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Mastocytosis: Immunophenotypical Features of the Transformed Mast Cells Are Unique Among Hematopoietic Cells

Hans-Peter Horny, MD^{a,*}, Karl Sotlar, MD^a, and Peter Valent, MD^b

^aInstitute of Pathology, Ludwig-Maximilians-Universität, Thalkirchnerstrasse 36, D-80337 Munich, Germany

^bDivision of Hematology and Hemostaseology, Department of Internal Medicine, Medical University of Vienna, Währinger Gürtel 18 – 20, A-1090 Vienna, Austria

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Introduction

Mastocytosis is regarded as a disease of bone marrow origin and is histologically characterized by compact tissue infiltrates of atypical mast cells that are never seen in reactive states.¹ Most patients with mastocytosis, in particular those with systemic disease, have transformed mast cells carrying an activating point mutation at codon 816 of *KIT*, usually *KITD816V*, and also show an elevated serum tryptase level.² The disease shows a broad spectrum of clinical features, including the typical skin lesion of urticaria pigmentosa, which are not found in all patients. Mastocytosis is listed among myeloproliferative neoplasms according to the World Health Organization classification.³ In this article the immunophenotypical features of mast cells are described. Based on these features, it is obvious that mast cells are not closely related to other myeloid cells. Using the knowledge on aberrantly expressed antigens by mast cells, the hematopathologist should be able to recognize the disease even in the presence of unusual morphologic findings or an associated hematologic non-mast cell lineage disease.

Classification and Diagnosis of Systemic Mastocytosis

Mastocytosis basically presents in two major variants: pure cutaneous disease usually occurring in children; and systemic mastocytosis (SM) showing infiltration of at least one extracutaneous organ, commonly the bone marrow, occurring in adults. Diagnosis of SM is based on the presence of one major and one or more out of four minor criteria. The central role of the hematopathologist is reflected by the fact that the only major diagnostic SM criterion is the demonstration of compact mast cell infiltrates in various extracutaneous

*Corresponding author. Europäisches Referenzzentrum für Mastozytose (ECNM), Institut für Pathologie, LMU, Thalkirchnerstrasse 36, München 80337, Germany. Hans-Peter.Horny@med.uni-muenchen.de.

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tissues. Moreover, minor criteria include two that can also be assessed by the hematopathologist: demonstration of prominent spindling of mast cells, and an aberrant immunophenotype of mast cells with expression of the lymphocyte-associated antigens CD2 and/or CD25. Because compact mast cell infiltrates can be absent in a significant proportion of patients with suspected SM, it is necessary to demonstrate at least three minor criteria to be able to establish a final diagnosis of SM.

Immunohistochemistry

Immunohistochemical analysis is strongly recommended in all patients with suspected SM for several reasons.⁴ First, detection and quantification of loosely scattered mast cells that often are difficult to count in conventional stains, such as Giemsa or toluidine blue, because of significant decrease or even absence of metachromatic granules, in particular in spindle-shaped or fibroblast-like mast cells. Second, detection of even very small compact mast cell infiltrates to demonstrate the major diagnostic SM criterion. Finally, detection of an atypical immunophenotype of mast cells, in particular expression of CD25, which can be found in more than 90% of cases of SM, in particular in indolent SM.

The following antibodies should be applied in every case of suspected mastocytosis, in particular when bone marrow trephine specimens are investigated: tryptase, CD117 (*KIT*), and CD25.⁵ Expression of CD2 is listed also as a minor diagnostic criterion but has proved to be of minor relevance for two reasons. First, CD2 has a significantly lower sensitivity compared with CD25; is not expressed in most cases with advanced disease, such as aggressive SM or mast cell leukemia; and is expressed only in about 50% to 60% of cases of indolent SM. Second, it is of minor relevance because of the constant presence of CD2-positive T cells within compact mast cell infiltrates, with the possibility of false-positive results.

All normal/reactive and neoplastic mast cells coexpress the antigens tryptase and CD117 (*KIT*), with a few exceptions regarding tryptase.⁶ Tryptase expression may be very low or even missing in about 1% to 5% of patients with mastocytosis resulting in an incomplete “CD117-only” immunophenotype of mast cells and the possibility of an erroneous diagnosis of a non-mast cell tumor with expression of CD117 (Fig. 1). A cell not expressing CD117 is not a mast cell.

The aberrant expression of various other hematopoietic antigens by the transformed mast cells in SM is of minor importance for the correct diagnosis but may easily lead to misdiagnoses of hematologic tumors other than mastocytosis.⁷ The following antigens may be expressed, although in varying frequency and intensity:

1. Myelomonocytic antigens: CD14, CD33, CD61, CD63, CD68, CD203c, 2D7, myeloperoxidase, naphthol AS-D chloroacetate esterase
2. Lymphoid antigens: CD2, CD9, CD25, CD26, CD30 (Fig. 2), CD52, CD79a
3. Stem cell-associated antigens: CD44
4. Natural killer cell-associated antigen: CD57 (Fig. 2)

5. Dendritic cell-associated antigens: CD35, CD123
6. Other antigens: CD99, SDF-1 (Fig. 3)

Regarding these above-mentioned aberrantly expressed antigens, the following differential diagnoses have to be considered:

1. CD14, CD68: Monocytic leukemias, histiocytoses
2. CD63, CD203c, 2D7: Basophilic leukemias
3. CD33, MPO, CAE: Myeloid neoplasms
4. CD2: T-cell neoplasms
5. CD9, CD25, CD37, CD52, CD79a: B-cell lymphomas (hairy cell leukemia)
6. CD30: Hodgkin lymphoma, ALK+ anaplastic large cell lymphoma, embryonal carcinoma
7. CD57: Natural killer cell neoplasms
8. CD35, CD123: Dendritic cell tumors
9. CD99: Ewing sarcoma, acute lymphoblastic leukemia

It has to be emphasized that immunophenotypical features exhibited by transformed mast cells may easily lead to wrong diagnoses. The cytomorphologic aspects of mast cells including features like broad pale nongranulated cytoplasm and bilobated or monocytoid nuclei primarily may raise suspicion of a monocytic or histiocytic tumor, a hairy cell leukemia, or even a clear cell carcinoma. The expression of such markers as CD14, CD68, or CD25 (in cases of suspected hairy cell leukemia) confirms the suspicion and the underlying SM is not detected. Moreover, the presence of metachromatic granules is not unique to mast cells but also is seen in basophilic granulocytes. Because the secondary metachromatic granules of basophils are water-soluble these cells cannot be seen in routinely processed tissue. The aberrant expression of basophil-associated antigens, such as 2D7, CD63, and CD203c, therefore is not of major significance in diagnostic hematopathology, in particular regarding that basophilic leukemias are extremely rare.

CD30⁺ SM

Expression of the activation antigen CD30 by transformed mast cells is intriguing and one of the major pitfalls in this field.⁸ CD30 is commonly known as a highly specific marker for Hodgkin lymphoma but is also seen in other hematopoietic and nonhematopoietic neoplasms, such as anaplastic large cell lymphoma, lymphomatoid papulosis, and embryonal carcinoma. The recent description of CD30 expression in a considerable number of mastocytoses enables one to assess or exclude this disease by application of diagnostic relevant markers, such as tryptase or CD117. In advanced SM including aggressive SM and mast cell leukemia the expression of CD30 is seen more often, in more mast cells, and in stronger intensity than in the more common SM subvariants with no or only low-grade malignant potential. In the following a prototypical case is briefly reported.

A 32-year-old man suffered from a Hodgkin lymphoma (mixed type) diagnosed in an axillary lymph node. Staging investigation of the bone marrow revealed disseminated nodular infiltrates consisting of lymphocytes, eosinophils, and large atypical cells expressing CD30 (see Fig. 2C, D). Thus, a diagnosis of generalized lymphoma (stage IV) was established. A slightly elevated serum tryptase led to reinvestigation of the bone marrow histology. The CD30-expressing cells reacted with antibodies against CD25, CD117, and tryptase, and a point mutation at codon 816 of *KIT* was also detected. This point mutation, however, was not found in the lymph node and morphologic investigation did not reveal nodal infiltrates of SM. Accordingly, the final diagnoses had to read as follows: localized Hodgkin lymphoma in an axillary lymph node, and isolated bone marrow mastocytosis with *KIT*D816T (which is a novel hitherto not described point mutation of *KIT*).

Some diagnostic relevant antigens in hematopathology have been found to be never expressed by mast cells and therefore can be used as cell markers in microdissection experiments, for example in cases of associated SM and non-mast cell hematologic neoplasm (SM-AHNMD) to determine whether or not the *KIT*D816V mutation is restricted to the mast cell compartment or has spread to other lineages. These antigens are the following: TdT, glycophorin A/C, CD3, CD7, CD10, CD15, CD20, CD34, CD38, and CD56.

Summary

Mastocytosis comprises an extremely heterogeneous group of diseases ranging from the absolutely benign solitary mastocytoma of the skin to the life-threatening mast cell leukemia. All variants of mastocytosis have been assigned to the group of myeloproliferative neoplasms in the World Health Organization classification system of tumors of the hematopoietic tissues. In neoplastic states, mast cells show an unusually broad spectrum of immunophenotypical aberrancies. The most important mast cell-related antigens used for diagnosis in histologic sections are tryptase and CD117 (*KIT*). In normal and reactive states all mast cells coexpress tryptase and CD117, but tryptase expression may be strongly decreased or even lost in rare cases of mastocytosis, thus leading to an incomplete “CD117-only” immunophenotype. Cells not expressing CD117 are not mast cells. CD25 is not expressed by normal/reactive but by transformed mast cells and has been found to be the most reliable immunohistochemical marker for diagnosis of mastocytosis. Moreover, mast cells may express a great variety of myeloid (eg, MPO, 2D7, CD203c, naphthol AS-D chloroacetate esterase), monohistiocytic (eg, CD14, CD68), stem cell-related (eg, CD44), and lymphoid (eg, CD2, CD30, CD52, CD79a) antigens in varying frequencies and intensities. Such immunophenotypical features of transformed mast cells may lead to severe diagnostic problems for the hematopathologist. This high degree of immunophenotypical atypia is not seen in myeloid cells even in neoplastic states and therefore strongly argues against the assumption that mastocytosis is a true myeloproliferative neoplasm.

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Key Points

- Mastocytosis is a hematologic disorder of bone marrow origin. The disease exhibits an unusual broad heterogeneity regarding clinical, morphologic, and immunologic features.
- Mast cells in reactive and neoplastic states almost always coexpress the antigens CD117 (*KIT*) and tryptase, irrespective of the stage of maturation or activation.
- Aberrant immunophenotypical features of mast cells are very common in mastocytosis, but only expression of the antigens CD2, CD25, and CD30 is used in the routine diagnostic work-up of the disease.
- The aberrant expression of various myelomonocytic, lymphoid, natural killer, and stem cell associated antigens by mast cells is unique among hematopoietic cells and not seen in other myelogenous neoplasms.
- Mastocytosis is not a myeloproliferative disorder as stated in the World Health Organization classification of tumors of hematopoietic origin but deserves a separate disease category.

Fig. 1. Systemic mastocytosis (intestinal type).

(A) Giemsa staining shows the typical picture of eosinophilic colitis with an abundance of mature eosinophils diffusely infiltrating the lamina propria mucosae (Giemsa, original magnification $\times 40$). (B) Immunostaining, however, reveals a compact band-like infiltrate consisting of medium-sized CD117-expressing round mast cells beneath the surface epithelium, which is intact (anti-CD117; ABC method, original magnification $\times 40$). (C) Most CD117-expressing cells, in particular those forming the band-like infiltrate, do not react with an antibody against tryptase. Note the presence of loosely scattered partly spindle-shaped tryptase-expressing cells, which are mast cells probably not belonging to the neoplastic clone (antitryptase/AA1; ABC method, original magnification $\times 20$). (D) The mast cell nature of the CD117-expressing cells is further underlined by the coexpression of CD25 (and the demonstration of the activating point mutation *KITD816V*) (anti-CD25; ABC method, original magnification $\times 40$). Diagnosis in this unusual case could read as follows: systemic (intestinal) mastocytosis associated with (obscured by) eosinophilic colitis, or systemic (intestinal) mastocytosis with marked eosinophilia mimicking eosinophilic colitis.

Fig. 2. Systemic mastocytosis involving the bone marrow.

(A) Indolent systemic mastocytosis exhibiting a typical compact infiltrate consisting of densely packed spindle-shaped mast cells with strong expression of tryptase (antitryptase/AA1; ABC method, original magnification $\times 20$). (B) The same case shows mast cells with an aberrant immunophenotype and coexpression of the natural killer/T cell-associated antigen CD57 (anti-CD57; ABC method, original magnification $\times 20$). (C) Isolated bone marrow mastocytosis exhibiting a compact infiltrate of mixed cellularity consisting of eosinophils, lymphocytes, and medium-to-large size atypical mast cells with strong aberrant expression of CD30. These histologic findings initially were interpreted as involvement of the bone marrow by Hodgkin disease in a patient with known Hodgkin disease of an axillary lymph node (anti-CD30; ABC method, original magnification $\times 40$). (D) The same case shows some loosely scattered CD30-expressing mast cells within the bone marrow stroma (anti-CD30; ABC method, original magnification $\times 40$). Hodgkin cells never occur as single cells but invariably show the typical cellular microenvironment with attraction of eosinophils, lymphocytes, and other “inflammatory” cells. Based on this picture alone a diagnosis of systemic mastocytosis could be established by an experienced hematopathologist.

Fig. 3. Systemic mastocytosis involving the bone marrow.

(*A, B*) Indolent systemic mastocytosis with multifocal bone marrow involvement. The mast cells in the compact and the diffuse infiltrate exhibit an aberrant immunophenotype with expression of two antigens: CD26 and SDF-1. Note the CD26/SDF-1 expression can also be seen on endothelial cells but not on hematopoietic cells other than mast cells. (*C, D*) Mast cell leukemia with diffuse-compact infiltration of the bone marrow by small-to-medium sized mast cells containing large numbers of metachromatic granules (*C*) but also expressing SDF-1. Normal blood cell precursors and fat cells are virtually absent (*A*, anti-CD26, ABC method, original magnification $\times 20$; *B* and *D*, anti-SDF-1, ABC method, original magnification $\times 20$; *C*, Giemsa, original magnification $\times 20$).