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ORIGINAL ARTICLE

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Neoplastic human tissue mast cells express the adhesion molecule CD44/HCAM

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Abstract Expression of the homing-associated cell adhesion molecule/HCAM (CD44) in normal/reactive and neoplastic human tissue mast cells (TMC) was determined immunohistochemically using the antibody DAKO-DF1485, which detects all isoforms of CD44. Studies were performed on 30 routinely processed specimens. Twenty of these, from bone marrow, skin, spleen, liver, lymph node and jejunal mucosa, contained infiltrates of TMC. These represented various types of generalized mastocytosis/systemic mast cell disease, including benign systemic mastocytosis, malignant mastocytosis and cutaneous mastocytosis. Ten specimens consisted of tissue with a marked reactive increase in TMC; most of these were lymph nodes with chronic nonspecific lymphadenitis and benign or malignant solid tumours. In all 30 specimens TMC exhibited an annular pattern of immunostaining, which was usually very strong. Both normal/reactive and neoplastic TMC exhibited consistent immunoreactivity with the antibody DAKO-DF1485, and this antibody may be of diagnostic value in the detection of atypical TMC associated with malignant mastocytosis. TMC and their neoplastic derivatives belong to a large family of mesenchymal and epithelial cells containing the principal surface receptor for hyaluronan.

Key words CD44 · Mast cell · Mastocytosis · Systemic mast cell disease

Introduction

During the investigation of human bone marrow in various reactive and neoplastic states (including systemic mastocytosis) with newly developed antibodies suitable for use on formalin-fixed, paraffin-embedded tissue at the Institute of Pathology, University of Tübingen, we noted strong reactivity of tissue mast cells (TMC) with DAKO-DF1485, which is directed against a trypsin-resistant epitope of the CD44 molecule (homing-associated cell adhesion molecule/HCAM). This incidental finding prompted us to perform a broader survey of the expression of CD44 by reactive and neoplastic TMC. CD44 is a widely distributed protein and is the principal cell surface receptor for hyaluronan, suggesting a role in cell–matrix interactions and cell migration [1, 14]. The expression of CD44 has been described in normal human TMC from various sites, and has recently also been found on a novel subset of human TMC, the cardiac mast cell, in patients with cardiomyopathy [10, 12]. CD44 is one of a relatively large number of well-characterized cell surface receptors expressed by human TMC, which include high-affinity IgE-binding sites (FcERI), the mast cell growth factor (MGF) receptor (CD117), the common β-chain of β1-integrins (CD29), the ICAM-1 antigen (CD54) and leucosialin (CD43) [9, 13].

Materials and methods

The investigations were performed on formalin-fixed, paraffin-embedded tissue specimens from the sites listed in Table 1. Twenty specimens were derived from patients with various types of mastocytosis, and ten specimens consisted of tissue with a reactive increase in TMC (mast cell hyperplasia). In all cases, the original diagnosis of mastocytosis had been based on the demonstration of cohesive tissue infiltrates of mast cells, which were identified by the presence of metachromatic granules in Giemsa-stained sections, strong staining by the naphthol AS-D chloroacetate esterase reaction [3, 4] and immunoreactivity for tryptase (personal unpublished finding). Bone marrow trephine biopsy specimens were mildly decalcified in edetic acid/EDTA for 24 h before paraffin embedding [2]. Sections were dewaxed and subjected to microwave treatment for three 5-min cycles. They were then immunostained with the monoclonal antibody DF1485 (DAKO Diagnostika, Hamburg, Germany) by the avidin–biotin peroxidase complex (ABC) method [5]. Optimum staining was obtained at a primary antibody dilution of 1:50.

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Table 1 Histopathological diagnoses of the cases investigated, and intensity of immunostaining of normal/reactive and neoplastic human tissue mast cells (*TMC*) by the antibody DF1485 (which detects CD44/HCAM; + weak annular staining of TMC, ++ strong annular staining of TMC, *AL* angioleiomyoma, *CL* chronic lymphadenitis, *CM* cutaneous mastocytosis, *CS* chronic sinusitis, *LU* leiomyoma uteri, *MCC* Merkel cell carcinoma, *MFH* malignant fibrous histiocytoma, *MM* malignant mastocytosis, *SM* systemic mastocytosis)

No.	Diagnosis	Tissue	TMC	Other cells stained
1	SM	Bone marrow	+	Lymphocytes
2	SM	Bone marrow	+	Lymphocytes
3	SM	Bone marrow	++	Lymphocytes
4	SM	Bone marrow	++	Lymphocytes
5	SM	Bone marrow	++	Lymphocytes
6	SM	Bone marrow	++	Lymphocytes
7	SM	Bone marrow	++	Lymphocytes
8	SM	Liver	++	Hepatocytes
9	SM	Liver	++	None
10	SM	Lymph node	+	Lymphocytes
11	SM	Lymph node	++	Lymphocytes
12	SM	Jejunum	++	None
13	MM	Bone marrow	++	Lymphocytes
14	MM	Bone marrow	++	Lymphocytes
15	MM	Bone marrow	++	Lymphocytes
16	MM	Spleen	++	Lymphocytes
17	MM	Spleen	++	Lymphocytes
18	CM	Skin	+	Keratinocytes
19	CM	Skin	++	Keratinocytes
20	CM	Skin	++	Keratinocytes
21	CL	Lymph node	+	Lymphocytes
22	CL	Lymph node	+	Lymphocytes
23	CL	Lymph node	++	Lymphocytes
24	LU	Tumour	++	Tumour cells
25	Myxoma	Tumour	+	None
26	MFH	Tumour	+	Tumour cells
27	AL	Tumour	++	None
28	Seminoma	Tumour	+	Lymphocytes
29	MCC	Lymph node	++	Lymphocytes
30	CS	Paranasal sinus	++	Epithelial cells, Lymphocytes

Results

Most of the TMC in all the specimens investigated exhibited annular (specific, membrane-associated) staining by DF1485 (Figs. 1, 2), irrespective of the diagnosis. In the bone marrow, TMC appeared to represent the vast majority of cells with strong expression of CD44 (Fig. 3). A small proportion of the TMC in bone marrow infiltrates of systemic and malignant mastocytosis exhibited strong granular staining of the cytoplasm (Fig. 3). The annular staining was usually much stronger in the TMC than in the lymphocytes in the surrounding tissue. Granulopoietic and erythropoietic cells were not stained in any of the bone marrow specimens investigated (Table 1).

Discussion

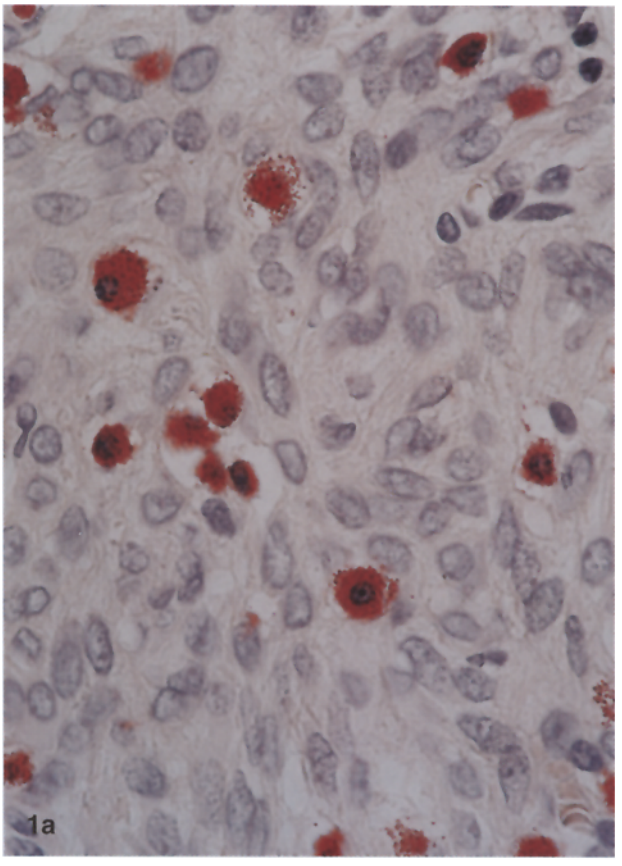
Our study shows that normal and neoplastic human TMC express the adhesion molecule CD44/HCAM, and that since TMC usually exhibit stronger staining by DAKO-DF1485 than other CD44-positive cell types (in particular lymphocytes), this antibody can be useful for the identification of both loosely scattered and densely accumulated TMC.

The highly atypical, neoplastic TMC that are otherwise very difficult to identify were also found to be consistent in their expression of CD44. We believe that the application of DAKO-DF1485 may be of diagnostic value in the identification of tissue infiltrates associated with malignant mastocytosis, particularly in the bone marrow, where TMC were found to make up the vast majority of strongly CD44-positive cells.

Fig. 1a, b Leiomyoma uteri with marked mast cell hyperplasia. **a** Very small but cellular leiomyoma consisting of whorls and bundles of spindle-shaped tumour cells without nuclear atypia. Note the abundance of large intratumoural mast cells, which can be identified easily by the deep red staining of their cytoplasm in this naphthol AS-D chloroacetate esterase stain. Naphthol AS-D chloroacetate esterase, ×800. **b** Immunohistochemical staining for CD44 reveals strong annular reactivity of virtually all the mast cells and much weaker annular staining of a considerable proportion of the tumour cells. DF1485/anti-CD44, ABC method, ×800

Fig. 2 Systemic mastocytosis with infiltration of the jejunum. The lamina propria of the mucosal layer is densely infiltrated by mast cells with annular, i.e., specific, immunoreactivity for CD44. Note that the epithelial cells are not stained. DF1485/anti-CD44, ABC method, ×560

Fig. 3a, b Systemic mastocytosis with infiltration of the bone marrow. DF1485/anti-CD44, ABC method. **a** A cohesive peritrabecular cluster of large, round to oval mast cells with annular immunostaining for CD44 dominates the picture. Marked fibrosis demarcates the mastocytic infiltrate from the bone. ×560. **b** In another case, many spindle-shaped mast cells with elongated nuclei are visible. In this exceptional case all but one of the mast cells exhibit strong granular staining of the cytoplasm (i.e., nonspecific staining) for CD44. ×800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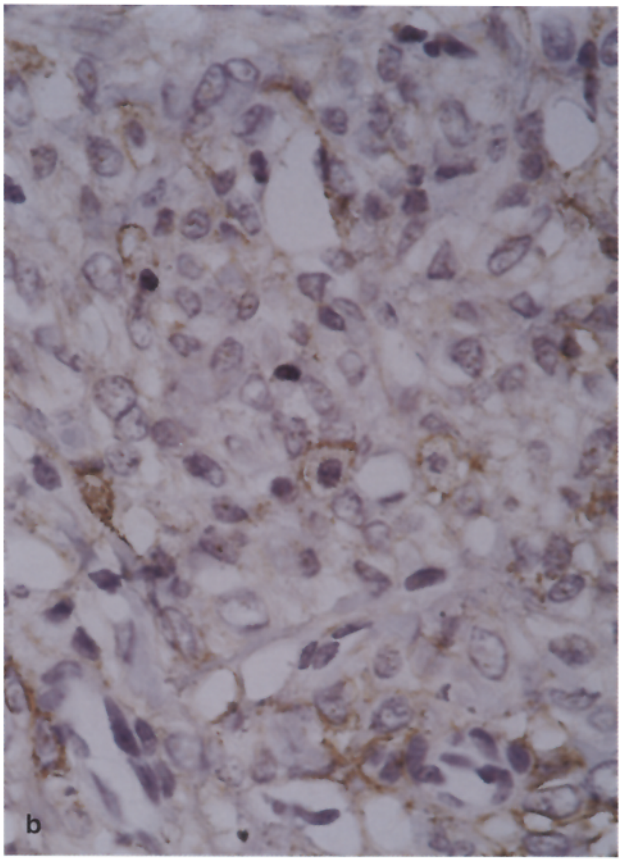
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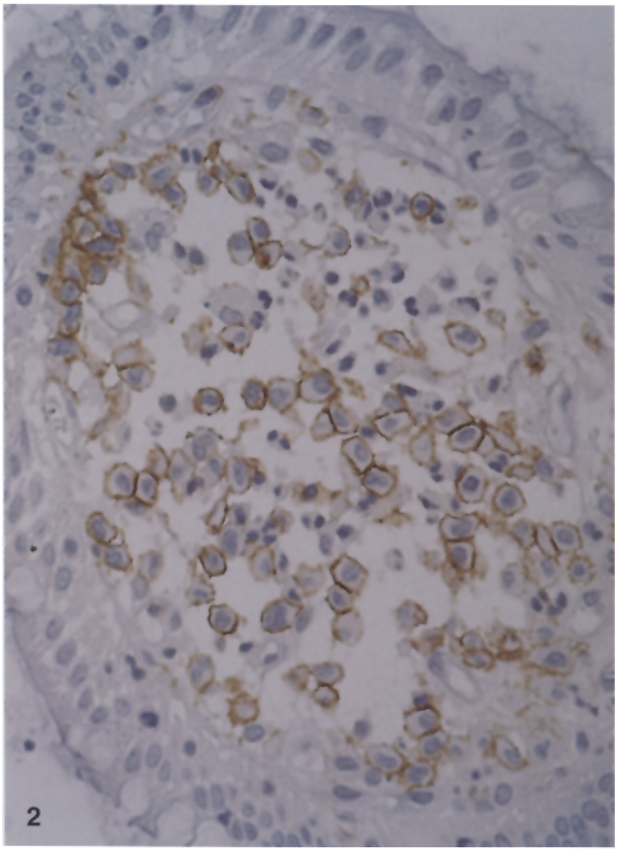
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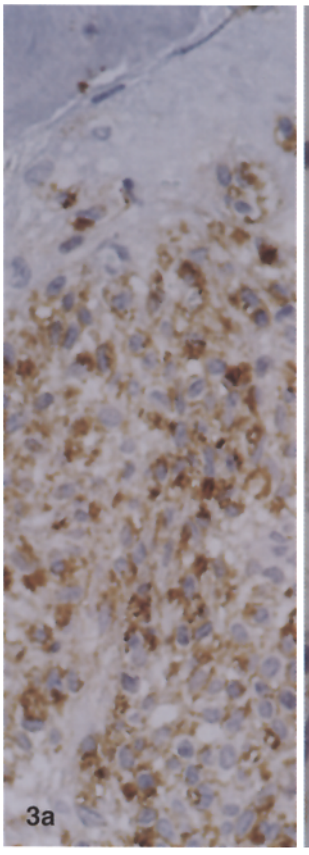
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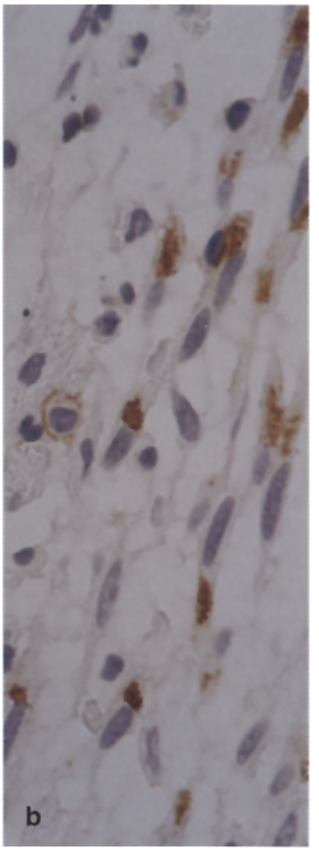
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As CD44 is a membrane-associated molecule, the strong granular cytoplasmic staining seen in a considerable proportion of both reactive and neoplastic mast cells was probably nonspecific. In a previous study we demonstrated that mast cell granules can bind certain antibodies nonspecifically through ionic linkage [7], and the same mechanism is likely to be responsible for the granular cytoplasmic staining of mast cells with DAKO-DF1485.

The exact reactivity of DAKO-DF1485 (CD44s, standard or haematopoietic isoform or CD44v, variant isoforms) is not known, but the staining pattern found in our study is compatible with reactivity with CD44s [11]. Moreover, immunohistochemical investigations on the same material with an antibody against the CD44 variant isoform v6 yielded no immunoreactivity of normal/reactive and neoplastic TMC (unpublished observations).

The strong immunohistochemical expression of the homing receptor-associated antigen CD44 by normal/reactive and neoplastic human TMC probably reflects the close interactions known to occur between these cells and the extracellular matrix. CD44 has been implicated in the migration and activation of leucocytes in immunosurveillance and inflammation, the formation of haematopoietic progenitor cells in the bone marrow, and the development of lymphoid organs. TMC have been shown to be involved in connective tissue metabolism, to exhibit an affinity for perivascular sites, and to accumulate during inflammation, the latter process requiring directed migration of mature TMC or their precursors [6, 8]. The significance of the strong, consistent expression of CD44 in TMC neoplasms remains a matter of speculation.

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