Monoclonal antibody (UCHL1) that recognises normal and neoplastic T cells in routinely fixed tissues

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SUMMARY UCHL1 is a murine monoclonal antibody that recognises a 180–185 kD determinant on CD4 (72%) and CD8 (36%) positive T cells. This antibody is effective in formalin fixed and paraffin embedded tissues, using the immunoperoxidase method. One hundred and forty three cases of malignant lymphoma were examined. Neoplastic cells in 100% of cases of *Mycosis fungoides* (n = 10), 83% of cases of peripheral T cell lymphoma (n = 25), and 78% of cases of (T-ALL) T acute lymphoblastic lymphoma (n = 9) were stained by this antibody. In addition, staining was seen in 100% of cases of malignant histiocytosis of the intestine (n = 13), a condition now thought to be a T cell lymphoma. Two cases of true histiocytic lymphoma were also positive. This antibody stained neither the neoplastic cells in a wide range of B cell lymphomas (n = 62) nor Reed-Sternberg cells in 16 cases of Hodgkin's disease. UCHL1 also stained neoplastic cells in four cases of granulocytic sarcoma. A panel of normal tissues was similarly studied. Staining was seen in normal T cells and mucosal intraepithelial lymphocytes, macrophages, mature myeloid cells, and endometrial stromal granulocytes.

UCHL1 is a monoclonal antibody that identifies T cells in formalin fixed paraffin embedded tissues, and should prove useful for diagnosing T cell lymphomas, especially when only formalin fixed tissue is available for diagnosis.

Many monoclonal antibodies to T cell related membrane antigens have been produced. These antibodies effectively stain T cells in cryostat sections of fresh frozen tissue, cell suspensions, and cytological preparations. To date there have been no reliable T cell markers for routinely fixed wax embedded tissue. This is a serious limitation as most routine histological material arrives in fixative. Furthermore, the quality of cryostat sections is inferior to that of paraffin embedded tissue. Unlike B cell lymphomas, there is no reliable method of assessing clonality in a malignant T cell proliferation. Diagnosis in these instances rests on showing the presence of T cell specific markers on the neoplastic population of cells, a population often difficult to identify with certainty in frozen sections. The use of monoclonal antibodies effective on paraffin embedded tissue will overcome these difficulties. We describe the use of a new monoclonal antibody (UCHL1),¹ which recognises a T cell related membrane antigen on normal and up to 86% of neo-

plastic T cells. Not only is this antibody an effective T cell marker in cryostat cut sections, but reliable membrane staining of T cells is seen in routinely fixed wax embedded tissue.

Material and methods

MONOCLONAL ANTIBODIES

UCHL1

This murine (IgG2a monoclonal antibody) was produced by conventional procedures using the spleen from a BALB/c mouse that had been extensively immunised with T cells from an IL-2 dependent T cell line (CA1).¹

SOURCE OF TISSUE

Normal tissues

Formalin fixed paraffin embedded normal tissues were taken from the surgical pathology files at this hospital (Table 1). Six fetal thymuses covering gestational ages from 20 weeks to full term were taken at necropsy within 72 hours of death.

Tonsils from routine tonsillectomies were fixed in

Table 1 Normal tissues examined using UCHL1

Tissue	Cytoplasmic staining	Membrane staining
Lymphoreticular		
Tonsil (n = 8)		
Lymph node (n = 10)	Smooth muscle, macrophages follicle centre cells	T cells, polymorphs, macrophages
Spleen (n = 2)		
Bone marrow (n = 6)		T cells, mature myeloid cells
Adult and fetal thymus (n = 7)	Hassall's corpuscles, smooth muscle	90% cortical thymocytes, 50% medullary thymocytes cortical blasts negative
Other tissues		
Lung (n = 1)	Alveoli, smooth muscle	Alveolar macrophages
Tongue (n = 1)	Epithelium, smooth muscle	
Stomach (n = 5)	Epithelium, smooth muscle	
Jejunum (n = 2)	Epithelium, smooth muscle	Intraepithelial lymphocytes
Ileum (n = 1)	Epithelium, smooth muscle	Intraepithelial lymphocytes
Colon (n = 2)	Smooth muscle	
Liver (n = 1)	Hepatocytes	
Gallbladder (n = 1)	Epithelium, smooth muscle	
Breast (n = 1)	Epithelium, smooth muscle, stroma	
Ovary (n = 1)	Smooth muscle	
Testis (n = 2)	Epithelium, smooth muscle	
Prostate (n = 1)	Smooth muscle	
Endometrium (n = 1)	Epithelium, smooth muscle	Stromal granulocytes
Cervix (n = 1)	Smooth muscle, squamous epithelium	
Synovium (n = 1)	Smooth muscle	
Nerve (n = 1)		
Thyroid (n = 2)	Epithelium, smooth muscle	
Parathyroid (n = 2)	Epithelium, smooth muscle	
Bladder (n = 1)	Epithelium, smooth muscle	
Placenta (n = 1)	Syncytiotrophoblast, cytotrophoblast	Hofbauer cells

unbuffered formol saline, neutral buffered formol saline, Bouin's fixative, mercuric fixatives, and formalins of pH ranging from 7.0 to 1.9.

Bone marrow smears from six patients undergoing routine haematological investigation were air dried

Table 2 Details of negative staining malignant B cell lymphomas studied

	No of cases
Nodal lymphomas	
Burkitt's lymphoma	4
Lymphoblastic (non-Burkitt)	1
Lymphocytic lymphoma	4
Lymphoplasmacytic lymphoma	2
Centrocytic lymphoma	4
Centroblastic and centrocytic	11
Centroblastic lymphoma	7
Immunoblastic lymphoma	4
	37
Extranodal lymphomas	
MLP*	4
<i>Centroblastic and centrocytic:</i>	
Stomach	5
Lung	1
Skin	1
Mediastinum	1
Testis	1
Tongue	1
Ileum	1
<i>Centroblastic:</i>	
Stomach	1
Ileum	2
Thyroid	1
Hairy cell leukaemia (spleen)	1
Plasmacytoma	5
	25

*Multiple lymphomatous polyposis of the intestine

for between one and 72 hours. If staining was not undertaken after this period they were stored at -70°C .

Neoplastic tissues

Tables 2-4 detail the routinely fixed and wax embedded lymph nodes and extranodal tissues studied. The cases were taken from the surgical pathology files at University College Hospital, the Institute of Laryngology and Otology London, and the Middlesex Hospital, London. All lymphomas were classified according to a modified Kiel classification.² The results of immunophenotyping in frozen tissue were available in 27 of 44 cases of T cell lymphoma,

Table 3 Details of Hodgkin's and non-B non-T lymphoreticular tumours and their reactivity with UCHL1

Type of tumour	No of tumour	No positive with UCHL1
True histiocytic	2	2*
Malignant histiocytosis of the intestine	13	13
Common acute lymphoblastic leukaemia	2	0
Granulocytic sarcoma	4	4†
<i>Hodgkin's disease</i>		
Lymphocyte predominant	1	0‡
Mixed cellularity	6	0‡
Nodular sclerosis	6	0‡
Lymphocyte depleted	3	0‡

*Weak focal membrane staining.

†Staining in mature myeloid cells only.

‡Reactive T cells positive, cytoplasmic staining in Reed-Sternberg cells.

Table 4 Details of T cell tumours studied using UCHL1

Histological type	No	UCHL1 positive	Total percentage positive
Lymphoblastic	9	7	78
<i>Mycosis fungoides</i>	10	10	100
T zone	2	2	100
Similar to angioimmunoblastic lymphadenopathy	3	3	100
Pleomorphic	15	12	79
Monomorphic	2	1	50
HTLV-1 positive	3	3	100
Total	44	38	86

26 of 62 cases of B cell lymphoma, both cases of true histiocytic lymphoma, and in two of 13 cases of malignant histiocytosis of the intestine.

STAINING METHODS

Paraffin and cryostat sections

UCHL1 was tested at various dilutions from 1/1 to 1/20. Subsequently, UCHL1 was used as a neat supernatant as the first layer in the indirect immunoperoxidase reaction. Paraffin sections cut at 3–4 μ m and cryostat sections at 6 μ m were stained by the indirect immunoperoxidase method, details of which have been described previously.³

Bone marrow smears

Marrow smears were either stained within 72 hours or

were stored at -70°C and thawed for staining. Smears were stained by the APAAP method according to Mason *et al.*⁴

Results

In both cryostat cut and wax embedded tissue positive staining with UCHL1 was characteristically crisp and ring like, outlining the cell membrane. This pattern was identical with that seen with other T cell membrane markers in cryostat sections. In addition, large cells in many cases gave diffuse cytoplasmic staining, and staining was seen in the connective tissue.

DILUTION

UCHL1 supernatant was effective at a range of dilutions from 1/1 to 1/20. The degree of background staining was reduced at a dilution of 1/5, but the proportion of T cells staining decreased in number. UCHL1 was therefore used as a neat supernatant throughout the study.

FIXATION

The optimum reactivity with UCHL1 was seen in those tissues fixed in neutral buffered formol saline. Staining was not as clear in tissues fixed in formalins in the pH range 7.0 to 3.5. Increasing non-specific background staining and poor membrane specificity

Fig. 1 Paraffin section from normal tonsil stained with UCHL1. Interfollicular T cells are recognised, together with scattered cells within a follicle centre. Mantle zone cells and follicle centre cells are negative. (Immunoperoxidase.) $\times 100$.

Fig. 2a Detail from ulcerated ileal tumour from a case of malignant histiocytosis of the intestine. Patient had long history of coeliac disease. Tumour cells are large with open nuclei, plentiful cytoplasm, and indistinct cytoplasmic borders. (Haematoxylin and eosin.) $\times 800$.

Fig. 2b Paraffin section from same case as Fig. 2a stained with UCHL1. Strong membrane staining is seen in neoplastic cells. Granulocyte with weaker membrane staining is also present. (Immunoperoxidase.) $\times 500$.

Fig. 3a Pleomorphic T cell lymphoma from patient with positive HTLV1 serology. Tumour is composed of bizarre mononuclear and multinucleated cells with convoluted nuclei and multiple nucleoli. (Haematoxylin and eosin.) $\times 500$.

Fig. 3b Paraffin section from same case as Fig. 3a stained with UCHL1. Crisp membrane staining is seen outlining individual neoplastic cells. (Immunoperoxidase.) $\times 500$.

Fig. 4a High power detail from case of angioimmunoblastic lymphadenopathy like T cell lymphoma. Note vascular background with small and medium sized convoluted T cells, scattered histiocytes, and occasional blast cells. (Haematoxylin and eosin.) $\times 500$.

Fig. 4b Paraffin section from same case as Fig. 4a stained with UCHL1. Small and medium T cells are recognised by antibody. Large scattered neoplastic blasts are clearly identified. (Immunoperoxidase.) $\times 500$.

were serious limitations when tissues fixed in formalins of pH < 3.1 were studied. Bouin's fixative and mercurial fixatives were wholly unsuitable at preserving the membrane antigen recognised by UCHL1. Proteolytic digestion with trypsin and the use of imidazole in the development of the peroxidase reaction both introduced unacceptably high non-specific background staining.

In necropsy tissue taken within 72 hours of death the cytology was poor, but the membrane antigen was still preserved. Bone marrow trephines that had been decalcified in formic acid for 24 hours following fixation were also found to give adequate results with UCHL1.

NORMAL TISSUES

Table 1 summarises the normal tissues studied and their reactivity with UCHL1. Lymphocytes in the T cell areas of normal tonsil (Fig. 1), spleen, and normal and reactive lymph nodes were recognised by UCHL1. In thymic tissue 90% of cortical thymocytes and about 50% of medullary thymocytes were stained at all ages studied. In contrast, the large cortical thymic blasts were negative. Membrane staining in mature myeloid cells and about 20% of macrophages was a constant finding in all the tissues sampled.

Bone marrow smears were stained by the APAAP method to overcome the high concentrations of endo-

genous peroxidase. Cells of megakaryocytic and erythroid lineages were uniformly negative. The cells of the myeloid lineage showed a gradation of staining intensity. The mature granulocytes stained most strongly, although never as intensely as the lymphocytes present. The strength of staining decreased with increasing immaturity, the promyelocytes giving only faintly positive results.

Diffuse cytoplasmic staining was seen in glandular epithelia of the upper gastrointestinal tract, endometrium, gallbladder, and breast. Cytoplasmic staining was also seen in squamous epithelium, transitional epithelium, hepatocytes, syncytiotrophoblast and cytotrophoblast, and all smooth muscle. The intensity of this staining varied from case to case and with the type of fixation. The importance of this staining is unknown, and its intensity was rarely strong enough to obscure the strong membrane staining of T cells. In all of the normal tissues studied lymphoid and a proportion of normal myeloid cells and macrophages were stained. Notable among these were mucosal intraepithelial T cells, endometrial stromal granulocytes, and placental Hofbauer cells.

NEOPLASTIC TISSUE

B cell lymphomas

UCHL1 did not stain the neoplastic cells in any of the cases listed in Table 2. Some large B cell lymphomas

of centroblastic and immunoblastic types, however, gave diffuse cytoplasmic staining with UCHL1.

Of the B cell neoplasms, those which failed to stain with the antibody were those of true centrocytic type and cases of multiple lymphomatous polyposis. These cases are interesting because they show cross reactivity with the T cell related marker CD5, recognised by antibodies such as Leu-1.^{5,6} In all cases reactive T cells were stained as well as a proportion of myeloid cells.

Hodgkin's disease

Tissue from 16 cases of Hodgkin's disease representing the major histological subtypes was examined (Table 3). Reed-Sternberg cells and their mononuclear variants, as well as the L and H cells of lymphocyte predominant nodular Hodgkin's disease, were not stained by UCHL1. Cytoplasmic staining was observed in many Reed-Sternberg cells. The reactive infiltrates of T cells were positive, including the rings of T helper cells disposed around the Reed-Sternberg cells.⁷ Reactivity with myeloid cells was also seen.

Leukaemic deposits and granulocytic sarcoma

Four cases of granulocytic sarcoma were examined; two cases from the gastrointestinal tract, one from the skin, and one from lymph node. Membrane staining with UCHL1 was seen in all cases. The staining was restricted to the more mature myeloid cells; these findings were similar to that seen in normal marrow. Soft tissue deposits from two cases of common acute lymphoblastic leukaemia showing a pre-B phenotype were also studied. Neither case was stained by UCHL1.

True histiocytic lymphoma (of monocyte or macrophage derivation)

Two cases of true histiocytic lymphoma were studied. Their immunophenotype had been determined on cryostat cut sections.⁸ UCHL1 gave weak focal membrane staining similar to that seen in reactive macrophages in normal tissues.

Malignant histiocytosis of the intestine

Thirteen cases of malignant histiocytosis of the intestine were examined. All of the cases expressed membrane staining with UCHL1 (Fig. 2). Two of the cases had shown a T cell immunophenotype determined on cryostat sections and a genetic rearrangement at the T cell receptor β chain locus.⁹

T cell lymphomas

Forty three cases of T cell lymphoma were studied (Table 4). Ten cases were of classical *Mycosis fun-*

goides of the erythrodermic and plaque stages, nine were T lymphoblastic lymphomas, and the remaining 25 encompassed a wide range of T cell lymphoma phenotypes. All cases of lymphoblastic lymphoma and 16 cases of peripheral T cell lymphoma had their immunophenotype determined on cryostat cut material. The remaining five cases of peripheral T cell lymphoma and all but two cases of *M fungoides* had been diagnosed on morphological grounds. Neoplastic cells in all of the cases of *M fungoides* were stained by UCHL1. Staining was seen in all three cases of HTLV-1 positive T cell lymphoma (Fig. 3) and seven of the nine cases of lymphoblastic lymphoma. UCHL1 stained the neoplastic cells in 18 of the 22 remaining cases of peripheral T cell lymphoma (Fig. 4).

Discussion

The approach to lymphoproliferative disorders has been greatly influenced by the advent of immunocytochemical techniques. Numerous polyclonal and monoclonal antisera to cells of B, T, and macrophage derivation are now available. Their principle disadvantage lies in their failure to stain routinely fixed histological material reliably. Some markers of the macrophage have been described,¹⁰ which are effective in paraffin embedded tissue, and recently the antibodies LN-1 and LN-2 have been described, which recognise B cells in B5 fixed tissues.¹¹ Antibodies to HLA-D region antigens, which are useful in the diagnosis of lymphoreticular tumours embedded in paraffin, have also been described.^{12,13} Using the immunogold technique¹⁴ it is possible to achieve limited success in fixed tissues with pan T reagents effective only on frozen sections. No reliable marker of T cells in routinely processed material, however, has yet been described.

Our results show that UCHL1 is a useful marker of normal and neoplastic T cells both in cryostat sections and in formalin fixed paraffin embedded tissue. The choice of fixative is important for the preservation of the antigen. Bouin's fixative, mercuric fixatives, and highly acidic formalins are unsuitable for its use.

The UCHL1 antigen seems to be expressed fairly early in T cell ontogeny. The staining of more mature T lymphoblastic lymphomas and some T lymphoblastic cell lines and the staining of common cortical thymocytes supports this observation. The T lymphoblastic lymphomas, which did not stain, showed early thymic phenotypes on frozen sections.⁵

UCHL1 seems to recognise a previously undetected antigen, which has been shown by immunoprecipitation to be a protein of molecular weight

180–185 kD. The leucocyte common antigen LCA and the lymphocyte function associated antigen LFA-1 identify determinants of similar molecular weight to UCHL1, but preclearing experiments show that they are not biochemically related to UCHL1; nor is UCHL1 the same as other antigens that identify subsets of helper and suppressor cells.¹ Attempts to correlate the immunophenotype determined on cryostat sections with staining for UCHL1 showed no common immunophenotype among the UCHL1 positive T cell tumours.

The most consistent finding was the expression of the CD3 and CD7 antigens in association with UCHL1. Those T cell lymphomas that did not stain with the antibody also showed poor expression of a large range of pan T and T lymphocyte subset specific antigens in frozen sections. For example, one case tested with seven T cell specific antibodies expressed only the CD2 antigen.

The staining results in malignant histiocytosis of the intestine are interesting. Recent work strongly suggests that this tumour, once thought to be of macrophage derivation, is a T cell lymphoma.⁹ Our results support this view. Although UCHL1 staining was also seen in true histiocytic lymphomas, the staining was far weaker than that seen in T cell lymphomas and malignant histiocytosis of the intestine.

The cross reactivity of the antibody with cells of mature myeloid and macrophage lineage is also interesting. Several other monoclonal antibodies, which recognise T cell related antigens, cross react with macrophages and cells of the myeloid series.¹⁵ For diagnostic purposes this cross reactivity should present no problems in the use of UCHL1, as long as the antibody is used as part of a panel of reagents.

In conclusion, UCHL1 is a reliable marker of normal and neoplastic T cells in formalin fixed paraffin embedded tissues and should prove to be a valuable addition to the limited range of antibodies effective in fixed tissues.

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