Signet ring cell lymphoma of T cell type

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SUMMARY A rare variant of non-Hodgkin's lymphoma, signet ring lymphoma of T cell phenotype (only the fourth to be reported) in a 75 year old man was studied by light microscopy, immunohistochemistry, electron microscopy and gene rearrangement studies. Ultrastructurally, a wider spectrum of cell size and nuclear shape was observed in this case than in the previously recorded cases. The morphology of the signet ring vacuoles was identical to that found in the commoner B cell signet ring lymphoma of clear vacuole type, and it is suggested that the vacuoles derive from multivesicular bodies.

The four cases reported so far have all presented with skin disease, and the limited evidence available suggests that the prognosis may be good.

Signet ring lymphoma is a rare histological variant of non-Hodgkin's lymphoma.^{1–5} The signet ring appearance is due to nuclear distortion by a cytoplasmic structure which is either a solid Russell body-like inclusion^{2,3} or a clear vacuole.^{1,4} The first examples of these lymphomas were recognised as being of B cell lineage by demonstration of immunoglobulin production, or because they were of follicular centroblastic/centrocytic type. Recently, however, two reports have been published which indicate that the clear vacuole type of signet ring appearance rarely occurs in T cell lymphoma.^{6,7} We report here a further case of cutaneous T cell signet ring lymphoma, with histological, immunohistochemical, ultrastructural and gene rearrangement studies.

Case report

A 75 year old white man presented with a 12 month history of a painless, slowly growing pimple on his chin. His general health was otherwise good. On examination he was thin but fit. He had two pale firm lesions (each 0.7 cm in diameter) on the left side of his chin, which were biopsied. On examination at referral to this hospital he was found to have a further small pink papillomatous lesion (0.25 cm in diameter) medial to the previous biopsy scar. He was also noted to have a single small mobile left axillary lymph node and a slightly enlarged smooth liver, but no splenomegaly. Tests showed mild iron deficiency anaemia

and a minimally raised alkaline phosphatase activity. In other respects his blood count and other biochemical variables were normal. Over the next three months the chin nodule grew slowly larger, and it was further excised and submitted fresh for histological examination. Fifteen months after presentation the patient remained well with no additional treatment.

Material and methods

LIGHT MICROSCOPY

Tissue was fixed in 10% buffered formalin, processed, and embedded in paraffin wax by conventional techniques. Sections were stained by haematoxylin and eosin, Gordon and Sweet's method for reticulin, periodic acid Schiff (PAS) with and without diastase pre-digestion and alcian blue (pH 2.5). Frozen sections were stained with oil red O for lipid. Small blocks were also processed into Spurr resin cut at 2 µm and stained with haematoxylin and eosin and Giemsa.

IMMUNOHISTOCHEMISTRY

Fresh tissue blocks embedded in OCT compound were snap-frozen in liquid nitrogen and sections were stained by the avidin-biotin technique with the antibodies listed in the table. Wax sections were also stained as indicated.

ELECTRON MICROSCOPY

Fresh tissue from the second biopsy specimen was cut into millimetre cubes, fixed in chilled 2.5% glutaraldehyde in 0.1M cacodylate buffer, and processed as described previously.⁸

Table

CD No	Antibodies	Source	Reactivity	Results (Proportion of cells staining)
1	OKT6	Ortho	Cortical Thymocytes Langerhans' cells	Negative
2	T11	Dako	} T cells	10–20%
3	OKT11	Ortho		
3	UCHT1	Unipath	} T cells	50–60%
4	OKT3	Ortho		
4	T4	Dako	} Macrophages Helper/inducer T cells	50–60%
4	OKT4	Ortho		
	Leu3a + b	Becton-Dickinson		
5	T1	Dako	} T cells	10–20%
	OKT1	Ortho		
7	T2	Dako	} T Cells	10–20%
8	T8	Dako		
	OKT8	Ortho	} Cytotoxic/suppressor T cells Splenic sinusoidal cells	10–20%
19	CD19	Dako		
	L26*	Dako	} B cells	Negative
45	LCA*	Dako		
45R	UCHL1†	Unipath	} Most white cells T cells	About 100%
45R	4KB5†	Dako		
	IgA/D/G/M†	Dako	} B cells	About 90%
	K and λ†	Dako		
	α-l-antichymotrypsin*	Dako	} B cells	Occasional cells
	α-l-antitrypsin*	Dako		
	OKT9	Ortho	} Many cells including T cells	Positive paranuclear dot
	OKT10	Ortho		
	Cam 5.2*	Becton-Dickinson	Anti-human transferrin	50–60%
			Anti-human stem cell	Negative
			Low molecular weight cytokeratin	Negative

Immunostaining performed on frozen tissue unless otherwise specified.

*Performed on wax sections.

†Performed on wax and frozen material.

DNA ANALYSIS

Fresh frozen tissue was subjected to immunoglobulin and T cell receptor gene rearrangement studies. DNA was digested with *EcoRI*, *Bam HI*, *Hind III* and *Bgl II* restriction enzymes. Hybridisation was performed with an immunoglobulin heavy chain joining region JH probe (C76R51A),⁹ a TCR β chain constant gene probe (Cβ),¹⁰ and a TCR γ chain joining gene probe (Jγ).¹¹

Results

LIGHT MICROSCOPY

The two biopsy specimens had similar appearances and contained well defined nodules in the dermis, separated from the overlying epidermis by a narrow zone of normal dermis (fig 1). There were three populations of lymphoid cells, arranged in a diffuse pattern with no evidence of follicle formation (fig 2). Most of the cells were medium to large with round, oval, or slightly bean-shaped nuclei with finely granular chromatin and one or two marginal nucleoli. The cytoplasm was scanty and faintly eosinophilic. The second population was of smaller cells with slightly irregular and hyperchromatic nuclei and scanty cytoplasm. Scattered throughout were cells with a single clear cytoplasmic vacuole, which distorted

the nucleus to a peripheral crescent, producing a signet ring appearance (figs 2 and 3). These cells accounted for some 3–5% of the population in the first biopsy specimen but 15–20% in the second. Also present was a smattering of eosinophils and occasional plasma cells. Moderate vascular proliferation was seen with some prominence of vascular endothelium. The vacuoles did not stain for mucin with PAS or alcian blue, or for lipid with oil red O on frozen section.

IMMUNOPHENOTYPING

Fresh frozen material was not available from the first biopsy specimen and immunostaining of wax sections was technically unsatisfactory. The results of immunoperoxidase staining of both fresh frozen and paraffin wax embedded material from the second biopsy specimen are shown in the table. In summary, the tumour cells (both large, small, and signet ring forms) stained variably for T cell markers but were uniformly negative for B cell markers, although a small population of polyclonal plasma cells was present. No immunohistochemical staining was seen within the signet ring cell vacuoles. The tumour cells also exhibited a paranuclear focus of positivity for α-l-antitrypsin. The lymphoma was classified as a peripheral T cell lymphoma with a signet ring appearance, not of mycosis fungoides or Sézary cell type.

Fig 1 Low power view showing diffuse lymphoid infiltrate and its separation from overlying epidermis by narrow zone of normal dermis. (Haematoxylin and eosin.)

Fig 2 Higher power view showing neoplastic lymphoid infiltrate with large cells, small cells, and cells with distorted peripheral nuclei and clear cytoplasmic vacuoles (signet ring cells). (Haematoxylin and eosin.)

ELECTRON MICROSCOPY

The main cell types were confirmed by electron microscopy (fig 4). Large cells, 9–15 μm across, had nuclear outlines which varied from irregular or polylobated to oval or round. There were occasional central or peripheral nucleoli and little marginal heterochromatin. The cytoplasm contained moderate numbers of mitochondria, a few narrow and elongated cisternae of rough endoplasmic reticulum, a Golgi body, free polyribosomes and an occasional lipid droplet. Some cells had moderate numbers of small dense lysosomes as well as dark-staining and light-staining multivesicular bodies; the latter were intermediate in size between the vacuoles of the smaller cells and multivesicular bodies of "conventional size".

The smaller cells, 6–10 μm across, also showed a varied nuclear outline but had more marginal heterochromatin than the larger cells, as well as a compact, often ring-shaped, nucleolus. The cytoplasm was similar except for the presence of mono- rather than polyribosomes. Some small cells contained a giant

vacuole up to 6 μm across bounded by a single membrane and containing spherical membranous microvesicles 50–60 nm in diameter (fig 5). Smooth endoplasmic reticulum and intermediate filaments were often found close to the vacuoles. These cells had small numbers of light and dark multivesicular bodies, also containing microvesicles similar in size and appearance to those in giant vacuoles (fig 6). The microvesicles in the light multivesicular bodies and giant vacuoles varied in number and were often peripheral, leaving a clear central area (fig 5). Those in dark multivesicular bodies were more closely packed and were more often admixed with elements of amorphous dense material (fig 6). Dense lysosomes were present in variable numbers.

Other similar small cells lacked a giant vacuole and contained varying numbers of small and large, light and dark multivesicular bodies, and lysosomes. These cells often had a reniform nucleus. A separate population of small cells had highly irregular ribbon-like nuclear profiles (fig 4). These never had a giant vacuole

Fig 3 High power view of signet ring rich area of lymphoma. (Haematoxylin and eosin.)

Fig 4 Low power electron micrograph showing range of same cell types as in fig 2. Inset: cells with ribbon-like and convoluted nuclei.

Fig 5 *Typical signet ring cell with large vacuole containing peripheral microvesicles.*

Fig 6 *Signet ring cell showing somewhat smaller vacuole than that seen in fig 5; light (L) and dark (D) multivesicular bodies and Golgi body (G).*

but contained an occasional small multivesicular body.

DNA ANALYSIS

The tissue gave germline immunoglobulin gene arrangements with the JH probe. Both the TCR β and TCR γ chain probes, however, gave rearranged bands on hybridisation, consistent with a T cell origin for the lymphoma.¹²

Discussion

Until recently all recorded examples of signet ring lymphoma have been of B cell lineage, and to date 27 cases have been described.⁶ These include two variants: those with a Russell body-like inclusion and those with a large clear vacuole. The B cell lineage of these lymphomas has been shown by their immunoglobulin production, including immunoglobulin staining of the inclusion or vacuole (IgM in the Russell body type and IgG in the clear vacuole type). The clear vacuole type is always of follicle centre cell origin and usually follicular in pattern.⁵

Since 1985 three examples of clear vacuole signet ring lymphoma of T cell phenotype have been documented,^{6,7} and the case recorded here is the fourth. In the case report by Grogan *et al*⁷ immunostaining for T cell markers of both the cell surface membrane and also within the cytoplasmic vacuole occurred. In all of these cases the ultrastructure of the clear vacuole was similar to that recorded for the commoner B cell type—that is, membrane-bound and containing microvesicles. In the B cell form some authors have claimed that the microvesicles contain immunoglobulin, inferring this from the positive, but usually weak, immunoglobulin staining observed in the vacuole at light microscope level.²⁻⁴ In a previous study of a B cell signet ring lymphoma, Harris *et al* were unable to obtain convincing staining in the vacuole, expressed uncertainty as to the immunoglobulin content of the microvesicles, and suggested that the vacuoles might be giant multivesicular bodies.¹ Although they are exceptionally large and often contain few microvesicles, they conform none the less to the definition of a multivesicular body as a single-membraned structure containing microvesicles.¹³

Our observations of a continuous size-distribution between giant vacuoles and smaller structures that could be taken to be multivesicular bodies and of a similarity of size, appearance, and distribution of microvesicles further support this interpretation. We suggest that “multivesicular bodies of giant vacuole type” occur as ultrastructurally similar forms in B and T signet ring cell lymphomas. As some multivesicular bodies are endocytotic and are concerned with mem-

brane recycling,¹⁴ the interpretation of giant vacuoles as multivesicular bodies would satisfactorily explain vacuolar staining for immunoglobulin and T cell antigens, both of which are found on the plasma membrane. In both B and T lymphomas, therefore, multivesicular bodies of giant vacuole-type may represent a defect in plasma membrane recycling.⁷ We are currently engaged in a detailed study of multivesicular bodies in non-Hodgkin's lymphomas to be published elsewhere.

The four T cell signet ring lymphomas so far recorded have shown some morphological variation. Ultrastructurally, our case shows a wider spectrum of cell size and nuclear shape than has been observed so far. Grogan *et al* also described the vacuolated neoplastic cells as large and irregular with little heterochromatin and prominent nucleoli.⁷ The cells of our case fitting this description, however, had only small multivesicular body-type inclusions, and the large vacuoles and the signet ring appearance were confined to the smaller cells. Small cells with ribbon-like nuclei reminiscent of cells in mycosis fungoides and Sézary's syndrome have not been mentioned in previous cases, but such cells may be a reactive component within a lymphoma.¹⁵

As indicated in a previous publication,¹ the differential diagnosis includes a signet ring carcinoma, round cell liposarcoma, and now both B and T cell variants of signet ring lymphoma. This indicates the need to include both B and T cell markers in any panel of antibodies when attempting to make the distinction, although the immunophenotype of the T cell lymphomas may be inconstant and anomalous.¹⁶⁻¹⁸ In our experience the demonstration of the T cell markers was better in frozen material than in paraffin wax embedded tissue, and this may be of practical importance when attempting to establish the diagnosis. This is the first case in which gene rearrangement studies have confirmed the T cell lineage of the lymphomatous signet ring infiltrate.

The three previously reported cases of T cell signet ring lymphoma and this fourth case had similar clinical features. They presented in elderly white men as long-standing nodular skin lesions predominantly on the head and neck. Three were treated with local or wholebody radiotherapy and combination chemotherapy with good response, despite nodal and even widespread disease (clinical stage IV in one case), with no evidence of disease recurrence after two and a half and four years in two cases.⁶ Our case, some 15 months after presentation, received no specific treatment (apart from the removal of the skin nodule) and has shown no evidence of disease progression.

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