EBM/11 reactivity in malignant histiocytosis

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SUMMARY A patient presented initially with a testicular mass, which on biopsy had morphological features consistent with malignant histiocytosis. The tumour cells labelled strongly with EBM/11, a murine monoclonal antibody with high specificity for cells of the human mononuclear phagocyte system. Subsequent clinical and laboratory studies confirmed the diagnosis. As poorly differentiated tumour cells reacted with EBM/11, this antibody may be useful in positively identifying malignant tumours with histiocytic differentiation from malignancies of other types where morphological detail alone is inconclusive in tumour classification.

Malignant histiocytosis, the malignant proliferation of cells of the mononuclear-phagocyte system, was first described by Robb-Smith as histiocytic medullary reticulosis.^{1,2} The characteristic clinical presentation and histological features were interpreted as indicating that the malignant cells were histiocytes. The diagnosis of this neoplasm depends on the ability of the pathologist to recognise the histiocytic nature of the proliferating cells. Although this is relatively easy in well differentiated examples, in poorly differentiated cases the diagnosis is difficult and it may be impossible, on morphological criteria alone, to distinguish this neoplasm from undifferentiated carcinoma, Hodgkin's disease, or diffuse large cell lymphoma. The use of the term histiocytic lymphoma by Rappaport³ has added to the confusion by blurring the distinction between large malignant lymphoid cells and malignant histiocytes. Using morphological criteria alone the immunological phenotype could be predicted in only 61% of diffuse large cell non-Hodgkin's lymphomata.⁴ To overcome these difficulties a variety of special techniques, histochemical and immunohistochemical, as well as electron microscopy, have been used to make a positive identification of malignant histiocytes.⁵ While these techniques have refined the diagnostic criteria of malignant histiocytosis they are neither specific^{5,6} nor sensitive.⁷

We describe a case of malignant histiocytosis which had most of the conventional morphological, ultrastructural, histochemical and immunohistochemical features of this tumour, but which also showed a strongly positive immunohistochemical reaction with

EBM/11, a recently described monoclonal antibody which has high specificity for cells of the human mononuclear-phagocyte system.⁸

Material and methods

TISSUE PREPARATION AND ANALYSIS

The bulk of the tissue submitted for histological examination was fixed in 4% formalin and processed by routine histological techniques. Paraffin wax sections (5 μ m) were stained with haematoxylin and eosin, methyl-green pyronin, Giemsa, periodic acid Schiff with and without diastase pretreatment, Perls's Prussian blue and Gomori's reticulin.

Material for transmission electron microscopy was fixed in buffered 4% glutaraldehyde at 4°C for four hours, fixed with osmium tetroxide, dehydrated in graded alcohols through propylene oxide and embedded in Emix (Emscope). Sections (0.5 μ m) were stained with toluidine blue, and ultrathin sections from selected fields stained with uranyl acetate and lead citrate were examined by transmission electron microscopy.

Portions of fresh tissue were snap frozen in liquid nitrogen and stored at -70°C. Sections (5 μ m) were cut on a cryotome, and air dried at 22°C overnight. Immunohistochemical staining was carried out using a panel of antibodies, the details of which are included in the table. Staining for lysozyme, α -1-antitrypsin (AAT), and α -1-antichymotrypsin (ACT) was performed on formalin fixed, paraffin wax embedded tissue; all other antibody or histochemical reactions were done on cryostat sections. Paraffin wax sections were trypsinised before primary antibody was applied. The alkaline phosphatase anti-alkaline phosphatase

Table Antibodies used in this study

Antibody	Species	Mono- (M) or Polyclonal (P) (clone)	Specificity	Dilution	Source	Reference
α -1-antitrypsin	Rabbit	P	α -1-antitrypsin	1:100	Behring	5 6
α -1-antichymotrypsin	Rabbit	P	α -1-antichymotrypsin	1:800	Dako	5
Lysozyme	Rabbit	P	Lysozyme	1:100	Behring	17 21
DAKO-EMA	Mouse	M (E29)	Human epithelial and mesothelial cells	neat	Dako	26
DAKO-LC	Mouse	M (PD7/26, 2B11)	Leucocyte common	neat	Dako	27
EBM/11	Mouse	M (EBM/11)	Human monocytes, macrophages	1:10	*NDP/Dako	11
KB90	Mouse	M	Macrophages/hairy cells	neat	*NDP	22
DAKO-Pan B	Mouse	M (To15)	B lymphocytes	neat	DAKO	22
DAKO T8	Mouse	M (DK25)	Human suppressor-cytotoxic T-cells	neat	DAKO	23
UCHT1	Mouse	M	T lymphocytes	neat	Dr P Beverly	30
Anti-Leu 3a	Mouse	M	Helper/inducer T lymphocytes	neat	Becton-Dickinson	31
Mac 387	Mouse	M	Macrophages/granulocytes some epithelium	1:10	DAKO	24

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(APAAP) technique was used when the primary antibody was monoclonal; where the primary antibody was polyclonal and with the monoclonal Mac 387, a two-stage indirect immunoperoxidase method was used.⁹ Histochemical reactions for acid phosphatase, α -naphthyl acetate, and chloroacetate-esterases were carried out using standard techniques.^{10 11}

CLINICAL FINDINGS AND LABORATORY INVESTIGATIONS

A 67 year old man presented with painless haematuria. He also complained of bruising easily for three weeks. Examination showed large ecchymoses over both calves and the right inguinal area. The only other abnormal finding was an enlarged right testis measuring $11 \times 8 \times 5$ cm. There was no lymphadenopathy or visceromegaly.

The full blood count showed a haemoglobin concentration of 12.0 g/dl, a white cell count of $13.7 \times 10^9/l$, and a platelet count of $51 \times 10^9/l$. The peripheral blood film was leucoerythroblastic and the bone marrow was abnormal. The coagulation screen (prothrombin time, kaolin cephalin clotting time, and fibrinogen titre) was normal. The biochemical profile showed a urea concentration of 11.3 mmol/l (2.5 – 6.7), creatinine of 144 μ mol/l (70 – 50), aspartate transferase activity 52 of IU/l (10 – 35), gamma-glutamyl transferase activity of 97 IU/l (15 – 40), urate of 579 IU/l (200 – 450) and a much increased acid phosphatase activity of 22 IU/l (1 – 5) and lactic dehydrogenase (LDH) 1205 IU/l (70 – 170). The chest radiograph and computed tomography scan of chest and abdomen showed no lymphadenopathy or visceromegaly. Immunoglobulins were normal with no M band on electrophoresis.

In view of the right testicular mass orchidectomy was done. On the basis of the bone marrow and testicular histology, the patient was given cytotoxic

drugs, CHOP (IV cyclophosphamide 1500 mg, IV adriamycin 100 mg, IV vincristine 2 mg and oral prednisolone 120 mg, daily for eight days) alternating at two weekly intervals with IV methotrexate 375 mg (with folinic acid rescue). He received two courses in the succeeding two months and although requiring supportive blood transfusions was relatively well. Following the start of chemotherapy serum acid phosphatase fell to normal values. One week after the second pulse of methotrexate he was readmitted with a fever of 39°C and haemorrhage. He was very pancytopenic (white cell count of $0.2 \times 10^9/l$, haemoglobin concentration of 9.7 g/dl, and platelet count of $12 \times 10^9/l$). In spite of treatment (folinic acid, supportive blood, and platelet transfusions and broad spectrum antibiotics) he remained pancytopenic and died one week later. Necropsy was performed three days later.

Results

On gross examination, the right testis was partially replaced by firm homogeneous white tumour, and where residual testicular tissue was recognisable it appeared to be infiltrated by tumour. The tunica was not penetrated.

Necropsy showed that there was no superficial or deep lymphadenopathy. The spleen was of normal size and the liver was slightly enlarged (1950 g). The lumbar vertebral bone marrow was red, soft, and gelatinous. The femoral bone marrow which extended down the entire length of the shaft was of similar appearance.

HISTOPATHOLOGY

The bone marrow aspirate and a trephine biopsy specimen showed a dense infiltrate, arranged in diffuse sheets of large abnormal mononuclear cells with prominent nucleoli (fig 1). The cytoplasm was abun-

Fig 1 Section of bone marrow trephine biopsy specimen showing replacement of the marrow by tumour similar to that in fig 2. A few residual haemopoietic foci can be seen. (Haematoxylin and eosin.)

dant and vacuolated and some cells showed haemophagocytosis.

The tumour in the testis was arranged in sheets of non-cohesive cells which in places completely obliterated the normal testicular architecture but which also extensively infiltrated around normal testicular tubules. In the left testis the normal structures were preserved, although widely separated by extensive interstitial infiltrates of tumour cells. The cells were large, containing a variable amount of pale eosinophilic cytoplasm, and in some erythrophagocytosis was seen (fig 2). The nuclei varied from ovoid to highly convoluted or multilobulated. Mitotic figures were abundant and many were atypical. The nuclei were vesicular with thick nuclear membranes, clumping of chromatin, and one or more nucleoli which were not prominent. Many cells contained cytoplasmic pigment, which stained as haemosiderin. The tumours in the bone marrow and the testis were of similar appearance.

The tissues obtained at necropsy showed poor morphological detail. The bone marrow was largely replaced by benign reactive histiocytes among which were scattered tumour cells. Haemopoiesis was considerably reduced with scattered regenerative islands

alone. This contrasts with the findings at the time of diagnosis. Large malignant cells were noted in the splenic red pulp and within the liver sinusoids. The preservation of tissues at necropsy was so poor that immunohistochemistry was not performed.

HISTOCHEMISTRY

The tumour cells showed variable, in some quite strong, α -naphthyl acetate esterase activity but no reaction for chloroacetate ASD esterase. There was very weak acid phosphatase activity which had a finely granular distribution.

ULTRASTRUCTURE

Tumour cells were moderately large with abundant cytoplasm and no intercellular junctions. Cell borders were irregular with numerous pseudopodial cytoplasmic projections and ruffling of the cell membrane. There was abundant dilated rough endoplasmic reticulum and a moderate number of lysosomes. Vacuoles containing residual bodies were plentiful and occasional phagocytosed erythrocytes were noted. Mitochondria were few. The nuclei showed deep indentation and folding with coarse clumping of chromatin and large nucleoli.

Fig 2 Representative field from the testicular tumour showing large poorly cohesive tumour cells with pronounced nuclear pleomorphism and abnormal mitotic figures. Tumour cell showing erythrophagocytosis is present (arrow). (Haematoxylin and eosin.)

Fig 3 Cryostat section of testicular tumour showing diffuse strong positivity of tumour cells with EBM/11. (Alkaline phosphatase-antialkaline phosphatase.)

IMMUNOHISTOLOGY

The tumour cells were negative for epithelial membrane antigen but were uniformly positive for leucocyte common antigen showing the characteristic "membrane" pattern of staining. Most tumour cells showed strong granular positivity for lysozyme. Occasional cells showed granular cytoplasmic positivity for AAT and ACT. All tumour cells showed strong cytoplasmic positivity for EBM/11 (fig 3) and in some the reaction was strikingly intense. Only focal cells were positive for KB90. Many tumour cells were strongly positive for Mac 387. Pan B- and pan T lymphocyte markers were negative in malignant cells, although there were numerous lymphocytes in the background which stained with T cell markers.

Discussion

Apart from the unusual presentation with a prominent tumour mass in the testis, this case had the typical histological features of malignant histiocytosis.^{2 12 13 14} The leucoerythroblastic blood picture indicative of extensive bone marrow disease was also typical. The atypical cells in liver and spleen represented residual

tumour but autolysis rendered these cells unsuitable for immunohistochemical study.

The morphological detail of the tumour shown by haematoxylin and eosin staining, although typical, was not specific, and such an appearance may be seen in malignant lymphoma or anaplastic carcinoma. The enzyme histochemical reactions of the tumour cells were typical of histiocytic malignancy but, once again, not specific. Acid phosphatase has been identified in malignant lymphoma¹⁵ and α -naphthyl acetate esterase has been reported in poorly differentiated carcinomas, some of which have also shown erythrophagocytosis.⁶ The ultrastructure was likewise typical but not pathognomic.^{13 16} Lysozyme, α -1-antitrypsin, and α -1-antichymotrypsin are not specific or particularly sensitive.^{5 7 13 17 18} Their usefulness in the tissue diagnosis of poorly differentiated tumours is thus limited.

The reaction with EBM/11 was strikingly positive even in the less well differentiated tumour cells. This contrasts with the other macrophage markers. KB90 gave a focal, weak positivity. Mac 387 also reacted strongly with some, but fewer, tumour cells. This antibody, however, is known to have lower specificity for mononuclear-phagocyte system cells than EBM/11.¹⁹ The immunohistological phenotype of this tumour, and in particular the positivity for EBM/11, with its high specificity for mononuclear-phagocyte system cells,⁸ strongly supports its histiocytic differentiation.

This is the first instance of a positive reaction of EBM/11 with cells of a malignant tumour. To date, about 200 cases of malignant lymphoma have been examined with EBM/11 and none has shown a positive reaction (K C Gatter, unpublished observations). All these lymphomas have shown other specific T or B lymphocyte markers. It is worth noting that in this case even less well differentiated tumour cells gave a positive reaction with EBM/11.

Care should be exercised in inferring histogenesis of tumour cells from the presence of markers detected by monoclonal antibodies. This does not, however, detract from their usefulness in establishing the differentiation phenotype of tumours such as that described in this paper and in assisting in the more precise definition of clinicopathological entities such as malignant histiocytosis or histiocytic medullary reticulosis.

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