, and 2 U Ampliqa Gold DNA Polymerase by using a PTC-200 programmable thermal cycler (MJ Research, Watertown, Mass).

PCR products were visualized on a 1% agarose gel (Cambrex, Rockland, Me) and digested with the *Hinf*I restriction enzyme (New England Biolabs, Beverly, Mass) for 1 hour at 37°C. Presence of the D816V *c-kit* mutation was then determined by restriction fragment length polymorphism (RFLP) analysis on the basis of the introduction of a new digestion site for the *Hinf*I enzyme.¹⁶ Digestion of the wild-type PCR fragment by the *Hinf*I enzyme results in 113-bp and 61-bp bands, whereas additional 99-bp and 14-bp bands result from further digestion of the 68-bp band in samples containing the mutation. Human mast cell leukemia cell line (HMC-1) HMC-1.2 and HMC-1.1 cells with and without the D816V *c-kit* mutation²⁵ were analyzed simultaneously with each experiment as positive and negative controls, respectively. Fragments were visualized and their intensities were quantified by a gel imaging system (Nucleovision; Nucleotech, San Carlos, Calif). As expected, the mutated band was detectable in the heterozygote state in various proportions. A distinct band corresponding to the mutated sample was clearly visualized when the intensity of the mutated band was at least 1% of the sum of the wild-type and mutated bands. Therefore, a sample was considered to carry the mutation if the intensity of the mutation band was at least 1% or higher.

RT-PCR and mutational analysis of cDNA

The cells were lysed in Trizol (Invitrogen, Carlsbad, Calif), and total RNA was extracted by using the RNeasy Mini Kit (Qiagen). cDNA was then synthesized from total RNA by using Moloney leukemia virus RT without Ribonuclease H activity and oligo (dT) primer (Invitrogen, Carlsbad, Calif). A 373-bp region of the *c-kit* gene was amplified by PCR in a final volume of 50 μL containing 1 mmol/L buffer, 200 μmol/L of each dNTP, 0.4 μmol/L of each primer, and 1 μL Advantage cDNA polymerase mix (BD Bioscience-Clontech, Palo Alto, Calif) using primers 5'-AAAGGAGATCTGTGAGAAT-AGGCTC-3' and 5'-AGCTCCCAAAGAAAAATCCATAGG-3'. PCR products were digested with the restriction enzymes *Hae* III and *Hinf* I (New England Biolabs) and electrophoresed on a 10% tris-borate EDTA polyacrylamide gel (Invitrogen, San Diego, Calif), and the gel was stained with ethidium bromide. Digestion of the wild-type PCR product with these enzymes yields fragments of 171 bp, 127 bp, 43 bp, and 31 bp. An A to T substitution in *c-kit* nucleotide 2468 (within the 171-bp fragment) causing D816V mutation

introduces a new *Hinf*I recognition site resulting in fragments of 157 bp, 127 bp, 43 bp, 31 bp, and 14 bp.

Single-cell PCR and sequencing

Isolated basophils were individually sorted into 96-well PCR plates containing 20 μ L PCR buffer (Promega, Madison, Wis) and proteinase K (0.2 mg/mL; Sigma) by fluorescence-activated cell sorting. Peripheral blood monocytes, neutrophils, and B cells were sorted on the basis of expression of CD14, CD15, and CD19, respectively, and their characteristic side-scatter patterns. Sorted cells were incubated for 1 hour at 55°C and 10 minutes at 95°C. Genomic DNA was preamplified by using random primers and 60 rounds of amplification with Taq polymerase, and the preamplified genomic DNA was subjected to nested PCR by using primers spanning codon 816 as described.²⁰ PCR products were purified with QIAquick PCR purification kit (Qiagen) and directly sequenced by using an ABI Prism 377 automated DNA sequencer (Applied Biosystems) and a Big Dye Terminator DNA sequencing kit (Applied Biosystems).

Flow-cytometric analysis

Expression of Kit (CD117) on basophils was determined by flow cytometry. Basophils were isolated from 12 patients with systemic mastocytosis and 12 controls, including 9 healthy individuals and 3 patients with unexplained flushing or anaphylaxis without mastocytosis. Immunofluorescence staining of purified basophils was performed by incubating 5×10^4 cells for 20 minutes at 4°C with a fluorescein isothiocyanate conjugate of goat antihuman IgE (Biosource), a phycoerythrin conjugate of CD117 (BD Bioscience), and CD123 (BD-Pharmingen) in 100 μ L PBS supplemented with 2 mmol/L EDTA and 0.1% FBS. The labeled cells were washed in PBS containing 0.1% FBS and analyzed on a flow cytometer (FACSCalibur; Becton Dickinson).

RESULTS

Analysis of the *c-kit* D816V mutation in basophils

If mast cells and basophils are derived from the same committed progenitor, one would expect to find the mutation more frequently in basophils than in other myeloid cells. To investigate this possibility, genomic DNA was isolated from peripheral blood basophils as well as monocytes and neutrophils from 33 patients with mastocytosis. In 12 of these patients (Table I; patients 1-12), it was also possible to enrich lesional mast cells from bone marrow aspirates by CD25 immunomagnetic selection, which yields a mixed population of mast cells and a subset of lymphocytes in which, between these 2 populations, only mast cells express Kit protein.^{26,27} Therefore, we had the opportunity to examine these neoplastic mast cells for the presence of the *c-kit* mutation by subjecting this CD25⁺ population to RT-PCR and RFLP analysis. Using this approach, the D816V *c-kit* mutation was detectable in the CD25⁺ bone marrow fraction in all 12 patients analyzed. The same mutation, however, was detectable only in the genomic DNA and mRNA of basophils in 1 of 12 patients so examined (patient 12). This patient had the D816V *c-kit* mutation detectable not only in basophils but also in monocytes and neutrophils (Fig 1, A). None of the remaining patients in this group of 12 carried the mutation in any additional cell

TABLE I. Detection of the D816V *c-kit* mutation in the genomic DNA of basophils, monocytes, neutrophils, and mRNA of CD25⁺ cells (mast cell fraction)

Patient	CD25 ⁺ cells	Basophils	Monocytes	Neutrophils
1	(+)	(-)	(-)	(-)
2	(+)	(-)	(-)	(-)
3	(+)	(-)	(-)	(-)
4	(+)	(-)	(-)	(-)
5	(+)	(-)	(-)	(-)
6	(+)	(-)	(-)	(-)
7	(+)	(-)	(-)	(-)
8	(+)	(-)	(-)	NA
9	(+)	(-)	(-)	(-)
10	(+)	(-)	(-)	(-)
11	(+)	(-)	(-)	(-)
12	(+)	(+)	(+)	(+)
13	NA	(+)	(+)	(+)
14	NA	(+)	(+)	(+)
15	NA	(+)	(+)	(+)
16	NA	(+)	(+)	NA
17	NA	(-)	(+)	NA
18	NA	(-)	(-)	(+)
19	NA	(-)	(-)	(-)
20	NA	(-)	(-)	(-)
21	NA	(-)	(-)	(-)
22	NA	(-)	(-)	(-)
23	NA	(-)	(-)	(-)
24	NA	(-)	(-)	(-)
25	NA	(-)	(-)	(-)
26	NA	(-)	(-)	(-)
27	NA	(-)	(-)	(-)
28	NA	(-)	(-)	(-)
29	NA	(-)	(-)	(-)
30	NA	(-)	(-)	(-)
31	NA	(-)	(-)	(-)
32	NA	(-)	(-)	(-)
33	NA	(-)	(-)	(-)

NA, Not analyzed.

lineage either at the genomic DNA or the mRNA level (see example, Fig 1, B). These results suggest the involvement of a pluripotential early progenitor that is able to give rise not only to mast cells but also to other lineages in the patient who had the mutation detectable in multiple cell lineages (Fig 2, B and D), whereas the remaining patients appeared to have an involvement of a cell that gives rise to mast cells but not basophils (Fig 2, C).

Examination of the mutational analysis results from the remaining 21 patients (patients 13-33), in whom a CD25 enrichment was not possible because of insufficient sample volume and low cell recovery, was also consistent with the lack of a shared committed progenitor between mast cells and basophils (Fig 2, A). Analysis of D816V *c-kit* mutation in this population identified an additional 4 patients (Table I; patients 12-16) who carried the mutation in the genomic DNA of their basophils. All of these patients also carried the mutation in their monocytes and/or neutrophils, also consistent with involvement of an early progenitor.

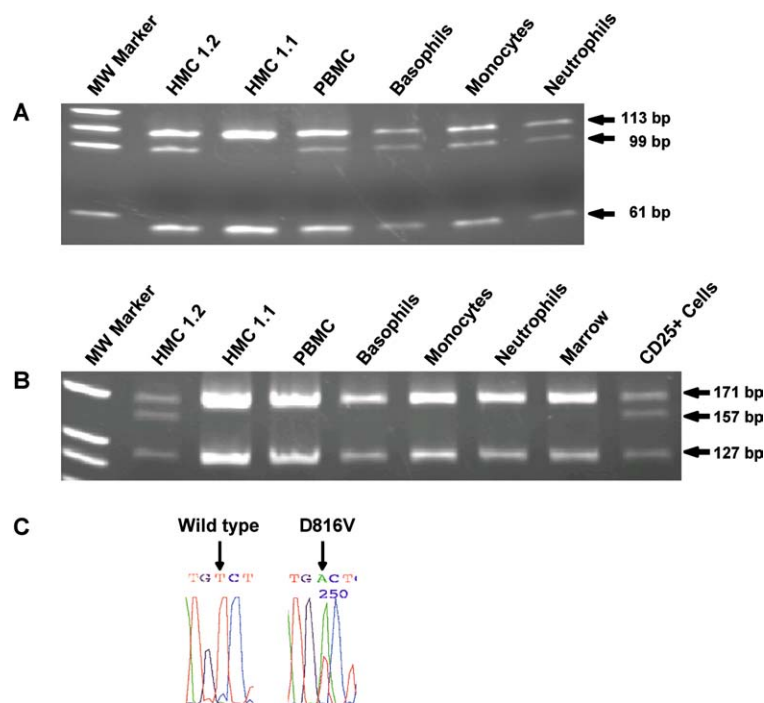


FIG 1. Demonstration of the D816V *c-kit* mutation in the genomic DNA (A, patient 15) and mRNA (B, patient 1) in basophils and other hematopoietic lineages in mastocytosis. The codon 816 A/T *c-kit* mutation is indicated by the presence of a 99-bp band in the genomic DNA and a 157-bp band in the mRNA (cDNA) samples. HMC-1.1 and HMC-1.2 cells were included as negative and positive controls, respectively. C, Demonstration of the D816V *c-kit* mutation in an individual basophil with both wild-type and mutated alleles (right panel) in patient 15 (T to A change in antisense sequence). MW, Molecular weight.

In addition, 1 patient each had the mutation detectable in monocytes (patient 17) and neutrophils (patient 18) but not in basophils. Overall, the D816V *c-kit* mutation was detectable in the genomic DNA of basophils in 5 of 33 (15%) patients. This compared with the presence of the mutation in monocytes in 6 (18%) and neutrophils in 5 of 30 (17%) patients. RT-PCR analysis with a set of primers designed for cDNA showed the presence of the D816V *c-kit* mutation at the mRNA level in basophils of all 5 patients. These results show that the incidence of the D816V mutation in basophil lineage is not higher compared with monocytes and neutrophils, again consistent with involvement of an early progenitor not exclusively shared with the mast cell lineage.

Single-cell mutational analysis of basophils

We next determined whether the extent of clonal expansion of the basophils carrying D816V *c-kit* mutation is comparable with that of the other lineages. If basophils and mast cells shared a common committed progenitor (Fig 2, B), that underwent neoplastic transformation, basophils would be expected perhaps to exhibit a relatively larger degree of clonal expansion in comparison with other cell lineages in a patient with mast cell proliferative disease. To address this question, we examined the *c-kit* mutation in the genomic DNA of individual basophils of a patient with smoldering systemic mastocytosis and

multilineage involvement and compared the frequency of the mutation in basophils with that of monocytes, neutrophils, and B cells. Single cell analysis using a 2-step nested PCR amplification of the *c-kit* genomic DNA revealed the presence of the mutation in 121 out of 137 individual basophils (88%; Fig 1, C). This compared with 119 of 136 (88%) monocytes, 93 of 105 (89%) neutrophils, and 122 of 166 (73%) individual B cells with the mutation. Thus, the extent of clonal expansion of basophils derived from the hematopoietic progenitor carrying the D816V *c-kit* mutation was similar to that of monocytes and neutrophils and was slightly higher than that of B cells. This outcome was not consistent with a shared committed progenitor (Fig 2, B).

Analysis of surface Kit expression

Cell surface expression of Kit in basophils has been reported to be increased in patients with allergic diseases compared with healthy individuals, and basophils displaying higher surface Kit levels have been proposed to share a common progenitor with mast cells.¹⁵ Thus, we next examined whether there is any difference in surface Kit expression of basophils in subjects with or without mastocytosis. Flow-cytometric analysis for CD117 (Kit) was performed in basophils obtained from 12 patients with different categories of mastocytosis, 3 patients with diagnoses other than mastocytosis, and 9 healthy individ-

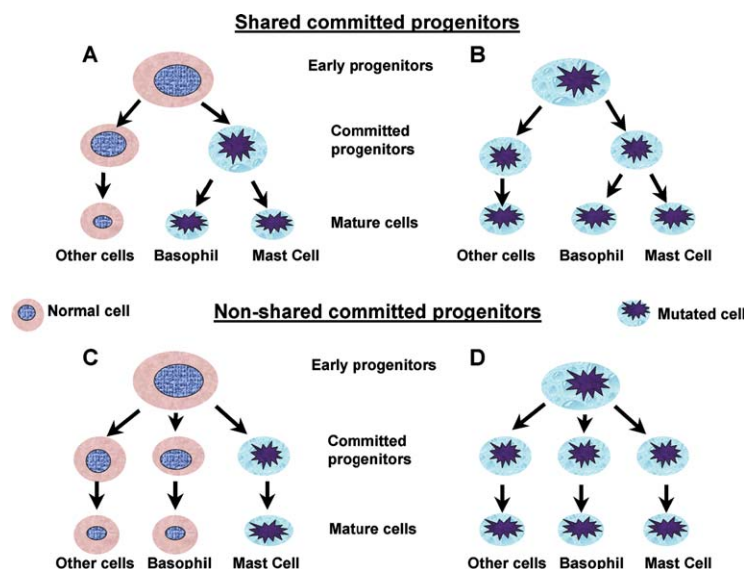


FIG 2. Possibilities of lineage involvement patterns according to hypotheses of shared and nonshared committed progenitors between mast cells, basophils, and other lineages in systemic mastocytosis. Depending on whether the initial mutation occurs in an early (multipotential; **B** and **D**) or a late (committed; **A** and **C**) progenitor, the mutation is observed in different patterns in individual patients. The mutation would be detected only in mast cells and basophils if a dual committed progenitor is affected (**A**).

uals. Basophils from all subjects were found to express low levels surface Kit, and no significant difference was found between groups (Fig 3).

DISCUSSION

Systemic mastocytosis is a clonal neoplastic disorder in adults usually associated with the gain of function *c-kit* D816V mutation whose pathologic hallmark is the presence of excessive numbers of mast cells in tissues.^{17,28} Detection of the D816V *c-kit* mutation presents a unique opportunity to examine lineage relationships of the hematopoietic cells derived from the same affected progenitor. Previous studies using this approach have reported the presence of the mutation variably in monocytes, neutrophils, eosinophils, erythrocytes, and B cells^{19,20,29-31} and provided evidence that systemic mastocytosis is a disorder of a hematopoietic progenitor cell in patients with an extensive disease variant termed *smoldering systemic mastocytosis*.^{18,21,32-34} No studies to date, however, have examined the presence of the *c-kit* mutation in basophils.

Using the D816V *c-kit* mutation as a marker to track lineage progeny derived from a progenitor cell is similar in concept to studies that examined the fate of hematopoietic cells artificially marked with retroviral vectors, which insert their genetic material randomly into the genome of the cells.^{35,36} Other examples of this approach in human disease are chronic myeloid leukemia³⁷ and a variant of hypereosinophilic syndrome³⁸ in which hematopoietic cell lineages were examined for the presence of *bcr-abl* and *FIP1L1-PDGFR* fusion genes, respectively. If

introduction of the mutation to the progenitor cells occurs randomly, the mutation may affect early and late mast cell progenitors. Fig 2 depicts possible outcomes for each level of such involvement. In the event that an early progenitor cell with the capacity to give rise to multiple cell lineages is affected, the mutation would be detectable not only in mast cells but also in other cell lineages derived from that progenitor (Fig 2, *B* and *D*). This was the case in 5 patients described in this report with multilineage involvement (Table I; Fig 1). On the other hand, if the mutation affects a later and more lineage-committed progenitor of the mast cells, then it would be detectable only in mast cells (Fig 2, *C*) or mast cells and their sister lineage derived from the same committed progenitor, such as the basophil (Fig 2, *A*). The lack of detection of the *c-kit* mutation in basophils in the absence of multilineage involvement argues against the presence of a dual mast cell–basophil progenitor (Fig 2, *A*) with restricted lineage commitment to these 2 cell types and for a pluripotential early progenitor in such cases (Fig 2, *D*). These results are in agreement with the *in vitro* colony assays in which incubation of human mononuclear cells with stem cell factor either alone or in combination with other cytokines yielded either pure mast cell colonies or mast cells mixed with multiple myeloid cells including blasts, promyelocytes, neutrophils, eosinophils, and basophils.^{11,12}

The results of the single cell analysis in 1 patient in this study revealed that presence of D816V mutation caused a similar extent of clonal expansion in basophils compared with other myeloid lineages, consistent with an early pluripotential progenitor (Fig 2, *D*), but less consistent with a shared committed progenitor (Fig 2, *B*). Whether the presence of the mutated *c-kit* gene in basophils results

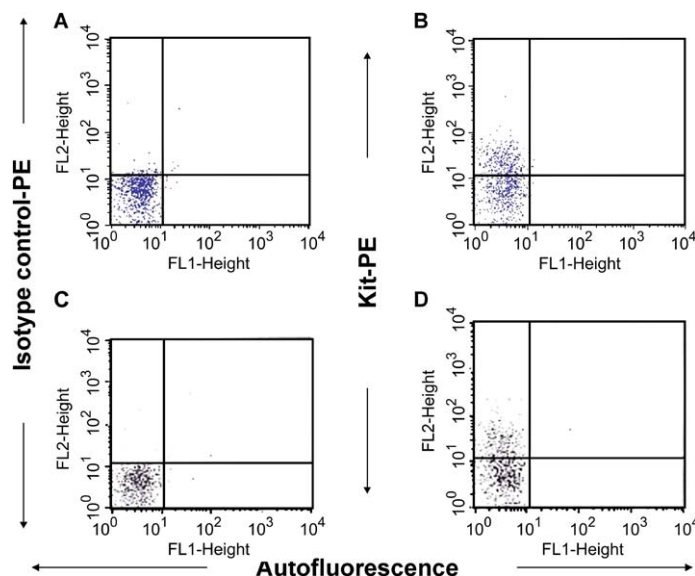


FIG 3. Flow-cytometric analysis of the Kit expression on the surface of basophils. Similar amounts of dim surface Kit expression were observed on basophils from patients with mastocytosis ($n = 12$; **A** and **B**) and subjects without mastocytosis (**C** and **D**). The latter population included healthy volunteers ($n = 9$) and patients with unexplained flushing or anaphylaxis but not mastocytosis ($n = 3$). **A** and **C**, Isotype controls; **B** and **D**, cells stained with CD117-phycoerythrin (PE).

in any functional alterations of basophils remains to be elucidated. The detection of the mRNA with the *c-kit* mutation as well as surface expression of Kit suggests that the mutated gene is transcribed and the message possibly translated in basophils. However, there was no difference in the amount of surface Kit expression between patients with mastocytosis and healthy controls (Fig 3). In addition, microscopic examination of Wright-Giemsa-stained basophil preparations from patients with mastocytosis and healthy subjects did not reveal any notable differences in basophil morphology.

The results of this study also provide clues to the lineage relationship between mast cells and monocytes. An earlier *in vitro* study suggested that human mast cells can be derived from cultures of mononuclear phagocytes³⁹; however, this finding has not been confirmed in other studies.¹² Another study showed that a CD34⁺/CD117⁺/CD13⁺ cell population could preferentially give rise to mast cells and monocytes when cultured in stem cell factor alone. A comparative analysis of the expression of 130 CD markers on the surface of basophils, monocytes, and mast cells showed that mast cells shared more surface markers with monocytes than basophils.¹³ This latter study suggests that conclusions on lineage relationships should not be based on single cell surface markers. Similar to the findings in basophils, clonal monocytes carrying the mutation were detected only in the presence of multilineage clonal involvement. These results support the hypothesis that mast cells are derived from a unique hematopoietic progenitor and not from basophils, monocytes, or bilineage-restricted progenitors.^{11,12}

In summary, the analysis of cell lineages for the D816V mutation allows 2 general conclusions. First, in some

patients, the mutation was detected only in mast cells. This observation is consistent with the conclusion that in some patients, the mutation occurs in a self-renewing population of cells that give rise only to mast cells (Fig 2, C). Second, when the mutation was found in basophils, it was also present in other lineages, consistent with the conclusion that the mutation occurred in an early progenitor giving rise to multiple cell lineages (Fig 2, B and D). Because there was no evidence for preferential expansion of basophils in such a situation, the possibility of a precursor committed to the basophil and mast cell lineage with the mutation taking place in an early progenitor (Fig 2, B) is unlikely. In no instance were patients identified in whom only mast cells and basophils harbored the D816V mutation, arguing strongly against a committed bipotential cell that could give rise to mast cells and basophils (Fig 2, A).

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