Definitive identification of human T cells in formalin fixed paraffin wax embedded tissue

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SUMMARY The use of a new monoclonal antibody, BF1, which reacts in routinely fixed tissues, directed against the β chain of the T cell antigen receptor was assessed to determine its reactivity in a variety of normal tissues as well as in a number of B and T cell tumours and other lymphoid disorders. It reacted with all five of the unequivocal T cell tumours tested. BF1 will have widespread use in the assessment of lymphoid tissues in patients with the acquired immune deficiency syndrome (AIDS) and the differential diagnosis of tumours.

The ability to identify lymphocytes derived from the human thymus (T lymphocytes) in tissues processed for routine histological examination might help elucidate the nature of a wide range of inflammatory and neoplastic conditions. As present, attempts to identify T cells are restricted by the lack of suitable monoclonal antibodies which are both T cell specific and reactive in processed material. Two monoclonal antibodies UCHL1 and MT1 have been widely studied. Neither of these is specific for T cells and both react with a range of other inflammatory cell types.¹⁻⁵ Analyses based on positive staining by these monoclonal antibodies are flawed by their lack of specificity.

An ideal T cell specific reagent would react with one of a handful of T cell specific molecules such as the proteins of the CD2 (T11), CD3/TCR complex, (the T cell antigen receptor and associated T3 proteins), or CD7 antigen. These are the only antigens which are present on essentially all T lymphocytes regardless of their state of differentiation and helper/suppressor subset.⁶ Antibodies to the T cell antigen receptor/CD3 complex react with the T lymphocyte equivalent of the B cell antigen receptor immunoglobulin (Ig), which is a definitive marker for the B cell lineage.⁷ As TCR/CD3 and Ig are intimately associated with the function of T and B cells, respectively, these proteins are only produced in the appropriate cell type.

The T cell antigen receptor complex consists of two major components, the antigen binding moiety, which is a dimer composed of an α and β subunit, and the CD3 complex, which is a group of four proteins loosely associated with the α/β dimer and which seem to transduce the change produced by antigen binding into an intracellular response.^{8,9} Monoclonal

antibodies which react with the variable portion of the β chain of the receptor have been described and used to identify the clonality of T cell proliferations.¹⁰ Unfortunately, these monoclonal antibodies do not react in fixed tissue and none of the many monoclonal antibodies to CD3 has been shown to do so.

Recently a monoclonal antibody BF1 has been described which reacts with all β chains of the conventional TCR α/β heterodimer.¹¹ This antibody reacts with denatured β chains in Western blots as well as with intact material from cells solubilised with detergent. It does not, however, react with an exposed antigenic epitope and so does not react with intact living cells. It will, however, react with such cells after brief fixation. This collection of observations led us to investigate whether BF1 reacts with human T cells in routine histological material.

Material and methods

Tissue samples were fixed in 10% formol saline, processed, and embedded in paraffin wax using standard methods. Some biopsy specimens were fixed in 0.02M phosphate 0.15M sodium chloride (PBS), pH 7.2, containing 10% formaldehyde.

Sections were cut and dried at 56°C for five minutes, then dewaxed in xylene for 10 minutes, and rehydrated through graded alcohols to water. Slides were immersed in Tris-buffered saline (0.15M sodium chloride, 0.05M Tris-hydrochloric acid, pH 7.6) (TBS) and warmed to 37°C in a water bath. Then they were transferred to a slide chamber containing 0.1% trypsin in 0.1% calcium chloride, pH 7.8, at 37°C for 30 minutes. Slides were washed in TBS for one minute and excess fluid removed without rubbing off the section.

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The primary antibody was then applied. BF1 was used as a 1/1000 dilution of ascitic fluid in phosphate buffered saline (0.15M sodium chloride, 0.02 M phosphate containing 10 mM NaN₃, 5% fetal calf serum, and 5% normal human serum). The negative control sections were incubated with a 1/1000 dilution of MPC 21 ascites, a mouse myeloma protein of the same subclass (IgG1) as BF1. A positive control using a monoclonal antibody to the leucocyte common antigen (Dako) was used at a dilution of 1/4. Incubation with antibody continued for two hours at room temperature.

The slides were washed three times in TBS, excess fluid removed and covered with the secondary antibody (affinity purified goat anti-mouse IgG (GAMig) Sigma, no M8642) at a dilution of 1/120 in TBS containing 5% NHS and incubated at room temperature for 45 minutes. Slides were washed as before and covered with alkaline phosphatase anti-alkaline phosphatase immune complex (APAAP Dako)¹² at a dilution of 1/15 in TBS 5 NHS and left at room temperature for 45 minutes. Slides were washed three times as before and the GAMig and APAAP steps were repeated three times for 15 minutes at each incubation with three washes between each step. After a final wash in TBS the enzyme substrate was added and incubated at room temperature for 15 minutes.

For a red reaction product the following substrate was used: 2 mg naphthol-AS-MX-phosphate (Sigma) was dissolved in 0.2 ml of dimethyl formamide, 9.8 ml 0.1M Tris buffer (pH 8.2) was added together with 20 µl of 1M Levamisole. To this 10 mg of fast red TR salt (Sigma) was added and the solution was filtered through filter paper directly on to the slides.

For a black reaction product 0.15 ml of a stock solution containing 40 mg of 5 bromo 4 chloro 3 indolyl phosphate (Sigma) in 6.7 ml methanol and 3.3 ml acetone was freshly added to 9 ml of 0.2 M Tris (pH 9.5) containing 1 mg of nitro blue tetrazolium (Sigma) and 0.02 ml of 2 M MgCl₂. This was added directly to the slides.

Finally, slides were washed in tap water and counterstained with Harris's haematoxylin for the red substrate, or neutral red if the black substrate was used, and coverslipped over an aqueous mountant.

Results

BF1 was applied to various normal and pathological tissues, and the results are shown in tables 1 and 2. BF1 reactivity was present in all the areas in which T cells are found by other methods in normal tissues.¹³⁻¹⁵ Although the number of disease states studied was small (table 2), it suffices to establish the principle that the monoclonal antibody will work under routine conditions and can be used to interpret difficult

Table 1

Normal tissues	(n =)	Pattern of staining
Lymph node	2	Most cells in interfollicular areas and a few cells in germinal centres positive
Tonsil	2	Similar to lymph node
Adult thymus	1	Most medullary and many cortical thymocytes positive
Fetal thymus	2	Similar to adult thymus
Spleen	2	Cells in periarteriolar lymphocyte sheath positive
Appendix	2	Epithelial cells negative, intraepithelial lymphocytes positive, lymphoid tissue similar to lymph node
Small intestine	1	Similar to appendix
Prostate	1	No staining
Lung	1	No staining except for occasional lymphocyte

Table 2

Other tissues	(n =)	Pattern of staining
T cell lymphomas		
Lymph node	5	Tumour cells positive
Skin	1	Tumour cells positive
B cell lymphomas	6*	Tumour cells negative, reactive T cells in tumour positive
Angioimmunoblastic lymphadenopathy (AILD)	2	Most of lymphoid cells positive
HIV positive lymphocyte depleted node	1	Few residual T cells positive
HIV positive with partial lymphocyte depletion	1	Positive cells present within T cell areas of node, many more positive cells than in depleted node

*Histological diagnoses: plasmacytoma n = 1, follicular centroblastic (n = 1), diffuse centrocytic/centroblastic n = 1, thyroid lymphoma (n = 1), extranodal lymphomas in HIV positive patients (n = 2).

problems such as the state of lymphoid tissues from patients with the acquired immune deficiency syndrome (AIDS) (figs 1 to 6).

Discussion

The results shown here are sufficient to establish that the anti-TCR β chain monoclonal antibody BF1 stains most cells in normal tissues processed by standard routine histopathology laboratory techniques. It reacted with all five of the unequivocal T cell tumours tested. In addition, it is a useful marker for the extent of T cell depletion in lymphoid tissues from patients with AIDS.

Because T cell antigen receptor proteins are unique to T cells, positive staining with BF1 is sufficient to identify cells as being of this lineage. Failure to react, however, may not be taken as definitive evidence that a cell is not a T cell. For example, although most primitive T cell leukaemias tested have rearranged the

Fig 1

Fig 2

Fig 3

Fig 4

Fig 1 Lymph node showing normal distribution of T cells in mantle zone and paracortex with some positive cells in germinal centre (high density in light zone—bottom right).

Fig 2 Angioimmunoblastic lymphadenopathy with pronounced typical vascular pattern and large numbers of βF1 positive cells.

Fig 3 Previously phenotyped (T3 positive TCR VB5 positive¹⁰) T cell lymphoma showing most cells to be positive for βF1.

Fig 4 Skin from patient with mycosis fungoides showing dense infiltrate of βF1 positive cells in upper dermis, and infiltrating epidermis.

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gene for the β chain and contain messenger RNA, for this protein no proof of actual protein production yet exists.¹⁶ Recently a "pre-T cell" equivalent to the pre-B cell has been described in the mouse thymus.¹⁷ This cell, which is probably the most primitive T cell precursor, produces β chain in its cytoplasm but does not seem to express it on the cell surface. This is analogous to the cytoplasmic expression of the IgM heavy chain in pre-B cells. Thus while it seems likely the BF1 will react with pre-T cells, this point requires confirmation.

Some T cell tumours may cease to produce one or more of their TCR components. This is rare but loss of immunoglobulin production does occur in multiple myeloma where 1–5% of tumours are non-secretory.¹⁸ The discovery of a new TCR heterodimer composed of γ and δ chains also suggests that there may be T cells which do not use the β chain protein.¹⁹ For reasons that are not yet clear, however, the tumours of this type have produced intracellular β chain as well as the γ/δ complex expressed on the cell surface.²⁰ The prevalence of CD3 positive, β negative cells is not known.

We did not study the effects of other fixatives and approaches to tissue processing, and there may, therefore, exist conditions under which BF1 does not react. These studies have been largely confined to lymphoid tissues, and BF1 may have totally unexpected cross reactions in other tissues.

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