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Chapter 7

Cell adhesion receptors in lymphoma dissemination

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submitted
Chapter 7

Introduction

Cell adhesion receptors play a key role in many biological processes including embryogenesis, hemostasis, inflammation, and tumor metastasis. In these processes, they coordinate cell-cell and cell-extracellular matrix (ECM) interactions as well as cell migration, and they act as co-regulators of cell growth and programmed cell death. In the immune system, adhesion molecules promote interactions of lymphocytes with antigen-presenting cells and target-cells required for the induction and regulation of an immune response and for effector cell function. Furthermore, adhesion molecules regulate leucocyte extravasation and lymphocyte homing by mediating adhesion to endothelium and to ECM components. As discussed in this paper, there is now ample evidence that the adhesion receptors, which direct the homing of normal lymphocytes also mediate the dissemination of their malignant counterparts, the non-Hodgkin's lymphomas (NHLs). In this way, these molecules contribute to lymphoma aggressiveness and determine the highly tissue-specific dissemination patterns of certain NHL-subtypes.

Lymphocyte Adhesion Receptors in the Regulation of Lymphocyte Homing.

Lymphocytes express members of a number of different adhesion receptor families, i.e. the immunoglobulin, the integrin, the selectin, and the CD44 families. The role of these adhesion molecules in lymphocyte interactions with other cells and with the ECM is complex and dynamic: Interaction of adhesion receptors with their ligands does not only lead to cell adhesion per se, but transduces signals into the cell regulating cytoskeletal organization, cell cycle progression, and programmed cell death (outside-in signaling). On the other hand, cytoplasmic signals, e.g. generated via the T-cell receptor, regulate the cell surface expression and functional activity of adhesion molecules (inside-out signaling). Selective expression and regulation of adhesion molecules on lymphocytes and endothelium forms the basis for tissue-specific lymphocyte homing. Table 1 summarizes some of the important features of the adhesion molecules involved in lymphocyte homing discussed in the present paper.
Table 1. Lymphocyte adhesion molecules and their role in homing*.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Expression in lymphocytes</th>
<th>Ligands</th>
<th>Interacting cells/substrates</th>
<th>predominant role(s) in homing</th>
</tr>
</thead>
<tbody>
<tr>
<td>α4β2 (LFA-1)</td>
<td>broad expression on T and B cells</td>
<td>ICAM-1, ICAM-2, ICAM-3</td>
<td>endothelium, HEV, DC, FDC, activated epithelium</td>
<td>broad function in lymphocyte homing</td>
</tr>
<tr>
<td>α4β1 (VLA-4)</td>
<td>broad expression on T and B cells</td>
<td>VCAM-1, fibronectin (CS-1)</td>
<td>activated endothelium, DC, FDC</td>
<td>homing to inflammatory sites</td>
</tr>
<tr>
<td>α4β7</td>
<td>naive T and B cells (low), memory T cell subset (high), peripheral B cell subset (low)</td>
<td>MAdCAM-1, (VCAM-1), (fibronectin)</td>
<td>endothelium intestinal mucosa, Peyser's patch and mesenteric lymph node HEV</td>
<td>gut-homing</td>
</tr>
<tr>
<td>αEβ7</td>
<td>intra-epithelial T-cells</td>
<td>E-Cadherin</td>
<td>epithelium</td>
<td>epitheliotropism</td>
</tr>
<tr>
<td>L-Selectin</td>
<td>resting T and B cells</td>
<td>PNAd, GlyCAM, CD34, (MAdCAM-1)</td>
<td>HEV</td>
<td>peripheral lymph node-homing</td>
</tr>
<tr>
<td>CLA</td>
<td>memory T-cell subset</td>
<td>E-selectin</td>
<td>endothelium (skin)</td>
<td>skin-homing</td>
</tr>
<tr>
<td>CD44 (multiple isoforms)</td>
<td>broad expression on T and B cells, splice variants on activated T and B cells</td>
<td>hyaluronate, collagen IV, fibronectin, osteopontin, FGF-2, HGF/SF, MIP-1β</td>
<td>ECM endothelium</td>
<td>homing to sites of inflammation - modulation of cell growth and motility.</td>
</tr>
</tbody>
</table>

*only molecules discussed in the present paper are listed
Figure 1. In the post-capillary venules selectin-sialomucin interactions mediate ‘rolling’ of lymphocytes on endothelium. Chemokines presented by proteoglycans on the endothelium can bind to the G-protein-coupled 7-membrane spanner chemokine receptor, and activate members of the integrin family on the surface of lymphocytes. Interaction of integrins to their ligands, results in a stable lymphocyte-endothelium adhesion and finally diapedesis.

Interaction of lymphocytes (and other leucocytes) with endothelium is a multistep process (Fig. 1). The initial step consists of a loose “tethering” engagement between the lymphocyte and the endothelium, leading to a rolling movement of the lymphocyte over the vascular endothelium of the post-capillary venule (Fig. 1). This step generally is mediated by molecules of the selectin family, which are strategically localized on the tips of the cell membrane’s microvilli, thus allowing for effective interaction of the selectin with its sialomucin ligand. However, other molecules such as the integrins $\beta_1$ and $\beta_7$, and CD44 can also mediate rolling. Lymphocyte rolling is transient and reversible, unless followed by a signal leading to activation of adhesion molecules of the integrin family. These molecules, i.e. $\alpha_4\beta_1$ (LFA-1), $\alpha_4\beta_7$ (VLA-4) and $\alpha_4\beta_7$, mediate stable adhesion and promote migration of lymphocytes across the vessel wall (Fig. 1). The extremely rapid integrin activation that has to take place in the blood stream in vivo is mediated by chemokines, as blockade of their G-protein-coupled receptors by pertussis toxin efficiently inhibits lymphocyte emigration from the blood. Recently, several chemokines like SDF-1, SLC (6-C-kine), BLC/BCA-1, MIP-3$\beta$, MIP-3$\alpha$, IP10 and Mig have been identified that are capable of mediating rapid (milliseconds) integrin dependent lymphocyte arrest under flow conditions. Consistent
Cell Adhesion Receptors in Lymphoma Dissemination

with their important regulatory role in homing, these chemokines display site-specific production as well as specificity for distinct lymphocyte subsets \(^{32,34,38}\). For example, the chemokine SLC is specifically expressed by HEV and selectively recruits naive T cells, expressing the chemokine receptor CCCR7, into the secondary lymphoid organs \(^{36}\). BLC/BCA, by contrast, is involved in the recruitment of B cell into B cell areas. It is specifically expressed in B-cell follicles, presumably by FDC. Disruption, of CXCR5, the receptor for BLC leads to a disturbed development of primary follicles and germinal centers in the spleen and Peyer’s patches \(^{37,39}\). In addition to chemokines, heparan sulfate proteoglycans (HSPG) expressed on endothelium or ECM may contribute to integrin activation and promote diapedesis by concentrating and presenting chemokines (Fig. 1). As specific modifications of heparan sulfate chains have been shown to determine growth factors binding specificity, chemokine interaction with HSPG may contribute an additional layer of specificity to the lymphocyte-endothelial cell interaction cascade \(^{40-42}\).

In the multi-step lymphocyte-endothelial cell interaction model described above (Fig. 1), specificity is determined by unique combinations of primary and secondary adhesion receptor pairs, as well as by chemokine mediated activation events. Important factors regulating the adhesion receptor profile of lymphocytes are prior antigenic stimulation and/or state of activation \(^{4,5,43}\). Imprinting of the lymphocyte at the site of antigenic experience leads to expression of a specific set of adhesion receptors on the lymphocyte involved in tissuespecific homing to the sites of primary antigenic stimulation. These ‘homing’ receptors permit interaction with endothelial area codes (‘vascular addressins’), crucial in tissue-specific recirculation of lymphocytes. Combinations of lymphocyte adhesion molecules and vascular addressins involved in tissue-specific homing are: \(\alpha_E\beta_7/\text{MAdCAM-1}\) for mucosa associated lymphoid tissues (MALT); cutaneous lymphocyte antigen (CLA)/E-selectin for skin; L-selectin/PNAd, for peripheral lymph nodes. Binding of \(\alpha_E\beta_7\) to E-cadherin expressed on epithelial cells, is important in positioning lymphocytes in epithelium (Fig.2).

Adhesion Receptors in Lymphoma Dissemination.

At least three sets of clinical observations indicate that conserved homing programs play an important role in the dissemination of NHLs \(^{44}\). First, while NHLs related to small resting lymphocytes usually are disseminated to multiple lymphoid sites at presentation, tumors related to activated lymphocytes (e.g. centroblasts and immunoblasts) are often initially localized and disseminate primarily to adjacent lymph nodes. These differences in dissemination propensity presumably reflect the recirculating versus sessile character of the normal counterparts of these NHLs. Second, extranodal NHLs arising in the MALT or the
skin disseminate preferentially to mucosal sites and skin, respectively. This strongly suggests that they make use of specific area codes, similar to those employed during normal lymphocyte homing. Third, several reports have documented specific lymphoma dissemination to sites of (micro)trauma and inflammation. This phenomenon could be explained by interaction of tumor cells with activated endothelium at the site of injury. In the following paragraphs, studies focussing on the role of specific adhesion receptors in lymphoma dissemination will be discussed.

Figure 2. Lymphocyte migration is strictly regulated by cell surface molecules on lymphocytes (lymphocyte homing receptors) and endothelium (vascular addressins). Random migration of naive T-cells throughout the body is mediated by the dual expression of α4β7 and L-selectin. CD44 and members of the integrin and selectin family expressed on activated T-cells can form a stable binding to activated endothelium. Migration of memory T-cells is limited to organs of primary antigenic stimulation. Selective expression of the lymphocyte homing receptors α4β7, L-selectin and CLA mediate, by binding to their vascular addressins, tissue specific homing to the mucosa, peripheral lymph node and skin respectively. Interaction of αEβ7 to E-cadherin, expressed on epithelial cells, is involved in positioning of lymphocytes in the epithelium of skin and mucosa. The non-Hodgkin's lymphomas more or less using these cell surface molecules in their dissemination are mentioned in boxes.
Selectins and their Carbohydrate Ligands

**L-selectin; peripheral lymph node homing** (Fig 2). L-selectin, like the other members of the selectin family, consists of lectin, epidermal growth factor, and short consensus repeat domains. L-selectin is expressed by lymphocytes, monocytes and neutrophils and is rapidly down-regulated upon cell activation. The first evidence for a selective role of L-selectin in lymphocyte homing to peripheral lymph nodes (PLN) came from experiments demonstrating that mAbs against L-selectin interfere with *in vitro* binding of lymphocytes to high endothelial venules (HEV) of PLN, but not to HEV of Peyer's patches. In accordance with this finding, inactivation *in vivo* of L-selectin, either by mAbs or by gene knockout, was found to result in a virtually complete inhibition of lymphocyte homing to PLN. The main ligands for L-selectin are the peripheral node addressins (PNAd). PNAd are selectively, but not exclusively, expressed on HEV in peripheral nodes. Monoclonal antibody (mAb) MECA-79, against PNAd, decreases lymphocyte adherence to PLN HEV by 60-90%. The MECA-79 epitope is a carbohydrate moiety that decorates a number of different protein backbones including CD34 and GlyCAM-1. a(1,3) Fucosyltransferase Fuc-TVIIA knockout mice, which are deficient in selectin ligands, also show a strongly reduced lymphocyte homing to PLN. In conclusion, both L-selectin and its ligands are needed for lymphocyte homing to PLN.

Studies of L-selectin expression on NHLs have shown that the vast majority of nodal lymphomas express L-selectin (table 2). This holds for low-grade and aggressive B cell lymphomas, as well as for T-cell lymphomas with a primary nodal localization. In extranodal lymphomas, however, a more heterogenous expression of L-selectin is found. Among extranodal lymphomas localized in the gastrointestinal tract, low-grade B-cell lymphoma of the MALT and malignant lymphomatous polyposis (MLP) do express L-selectin (table 2). However, in both tumor types, the mucosal homing receptor α4β7 is also expressed. In MLP, a variant of mantle cell lymphoma, this double 'homing phenotype' is generally accompanied by widespread lymphoma dissemination to both mucosal sites and PLN. By contrast, low-grade B-cell lymphomas of the MALT, although also co-expressing PLN (L-selectin) and mucosal (α4β7) homing receptors, do not usually involve PLN. For PLN dissemination of these lymphomas, other steps in the lymphocyte-endothelial interaction cascade, e.g. involving integrins or chemokine signals, could be rate limiting. Alternatively, the absence of Helicobacter Pylori antigens in the PLN, which may promote tumor cell growth and survival, might explain the low incidence of PLN involvement in low-grade MALT lymphomas.

In contrast to the above mentioned tumors, aggressive mucosal B-cell and T-cell lymphomas only occasionally express L-selectin. Similarly, primary cutaneous T cell
Table 2. Expression of adhesion molecules in non-Hodgkin’s lymphomas.

<table>
<thead>
<tr>
<th>REAL classification</th>
<th>L-selectin</th>
<th>α4β7</th>
<th>CLA</th>
<th>αEβ7</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-cell CLL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- nodal</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- nodal</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- GI-tract (MLP)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Marginal zone B-cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| - GI-tract (low grade MALT) | + | + | - | -/+
| Follicle centre lymphoma| | | |
| - nodal             | +/-       | -    | -   | -    |
| Diffuse large B-cell|           |      |     |      |
| lymphoma            |           |      |     |      |
| - nodal             | +/-       | -    | -   | -    |
| - GI-tract          |           |      |     |      |
| Peripheral T-cell   |           |      |     |      |
| lymphoma            |           |      |     |      |
| - nodal             | +         | -    | -   | -    |
| - GI-tract          | -         | +/-  | -   | +/-  |
| - skin (MF)         | +/-       | -    | +   | +/-  |
| Anaplastic large cell|          |      |     |      |
| lymphoma            |           |      |     |      |
| - nodal             | -         | +/-  | +/- | -    |
| - skin              | +/-       | -    | +   | +/-  |

+ reported cases all or almost all positive; +/- variable reports, most cases positive; -/+ variable reports, most cases negative; - reported cases negative or sporadically positive.

lymphomas express low levels of L-selectin \(^58\) (table 2). The paucity of L-selectin on most extranodal lymphomas may contribute to their low propensity to disseminate to PLN.

Cutaneous lymphocyte antigen (CLA): skin homing. Cutaneous lymphocyte antigen (CLA) is a carbohydrate antigen that is closely related to the sialyl Lewis X antigen (sLex) \(^2,59,60\). CLA is present on a minor subset (10-15%) of memory T lymphocytes in the peripheral blood, tonsils and lymph nodes \(^60,61\). However, of the T cells in the normal and inflamed skin, 40% to >90%, respectively, are CLA positive \(^60,62,63\). CLA mediates skin homing via interaction with E-selectin, which is constitutively expressed on skin endothelium \(^2,64\) (Fig. 2).

Interestingly, cutaneous T cell lymphomas, mycosis fungoides as well as other subtypes, express CLA \(^60,65,66\). By contrast, they do not express the mucosal homing receptor α4β7,
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(Drillenburg unpublished observation). Vice versa, gastrointestinal T-cell lymphomas consistently lack CLA but express the mucosal homing receptor α4β7 (table 2). Hence, like on their normal counterparts, i.e. the skin and gut homing memory-T cell subsets, CLA and α4β7 expression on NHL is mutually exclusive. This highly selective expression of CLA on cutaneous T cell lymphomas strongly suggests that CLA is mediating the skin-specific dissemination of these lymphomas in vivo. (Fig.3).

Integrins

Integrins are heterodimeric proteins consisting of non-covalently associated α- and β-subunits 18. Most of the eight β-chains can associate several of the fifteen different α-chains that have so far been discovered. Only a limited number of these integrin molecules are expressed on lymphocytes. They mediate interactions of lymphocytes with a variety of cells including endothelium, epithelium, dendritic cells, FDC, and the ECM 1,2,6,8. Integrins expressed on lymphocytes are not constitutively active but their function is activation dependent 1,3,18.

LFA-1 (αLβ2). LFA-1 is one of the most important integrins of the immune system. It is involved in a wide variety of lymphocyte interactions with other cells including adhesion to endothelium 1,3,4,4,69, binding to APC 70,71, FDC 72, and epithelium 73. The ligands for LFA-1 on endothelium are the intercellular adhesion molecules (ICAM)-1, -2 and -3 21,74,76. LFA-1 interaction with ICAM-1 plays a major role in lymphocyte adhesion and transmigration through HEV 3,21,77,78. Defective expression of the β2-chain leads to impaired emigration of leucocytes from the blood to sites of inflammation, resulting in a lethal immunodeficiency, called leucocyte adhesion deficiency type I (LAD-I) 79.

LFA-1 has been shown to support in vitro invasiveness of T cell hybridomas and lymphoma cell lines in hepatocyte and fibroblast monolayers and promotes experimental metastasis in nude mice 80-83. In human lymphomas, expression of LFA-1 is closely related to lineage derivation and stage of differentiation 84. However, notwithstanding its important role in lymphocyte adhesion and migration, LFA-1 expression on NHLs does not predict clinical behavior 84-86. Conceivably, functional overlap between LFA-1 and α4β7 causes redundancy of LFA-1 in interactions relevant for lymphoma dissemination, including those with endothelium. Furthermore, as the function of LFA-1 is activation dependent, its expression does not directly reflect function.

Integrin α4β1 and other β1 integrins. The leucocyte integrin α4β1 (VLA-4) is unique among
\( \beta_1 \) integrins since it is not only involved in cell-matrix but also in cell-cell interaction. It can bind the CS-1 domain of fibronectin as well as vascular-cell adhesion molecule-1 (VCAM-1). The latter molecule is constitutively expressed on FDC and is induced on endothelium at sites of inflammation.

\( \alpha_4 \beta_1 \) plays an important role in lymphocyte migration to sites of inflammation, including inflamed joints in rheumatoid arthritis, and experimental allergic encephalitis (EAE) lesions in the brain. During germinal center reactions, \( \alpha_4 \beta_1 \), in concert with LFA-1, mediates the binding of germinal center lymphocytes to FDC. Apart from establishing physical contact, outside-in via \( \alpha_4 \beta_1 \) (and LFA-1) presumably contributes to the B-cell selection process in the germinal center by inhibiting apoptosis of germinal center B cells.

Recently, \( \alpha_4 \) knock-out mice were reported to have severe defects in T-cell development as well as in mucosal homing. By using chimeric mice, it was shown that \( \alpha_4 \) null lymphocytes are unable enter Peyer’s patches, while homing to lymph nodes and spleen was not disturbed. These findings demonstrate the importance of \( \alpha_4 \) in gut homing (see below). In human NHL, \( \alpha_4 \beta_1 \) is widely expressed in a pattern that matches the expression on related stages of normal lymphocyte differentiation. Like binding of normal germinal center B cells, binding of the tumor cells of follicle center cell lymphomas to FDC is mediated by \( \alpha_4 \beta_1 \). Progression of this tumor from a follicular to a diffuse phenotype is associated with loss of FDC from the tumor, rather than with down-regulation of \( \alpha_4 \beta_1 \).

Other \( \beta_1 \) integrins expressed on lymphocytes are \( \alpha_1 \beta_1 \), \( \alpha_5 \beta_1 \), and \( \alpha_6 \beta_1 \). These integrins mediate interactions with ECM components including collagen, fibronectin, and laminin, and may play an important role in lymphocyte migration through the ECM. Entry of lymphocytes into epithelia has been suggested to require \( \alpha_4 \beta_1 \) interaction with laminin-5 in basement membrane.

**Integrin \( \alpha_4 \beta_7 \); mucosal homing.** The integrin \( \alpha_4 \beta_7 \) mediates lymphocyte homing to the intestinal mucosa by binding to MAAdCAM-1, a vascular addressin selectively expressed on mucosal endothelium. MAAdCAM-1 is an immunoglobulin family member with domains that display homology to the adhesion receptors ICAM-1 and VCAM-1, as well as to another mucosa associated Ig-family member, IgAl. In mice, \( \alpha_4 \beta_7 \) is the dominant lymphocyte receptor for MAAdCAM-1 and for regulating lymphocyte homing to mucosal sites. In man, \( \alpha_4 \beta_7 \) appears to have a similar function. It is expressed on mucosal lymphocytes, and moreover, it is present on a subset of peripheral blood memory T-cells with gut homing properties. In \( \beta_7 \)-knockout mice, the formation of mucosa-associated lymphoid tissues is severely impaired. \( \beta_7 \)-deficient lymphocytes failed to stably arrest and transmigrate at Peyer’s patch HEV, although the initial rolling was intact. Recently, the human MAAdCAM-1 has been cloned, like its murine equivalent it is preferentially expressed in the
Figure 3. Cell adhesion receptors and vascular addressins. α4β1 (A) / MAdCAM-1 (B) expression in a low grade B-cell lymphoma of MALT type (stomach; arrow: epithelial structures), and CLA (C) / E-selectin (D) expression in a cutaneous T-cell lymphoma.
We have studied the expression of the $\alpha_4\beta_7$ mucosal homing receptor in NHLs. In malignant lymphomatous polyposis (MLP), an unusual presentation of non-Hodgkin's lymphoma of mantle cell type, characterized by multifocal involvement of the gastrointestinal tract, expression of $\alpha_4\beta_7$ was present in 7 out of 8 cases. By contrast, all cases of nodal mantle cell lymphoma tested were $\alpha_4\beta_7$ negative. Furthermore, we observed that most cases of low grade B-cell lymphomas of the MALT and intestinal T-cell lymphomas express $\alpha_4\beta_7$, while expression on lymphomas with a non-mucosal primary localizations was uncommon. These findings suggest that $\alpha_4\beta_7$ plays an important role in determining the characteristic mucosal dissemination often found in lymphomas of the MALT (table 2). Interestingly, Dogan et al. recently reported that low grade B-cell lymphomas of the MALT upregulate $\alpha_4\beta_7$, after Helicobacter pylori induced T-cell dependent proliferation of neoplastic cells.

**$\alpha E\beta_7$: interaction with epithelium.** In the gut, the integrin $\alpha E\beta_7$ is found on nearly all intestinal intraepithelial lymphocytes and on approximately 50% of the T-cells in the lamina propria. $\alpha E\beta_7$ can bind E-cadherin on epithelial cells and in this way mediates the positioning of lymphocytes in the epithelium (Fig. 2). Expression of $\alpha E\beta_7$ is present on celiac disease associated intestinal T cell lymphomas. Furthermore, $\alpha E\beta_7$ was found on the intra-epidermal malignant T-lymphocytes in mycosis fungoides. In advanced stages with loss of epitheliotropism, there was a decrease expression of $\alpha E\beta_7$, suggesting a direct involvement of $\alpha E\beta_7$ in the process of epitheliotropism. Consistent with this notion, we recently reported a strong expression of $\alpha E\beta_7$ in Pagetoid reticulosis, a rare form of cutaneous T-cell lymphoma characterized by an extreme epitheliotropism resulting in a pagetoid pattern of lymphocyte infiltration between keratinocytes.

**The CD44 family**

CD44 represents a family of glycoproteins encoded by a single gene on the human chromosome 11 (Fig.4). Members of this family show a broad tissue distribution and have been implicated in a number of important biological processes, including lymphocyte homing and activation, hematopoiesis, and tumor progression and metastasis. The CD44 gene consists of 19 exons. Structural and functional diversity of CD44 is generated by alternative splicing mRNA-splicing involving 10 exons encoding domains of the extracellular portion of the CD44 molecule. In addition to variable exon usage, variations in glycosylation contribute to the diversity of CD44. Most CD44 expressing cells express the 'standard' CD44 (CD44s) isoform translated from
Figure 4. Schematical representation of the CD44 gene and its encoded proteins. The extracellular domain and cytoplasmic tail of CD44 isoforms vary in size as the result of alternative splicing. The alternatively spliced exons are indicated by open boxes. The human v1 exon contains a stop codon. In the model of the protein, all putative glycosylation sites are indicated: O-glycosylation (open circles); N-glycosylation (closed circles); chondroitin sulfate (open squares); heparan sulfate (rod). As indicated, the heparan sulfate binding site in exon v3 has the ability to bind growth factors. In addition, the HA-binding sites (double line); the disulfide bonds (S-S); the ankyrin binding site (-----); the Ezrin binding site (- - - -); the phosphorylation sites (P); and the putative interaction sites for SRC-family kinases, are indicated.
an mRNA species containing none of the 'variant' (v) exons. On hematopoietic cells and lymphocytes this 85-95 kD molecule is the principle CD44 isoform. Larger CD44 isoforms containing various combinations of variant exons are preferentially expressed on epithelial cells, but they can also be expressed on activated lymphocytes and aggressive malignant lymphomas. During lymphocyte ontogeny and activation the expression of CD44 is strictly regulated, suggesting an important functional role for CD44. Indeed, CD44 has been reported to be involved in a variety of lymphocyte functions including lymphopoiesis, lymphocyte activation, and lymphocyte migration and homing. In the latter process, CD44 mediates lymphocyte binding to high endothelial venules, lymphocyte rolling, and migration to inflammatory sites. In T-cells, outside-in signaling through CD44 can costimulate T-cell receptor mediated proliferation, cytokine release, and integrin activation. Cross-linking of CD44 on T cells leads to protein tyrosine kinase activation. CD44 is physically and functionally associated with the src-family tyrosine kinase p56 in these cells.

A number of experimental and clinical studies suggest an important role of CD44 in the biological behavior of NHLs and indicate that CD44 represents a clinically useful marker predicting disease outcome in NHLs. In a nude mouse model, CD44s (but not CD44v8-10) enhanced the growth and metastatic capacity of Burkitt's lymphoma cell lines. The capacity of the tumor cells to bind hyaluronate (HA) via CD44s presumably plays an important role in these tumor promoting effects of CD44. HA may enhance tumor cell growth by providing a molecular bridge facilitating tumor cell attachment to the extracellular matrix. The matrix could serve as a scaffold for tumor cell growth and as a reservoir for growth and/or motility factors with biological effects on the tumor cells. In this context, it is of interest that CD44 variants containing exon v3 can be decorated with heparan sulfate (HS) side-chains, and by this virtue, can serve as receptors for heparin binding growth factors, including MIP-1β, FGF-2 and HGF/SF. Presentation of HGF/SF to its high-affinity receptor c-Met by CD44-HS, was recently shown to promote signaling, and may enhance tumor growth and motility.

Clinical studies support an important role of CD44 in the biology of human NHLs. In diffuse large cell lymphomas (DLCL) of the B-lineage, we observed a strong correlation between CD44 expression and lymphoma dissemination. Furthermore, CD44 expression is an unfavorable prognosticator in these tumors. Similar findings were reported by the group of Jalkanen for high grade B-cell lymphomas. In a recent study, we have explored the prognostic value of CD44 in a group of 276 patients, diagnosed as having DLCL according to the criteria of the REAL-classification. In this multicenter population based study group, expression of CD44s was a powerful prognosticator for overall survival and disease free survival in patients with localized disease. In a multivariate comparison with
the clinical parameters of the International Prognostic Index (IPI), which are currently used to predict prognosis in DLCL, CD44s expression was the major prognosticator for the subgroup of patients with localized nodal disease.

In addition to CD44s, NHLs may express CD44 isoforms containing variant exons. These larger variants are predominantly expressed on a subgroup of aggressive lymphomas. They often include exon v6, which was reported confers metastatic behavior in rat carcinoma cell lines. In a study by Stauder and colleagues in a mixed group of high-grade NHLs, including patients with precursor B-cell- and Burkitt’s-lymphomas, as well as DLCL, CD44v6 was found to be an independent prognosticator predicting tumor related death. In our own study concentrating on a single diagnostic group, i.e. DLCL, CD44v6 had no prognostic value, while CD44s was an important prognosticator.

Conclusion

A large body of evidence indicates that adhesion receptors do not only regulate normal lymphocyte trafficking but also play a pivotal role in the dissemination of non-Hodgkin’s lymphomas (NHLs). Cutaneous lymphocyte antigen (CLA), α4β7, αEβ7, and L-selectin, homing receptors mediating the tissue-specific positioning of normal lymphocytes in the skin, mucosa, epithelium, and peripheral lymph nodes, respectively, are selectively expressed on lymphomas evolving at these sites. By interacting with vascular addressins they mediate tissue-specific lymphoma dissemination. CD44, a family of adhesion receptors involved in both lymphocyte migration and tumor metastasis, promotes lymphoma dissemination by interacting with ECM components, particularly with hyaluronate. In addition, binding of growth and motility factors by heparan sulfate proteoglycan forms of CD44 may play a role. Expression of CD44 on localized aggressive NHLs is an independent unfavorable prognosticator. Taken together, these findings offer a framework for the understanding of tumor dissemination in NHL. In view of the similarities between lymphocyte behavior and the metastatic behavior of solid tumors, lymphoma dissemination may serve as a paradigm for tumor metastasis in non-lymphoid tumors.

References


19. Butcher EC. Leucocyte-endothelial cell recognition: three (or more) steps to specificity or diversity. Cell 1991; 67:1033


role for microvillous receptor presentation in leukocyte adhesion. Cell 1995; 82:989


34. Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 1990; 347:669


36. Gunn MD, Tangemann K, Tam C, Cyster JG, Rosen SD, Williams LT. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. Proc Natl Acad Sci USA 1998; 95:258


38. Legler DF, Loetscher M, Stuber Roos R, Clark-Lewis I, Baggiolini M, Moser B. B cell-attracting chemokine 1, a human
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54. Pals ST, Meijer CJLM, Radaszkiewicz T. Expression of the human peripheral lymph node homing receptor (LECAM-1) in nodal and gastrointestinal non-Hodgkin's lymphomas. Leukemia 1991; 5:628


57. Möller P, Eichelmann A, Mechtersheimer G, Koretz K. Expression of β-1 integrins, H-CAM (CD44), and LECAM-1 in primary gastrointestinal B-cell lymphomas as compared to the adhesion receptor profile of the gut associated lymphoid system, tonsil and peripheral lymph node. Int J Cancer 1991; 49:846


63. de Boer OJ, Horst E, Pals ST, Bos JD, Das PK. Functional evidence that the HECA-452 antigen is involved in the adhesion of human neutrophils and lymphocytes to tumour necrosis factor-alpha-stimulated endothelial cells. Immunology 1994; 81:359

64. Berg EL, Yoshino T, Rott LS, Robinson MK, Warnock RA, Kishimoto TK, Picker L, Butcher EC. The cutaneous lymphocyte antigen is a skin homing receptor for the vascular lectin endothelial cell-leucocyte adhesion


84. Horst E, Radaszkiewicz T, Hooftman-den Otter A, Pieters R, van Dongen JJM, Meijer CJKLM, Pals ST. Expression of the leucocyte integrin LFA-1 (CD11a/CD18) and its ligand ICAM-1 (CD54) in lymphoid malignancies is related to lineage derivation and stage of differentiation but not to tumor grade. Leukemia 1991; 5:848


92. Koopman G, Keehnen RM, Lindhout E, Zhou DF, de Groot C, Pals ST. Germinal center B cells rescued from apoptosis by CD40 ligation or attachment to follicular dendritic cells, but not by engagement of surface immunoglobulin or adhesion receptors, become resistant to CD95-induced apoptosis. Eur J Immunol


97. Briskin MJ, McEvoy LM, Butcher EC. MAdCAM-1 has homology to immunoglobulin and mucin-like adhesion receptors and to IgAl. Nature 1993; 363:461

98. Holzmann B, McIntyre BW, Weissman IL. Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an \( \alpha \) chain homologous to human VLA-4\( \alpha \). Cell 1989; 56:37


100. Farstad IN, Halstensen TS, Lazarovits AI, Norstein J, Fausa O, Brandtzaeg P. Human intestinal B-cell blasts and plasma cells express the mucosal homing receptor integrin \( \alpha_4 \beta_7 \). Scand J Immunol 1995; 42:662


107. Simonitsch I, Vole-Platzer B, Mosberger


112. Stamenkovic I, Amiot M, Pesando JM, Seed B. A lymphocyte molecule implicated in lymphocyte homing is a member of the cartilage link protein family. Cell 1989; 56:13


114. Stamenkovic I, Aruffo A, Amiot M, Seed B. The hematopoietic and epithelial forms of CD44 are distinct polypeptides with different adhesion potentials for hyaluronate-bearing cells. EMBO J 1991; 10:343


122. Wielenga VJM, Heider KH, Offerhaus GJA, Adolf GR, van den Berg FM, Ponta

123. Screaton GR, Bell MV, Jackson DG, Cornelius FB, Gerth U, Bell JI. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. Proc Natl Acad Sci USA 1992; 89:12160


137. Taher TEL, Smit L, Griffioen AW,


