CASE REPORT

"Occult" mastocytosis with activating *c-kit* point mutation evolving into systemic mastocytosis associated with plasma cell myeloma and secondary amyloidosis

K Sotlar, W Saeger, F Stellmacher, J Stahmer, S Jäckle, P Valent, H-P Horny

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A case of a 70-year-old man presenting with exsudative enteropathy due to light-chain-associated amyloidosis is reported. The diagnosis of systemic mastocytosis associated with IgG/λ plasma cell myeloma and secondary generalised amyloidosis was carried out by morphological evaluation of bone marrow biopsy. The *c-kit* point mutation D816Y was detected by molecular analysis. Two years before, a cystadenolymphoma of the left parotid gland had been removed. A moderate increase of loosely scattered spindle-shaped mast cells, a subpopulation of them expressing CD25, an antigen that is not expressed by normal or reactive mast cells, was shown by retrospective analysis carried out on an intraparotid lymph node. The *c-kit* mutation D816Y was shown by the molecular analysis of the lymph node. In summary, the notion that systemic mastocytosis may very rarely be associated with B cell neoplasms and that neoplastic mast cell infiltrates may be obscured because of only a minimal increase of atypical mast cells, which are outnumbered by other non-neoplastic cells in the same tissue, is supported by this case. This finding was preliminarily termed "occult" mastocytosis.

Mastocytosis comprises a heterogeneous group of disorders characterised by abnormal proliferation and accumulation of mast cells in various tissues and organs. The World Health Organization (WHO) classification includes seven main categories of mastocytosis, namely cutaneous mastocytosis, indolent systemic mastocytosis usually presenting with cutaneous involvement, systemic mastocytosis with an associated clonal haematological non-mast cell lineage disease (SM-AHNMD), aggressive systemic mastocytosis, mast cell leukaemia, mast cell sarcoma and extracutaneous mastocytoma.¹ For the discrimination of systemic mastocytosis from cutaneous mastocytosis and other pathological conditions with a marked increase of reactive mast cells, certain criteria were defined: the major diagnostic criterion is the histological evidence of multifocal compact tissue infiltrates consisting of at least 15 mast cells. Minor criteria include the demonstration of (a) >25% spindle-shaped mast cells; (b) *c-kit* mutations at codon D816V; (c) expression of CD25 or CD2 by mast cells; and (d) a persistently raised serum total tryptase level (>20 ng/ml).² A diagnosis of systemic mastocytosis should only be made when either the major criterion plus at least one of the minor criteria or at least three minor criteria are fulfilled.² A considerable number of patients with systemic mastocytosis will develop an AHNMD. Most of these are myeloid malignancies, for example, acute myeloid leukaemia, myeloproliferative or myelodysplastic syndromes. By contrast, only

very few cases of an associated lymphoproliferative disorder have been reported in systemic mastocytosis.^{3–5}

We here report on the unique case of a 70-year-old man presenting with exsudative enteropathy caused by light-chain-associated amyloidosis because of systemic mastocytosis with associated plasma cell myeloma (SM-AHNMD) diagnosed in the bone marrow. Interestingly, molecular analysis showed the uncommon *c-kit* mutation D816Y not only in the actual bone marrow but also in an intraparotid lymph node removed 2 years earlier on the occasion of the resection of a cystadenolymphoma.

CASE REPORT

A 70-year-old man presented with a history of chronic diarrhoea and a weight loss of about 30 kg during 4 months. Two years earlier, a cystadenolymphoma of the left parotid gland and an intraparotid lymph node exhibiting signs of chronic non-specific lymphadenitis had been removed. Mild anaemia (12.2 g/dl), leucocytosis (13 600/μl), hypoproteinaemia (4.2 mg/l) and proteinuria (1.814 mg/24 h) were observed. Electrophoresis indicated Bence-Jones proteins. Radiological examination, however, showed no osteolytic foci. Sonography of the abdomen disclosed massive ascites and densification of the renal parenchyma highly suspicious of amyloidosis. Hepatosplenomegaly or lymphadenopathy was not present. Histological investigation of the duodenal mucosa showed (secondary) light-chain-associated amyloidosis. Subsequent histological and immunohistochemical evaluation of a bone marrow trephine biopsy specimen was carried out, which showed a diffuse increase of plasma cells with light chain restriction of λ type, enabling the diagnosis of plasma cell myeloma with mild infiltration of the bone marrow. As amyloid deposits were detected in the wall of small intramedullary blood vessels, the diagnosis of generalised amyloidosis could be established. Unexpectedly, a focal increase of mast cells was also seen in the bone marrow. Immunohistochemical expression of CD25 in mast cells and the presence of a *c-kit* codon 816 mutation led to the final diagnosis of SM-AHNMD or plasma cell myeloma. The patient was treated with pantoprazol and glucocorticoids. As no sufficient oral nutrition could be established, an alimentary fistula was installed. The patient died with signs of bronchopneumonia after a total duration of the disease of only about 7 months. An autopsy was not conducted.

MATERIALS AND METHODS

The tissues investigated were removed from the left parotid gland, including an intraparotid lymph node (2001), from

Abbreviations: AHNMD, associated clonal haematological non-mast cell lineage disease; PNA, peptide nucleic acid; SM-AHNMD, systemic mastocytosis associated clonal haematological non-mast cell lineage disease

Figure 1 Systemic mastocytosis associated with plasma cell myeloma. (A) Duodenal or intestinal amyloidosis: The duodenal mucosa shows a normal structure of the villi with an intact surface epithelium. The lamina propria is filled with a hyaline dense material exhibiting kongophilia. Lymphoplasmacellular cells are loosely scattered throughout, without forming dense infiltrates. No mast cell infiltrates can be seen (Kongo red, 100 $\times$). (B) Hypercellular bone marrow with a sharply demarcated mast cell infiltrate (Giemsa, 200 $\times$). (C) Higher magnification shows a prominent spindling of mast cells containing an abundance of metachromatic granules (Giemsa, 630 $\times$). Note the increase in sea-blue histiocytes as a non-specific sign of increased cellular turnover is seen. Immunohistochemical stainings show (D) a strong granular cytoplasmatic reactivity of the mast cells for anti-tryptase (100 $\times$) and (E) an anular (membrane-associated) reactivity for anti-CD117 (KIT, 400 $\times$). (F) Immunohistochemical staining of a bone marrow biopsy specimen with an antibody against the antigen CD38 associated with plasma cells shows a moderate increase of loosely scattered slightly pleomorphic plasma cells by strong anular (membrane-associated) reactivity. Note that the number of plasma cells here does not exceed 20% of all nucleated bone marrow cells (anti-CD38, 400 $\times$).

the duodenal mucosa (2003), and from the bone marrow (2003). All tissue specimens were fixed in 5% neutral buffered formalin and embedded in paraffin wax. Before embedding in paraffin wax, the bone marrow biopsy specimen was mildly decalcified in edetic acid. Tissue sections were stained with antibodies directed against various antigens related to plasma cells and mast cells, including CD38, CD56, λ and κ light chains, tryptase, chymase, CD25 and CD117.⁶

Five 8- μ m-thick sections were cut from paraffin blocks of the intraparotid lymph node and biopsy of the bone marrow was carried out. Total genomic DNA was extracted as described previously.⁷ Peptide nucleic acid (PNA)-mediated PCR clamping and melting point analysis on 100 ng of total DNA was investigated for the presence of *c-kit* exon 17 mutations as described previously.⁸ Single mast cells stained with anti-tryptase from the preauricular lymph node and the bone marrow were microdissected by laser pressure catapulting on a PALM Robot MicroBeam system and pooled to a total of 150 cells per PCR tube. The method has been described in detail previously.⁹ In this study, hybridisation probes were used as described for PNA-PCR.⁸ Direct sequencing of amplification products was carried out by the

dye-deoxy terminator method on a 377 ABI Prism Sequencer (PE Applied Biosystems, Weiterstadt, Germany.).

RESULTS

Histological and immunohistochemical findings

The duodenal mucosa showed a preserved architecture. The histological picture was dominated by diffuse deposits of amyloid within the broad-ended lamina propria (fig 1A). Amyloid proved to be immunoreactive for immunoglobulin light-chain λ but negative for antibodies against amyloid A and amyloid transthyretin related. Infiltrates of plasma cell myeloma or mastocytosis were not found. A diagnosis of light-chain-associated amyloidosis with exsudative enteropathy was confirmed.

The microarchitecture of the bone marrow was partially destroyed. Sharply demarcated foci consisting of spindle-shaped mast cells with abundant metachromatic granules were easily detectable (fig 1B,C). Immunohistochemically, the mast cells expressed the antigens tryptase (fig 1D), KIT (CD117; fig 1E) and CD25. Hypercellular marrow areas contained an intact haematopoiesis and, moreover, also showed an increase in slightly pleomorphic plasma cells,

which were either loosely scattered throughout or formed small perivascular aggregates. The number of CB38-positive plasma cells did not exceed 20–30% of all nucleated marrow cells (fig 1F). The plasma cells showed immunoglobulin light-chain restriction of λ type. The walls of small blood vessels contained amyloid deposits. A diagnosis of SM-AHNMD and generalised secondary amyloidosis of light chain λ was confirmed.

A retrospective morphological and immunohistochemical analysis of an intraparotid lymph node showed signs of chronic non-specific lymphadenitis and a marked increase in loosely scattered mast cells. Immunohistochemically, a minor proportion of tryptase-positive mast cells apparently reacted with anti-CD25. Compact mast cell infiltrates were not detected. The diagnosis of chronic non-specific lymphadenitis with mast cell hyperplasia and a few immunophenotypically abnormal (CD25+) mast cells was established. The criteria for diagnosis of mastocytosis, however, were not fulfilled.

Detection of *c-kit* mutation D816Y in tissue sections and microdissected mast cells

Melting point analysis of total genomic DNA from tissue sections of the preauricular lymph node and the bone marrow amplified by PNA-mediated PCR clamping showed a dominant wt-specific signal at about 59°C and, in addition, a smaller aberrant melting peak at about 55°C, indicating the presence of a *c-kit* mutation (fig 2). Direct sequencing of PCR products showed the presence of a G→T transversion at position 7175 of the *c-kit* gene, leading to an amino-acid exchange of asparagine for tyrosine at codon 816 (D816Y).

Three PCR tubes containing 150 mast cells each were collected from the tissues of the preauricular lymph node and the bone marrow by pooling single microdissected tryptase-stained mast cells. Nested PCR amplification showed PCR products from each of the six tubes. Melting point analysis showed aberrant melting peaks at about 55°C for all three tubes containing bone marrow mast cells and for one of the three tubes containing mast cells microdissected from the preauricular lymph node. Amplification products of the remaining two tubes showed wt-specific melting points. Direct sequencing of amplification products showing aberrant melting peaks again revealed the *c-kit* mutation D816Y in both tissues.

DISCUSSION

Most AHNMDs in patients with systemic mastocytosis are myeloid neoplasms.^{5–10} By contrast, only a very few cases of associated lymphoproliferative disorders have been reported.^{3–5 11 12} Here we report on the unique case of a 70-year-old man presenting with exsudative enteropathy due to light-chain-associated amyloidosis and plasma cell myeloma associated with SM-AHNMD, the latter being an unexpected histological finding in a bone marrow biopsy specimen that was removed for staging procedures.

Diagnosis of mast cell neoplasia as an essential part of the SM-AHNMD complex in our experience is always unexpected because, as in the present case, symptoms indicating mast cell disease, in particular pathognomonic skin lesions of cutaneous mastocytosis (urticaria pigmentosa), are missing. Regarding bone marrow morphology in most other patients of SM-AHNMD, diffuse dense myelomonocytic or blast cell infiltrates obscure or even distort pre-existing compact (diagnostic) mast cell infiltrates. This phenomenon was recently described in a patient with acute myeloid leukaemia M2 and t(8;21), in whom compact mast cell infiltrates were found only in the second bone marrow biopsy specimen removed after induction chemotherapy with a marked aplasia of blast cells and haematopoiesis.¹³ In this patient, only the detection of an activating *c-kit* mutation, as a minor diagnostic criterion, allowed the identification of systemic mastocytosis in the initial bone marrow biopsy specimen. Also, an intraparotid lymph node had been removed together with a cystadenolymphoma 2 years before the diagnosis of SM-AHNMD. Surprisingly, the lymph node contained the same uncommon type of activating mutation

Take-home messages

- This is the first published case of systemic mastocytosis associated with exsudative enteropathy due to amyloidosis caused by plasma cell myeloma.
- Systemic mastocytosis may be preceded by an "occult" phase in which there is only minimal tissue infiltration but presence of activating point mutations of *c-kit*.

Figure 2 Melting point analysis of amplified DNA from total DNA of the bone marrow (BM, black lines with "•") and of an intraparotid lymph node (LN, black lines with "x"). HMC-1 DNA heterologous for the KIT mutation D816Y served as a positive control (grey lines). Melting curves derived from conventionally amplified (no peptide nucleic acid (PNA)) DNA are depicted as broken lines, and those derived from amplification products after PNA-mediated PCR clamping are shown as continuous lines. The arrow indicates a shoulder in the melting curve of conventionally amplified DNA from the bone marrow suspicious for the presence of a *c-kit* mutation. The mutation D816Y is then disclosed by PNA-mediated PCR clamping.

(D816Y) as detected in the bone marrow, although mastocytosis could not be diagnosed on the basis of morphology alone.

In this regard, it is well known that activating mutations in the catalytic domain of the tyrosine kinase receptor KIT cause autophosphorylation of the receptor and ligand-independent growth of mast cells, and are therefore considered to be associated with the evolution of mast cell neoplasias.¹⁴ One of these mutations, namely D816V, was first detected in the mast cell leukaemia-derived cell line HMC-1,¹⁵ but was later also found in most cases of systemic mastocytosis, including SM-AHNMD.^{5, 16, 17} Other activating *c-kit* mutations, for example, D816Y, D816H and D816F have been described in systemic mastocytosis and UP,^{18–20} and were shown to be of somatic origin.²¹ The finding of “occult” (molecular) mastocytosis with point mutation of the *c-kit* proto-oncogene preceding morphological manifestation of systemic mastocytosis by at least 2 years is unique and is therefore difficult to interpret. Undoubtedly, mutated (clonal) mast cells were present at least 2 years before final morphological evidence of mastocytosis was available. On the basis of the molecular findings, it could be speculated that only a (minor?) proportion of the total mast cells exhibited this type of point mutation. We are aware of only one more patient with molecular indication of mastocytosis before morphological manifestation of systemic mastocytosis (unpublished observation). This male patient had undergone prostatectomy because of nodal-negative adenocarcinoma of the prostate. After a disease-free survival of about 10 years, a trephine biopsy of the left iliac crest was carried out because of chronic refractory anaemia. This led to the diagnosis of systemic mastocytosis, probably (isolated) bone marrow mastocytosis as cutaneous lesions of urticaria pigmentosa were missing and there was no organomegaly or lymphadenopathy. Retrospective analysis of pelvic lymph nodes yielded no morphological signs of mastocytosis. In particular, there were no compact mast cell infiltrates or a coexpression of CD25 by mast cells positive for tryptase. Molecular analysis, however, showed the presence of the *c-kit* point mutation D816Y, which had been seen before in the bone marrow of the patient and was also present in this case.

In summary, we identified an apparently very small subpopulation of patients with overt (morphological) systemic mastocytosis affecting the bone marrow preceded by occult disease without morphologically diagnosable mastocytosis but with an unusual type of point mutation (D816Y) of the *c-kit* proto-oncogene of up to 10 years of duration. The frequency of these *c-kit* point mutations in the prediagnostic phase of mastocytosis remains a matter of speculation, but can only be assessed incidentally with molecular biological methods.

Authors' affiliations

K Sotlar, Institute of Pathology, University of Tübingen, Tübingen, Germany

W Saeger, Marienkrankenhaus Hamburg, Hamburg, Germany

F Stellmacher, **H-P Horny**, Institute of Pathology, University of Lübeck, Lübeck, Germany

J Stahmer, **S Jäckle**, Clinic of Internal Medicine, Reinbek Hospital St Adolf, Reinbek, Germany

P Valent, Department of Internal Medicine I (AKH), Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria

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Correspondence to: Prof H-P Horny, Institute of Pathology, University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany; horny@patho.mu-luebeck.de

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