Signet ring cell lymphoma: a rare variant of follicular lymphoma

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SUMMARY A case of signet ring cell lymphoma, the eighth in published reports is recorded. This rare tumour is a variant of follicular lymphoma which may be mistaken for metastatic carcinoma. The case has been studied by light microscopy, immunohistochemistry and electron microscopy and confirms that this sub-group has rather uniform characteristics. Observations on the possible origins of the vacuoles are presented.

Since 1978, six cases of malignant lymphoma in which signet ring cells were a prominent histological feature have been recorded in published reports in the USA1 2 3 and one further case with a few such cells has also been recorded.2 One case has been recorded in Australia.4 This is a little known and rare morphological variant of lymphoma whose histological appearances may be misinterpreted if the pathologist is unaware of its existence. For this reason we wish to record the first case to appear in a British publication.

Case report

A 57-year-old man presented at another hospital with a three month history of a swelling in the left groin. His general health was excellent and he was otherwise asymptomatic. A right-sided hydrocele had been drained 18 months earlier but there was no other previous medical history. A biopsy of the swelling was done and he was referred to this hospital for treatment.

On examination he was a fit man. There was a recent biopsy scar in the left groin with a mass of residual lymph nodes deep to it, but no other peripheral lymphadenopathy was detected. The liver and spleen were not enlarged. There was a right-sided hydrocele but both testes were normal to palpation. A small basal cell carcinoma, proven by biopsy, was present on the left outer canthus.

Investigations were as follows: haemoglobin 16-8 g/dl, white cell count 6.9 × 10⁹/l (6900/mm³), platelet count 254 × 10⁹/l (254 000/mm³). Biochemical profile normal. Serum protein electrophoresis normal. Serum immunoglobulin concentrations: IgG 12 g/l (normal 8-0-16-0), IgA 1.9 g/l (normal 1-2-4-0), IgM 1.3 g/l (normal 0-5-1-6). Twenty-four hour urine immunoassay: 1.2 l of urine contained 6.3 mg of kappa light chains and 0.9 mg of lambda light chains (normal <200 mg/l). Bone marrow aspirate and trephine—normal. Chest x-ray examination showed some cardiac enlargement but was otherwise normal. Intravenous urogram—normal. Computer-assisted tomography of lower chest and abdomen did not show evidence of lymphadenopathy. In view of the difficulty in interpreting the first biopsy a further biopsy of the inguinal mass was done.

After diagnosis a radical course of radiotherapy was given, the field encompassing the para-aortic, iliac and inguinal node areas. A central mid-plane dose of 3500 cGy was given in 20 fractions over 28 days. By the conclusion of the radiotherapy there had been almost complete clinical resolution of the affected inguinal nodes. The basal cell carcinoma also received radiotherapy.

Material and methods

LIGHT MICROSCOPY

Tissue was fixed in 10% buffered formalin, processed and embedded in paraffin wax by conventional techniques. Sections (5 µm) were then stained by haematoxylin and eosin (H and E), Gordon and Sweet’s method for reticulin fibres, PAS, diastase-PAS and Alcian blue. Stains for acid phosphatase were done using a modification of the method of Yam, Li and Lam.5

Wax sections were also stained by the peroxidase-antiperoxidase technique for IgG, IgM, IgA, lambda
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and kappa light chains, and for muramidase. Frozen sections of the formalin-fixed tissue were stained for lipids by oil red O, Sudan black and Nile blue sulphate.

Touch preparations from the cut surface of the fresh lymph node were air-dried, fixed in 10% formalin and stained by H and E and Giemsa. Small blocks were also embedded in Spurr resin, sectioned at 2 μm and stained by H and E.

ELECTRON MICROSCOPY
Fresh tissue from the second lymph node biopsy was minced into 1 mm cubes and fixed in chilled 4% glutaraldehyde in Millonig's buffer. After rinsing in buffer and secondary fixation in 1% osmium tetroxide, the specimens were dehydrated in acetone and 1, 2-epoxypropane and embedded in TAAB 812. An LKB Ultrotome III was used to cut 1 μm survey sections which were stained with toluidine blue in aqueous 1% borax; ultra-thin sections were stained in aqueous uranyl acetate, then lead citrate and examined in a Kratos ex-clinical 275 electron microscope.

Results

LIGHT MICROSCOPY

Original biopsy of femoral lymph node This node was extensively necrotic and, beyond suggesting that it might well be the site of lymphoma, no firm diagnosis was made.

Second biopsy of femoral lymph node The normal nodal architecture was destroyed. Approximately 80% of the node was replaced by lymphoma of diffuse pattern but at the periphery a rim of neoplastic follicles was apparent (Fig. 1). Slight extension of lymphoma cells into perinodal tissues was present. In the areas of diffuse pattern, a high proportion of the cells were of signet ring morphology. They contained cytoplasmic vacuoles of variable size, the largest compressing the nucleus to a peripheral crescent (Figs. 2 and 3). The other cells in this area were mainly small centrocytes (small cleaved cells) having characteristic irregular nuclei with absent or inconspicuous nucleoli and sparse

Fig. 1 Neoplastic follicles are seen to the left with lymphoma of diffuse pattern to the right. Haematoxylin and eosin × 58
Fig. 2  Lymphoma of diffuse pattern containing many vacuolated cells. Haematoxylin and eosin × 232

Fig. 3  Resin-embedded sections (2 μm thick) showing the signet-ring morphology of the lymphoma cells. The nuclei of the non-vacuolated cells have an irregular (cleaved) shape. Haematoxylin and eosin × 1178

cytoplasm (Fig. 3). These features were especially clearly demonstrated in 2 μm thick resin-embedded sections (Fig. 3) and in touch preparations. The peripheral follicles were also composed of a uniform population of small centrocytes (Fig. 4); they lacked phagocytic histiocytes and had somewhat ill-defined margins. Signet ring cells were not found in the follicles.

Frozen sections of the formalin-fixed tissue stained with oil red O, Sudan black and Luxol fast blue were negative. PAS and Alcian blue stains on wax sections were negative in the vacuoles of the signet ring cells and elsewhere.

Immunoperoxidase stains for IgG, IgA, IgM, kappa and lambda light chains showed small to moderate numbers of cells which were positive for IgG and kappa light chains but no convincing staining was seen within the vacuoles.

Stains for acid phosphatase and muramidase were negative in the vacuoles.

The final histological diagnosis was malignant lymphoma, centrocytic, follicular and diffuse pattern-signet ring cell variant.

Electron Microscopy
The blocks studied by electron microscopy all came from lymphomatous tissue of diffuse pattern. Two main cell types were identified, namely, typical
centrocytes (small cleaved follicle centre cells) and signet ring cells. Most of the centrocytes were small (4-9 μm) and characterised by irregularly shaped nuclei with “blebs” and complex outfoldings of the nuclear envelopes corresponding to the nuclear pockets described by Henry as being typical of centrocytes (Fig. 5). Chromatin was condensed peripherally and there were also small clumps throughout the nucleus. Well-defined nucleoli with annular profiles were often present. The cytoplasm contained a variable amount of rough endoplasmic reticulum (RER) which formed elongated cisternae; in some cells the RER was quite well-developed. Monoribosomes were plentiful. In other aspects the cytoplasmic organelles were unremarkable. A small number of centrocytes were larger (6-11 μm) and had nuclei with less condensed chromatin; in these cells polyribosomes were plentiful.

The signet ring cells were identified as being of centrocytic lineage since, apart from the presence of their cytoplasmic vacuoles, they were ultrastructurally identical to the typical centrocytes. In particular the nuclei, although usually deformed to a crescentic of annular outline, often displayed typical nuclear pockets (Fig. 6).

Usually each cell possessed a single large vacuole (7-11 μm diam) but in some a number of smaller vacuoles (1-5 μm diam) was present (Fig. 7). The vacuoles were limited by a single smooth membrane although sometimes the membrane was absent, presumably due to imperfect fixation. In most instances the vacuolar contents were uniformly structureless and electron-lucent with occasional membranous structures either traversing the vacuole or forming closed loops within it (Figs. 5 and 7); these may have been derived by artefactual “stripping-off” of the limiting membrane or may be myelinoid figures produced by glutaraldehyde fixation. Some vacuoles contained vesicles (30-40 nm diam); in small vacuoles the vesicles tended to be numerous (Fig. 8) but in large vacuoles the vesicles were usually absent or few in number.

Multivesicular bodies (MVBs) were frequently observed adjacent to the vacuoles (Fig. 9) but were also present at sites distant from them. Most of the MVBs were round or oval and 200-250 nm in greatest diameter, conforming to the usual descriptions of this organelle; however, an occasional MVB was larger and irregular in shape (Fig. 10). The internal vesicles of the MVBs were approximately 35 nm diameter and were of identical size and appearance to those found in the vacuoles.

RER was often found intimately associated with the outer aspect of the vacuolar membrane but not in continuity with it (Figs. 5 and 6).

Discussion

Three main areas of interest exist in discussing this curious tumour.
Fig. 5  A non-vacuolated centrocyte (right) with nuclear bleb (*), and a signet ring cell with a vacuole containing loops of membrane. RER partly encircles the vacuole. Uranyl acetate and lead citrate × 19 000

Fig. 6  A signet ring cell with extensive nuclear proliferation (*) and a vacuole containing membranous loops (arrowhead) and associated RER (arrow). Uranyl acetate and lead citrate × 22 430

**Diagnostic problems**

These can be illustrated by outlining the diagnostic events in the present case. On first examination of the wax sections of the second biopsy the vacuoles were noted and we first thought that, because of their empty appearance, they might well be fixation...
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Fig. 7 Signet ring cells showing extremes of vacuole size. One cell (arrowhead) has two small vacuoles. Another (**) has a vacuole traversed by a linear membranous element. Uranyl acetate and lead citrate × 2480

Fig. 8 Small vacuole containing many small light-centred vesicles. Uranyl acetate and lead citrate × 47 520

artefacts. Study of the touch preparations and 2 μm thick resin sections showed that this was untrue and it was then suggested that the signet-ring cells might represent either metastatic mucin-producing carcinoma or, as a remote possibility, liposarcoma. The negative results with mucin and fat stains eliminated these possibilities. Close examination of the cytology of the tumour cells together with the recognition of the neoplastic follicles then led to the correct diagnosis.

The possibility of making the wrong diagnosis is further emphasised by Vernon et al.3 whose case was originally misdiagnosed as poorly differentiated adenocarcinoma.
The place of signet ring cell lymphoma in the general classification of lymphomas

On the limited evidence available signet ring lymphoma occupies a well-defined niche. The cases so far reported, with one exception, have been follicular lymphomas with a predominantly cleaved (centrocytic) cell population—that is, in the Rappaport classification: nodular, poorly differentiated, lymphocytic. Further similarities also exist in the immunoglobulin content of the tumour cells of the follicular cases as demonstrated by immunoperoxidase stains. In five of the eight reported cases (including this one) the heavy chain has been IgG and in three cases none was demonstrated; in four lambda light chain has been found, two contained kappa light chain and in two light chains were not found.

It thus appears that signet ring lymphoma is, on available evidence, an unusual variant of follicular centrocytic lymphoma with a rather constant pattern.

Fig. 9 A vacuole and associated MVBs (arrows), one appearing to fuse with the vacuole (arrowhead); *—nuclear blebs; L = lysosome. Uranyl acetate and lead citrate $\times 31025$

Fig. 10 Detail of a large irregular MVB near a vacuole (v). Uranyl acetate and lead citrate $\times 24150$
of cytoplasmic immunoglobulin production.

The possible exception to this generalisation is represented by case 2 of Van den Tweel et al. This case was diagnosed as immunoblastic sarcoma and although signet ring cells were present they were very few in number and a more prominent feature was the presence of many Russell's bodies.

The origin and nature of the vacuoles
Six cases from the previous reports have been studied by electron microscopy. In five the vacuoles have been observed to be membrane-bound and all six have contained variable numbers of intravacuolar microvesicles. Vernon et al. state that the limiting membrane of the vacuoles is continuous with adjacent smooth endoplasmic reticulum, although this feature is not illustrated. We have not been able to confirm this observation but we have noted a relation with the RER, although continuity between vacuole membrane and RER was not found. This ultrastructural pattern does not, however, resemble the accumulations of RER which formed the cytoplasmic inclusions in cases IP, RW and MS of Kim et al.

Previous workers with the exception of Moir also agree that immunoperoxidase stains demonstrate the presence of immunoglobulin within the signet-ring cells although they comment that staining is usually confined to a narrow peripheral rim of the vacuoles. The consensus thus appears to be that the vacuoles are accumulations of intracellular immunoglobulin, and Vernon et al. suggest that it is stored within the microvesicles. Previous authors have thus tended to consider the signet ring lymphomas as being in some way analogous with lymphomas in which Russell's bodies are prominent and have implied that the two types of inclusion are related phenomena, although ultrastructurally they are quite different.

Our own findings differ somewhat from most previously reported observations. The immunoperoxidase technique did not convincingly demonstrate intravesicular immunoglobulin agreeing with the results of Moir. The suggestion that the intravacuolar vesicles contain immunoglobulin might be compatible with these findings, since in our case these vesicles were sparse, many vacuoles appearing entirely empty. The small vacuoles containing many vesicles would be expected to stain for immunoglobulin and such vacuoles may perhaps have been present, but not recognisable by light microscopy, in those cells staining positively for immunoglobulin.

However, we are not convinced that the intravacular vesicles are, in fact, stored immunoglobulin and our ultrastructural observations suggest that they may be derived from MVBs. The evidence for this lies in the frequent proximity of MVBs to the vacuoles and the identical size and structure of the vesicles in the MVBs and the vacuoles although only one example where an MVB appeared to be in continuity with a vacuole could be found (Fig. 9). MVBs are a form of lysosome and structures interpreted as giant MVBs by Ghadially were reported in multiple myeloma by Sorenson (1961) and in reticulosarcoma cells by Vasquez, Pavlovsky and Bernhard. Thus, it might be that the vacuoles in signet ring cell lymphomas are also giant MVBs, although our negative results with stains for lysosomal enzymes are against this hypothesis.

Addendum
Since this paper was accepted for publication a further case of signet ring cell lymphoma has been reported from Italy. However, in this case the cellular inclusions were of the "Russell's body" type.

References