

Brief report

Demonstration of an aberrant mast-cell population with clonal markers in a subset of patients with “idiopathic” anaphylaxis

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Idiopathic anaphylaxis remains a perplexing disorder in which existing prophylactic therapy is inadequate. In this prospective study, we sought to determine whether patients with idiopathic anaphylaxis might have evidence for a clonal disorder of mast cells related to mastocytosis and for which novel targeted therapies might be considered. We report 12 patients with “idiopathic” anaphylaxis who did not exhibit either urticaria pigmentosa or the characteristic bone marrow

biopsy finding of multifocal mast-cell aggregates observed in systemic mastocytosis. Of these 12 patients, 5 had evidence of 1 or more minor criteria for mastocytosis. *C-KIT* mutational analysis was positive for the 816D>V activating mutation in 3 of 3 patients in CD25⁺ bone marrow cells where the analysis was performed. These results demonstrate the presence of an aberrant mast-cell population carrying clonal markers in a subset of patients diagnosed with “idiopathic”

anaphylaxis, who may respond to inhibitors targeting mutated *C-KIT*. This intramural clinical trial was conducted in 2003 and 2004 and was registered at <http://clinicalcenter.nih.gov> with a study number 03-I-0010. Since the study is now closed, it is no longer available online. (Blood. 2007;110:2331-2333)

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Introduction

The cause of anaphylaxis remains unexplained in up to two-thirds of patients. The result is that the diagnosis of idiopathic anaphylaxis is assigned to the patient.¹ Because idiopathic hypotensive episodes are a feature of mastocytosis,²⁻⁵ a clonal proliferative disorder of the mast-cell lineage, we asked the question of whether idiopathic anaphylaxis might in some cases be associated with a similar clonal disorder of mast cells.

We thus determined clonal markers of mast-cell disease such as immunophenotypic or morphologic abnormalities and the 816D>V (D816V) *c-kit* mutation associated with systemic mastocytosis in this study, which enrolled patients with idiopathic anaphylaxis as well as those with a confirmed or probable diagnosis of mastocytosis. As will be shown, a group of patients with recurrent anaphylaxis without urticaria pigmentosa (UP) and multifocal bone marrow mast-cell aggregates exhibit an aberrant mast-cell population carrying the *c-kit* D816V mutation.

sodes accompanied by the presence of one or more symptoms suggestive of systemic mast cell degranulation, such as flushing, tachycardia, abdominal pain, and diarrhea. All patients underwent a medical evaluation including a bone marrow biopsy and aspirate to investigate presence of the World Health Organization (WHO) diagnostic criteria for mastocytosis.⁶

Mast cells in bone marrow biopsy sections were identified by immunohistochemical staining for tryptase.⁷ Mast cells in bone marrow aspirates were identified by flow cytometry as a CD117^{high}, IgE⁺ population as described.^{8,9} Mutational analysis of the *C-KIT* gene was performed by reverse transcription–polymerase chain reaction (RT-PCR) and restriction fragment–length polymorphism analysis of the PCR products in bone marrow aspirate mononuclear cells as described.¹⁰ In some patients, bone marrow mast cells were enriched based on their surface CD25 expression using anti-human CD25 paramagnetic beads according to the manufacturer’s instructions (Miltenyi Biotec, Auburn, CA).¹¹

Associations between variables were evaluated by Spearman correlation coefficient, and group differences by Wilcoxon rank-sum test.

Patients and methods

A total of 72 consecutive patients referred to the National Institutes of Health (NIH) Clinical Center in a 17-month period were enrolled in this prospective study after signing an informed consent in accordance with the Declaration of Helsinki. The study was approved by the National Institute of Allergy and Infectious Diseases (NIAID) intramural Institutional Review Board (IRB). The study population consisted of patients referred with the diagnosis of idiopathic anaphylaxis or confirmed or probable mastocytosis. Anaphylaxis was defined by self-limited syncopal or near-syncopal epi-

Results and discussion

We sought to examine the prevalence of clonal mast-cell disease in patients with the diagnosis of recurrent idiopathic anaphylaxis, and to determine laboratory or clinical parameters that would help to identify this patient group. We enrolled 72 consecutive patients (44 women and 28 men with a median age of 46 years [range, 22-69 years]) referred to the NIH Clinical Center with a diagnosis of confirmed or suspected systemic mastocytosis or idiopathic anaphylaxis (Table 1). Of those 72 patients, 36 (18 women, 18 men) had

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confirmed cutaneous or systemic mastocytosis and did not suffer from recurrent hypotensive episodes. Mutational analysis of the *C-KIT* gene was carried out in 22 patients in this group and was found to be positive for the 816D>V mutation in 18 (82%).

A total of 24 patients (18 women, 6 men) had a history of recurrent anaphylactic episodes. Among patients with anaphylaxis, 8 patients had the diffuse maculopapular hyperpigmented rash of UP, the characteristic skin finding of mastocytosis. Among 16 patients with recurrent anaphylactic episodes who did not have UP, 4 had characteristic multifocal mast-cell infiltrates in their bone marrow biopsy diagnostic of systemic mastocytosis. Thus, a total of 12 (50%) of 24 patients with recurrent syncopal episodes had evidence of cutaneous mastocytosis and/or multifocal bone marrow mast-cell aggregates, the histopathologic hallmark and the major diagnostic criterion of systemic mastocytosis. The 816D>V *C-KIT* mutation was detectable in bone marrow of 7 (88%) of 8 patients analyzed in this group, similar to the patients with mastocytosis without anaphylaxis. The frequency of detection of *c-kit* mutation in this study is consistent with a recent report that showed the mutation in 93% of patients,¹⁴ and suggests that 816D>V mutation alone is not a predictor of anaphylactic episodes in patients with mastocytosis.

The remaining 12 of the 24 patients with recurrent anaphylaxis lacked both UP skin lesions and multifocal mast-cell aggregates in the bone marrow biopsy. On further analysis, 5 (42%) of these 12 patients (3 women, 2 men; 95% confidence interval, 0.15-0.72) had evidence of 1 or more minor criteria for mastocytosis (Table 2). All 5 patients had aberrant expression of CD25 on the surface of mast cells. In addition, inspection of the tryptase-stained bone marrow biopsy specimen or a Wright-Giemsa-stained aspirate smear showed evidence of morphologically aberrant mast cells, such as those with elongated shapes and hypogranulation, in all 5 patients. A total of 2 patients had serum tryptase levels greater than 20 ng/mL (Table 2). The 816D>V *C-KIT* mutation was detectable in bone marrow mononuclear cells enriched for CD25⁺ cells containing the aberrant mast-cell population in 3 of 3 patients examined. Of note, examination of unsorted bone marrow mono-

Table 1. Frequency of the 816D>V *c-kit* mutation in patients with recurrent anaphylaxis in comparison to two control groups

Patient group	n	<i>c-kit</i> mutation, no. (%) [*]	CD25 ⁺ , no. (%)
Mastocytosis†	48	25/30 (83)	39/45 (87)
Without anaphylaxis	36	18/22 (82)	29/34 (85)
With anaphylaxis	12	7/8 (88)	10/11 (91)
"Idiopathic" anaphylaxis‡	12	3/6 (50)	5/12 (42)
With clonal mast cells§	5	3/4 (75)	5/5 (100)
True idiopathic	7	0/2 (0)	0/7 (0)
Nonmastocytosis, nonanaphylaxis	12	0/5	0/10

Data on mast-cell CD25 expression, another marker of clonal mast-cell disease, are provided for comparison.

^{*}816D>V except in 2 patients with mastocytosis (with or without anaphylaxis) who had either a 816D>Y or a 522F>C mutation. Clinicopathologic features of the patient with 522F>C mutation has been reported in detail.¹² All mutational analyses were performed on bone marrow aspirates. Nucleic acid isolation from paraffin-embedded bone marrow biopsy tissue (fixed in B-plus) of 6 patients with anaphylaxis was attempted,¹³ but was not successful.

†Patients readily diagnosed with mastocytosis due to UP skin lesions and/or multifocal mast-cell clusters in the bone marrow.

‡Patients with neither urticaria pigmentosa nor mast-cell clusters in bone marrow, who were referred for evaluation of multiple recurrent anaphylactic episodes. Further study of bone marrow mast cells for minor diagnostic criteria in this subgroup revealed 5 patients with various clonal mast-cell markers as reported in detail in Table 2. The remaining 7 patients in this subgroup had no clonal mast-cell markers, thus representing the true idiopathic population.

§See Table 2.

Table 2. Pathologic findings in patients with anaphylaxis with a clonal mast-cell disorder

Patient	Sex	Age at study, y	Tryptase, ng/mL	CD25 ⁺ MC	816D>V <i>c-kit</i> mutation [*]	Aberrant MC morphology
1	F	46	7.92	+	+	+
2	F	44	21.5	+	+	+
3	F	31	128	+	+	+
4	M	45	12.7	+	-	+
5	M	42	8.11	+	ND	+

None of the patients had cutaneous mastocytosis or mast-cell clusters fulfilling the major WHO diagnostic criterion for systemic mastocytosis. MC indicates mast cells; M, male; F, female; +, present; -, not present; and ND, not determined.

^{*}Mutational analysis was carried out in CD25⁺ sorted cells in patients 1 through 3, and unsorted bone marrow mononuclear cells in patient 4.

nuclear cells in these 3 patients yielded a positive result in only 1 patient, suggesting that CD25 enrichment increased the sensitivity of the detection of the *C-KIT* mutation. CD25 subpopulation analysis for the *C-KIT* codon 816 mutation could not be carried out in the remaining 2 patients in whom only 2 diagnostic criteria were demonstrated due to sample limitations. These results show that an aberrant mast-cell population carrying clonal mast-cell markers is detectable in a subgroup of patients with idiopathic anaphylaxis. Some anaphylactic attacks followed yellowjacket hymenoptera stings in 1 patient (patient 5; Table 2). The findings in this patient were consistent with a recent report of subdiagnostic accumulation of mast cells in patients with recurrent anaphylaxis after hymenoptera stings.¹⁵ No stimuli that consistently brought on hypotensive episodes in other patients were identified.

A total of 7 patients with anaphylaxis (6 women, 1 man) had neither UP nor an aberrant bone marrow mast-cell population, and were diagnosed as having idiopathic anaphylaxis. An additional group of 12 patients of the 72 patients entered into this study had no syncopal or presyncopal events required to include them into the group with a history of recurrent anaphylactic episodes; however, they did have symptoms such as recurrent flushing, tachycardia, urticaria, or angioedema, which led their referral physicians to consider the diagnosis of mastocytosis. These patients had no diagnostic evidence of mastocytosis or of a clonal mast-cell disorder.

Serum IgE levels inversely correlated with serum tryptase¹⁶ in the full cohort ($r = -0.46$; $P < .001$), and patients with mastocytosis had significantly lower total serum IgE than those without mastocytosis (medians, 10 vs 84 IU/mL; $P < .001$). Patients with anaphylaxis as a group tended to have higher total IgE levels regardless of the etiology when compared with those without anaphylaxis, although this did not reach statistical significance (medians, 22.5 vs 12 IU/mL; $P = .08$). This may be pertinent to the pathogenesis of anaphylaxis, as IgE, even in its monomeric form, can potentiate mast-cell activation,¹⁷⁻¹⁹ and presence of a mutated *c-kit* molecule may further augment this process through convergent downstream signal-transduction pathways.^{20,21}

In conclusion, an aberrant bone marrow mast-cell population carrying the clonal markers found in mastocytosis was detected in a high percentage of patients with recurrent anaphylaxis in this study. These findings provide a basis for the etiology of anaphylactic episodes in a significant subset of patients with "idiopathic" anaphylaxis.

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Authorship

Contribution: All authors contributed significantly to the manuscript; C.A. designed the study and performed the research, collected data, and wrote the paper; L.M.S. collected data and performed research; C.N.K. performed research; N.K.S. collected and analyzed data; E.B. analyzed data; P.N.

performed research; and D.D.M. performed research, provided funding for the study, critically reviewed the data, and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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