



## Expression of adhesion molecules in chronic B-cell lymphoproliferative disorders

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### Abstract

**Background and Objective.** Abnormalities in the expression of cell adhesion molecules (CAM) are thought to influence the patterns of intranodal growth and hematogeneous spread of malignant cells in chronic lymphoproliferative disorders (LPD). Therefore, the characterization of CAM phenotypic profiles of the neoplastic clones in LPD may help to identify distinct subtypes with prognostic implications. In this work we sought to investigate whether the expression of CAM by circulating malignant cells in patients with B-cell LPD differed from that of normal peripheral blood B-lymphocytes (PBL) and whether the observed phenotypic patterns could be correlated to other biological and clinical parameters of known clinical relevance.

**Design and Methods.** Peripheral blood mononuclear cells were obtained from 148 patients with B-cell chronic lymphocytic leukemia (B-CLL), 52 with B-cell non-Hodgkin lymphomas (NHL) and 10 with hairy cell leukemia (HCL). The expression of CAM was analyzed by flow cytometry using monoclonal antibodies against CD49d, CD29, CD11a, CD11b, CD11c, CD18, CD62L, CD54 and CD44.

**Results.** All CAM were detected in normal peripheral blood B-lymphocytes, except CD11c and CD54, which were present in only a minority of cells. Fluorescence mean channel values (FMC) showed that all molecules, with the exception of CD44, were expressed with dim intensity. Emerging patterns of CAM expression, as assessed by FMC values, were observed in different LPD: thus, B-CLL is characterized by a very low expression of CD49d/CD29 and  $\beta_2$  integrins. In this disorder, CD49d/CD29, CD11a, and CD54 increase with tumor burden; NHL show high expression of CD29 and CD54; strong expression of all molecules (except CD11a) was found in HCL, particularly CD11c (FMC values 60 times higher than normal). CD62L was faintly expressed in all diagnostic groups, whereas CD11c showed consistently high FMC values.

**Interpretation and Conclusions.** The data shows that the phenotypic characterization of LPD can be further refined by the analysis of their patterns

of CAM expression which may help to identify distinct subsets within each nosological group.  
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Key words: cell adhesion molecules, chronic lymphocytic leukemia, non-Hodgkin's lymphoma

Chronic lymphoproliferative disorders of B-cell lineage are a heterogeneous group of neoplasms that range from B-cell chronic lymphocytic leukemia without lymph node involvement, to low-grade non-Hodgkin lymphomas with minimal leukemic expression.<sup>1</sup> This dichotomy is nevertheless not absolute, since advanced-stage NHL frequently develop an overt leukemic phase and CLL patients are commonly seen with generalized lymphadenopathies, hepatomegaly and/or splenomegaly.<sup>1,2</sup>

Although the determinants of the biological behavior of LPD are still unknown, it is generally accepted that quantitative and qualitative abnormalities in the expression of CAM may account for the patterns of intranodal growth and hematogeneous spread of malignant cells.<sup>3-5</sup> In fact, the trans-endothelial migration of lymphocytes and their localization in lymphoid organs depends on complex mechanisms of intercellular adherence that are largely mediated by specific cell-surface adhesion molecules and their ligands, belonging to the integrin family, the family of the immunoglobulin (Ig) supergene related molecules, the selectin family and CD44, the *lymphocyte homing receptor*.<sup>6,7</sup>

Abnormalities in the expression of most of these molecules have been recently reported in several types of B-cell LPD. They may be relevant to the understanding of the clinical behavior of these disorders and may prove to be useful as prognostic indicators. Within the  $\beta_2$  integrins, the expression of LFA-1 (CD11a/CD18) has been associated with a low probability of hematogeneous dissemination, and with a nodular pattern of lymph node involvement in low-grade lymphomas.<sup>3,8,9</sup>

On the other hand, NHL with a predominant

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nodular pattern of growth and an infrequent leukemic behavior, as well as CLL patients with large lymphadenopathies and splenomegaly, exhibit a high expression of other molecules like CD11b and CD54.<sup>4,10</sup> A strong expression of CD44 and CD62L has also been associated with progressive leukocytosis and lymphoma dissemination, and correlates to a bad prognosis.<sup>5,11,12</sup>

Although the expression of cell adhesion molecules has been carefully characterized in recent years, some of the results concerning a possible correlation between surface phenotypes and the clinical behavior of the malignant clones are conflicting. Furthermore, the presence of different patterns of CAM expression in various specimens of the same patient points to an intra-sample variability that may depend on the specific micro-environment, the stage of maturation and activation or even on the role of a particular CAM. This fact suggests that CAM expression in lymphoid cells may reflect a particular movement momentum, supporting our previous observations in the functional compartments of lymph nodes and tonsils.<sup>13</sup>

In this work we analyzed the expression of 9 different adhesion molecules in the bone marrow and/or peripheral blood lymphocytes from 210 patients suffering from B-cell LPD in leukemic phase. We also compared the patterns of CAM expression with those of normal circulating B-lymphocytes. The identification of specific phenotypic profiles of CAM expression among different types of chronic B-cell LPD may contribute to a more accurate diagnosis of these conditions and may prove to be useful in predicting their clinical behavior.

## Materials and Methods

### Patient population

Two hundred and ten patients with chronic B-cell LPD (148 B-CLL, 10 HCL and 52 NHL with leukemic expression) from the Hematology Department of the Instituto Português de Oncologia de Francisco Gentil (Lisbon), Hospital de Santa Cruz (Lisbon) and Hospital Distrital de Leiria (Leiria) were studied between January 1995 and December 1996. The overall median age of the patients was 65 years (ranging from 31 to 84 years), with a male to female ratio of 1:1.3. The mean values of peripheral lymphocyte counts at diagnosis were  $29 \times 10^9/L$  in B-CLL patients,  $13 \times 10^9/L$  in NHL and  $4.8 \times 10^9/L$  in HCL.

The diagnosis of B-CLL was based on well accepted criteria,<sup>14</sup> and patients classified according to the staging criteria of Rai<sup>15</sup> and Binet.<sup>16</sup> HCL and NHL were diagnosed on basis of clinico-pathological, morphological and immunological features.<sup>17</sup> Peripheral blood samples obtained from ten healthy adult volunteers were used as controls.

### Cell staining and monoclonal antibodies

Peripheral blood and bone marrow samples collected from patients and normal donors were processed and analyzed by flow cytometry within the first 24 hours.

For cell staining,  $2 \times 10^6$  nucleated cells were incubated with 15  $\mu L$  of the relevant mouse monoclonal antibodies (15 min. at room temperature in the dark), lysed with Becton-Dickinson FACS lysing solution (according to the manufacturer indications) and then washed and resuspended in phosphate-buffered saline (PBS) solution. For the characterization of the surface immunoglobulin ( $\kappa$  and  $\lambda$  light chains), cells were washed three times before incubation with the specific monoclonal antibodies (moAb). The moAb used in this study are described in Table 1.

In order to better differentiate CLL from other forms of LPD, the CLL diagnostic scoring system proposed by Matutes *et al.*<sup>18</sup> was applied. This system is based on the proportion of cells expressing CD5, CD23 and FMC7, as well as on the intensity of expression of CD22 and surface immunoglobulin. The difference between CLL and NHL was found to be highly significant ( $p < 0.0001$ ), confirming the value of this scoring system.<sup>19</sup>

The CAM investigated in this study were:  $\beta_1$  inte-

**Table 1. Monoclonal antibodies.**

MoAb	Fluoro-chrome	Isotype	Specificity	Source
Leu-1	FITC	IgG2a	CD5	BD
Leu-12	FITC/PE	IgG1	CD19	BD
CALLA	FITC	IgG2a	CD10	BD
Leu-16	PE	IgG1	CD20	BD
Leu-14	PE	IgG2b	CD22	BD
HLA-DR	PE	IgG2a	HLA-DR	BD
Rec.TAC/IL-2	PE	IgG1	CD25	BD
9P25/I0B8	FITC	IgG1	CD23	IMM
FMC7	FITC	IgM	FMC7	IMM
$\kappa$ light chain	FITC	IgG1	$\kappa$ light chain	BD
$\lambda$ light chain	PE	IgG1	$\lambda$ light chain	BD
25.3/IOT16	FITC	IgG1	CD11a (LFA-1, $\alpha_L$ )	IMM
Bear1/IOM1	PE	IgG1	CD11b (Mac-1, $\alpha_M$ )	IMM
Leu-M5	FITC	IgG2b	CD11c (p150.95, $\alpha_X$ )	BD
7E4	FITC	IgG1	CD18 ( $\beta_2$ chain)	IMM
K20/IOT29	FITC	IgG2a	CD29 (VLA-4, $\beta_1$ )	IMM
J.173/IOL44	FITC	IgG1	CD44 (H-CAM)	IMM
HP2.1/IOP49d	FITC	IgG1	CD49d (VLA-4, $\alpha_4$ )	IMM
84H10/IOL54	FITC	IgG1	CD54 (ICAM-1)	IMM
DREG56	FITC	IgG1	CD62L (LECAM-1)	IMM

Abbreviations: FITC, fluorescein isothiocyanate; PE, phycoerythrin; BD, Becton-Dickinson (San José, CA, USA); IMM, Immunotech (Marseille-Luminy, France).

grins, CD49d (VLA- $\alpha_4$ ) and CD29 (VLA- $\beta_1$ );  $\beta_2$  integrins, CD11a (LFA-1  $\alpha_L$ ), CD11b (Mac-1  $\alpha_M$ ), CD11c (p150,95  $\alpha_X$ ) and CD18 ( $\beta_2$  chain); selectin family, CD62L (LECAM-1); immunoglobulin superfamily, CD54 (ICAM-1); CD44 (H-CAM) (Table 1).

### Immunophenotypic analysis

Two color immunofluorescence analyses were performed using a FACScan flow cytometer (Becton-Dickinson Immunocytometry Systems, San José, Ca, USA). Negative controls were provided by cells incubated with an irrelevant moAb of an identical isotype, combined with the same fluorochrome (Simultest Control, BDIS, San José, Ca, USA).

The cytometer was calibrated in order to obtain a fluorescence mean channel (FMC) between 2 and 4 for the negative cells. A lymphocyte gate was established based on known forward and right angle side-scatter lymphocyte characteristics. In order to focus the analysis on the B-lymphoid cell population, the evaluation of the expression of the adhesion molecules was restricted to the CD19<sup>+</sup> cells, using a double-labelling combination of CAM-FITC/CD19-PE, except for CD49d and CD11c, where the two fluorochromes were used in a reverse order.

For each sample, the expression of each particular molecule was defined as positive when identified in more than 20% of the cells. Furthermore, the analysis was also based on the identification of the fluorescence mean channel for each CAM and the results were expressed as a ratio between the observed FMC and the FMC of normal B lymphocytes (FMC ratio, or FMCR), as previously described.<sup>20</sup>

### Statistical analysis

To determine statistical significance, the paired Student's t-test was used and the null hypothesis rejected at the  $p < 0.05$  level.

## Results

### Expression of CAM in normal peripheral blood B lymphocytes

The expression of CAM in normal PBL is summarized in Table 2. Most of the analyzed CAM are expressed in normal B cells. The only exceptions are CD11c and CD54, which were detected in only 19% and 17% of B cells, respectively. When assessed by FMC analysis, all molecules are expressed with dim intensity (FMC ranging from 7 to 54), with the exception of CD44 which shows a FMC of 351.

### Comparison of the expression of CAM in bone marrow and PBL

The expression of the 9 adhesion molecules in bone marrow was compared to that of PBL sam-

ples in 28 patients (19 B-CLL, 7 NHL and 2 HCL). The expression of CAM was found to be similar in PBL and bone marrow cells in all patients ( $p \geq 0.15$ ). Accordingly, subsequent studies on the expression of CAM were performed exclusively in PBL samples.

### Expression of VLA-4 by circulating neoplastic B cells

In CLL patients, both CD49d and CD29 are weakly expressed when compared to normal B lymphocytes ( $p < 0.0001$ ) (Table 3). Our results show that the intensity of expression of CD49d increases with tumor burden. In fact, patients in Binet stage

**Table 2. Expression of adhesion molecules in normal peripheral blood B lymphocytes.**

Family of CAM	CAM	FMC* (Mean $\pm$ sem $^\circ$ )	Positive cells (% $\pm$ sem $^\circ$ )
$\beta_1$ integrins	CD49d	36.1 $\pm$ 6.6	97.9 $\pm$ 2.6
	CD29	18.4 $\pm$ 3.6	70.7 $\pm$ 9.0
$\beta_2$ integrins	CD11a	22.6 $\pm$ 4.0	94.4 $\pm$ 5.0
	CD11b	12.8 $\pm$ 2.8	40.1 $\pm$ 9.4
	CD11c	20.9 $\pm$ 6.0	19.2 $\pm$ 5.4
	CD18	21.3 $\pm$ 2.6	89.5 $\pm$ 6.5
Ig supergene	CD54	7.0 $\pm$ 1.5	17.6 $\pm$ 7.0
Selectin	CD62L	54.2 $\pm$ 27.0	88.3 $\pm$ 9.8
	CD44	351.4 $\pm$ 39.9	99.5 $\pm$ 1.3

\*FMC: fluorescence mean channel;  $^\circ$ sem= standard error of the mean.

**Table 3. Expression of CAM in lymphoproliferative disorders.\***

Family of CAM	CAM	CLL (n=148) (mean $\pm$ sem $^\circ$ )	NHL (n=52) (mean $\pm$ sem $^\circ$ )	HCL (n=10) (mean $\pm$ sem $^\circ$ )
$\beta_1$ integrins	CD49d	0.33 $\pm$ 0.04	1.02 $\pm$ 0.10	1.12 $\pm$ 0.12
	CD29	0.86 $\pm$ 0.05	1.52 $\pm$ 0.14	2.99 $\pm$ 0.32
$\beta_2$ integrins	CD11a	0.41 $\pm$ 0.03	0.95 $\pm$ 0.14	0.81 $\pm$ 0.14
	CD11b	0.38 $\pm$ 0.03	0.64 $\pm$ 0.09	1.21 $\pm$ 0.20
	CD11c	2.36 $\pm$ 0.20	2.82 $\pm$ 0.75	62.14 $\pm$ 14.06
	CD18	0.38 $\pm$ 0.03	0.94 $\pm$ 0.16	2.09 $\pm$ 0.51
Ig supergene	CD54	0.85 $\pm$ 0.04	2.43 $\pm$ 0.34	2.61 $\pm$ 0.29
Selectin	CD62L	0.55 $\pm$ 0.04	0.48 $\pm$ 0.05	0.31 $\pm$ 0.11
	CD44	0.85 $\pm$ 0.03	1.00 $\pm$ 0.09	3.41 $\pm$ 0.54

\*Results are expressed as FMCR;  $^\circ$ sem= standard error of the mean.

A (low tumor burden) have a very low expression of CD49d (FMCR = 0.19), which differs significantly ( $p < 0.05$ ) from that in patients with stages B and C (FMCR = 0.38 and FMCR = 0.48, respectively) (Table 4). It should be noted, however, that the intensity of the expression of CD49d in patients with high tumor burden, remains lower than that in normal PBL. Concerning the expression of CD29 in CLL, a similar trend is observed when comparing the intensity of expression in patients in stage A, with those in stages B and C ( $p \leq 0.02$ ) (Table 4). The expression of this molecule also differed significantly between patients with and without splenomegaly (FMCR =  $0.72 \pm 0.05$  and FMCR =  $1.25 \pm 0.15$ , respectively). The difference between these two groups is statistically significant ( $p = 0.003$ ).

In contrast with CLL, the expression of CD49d in patients with NHL does not differ from that of normal B-cells ( $p = 0.85$ ), although the fluorescence intensity of CD29 is higher than normal in this group of patients ( $p < 0.001$ ) (Table 3).

In HCL, both molecules are expressed with a brighter intensity than in normal PBL (FMCR = 1.12 and FMCR = 2.99, respectively). These differences are statistically significant ( $p < 0.0001$ ).

#### Expression of the $\beta_2$ integrins by circulating neoplastic B cells

The neoplastic B cells exhibit a very low expression of CD11a/CD18 in CLL patients (Table 3). These molecules are virtually undetectable in most cases, with less than 20% positive cells and a FMCR of 7. This pattern appears to be characteristic of CLL (FMCR of 0.41 and 0.38 for CD11a and CD18, respectively). We also observed a low expression of CD11b in this disease, with a FMCR of

0.38. By contrast, CD11c is strongly expressed in CLL patients, differing significantly from normal PBL (FMCR = 2.36,  $p < 0.0001$ ). These abnormalities of  $\beta_2$  integrins expression in CLL were found to be independent of clinical Binet stages, with the exception of CD11a, when comparing the group of patients in stage A with those in stages B and C ( $p = 0.01$ ), thus reflecting a possible relationship with tumor burden (Table 4).

In NHL patients, the expression of CD11a/CD18 does not differ from that of normal PBL (Table 3). In contrast, the expression of CD11b is dimmer than in normal PBL (FMCR = 0.64,  $p = 0.003$ ), but to a lesser extent than in CLL (Table 3).

HCL patients show a peculiar  $\beta_2$  integrin pattern. CD11a expression is weaker than in normal B lymphocytes, with a FMCR of 0.81, which is intermediate between normal and CLL cells (FMCR of 1.00 and 0.41, respectively). CD11b expression is stronger than in normal cells ( $p < 0.0001$ ), as opposed to what is observed in other LPD. In these patients, CD11c exhibits the highest intensity observed in LPD, with a FMCR of 62.14. The CD18 expression is also stronger in HCL cases than in normal PBL ( $p < 0.0001$ ).

#### CD54 expression

CD54, the CD11a/CD18 ligand, is expressed in less than 20% of cells, both in CLL and normal PBL. Nevertheless, CLL patients in Binet stage A exhibit a dimmer expression of CD54, when compared to normal cells (FMCR = 0.72) (Table 4). Patients in stages B and C show a FMCR of 0.96 and 1.14, respectively. This latter value differs significantly from that of neoplastic cells from patients in stage A ( $p = 0.003$ ) (Table 4).

The expression of this Ig related CAM in NHL patients is higher than in normal PBL (FMCR = 2.43,  $p = 0.0002$ ) (Table 3). In this particular group of patients, we observed an inverse relationship between the lymphocyte counts and CD54 expression. When comparing the expression of CD54 in NHL patients with circulating neoplastic B-cells of  $< 10 \times 10^9/L$  ( $n=23$ ) with those in whom lymphocytosis exceeded  $10 \times 10^9/L$  ( $n=21$ ), the observed CD54 FMCR differed significantly (3.01 and 1.37, respectively,  $p = 0.01$ ).

#### CD62L expression

In this study, CD62L expression was lower than in normal PBL in all groups of patients. Although expressed with dim intensity, the FMCR values were higher in CLL than in NHL or HCL patients (0.55, 0.48 and 0.31, respectively) (Table 3). In CLL patients, no differences were observed among patients in different Binet stages (Table 4).

#### CD44 expression

The intensity of CD44 expression was similar in normal PBL, CLL and NHL neoplastic B-lympho-

**Table 4. Expression of CAM according to the Binet stages in CLL patients.\***

Family of CAM	CAM	Binet stage		
		A (n=58) (mean $\pm$ sem $^\circ$ )	B (n=54) (mean $\pm$ sem $^\circ$ )	C (n=36) (mean $\pm$ sem $^\circ$ )
$\beta_1$ integrins	CD49d	0.19 $\pm$ 0.04	0.38 $\pm$ 0.08	0.48 $\pm$ 0.10
	CD29	0.67 $\pm$ 0.06	0.94 $\pm$ 0.12	1.02 $\pm$ 0.13
$\beta_2$ integrins	CD11a	0.32 $\pm$ 0.04	0.47 $\pm$ 0.08	0.46 $\pm$ 0.06
	CD11b	0.33 $\pm$ 0.03	0.39 $\pm$ 0.05	0.48 $\pm$ 0.17
	CD11c	2.06 $\pm$ 0.32	2.41 $\pm$ 0.60	1.92 $\pm$ 0.49
	CD18	0.30 $\pm$ 0.04	0.45 $\pm$ 0.05	0.36 $\pm$ 0.08
Ig supergene	CD54	0.72 $\pm$ 0.06	0.96 $\pm$ 0.09	1.14 $\pm$ 0.12
Selectin	CD62L	0.55 $\pm$ 0.08	0.44 $\pm$ 0.11	0.66 $\pm$ 0.13
	CD44	0.84 $\pm$ 0.06	0.86 $\pm$ 0.05	0.87 $\pm$ 0.07

\*Results are expressed as FMCR;  $^\circ$ sem = standard error of the mean.

cytes. By contrast, a very bright expression was exhibited by HCL lymphoid cells. In this group of patients the observed FMCR was 3.41, significantly different from the values found in both CLL and NHL patients ( $p < 0.0001$ ) (Table 3).

In summary, the present study shows that various abnormalities in CAM expression may be observed in LPD (Figure 1). While common patterns of expression may be identified in different subtypes of LPD, like the dim intensity of CD62L or the overexpression of CD11c, other CAM profiles like CD49d/CD29, CD11a/CD18 and CD54 vary according to the subtype of disease. These observa-

tions allow the definition of specific immunophenotypic patterns of CAM for each nosological entity. Consequently, CLL may be defined by the low expression of both  $\beta_1$  and  $\beta_2$  integrins. The intensity of expression of CD49d/CD29, CD11a and CD54 in this disease seems related with the tumor burden, while the expression of CD29 is high in those patients with splenomegaly. NHL can be defined by a bright expression of CD29 and CD54; this latter molecule exhibits an inverse relation with the circulating lymphocyte counts in this group of patients. Finally, HCL is characterized by the strong expression of all CAM, with the exception of

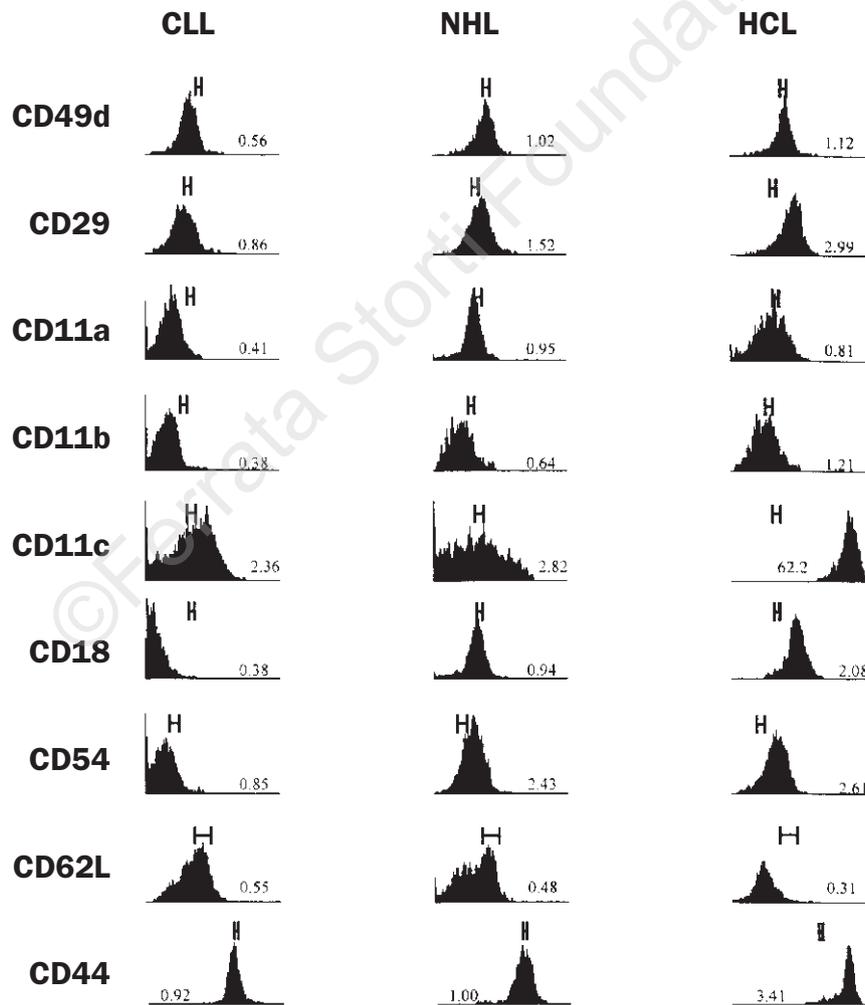


Figure 1. Diagram representing the histograms of the different CAM profiles exhibited by each group of patients. These histograms correspond to CD19 gated cells. The H symbol represent the mean±sem of the control B-lymphocytes, and the number written in each histogram represents the fluorescence mean channel ratio (FMCR) of each target population.

CD11a.

## Discussion

Surface molecules mediating cell-cell and cell-extra-cellular matrix interactions are good candidates to explain certain functional characteristics of different forms of lymphoproliferative disorders, like their ability to involve lymph nodes, spleen and extra-lymphoid tissues or, in contrast, to maintain a predominant leukemic expression with diffuse marrow infiltration and circulating neoplastic cells. Additionally, variations in the expression of CAM by the neoplastic cells during the course of the disease, may reflect modifications of the potential for leukemogenic spread and/or extra-lymphoid tissue invasion, with possible clinical and prognostic value.

In this study we have investigated the expression of nine different adhesion molecules by peripheral blood neoplastic B-cells of 210 patients diagnosed of CLL (148), NHL (52) and HCL (10). We observed that lymphoproliferative disorders are characterized by distinct patterns of CAM expression. CLL exhibits a very dim intensity of all  $\beta$  integrins as compared to controls and other pathological groups (Table 3). NHL is better characterized by a generalized dim  $\beta$ -integrin expression, in parallel with a bright expression of CD29 and CD54. HCL may be defined by an overexpression of all adhesion molecules studied, with the exception of CD11a.

We have based the present analysis in both the FMC for each antigen and in its relation to an internal control (normal PBL), expressing the results as a quotient between these two values (FMCR). In fact, the evaluation of CAM expression largely depends upon the detection systems used, and positive or negative flow cytometric results may depend on the surface intensity of each molecule, rather than in its presence or absence. Initial reports in literature were based on the percentage of positive cells for each antigen, or on the relative fluorescence intensity of each marker (defined as the mean channel of the logarithmic fluorescence histogram), resulting in wide variability of parameters of analysis set by the operator. Since the mean fluorescence intensity of most adhesion molecules are close to the negative control, and since they show a continuous range of expression at the cell surface, slight variations in the definition of cut-off values, always arbitrary, may significantly influence results when expressed as percentages of positive cells. While results expressed as the FMC may be less operator-dependent, they can still be influenced by the fluorochromes used and the calibration settings of the cytometer. Difficulties in the standardization of flow cytometric analysis may hamper comparison between different studies and help to explain some conflicting published data.

We believe that our analysis method for each CAM, based on the ratio between the FMC of a pathological sample and the FMC of normal B-lymphocytes, may overcome some of these problems.

The low expression of the  $\beta$ -integrins in both CLL and in NHL observed in this study agrees, in general, with previous reports.<sup>3,4,21-26</sup>

In CLL, we observed a relationship between the expression of CD49d/CD29 by neoplastic cells and the Binet clinical stages (see Table 4); the intensity of expression of these molecules is also related to the presence of splenomegaly within this group of patients.

Although the expression of CD49d in NHL is similar to that of normal B-cells, the intensity of expression of CD29 is significantly increased. Furthermore, the intense expression of this CAM is mainly observed in those disorders with a higher incidence of lymphadenopathies and splenomegaly (NHL and CLL with palpable spleens). These observations, which agree with previously reported studies,<sup>25,26</sup> suggest that the  $\beta_1$ -integrin family of CAM plays a relevant role in the localization of neoplastic B-lymphocytes within the lymphoid organs. From a clinical point of view, the relevance of CD49d/CD29 in the formation of neoplastic follicles is reinforced by its higher expression in follicular than in diffuse NHL.<sup>23</sup> In fact, it is presently accepted that CD49d/CD29 has an important role in the adhesive properties of both leukemic and normal B-cells.<sup>27,28</sup> The interactions of CD49d/CD29 with both VCAM-1, at the earlier stages of normal B-cell differentiation, and fibronectin, at the stages of terminal differentiation, are believed to be essential for the normal B-cell development.<sup>29</sup> Furthermore, anti-CD49d/CD29 antibodies are able to inhibit all steps of normal B-cell lymphopoiesis.<sup>30</sup>

A consistent observation in this study was the very low expression of CD11a/CD18 by neoplastic B-cells in CLL, which is in agreement with the results of previous reports.<sup>3,8,21,26</sup> The data presented here confirm that this pattern of expression is characteristic of B-cell CLL and allows its distinction from other forms of LPD. Additionally, we observed a close relationship between CD11a expression and the tumor burden, indicating that this molecule may have a role in the adhesion of B-cells to lymphoid tissues. The low expression of CD11a/CD18 in CLL patients may also help in the differential diagnosis with small lymphocytic lymphoma, that is always CD11a/CD18 positive.<sup>8</sup> The patterns of expression of CD11a/CD18 may account for the differential anatomic compartmentalization of these otherwise morphological and immunophenotypically similar lymphoid malignancies.

Among  $\beta_2$  integrins, a very weak expression of CD11b was persistently observed, well below the level of normal PBL, both in CLL and NHL. Based on the recent knowledge that CD11b/CD18 may be

involved in the mechanisms of leukocyte apoptosis,<sup>31</sup> it is conceivable that the deficiency of CD11b expression in certain forms of LPD, may interfere with the induction of apoptosis in these disorders.

In the present study we observed a low expression of CD54 in stage A CLL patients, that is higher in patients with lymphadenopathies and/or organomegalies (stages B and C). By contrast, a strong expression of CD54 was observed in NHL patients, varying inversely with the lymphocyte blood counts. These findings suggest that CD54 may play a role in the tropism of neoplastic B lymphocytes to the lymphoid tissues. A relationship between the expression of either membrane-bound or soluble CD54 and the tumor burden has already been reported by several authors in CLL.<sup>10,32-34</sup> Recently, it has been demonstrated that the serum levels of specific CAM expressed by malignant B-lymphocytes, may be used as useful markers of tumor mass and relevant prognosis indicators.<sup>35,36</sup>

The low expression of CD62L in LPD, that seems independent of both diagnosis and tumor burden, may reflect a basic abnormality of circulating neoplastic B-lymphocytes and can be related to the role of this molecule in the cellular attachment capacity to endothelial cells and high endothelial venules (HEV), as part of their migration process.<sup>37,38</sup>

In summary, the data presented here helps to clarify the quantitative abnormalities of CAM expression by neoplastic B-lymphocytes and confirm that specific patterns may be of value to refine the diagnosis of B-cell lymphoproliferative disorders.

### Contributions and Acknowledgments

*PJMdSL participated in the conception and design of the study, coordinated all the cytometric assays, carried out the analysis of the results and collaborated in the writing of the paper. MTSDF and AMCP were responsible for all the cytometric assays and data handling. MRLGdS collaborated in the analysis of results and reviewed the manuscript, contributing to its intellectual content. MEMRRCJ and RJSMDc were involved in the clinical assessment of patients and revised the manuscript. ABdSP was the principal investigator, formulated the design of the study and wrote the paper.*

### Funding

*This work was supported by the JNICT grant PBIC/SAU/1542/92.*

### Disclosures

*Conflict of interest: none.  
Redundant publications: no substantial overlapping with previous papers.*

### Manuscript processing

*Received on August 25, 1997; accepted on November 3, 1997.*

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