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Original Paper

Comparative Expression of Major Histocompatibility Complex (MHC) Antigens on CD5$^+$ and CD5$^-$ B Cells in Patients with Chronic Lymphocytic Leukaemia (CLL)

A.M.E. Nouri,[1] S. Smith,[2] A.C. Newland[2] and M.G. Macey[2]

[1]Departments of Medical Oncology; and [2]Haematology, London Hospital Medical College, Turner Street, London E1 2AD, U.K.

The aim of this study was to investigate the expression of major histocompatibility complex (MHC) antigens on CD5$^+$ and CD5$^-$ B cells of 13 patients with chronic lymphocytic leukaemia (CLL). This was carried out using a series of monoclonal antibodies (MAbs) against polymorphic and monomorphic class I and class II antigens, as well as to the transferrin receptor and assessed by flow cytometry and direct and indirect immunofluorescence. The expression of these molecules was assessed as mean fluorescent intensity (MFI). The results showed that cells from all 13 individuals expressed monomorphic class I antigens. The number of cases expressing polymorphic HLA-Bw6, -Bw4, -B7, -B27 and -A2 class I antigens on CD5$^-$ B cells was 11 (85%), 6(46%), 2(15%), 1(8%), 3 (23%), respectively, which was consistent with the expected population frequency distributions of these antigens. For each of the class I antigens on CD5$^+$ and CD5$^-$ B cells, the ratio of the MFI was greater than 1 in 12 of 13 cases. For the transferrin receptor (CD71), this ratio was also almost always greater than 1. These results indicate that, unlike solid tumours where the loss or abnormal expression of class I and II antigens is a frequent event, the expression of class I antigens in CLL patients seems to be normal. This indicates that the loss of these antigens cannot provide the leukaemic cells with a selective advantage to escape immunological detection.

Key words: CLL, leukaemia, MHC antigens, immune system
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INTRODUCTION

THE ROLE of the immune system and consequently that of the major histocompatibility complex (MHC) antigens, in discriminating self from non-self, is well established [1]. During the past few decades, various reports in support of such an assertion have been documented. These include the demonstration of the critical role of MHC antigens in resistance to viral infection and rejection of tumours in experimental animals [2, 3]. The most significant evidence in favour of immune surveillance is the finding of frequent abnormal expression of MHC molecules in a variety of human tumours, including breast [4], lymphoma [5], colon [6, 7] and bladder [8] and the finding that the treatment of cancer patients with cytokines such as interferon α, known to induce MHC antigens [9], can result in a partial or complete remission in a significant minority of melanoma and renal carcinoma patients [10, 11].

In leukaemic patients, the search for a tumour specific antigen(s), a possible target for immune attack, has been unsuccessful. However, there have been a number of reports implicating the role of the immune system in the pathogenesis of the disease in these patients. Apperley and colleagues [12] and later Hughes and associates [13] reported that patients receiving allogeneic bone marrow transplants (BMT) were at a higher risk of leukaemic relapse when there was a high degree of T cell contaminant in the grafted cells. Interestingly, Sosman and colleagues [14] and later studies by Jahn and associates [15] showed that CD3$^+$ CD8$^+$ T cell lines isolated from acute lymphoblastic leukaemia patients could kill leukaemic cells but not remission lymphocytes from the same patients or natural killer (NK) tumour target cells. In

Correspondence to A.M.E. Nouri.
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another study, Lim and colleagues [16] showed that T cells from leukaemic patients had elevated levels of cytoplasmic serine esterase granules in their T cells and increased circulating soluble interleukin-2 (IL-2) receptors and CD8 molecules.

Chronic lymphocytic leukaemia (CLL) is the most common lymphoid leukaemia. It is a clonal proliferation of a subset of B cells expressing the CD5 antigen. The aim of this study was to establish whether $CD5^+$, B CLL cells use the abnormal expression of MHC antigens as a means of evading immunosurveillance.

MATERIALS AND METHODS

Patient material

Blood samples anticoagulated with ethylene diamine tetraacetic acid (EDTA) were collected from 13 patients with CLL classified according to the guidelines of the European Haematology Association. Samples from newly diagnosed patients not receiving chemotherapy were cryopreserved for subsequent analysis in batches.

Primary and labelled antibodies

The monoclonal antibodies (MAbs) used as primary reagents in the form of tissue culture supernatants (together with their specificities) were as follows: W6/32 (all beta 2m-associated HLA-A,B,C antigens) [17], 116.5.28 and 126.39 (Bw4 and Bw6 antigens) which were gifts from Dr Gelsthorpe. ME1 (HLA-B7) which was a gift from A. McMichael (Oxford), BB7.2 (HLA-A2) [8], TAL14.1 (DR antigens) which was a gift from Dr J. Bodmer (ICRF) and L243 (HLA-DR) [18].

Phycoerythrin (PE)-conjugated CD5 was obtained from Becton Dickinson (Oxford, U.K.) fluorescein isothiocyanate (FITC)-conjugated rabbit antimouse conjugated immunoglobulins were from Dako (Basingstoke, U.K.) and Quantum Red (QR)-conjugated CD 19 was from Sigma (Poole, U.K.). The same batches of antisera were used throughout the study.

Cell labelling

Blood (100 µl) was incubated with 10 µl of anti-HLA antibody for 15 min at room temperature, then washed in Hank's Balanced Salt Solution (HBSS) prior to the addition of 5 µl of FITC antimouse immunoglobulin. After incubation for 10 min, the cells were washed in HBSS then incubated for 10 min with 10 µl of mouse immunoglobulin. The cells were washed with HBSS then incubated with 10 µl each of PE CD5 and QR CD19 for 10 min prior to processing in the Q-prep work station (Coulter, Luton, U.K.) then analysed by flow cytometry.

Flow cytometric analysis

Prepared cells were analysed on a FACScan analyser (Becton Dickinson) using the LysysII program and Consort 3.2 software. Lymphocytes were identified based on their forward and side scatter properties (region 1, Figure 1a). The log PE and QR fluorescence associated with region 1 was displayed on a dual parameter histogram (Figure 1b), $CD5PE^+$ $CD19QR^+$ (region 2) and CD5 – PE $CD19^+$ QR (region 3) lymphocytes were identified. The FITC fluorescence associated with regions 2 and 3 were then displayed on histograms of log fluorescence on the x-axis and cell count on the y-axis (Figure 1c). For each analysis, 10 000 events were

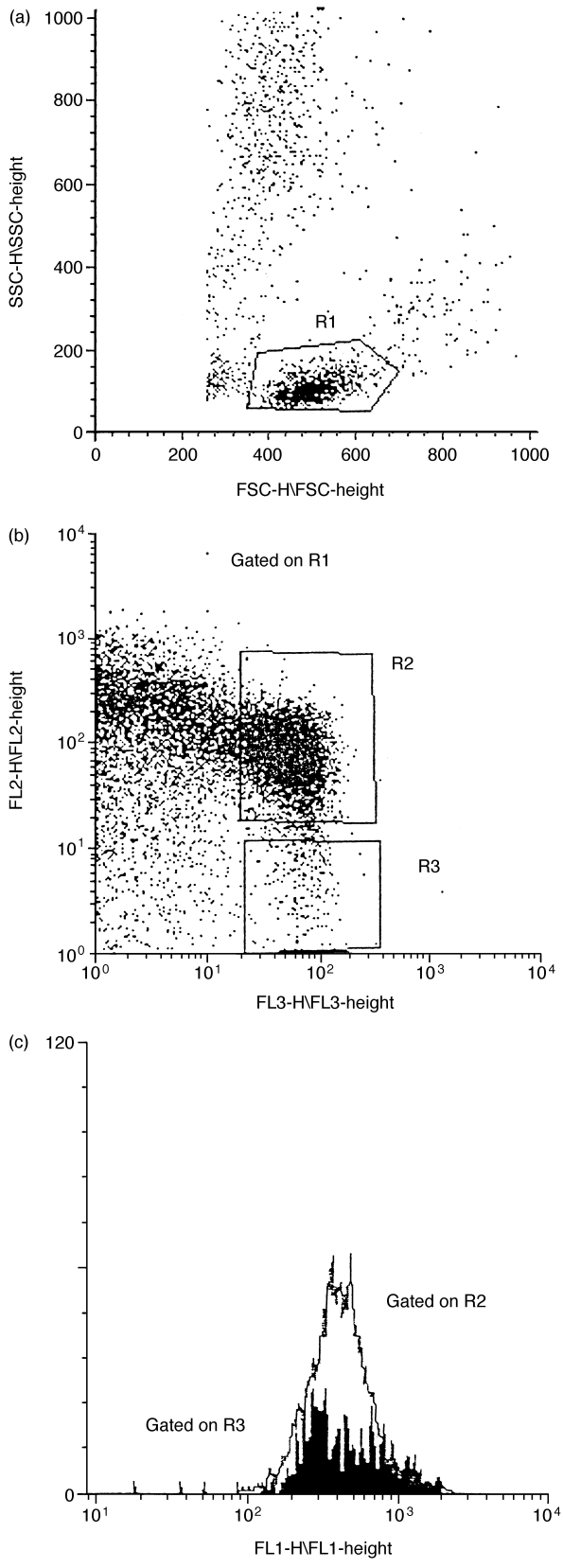

Figure 1. (a) Lymphocytes were gated in region R1 based on their forward and side scatter properties. The orange and red fluorescence associated with the lymphocytes in region R1 is shown in (b). $CD19^+$ $CD5^+$ lymphocytes were gated in region R2 and $CD19^+$ $CD5^-$ lymphocytes were gated in region R3. The green fluorescence intensity of cells in R2 and R3 is shown in (c).

accumulated and the percentage positive cells and mean fluorescence intensity recorded. Spectral compensation was carried out on single and dual labelled samples before analysis of triple labelled lymphocytes. The flow cytometer was calibrated prior to use with Fluorospheres (Dako).

Statistical analysis

Differences between antigen expression on $CD5^+$ B cells and $CD5^-$ B cells were determined using a paired Student's *t*-test.

RESULTS

Monomorphic and polymorphic class I and class II antigen expression on $CD5^-$ B cells

$CD5^-$ B cells in all 13 samples from CLL patients expressed class I and class II antigens, but did not express detectable levels of CD71. Monomorphic class I (as detected by W6/32 MAb) showed positive staining in all cases. The number of cases showing positivity for polymorphic HLA-Bw6, -Bw4, -B7, -B27 and -A2 antigens was 11 (85%), 6 (46%), 2 (15%), 1 (8%) and 3 (15%), respectively (Table 1). In all cases, except -Bw6 which had slightly higher than the normal frequency, these were the expected frequencies.

Mean fluorescent intensity (MFI) measurement of antibodies to class I and class II antigens on $CD5^+$ and $CD5^-$ B cells

The MFIs for the binding of MAbs to class I and II antigens on $CD5^-$ and $CD5^+$ B cells were compared. Table 2 shows that for the class I antigens, the MFIs on $CD5^-$ cells of all 13 cases, ranged from 18 to 94 with a mean standard deviation (SD) of 34 ± 23. The $CD5^+$ B cells of the same individuals had MFIs which ranged from 26 to 110 with mean $\pm$ SD of 43 ± 21. The corresponding values for class II antigens were 63 ± 43 and 86 ± 41, respectively. MFIs for HLA-Bw6 and Bw4 are also shown in Table 2. The MFIs of $CD5^-$ and the corresponding $CD5^+$ B cells were significantly different for each antigen type. These results indicate that generally the MFIs of $CD5^+$ B cells were greater than the MFIs of the corresponding $CD5^-$ B cells for monomorphic and polymorphic class I antigens, HLA-Bw4 and -Bw6 (case 5 showed higher MFIs on $CD5^+$ than on $CD5^+$ cells for class I antigens). A similar profile was obtained for the expression of the CD71 molecule (Table 2). In almost all cases, the MFI ratios of $CD5^+/CD5^-$ cells was greater than 1.

Table 1. *Pattern of expression of polymorphic class I antigens in $CD5^-$ B cells from chronic lymphocytic leukaemia patients*

Case	Bw6	Bw4	B7	A2	B27
1	+	–	–	–	–
2	+	+	–	+	–
3	+	–	–	–	–
4	+	+	–	–	–
5	+	–	–	–	–
6	+	–	–	–	–
7	+	–	+	–	–
8	–	+	–	+	–
9	+	–	–	–	–
10	–	+	+	+	+
11	+	+	–	–	–
12	+	+	–	–	–
13	+	–	–	–	–
No. +ve	11	6	2	3	1
% +ve	85	46	15	23	8

Results of flow cytometric analysis are presented as + and – .

DISCUSSION

These results suggest that, unlike other tumours, the expression of class I antigens on CLL cells is not reduced and, hence, could not provide the leukaemic cells with a selective advantage for escaping immune detection mechanism(s).

The demonstration of a high frequency of various malignancies in immunosuppressed patients has been the most convincing evidence in support of an immunosurveillance hypothesis. This has been reinforced by the observation of the frequent loss or presence of defective MHC antigens in many human cancers, including breast [4], colon [6] and bladder [7, 8]. These abnormalities have led to the suggestion that such defects could allow tumour clones a selective advantage to escape immunological detection. Indeed, Spier and colleagues [19] reported that tumour cells frequently lose their MHC antigens in non-immune suppressed patients, whilst in immunosuppressed patients, such losses are rather rare events, suggesting that tumour cells in these patients do not need to evade the immune system to survive.

The findings of this investigation showed that there was no case where deficiencies in class I antigens as detected by MAbs specific for monomorphic or polymorphic antigens, could be demonstrated. Indeed in 12 of 13 cases (case 5 excepted), the intensity of class I antigens was greater on $CD5^+$ B cells when compared with the corresponding $CD5^-$ B cells. MAbs to screen all the possible polymorphic antigens were not employed, but the available data do not support the notion that the loss of HLA antigens was a major mechanism for the leukaemic lymphocytes to evade immune detection.

In recent years, many investigators have reported the presence of so-called tumour specific antigen(s) [20, 21], many of which could be oncogene products or overexpressed self antigens. Tumour infiltrating lymphocytes (TILs) are believed to be critical to allow these antigens to be recognised by the immune system and be used as a vehicle for discriminating between self and non-self in the context of MHC antigens. This is particularly interesting considering the findings of Spier and colleagues [19] who showed that unlike immunosuppressed individuals, where there were no T cell infiltrates in tumours, a large number of TILs could be seen in tumour biopsies of immunocompetent patients with B cell lymphomas.

The generation and expansion of TILs, particularly from patients with solid tumours, has been reported for some time [22, 23]. In our study [23], TILs expanded *in vitro* by IL-2 from tumour biopsies of patients with bladder cancer, showed defective killing against autologous tumour cells. We proposed that the defect could be due to the abnormal expression of polymorphic class I antigens seen on the tumour cells. We also reported that in leukaemic patients *in vitro* IL-2-activated lymphocytes showed a gradual increase in the percentage of $CD3^+$ cells and this increase paralleled the decrease in the number of leukaemic, CD10/CD19 expressing cells [24]. We hypothesised that the decrease in tumour cell number could be either due to tumour cell apoptosis or to killing by the IL-2-activated T cells in the MHC restricted fashion.

In all 13 cases investigated in this study, class II antigens were expressed on $CD5^-$ B cells, a finding which confirms

Table 2. Mean fluorescent intensities of class I and II antigens for paired CD5$^-$ and CD5$^+$ B cells of a cohort of chronic lymphocytic leukaemia patients

	Class I		Class II		Class I Bw4		Class I Bw6	
Case	CD5$^-$	CD5$^+$	CD5$^-$	CD5$^+$	CD5$^-$	CD5$^+$	CD5$^-$	CD5$^+$
1	27	35	39	46	na	na	329	334
2	26	41	29	47	49	94	21	39
3	26	41	44	70	52	77	na	na
4	18	27	170	63	51	154	19	24
5	75	44	49	67	24	43	na	na
6	21	50	89	22	40	93	na	na
7	23	36	40	123	38	167	na	na
8	22	26	46	75	na	na	23	25
9	44	55	39	89	120	142	na	na
10	94	110	141	179	na	na	47	58
11	21	31	42	103	47	72	17	19
12	21	35	42	121	25	69	15	30
13	28	40	56	118	44	120	na	na
Mean ± S.D.	34 ± 23	43 ± 21	63 ± 43	86 ± 41	65 ± 59	124 ± 79	24 ± 11	32 ± 14
P value*	< 0.001		< 0.001		0.001		0.024	

na, not available, S.D. standard deviation. *Student's *t*-test.

our earlier report [25]. The CD5$^+$ B cells showed a higher expression of class II antigens in all cases except two (cases 4 and 6), than the CD5$^-$ B cells and the ratios of MFIs for CD5$^+$ and CD5$^-$ B cells were greater than unity. These observations are in accordance with the findings of Lee and Oliver [26] who showed high expression of class II antigens in patients with acute myeloid leukaemia. These authors went on to show that despite the high expression, the cells failed to function normally as antigen presenting cells, although this could be corrected by co-stimulation of remission lymphocytes with autologous blasts plus allogeneic cells expressing class II antigens to induce HLA-restricted cytotoxicity [27]. In a more recent report, Hirano and associates [28], investigating various cell surface molecules, also reported the high expression of class II antigens in more than 80% of these leukaemic patients, although the exact significance of these findings remains to be established. The report by Guerry and colleagues [29] showed that in melanoma, despite the high expression of class II, these antigens failed to engage T helper cells as they could not act as restriction elements for T cell activation. Further experiments are planned to discern if there is a similar process occurring in our CLL patients.

It has long been established that one common feature of many tumour cells is the high expression of the transferrin receptor (CD71) [30]. The findings of this investigation indicated that the levels of CD71 were very low or undetectable on CD5$^-$ B cells. However, the expression on the corresponding CD5$^+$ B cells was very much higher in almost all cases, with CD5$^+$/CD5$^-$ ratios of up to 75. This indicated that like MHC antigens, CD71 expression was also unregulated on the CD5$^+$ B cells, consistent with their higher proliferative nature.

In conclusion, the findings of this investigation have shown that the cell surface molecules HLA and CD71 were expressed on CD5$^+$, leukaemic B cells. Although no firm conclusion could be drawn from the results as they do not cover all the polymorphic class I antigens, it is tempting to argue that in these patients the loss of MHC antigens does not appear to play a critical role in the evasion of CD5$^+$ B cells from immunosurveillance.

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