Aggressive systemic mastocytosis with sarcoma-like growth in the skeleton, leukemic progression, and partial loss of mast cell differentiation antigens

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Introduction

Systemic mastocytosis (SM) is a clonal disease of mast cells (MC) and their progenitors.[1] The hallmark of the disease is the multifocal accumulation of MC in one or more extracutaneous organs. The clinical course and prognosis in SM vary among patients.[1,2] Based on WHO criteria, 4 variants of SM have been defined: indolent systemic mastocytosis (ISM), SM with an associated clonal hematologic non-MC-lineage disease, aggressive SM (ASM), and mast cell leukemia (MCL).[1] ASM is a high grade MC disease in which MC infiltration is associated with clinically relevant impairment of organ function.[1,2] The most commonly affected organ systems are the bone marrow, liver, and the skeletal system.[2]

The histology of the bone marrow in ASM shows a variable degree of infiltration with atypical (immature) MC.[1-4] In most cases, MC are immature, often with bi- or multi-lobed nuclei or a blast-like morphology.[2-5] Sometimes, such immature MC can be confused with monocytic cells.[5] Therefore, it is of great importance to apply immunohistochemical markers to identify and analyze MC in such cases.

The key marker recommended for immunohistochemical detection and enumeration of MC in SM is tryptase.[1-4] In fact, tryptase is expressed at all stages of MC development and all subsets of MC.[1-6] Moreover, tryptase has been described to be expressed in neoplastic MC in all variants of SM including ASM.[1-4] The second major serine protease of MC, chymase, is only expressed in a subset of normal tissue MC,[6] whereas in most patients with advanced MC neoplasms, MC are chymase-negative cells.[7] However, neoplastic MC also express KIT and several other leukocyte antigens such as CD44, CD63, or histidine decarboxylase.[4,7-10] In immature myeloid neoplasms, the application of such markers, especially KIT, is often required to differentiate MC from basophils or tryptase-positive blasts. Another diagnostic marker is CD25.[1,2,8,10] This antigen is specifically expressed in neoplastic MC in SM, but not in normal/reactive MC.[8,10]

Partial or complete loss of a differentiation antigen in neoplastic cells is a rare but well recognized phenomenon, and may obscure the diagnosis. We report on a case with ASM with extensive sarcoma-like growth of MC in the skeleton, in which MC showed an abnormal phenotype with partial loss of tryptase.

Case Report and Results

Case Report. A 34-year-old female patient presented in June 2005 with a 3-year-history of unexplained osteopathy. In 2003, surgery had been performed because of suspected osteomyelitis of the right femur. The histology at that time did not show a conclusive result. Consecutively, the clinical symptoms of osteopathy worsened and a tumor in the right femur with associated osteolysis developed. In addition, the patient suffered from skin-flushes and diarrhoea. Computed tomography (CT) revealed multiple osteolytic lesions in the entire skeleton, which led to the suspicion of a metastatic tumor. All other organ systems appeared normal on CT scans. The blood count showed mild normocytic anemia (hemoglobin 11.3 g/dL) and mild thrombocytosis (486,000/μL). The leukocyte count was 7,210 per μL. The differential blood count was normal. Liver enzymes, serum calcium, alkaline phosphotase, and lactate dehydrogenase were within the normal range. However, serum tryptase levels were found to be markedly elevated (256 ng/mL). In consecutive examinations, the diagnosis of ASM with involvement of the skeleton, was established.

Histology and immunohistochemical (IHC) examinations of the bone lesion. Histologic examination of the tumor lesion (right femur) revealed a sarcoma-like infiltration of the bone with immature atypical cells. Most cells were medium-sized and showed a non-granulated cytoplasm and atypical nuclei. These nuclei were often bi- or poly-lobed with irregular to fine chromatin. Some of these cells appeared to have spindle-shape morphology, and some were found to be giant cells. Immunohistochemistry was performed according to an established protocol.[3,4,9] A specification of antibodies used and the staining techniques applied are shown in Table 1. As assessed by routine IHC-staining, the cells in the tumor lesion were found to express CD2, CD25, CD43, CD45, and CD68R, but did not express CD14, CD30, CD34, CD42b, or CD61 (Table 2). Unexpectedly, neoplastic cells did not react with an antibody against tryptase (Figure 1A; Table 2). Moreover, these cells stained negative for mast cell chymase, myeloperoxidase (MPO), lysozyme, chloroacetate esterase (CAE), and basogranulin (Table 2). As assessed by Ki67-staining, about 5% of all cells were found to be proliferating cells. Interestingly, neoplastic cells expressed the survival-related antigens Mcl-1 and Bcl-2 (Table 2).

Detection of mast cell antigens by a highly sensitive staining protocol. Expression of CD2, CD25, and CD68R as well as the elevated tryptase level raised the suspicion of a highly aggressive MC tumor (MC-sarcoma) despite lack of CAE, tryptase, and chymase determined by IHC-staining. To confirm the identity of MC, we therefore repeated several IHC-stains (including tryptase, chymase, CD34) using a highly sensitive staining-protocol employing biotinylated goat-anti-rabbit or goat-anti-mouse secondary antibodies, enzyme-labeled streptavidin, and AEC as chromogen (all from Biocare Medical, Pike Lane, Concord, CA). In addition, we extended the

Table 1. Specification of antibodies.

Antibody	Clone	Antigen retrieval	Dilution	Animal source	Company
Tryptase	G3	-	1:1,000	m	Chemicon
Chymase	B7	Proteinase	1:1,000	m	Chemicon
HDC	polyclonal	MW	1:500	r	Progen
Mcl-1	S-19	MW	1:200	r	Santa Cruz
Bcl-2	124	MW	1:80	m	DAKO
Mib-1	Ki67	MW	1:50	m	DAKO
Lysozyme	polyclonal	MW	1:1,500	r	DAKO
Myeloperoxidase (MPO)	polyclonal	MW	1:400	r	DAKO
Basogranulin	BB1	Proteinase	1:500	m	A.F.Walls*
CD2	AB75	AUTO	1:40	m	Novocastra
CD14	7	MW	1:100	m	Novocastra
CD25	4C9	MW	1:50	m	Novocastra
CD30	BerH2	MW	1:80	m	DAKO
CD34	QBend/10	MW	1:50	m	Immunotech
CD42b	42C01	MW	1:50	m	Neomarkers
CD43	MTI	MW	1:400	m	Biotest
CD44	DF1485	MW	1:50	m	DAKO
CD45	2B11+PD7/26	MW	1:20	m	DAKO
CD61	2f2	MW	1:200	m	Novocastra
CD63	NKI/C3	MW	1:200	m	Novocastra
CD68R	PG-M1	Proteinase	1:100	m	DAKO
CD117/KIT	polyclonal	MW	1:500	r	DAKO

*MW, microwave oven; AUTO, autoclave pretreatment; m, mouse; r, rabbit. *The antibody BB1 directed against basogranulin was kindly provided by Dr. A. F. Walls (University of Southampton, U.K.).*

Table 2. Expression of leukocyte antigens in neoplastic mast cells.

	Immunohistochemistry (IHC)			*ICC*
	tumor		*bone marrow (bm)*	*bm MNC*
	routine staining	*super-sensitive*	*routine staining*	*cytospins*
Tryptase	-	++	+	±
Chymase	-	-	-	-
HDC	±	++	+	++
CD117/KIT	++	++	+	++
CD25	+	+	+	±
CD34	-	-	-	-
CD63	+	++	+	++
BB-1	-	-	-	-
CD44	n.d.*	++*	++*	++
Mcl-1	+	n.d.	+	n.d.
Bcl-2	+	n.d.	n.d.	n.d.
CD2	+	n.d.	n.d.	n.d.
MPO	-	n.d.	n.d.	n.d.
CAE	-	n.d.	n.d.	n.d.
Lysozym	-	n.d.	n.d.	n.d.
CD43	+	n.d.	n.d.	n.d.
CD45	+	n.d.	n.d.	n.d.
CD68R	+	n.d.	n.d.	n.d.
CD14	-	n.d.	n.d.	n.d.
CD30	-	n.d.	n.d.	n.d.
CD42b	-	n.d.	n.d.	n.d.
CD61	-	n.d.	n.d.	n.d.

*ICC, immunocytochemistry; bm, bone marrow; n.d., not determined; *for CD44, routine staining is performed using the super-sensitive staining protocol. Score of antibody staining reactivity: ++, strongly reactive; +, reactive; ±, weakly reactive; -, no reactivity found.*

panel of markers (CD44, CD63, KIT/CD117, histidine decarboxylase=HDC) (Table 1). Using these markers and the highly sensitive staining-protocol, neoplastic cells were found to express clearly detectable levels of tryptase (Figure 1B), whereas the cells remained chymase-negative and BB1-negative in the highly sensitive stain. The identity of neoplastic MC was confirmed by positive staining for KIT/CD117 and HDC (Figure 1C and 1D; Table 2). Moreover, we were able to show that neoplastic cells express CD44 and CD63 (Table 2).

Immunohistochemistry and immunocytochemistry of bone marrow cells. The consecutive staging included a bone marrow trephine biopsy (June 2005) with histology and immunohistochemistry. In this investigation, a discrete (focal plus diffuse - approximately 5%) infiltration of the bone marrow with atypical MC was found. These MC were mostly immature cells, often with bi- or polylobed nuclei. Surprisingly, in contrast to MC in the tumor lesion, MC in the bone marrow sections stained clearly positive for tryptase by conventional immunohistochemistry (Table 2). In addition, these MC were found to express KIT, CD25, CD44, and CD63, but did not react with antibodies against chymase, BB1, or CD34 (Table 2).

Immunocytochemistry was performed at the time of leukemic progression using neoplastic MC isolated from bone marrow aspirates. In these aspirates, a significant proportion of cells (43%) were found to be atypical immature MC (Figure 2A and 2B). Neoplastic MC were enriched using Ficoll and then spun on cytospin slides. Immunocytochemistry was performed using antibodies depicted in Table 2. In these experiments, leukemic MC were found to display tryptase (Figure 2C) as well as KIT, HDC, and CD63 (Figure 2D-2F). In addition, MC were found to express CD25 and CD44, but did not express chymase or CD34 (Table 2).

Cytogenetic and molecular analysis. Cytogenetic analysis of bone marrow mononuclear cells showed a normal karyotype (46,XX). We also examined bone marrow cells and malignant cells derived from the primary tumor site for the presence of *KIT* D816V as described.[11] However, no *KIT* mutation at codon 816 was detected. In addition, we were also unable to detect any leukemia-specific fusion genes by multiplex-PCR (*not shown*).

Clinical course and response to therapy. After the diagnosis ASM was established, the patient received interferon-alpha (IFNα) and prednisone. IFNα was intially administered at 3 million units per week, and later at 5 million units per day. In addition, she received pamindronate (90 mg i.v. every 4 weeks). However, no improvement was noted. Rather progression of disease was recorded

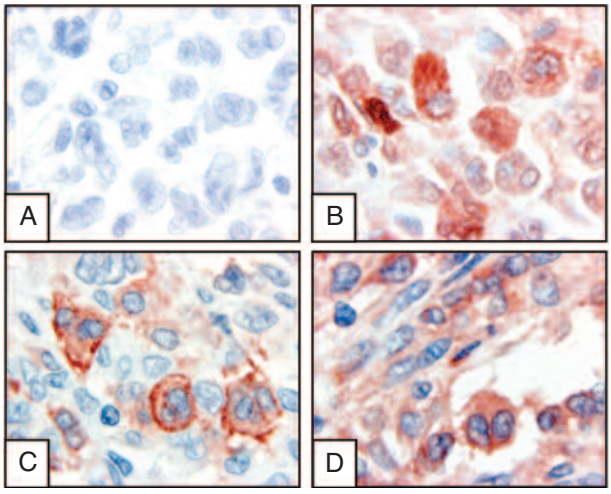

Figure 1. Immunohistochemical detection of mast cell antigens in neoplastic cells. Neoplastic cells detected in the tumor lesion were found to be immature cells, several of them exhibiting bi- or polylobed nuclei. By routine staining, these cells did not react with an antibody against tryptase (A). However, when a highly sensitive staining protocol was applied, these neoplastic cells were found to stain positive for tryptase (B). In addition, these neoplastic mast cells were found to react with antibodies directed against KIT (C) and against histidine decarboxylase (HDC) (D), confirming their identity. The staining techniques applied are described in the text.

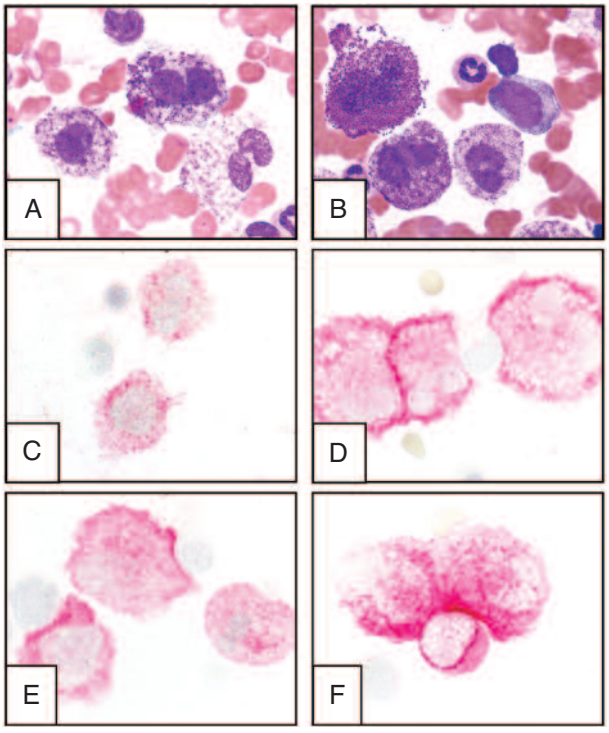

Figure 2. Morphology and immunocytochemical staining reaction of neoplastic mast cells at the time of leukemic progression. A, B: As assessed by Wright-Giemsa-staining, the bone marrow smear was found to contain a significant number of immature mast cells, leading to the diagnosis of mast cell leukemia. As assessed by immunocytochemistry, these cells were found to stain positive for tryptase (C), KIT (D), histidine decarboxylase (HDC) (E), and CD63 (F).

with an increase in serum tryptase (401 ng/mL). Therefore, therapy with cladribine (2CdA) was initiated in September 2006. The dose and schedule of 2CdA were the same as that published previously.[12] The patient received 3 courses of 2CdA. Thereafter, restaging was performed and revealed progressive disease with a huge increase of highly atypical MC in bone marrow smears (>20%), consistent with the diagnosis of MCL. In addition, tryptase levels further increased (489 ng/mL). The patient was then treated with polychemotherapy using high dose cytarabine and fludarabin, and a search for a stem cell transplant donor was intitiated.

Discussion

The diagnosis of systemic mastocytosis is primarily based on a thorough histologic and immunohistochemical investigation of the bone marrow.[1-4] The standard marker for detection of MC in bone marrow-sections is tryptase. This enzyme is usually expressed in all subtypes of MC and in all variants of mastocytosis.[1-4,6,7] We describe an unsual case of ASM in whom a sarcoma-like spread of MC in the skeleton and progression to MCL were documented. In this unusual case, we were unable to define the identity of neoplastic MC by tryptase-staining using a standard-routine-protocol. However, the application of additonal MC-markers confirmed the diagnosis ASM, and MC were found to express low levels of tryptase by applying a super-sensitive staining-protocol.

During de-differentiation of neoplastic cells, several differentiation-related antigens may be lost. In case of advanced MC-neoplasms, it is well known that neoplastic cells often lack chymase and high-affinity IgE-receptors,[13] two antigens that are usually expressed in mature tissue MC. Thus, lack of chymase and IgE receptor in neoplastic MC is a well recognized phenomenon. However, to the best of our knowledge, loss or lack of tryptase in neoplastic MC in SM has so far not been described. In the present study, the lack of tryptase in neoplastic MC was thus judged as extremely unusual phenomenon, and several different control experiments were performed to clarify whether these cells are indeed MC and wether tryptase is expressed at very low levels or is indeed absent in malignant cells. As determined by a highly sensitive staining protocol, these neoplastic cells were found to display very low levels of tryptase.

A remarkable observation was that in the bone marrow, neoplastic MC expressed substantial amounts of tryptase. From this observation, it may be concluded that only a subset of MC, namely those infiltrating into the bone, displayed lower levels of tryptase. This phenomenon may be explained by enhanced secretion of the enzyme by bone-invading neoplastic MC or by the fact that only very immature tryptase-negative MC were capable of invading the bone. The observation that bone marrow MC expressed measurable levels of tryptase also explains why serum tryptase levels were clearly elevated despite the almost complete lack of the

enzyme in bone-invading MC.

Apart from tryptase, a number of other immunohistochemical markers for MC in SM have been described. Among these are KIT, histidine decarboxylase (HDC), and CD63.4,7,9,10 In the present study, we were able to show that neoplastic MC co-express KIT and HDC, a marker-combination that is usually only displayed by MC. In addition, neoplastic cells expressed CD63. All in all, these results are clearly indicative of the presence of MC.

Mast cell sarcoma and MCL are high grade MC neoplasms characterized by enhanced survival and proliferation of MC.[1,2] In the present study, we were able to show that 5% of all MC express Ki67, which indicates a high proliferative capacity. In addition, we were able to show that neoplastic MC co-express Bcl-2 and Mcl-1, two members of the Bcl-2 family, that act antiapoptotic in diverse neoplastic cells including neoplastic MC.[14,15] All in all, these data are consistent with a highly malignant phenotype of MC that was found to correspond with the adverse clinical course in this patient.

In most patients with SM including ASM and MCL, neoplastic cells display mutations in codon 816 of the *KIT* proto-oncogene.[1,2,11,16] However, in our patient, no *KIT* mutation at codon 816 was detectable. This observation may point to other defects contributing to malignant cell growth in our patient. However, no major cytogenetic defect and no leukemia-related gene-defect could be detected in neoplastic cells in this patient.

In summary, we report on an unusual case of ASM with sarcoma-like growth of MC in the skeleton, and progression to MCL within short time despite therapy with IFNα and 2CdA. In this patient, the diagnosis was initially obscured by the atypical clinical presentation and the partial loss of tryptase. We recommend the application of an extended panel of MC-related antigens including KIT and HDC, for such cases in order to establish the correct diagnosis.

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