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Venular endothelium binding molecules CD44 and LECAM-1 in normal and malignant B-cell populations. A comparative study

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Summary. Lymphocytes leave the blood via post-capillary venules by binding initially to their specialized endothelium. CD44 is a 80–90 kDa hyaluronate-binding glycoprotein involved in binding to endothelium of high endothelial venules (HEV). LECAM-1 is a 75–85 kDa glycoprotein with lectin activity interacting with human peripheral lymph node vascular addressin (PNAd) on HEV. This immunohistochemical study shows that CD44 and LECAM-1 are essentially coordinately expressed on B-lymphocytes. The mode and level of CD44/LECAM-1 expression dissect the peripheral B-cell development into stages that are closely linked to morphologically defined B-cell compartments. Although statistically correlated in B-cell leukaemias ($p < 0.0009$) and extranodal B-cell lymphomas ($p < 0.003$), expression of both molecules was less stringently coordinated in 127 B-cell neoplasms examined. B-cell chronic lymphocytic leukaemia, hairy cell leukaemia and mantle zone lymphoma were CD44/LECAM-1 positive, thus corresponding to their reactive counterparts. Correspondingly, follicular centre cell-derived lymphomas were devoid of both markers. Conversely, CD44 and LEC-AM-1 were infrequently detectable in extranodal malignant B-cell neoplasms, irrespective of their maturational state. Presence versus absence of CD44 and LECAM-1, alone or together, determined neither the leukaemic versus aleukaemic state nor the nodal versus extranodal tumour-forming phenotype of a B-cell tumour.

Key words: CD44 – Leucocyte-endothelial cell adhesion molecule-1 – B-lymphocytes – B-cell neoplasms

Introduction

The ontogeny of normal B-lymphocytes is characterized by a succession of mobile and stationary phases. Naive B-cells leave the bone marrow, circulate, adhere to specialized venular endothelium to enter the lymphoid tissues and, on antigen contact, settle for clonal expansion. Early activation occurs in the mantle zone; cell cycle progression, together with somatic hypermutation, takes place in the centre of lymph follicles (Liu et al. 1992). Thereafter, B-lymphocytes either develop to memory cells, which circulate, or progress to plasma cells which, during maturation, move to the vicinity of a sinusoid (or in mucosa-associated lymphoid tissue, to the lamina propria) to settle for terminal differentiation. Reactivation of memory cells may also be confined to a specialized microenvironment, the so-called extrafollicular (EF) area (Liu et al. 1988; Zhang et al. 1988). This region carries different names in different lymphoid organs: the marginal zone in the spleen; dome region in the intestinal mucosa; intraepithelial B-cell compartment in the tonsil; medullary B-cell compartment of the thymus. In the lymph node, this region corresponds to the peri- and intrasinusoidal region adjacent to the lymph follicles (Möller and Mielke 1989). As molecular substrates for the environmental tropism of lymphocytes, adhesion receptors have been found on these cells that may contribute to programmed lymphocyte extravasation, currently called homing, and extravascular migration, by virtue of recognizing cell-type-specific determinants on, say, the endothelium of high endothalial venules (HEV). Two molecules which seem to be crucial for leaving the bloodstream and entering lymphatic tissues are CD44 and leucocyte-endothelial cell adhesion molecule-1 (LECAM-1).

The CD44 molecule is referred to as Hermes class of homing receptor, H-CAM, Pgp-1, In(LU)-related p80, B-cell p80, and extracellular matrix receptor III (ECM-III) (Haynes et al. 1989; Picker et al. 1989). Ongoing work on molecular cloning and immunochemistry of CD44 reveales that antibodies of this cluster define an immunologically related but structurally very diverse family of membrane proteoglycans and glycoproteins (Culty et al. 1990). The classical form of CD44 is the 80–90 kDa hyaluronate binding molecule involved in HEV-binding designated CD44H (H for haematopoiet-

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ic) (Stamenkovic et al. 1991; Sy et al. 1991). Various isoforms of CD44 have been described which have one (or more) additional extracellular domain(s), one of which – made up of 132 additional amino acid residues – gives rise to a 130–150 kDa protein core molecule designated CD44E (E for epithelial) (Brown et al. 1991; Stamenkovic et al. 1991) or CD44R1 (Dougherty et al. 1991). Further splice variants called CD44R2 or vCD44 (Günthert et al. 1991) differ in the size of this additional extracellular domain, which extends the protein core by between 69 (CD44R2, Dougherty et al. 1991) and 338 (Hofmann et al. 1991) amino acid residues. The diversity of CD44 molecules might be further enhanced by differential substitution of the core protein with various carbohydrate substitutes like sulphated asparagine-linked glycopeptides, chondroitin sulphate, heparan-sulphate glucosaminoglycans and O-linked mucins and polylactosamins (Brown et al. 1991). The chondroitin-sulphate carrying form of CD44 was shown to bind to type I collagen and to fibronectin (Jalkanen and Jalkanen 1991; Faasen et al. 1992). CD44 isoforms are differentially expressed in B-lymphocytes and several other cell types (Picker et al. 1988; Horst et al. 1990a; Collado et al. 1991; Ryan et al. 1991), but knowledge in this field still is very incomplete. Recent data on myeloid and lymphoblastoid B-cell lines (Dougherty et al. 1991; Hofmann et al. 1991) challenge the current conceptual dichotomy of a haematopoietic and an epithelial form of CD44.

LECAM-1, also known as "lymph node homing receptor" on lymphocytes, gp90^{MEL} (Geoffrey and Rosen 1989), Leu-8, or LAM-1, is a 75–85 kDa glycoprotein with calcium-dependent lectin activity and is thus a member of the so-called selectin family of surface molecules (Tedder et al. 1989, 1990; Imai et al. 1990; Lanier and Loken 1984). Like other selectins, LECAM-1 has an N-terminal C-type lectin domain juxtaposed with an epidermal growth factor domain and complement regulatory protein-like units (Liu et al. 1988; Bevilacqua et al. 1989; Johnston et al. 1989; Siegelmann et al. 1989; Picker et al. 1991). The lectin domain of LECAM-1 is the structure interacting with the recently described human peripheral lymph node vascular addressin (PNAd) (Berg et al. 1991). The binding property for the endothelial-leukocyte adhesion molecule (ELAM-1) and for CD62 seems to be restricted to a sialyl Lewis x-carrying LECAM-1 isoform exclusively expressed on granulocytes (Picker et al. 1991). On B-cells, LECAM-1 is differentially expressed along the differentiation pathway (Lanier and Loken 1984; Kansas et al. 1985; Kansas and Dailey 1989; Bührer et al. 1990; Murakawa et al. 1991).

Investigating normal lymphoid organs with monoclonal antibodies against these adhesion molecules, we confirm and extend observations by others (Picker et al. 1988; Horst et al. 1990a; Collado et al. 1991; Ryan et al. 1991; Kansas et al. 1985; Kansas and Dailey 1989; Bührer et al. 1990; Murakawa et al. 1991) in showing that both CD44 and LECAM-1 dissect the peripheral B-cell development into stages that are closely linked to morphologically defined B-cell compartments and, at the same time, define cytologically discernible B-cell types. We report here that both antigens are generally coordinately expressed, with only minor differences within the gut-associated lymphoid tissue and plasma cell compartment. This study further aims at the analysis of CD44 and LECAM-1 expression in a broad spectrum of B-cell neoplasias, including B-cell leukaemias, nodal B-cell lymphomas, extranodal, epithelium-associated lymphomas, and multiple myeloma.

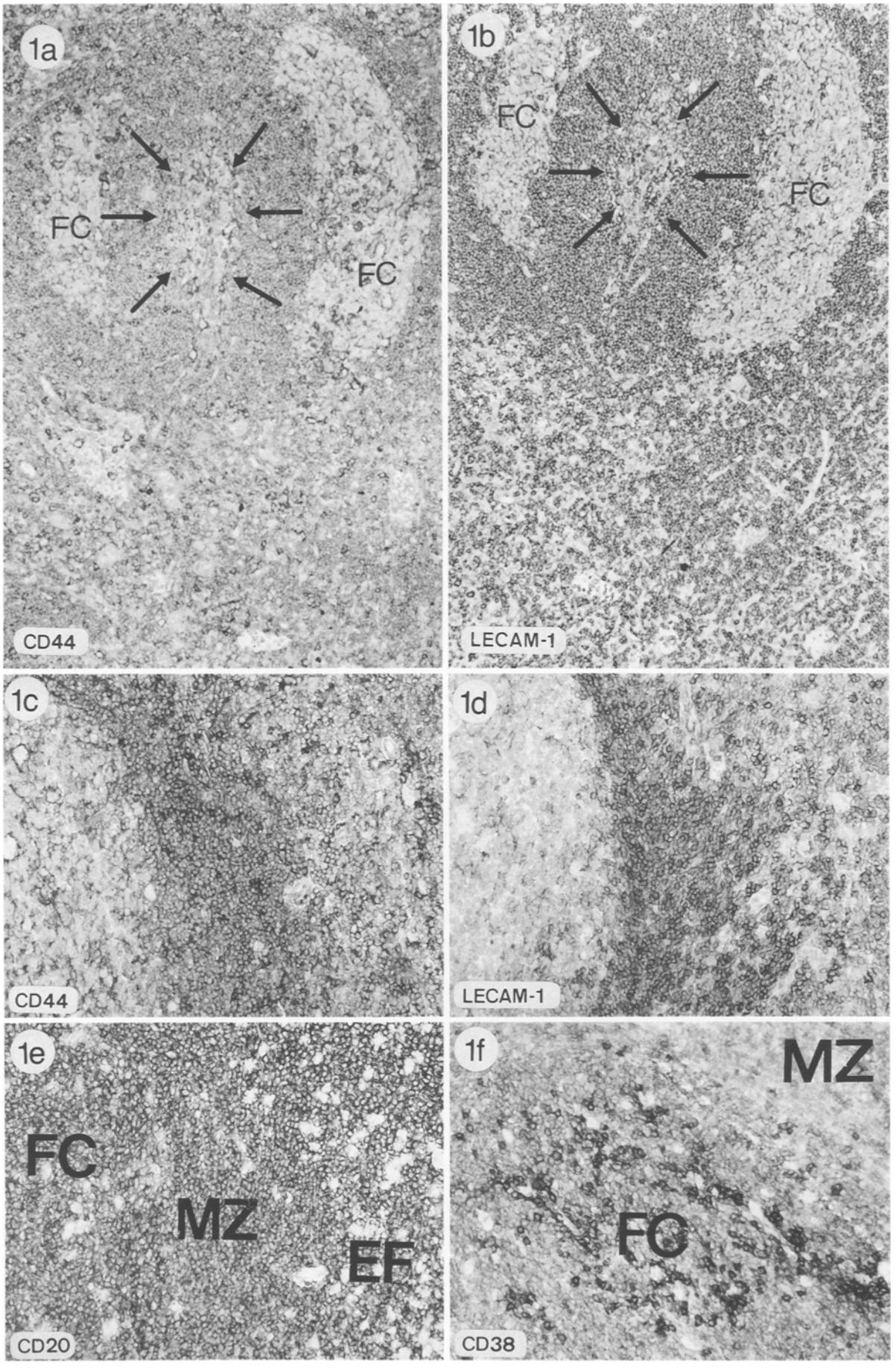
Materials and methods

In order to determine the physiological expression of CD44 and LECAM-1 in B-cell subpopulations of the normal lymphoid system, juvenile spleens, cervical and abdominal lymph nodes (including several specimens affected by toxoplasmic or non-specific lymphadenitis) and infantile thymi obtained during cardiotomy were chosen as reference organs. Previously immunophenotyped B-cell neoplasms were drawn from our bank of fresh frozen tumour tissue. The series examined included 4 lymph nodes involved in ($CD10^+$ $cIgM^+$) pre B-acute lymphoblastic leukaemia (ALL), 10 lymph nodes involved in chronic B-lymphocytic leukaemia (CLL), 7 spleens involved in hairy cell leukaemia (HCL), 4 lymph nodes showing mantle zone lymphoma (MZL) as described by Weisenburger et al. (1982), 35 lymph nodes affected by different types of follicle centre cell lymphoma (FCCL) which had been (sub)classified using the updated Kiel classification of non-Hodgkin's lymphomas (Stansfeld et al. 1988), 10 lymph nodes showing Burkitt's lymphoblastic lymphoma, 31 primary gastro-intestinal B-cell lymphomas (MALT), extensively immunophenotyped as described in detail elsewhere (Mielke and Möller 1991). For the purpose of this study, the gastro-intestinal lymphomas were subdivided into "low- and high-grade malignant variants", following the criteria given by Isaacson et al. (1983), 20 specimens of thymic B-cell lymphoma (Möller et al. 1986, 1987, 1989a, b) which were either mediastinal masses or secondarily infiltrated lung tissues, and 6 multiple myelomas. The diagnosis of each tumour type was established on the basis of histological routine stains (haematoxylin and eosin, Giemsa, and silver stains) of paraffin sections and of immunohistochemical data comprising CD10, CD19, CD20, CD22, CD38 and immunoglobulin expression (data not shown).

From the quick-frozen tissues serial frozen sections of about 1 cm^2 in area and 4–6 µm in thickness were air-dried overnight, fixed in acetone for 10 min at room temperature, and immunostained immediately or stored at −20° C for 1–3 weeks.

CD44(A3D8) (isotype IgG_1) was supplied by Dianova, Hamburg, Germany. Immunoprecipitation of lysates of tonsillar lym-

Fig. 1a–f. Toxoplasmic lymphadenitis: serial sections. **a, c** While MZ B-cells mostly carry CD44 at high levels, follicle centre B-cells (FC) are CD44 negative (positive staining is confined to DRC). The area marked by *arrows* contains a focus of monocytoid B-cells which are weakly positive. CD44 is broadly expressed within the T-cell and histiocytic populations. **a** ×86; **c** ×136. **b, d** The pattern of LECAM-1 expression essentially corresponds to that of CD44; however, histiocytes are LECAM-1 negative. Staining within follicle centres is confined to DRC. Monocytoid B-cells are weakly LECAM-1 positive. **b** ×86, **d** ×136. **e** Serial section adjacent to **d** stained for the pan-B antigen CD20 using CD20(L26) reveals that the perifollicular lymphoid cells are B-cells. ×136. **f** The CD38 antigen as detected via CD38(OKT10) is expressed at low levels on FC B-cells and at high levels in plasma cells. This serial section together with **c** and **d** demonstrates that intrafollicular plasma cells are CD44/LECAM-1 negative. ×136. Immunoperoxidase staining of frozen sections, faint haematoxylin counterstain; same technique for all photographs


1a
FC
FC
CD44
1b
FC
FC
LECAM-1
1c
CD44
1d
LECAM-1
1e
FC
MZ
EF
CD20
1f
MZ
FC
CD38

phocytes with A3D8 gave rise to a 85–95 kDa band (Picker et al. 1989) indicating that (at least) the classic form of the CD44 molecule is recognized by this antibody. Anti-Leu8 (isotype IgG2a) (Lanier and Loken 1984), recognizing LECAM-1, was obtained from Becton-Dickinson, Mountain View, Calif., USA. Positive control antibodies CD20(L26) and CD38(OKT10) were obtained from Dakopatts, Copenhagen, Denmark and Ortho Diagnostics, Raritan, NJ, USA, respectively. A polyclonal biotinylated sheep antibody to mouse Ig (reactive with all mouse isotypes) and a streptavidin-biotinylated peroxidase complex, all obtained from Amersham, High Wycombe, UK, served as a detection system for the primary antibodies.

Staining methods are described in detail elsewhere (Mielke and Möller 1991). The monoclonal antibodies were used in a protein concentration of about 10 µg/ml. The substrate for the peroxidase reaction was 3-amino-9-ethylcarbazole and resulted in an intense red precipitate. The sections were faintly counterstained with Harris' haematoxylin. In each individual case negative controls were performed without the primary antibody and, in a limited number of cases, by employing several irrelevant murine monoclonal antibodies of IgG_1 and IgG_{2a} isotypes and directed against non-human antigens. No staining was observed, except for the reaction of granulocytes whose endogenous peroxidase was not destroyed.

Strongly stained stromal cells and/or endothelial cells and/or T-lymphocytes and histiocytes, always present in combinations characteristic of the respective antigen under study, served as intrinsic positive controls. They were taken as internal markers for maximum reactivity, which was regarded as "high antigenic density" and symbolized " + + + ". A definitely weaker staining intensity of the (neoplastic) B-cell population was characterized as "intermediate antigenic density" and symbolized " + + "- A very weak but clearly discernible staining was regarded as "low antigenic density" and symbolized " + ". The absence of antigen was symbolized " − ". Whenever the staining intensity within a (neoplastic) B-cell compartment was heterogeneous, a simple semiquantitative statement was made. For comparative visualization of the entire B-cell population, CD20 immunostaining (Fig. 1e) in combination with CD38 staining (Fig. 1f) was used.

For statistical analysis Fisher's exact test was applied.

Results

The patterns of CD44 and LECAM-1 expression in B-cells of normal spleen, tonsil, gut, lymph node and thymic medulla are given in Table 1. CD44 was expressed in high levels in a subset of the plasma cell (PC) compartment but not in PC within the follicle centre (FC). CD44 was further expressed at intermediate to high levels on lymphocytes of the mantle zone (MZ), in intermediate level on EF B-cell and on thymic medullary B-cells. There were no major differences in antigenic densities of each B-cell compartment in different lymphoid tissues except for the EF B-cell population in lymph nodes. The small lymphocytic/centrocytoid variant of nodal EF B-cells was CD44 positive at intermediate levels, whereas so-called monocytoid B-cells in toxoplasmic lymphadenitis were CD44 negative. Centrocytes and centroblasts were CD44 negative. Follicular dendritic cells (DRC) expressed CD44 at an intermediate and low level in the apical and basal region of the FC, respectively. Outside the B-cell system, a considerable number of T-cells and histiocytic cells were CD44 positive (Fig. 1a, c).

While it was clear that fewer non-B cells were LECAM-1 positive, the expression of LECAM-1 (Fig. 1b, d) in the B-cell compartments closely corresponded to that of CD44, with only minor differences. Unlike the results for CD44, the gastrointestinal EF compartment contained a subpopulation of LECAM-1 negative cells. Furthermore, the antigenic density of the LECAM-1 positive PC subset was clearly low compared to the amount of CD44 expressed by these cells.

Table 1. Expression of CD44 and LECAM-1 in distinct B cell types confined to microtopographic areas of different organs and in dendritic reticulum cells

		CD44	LECAM-1
Dendritic reticulum cells	Apical	+ + [a]	+ +
	Basal	+	−
Follicle centre		−	−
Mantle zone		+ +/+ + +	+ + +
Extrafollicular compartment	Spleen	+ +	+ +
	Tonsil	+ +	+ + +
	Gut	+ +	−/+ +
	Node	− or + + [b]	− or + + [b]
	Thymic medulla	+ +	+ + +
Plasma cell compartment		−/+ + +	−/+

[a] +, Low; + +, intermediate; + + +, high antigenic density; −, no antigen detectable; x/y, admixture of two subsets differing in antigenic density; [b] according to the size of the cells (cf. Results)

The data on CD44 and LECAM-1 expression in different types of B cell neoplasms are summarized in Table 2. B-cell leukaemias showed an essentially coordinate expression of CD44 and LECAM-1. ALLs were CD44/LECAM-1 negative (Fig. 2a, b) while CLLs were positive. HCL expressed CD44 at low levels and LECAM-1 at variable, mostly intermediate levels (Fig. 3). There was a single exception which was weakly CD44 positive but lacked LECAM-1. Taken together, the mode of CD44 and LECAM-1 expression in nodal lymphomas mimicked that observed in reactive states. Thus, except for one LECAM-1 lacking case, MZL were strongly positive for both molecules. According to centrocytes and centroblasts, presence of one or both antigens was infrequent in FCCL and in Burkitt's lymphoma (which is known to share numerous features with follicle-derived tumours and normal FC B-cells). This group, however, contained several cases that expressed either high amounts of CD44 in the absence of LECAM-1 or both antigens in high or moderate levels. LECAM-1 positivity in the absence of CD44 was encountered in 4/15 centroblastic/centrocytic lymphomas and in a subset of neoplastic cells of a lymphoblastic lymphoma. In FCCL, no association between the modes of expression of both CD44 and LECAM-1 and the growth pattern (follicular versus diffuse) emerged (data not shown). Among the extranodal B-cell neoplasms, absence of one or both receptor molecules prevailed, especially in gastro-intestinal lymphomas. Presence of both antigens in this group of lymphomas was exceptional; the 4 out of 31 cases which were CD44/LECAM-1 double positive morphologically and immunologically corresponded to the MZ (Fig. 4a, b) or the EF stage of B-cell differentiation (Mielke and Möller 1991). A differentiation-associated down-regula-

Table 2. Adhesion receptor profile of B-cell neoplasms

Type	*n*	CD44				LECAM-1			
		−	+	++	+++	−	+	++	+++
B-cell leukaemias									
Acute B-lymphoblastic leukaemia	4	4	0	0	0	4	0	0	0
Chronic B-lymphocytic leukaemia	10	0	1	8	1	0	1	0	9
Hairy cell leukaemia	7	0	6	1	0	1	1	4	1
Nodal B-cell lymphomas									
Mantle zone lymphoma	4	0	0	0	4	1	0	0	3
Follicle centre cell lymphoma									
− Centroblastic/centrocytic	15	12	2	0	1	9	5	1	0
− Centrocytic	8	3	3	2	0	8	1	0	0
− Centroblastic	12	4	3	0	5	8	2	1	1
Burkitt's lymphoma	10	9	1	0	0	9	1	0	0
Extranodal B-cell lymphomas									
Primary gastro-intestinal lymphoma									
− Low grade	13	7	4	2	0	8	4	1	0
− High grade	18	10	1	2	0	10	3	0	0
Thymic B-cell lymphoma	20	10	6	3	1	12	4	3	1
Multiple myeloma	6	2	0	2	2	3	1	1	1

+, Low; ++, intermediate; +++, high antigenic density; −, no antigen detectable

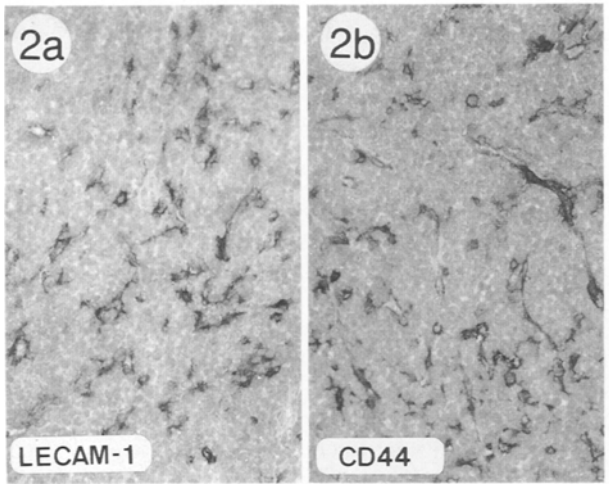


Fig. 2a, b. Acute B lymphoblastic leukaemia. The neoplastic cells express neither CD44 (**a**) nor LECAM-1 (**b**); the reliability of the immune reaction is confirmed by the strongly stained stromal cells; ×128

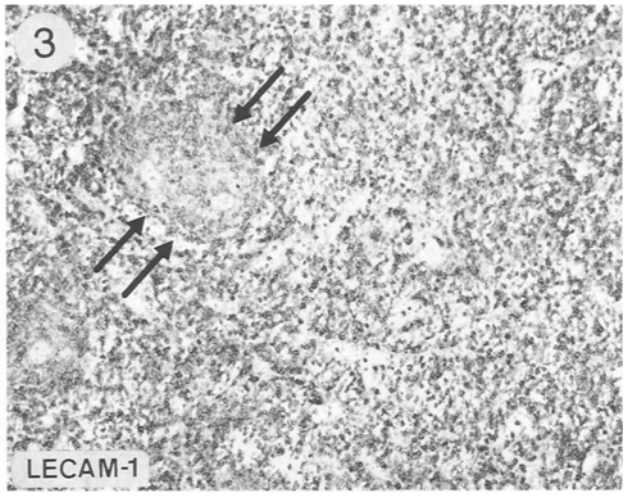


Fig. 3. LECAM-1 expression in hairy cell leukaemia. The tumour cells, which are diffusely distributed in the red pulp of the spleen, strongly express LECAM-1, while the staining intensity (i.e. antigenic density) of cells of the periarteriolar lymph sheaths (*arrows*) is definitely lower. ×64

tion of CD44 and LECAM-1 can be assumed for cases for which morphology and/or immunophenotype (Mielke and Möller 1991) suggested an FC stage of development. There were, however, three lymphocytoid/centrocytoid cases that (in terms of differentiation) exhibited an aberrant lack of both molecules. In tumours with hybrid immunophenotype including an FC aspect, CD44 and LECAM-1 were predominantly absent. The mode of expression of both molecules differed in 3 out of 31 gastro-intestinal B-cell tumours (Fig. 5a, b). Thymic B-cell lymphoma, which morphologically and immunophenotypically shows considerable resemblance to EF B-cells (Poletti et al. 1988), did less so concerning the mode of CD44 and LECAM-1 expression. In contrast to reactive EF cells (or, more precisely, thymic medullary B-cells), CD44 and LECAM-1 were coordinately present in only 3 out of 20 thymic lymphomas, and 8 tumours were even negative in both respects. Non-coordinate expression to a substantial degree was seen in 2 cases which clearly expressed LECAM-1 in the absence of any detectable CD44. The phenotype of multiple myeloma proved again to be heterogeneous. The strong and coordinate

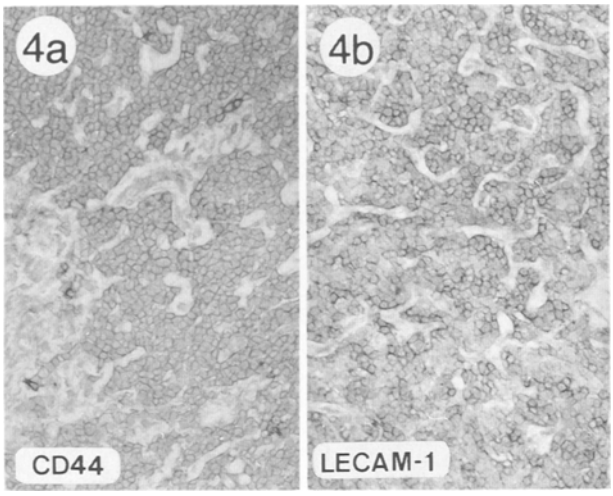


Fig. 4a, b. Primary gastric B-cell lymphoma of lymphocytic cytology. This tumour had a mantle zone immunophenotype (case 1 in Mielke and Möller 1991) and correspondingly coexpressed CD44 (**a**) and LECAM-1 (**b**). ×128

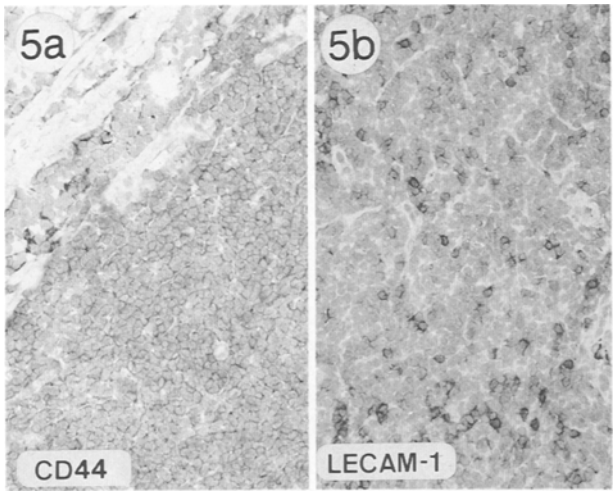


Fig. 5a, b. Primary gastric B-cell lymphoma of centrocytoid morphology. This tumour had an immunophenotype corresponding to that of extrafollicular B-cells (case 8 in Mielke and Möller 1991). Corresponding to the non-neoplastic counterpart, CD44 (**a**) is expressed by the tumour cells. **b** Since a subset of extrafollicular B-cells of the gut-associated lymphoid system is LECAM-1 negative (Table 1), this gastric lymphoma might be devoid of LECAM-1 due to physiological down-regulation. ×128

expression of both receptor molecules characteristic for most PC was detectable in one single case, while 2 other myelomas lacked both structures.

The mode of CD44 and LECAM-1 expression, which turned out to be essentially coordinate in reactive states of B-cell development, was statistically correlated in B-cell leukaemias ($p < 0.0009$) and extranodal B-cell lymphomas ($p < 0.003$). Although MZL and Burkitt's lymphoma showed a coordinate expression or lack of both molecules, respectively, this was not the case in FCCL. Therefore, we did not find any significant correlation in nodal lymphomas taken together.

Summing up, ALL were devoid of both molecules, and CLL and MZL, which may be regarded as malignant counterparts of the MZ, correspondingly expressed CD44 and LECAM-1 at high levels. HCL, which resembles splenic marginal zone (EF) cells, accordingly showed low and intermediate levels of both antigens. FCCL had the propensity to be devoid of both markers. The same was true for extranodal malignant B-cell neoplasias in general, however, irrespective of their maturational state. This indicates that the linkage between differentiation and CD44/LECAM-1 expression is only weak in this clinical subgroup of B-cell lymphomas. However, presence versus absence of CD44 and LECAM-1 alone or together did not per se determine the leukaemic versus the aleukaemic, nodal or tumour-forming phenotype of a B-cell tumour. On the one hand, ALL lacked both molecules and on the other, thymic B-cell lymphoma, which is obligatorily aleukaemic and shows lymph node involvement only exceptionally, expressed these molecules in quite a number of cases.

Discussion

During B-cell ontogeny, CD44 is expressed in high amounts during the very early phase; CD44 levels are likewise high on peripheral blood B-cells and MZ B-cells; CD44 is transiently abrogated during the pre-B-cell and germinal centre stage of development and is re-expressed at the PC stage (Picker et al. 1988; Horst et al. 1990a; Collado et al. 1991; Ryan et al. 1991). Our observation that EF B-cells (particularly monocytoid B-cells in toxoplasmic lymphadenitis) consistently carry lower levels of CD44 than major MZ B-cell subsets is new and cannot be further explained at present. However, all these findings together make CD44 a differentiation antigen.

On B-cells, LECAM-1 is differentially expressed along the developmental pathway (Lanier and Loken 1984); it is up-regulated when the B-cell acquires CD20 (Kansas et al. 1985) and is transiently down-regulated and/or shed during the follicle centre blast stage (Kansas and Dailey 1989; Poletti et al. 1988) as well as after activation in vitro (Kansas and Dailey 1989; Bührer et al. 1990). We have shown that EF B-cells express LECAM-1 at varying levels, differing among the EF compartments in spleen, lymph node, and thymic medulla. At present, there is no explanation for these (quantitative?) differences among EF B-cells of different sites. Effects of anti-LECAM-1 antibodies suggest that this molecule plays a role not only in the tissue sequestration of lymphocytes by binding to HEV but also in regulating T- and B-cell differentiation (Kanof et al. 1987; Takada et al. 1989; Murakawa et al. 1991).

The CD44 gene is located on *11p* (Goldstein et al. 1989) and the LECAM-1 gene has been assigned to *1p* (Watson et al. 1990). Knowledge on the regulation of both adhesion receptor genes is still scarce (for review see Michl et al. 1991), particularly in human B-cells. In mice, the polyclonal in vitro stimulation of splenic B-cells via surface immunoglobulin (sIg) led to an increase

in murine CD44 protein levels, while lipopolysaccharide, phorbolesters, and interleukin-4 failed to significantly up-regulate CD44 (Camp et al. 1991). Since sIg activation gives rise to activated B-blasts, these data are incompatible with the above cited in situ data indicating an activation-associated down-regulation of CD44. Down-regulation (and shedding, Spertini et al. 1991a) of LECAM-1 on B-cells was shown after stimulation with anti-μ and B-cell growth factor (Kansas and Dailey 1989) or with mitogen (Tedder et al. 1990). Furthermore, it was shown that the affinity of LECAM-1 as a receptor can transiently be increased through cell-specific activation signals (Spertini et al. 1991b). Our observation of an overall correspondance of CD44 and LECAM-1 expression in normal B-cells with microheterogeneity suggests that important maturational stimuli may act similarly on both genes while gene expression might be modulated by factors acting locally. In B-cell neoplasmas, expression of CD44 and LECAM-1 was less stringently correlated. Minor differences in relative antigenic densities were far less frequent than equal expression. However, they were encountered more often (especially in CLL and HCL) than a clear-cut non-coordinate expression of CD44 or LECAM-1. In this latter case, high or intermediate levels of CD44 were more often associated with absence of LECAM-1 than vice versa. These findings further substantiate the assumption of a fine tuning mechanism modulating the actual number of CD44 and LECAM-1 molecules on a B-cell. By contrast, major discrepancies are more likely to be the consequence of structural defects which, at least for the CD44 gene, may be rare in lymphomas and leukaemias (Kee et al. 1991).

CD44 was found in such types of B-cell leukaemias and nodal lymphomas that are currently considered to correspond to developmental stages during which CD44 is expressed (Picker et al. 1988; Pals et al. 1989). Moreover, tumours mimicking CD44-negative stages consistently (e.g., ALL) or predominantly (e.g., FCCL) lacked this structure. LECAM-1 expression in B-cell neoplasms has been studied previously by several authors (Garcia et al. 1986; Michie et al. 1987; Strickler et al. 1988; Carbone et al. 1988; Kimby et al. 1989; Spertini et al. 1991a) who agreed upon the almost regular expression of this molecule in CLL. Our own observation of strong LECAM-1 expression in CLL and intermediate levels in HCL is in very good correspondence with the B-cell types from which these leukaemias are most likely derived, namely MZ and EF B-cells. Similarly, the majority of so-called MZL were found to be LECAM-1-positive (Strickler et al. 1988). The overall paucity of LECAM-1 in FCCL (already described by Carbone et al. 1988) also corresponds to the putative normal counterpart, FC B-cells. Apart from these analogies, substantial discrepancies between developmental stage and mode of CD44/LECAM-1 expression were found in both directions. Thus, an inadequate *hyper*expression of both molecules may be assumed for two nodal FC lymphomas of centroblastic subtype; an inadequate *hypo*expression/loss of both receptors can be supposed for a considerable proportion of extranodal lymphomas, including gastro-intestinal lymphomas (Pals et al. 1991), and particularly for thymic B-cell lymphoma. In thymic B-cell lymphoma, especially in cases with secondary lung involvement (data not shown), CD44 and LECAM-1 were more frequently detectable than in FCCL. Nevertheless, the EF derivation of thymic B-cell lymphoma which we claim for this tumour type (Möller et al. 1989a), is not convincingly comprehensible from the present data. The (in terms of differentiation inadequate) lack of CD44 in 10 and LECAM-1 in 11 out of 20 thymic B-cell lymphomas of our series confirms LECAM-1 data by Menestrina et al. (1986) and Borgonovo Brandter et al. (1989).

In diffuse large B-cell lymphoma, inadequate ("abnormal") CD44 expression was reported to be associated with more disseminated disease (Pals et al. 1989; Horst et al. 1990b). Correlation of a CD44 positive phenotype with poor prognosis was suggested by Jalkanen et al. (1990), Pals et al. (1989) and Horst et al. (1990b). This view is challenged by our finding of a complete lack of CD44 in all B-ALL. We agree with Picker et al. (1988) in their statement that this molecule is not in itself indicative of a disseminative phenotype in B-cell neoplasms, since we found CD44 expressed in about half of the thymic B-cell lymphoma cases studied. Thymic B-cell lymphoma, in fact, is a special variant of diffuse, medium to large B-cell lymphoma (Möller et al. 1986, 1987, 1989a, b) which, although locally invasive and frequently involving adjacent organs and tissues, very rarely – if at all – disseminates. We confirm the frequent but inconsistent lack of LECAM-1 in gastro-intestinal B-lymphomas (Pals et al. 1991) suggesting that LECAM-1 plays a role in dissemination. However again, B-ALL as a highly disseminative disease lacks LECAM-1. How then do B-ALL cells (and their normal pre-B-cell counterparts) enter the lymphoreticular system? It is not excluded that lymph node invasion by B-cell precursors and lymph node involvement in acute B-lymphoblastic leukaemia lacking both CD44 and LECAM-1 might be mediated by other HEV receptors like VLA-4 (CDw49d/CD29) (Möller et al. 1992). Further, how should we explain the typical CD44/LECAM-1-negative FCCL? It seems conceivable that CD44/LECAM-1-positive clonogenic precursor cells exist in such a malignancy that circulate, leave the circulation and, by doing so, receive signals delivered by the invaded microenvironment that down-regulate these molecules.

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