marrow despite extensive investigation. It is possible that a cell-mediated immune response to soluble tumour-related antigens is the pathogenetic mechanism for granuloma formation in this case.

This case illustrates the difficulty of demonstrating infiltrating lobular breast carcinoma in bone marrow using routine haematoxylin and eosin stains. It shows the value of immunohistological techniques. Demonstration of infiltration can have implications for prognosis and patient management.⁴ It also describes the formation of epithelioid granulomas in the bone

marrow in response to micrometastases of lobular breast carcinoma.

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Leu-M1 immunoreactivity and phaeochromocytoma

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Abstract

The aim was to evaluate Leu-M1 immunoreactivity as a prognostic factor in phaeochromocytoma. Anti-Leu-M1 monoclonal antibodies were used to determine the Leu-M1 immunoreactivity in 17 histologically confirmed phaeochromocytomas from 15 patients, using an avidin-biotin technique. Ten patients had a sporadic phaeochromocytoma, and five had multiple endocrine neoplasia type 2A (MEN 2A). Malignancy was diagnosed in three patients by the presence of metastases. Leu-M1 immunoreactivity was shown in 12 (70.5%) phaeochromocytomas. Three patterns of arrangement were observed: isolated (scattered positive cells) (n = 3); focal (aggregates of positive cells) (n = 5), and diffuse patterns (dispersed positive cells) (n = 4). Two cases of malignant phaeochromocytoma were positive (one

focal and one isolated pattern). All cases of MEN 2A showed immunoreactivity, although no characteristic pattern was prevalent. A diffuse pattern was observed in all phaeochromocytomas longer than 7 cm. In conclusion, Leu-M1 expression is frequent in phaeochromocytoma. However, Leu-M1 immunoreactivity seems to be useless in predicting malignant behaviour and to be influenced mainly by tumour size.

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Keywords: Leu-M1 antigen; phaeochromocytoma; multiple endocrine neoplasia.

Phaeochromocytoma is an infrequent tumour derived from chromaffin tissue. It occurs sporadically but in 10% of cases it is associated

Table 1 Clinicopathological characteristics of patients studied

<i>Clinicopathological characteristic</i>	<i>Case number</i>		3	4	5	6	7	8	9 ^a	10	11 ^a	12B	13B	14	15 ^a
	1	2													
Age (years)	38	33	67	41	48	34	47	33	39	54	28	25	19	58	23
Sex	M	F	F	F	F	M	M	M	F	F	F	M	F	F	F
Type	S	S	S	S	S	S	S	S	S	S	MEN	MEN	MEN	MEN	MEN
Clinical manifestations	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+
Size (cm)	3.5	2.7	4.5	7.8	5	6.3	8	6	5.5	3.5	5.4	2.5/4	9.5/3	10	4
CM (μmol/24 hours)	3.4	4.9	5.9	1.4	8.4	4.1	5.4	12	1.9	22.3	16.1	19.6	1.7	6	3.8
VMA (μmol/24 hours)	62.6	58	79	272	110	55.7	129	34	56	85	340	359	22	193	40.3
Leu-M1 pattern (intensity)	F (++)	—	—	D (+++)	—	F (++)	D (++)	I (+)	—	—	I (+)	F/F (+/++)	D/I (+++/++)	D (+++)	F (++)
Inflammatory component (Leu-M1 intensity)	—	—	—	—	—	—	+	—	++	—	+++	—	—	—	+++

*Malignant phaeochromocytomas.

B = bilateral; S = solitary; I = isolated; F = focal; D = diffuse; CM = urinary catecholamines (fluorometric assay, normal range 0.1-0.5 µmol/24 hours); VMA = urinary vanillylmandelic acid (colorimetric assay, normal range 3.6-52 µmol/24 hours).

Figure 1 Leu-M1 immunostaining of pheochromocytoma cells. Three patterns of arrangement can be observed: (A) isolated, showing scattered positive cells; (B) focal, showing aggregates of positive cells; and (C) diffuse, showing dispersed positive cells (original magnification $\times 280$).

with certain familial disorders. In multiple endocrine neoplasia type 2A (MEN 2A), about 40% of carriers have pheochromocytoma.^{1 2} Hypertension is the most common feature of pheochromocytoma, although patients may show a wide range of symptoms at presentation which are generally ascribed to the excessive circulating catecholamines released from the tumour. The majority of pheochromocytomas are benign, a distinction which cannot be made

histologically; malignancy requires evidence of local invasion or metastatic spread. Furthermore, the survival of patients with malignant tumours is difficult to predict on the basis of clinical, biochemical, or histopathological characteristics.¹ These facts have led to studies of prognostic factors that might predict future malignancy.^{3 4}

Recently, it has been proposed that Leu-M1 (CD15), a human myelomonocytic antigen, is useful as a prognostic factor in papillary and medullary thyroid carcinoma.^{5 6} As medullary carcinoma is associated with pheochromocytoma in MEN 2 and both are derived from chromaffin cells, Leu-M1 expression might also be observed in pheochromocytoma and be related to malignant behaviour. We studied the immunoreactivity of Leu-M1 in pheochromocytoma to investigate its relation with several clinicopathological variables and to determine whether it could have a practical use as an index of malignancy.

Methods

A series of 17 histologically confirmed pheochromocytomas from 15 patients (six men and nine women; mean age 39 years) was studied. Clinical and pathological records were surveyed to obtain the following data: age, sex, presence or absence of symptoms, type of disease (MEN or sporadic), levels of catecholamines and vanillylmandelic acid in a 24 hour urine collection, size of the tumour, and presence of local invasion or metastases (table 1).

Specimens were fixed in formalin, embedded in paraffin wax, and stained with haematoxylin and eosin for routine histological examination. Representative sections were immunohistochemically evaluated with anti-Leu-M1 monoclonal antibodies (CD15, Becton Dickinson) using the avidin-biotin complex technique (Dako) for identification of cytoplasmic antigen. Biotinylated affinity purified anti-mouse antibody and avidin-biotin peroxidase complex were used. The substrate colour reaction product was developed with 3-amino-9-ethylcarbazol (Dako). Appropriate positive controls (tonsils) were used for this antibody.

Immunostaining of the tissue was evaluated by three investigators who were blind to the light microscopic morphology and clinical history of the patients. Antibody staining was scored in a semiquantitative manner to determine the intensity of cytoplasmic immunoreactivity and arrangement pattern. Twenty high power fields were studied in all cases.

Results

Ten patients had sporadic pheochromocytoma and five had MEN 2A. Clinical manifestations were observed in 13 patients. Hypertension and headache were the presenting features in all but one patient who was admitted because of heart failure. High levels of urinary catecholamines were observed in all patients and increased vanillylmandelic acid excretion was observed in 12 cases. Three patients had a malignant pheochromocytoma (two MEN

cases and one sporadic case) confirmed by the presence of liver metastases. Bilateral pheochromocytomas were demonstrated in two patients with MEN 2A. The size of the tumour fluctuated between 2.5 and 10 cm (mean 5.3 cm).

Immunoreactivity for Leu-M1 antigen was observed in 12 (70.5%) of 17 pheochromocytomas, with three patterns of arrangement. Three tumours showed an isolated pattern with positivity in scattered cells; five tumours presented a focal pattern with aggregates of positive cells; and a diffuse pattern with dispersed positive cells was observed in four tumours (fig 1). Only two cases of malignant pheochromocytoma showed Leu-M1 immunoreactivity presenting focal and isolated patterns of arrangement. Leu-M1 was expressed in all specimens of MEN 2A although no characteristic pattern was prevalent (two isolated, three focal, two diffuse pattern). All cases with tumour size greater than 7 cm expressed intense immunoreactivity with a diffuse pattern.

The inflammatory tissue accompanying tumour cells was significant in malignant pheochromocytomas, and intense positivity for Leu-M1 antigen in myelomonocytic cells was observed. No benign cases showed inflammation except for one case in which there were a few inflammatory cells.

Discussion

The Leu-M1/CD15 antigen was initially detected in the human histiocytic cell line and it is expressed in approximately 90% of granulocytes and monocytes. Several studies have shown that immunoreactivity for the Leu-M1 antigen could be observed consistently in the neoplastic cells from patients with Hodgkin's disease. This led us to think that it could be useful in the differential diagnosis of Hodgkin's disease.⁷ However, more recent studies have shown that Leu-M1 antigen is expressed in a high percentage of T cell lymphomas and adenocarcinomas whereas it is rare in sarcomas, mesotheliomas, and melanomas. Therefore, it seems that its main value remains in the differential diagnosis of carcinoma.⁸

A few years ago, Schröder *et al*⁶ suggested that Leu-M1 immunoreactivity could be an important independent prognostic factor in papillary and medullary carcinomas of the thyroid. These investigators observed that the risks of recurrence and mortality were 3 and 4.5 times greater, respectively, for patients with medullary thyroid carcinoma without haematogenous spread but with high Leu-M1 immunoreactivity when compared with patients with low or absent Leu-M1 expression. As medullary carcinoma is associated with pheochro-

mocytoma in MEN 2, and both derive from chromaffin cells, intense Leu-M1 expression could also be related to aggressive behaviour in pheochromocytoma. On this basis, Leu-M1 expression should be more intense in malignant tumours.

In our study, we observed immunoreactivity for Leu-M1 in 70.5% of pheochromocytomas. To our knowledge, Leu-M1 immunoreactivity in pheochromocytoma has not previously been recorded. Nevertheless, the results are not surprising as this percentage of positivity is similar to those observed in other larger series of adenocarcinomas.⁸ However, from our results it seems that neither Leu-M1 immunoreactivity nor the pattern of arrangement has any relation with the clinicopathological characteristics considered in the study except tumour size. Surprisingly, and for unknown reasons, tumours larger than 7 cm presented a peculiarly diffuse pattern.

Logically, inflammatory cells identified within chromaffin tissue also showed positivity for Leu-M1, mainly on account of myelomonocytic cells. Curiously, this inflammatory component was observed in all three cases of malignant pheochromocytoma but in only one case of benign tumour. Although, to our knowledge, the characteristics of the inflammatory tissue are not regarded as a factor of prognostic importance,^{3,4} the relatively small size of our series prevents us from reaching a firm conclusion on this, and a larger study addressing this point would be warranted.

We conclude that Leu-M1 immunoreactivity is frequent in pheochromocytoma but it seems to be useless as an index of malignant behaviour and to be influenced mainly by tumour size.

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