Malignant histiocytosis of the intestine: report of three cases with immunological and cytochemical analysis

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SUMMARY Tumour cells from three cases of malignant histiocytosis of the intestine (MHI) have been studied immunologically and cytochemically. The cells did not form E rosettes and showed weak Fcγ surface receptors. They contained non-specific esterase and acid phosphatase in diffuse granular distribution. In one case tumour cells showed no staining by an immunoperoxidase technique with two monoclonal anti-T cell antibodies but positive staining with a monoclonal anti-Ia-like antibody. The malignant cells from all three cases could be shown to contain alpha-I-antitrypsin. These are the first cases of MHI to have been studied in this way and the results confirm the true histiocytic—that is, monocyte/macrophage, derivation of the tumour cells in this disease.

In 1978 Isaacson and Wright first suggested that primary gastrointestinal lymphoma complicating coeliac disease was a specific histopathological entity.1, 2 In their view the great majority, if not all, of these cases which had been variously labelled Hodgkin’s disease, reticulum cell sarcoma and immunoblastic sarcoma were a variant of malignant histiocytosis which appeared to arise in the intestine. They called this disease malignant histiocytosis of the intestine (MHI) to distinguish it from the classical form of malignant histiocytosis as defined by Byrne and Rappaport.3 In view of the immunological setting in which the disease occurs and the wide variation in both its macroscopic and microscopic appearances others have found this concept difficult to accept.4 Central to this controversy is the precise characterisation of the malignant cells. Given the nature of these cases which often present as surgical abdominal emergencies or with other features which are not necessarily suggestive of lymphoma, it has proved difficult to obtain unfixed tissue for histogenetic studies. A recent opportunity to obtain fresh tissue from three such cases has, for the first time, permitted us to carry out a detailed study of the immunological, immunohistochemical and cytochemical properties of the malignant cells comprising the disease we call MHI.

Case reports

CASE 1
A 46-year-old man who had had intermittent diarrhoea for four years was admitted complaining of severe diarrhoea of six weeks duration accompanied by a 15 lb (6.8 kg) weight loss. On examination there was clubbing of the fingers and dehydration but no other abnormality and specifically no lymphadenopathy or hepatosplenomegaly. Laboratory investigations revealed a haemoglobin of 13·2 g/dl with normal white cell and platelet counts. Lactic dehydrogenase was 161 IU/l (normal 30-90), alkaline phosphatase 138 IU/l (normal 20-90) and albumin 29 g/l (normal 35-47). Serum calcium was 2·0 mmol/l (normal 2·25-2·62). Barium meal suggested neoplastic infiltration of the small intestine and a laparotomy was performed. At laparotomy the small bowel was flaccid and atrophic in appearance and showed brown discoulouration proximally. There was no other abnormality noted and full thickness biopsies of jejunum and ileum were taken. The biopsies showed the characteristic changes of coeliac disease with villous atrophy maximal in the jejunum. The “early lesion”5 of malignant histiocytosis of the intestine (MHI) were present. Bone marrow aspirate showed malignant histiocytes some of which showed haemophagocytosis. The patient was started on a gluten-free diet with dramatic
remission of his diarrhoea and peroral jejunal biopsy
six weeks after the laparotomy showed marked
improvement of villous architecture and reduction
in lamina propria plasma cells. After this initial
improvement the patient developed swinging pyrexia
followed by generalised lymphadenopathy. A
cervical lymph node biopsy was performed two
months after admission and submitted fresh to the
laboratory. He was then started on chemotherapy.

CASE 2
A 66-year-old woman presented with upper small
intestinal obstruction in 1979. She had a 27-year
history of intermittent iron deficiency anaemia.
Laboratory investigations were normal apart from a
haemoglobin of 10 g/dl with microcytosis of red
cells. At laparotomy a stricture with two adjacent
tumour nodules was identified 25cm from the
duodenojejunal junction and this was resected
together with enlarged mesenteric lymph nodes.
Pathological diagnosis was malignant histiocytosis
of the intestine. The patient made a good post-
operative recovery but refused any further investiga-
tions or treatment. She remained reasonably well
for two years after the initial surgery but was then
readmitted with a one-month history of diarrhoea,
abdominal pain and distention. Ascites was noted
on examination and malignant cells were identified
in the ascitic fluid. A barium meal showed a perfo-
ration in the upper duodenum. At laparotomy
there was diffuse peritonitis with a perforation of
the duodenum. The duodenal perforation was biopsied
and oversewn and biopsies were taken of large,
haemorrhagic, mesenteric lymph nodes and sub-
mitted fresh to the laboratory. The patient steadily
deteriorated and died nine days after operation. A
post-mortem was performed.

CASE 3
A 53-year-old man with a five-year history of inter-
mittent diarrhoea was admitted complaining of
anaesthesia and malaise of five months duration
accompanied by a 14lb (6.24 kg) weight loss. Examina-
tion revealed a lower abdominal mass. Pertinent lab-
atory investigations included a haemoglobin of
10.3 g/dl with normal white cell and platelet counts
and liver function tests which were grossly deranged.
At laparotomy there were multiple tumour nodules in
the small intestine with a large stricturing mass of the
distal ileum accompanied by enlarged mesenteric
lymph nodes. This mass was resected and a liver
biopsy performed. Histology showed MHI with
liver involvement. The patient made a good post-
operative recovery and subsequent bone marrow
aspirate and trephine were normal. A peroral jejunal
biopsy showed villous atrophy. Chemotherapy was
commenced with dramatic improvement which was
sustained for six months. The patient then deterio-
rated with recurrence of intra-abdominal disease and
the appearance of overwhelming numbers of
malignant cells in the bone marrow, an aspirate of
which was submitted fresh to the laboratory. The
disease was no longer responsive to chemotherapy
and the patient died after a hypotensive episode. A
post-mortem was performed.

Material and methods

Sections of formalin-fixed paraffin-embedded tissue
from all three cases were examined using routine
histopathological stains and the PAP immuno-
peroxidase technique after trypsin treatment. The
antigens stained for included the three major Ig
heavy chains, K & λ light chains, J chain, lysozyme
and alpha-1-antitrypsin (α1-AT). Details of the
methods used, and controls, have been previously
described.1–8

Frozen sections of lymph node from case 1 were
acetone-fixed and stained by the method of Stein9
with monoclonal antibodies directed towards
T lymphocytes (UCH T10 and S33, unpublished,
gifts from Dr P Beverly, ICRF Human Tumour
Immunology Unit, University College, London) and
with DA-2,11 a monoclonal antibody directed to-
towards a non-polymorphic determinant of HLA-DR
(generously provided by Dr M Crompton, ICRF,
Lincoln's Inn Fields, London).

Fresh lymph node biopsy tissue from cases 1 and 2
was finely minced in RPMI 1640 containing 10% fetal
serum and bone marrow aspirate from case 3
was collected in the same medium. Mononuclear
cells from the three cases were prepared by centri-
fugation through ficoll-triosil as described pre-
viously.12 All marker tests were performed on the
washed mononuclear cells and rosetted preparations
were cytocentrifuged and stained to permit the
identification of neoplastic cells. Detailed rosetting
techniques have been described elsewhere.13 Briefly,
mononuclear cells were rosetted with washed sheep
red blood cells (RBC) at 37°C followed by a period on
ice (E rosettes) or assayed for receptors for the Fc
portion of IgG (Fcy) and IgM (Fcμ) by rosetting with
appropriately sensitised ox RBC. Ox cells were
sensitised with a subagglutinating dose of an IgM
or IgG fraction of a rabbit anti-ox RBC serum
prepared in this laboratory. To facilitate the reaction
Fc rosetting was carried out on cells that had been
incubated at 37°C overnight in the absence of
human serum. Viable cells from cases 1 and 3 were
stained for surface immunoglobulins (sIg) using a
FITC-conjugated rabbit antiserum to polyclonal
human immunoglobulins (kindly provided by Professor GT Stevenson of the Tenovus Research Laboratory, Southampton General Hospital).

Rosetted and unrosetted cytocentrifuge preparations were fixed for the demonstration of non-specific esterase (NSE) and ASD chloracetate esterase by immersion in formol acetone for 30 s followed by a water rinse and for acid phosphatase by fixation in formalin vapour. The three enzyme activities were demonstrated by the method of Yam et al and Hayhoe respectively. In cases 2 and 3 staining for NSE was performed both with and without sodium fluoride (NaF) inhibition.

Results

The immunological, cytochemical and immunohistochemical results are summarised in the Table.

**Pathological Observations**

The operative small intestinal biopsies in case 1 showed severe villous atrophy and crypt hyperplasia of the jejunum with similar, but less marked changes, in the ileum. Brown pigment was present in the muscle cells of the jejunum and in both biopsies characteristic early lesions of MHI were evident in the form of destroyed crypts with surrounding histiocytic aggregates. The cervical lymph node was replaced by a pleomorphic large cell lymphoma with numerous multinucleated cells (Fig. 1).

In case 2 the intestinal lymphoma initially resected consisted of sheets of primitive, rather monomorphic, cells with little cytoplasm and nuclei containing large prominent nucleoli (Fig. 2). Uninvolved intestine showed villous atrophy and crypt hyperplasia with focal early lesions similar to those described in case 1. Tumour infiltrates the sinuses of the mesenteric lymph nodes. Biopsies from the duodenal perforation taken at the second laparotomy showed villous atrophy and crypt hyperplasia of the mucosa and non-specific chronic inflammation in the ulcer edges and base. The resected lymph nodes showed foci of haemorrhagic necrosis and the characteristic sinusoidal infiltrate of malignant histiocytosis. At necropsy there was peritonitis and bronchopneumonia with widespread infiltration of tissues by non-adherent pleomorphic malignant cells, many of which showed erythrophagocytosis. Intrasinusoidal infiltrates were present in lymph nodes, spleen, liver and adrenal glands and malignant cells were also seen in bone marrow smears.

The intestinal tumour in case 3 consisted of sheets of large cells with abundant cytoplasm and nuclei containing prominent nucleoli (Fig. 3). Tumour cells replaced mesenteric lymph nodes and infiltrated the sinusoids of the liver. The bone marrow aspirate, obtained shortly before death, consisted almost entirely of malignant cells. At post-mortem there was a large mass of tumour in the small bowel mesentery and an enlarged liver (2150 g) and moderately enlarged spleen (285 g). Histology showed characteristic infiltrates of non-adherent tumour cells in mesenteric lymph nodes, liver sinusoids and spleen.

**Properties of tumour cells from three cases of MHI**

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E — E rosettes, ASDCA — ASD chloracetate esterase, AP — acid phosphatase, Lys — lysozyme, * weakly positive, i polytypic IgG.
INVESTIGATION OF SURFACE RECEPTORS
In cases 1 and 3 tumour cells demonstrated no surface immunoglobulin after incubation for 24 h. The malignant cells of all cases did not form E rosettes but showed weak rosetting with IgG-coated ox cells (Fig. 4). There was no rosetting with IgM-coated ox cells and no phagocytic activity was demonstrated.

CYTOCHEMISTRY
The tumour cells of all cases stained positively for NSE and in cases 2 and 3 this positive staining was inhibited by NaF (Fig. 5). Stains for ASD chloroacetate esterase were negative, while stains for acid phosphatase were positive (Fig. 6).

IMMUNOHISTOCHEMISTRY
The tumour cells in case 1 were shown to contain polytypic IgG (κ and λ chains present) while those in the other two cases were negative for all immunoglobulins. The cells in all three cases were negative for J chain and lysozyme but stained positively for α1-AT with characteristic granular staining often tucked into the nucleus (Fig. 7).

Frozen sections of case 1 showed positive staining of the surface of large numbers of lymphocytes with monoclonal antisera to T cells (UCH T1 & S33) while tumour cells were negative. Monoclonal antiserum to HLA DR (DA 2) stained numerous cells in the sections including obvious pleomorphic tumour cells.

Discussion
Previous studies of fresh material from cases of MHI have been limited to histochemical staining of
frozen sections. These studies, which showed NSE and acid phosphatase in tumour cells of three cases did not entirely exclude the possibility of a T cell origin of these malignancies since T cells can show cytochemical reactions similar to histiocytes.

T cells may also show Fcγ surface receptors and a recent report of two cases of Tγ cell lymphoma mimicking malignant histiocytosis morphologically further emphasises the pitfalls in the diagnosis of histiocytic malignancies. The malignant cells in these two cases, however, formed E rosettes and showed T cell cytochemical reactions. There can be little
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doubt of the histiocytic origin of the malignant cells in the three cases we describe in this paper since they failed to form E rosettes, showed Fcγ surface receptors and stained diffusely for NSE and acid phosphatase with inhibition of NSE staining by NaF in two of the cases. The identity of the cells in case 1 is further confirmed by their failure to react with two monoclonal antibodies to T cells and their positive reaction with monoclonal anti Ia-like antibody (DA 2) in the absence of surface immunoglobulin. The demonstration of phagocytosis both in tissue sections and cell suspensions has been stressed as a property of malignant histiocytes. While we have frequently observed phagocytosis in sections of MHI this has by no means been an invariable finding in our cases. Phagocytosis tends to be observed in better differentiated cells, and its presence is quite inconstant. In case 2 for example, there was no hint of phagocytosis in any of the surgically removed tumour tissue but it was floridly present in necropsy sections. While we do not specifically test for phagocytosis in cell suspensions we might have expected to observe it in Rossetted preparations, particularly those utilising IgG-coated ox RBC. Just as the demonstration of phagocytosis is not incontrovertible evidence for the histiocytic nature of malignant cells, neither, in our view, does the failure to demonstrate it exclude a histiocytic malignancy.

The considerable clinical and pathological differences between the three cases reported here emphasise the wide clinical spectrum of this disease and the great variation in both its macroscopic and microscopic appearances. Identification of the histiocytic nature of the malignant cells is, thus, central to the concept that MHI is a specific entity associated with coeliac disease. While this is best achieved using fresh material, difficulty in obtaining fresh specimens has led us to search for reliable immuno-histochemical markers of macrophages that could be applied to formalin-fixed paraffin-embedded tissues from these cases. We have found that positive staining for α2-AT, as demonstrated in these three cases, is a highly reliable and specific marker of both benign and malignant histiocytes. This material which has been shown to be synthesised by monocyte/macrophage cells is found consistently in the tumour cells of MHI.

As well as providing confirmatory evidence of their histiocytic derivation these cases strongly support the suggestion that the villous atrophy in MHI is indicative of true coeliac disease, rather than a secondary response to the malignant neoplasm. There was objective evidence of gluten sensitivity in case 1 and the long history of recurrent iron deficiency anaemia in case 2 is characteristic of coeliac disease. We would stress the importance of obtaining fresh tissue from more of these cases, not only to verify the above findings but also to permit cytogenetic studies and to search for possible associated viruses.

We are grateful to Dr John Howell of Poole General Hospital for help in obtaining tissue from case 1, and to Dr W Hindle for permission to publish this case. We are also grateful to Mr R Lane of the Royal Hampshire County Hospital, Winchester, for help in obtaining fresh tissue from case 2 and his permission to publish the case.

References
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