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High endothelial venules and cell adhesion molecules in B-cell chronic lymphocytic leukaemia and related low grade B-cell lymphoma/leukaemia: II. Expression of cell adhesion molecules in lymph nodes biopsies

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Summary. The expression of cell adhesion molecules (LECAM-1, LFA-1, VLA-4, ICAM-1 and CD44) of lymph nodes from B-cell chronic lymphocytic leukaemia (B-CLL; mature: 16 cases, immature: 8), lymphocytic lymphoma (LL; M:5, IM:2) and reactive lymph nodes (10) were investigated in frozen tissue sections. In order to assess the B- and T-cell compartments of the lymph nodes some additional markers CD3, CD20, CD45 RO were used, and for follicular dendritic reticulum cells (FDCs) CD35. The expression of the LECAM-1 molecule was correlated to the expression of activation (CD23, CD25) and proliferation (Ki67) markers. Findings, in accordance with the relevant data of the literature, indicate that B-CLL and LL show and identical adhesion profile to the mantle zone of the germinal centres of reactive lymph nodes; namely, they were $CD44^+$/$LECAM\text{-}1^+$/$VLA\text{-}4^+$. The T-zones of the reactive lymph nodes were characterized by an $LFA\text{-}1^+$ and the follicles by an $ICAM\text{-}1^+$ pattern, while in B-CLL and LL cases the $LFA\text{-}1^+$ and $ICAM\text{-}1^+$ were detected without coexpression of these molecules in only a small number of cases. The ratio and location of $CD3^+$ and $LFA\text{-}1^+$ cells was very similar in lymph nodes from B-CLL and LL, and from this fact may arise the suspicion that $LFA\text{-}1^+$ reported in LL cases derive from the sometimes significant T-cell compartment of the diseased lymph nodes. The LECAM-1 molecule did not show any correlation with the investigated activation/proliferation markers.

Key words: B-CLL, Lymphocytic lymphoma, Adhesion molecules, Activation/proliferation

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Introduction

As is indicated in the accompanying report of this issue, the adhesion profile of peripheral blood B-cells in chronic lymphocytic leukaemia (B-CLL) has been widely investigated and has revealed some differences in individual cases (Maio et al., 1990; Spertini et al., 1991a; Baldini et al., 1992; Rossi et al., 1993).

Reports about the adhesion profile of lymph node infiltrates in B-CLL are sparse, and this fact may be attributed to the diverse opinion about the importance of biopsy of the enlarged lymph nodes (Rossi et al., 1989; Kelényi, 1993), and as a consequence in many centres no substantial number of frozen samples are available for such studies.

However, studies of adhesion molecules in lymph node infiltrates are also important since beside lymphocyte-endothelial (blood lymphocytes-high endothelial venules -HEVs) interactions some other cell-cell and cell-matrix interactions may significantly contribute to dissemination of lymphomas/leukaemias.

In the second part of this study, the expression of the L-selectin molecule (LECAM-1), two important integrins (LFA-1/CD11a and VLA-4/CDw49), a molecule of the immunoglobulin superfamily (ICAM-1/CD54) and the hyaluron-binding molecule («homing receptor»: CD44) were analyzed. The question to be answered was whether the L-selectins showed any correlation with the expression of some activation/proliferation markers (CD23, CD25 and Ki67 antigen).

Materials and methods

Materials

Frozen lymph node biopsy samples (stored at -70 °C) was obtained from the material (from 1st of January,

1989 until 1st of June, 1993) of the Department of Pathology, University Medical School of Pécs (Lymphoma Reference Centre). The histological diagnoses and assortment of cases into subgroups of maturation were based on the same criteria used in part I (Stansfeld et al., 1988; Kelényi, 1993).

According to histological diagnosis the material comprised of 16 cases of B-CLL, pseudofollicular, mature (PF, M; male/female: 15:1, average age: 59.3 yrs), 8 cases of B-CLL, PF, immature (IM; 5/3, 66 yrs), lymphocytic lymphoma (LL, M; 4:1, 70.8) and 2 cases of LL, IM (1:1, 70 yrs). 10 cases of reactive lymph nodes stored in similar conditions served as controls.

Methods

The unfixed tissue was put into isopentane, frozen by liquid nitrogen and stored until use at - 70 °C.

4-6 µm-thick frozen sections were made by a cryostat (Tissue-Tek II).

All the procedures of immunohistochemistry were performed at room temperature, and a Tris-HCl-NaCl (TBS) buffer of 150mM, pH 7.3, was used. Briefly, slides were fixed in acetone for 10 minutes. The sources and specificity of monoclonal antibodies (MoAbs) applied in this study are summarized in Table 1. To visualize the binding-sites, a peroxidase-conjugated rabbit anti-mouse-Ig was used (30 minutes; DAKO, Sweden), and as the substrate of the peroxidase, 3-amino-9-ethyl-carbasole was used. The procedure was finished with a haematoxylin counter-staining. The specificity of the reactions were tested by omitting the primary MoAb.

Biopsy samples of B-CLL and LL cases were regarded as positive if more than 25% of the cells were labelled with the investigated MoAb (Stauder et al., 1989).

The following symbols were used to characterize the intensity of positive staining with anti-LECAM-1, anti-CD44 and anti-VLA-4 MoAbs: +++, intensive staining; ++, moderate intensity; and +, weak, although definite staining (Möller et al., 1992a,b).

The percentage of Ki67-positive cells was determined by the method of Simpson et al. (1992). The microscope field was bisected by a linear eyepiece micrometer, and the number of cells (n) intercepted by this line, as well as the Ki67-positive cells were counted. The cell count (A) was estimated by the formula of $A = r^2D$ ($r = n/2$). The measurement was performed in 10 consecutive fields, and for the estimation of the cell-content the data of the 1x, 5x and 10x fields were used.

The ratio of CD3- and LFA-1-positive cells was calculated with a similar method; in 10 consecutive fields the number of CD3 and LFA-1 positive cells were registered and related to all the cells bisected by the eyepiece micrometer.

Data were evaluated statistically by using a two-sample t-test.

Table 1. Antibodies applied in the study.

ANTIBODY	SOURCE	SPECIFICITY	DILUTION
CD3 (OKT3)	ORTHO	T	1:50
CD20 (L26)	DAKO	B	1:100
CD45RO (UCHL-1)	DAKO	T, B, Mo, Ma	1:100
CD23	Immunotech	act.B, FDC	1:25
CD25	Immunotech	IL-2, act.Ma	1:25
Ki67	DAKO	act. cells	1:25
CD35	DAKO	FDC, B, RBC	1:50
CD44 (H90)	A. Bernard	leuk., RBCs	1:100
LECAM-1	Immunotech	L-selectin	1:25
CD11a	Immunotech	LFA-alfa	1:50
CDw49	Immunotech	VLA-4	1:25
CD54	Immunotech	ICAM-1	1:25

T: T-cell, B: B-cell, Mo: monocyte, Ma: macrophage, FDC: follicular dendritic reticulum cells, act: activated, leuk: leukocyte, RBC: red blood cell.

Results

Reactive lymph nodes

Data about the adhesion profile of reactive lymph nodes are presented in Table 2.

Compartments of reactive lymph nodes were characterized by certain settings of the adhesion molecules. The germinal centres were LFA-1/CD11a- (Fig. 1) and ICAM-1/CD54-positive, in the mantle zones an intensive CD44-, LECAM-1-, and VLA-4/CDw49-positivity was seen (Fig. 2), while in the paracortical T-zones an intense LFA-1-positivity was observed (Fig. 1).

In the germinal centres the follicular dendritic reticulum cells (FDCs) showed a CD54-labelling very similar to the staining seen with with anti-CD35 MoAb; i.e. the meshwork of dendritic processes were clearly revealed by both stainings.

The HEVs and other small vessels with flat endothelium (SVFEs) showed a strong positivity with anti-CD54 MoAb.

B-CLL and LL

In connection with the adhesion profile of lymph nodes with B-CLL and LL, readers are referred to Table 3.

No differences were detected in the adhesion profile of B-CLL and LL cases, and this was also similar in

Table 2. Adhesion profile of reactive lymph nodes.

	CD44	LECAM-1	VLA-4/ CDw49	LFA-1/ CD11a	ICAM-1/ CD54
Follicles	-	-	-	+++/++/+	+++/++/+
Mantle zone	++/+++	++/+++	++	-/+	-
Paracortex	++/+++	-/+	+/-	+++	-
Vessels*	-	-	-	-	+++

*: Vessels, HEVs and SVFEs.

mature and immature subgroups.

Staining with anti-CD44 MoAb was positive in every case, while the LECAM-1 molecule was detected in 81% of cases; the relevant figure for VLA-4/CDw49 positivity was 94% (Fig. 3).

Cells were positive for the LFA-1/CD11a molecule in

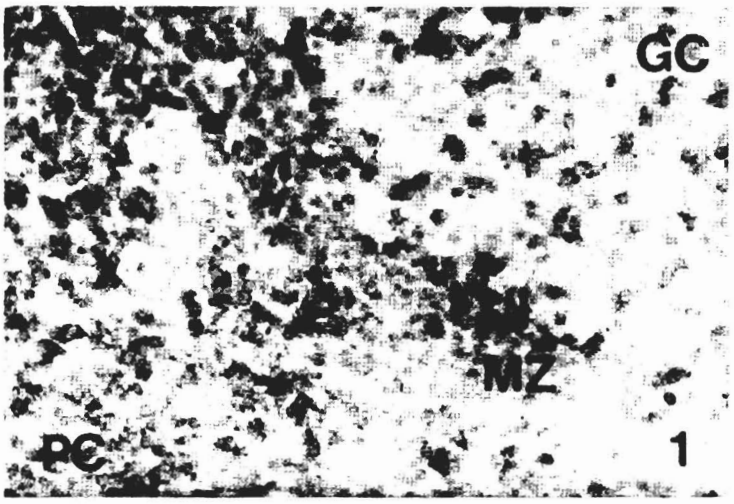

Fig. 1. Reactive lymph node. Pattern of LFA-1 (CD11a) reaction. Only a few cells expressing LFA-1 are scattered within the germinal centre (GC; clear area at the upper right portion) and within the paracortex (PC, left lower portion). The mantle zone (MZ) separating the two areas is indicated by dark (strongly heterochromatinized nuclei), but unreactive cells. Immunoperoxidase reaction, haematoxylin counterstaining. x 200

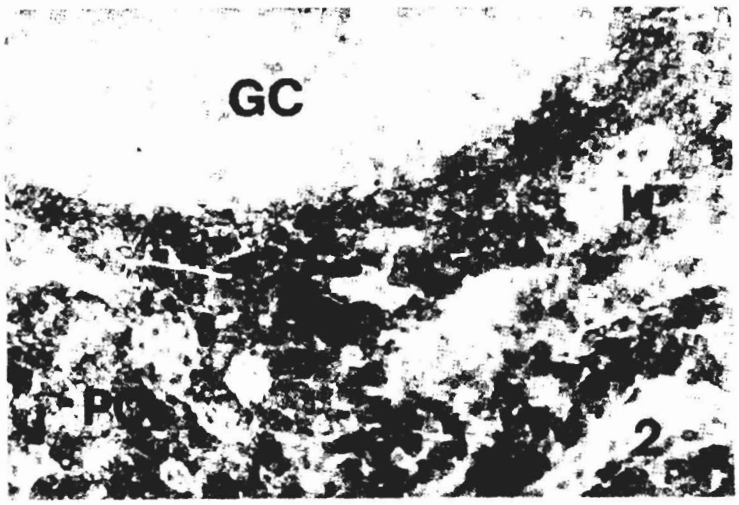

Fig. 2. Reactive lymph node. The CD44 antigen is expressed by the mantle zone (MZ) and paracortical (PC) T-cells equally well. Germinal centres: GC. Note (Table 2) that mantle zone lymphocytes are characterized by a CD44$^+$/LECAM-1$^+$/VLA-4$^+$. Immunoperoxidase reaction, haematoxylin couterstaining. x 200

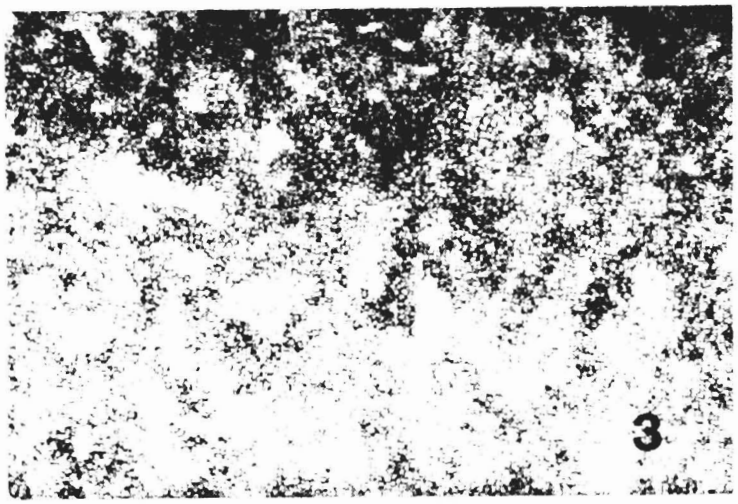

Fig. 3. B-CLL. A diffuse staining of the nodal infiltration with anti-LECAM-1 MoAb. The adhesion profile of B-CLL cells in a great majority of cases is identical to that of the mantle zone lymphocytes (see Fig. 2, Tables 2, 3). Immunoperoxidase reaction, haematoxylin counterstaining. x 100

a low number of samples (Table 3), and the proportion and distribution of CD3- (Fig. 4a) and LFA-1/CD11a- (Fig. 4b) positive cells was very similar. Taking into acount all the investigated cases, the percentage of the labelled cells was 18.5±8% for CD3 and 20.0±8% for LFA-1/CD11a (mean ± SD). From some representative samples consecutive series were made to demonstrate the similarity of CD3 and LFA-1/CD11a labelling (Figs. 4a,b).

Lymphocytes showed a weak positivity with anti-CD54 (ICAM-1) MoAb in only a low number of cases (12% of all the samples), while vessels (HEVs and SVFE) were uniformly positive with this MoAb.

Cells stained poorly with anti-Ki67 MoAb (mean ± SD: 3.5±2.8%; range: 0.1-10%), and statistically no difference was found in the number of Ki67-positive cells between LECAM-1-positive and negative groups (mean: 3% versus 3.6%). Moreover, the Ki-67 positivity

Table 3. Adhesion profile of B-CLL and LL lymph nodes.

	CD44				LECAM-1				VLA-4/CDw49				LFA-1/CD11a	ICAM-1/CD54
	-	+	++	+++	-	+	++	+++	-	+	++	+++	%,*	*
B-CLL, PF-M	0	0	7	9	4	6	5	1	3	5	7	1	28±5 (5/16)	(2/16)
BC-LL, PF-IM	0	1	4	3	1	4	3	0	1	4	2	1	31±3 (5/6)	(1/8)
LL-M	0	0	4	1	0	2	3	0	0	4	1	0	21±1 (2/5)	(1/5)
LL-IM	0	0	2	0	1	0	0	1	0	1	1	0	24±4 (2/2)	(0/2)
All cases	0	1	17	13	6	12	11	2	4	14	11	2	27±5 (12/31)	(4/31)

PF: pseudofollicular; LL: lymphocytic lymphoma; M: nature; IM: immature; %: mean ± SD of positive cases; *: number of cases per all the investigated cases.

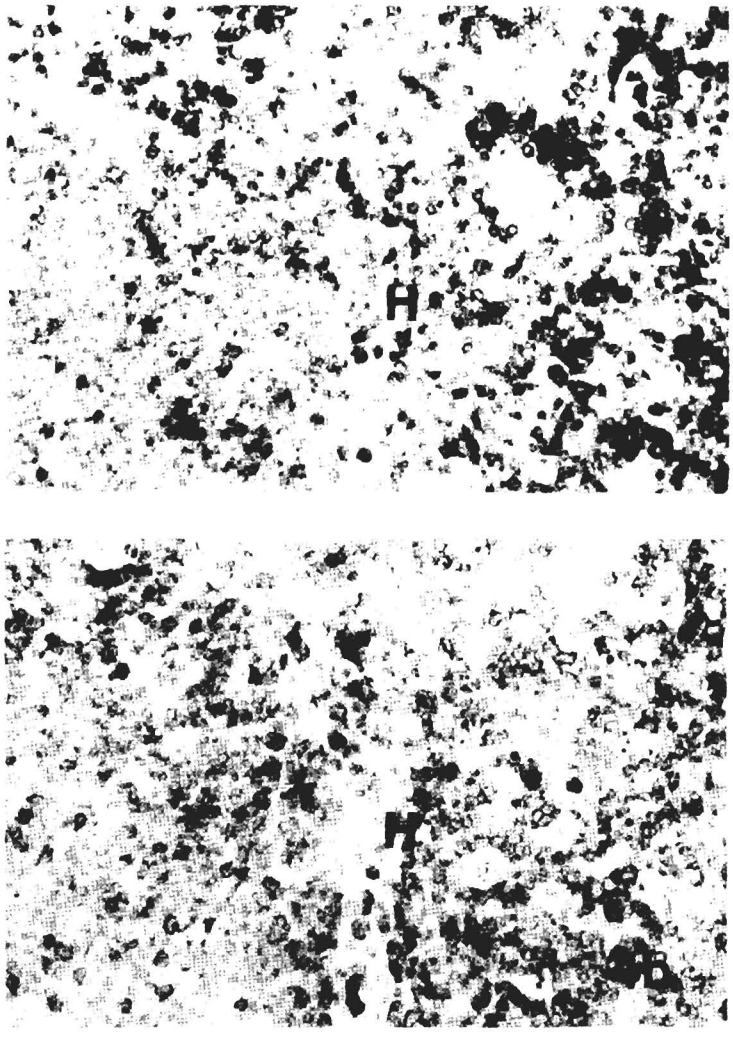

Fig. 4. B-CLL. Consecutive samples of the same node reveal a very similar staining (in number and pattern) with anti-CD3 (pan T marker) **(a)** and anti-CD11a/LFA-1 (integrin molecule) **(b).** Readers are oriented by a HEV (H) transecting the photograph.. Immunoperoxidase, haematoxylin counterstaining. x 200

was close in mature and immature subgroups (B-CLL, PF, M: 4%, B-CLL, IM: 3.6%, LL, M and IM: 2.4%).

A highly variable staining was seen with anti-CD23 MoAb (the number of positive cells varied from 20% to a diffuse positivity of 80-90% of cells) without any correlation to the LECAM-1 positivity.

No samples were positive with CD25.

Discussion

The morphological features and immunological findings are very similar in B-CLL and LL, and the basic differences is whether the malignant cells are present or not in the blood (Stansfeld, 1992). However, there are cases with prior aleukaemic manifestation which later transform into B-CLL, as well as B-CLL cases with low white blood cell count (Batata and Shen, 1993).

Inghirami et al. (1988) explained these variables with the differences observed in LFA-1-integrin expression. According to their investigations the expression of LFA-1 was low (29%: number of positive cases per all the investigated cases) in B-CLL compared to «small cell lymphocytic lymphoma» (SLL) where this figure was high (92%). Based on these findings, it was assumed that the presence of LFA-1 on SLL cells was responsible for their preferential localization in the lymph nodes and spleen, while the absence of LFA-1 molecules, and as a consequence the loss of cell-to-cell adherence, were accounted for with their propensity to invade the peripheral blood.

Stauder et al. (1989) could detect LFA-1 in 70% of their B-CLL cases, while in immunocytomas (ICs) only 55% of cases were regarded as positive.

The present findings are in agreement with Miedema et al. (1985) i.e. the expression of LFA-1 molecules in tissue infiltrates and peripheral blood is low both in B-CLL and LL cases. However, the relevant figures of the present study (number of positive per all the investigated cases) were relatively high: 41% (B-CLL); and 57% (LL). In the individual cases regarded as positive, the number of labelled cells hardly exceeded the criterion chosen for positivity (> 25%), and the number of LFA-1 and CD3 (T-cells) and their localization was very similar. These findings suggest that most of the cells labelled with anti-CD11a (LFA-1, α-chain) represent T-cells in the lymph node infiltrates of B-CLL and LL. Moreover, the phenotype of B-CLL cells is identical with lymphocytes of mantle zone origin an area which -in accordance with the data from the literature- was LFA-1 negative.

In the present study, cells of germinal centres and vessels (HEVs and SVFEs as well) of reactive lymph nodes expressed ICAM-1 adhesion molecule strongly. In B-CLL/LL nodes, HEVs and SVFEs were also uniformly positive, while the tumour cells infiltrating the nodes remained unstained, or only a very small number of cells expressed ICAM-I weakly.

Stauder et al. (1989) found only 20% of B-CLL cases ICAM-1 positive, and did not succeed in demonstrating any correlation between the expression of LFA-1 and ICAM-1 adhesion molecules. In a small B-CLL-series (6 cases) of Horst et al. (1990) there was no ICAM-1-positive case.

These facts argue against the putative role of LFA-1 and ICAM-1 molecules (receptors and ligands) in establishing cohesive interactions (cell-cell contacts) in the nodal infiltrates of B-CLL and LL.

Considering the other investigated adhesion molecules, B-CLL/LL lymph node infiltrates were characterized by a CD44/LECAM-1/VLA-4 positive pattern reflecting the adhesion profile of the mantle zones of reactive lymph nodes, which corresponds to data communicated recently by Möller et al. (1992a,b) and Zutter (1991).

The findings of this study are salient, since only a few studies deal with the adhesion profile of B-CLL lymph nodes. Surveying the literature in connection with LECAM-1 expression of B-CLL lymph nodes, 24 cases were found, while the VLA-4 expression was analyzed only in 9 cases (Michie et al., 1987; Carbone et al., 1988; Möller et al., 1992a,b). The 5 LECAM-1-negative B-CLL cases of this study indicate that LECAM-1 (L-selectin) is not invariably expressed in B-CLL/LL lymph node infiltrates, and, in agreement with Michie et al. (1987) it demonstrates that LECAM-1-negative B-CLL/LL cases exist.

However, it is well known that adhesion receptors are easily modified by certain activation/proliferation signals (Hamann et al., 1988; Buhrer et al., 1990, 1992). In the study by Stauder et al. (1989), the authors could not demonstrate any correlation between the expression of adhesion molecules LFA-1/ICAM-1 and the proliferation marker Ki67. Spertini et al. (1991b) revealed that the function of L-selectins (LECAM-1-mediated endothelial binding) is regulated in three different ways: expression of the receptor on distinct lymphocyte subpopulations; transient activation-induced increase in receptor function; and subsequent shedding of the receptor. The relationship of LECAM-1 expression and activation/proliferation signals (expression of CD23, CD25 and Ki67) in lymph node infiltrates of this study were very similar to those of the study of Stauder et al. (1989) with LFA-1/ICAM-1 and Ki67. No correlation could be demonstrated between the LECAM-1 expression and the investigated activation/proliferation markers. Moreover, it was remarkable that no difference was found in the expression of Ki67 in mature and immature subgroups.

Conclusively, lymph node infiltrates in B-CLL and LL do not seem to show any difference in the expression of the investigated adhesion molecules (LFA-1, ICAM-1, CD44, LECAM-1 and VLA-4), and the plain detection of certain activation/proliferation markers does not explain the differences in the expression and function of these adhesion molecules in particular cases. Simultaneous investigations on adhesion profile and function of peripheral blood lymphocytes and lymph node infiltrates, together with the parameters of actual

dissemination, will elucidate these variables.

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