CD79a: A Novel Marker for B-Cell Neoplasms in Routinely Processed Tissue Samples

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The CD79 molecule, comprising two polypeptide chains, mb-1 (CD79a) and B29 (CD79b), is physically associated in the B-cell membrane with immunoglobulin. It transmits a signal after antigen binding and may, therefore, be considered the B cell equivalent of CD3. It appears before the pre–B-cell stage, and the mb-1 (CD79a) chain can still be present at the plasma cell stage. In this report, we describe a new anti-CD79a monoclonal antibody, JCB117, which reacts with human B cells in paraffin embedded tissue sections, including decalcified bone marrow trephines. When tested on a total of 454 paraffin embedded tissue biopsies, gathered from a number of different institutions, it reacted with the great majority (97%) of B-cell neoplasms, covering the full range of B-cell maturation, including 10 of 20 cases of myeloma/plasmacytoma. It is of interest that the antibody labels precursor B-cell acute lymphoblastic leukemia samples, making it the most reliable B-cell marker detectable in paraffin-embedded specimens in this disorder. All neoplasms of T cell or nonlymphoid origin were negative, indicating that antibody JCB117 may be of value to diagnostic histopathologists for the identification of B-cell neoplasms of all maturation stages.

IMMUNOGLOBULIN present in the plasma membrane of human B cells lacks a cytoplasmic region of sufficient size to react with other molecules, and for many years the means by which a signal is transmitted to these cells after antigen binding remained unclear. This question has now been resolved with the demonstration that a dimeric molecule with a substantial intracytoplasmic region is physically associated with membrane Ig in mammalian B cells.[1,2] This molecule comprises two polypeptides, encoded by the *mb*-1 and *B*29 genes, and is associated with at least one tyrosine kinase (lyn). Induction of tyrosine kinase activity after antigen binding causes phosphorylation of the mb-1/B29 dimer, and also of other molecules, thereby initiating intracellular signaling.[3]

The Fifth Workshop on Leukocyte Differentiation Antigens (Boston, MA, November 1993) identified four submitted reagents as recognizing the mb-1/B29 dimer. The molecule was designated CD79, the two component chains being referred to as CD79a (mb-1) and CD79b (B29). It appears from the Workshop, and from other studies, to be restricted in its expression to B cells, whether normal or neoplastic.[4,5] CD79a and CD79b chains can be expressed early in B-cell maturation, before cytoplasmic μ chain, which does not appear until the pre–B-cell stage.[5] In pre-B cells, the CD79 dimer is associated with the pre–B-cell receptor, consisting of μ chain and the pseudo–light chain complex.[6] In keeping with these observations, almost all acute lymphoblastic leukemias of precursor B cell type are positive when tested with CD79a (mb-1) antibodies.[5,7]

The first antibodies to be raised against human CD79 (mb-1/B29) reacted poorly or not at all with routinely fixed paraffin embedded tissue.[4,8] In the present report, we describe a new antibody against the CD79a (mb-1) polypeptide, which detects its target in routinely processed tissue. Extensive testing confirmed its reactivity with almost all neoplasms of B-cell origin tested in this type of material, including many acute lymphoblastic leukemia samples. Given that the diagnosis of these tumors can present difficulties for the pathologist, this antibody is of potential practical value.

MATERIALS AND METHODS

Tissue and cell samples. Routinely processed paraffin-embedded tissue biopsy samples were obtained from the surgical pathology files of the authors' institutions. The majority of soft-tissue biopsy samples had been fixed in a conventional formalin-based fixative, but approximately 60 had been fixed in B5 fixative. Bone marrow trephines were fixed in acetic formalin (11 cases),[9] or in B5 fixative followed by decalcification with EDTA (86 cases). Lymphomas were categorized according to the Revised European American Lymphoma classification.[10] Peripheral blood mononuclear cells were obtained from normal donors by a gradient centrifugation of heparinized samples.

Cell lines. B-cell lines were obtained from stock held in the principal investigator's laboratory. For biochemical experiments, the human Burkitt lymphoma cell line Ramos was used, which expresses Ig μ chains in association with λ light chains.[1]

Production of recombinant mb-1 protein. A soluble form of the extracellular IgSF domain of human mb-1 was produced as a chimaeric protein containing domains 3 and 4 of rat CD4,[11] using cDNA from Dr N. Sakaguchi (Faculty of Medicine, Tottori University, Tottori, Japan), for which a partial sequence is published.[12] Design of the construct encoding human mb-1 was based on the cDNA sequence in the EMBL/GENBANK database (accession no. M74721).[13] Recombinant mb-1/CD4d3 + 4 was immunoaffinity purified with a CD4 monoclonal antibody (MoAb), yielding 70% monomers and 30% dimers, and then further purified by gel filtration on Superose 12 (Pharmacia, Milton Keynes, UK). Monomeric mb-1/CD4d3 + 4 was used for immunization.

Production of antibody JCB117. This MoAb came from an experiment in which a murine hybridoma was prepared using standard

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techniques,[14] following four immunizations with a total of 200 μg recombinant mb-1 protein. Initial screening was performed on cryostat sections of human tonsil tissue, and a cell line producing antibody designated JCB117, which stained mantle zone B cells and plasma cells in an identical fashion to previously characterized anti-CD79a antibodies,[4] was established after cloning.

Immunostaining. Tissue sections from paraffin-embedded blocks were cut onto slides previously coated with Silane and then dried at 60°C overnight. The sections were dewaxed in Histoclear, rehydrated, and washed in tap water for 2 minutes before being placed on a glass rack in a microwave-resistant dish and fully covered with 0.1% sodium citrate. The dish was covered with film wrap in which two holes has been pierced and microwaved at 700 W for 4 minutes, ensuring that slides remained completely covered. The dish was then microwaved for a further 4 minutes at 700 W and allowed to stand for 15 minutes before removing the slides and rinsing them in tris buffered saline (TBS; 0.5 mol/L TRIS, pH 7.6, diluted 1:10 in 0.15 mol/L NaCl) for 5 minutes. The sections were then incubated with MoAb JCB117 and stained either by the alkaline phosphatase:anti-alkaline phosphatase (APAAP) method[15] or by the avidin biotin complex (ABC) procedure, using a Ventana automatic immunostainer (Ventana Instruments, Arizona).

Biochemical techniques. For cell surface radioiodination, Ramos cells were suspended in phosphate-buffered saline (PBS) and labeled with $Na^{125}I$ (Amersham Co, Amersham, UK) at a ratio of 1 mCi per 25×10^6 cells, using lactoperoxidase as a catalyst. Cells were lysed in immunoprecipitation buffer (IPB), consisting of 10 mmol/L triethanolamine-HCl, pH 7.8, 0.15 mol/L NaCl, 5 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 0.02 mg/mL ovomucoid trypsin inhibitor, 1 mmol/L Na-*p*-tosyl-L-lysine chloromethyl ketone, and 0.02 mg/mL leupeptin, supplemented with 1% Nonidet P-40 (NP-40) as detergent. For protein denaturation, as mentioned in Results, 0.5% sodium dodecyl sulfate (SDS) was added to the lysate, followed by heating for 5 minutes at 68°C. Next, the lysate was diluted fourfold with IPB containing 1.5% NP-40. For subsequent reductive alkylation, the lysates were incubated for 30 minutes at 45°C in the presence of 2 mmol/L dithiothreitol, followed by incubation for 30 minutes at room temperature with 20 mmol/L iodoacetamide. Immunoprecipitation was performed essentially as described.[1] After centrifugation, denaturation, and preclearing, equal parts of the lysate were incubated with protein A sepharose CL 4B beads (Pharmacia, Uppsala, Sweden) that had been precoated with rabbit-antimouse Ig and either the JCB117 MoAb (equivalent to 0.5 mL culture supernatant), or the HM57 anti-CD79a MoAb.[4] Specific immunoprecipitates were washed in IPB with 1% NP-40, resuspended in SDS sample buffer, and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% to 15% gradient gel.

For Western blotting, Ramos cells were lysed in IPB with 1% NP-40 at 10^8 cells per 500 μL, the lysate was centrifuged for 15 minutes at 13,000*g*, split in two parts and 125 μL three times concentrated SDS sample buffer with or without 5% β-mercaptoethanol was added to each part of the lysate.[16] Samples, the equivalent of 5×10^6 cells per lane, were separated by SDS-PAGE on a 10% gel and electrophoretically transferred to nitrocellulose. Membranes were blocked in PBS/0.2% Tween 20 with 5% nonfat dry milk, incubated with 1:4 diluted culture supernatant of JCB117, 1:1,000 diluted ascites fluid of HC-10 anti-major histocompatibility complex (MHC) class I MoAb or 1:1,000 diluted normal mouse serum, washed with PBS/0.2% Tween 20 and incubated with peroxidase-conjugated goat-antimouse Ig (Tago Immunologicals, Burlingame, CA). Membranes were washed and visualization was performed by enhanced chemiluminescence (Amersham, UK).

Flow cytometry. Cells were analyzed for reactivity with antibody JCB117 as described previously.[17] After labeling, cells were fixed in 1.5% formaldehyde in PBS, and analyzed in a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) using Lysis II software.

RESULTS

Biochemical characterization of antibody JCB117. When antibody JCB117 was tested by immunoprecipitation from NP-40 lysates, only trace amounts of CD79 dimer were isolated (Fig 1A), whereas the control anti-CD79a (MoAb HM57) precipitated a significant amount of disulfide linked CD79a/b heterodimer from an equal part of the lysate (Fig 1A). The reactivity of antibody JCB117 was slightly improved after mild denaturation of the CD79a chain by addition of 0.5% SDS to the lysate and heating at 68°C (Fig 1B), but it was still suboptimal compared with reactivity of the control antibody HM57. The samples that had been used for these immunoprecipitations were then subjected to reductive alkylation, to disrupt interchain and intrachain disulfide bonds, and a further immunoprecipitation with the same antibodies was performed. Whereas antibody HM57 had removed most antibody-reactive material from the relevant part of the lysate in the first precipitation (Fig 1C), JCB117 precipitated slightly more CD79a chain from the reduced and alkylated lysate than from the lysate that had merely been denatured (compare the JCB117 lanes in Fig 1A and 1B). However, JCB117 only recovered a fraction of the total amount of CD79a protein present in this part of the lysate.

In contrast with the poor reactivity by immunoprecipitation, antibody JCB117 clearly detected the CD79a (mb-1) polypeptide chain in a Ramos B cell lysate by Western blotting, indicating that the relevant epitope can be recovered after denaturation of the CD79a chain with SDS and heating at 100°C. Reactivity was found with the single CD79a chain under reducing conditions, as well as with the CD79a/b dimer under nonreducing conditions.

Immunocytochemical reactivity. Antibody JCB117 was used to stain a range of paraffin-embedded tissue sections, including both reactive and neoplastic lymphoid tissue samples (Table 1). Almost all B-cell neoplasms were labelled by the antibody, whereas no T-cell or myeloid neoplasms were positive. Staining was consistently strong on small cell neoplasms, but large B-cell neoplasms and follicular lymphomas tended to be weaker. One point of interest was the strong reactivity against acute lymphoblastic leukemias of non–T-cell type (Fig 2).

Normal plasma cells in tissue samples were stained strongly (Fig 3), in keeping with the previously documented expression of mb-1/CD79a in these cells.[4] Half of the myeloma/plasmacytoma cases tested also gave positive reactions (Fig 3), as did the plasma cells deriving from the neoplastic clone in a case of gastrointestinal (mucosa associated lymphoid tissue [MALT]) lymphoma (Fig 3).

The paraffin-embedded tissue tested had been fixed in formalin, B5, or acetic formalin. It should be noted that the latter fixative is an optimal fixative for a range of leukocyte antigens in marrow trephines, but that harsher decalcification techniques might possibly denature the epitope recognized by antibody JCB117 (as they do many other antigens). A total of more than 50 biopsy samples fixed in Bouin's fixative (a fixative used principally in France) have recently been analyzed (Prof G. Delsol, personal communication, January

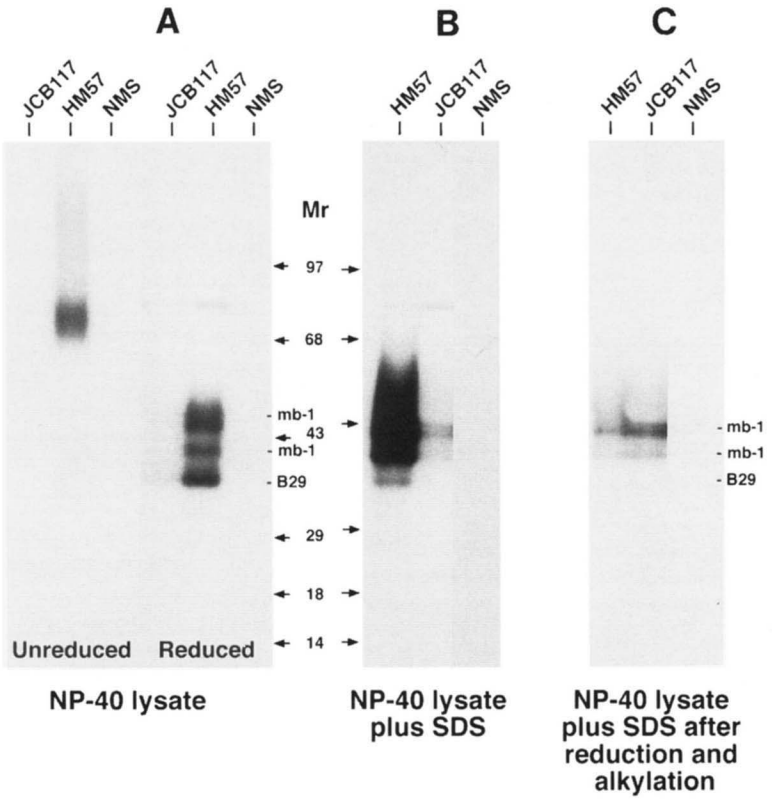

Fig 1. Reactivity of antibody JCB117 by immunoprecipitation from lysates of surface iodinated human B cells (Ramos cell line). (A) The antibody fails to immunoprecipitate CD79a (mb-1) from an NP-40 lysate, whereas the control antibody HM57 gives the expected reaction. Normal mouse serum (NMS) provides a negative control. (B) After treatment of the NP-40 lysate with SDS some weak reactivity of JCB117 with CD79a (mb-1) is observed. (C) The lysates shown in (B) were reduced and alkylated, and some residual CD79 (mb-1) could then be immunoprecipitated by JCB117.

1995) with equally good results, indicating that the antibody is also compatible with this fixative.

The antibody was tested by immunofluorescence flow cytometry against normal peripheral blood lymphoid cells and against mature and immature B-cell lines (Ramos, Raji, and Nalm-1), and was consistently negative, indicating that the epitope that it recognizes is not present, or is not accessible, on living cells.

Table 1. Reactivity of Anti-CD79a (mb-1) Antibody JCB117 With Paraffin-Embedded Samples of Hematopoietic Neoplasms

B-cell neoplasms		
Lymphoblastic lymphoma/leukemia		41/41
Small lymphocytic lymphoma/CLL		28/28
Lymphoplasmacytoid lymphoma		36/36
Mantle cell lymphoma		17/17
Follicular lymphoma		53/53
MALT lymphoma		29/29
Hairy cell leukemia		15/15
Myeloma/plasmacytoma		10/20
Large cell lymphoma		95/95
Burkitt's lymphoma		7/7
Anaplastic large cell lymphoma		13/15
	Total	344/356 (97%)
T-cell and nonlymphoid neoplasms		
Lymphoblastic lymphoma/leukemia		0/9
Mycosis fungoides		0/10
Peripheral T-cell lymphoma		0/32
Angioimmunoblastic T-cell lymphoma		0/8
Anaplastic large cell lymphoma		0/11
Acute myeloid leukaemia		0/28
	Total	0/98

DISCUSSION

The high degree of specificity of the CD79 (mb-1/B29) molecule for B cells is in keeping with its important functional role in the antigen receptor complex, which initiates a cellular response following antigen binding. It may be considered as playing a role in B cells very similar to that of CD3 in T cells (which is also physically linked to antigen receptors), and this presumably accounts for its comparable degree of cell lineage specificity.

Despite many attempts over more than a decade to raise antibodies specific for human B-cell antigens, the CD79 molecule escaped recognition by this means. It was first identified in animals by biochemical investigation of molecules required for insertion of Ig into the surface of B cells,[18-20] and simultaneously by cloning cDNA specific for B cells by the subtraction library technique.[11] Shortly afterwards, van Noesel et al[21] reported that a heterodimeric molecule (comprising polypeptides of 37 and 47 kD Mr) could be immunoprecipitated from human B cells in association with Ig. It was subsequently shown that these two chains reacted with antibodies raised against synthetic amino acid sequences from the mb-1 and B29 gene products.[1,8]

The fact that the random production of MoAbs against fresh human B cells, an approach that has identified many B-cell–restricted antigens, such as CD19, CD20, CD22, and CD37, was not the means by which CD79 was defined is worthy of note. Of the four antibodies in the Fifth Workshop

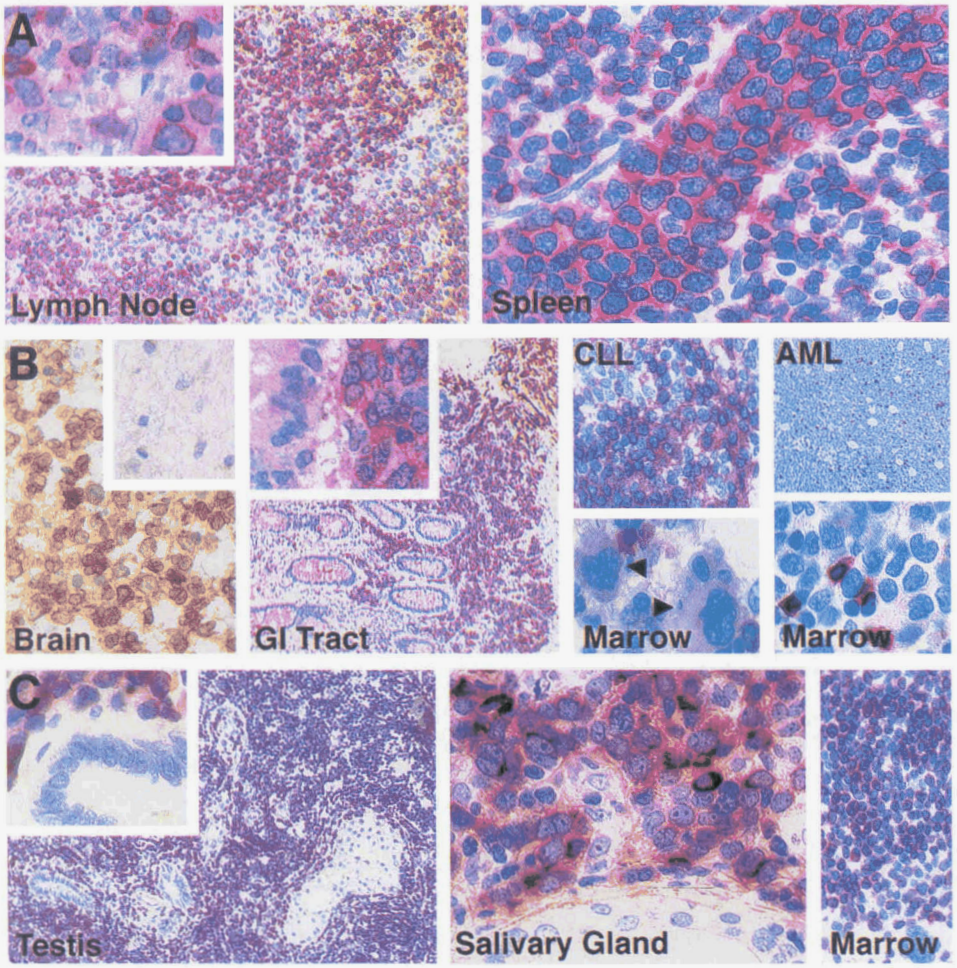

Fig 2. Reactivity of antibody JCB117 with paraffin embedded tumor biopsies. (A and B) Antibody JCB117 detects the CD79a polypeptide in neoplastic B cells in lymph node (large cell lymphoma), spleen (hairy cell leukemia), brain (large cell lymphoma), gastrointestinal tract (MALT lymphoma), and marrow trephine (chronic lymphocytic leukemia). The inset illustration for the central nervous system lymphoma shows adjacent normal brain. Normal megakaryocytes are arrowed in the chronic lymphocytic leukemia sample. A case of acute myeloid leukaemia (AML) is unstained, except for normal B cells. (C) Antibody JCB117 also detects the CD79a polypeptide in immature B cells as illustrated by three examples of precursor B-cell acute lymphoblastic leukemia in testis, salivary gland, and marrow trephine. All sections stained by the APAAP immunoalkaline phosphatase procedure with the exception of the brain biopsy (indirect immunoperoxidase technique).

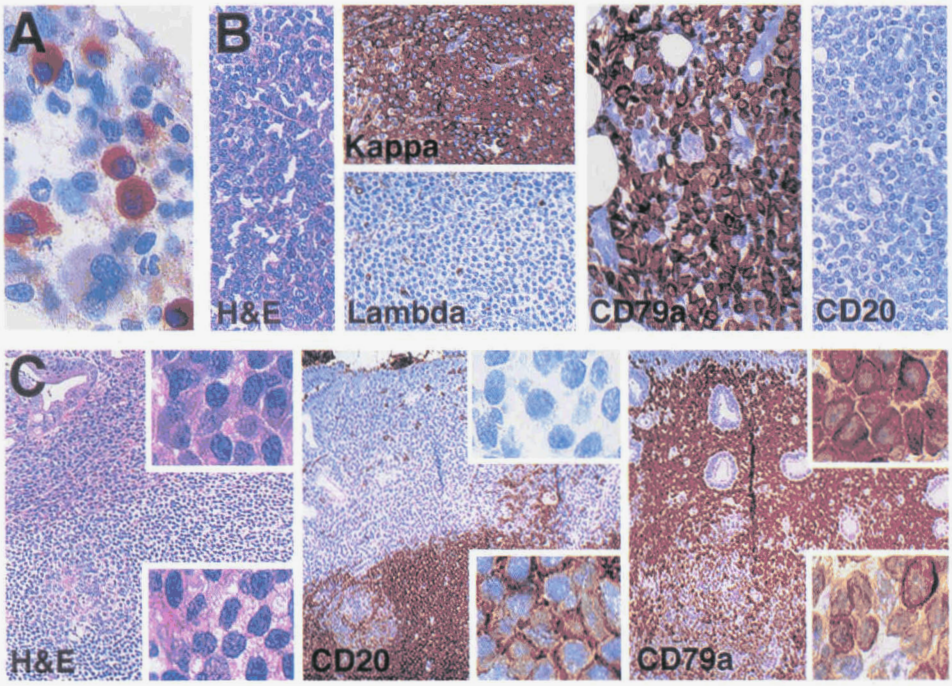

Fig 3. (A) Antibody JCB117 detects CD79a in normal plasma cells, as seen in this marrow trephine section. APAAP technique. (B) It also labels some plasma cell neoplasms (Table 1), as illustrated by this cutaneous plasmacytoma (expressing monoclonal kappa light chains), which is positive for CD79a (antibody JCB117), but CD20-negative, in keeping with the loss of CD20 by normal plasma cells. ABC immunoperoxidase technique. (C) A gastrointestinal B-cell lymphoma (MALT lymphoma) is shown in which two cellular compartments could be identified. In the deep mucosa neoplastic centrocyte-like cells typical of this neoplasm are seen (shown at high magnification in the lower inset illustrations). They are positive for both CD20 and CD79a (antibody JCB117). In contrast, the upper mucosa is densely infiltrated with monoclonal plasma cells (upper inset illustrations), representing terminally differentiated neoplastic B cells, which are positive for CD79a (antibody JCB117) but CD20-negative. ABC immunoperoxidase technique.

Table 2. Human B-Cell–Associated Antigens Detectable in Paraffin-Embedded Tissue Sections

Antigen	Antibodies	Comments	Reference No.
CD20	Monoclonal (L26)	Also found on some T cells and myeloid blast cells	25-26, 35
CD79a (mb-1)	Monoclonal (JCB117)	B-cell specific	This report
CD38	Monoclonal (AT13/5)	Also found on plasma cells and T cells	34, 36
CD45RA	Several monoclonals	Antigen also found on some T cells	37
CD74	Several monoclonals	Invariant chain of MHC class II molecule. Also found on macrophages, some T cells, etc	27, 28
CDw75	Several monoclonals	Carbohydrate antigen, also present on some T cells	27, 28

on Leukocyte Differentiation Antigens that detected the CD79 molecule, none was raised against living cells. One antibody (SN8), recognizing the *B29*/CD79b chain, was produced by immunization with a membrane antigen preparation from human B cells[22]; the other three were all generated against synthetic peptide sequences from the intracytoplasmic portion of the molecule.[1,8] We are only aware of one other monoclonal CD79 reagent (an anti-B29 antibody[23,24]) and this was raised against a biochemically purified CD79 preparation, rather than against living cells.

These findings suggest that the CD79 (mb-1/B29) dimer is masked on the surface of B cells. The present study supports this view: antibody JCB117 must detect the extracellular portion of the mb-1 (CD79a) polypeptide, because it was raised against recombinant protein containing this domain, but it is nevertheless unable to detect its target on fresh human B cells by flow cytometry. When tested by immunoprecipitation (Fig 1A) it also performed poorly (relative to another monoclonal CD79a antibody, HM57, which reacts with the intracytoplasmic tail of the polypeptide), even after the CD79 dimer had been separated from Ig and dissociated into its constituent chains. Nevertheless, it did react by Western blotting and we favor the idea that the epitope detected by JCB117 is only created on CD79a after the polypeptide has been denatured. However, a definitive conclusion on why it fails to react with living cells cannot be reached on the basis of the present data.

When the results obtained in this report are reviewed in the context of immunocytochemical detection of B cells in routinely processed tissue samples, it is evident that antibody JCB117 represents a valuable addition to the range of reagents that can be used for this purpose. The antibody stained the great majority of B-cell neoplasms, the exceptions being two of 15 B-cell anaplastic large cell lymphomas and 10 of 20 myelomas/plasmacytomas. In the context of the two former cases, there were no phenotypic features that distinguished them from the JCB117-positive B-cell anaplastic large cell lymphomas. We did not review the phenotypes of the cases of myeloma and plasmacytoma, but the fact that some of these neoplasms are positive for CD79a and others negative has been observed previously.[4,5,8]

Table 2 summarizes the antibodies currently available for this purpose, and it will be seen that only one other reagent, antibody L26,[25] directed against an intracellular epitope on the CD20 molecule,[26] is comparable in terms of its specificity for B cells, because other MoAbs currently used to detect B cells in paraffin sections react with cells other than B cells. However, JCB117 is in several respects an improvement on L26, which labels dendritic reticulum cells[27] (on occasion obscuring its reactivity with B cells) and some T cells.[28] This latter reactivity may be related to reports that CD20 is expressed at low levels on a minority of normal T cells[29-31] and on some human T-cell neoplasms.[30,31] Furthermore, L26 is reported to react with neoplastic cells in cases of acute myeloid leukaemia.[32]

More importantly, the CD79a polypeptide appears earlier in B-cell maturation than CD20, which is not expressed until the late pre–B-cell stage.[33] In the present study, all precursor B-cell neoplasms were labeled by antibody JCB117, in keeping with previous results.[4,5,7] Ten of these samples were also analyzed with antibody L26 and only four were positive, in keeping with the absence of CD20 from many cases of leukemia of this type. As a consequence, antibody JCB117 is the only reagent of which we are aware that has such a high frequency of reactivity with lymphoblastic leukemia of precursor B-cell type (ie, the great majority of non–T-cell lymphoblastic leukemias) in routinely processed biopsy material. Diagnostic problems posed by small cell tumors in pediatric pathology, for which the differential diagnoses include neuroblastoma and rhabdomyosarcoma, thus provide an obvious application for this new antibody.

It should be noted that detection of the epitope on human B cells by JCB117 in fixed paraffin-embedded tissue requires prior heating of the tissue by microwave irradiation. This approach to the unmasking of cellular antigens is proving of great value in diagnostic histopathology because of the large number of antigens that can be retrieved in this way.[34,38-41]

In conclusion, we describe an antibody that can be used as a reliable reagent by the diagnostic pathologist for the detection of both normal and neoplastic B cells in routinely processed tissue samples. It should be noted that the surgical pathology samples studied came from five different institutions in Europe and the United States, but that no differences were noted in the behavior of the antibody. This suggests that local differences in tissue processing methods have no deleterious effects on its reactivity. Therefore, the antibody appears to be a robust reagent in terms of the biopsy material on which it can be used. In the recently published Revised European American Classification of Lymphoma, CD79a (mb-1) was identified as an important marker for the identification of B lineage neoplasms,[9] and an antibody that can detect this antigen in paraffin sections is of clear utility. It may also be of value to the immunologist for the biochemical analysis (by Western blotting) of components of the B-cell antigen receptor complex.

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