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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: I. High endothelial venules and lymphocyte migration

G. Csanaky
Department of Pathology, University Medical School of Pécs, Pécs, Hungary

Summary. The high endothelial venule (HEV)-content and the lymphocyte migration index (LMI) of reactive lymph nodes and lymph nodes from patients with B-cell chronic lymphocytic leukaemia (B-CLL), as well as some related B-cell malignancies (lymphocytic lymphoma -LL-, prolymphocytic leukaemia -PLL-) were determined and statistically analyzed. The HEV-content and the LMI were significantly higher in reactive lymph nodes than in the low grade B-cell lymphomas and leukaemias ($p< 0.001$). The number of HEVs among lymphoma/leukaemia cases was the highest in LL independently of the maturation. However, the maturation of the process seems to determine the intensity of lymphocyte migration; i.e. a significantly higher LMI was found in mature (B-CLL, PF, mature; M and LL, M) than in immature subtypes (B-CLL, PF, immature -IM-, diffuse -D- and LL, IM) subtypes (levels of significance varied from $p< 0.05$ to $p< 0.001$). Based on these findings, a more intense migration of lymphocytes from blood to peripheral lymph nodes may be supposed in LL than in B-CLL, thus explaining the nodal sites of involvement in LL and the peripheral blood in B-CLL. Within the same histological categories the morphometric features in mature subtypes may implicate an enhanced HEV-lymphocyte interaction when compared to the immature subtypes.

Key words: B-cell lymphomas/leukaemias, High endothelial venules, Lymphocyte migration, Dissemination

Introduction

The high endothelial venules (HEVs) play a crucial role in lymphocyte recirculation, namely lymphocytes of the blood enter secondary lymphoid organs via the HEVs (Gowans, 1957; Gowans and Knight, 1964). HEVs are located in the paracortex (T-zones) of the lymph nodes, and they can be easily recognized even in routine histological preparations (Miller, 1969).

For a long while, histopathologists had paid very little attention to HEVs and other small vessels of lymph nodes apart from some lesions characterized by angioproliferative changes: angioimmunoblastic lymphadenopathy/lymphoma (Fizzera et al., 1975; Lukes and Tindle et al., 1975); T-zone lymphoma and pleomorphic T-cell lymphoma (Lennert, 1981; Lennert et al., 1982). However, the presence and function of HEVs may be very important in lymphoma/leukaemia dissemination, since neoplastic cells using the same receptors and ligands involved in lymphocyte-endothelial interaction may propagate via the HEVs from the blood into HEV-bearing organs (Stoolman, 1993).

In B-cell chronic lymphocytic leukaemia (B-CLL) the function of cell adhesion molecules and a dissemination process based on the adhesive interactions mediated by these molecules (homing receptors) seem to be well established (Maio et al., 1990; Spertini et al., 1991; Baldini et al., 1992; Rossi et al., 1993; Csanaky et al., 1994). B-CLL cases show highly variable patterns of leukaemia dissemination, and the differences in anatomic sites of involvement (nodal versus blood) are even more striking, if B-CLL cases are related to some other low grade B-cell lymphomas/leukaemias such as the lymphocytic lymphoma (LL) and prolymphocytic leukaemia (PLL) (Stoolman, 1993).

Prior studies reported a well preserved angiostructure, and -compared to other B-cell non-Hodgkin lymphomas- a high number of HEVs in B-CLL lymph nodes (Söderström and Norberg, 1974; Pajor et al., 1990).

In the present study HEV-content (number of HEVs per unit area) and lymphocyte migration (lymphocyte migration index -LMI-: number of lymphocytes found in the wall of HEVs related to the number of endothelial cells lining the venules) of nodal infiltrations in B-CLL, LL and PLL were investigated, and the variables

Offprint requests to: G. Csanaky, M.D., Department of Pathology, University Medical of Pécs, Pécs, Szigeti u. 12, H-7643 Hungary

Table 1. Statistical data of HEV-morphometry.

LMIs	HEVs						
	Reactive n= 11 (39.3)[a]	CLL-PF-M n=27 (65.1)	CLL-PF-IM n=18 (61.5)	CLL-D n= 10 (69.6)	PLL n= 4 (61)	LL-M n= 15 (70.2)	LL-IM n=9 (67.3)
Reactive	----------	p= 12.413***	p= 10.101***	p= 10.665***	p= 6.923***	p= 7.871***	p= 10.566***
CLL-PF-M	p= 4.437***	----------	p= 1.288 NS	p= 1.605 NS	p= 1.322 NS	p= 2.279 NS	p= 0.751 NS
CLL-PF-IM	p= 8.006***	p= 3.423**	----------	p= 2.103*	p= 1.887 NS	p= 1.002 NS	p= 1.813 NS
CLL-D	p= 5.795***	p= 2.203*	p= 0.612 NS	----------	p= 0.254 NS	p= 2.923 NS	p= 0.854 NS
PLL	p= 3.734**	p= 1.148 NS	p= 1.127 NS	p= 0.465 NS	----------	p= 2.087 NS	p= 0.832 NS
LL-M	p= 2.634*	p= 2.257*	p= 6.634***	p= 4.629***	p= 2.965**	----------	p= 2.314*
LL-IM	p= 4.344***	p= 0.308 NS	p= 3.876***	p= 2.358*	p= 1.600 NS	p= 2.703*	----------

D: diffuse; IM: immature; LL: lymphocytic lymphoma; M: mature; PF: pseudofollicular; PLL: prolymphocytic leukaemia; [a]: age average; NS: not significant. The significant differences are indicated by asterisks: *, p< 0.05; **, p< 0.01; ***, p< 0.001

observed in different subtypes were statistically analyzed.

Materials and methods

Materials

Biopsy samples came from the files (1990-1993) of the Department of Pathology, University Medical School of Pécs (Lymphoma Reference Centre). The classification was based on the «Updated Kiel Classification» (Stansfeld et al., 1988), and the samples were assorted into subgroups according to cytological maturity of the pseudofollicles (proliferation centres) (Kelényi, 1993). The number of samples (according to histological subtypes) and of the «controls» (reactive lymph nodes) are presented in Table 1.

Methods

Only sections with an area larger than 100 mm^2 were studied.

Paraffin-embedded lymph node biopsy material was used, and 4 µm-thick sections were stained with PAS.

The sections were covered with a graticulate with 1 mm^2 squares, and the HEVs were counted randomly at x600 magnification in at least 25 squares (Pajor et al., 1990).

Vessels were regarded as HEVs if they met the criteria set up by Freemont and Jones (Freemont, 1983; Freemont and Jones, 1983). Briefly: (a) vessels lined by cuboidal endothelial cells with large and pale nuclei; (b) the presence of strongly PAS-positive basement membrane; and (c) presence of lymphocytes between the endothelial cells or between endothelial cells and basement membranes.

The passage of lymphocytes through HEVs was assessed by LMI which referred to the ratio between the nuclei of lymphocytes observed in the wall of HEVs and those of endothelial cells. The LMIs were calculated as an average of at least 20 HEVs (Csanaky et al., 1991a).

The results were statistically analyzed by a two-sample t-test.

Results

HEV-content of the lymph nodes

In reactive lymph nodes the number of HEVs per unit area (10.16±1.79/mm^2) was significantly higher than in all the other subgroups (B-CLL and related lymphomas/leukaemias). A significantly higher number of HEVs was found (4.67±1.70/mm^2) in LL, M subgroup than in any other related lymphomas/leukaemias (B-CLL, PF, M and IM, B-CLL, D, PLL and LL, IM). A significant difference was also observed between B-CLL, PF, IM (4.12±1.31/mm^2) and B-CLL, D (2.79±1.30/mm^2). The number of HEVs per unit area in other subgroups was as follows, B-CLL, PF, IM; 3.58±1.29/mm^2; PLL: 2.56±2.12/mm^2; and LL-IM; 3.23±0.86/mm^2.

HEVs per unit areas (mean±SD) are illustrated in Fig. 1.

LMI

The highest values of LMI were detected in reactive

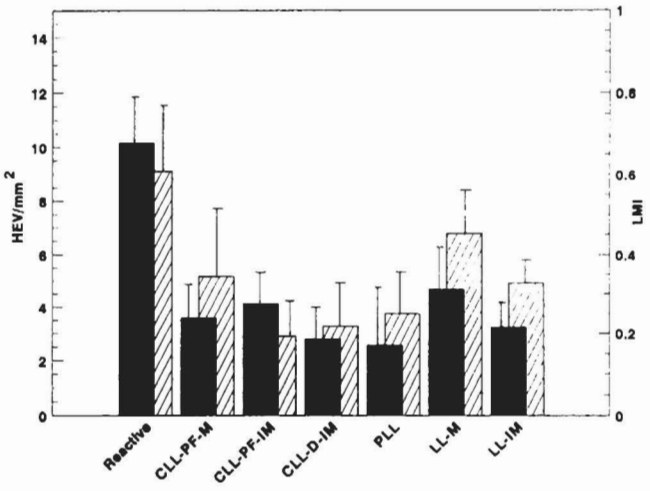

Fig. 1. HEVs per unit (dark bars) area and LMI (striped bars) in B-CLL, PLL and LL (mean±SD). Statistical data are presented in Table 1.

lymph nodes (0.60±0.17; Figs. 1, 2), in LLs (M: 0.45±0.12 and IM: 0.32±0.07) and in B-CLL, PF, M (0.34±0.16). Statistically, the LMIs were significantly higher in reactive lymph nodes than in any groups of the investigated lymphomas/leukaemias (Fig. 1). The differences were significant if the relevant figures of LL (M and IM) and B-CLL, PF, M were compared to those of B-CLL, PF, IM (0.19±0.08) and B-CLL, D (0.21±0.12). The LMI observed in PLL (0.25±0.10) were also significantly smaller when compared to LL, M (Fig. 3).

Statistical data are shown in Table 1.

Discussion

The angiostructure of Hodgkin's lymphomas was stable in the study of Möller and Lennert, while lymphocyte migration (LMI) was parallel with the swelling of the endothelial cells of HEVs (Möller and Lennert, 1984). These findings are in accordance with the fact that HEVs represents an «induced phenotype», and adhesiveness of HEVs depends on local microenvironmental factors (Csanaky et al., 1991b).

HEVs were demonstrated in Sezary's syndrome and T-CLL (Robb-Smith and Taylor, 1981). Kittas et al. (1985) in their comprehensive study, found differences in the HEV-content of low and high grade T-cell non-Hodgkin's lymphomas. The HEV-content was high in the low grade T-cell lymphomas/leukaemias (T-CLL and Sezary's syndrome), while the relevant figures were low in high grade tumours (T-immuno and -lymphoblastomas).

55 cases of B-cell non-Hodgkin's lymphomas were analyzed by Pajor et al. (1990) and the findings were very similar to T-cell non-Hodgkin's lymphomas. Namely, the number of HEVs per unit area was high in B-CLL, immunocytomas (lymphoblasmocytoid -LPOID-, lymphoplasmocytic -LP-) and centroblastic/centrocytic (CB/CC) lymphomas, while in high grade lymphomas (immunoblastoma -IB- and centroblastoma -CB-) the relevant figures were low. Ree and Leone (1978) attributed a prognostic relevance to the HEV-content of the extrafollicular areas in tumours of follicular origin, i.e. the highest actuarial survival rate was registered in cases with a high number of HEVs in the extrafollicular areas.

Söderström and Norberg (1974), based on an analysis of a limited number of cases, regarded CLL as an entity with a low number of HEVs. However, the HEV content and LMIs of B-CLL and other related lymphomas/leukaemias was lower than that of the reactive lymph nodes, our findings indicate that B-CLL and the related malignancies contain a significant number of functionally active HEVs. In Söderström and Norberg's study (1974), differences were reported between leukaemic and aleukaemic cases with regard to the HEV-

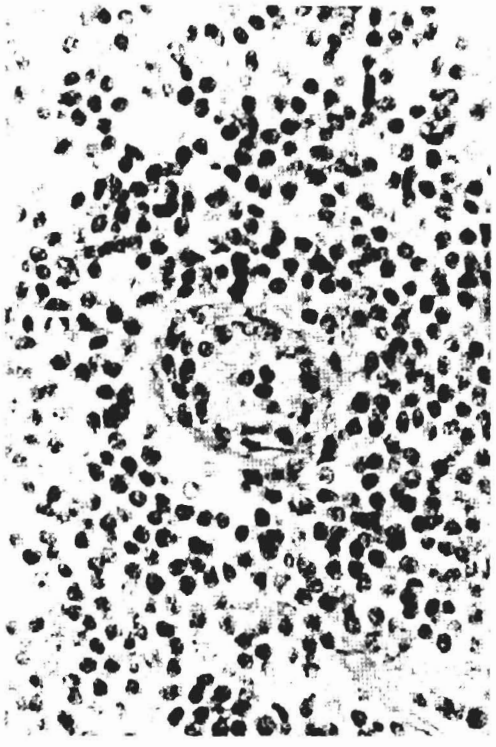

Fig. 2. A HEV from a reactive lymph node. Many small lymphocytes (dark heterochromatinized nuclei) are found between the endothelial cells (large, pale nuclei) and between the endothelial cells and the basement membrane. PAS. x 400

Fig. 3. A HEV from a case of LL, M. Preserved angiostructure. The migration of lymphocytes is inconspicuous. PAS. x 200

content. The high number of HEVs with preserved structure and the higher LMIs in LLs compared to B-CLL and PLL cases support the notion (Inghirami et al., 1988) that an intense migration from the blood into the lymph nodes may be responsible for the aleukaemic state of this entity. Moreover, the higher number of LMIs observed in mature subgroups (CLL, PF, M and LL, M) may reflect that signals of activation/proliferation down-regulate some of the adhesion receptors (Kimby et al., 1989) and result in a reduced migration of leukaemic cells from the blood into the lymph nodes. However, the regulatory effect of activation/proliferation signals on adhesion molecules holds widely; no direct evidence has been produced to prove them to be operative in B-CLL (see the accompanying report of this issue).

The studies of adhesion receptors in lymphomas/leukaemias (Brandley et al., 1987; Yednock et al., 1987; Spertini et al., 1991; Csanaky et al., 1993a,b; Stauder et al., 1993) should be supplemented by some simple morphometric studies in order to obtain an insight into the mechanism of lymphoma/leukaemia dissemination. In the future, a better histological delineation of the maturation subgroups, comparisons of the morphometric features and the adhesion profiles with simultaneous proliferation marker analyses (antibodies operative on paraffin-embedded material and AgNOR studies) will enable us to give a more comprehensive interpretation of the morphometric parameters (HEV content and LMI) on B-CLL and related malignancies.

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