CD43 expression in B cell lymphoma

J Treasure, A Lane, D B Jones, D H Wright

Abstract

Aims: To determine the expression of CD43 in frozen sections in a range of B cell lymphomas.

Methods: The monoclonal antibody WR14, clustered provisionally in the Fourth Leucocyte Typing Workshop as a CD43 reagent, was investigated by epitope blocking studies on formalin fixed reactive lymph node tissue, using the established CD43 antibody MT1, to validate its use as a CD43 reagent. CD43 expression was studied in 131 immunophenotypically defined B cell lymphomas, including lymphocytic lymphoma (Lc, n = 13), centrocytic lymphoma (Cc, n = 14), and a range of follicle centre cell lymphomas (FCC) including centroblastic/centrocytic follicular (CbCcF, n = 48), centroblastic diffuse (CbD, n = 39), centroblastic/centrocytic diffuse (CbCcD, n = 4), centroblastic follicular and diffuse (Cb FD, n = 3) and centroblastic/centrocytic follicular and diffuse (CbCc FD, n = 1). Nine lymphomas of mucosa associated lymphoid tissue (MALT) were also examined.

Results: Epitope blocking studies showed that WR14 is a CD43 reagent that binds to an epitope identical with or close to that recognised by MT1. Eleven of 13 (84%) cases of Lc and 11 of 14 (78%) cases of Cc expressed CD43; 87 of 95 (91%) cases of FCC did not. All eight low grade lymphomas of MALT were negative. One high grade lymphoma, transformed from a low grade MALT lymphoma, was positive for CD43. The expression of CD43 by tumours of B cell lineage was associated with the expression of CD5 ($p < 0.001$) although either antigen could occasionally be found in the absence of the other.

Conclusion: CD43 reagents can be used in conjunction with CD5 antibodies for the immunophenotypic discrimination of follicle centre cell lymphomas from non-follicle centre cell lymphomas.

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CD43(sialophorin/leukosialin/glycoprotein 115) is a heavily glycosylated protein normally expressed by T lymphocytes, natural killer cells, and most myeloid cells, with weak expression in epithelial and synovial cells, fibroblasts, and chondrocytes.¹ In peripheral blood most B lymphocytes do not express CD43,² but it seems to be expressed as a

surface marker very early on in B cell ontogeny.¹ Evidence obtained in vitro suggests that CD43 may be expressed on B cells after activation.³ CD43 expression has been documented in lymphocytic lymphoma of B cell origin and B-CLL. Stross *et al* have described CD43 expression in five of six paraffin embedded biopsy specimens from patients with CLL and lymphocytic lymphoma, and also in 11 of 15 blood smears from patients with B-CLL.⁴ This suggests that the expression of CD43 parallels, at least in part, the expression of CD5.

Some authors have observed CD43 expression in a proportion of B cell lymphomas in formalin fixed, paraffin wax processed tissues, but the results have varied greatly, with positivity ranging from 0%,⁵ to 21%⁶ for follicle centre cell lymphomas and from 22%⁷ to 100%⁵ for CD5 positive neoplasms. Such variability may be attributable in part to the sensitivity of the antigen to fixation effects. To date, however, little information has been available concerning CD43 expression in frozen sections of B cell lymphomas.

The monoclonal antibody WR14 has been used in our routine diagnostic B cell panel for five years. In the Fourth Leucocyte Typing Workshop WR14 was provisionally clustered as a CD43 reagent¹ and we have performed epitope blocking studies to provide confirmatory evidence of this.

We reviewed our accumulated data concerning expression of CD43, demonstrated by WR14 in frozen section immunohistochemistry, in a range of 131 confirmed B cell lymphomas, and we now report the results in association with histological classification and previously determined immunophenotype.

Methods

Cases (n = 131) of immunophenotypically defined B cell lymphoma for which both paraffin wax processed material and frozen

University
Department of
Pathology,
Southampton General
Hospital, Tremona
Road, Southampton,
SO9 4XY

J Treasure
D B Jones
D H Wright

Department of
Regional Immunology
A Lane

Correspondence to:
D B Jones

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Table 1 Monoclonal antibodies used*

WR14	CD43	T, some B, myeloid, natural killer ¹
WR17	CD37	pan B ⁸
HD37	CD19	pan B ⁹
SHCL-1	CD22	pan B ¹⁰
OKT1	CD5	T + B-cell subset ¹¹
RFAL-1	CD10	CALLA ¹²
E11	CD35	(CR1, C3b receptor) follicular dendritic cells ¹³
UCHT-1	CD3	Mature T ¹⁴
OKT3	CD3	Mature T ¹¹

* Together with immunoglobulin light and heavy chain antibodies.

Figure 1 Histogram showing relative numbers of lymphomas which are CD43 positive (solid) and CD43 negative (shaded).

sections were available, were obtained from the files of Southampton General Hospital. Histological diagnosis, made according to the Kiel classification, was confirmed in routinely prepared sections without knowledge of the CD43 status. Frozen sections stained for a panel of monoclonal antibodies (table 1) were reviewed in all cases, and B cell lineage was confirmed by the use of CD37, CD19, and CD22. The results of staining for CD5 were available in 91 of the cases.

WR14 was raised following fusion of NS-O myeloma cells with splenocytes from a Balb/c mouse immunised with T-CLL cells.¹⁵

WR14 antibody, at a concentration of 5 mg/ml in 0.1 M NaHCO₃ (pH 8.5), was biotinylated by mixing with biotin (Sigma B2643) dissolved in DMSO at 25 mg/ml, to give a ratio of 1 mg biotin to 10 mg antibody (10%). After incubation at room temperature for four hours the reaction was stopped with 5 µl ethanolamine. Unwanted reactants were removed by extensive dialysis against PBS.¹⁶

Biotinylated WR14 was applied to sections of a formalin fixed reactive lymph node with or without prior application of the unlabelled CD43 monoclonal antibody MT1.⁵ The ability of MT1 to block WR14 binding was tested at a WR14 concentration of 1/200, a titre known to give good staining of lymphocytes in formalin

fixed, paraffin wax embedded tissue, with blocking steps from 1/50 to 1/400.

Results

Directly biotinylated WR14 antibody gave strong positive staining in reactive lymph node at a range of concentrations. In the presence of the unlabelled CD43 reagent MT1, however, the activity of directly biotinylated WR14 was blocked, indicating that WR14 is a CD43 reagent which binds to an epitope identical with, or close to, that recognised by MT1.

CD43 expression was seen in 11 of 13 (84%) Lc and 11 of 14 (78%) Cc (fig 1) but in only 9% of the 95 FCC: CbCcF two of 48, CbD three of 39, CbCcD none out of four, CbFD two of three and CbCc FD one of one. The eight low grade MALT lymphomas were all CD43 negative. One high grade tumour, which had transformed from a low grade MALT lymphoma, showed strong CD43 staining of all cells.

There was a significant positive correlation between the expression of CD43 and CD5 ($p < 0.001$) (table 2). In a few cases (13%) CD5 and CD43 were not coexpressed.

Discussion

There have been few studies of paraffin wax processed material on the expression of CD43 on neoplastic B cells. The reported results vary considerably. In follicle centre cell lymphomas most authors have reported negative results with CD43 reagents,^{5,17-20} but positive staining has been reported in a small number of cases^{4,6,21}; 15 cases from our own files in which MT1 staining in paraffin wax was available were all negative, including one in which WR14 expression was shown in frozen tissue. In lymphocytic lymphomas weak positive staining has been observed in all cases ($n = 7$),⁵ or in only 50% ($n = 12$)²²; in three cases from our files two cases showed positive staining in some of the cells. All 12 cases of centrocytic lymphoma in Poppema's series⁵ showed weak positive staining, but three cases reported by Dobson²¹ were negative, and 11 of 12 cases in this department were also negative in paraffin wax processed material, including two in which WR14 staining was positive in frozen sections. In view of the variable results obtained in paraffin wax studies this frozen section study of a large series of cases gives a useful baseline assessment of rates of CD43 expression.

Several authors have observed that CD43 antibodies reactive in formalin fixed, paraffin processed material, such as MT1, stain neoplastic B cells more weakly than the accompanying reactive T lymphocytes^{4,5,7,17} and thus the sensitivity of the antigen detection system and the conditions of fixation may be important limiting factors.²³ Ng *et al* reported that whereas all of 55 T cell lymphomas were positive in frozen sections, only 69% stained positively in paraffin processed material.⁷ They showed an inverse correlation between fixation time and positivity rate. Our study of overall

Table 2 Relation between CD43 and CD5

	Total cases in which CD5 available	CD43+ CD5+	CD43+ CD5-	CD43- CD5+	CD43- CD5-
Lc	8	6	2	0	0
Cc	7	5	2	0	0
CbCcF	36	0	0	0	36
CbD	25	0	2	3	20
CbCcD	4	0	0	0	4
Cb FD	3	0	2	0	1
CbCcFD	1	1	0	0	0
MALT, LG	7	0	0	1	6
Total	91	12	8	4	67

Significantly associated, $\chi^2 = 31.8$; $p < 0.001$.

Figure 2 (A-C) Lymphocytic lymphoma showing expression of CD43 (A) and CD5 (B), but negative staining for CD10 (C). Scattered reactive T lymphocytes show stronger CD43 expression than positive tumour cells in (A). (D-F) Centrocytic lymphoma, also positive for CD43 (D) and CD5 (E) but negative for CD10 (F).

Figure 2 (G-I) Follicle centre cell lymphoma, negative for CD43 (G) and CD5 (H) but positive for CD10 (I).

CD43 expression in frozen tissue avoided false negative results due to fixation effects.

The monoclonal antibody WR14 has been used in our diagnostic B cell frozen section panel for five years. Studies reported in the Fourth Leucocyte Typing Workshop, together with our confirmatory epitope blocking studies, indicate that WR14 has CD43 reactivity and it has therefore been possible to review the expression of CD43, as detected by WR14 frozen section immunostaining, in a large range of B cell lymphomas.

The expression of CD43 by normal T lymphocytes can occasionally cause difficulty in interpretation of staining results, particularly where a heavy reactive T cell infiltrate is present. We were able to distinguish between CD43 positive reactive T lymphocytes and neoplastic B lymphocytes, both on morphological grounds, and with the use of parallel CD3 staining.

We have shown that most lymphocytic and centrocytic lymphomas express CD43 (84% and 78%, respectively): most FCC lymphomas are CD43 negative (Fig 1). A slightly higher proportion of centroblastic diffuse lymphomas (three of 36) than CbCcF lymphomas (two of 46) gave positive results. This may be explained by the higher grade nature of these lesions, bearing in mind that CD43 may be expressed on the surface of B cells following activation.¹

All eight low grade MALT lymphomas were CD43 negative. These tumours typically show absence of CD5 expression,²⁴ and the absence of CD43 expression adds to the evidence that the centrocyte-like cells of MALT lymphoma are immunophenotypically distinct from the neoplastic cells of centrocytic lymphoma. The only positive tumour of MALT in our series was a high grade tumour showing the features of transformation.

Stross *et al* have previously described an association between CD43 and CD5 expression in a small series of paraffin wax processed, formalin fixed lymphocytic lymphoma and CLL, and in blood films from patients with CLL.⁴ Various authors have reported that CD5 is present on only a minority of follicle centre cell lymphomas.²⁵⁻²⁷ Our data, based on a large series of phenotypically defined tumours of the B cell series, confirm that the correlation between CD5 and CD43 is significant, and show that while either antigen can occasionally be found in isolation, the use of antibodies to both CD5 and CD43 in a diagnostic panel can provide valuable confirmatory information concerning B cell sublineage.

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