Immunoreactivity of neoplastic and non-neoplastic monocytoid B lymphocytes for DBA.44 and other antibodies

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Abstract

Aims—To evaluate the immunoreactivities of neoplastic and non-neoplastic monocytoid B cells (MBC) and compare them with hairy cell leukemia (HCL) and mantle cell lymphoma (MCL).

Methods—An immunohistochemical study of paraffin wax embedded sections was done on surgically resected specimens of spleens with MBC clusters from patients with gastric cancer (14 cases), tonsils (five cases), and lymph node (two cases) showing lymphoid follicular hyperplasia (LFH), submandibular lymph nodes containing MBC in Sjögren's syndrome (one case). Extranodal organs affected by MCL (three cases) and monocytoid B cell lymphoma (MBCL) (seven cases), and spleens from HCL (four cases) were also studied. These specimens were fixed in 10% formalin and routinely processed for paraffin wax embedding. Fresh spleen specimens from patients with liver cirrhosis (one case) and gastric cancer (seven cases) were snap frozen.

Results—Mantle zone lymphocytes were DBA.44, CD74 positive and showed a weaker reaction for CDw75 than marginal zone lymphocytes and MBC, which were almost DBA negative. In neoplastic diseases tumour cells in MCL were DBA.44, CD74, and CDw75 positive. MBCL showed a positive reaction for CD74 and CDw75, but positivity for DBA.44 was observed in only one of seven cases. The HCL specimens, all positive for DBA.44, showed a weaker reaction for CD74 and a stronger reaction for CDw75 than either MCL and MBCL specimens.

Conclusion—These results show that mantle zone lymphocytes and MCL more closely matched HCL for reactivity to DBA.44 than MBC and MBCL. Reactivities for DBA.44 and CDw75 were greater in MBCL compared with its non-neoplastic counterpart, MBC.

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“Monocytoid” cells or “immature sinus histiocytes”, present in the lymph nodes of patients with toxoplasmic lymphadenitis, Sjögren's syndrome, or AIDS, are B cell in nature, and termed monocytoid B cells (MBC).¹⁻⁴ Our recent study showed that MBC occur in the spleens of older patients with gastric cancer⁵ and extranodal organs affected by auto-

immune disorders, such as Hashimoto's thyroiditis.⁶ A malignant counterpart of MBC has subsequently been recognised—monocytoid B cell lymphoma (MBCL).⁷

The B cell character of hairy cell leukemia (HCL) has been well established by immunological and immunogenetic studies.^{8,9} However, the normal counterpart of HCL cells and its stage in the continuum of B cell differentiation remains unknown. HCL cells characteristically express an antigen (CD11c) usually found on monocytes/macrophages recognised by Leu-M5, together with B cell antigens.¹⁰ Immunophenotypic similarities between HCL cells and MBC have been reported.^{11,12}

Monoclonal antibody DBA.44, which is raised against a diffuse large cell lymphoma cell line in normal Balb/C mice, reacts with a subpopulation of B lymphocytes in the mantle zone of lymphoid follicles.¹³ Within the group of low grade B cell lymphomas DBA.44 reacted principally with HCL. In this study the immunoreactivities of neoplastic and non-neoplastic MBC to DBA.44 and other antibodies were evaluated and compared with HCL and mantle cell lymphoma (MCL).

Methods

Surgically resected spleens from patients with gastric cancer (n = 14), tonsil of lymphoid follicular hyperplasia (LFH) (n = 5), lymph nodes of LFH (n = 2), submandibular lymph node of Sjögren's syndrome (n = 1), extranodal organs affected by MCL (n = 3) and MBCL (n = 7), and spleens from HCL (n = 4) were investigated. The sex ratio and median age of patients was as follows: gastric cancer, 2:3:1 and 49 years; LFH of tonsil, 3:1 and 34 years; LFH of lymph nodes, 1:1 and 28 years; Sjögren's syndrome, all female and aged 43 years; MCL, 1:2 and 53 years; MBCL, all female and aged 53 years; and HCL, 3:1 and 49 years, respectively.

All patients with HCL presented with severe splenomegaly, absent or only a mild superficial lymphadenopathy, and circulating leukaemic cells with a hairy appearance as detected by phase contrast microscopy. Immunohistochemical staining showed that the leukaemic cells were acid phosphatase positive because they were resistant to tartrate. Immunologically, these cells had surface immunoglobulins and were positive for CD11c, CD20, and CD25. Specimens were fixed in 10% formalin and routinely processed for paraffin wax embedding. Sections, cut at

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Table 1 Antibody panel

Antibody	Primary immunoreactivity	Source
<i>Frozen sections</i>		
CD5 (Leu1)	T cell, mature B cells	Beckton Dickinson (Mountain View, California)
CD11c (LeuM5)	Monocytes, granulocytes, hairy cell leukemia	Beckton Dickinson
CD24 (BA-1)	Mantle zone cells, granulocytes	Hybritech, Hialeah, Florida
DBA.44	Mantle zone cells, some immunoblasts, hairy cell leukemia	Dakopatts (Copenhagen)
<i>Paraffin wax sections</i>		
CD3	T cells	Dakopatts
CD15 (LeuM1)	Monocytes, myeloid cells, some epithelial cells	Beckton Dickinson
CD20 (Mx-PanB)	B cells	Kyowa Medex (Tokyo)
CD43 (MT1)	T cells	Bio-Science (Emmenbrucke)
CD45RA (4KB5)	B cells, monocytes	Dakopatts
CD45RO (UCHL-1)	T cells	Dakopatts
CD74 (LN2)	B cells, interdigitating reticulum cells, Reed-Sternberg cells	Techniclone
CDw75 (LN1)	Germinal centre cells	Techniclone
MB-1	B cells	Bio-Science
DBA.44	Mantle zone cells, hairy cell leukemia	Dakopatts
CD68 (KP-1)	Monocytes, granulocytes	Dakopatts
CD68 (PGM1)	Monocytes	Dakopatts
EMA	Epithelial cells	Dakopatts
S-100	Langerhans' cells	Dakopatts

4 µm, were stained with haematoxylin and eosin and immunoperoxidase (avidin biotin complex (ABC) method). Fresh spleen specimens from patients with liver cirrhosis (one case) and gastric cancer (seven cases) were snap frozen in liquid nitrogen (−180°C) and stored at −80°C until use for immunohistochemistry. The monoclonal antibodies used in this study are listed in table 1. The effect of treatment on tissue sections by microwave oven heating (five minutes) was estimated.

Results

The results of the immunohistochemical study of reactive states are summarised in table 2. In the frozen and paraffin wax embedded sections of spleen, mantle zone lymphocytes showed a stronger reaction for DBA.44 than parafollicular cells. Recently, we reported the occurrence of MBC in the spleen of elderly patients with gastric cancer.⁵ All the paraffin wax embedded spleens from 14 patients with gastric cancer contained clusters of monocytoid B cells, which were completely non-reactive with DBA.44 but were positive for CD20, MB-1, CD45RA, CD74, CDw75, and negative for CD43, CD45RO, CD15, CD68, and epithelial membrane antigen (EMA) (fig 1). In the tonsil and lymph nodes

showing LFH, positively reacting cells for DBA.44 were exclusively found in the mantle zone, which were CD20+, MB-1+, CD45RA+, CD74+, CDw75−. In one case of LFH of tonsil many small cleaved cells, together with non-cleaved cells in the germinal centres, positively stained for DBA.44. Monocytoid cells appearing in the lymph node of Sjögren's syndrome were DBA.44−, CD20+, MB-1+, CD74+, CDw75+. Proliferating cells in MCL were positive for DBA.44, though a few cells were positive in one case and CD20+, MB-1+, CD45RA+, CD74+, CDw75+ in all cases. The intensity of staining for DBA.44 in MCL was similar to that in mantle zone lymphocytes, but was rather weaker than that in HCL.

Proliferating cells in one of seven cases of MBCL showed a positive reactivity for DBA.44 and were CD20+, MB-1+, CD45RA+, CD74+, and CDw75+ (fig 2). The microwave heating of tissue sections strengthened the intensity of the positive reaction, though the number of positive cases for DBA.44 did not increase. All cases of HCL reacted positively for DBA.44 and were positive for CD20, MB-1, and CD45RA (fig 3). The reactivity of the proliferating cells in HCL for CD74 was weaker but stronger for CDw75 than those of MCL and MBCL.

Table 2 Results of immunohistochemistry in reactive states

Material	Immunoreactivities of lymphoid cells		
	Mantle zone	Marginal zone	Monocytoid cells
<i>Fresh frozen sections</i>			
Spleen of gastric cancer (n = 7)	DBA.44+, CD11c−, CD5+, CD24+,	DBA.44+, CD11c−, CD5−, CD24+	
<i>Paraffin wax sections</i>			
Spleen of gastric cancer (n = 14)	Numerous DBA.44+ CD20+, MB-1+, CD45RA+, CD74+ CDw75−, CD43−, CD45RO−	A few DBA+ cells CD20+, MB-1+, CD45RA+, CD74+ CDw75+, CD43−, CD45RO−	DBA.44−, CD20+, MB-1+, CD45RA+ CD74+, CDw75−, CD43−, CD45RO−
LFH of tonsil and lymph node (n = 7)	DBA.44+ CD20+, MB-1+, CD45RA+, CD74+ CDw75−, CD43−, CD45RO−		
Monocytoid cells in Sjögren's syndrome (n = 1)	DBA.44+ CD20+, MB-1+, CD45RA+, CD74+ CDw75−, CD43−, CD45RO−		DBA.44−, CD20+, MB-1+, CD45RA+ CD74+, CDw75−, CD43−, CD45RO+

Figure 1 Monocytoid cells near the sheath artery of the spleen (A) haematoxylin and eosin. Monocytoid cells showing a positive reaction for CD20(B), but not for DBA.44 (C) (ABC method).

(A)

(B)

(A)

Figure 2 Several cells in monocytoid B cell lymphoma showing surface staining for DBA.44 (ABC method).

Discussion

Morphological similarities between HCL cells and MBC and its neoplastic counterpart MBCL have promoted several immunological and immunohistochemical comparisons of these cells.^{9-10,12} Traweek *et al* reported that HCL was CD5⁻, CD3⁻, CD2⁻, CD19⁺, CD20⁺, CD11c⁺, CD25⁺, and PCA-1⁺.¹⁴ Except for CD25(Tac) negativity, MBCL showed a similar pattern of reactivity to HCL.^{11,14} The circulating MBCL cells exhibited strong acid phosphatase activity that was tartrate sensitive, which does not occur in HCL cells. In paraffin wax sections Stroup and Sheibani reported similar reactivities between HCL and MBCL cells: CD45RA⁺, CD20⁺, CDw75⁺, CD74⁺, LN3⁺, MB2⁺, CD45RO⁻, CD43⁻, and CD15⁻.¹² These authors suggested that the origin of MBCL is a site closely adjacent to the origin of HCL.^{12,14} The present immunohistochemical findings in MBCL and HCL are in general agreement with those of previous studies, which indicate positivity for CD20, MB-1, CD45RA, CD74, and CDw75, and negativity for monoclonal antibodies for macrophages and T lymphocytes. Reactivity for CD74 was rather stronger in MBCL cells than in HCL cells.

The monoclonal antibody DBA.44 is active on paraffin wax embedded specimens and reacts with HCL cells.¹³ Indeed, the proliferating cells in all our cases of HCL stained positively for DBA.44. Recently, Hounie *et al* reported that DBA.44 showed the strongest membrane staining in the tumour cells of centroblastic, immunoblastic, and MBC type among cases of node-based lymphomas.¹⁵ However, only one of seven cases of MBCL in the present study was reactive with DBA.44. Buffered formalin and Bouin's solution were

Figure 3 (A) Spleen invaded by hairy cell leukemia haematoxylin and eosin. (B) Hairy cell leukemia cells show distinct surface staining for DBA.44 (ABC method).

reported to be the optimal fixatives for DBA.44 staining.¹⁵ The decreased staining propensity of our cases of MBCL might have been attributable to the type of fixative used—10% formalin.

All cases of MCL were positive for DBA.44. This antibody is known to be reactive with mantle zone lymphocytes, the normal counterpart of MCL. However, the immunoreactivities of MCL (CD5+, CD11c-) were quite different from those of MBCL and HCL (CD5-, CD11c+).¹⁴ In LFH of lymph node and tonsil DBA.44 had its strongest reaction for mantle zone lymphocytes. The MBC in lymph nodes of toxoplasmic lymphadenitis and HIV associated lymphadenitis have been reported to show weak positive staining for DBA.44.¹⁵ In the present study the MBC in lymph nodes affected by Sjögren's syndrome were not reactive with DBA.44, which might also have been caused by decreased staining propensity due to 10% formalin fixation.

There have been suggestions that HCL might originate from the splenic marginal zone,^{16,17} but a normal counterpart of the HCL cell has not yet been substantiated. On frozen sections, the marginal zone lymphocytes in the spleen were not reactive with CD11c and CD25 (Tac),¹⁸ but these were positive in HCL specimens. Some studies have suggested that marginal zone cells occur in the lymph node,¹⁹ but others refute this. In the present immunohistochemical study on frozen sections of spleen the lymphocytes in the mantle zone were DBA.44+, CD11c-, CD5+, CD24+ and in the marginal zone were DBA.44+, CD11c-, CD5-, CD24+. In the paraffin wax sections numerous cells positive for DBA.44 were found in the mantle zone but only a few DBA.44 positive cells in the marginal zone. These findings agree with those of the previous report.¹⁵

Our recent study of the spleen in patients with gastric cancer showed the presence of MBC, especially in older patients.⁶ We discussed the possibility that MBC in the spleen could be a non-neoplastic counterpart of HCL. However, the MBC observed in the spleens of the present 14 patients with gastric cancer did not react positively for DBA.44.

In conclusion, this study has shown that DBA.44 reacted more strongly with MCL than MBCL. Non-neoplastic MBC were usually negative for DBA.44, but a positive reaction was seen in neoplastic MBC(MBCL), suggesting an increased reactivity for neoplastic cells.

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Over the years, I have noticed that it is uncommon for writers of WTBFHIIOP letters ever to admit honestly their mistakes even when these are pointed out to them in unambiguous terms, preferring to deny, obfuscate, or side-step their errors. Perhaps the problem lies with the fact that letters and authors' replies are not subject to peer review, merely to the perfunctory scrutiny of an over-worked editor. Perhaps that is how this one got through or am I to expect an ITWFHIIIML reply?

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¹ O'Brien DS. I read with interest the paper by . . . [letter]. *J Clin Pathol* 1994;47:868.

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Correction

In the October issue of the journal a printing error occurred in the paper by Ohsawa M, Kanno H, Machii T, Aozasa K (*J Clin Pathol* 1994;47:928-932). Figure 1 was labelled incorrectly as (A), (B), (A) but should read (A), (B), (C). The correct version of fig 1C is reproduced here.

Figure 1 Monocytoid cells near the sheath artery of the spleen (A) haematoxylin and eosin. Monocytoid cells showing a positive reaction for CD20 (B), but not for DBA.44 (C) (ABC method).

(C)