Biochemical Pharmacology, Vol. 59, pp. 455–465, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(99)00266-X

COMMENTARY

A Role for the Cell Adhesion Molecule CD44 and Sulfation in Leukocyte–Endothelial Cell Adhesion during an Inflammatory Response?

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ABSTRACT. CD44 is a widely expressed cell adhesion molecule that has been implicated in a variety of biological processes including lymphopoiesis, angiogenesis, wound healing, leukocyte extravasation at inflammatory sites, and tumor metastasis. The adhesive function of CD44, like other molecules involved in inducible adhesion, is tightly regulated. Post-translational modifications, isoform expression, aggregation state, and protein associations all can affect the ligand binding properties of CD44, and these can vary depending on the cell type and the activation state of the cell. The most extensively characterized ligand for CD44 is hyaluronan, a component of the extracellular matrix. Interactions between CD44 and hyaluronan can mediate both cell–cell and cell–extracellular matrix adhesion. In the immune system, both the selectin molecules and CD44 have been implicated in the initial binding of leukocytes to endothelial cells at an inflammatory site. Sulfation is required for selectin-mediated leukocyte–endothelial cell interactions, and, recently, inducible sulfation also was shown to regulate CD44-mediated leukocyte adhesion to endothelial cells. Sulfation, therefore, may be important in the regulation of cell adhesion at inflammatory sites. In this commentary we have reviewed the molecular aspects of CD44 and the mechanisms that regulate its binding to hyaluronan. In addition, we have summarized the role of CD44 and hyaluronan in mediating leukocyte–endothelial cell interactions and have discussed how this interaction may be regulated. Finally, we examined the potential role of sulfation as an inducible means to regulate CD44-mediated leukocyte adhesion and as a more general mechanism to regulate leukocyte–endothelial cell interactions. BIOCHEM PHARMACOL **59**;5:455–465, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. CD44; sulfation; TNFα; inflammation; leukocyte–endothelial cell interactions; cell adhesion

CD44—STRUCTURE

CD44 is expressed on many cell types, including leukocytes, fibroblasts, endothelial cells, and epithelial cells. The most prevalent form of CD44 is an 85- to 90-kDa glycoprotein referred to as CD44H or CD44s, which represents the major form on leukocytes and fibroblasts. Alternative splicing of at least 10 exons (v1–10) gives rise to multiple isoforms of CD44 (CD44v), which add additional sequence to the extracellular membrane-proximal region of CD44 [1]. CD44v isoforms are less abundant but can be found on endothelial cells, epithelial cells, activated lymphocytes, and some tumor cells. Figure 1 illustrates the salient features of the CD44 molecule [2, 3]. For a detailed review of the structure and molecular function of CD44, see Ref. 4. The amino terminal extracellular domain of CD44 (amino acids 21–182, numbered according to Ref. 3) contains a region with sequence identity to the Link module, a protein domain present in a family of extracellular proteins that bind HA† [3, 5]. Determination of the structure of one of these Link module-containing hyaluronan binding proteins, TSG-6, revealed a structure very similar to that of C-type lectins [6]. A C-type lectin domain is present in the amino terminal region of the selectins, a family of cell adhesion molecules that play an important role in the initial attachment of leukocytes to specialized or activated endothelial cells [7–9]. Mutagenesis studies have implicated the amino terminal region of CD44 in binding to HA [10, 11]. The Link homology region of CD44 contains the majority of N-linked glycosylation sites, and modification of these sites has been shown to affect the HA binding ability of CD44 (reviewed in Refs. 4 and 12).

The membrane-proximal region (amino acids 183–268) is the least conserved region of CD44, having only ~35% sequence identity between mouse and human. It contains multiple sites for O-linked glycosylation, two conserved SG

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† *Abbreviations:* CS, chondroitin sulfate; CS-A, chondroitin 4 sulfate; ECM, extracellular matrix; GAG, glycosaminoglycan; HA, hyaluronan; HEV, high walled endothelial venule(s); HS, heparan sulfate; HUVEC, human umbilical vein endothelial cell(s); Ig, immunoglobulin; IL, interleukin; mAb, monoclonal antibody; MIP-1α and β, macrophage inflammatory protein 1α and β; PHA, phytohemagglutinin; and TNFα, tumor necrosis factor α.

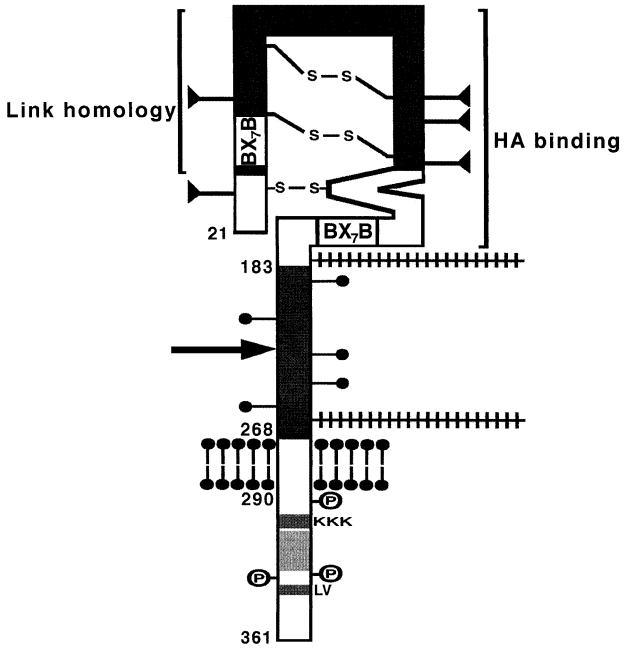

FIG. 1. Schematic diagram of the CD44 molecule. BX_7B is a motif known to play a role in HA binding. B represents a basic amino acid, and X is a neutral or basic amino acid [2]. The amino acids are numbered according to the human CD44H sequence [3]. The Link homology domain and HA binding region are as indicated, and the shaded region from 183 to 268 represents the membrane-proximal region. Potential disulfide bonds are as indicated (S—S). (▼) represents N-linked glycosylation, and (●) indicates potential O-linked glycosylation; (+++) represents GAG addition at the two conserved sites. The large arrow indicates the point of insertion for the additional sequence responsible for generating the CD44v isoforms. The cytoplasmic domain shows three regions, one (amino acids 298–300, KKK) that has been implicated in binding the ezrin, radixin, and moesin (ERM) proteins, a second (amino acids 304–318) that has been implicated in binding ankyrin, and a third LV motif (amino acids 331–332) that has been implicated in basolateral sorting. Potential serine phosphorylation sites are indicated with a P.

motifs for GAG addition, and the site of insertion for the additional amino acid sequence present in the CD44v isoforms. The inclusion of additional sequence as well as GAG addition and O-linked glycosylation all can affect the HA binding ability of CD44 (reviewed in Refs. 4, 12, and 13).

The transmembrane region of CD44 can influence HA binding by promoting the self-association of CD44 [14, 15]. Various cytoplasmic domain deletions of CD44 expressed in T cell lines or in transfected COS cells also can reduce or abolish HA binding [16–20] and can prevent migration of transfected melanoma cells on HA [21]. The cytoplasmic tail of CD44 can affect the localization of CD44 within a cell. In confluent epithelial cell cultures, CD44 is excluded from the apical region and localizes to the basolateral surface [22]. This localization is mediated by a dipeptide, LV, present in the cytoplasmic domain [23].

CD44—FUNCTION

CD44 has been implicated in embryogenesis, lymphopoiesis, lymphocyte activation, progenitor homing, angiogenesis, wound healing, leukocyte rolling and extravasation at inflammatory sites, and tumor metastasis. However, its precise role in these events is unclear, particularly in light of the surprising finding that CD44 gene knockout mice appeared normal. The mice showed no obvious developmental or functional abnormalities, and analysis of immune cell development and function did not reveal any significant defects [24]. Perhaps, like other cell adhesion molecules, CD44 has a redundant role, or perhaps its function can be compensated for by other molecules in the development of the CD44 gene knockout mouse. The potential function of CD44 in many of the above processes likely relates to its ability to participate in cell–cell adhesion and cell–ECM interactions. In many cases, these interactions are mediated by an interaction between CD44 and HA [25–27]. The importance of interactions between CD44 and HA is also inferred from studies of tumor cell growth and metastasis, where dysregulation of CD44–HA interactions occurs (reviewed in Refs. 13, 28, and 29). In addition to mediating cell adhesion events, CD44 has also been implicated in cell migration. Upon HA binding, CD44 can signal the migration of melanoma cells and of fibroblast cells during wound repair [21, 30]. CD44 can also mediate microvascular endothelial cell migration on fibrinogen during wound repair [31]. However, some aspects of CD44 function, such as its involvement in the migration of progenitor T cells to the thymus, may not involve an interaction with HA [32, 33].

CD44—LIGANDS

CD44 is presently the most extensively characterized cell surface hyaluronan receptor, and hyaluronan is the most extensively characterized CD44 ligand. Other molecules, such as serglycin [34, 35] and CS-modified invariant chain [36], have been identified that bind to CD44 via their CS side chains. Certain isoforms of CD44 (CD44v4–7, CD44v4–10, and CD44v10) can bind to CS-A [37–39], and mutation in the HA binding region abolishes this binding [37]. Osteopontin, a cytokine-like molecule with adhesive and migratory functions, can bind to integrin molecules and to specific CD44v isoforms [40]. CD44v isoforms may co-operate with β1 integrins to bind osteopontin [41]. CS-modified forms of CD44 also have been reported to bind other β1 integrin ligands such as fibronectin [42] and collagen XIV [43]. CD44 also can mediate melanoma cell migration and invasion on type I and type IV collagen [44, 45]. Modification of CD44 by CS and HS GAGs complicates the function of CD44, as it provides additional binding sites for molecules such as MIP-1β [46], matrix metalloproteinase 9 [47], and heparin binding growth factors [48], which may themselves play a role in cell adhesion, migration, and invasion.

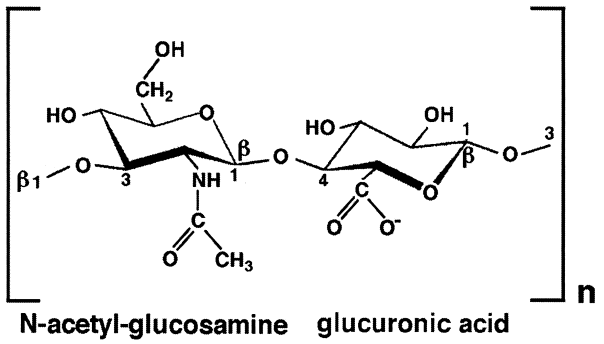

FIG. 2. Structure of the repeating disaccharide of hyaluronan. n is the number of repeating disaccharides and can range from 1 to over 1000.

HA is a high molecular weight GAG made up of repeating disaccharides of β(1→4)-D-glucuronic acid-β(1→3)-*N*-acetyl-D-glucosamine (Fig. 2). HA is the only GAG that is not sulfated or attached covalently to a membrane protein. HA is found in virtually all tissues of vertebrates as a component of the ECM and is present in large amounts in cartilage and synovial fluids. HA has received considerable attention in recent years due to its profound influence on cell behavior. The HA level within the ECM is strictly regulated by cellular hyaluronidase and receptor-mediated endocytosis of HA. The ECM becomes enriched in HA coincident with periods of rapid cell proliferation, aggregation, and migration in the processes of embryogenesis, wound healing, tissue regeneration and remodeling, as well as during tumor cell invasion (reviewed in Refs. 49 and 50). A delayed-type hypersensitivity reaction in the skin also transiently increases HA expression [51]. These HA-induced effects on cells are supported and directed by cell surface HA-binding proteins, such as CD44 [52].

CD44–HA-MEDIATED CELL ADHESION

CD44–HA-mediated adhesion has been observed between a T cell line and an endothelial cell line [16], between phorbol myristate acetate-activated T cells and human gingival fibroblasts [53], and between a B-cell hybridoma and a bone marrow stromal cell line [25]; and hematopoietic progenitors have been shown to bind HA [54]. In addition, there are several examples of leukocytes and leukocytic cell lines binding to cultured endothelial cells under static or flow conditions. These will be discussed in more detail later. In the above examples, HA is thought to act as a bridge to mediate CD44–CD44-dependent adhesion. This CD44–HA–CD44 interaction is thought to occur by one cell presenting surface-bound HA (anchored by cell surface CD44), which then is recognized by CD44 on the opposing cell.

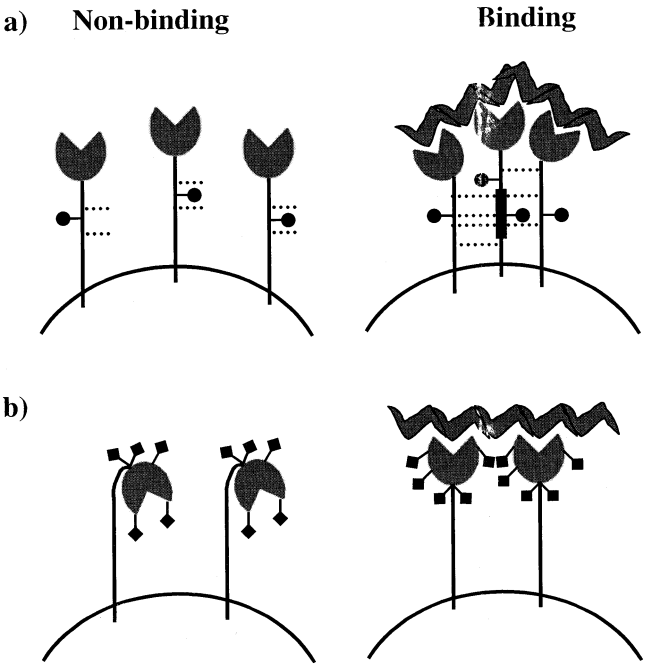

FIG. 3. Two possible models to explain the induction of HA binding by CD44: (a) the aggregation model, and (b) the conformational model. The non-binding and HA binding states are as shown. The inclusion of additional sequence present in the CD44v isoform is indicated by the black box, (●) represents potential O-linked glycosylation, (♦) represents N-linked glycosylation, and (···) represents GAG chains.

REGULATION OF HA BINDING ABILITY OF CD44

The ability of CD44 to bind HA is tightly controlled in a cell type- and activation state-specific manner, such that not all cells that express CD44 will bind HA [55, 56]. Although many cell lines can bind HA constitutively, normal resting leukocytes, which express CD44, do not. CD44 can exist in an inactive form unable to bind HA or an active form capable of binding HA. Conversion from the inactive to the active form can occur in cells in response to appropriate stimuli. While many factors have been shown to affect the HA binding ability of CD44, the biochemical changes occurring in response to physiological stimulators are just now being unraveled. Studies have shown that expression of CD44v isoforms in addition to associations and post-translational modifications to the extracellular domain, the transmembrane domain, and the cytoplasmic domain of CD44 all can influence CD44 binding to HA (reviewed in Refs. 4 and 13). Glycosylation changes in particular can affect HA binding (reviewed in Refs. 12 and 13). Given the plethora of factors that can affect the HA binding ability of CD44, we would like to suggest that all of these factors regulate HA binding via one of two mechanisms: (a) by affecting the aggregation state of CD44, or (b) by altering the conformation of CD44 (see Fig. 3).

The presence of CD44v isoforms facilitates CD44 aggregation in the membrane and induces HA binding [57]. The

fact that some CD44v isoforms can bind CS [37–39] suggests that GAG modification of CD44 could enhance CD44-CS/CD44v interactions, which also may facilitate aggregation. Data showing that modification of O-linked glycosylation of CD44 affected its ability to bind HA on cells, but not on a soluble CD44-Ig chimera, support the idea that O-linked glycosylation may affect the aggregation state of CD44 in the cell membrane [58]. HA binding also can be enhanced by the self-association of CD44H via its transmembrane region [14, 15], by artificial dimerization [19], and by dimerization by mAb [59]. We hypothesize that these changes, primarily occurring in the membrane-proximal region of CD44, affect HA binding by affecting the aggregation state of CD44. Conversely, post-translational modifications of CD44 could also alter the conformation or accessibility of the HA binding domain of CD44. Changes to N-linked glycosylation, which occurs primarily within the HA binding domain, may be predicted to act in this way (see Fig. 3).

CD44 EXPRESSION AND HA BINDING ABILITY OF LEUKOCYTES AND ENDOTHELIAL CELLS

Resting leukocytes express CD44 but do not normally bind HA. However, lymphocytes, isolated T cells, or T cell clones can be induced to bind HA after antigen-, superantigen- or mitogen-induced activation *in vitro* [60–62] and alloantigen or superantigen stimulation *in vivo* [63–65]. This was demonstrated either by leukocyte rolling on an immobilized HA substrate or by adhesion to HA. Often, the observed HA binding occurred in a subpopulation of activated cells and was transient. The percentage of HA-binding spleen cells was maximal at day 7 after *in vivo* alloantigen stimulation, and this returned to zero by day 12 [64]. B cell lines also can be shown to roll on immobilized HA [61], and *in vitro* culture of B cells in IL-5 induced a subpopulation to bind HA [66]. Lymphocyte activation, therefore, seems to induce a subpopulation of cells to bind HA, although this phenomenon appears to be short-lived. Exposure of peripheral blood monocytes to inflammatory cytokines such as TNFα and IL-1 induced HA binding in a population of cells in a CD44-dependent manner, demonstrating that monocyte activation also can result in the induction of HA binding by CD44 [67, 68].

Analysis of several cultured endothelial cells derived either from large vessels, such as HUVEC, or from the microvasculature, such as human dermal microvascular endothelial cells, demonstrates that these cells express CD44 but do not bind HA. Treatment of these cells with inflammatory cytokines, such as TNFα, resulted in the up-regulation of CD44 expression, but only endothelial cells derived from the microvasculature were induced to bind HA [69, 70*]. Cultured cells derived from lymph node HEV and the SV-40 transformed murine lymph node endothelial cell line SVEC4–10 expressed surface-bound HA [27], and this was increased after a 4-hr treatment with TNFα, IL-1β, or lipopolysaccharide [70]. Up-regulation of surface HA was not due to changes in mRNA for either HA synthase or HA degradative enzymes [70]. Histologically, HA was present in the intercellular spaces of vascular endothelial cells in skin capillaries [71].

Analysis of CD44 isoforms expressed in HUVEC that did not bind HA revealed the expression of only CD44H [48]. In another study, CD44H- and CD44v3-containing isoforms were detected, although in this case HA binding was not assessed [72]. Analysis of wound microvascular endothelial cells revealed the presence of CS-modified CD44H and CD44v3-containing isoforms [31], suggesting that there may be a correlation between CD44v isoform expression or CS modification and HA binding.

REGULATION OF HA BINDING IN LEUKOCYTES

In most cells, there is a threshold level of CD44 required before HA binding is observed. However, an increase in CD44 levels alone is insufficient to induce HA binding. Antigen-induced T cell activation increases CD44 expression and transiently induces HA binding in a subpopulation of T cells. However, after *in vivo* allogeneic stimulation, no evidence was found for the expression of CD44v isoforms in splenic cells [64]. In a second example, antigenic stimulation induced the transient expression of CD44v6-containing isoforms in T cells, B cells, and macrophages [73]. CD44v isoforms, including v6-containing isoforms, also were reported to be up-regulated after antigen- or mitogen-stimulated T cell activation [74]. Leukocyte activation also may result in glycosylation changes. Induction of HA binding after culture of B cells in IL-5 correlated with a decrease in N-glycosylation of CD44 [75]. Differences in N-linked glycosylation and phosphorylation of CD44 have been observed between resident (normal) and elicited (activated) macrophages, although in this case HA binding was not examined [76]. In another example, no gross molecular weight changes were observed after superantigen activation of lymph node T cells, suggesting no dramatic changes in the glycosylation state of CD44 [62]. A less common post-translational modification, sulfation, was found to be responsible for the TNFα-induced HA binding in a myeloid cell line [77]. This induction of HA binding was also responsible for adhesion of the myeloid cells to an SVEC4–10 endothelial monolayer. TNFα induced the sulfation of CD44, and inhibition of sulfation by chlorate treatment prevented HA binding by CD44. Although the sulfated moiety was not identified, preliminary evidence suggests carbohydrate sulfation (Maiti A and Johnson P, unpublished data). The idea of sulfation as an inducible means of regulating CD44-mediated HA adhesion on monocytic cells in response to inflammatory cytokines such as TNFα is an attractive one, as sulfation has been shown to be crucial for selectin-mediated leukocyte adhesion to specialized HEV in the lymph nodes.

* Yarwood H and Isacke CM, unpublished results. Cited with permission.

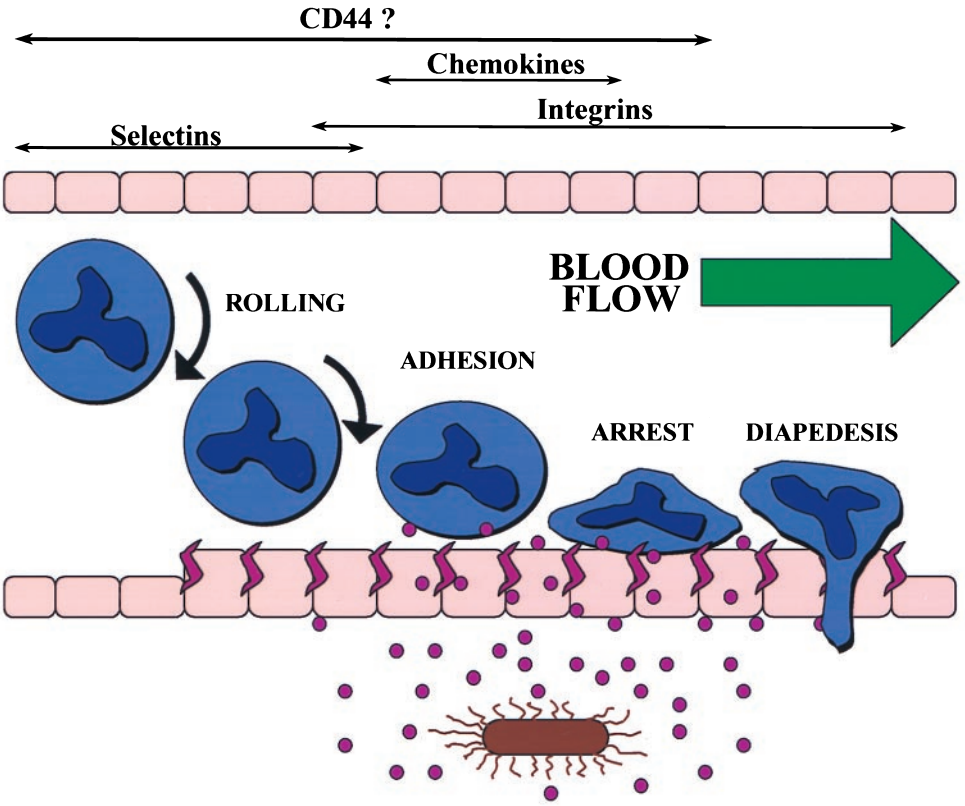

FIG. 4. Diagram of leukocyte adhesion to activated endothelium in a capillary at an inflammatory site. The molecules implicated at each stage are indicated above. The bacterium represents the cause of the inflammatory response, and the circles (●) represent the release of inflammatory agents such as chemokines at the site of infection. HA is associated with the activated endothelium.

LEUKOCYTE–ENDOTHELIAL CELL INTERACTIONS

In the immune system, naive T and B lymphocytes recirculate between the blood and the secondary lymphoid tissue. This lymphocyte trafficking or homing requires lymphocyte binding to HEV in the lymph nodes [78]. Lymphocytes first are slowed down by carbohydrate–lectin interactions between the lymphocyte and the specialized endothelial cell. The selectin molecules play an important role in leukocyte rolling on HEV, which then allows the leukocytes to bind via the integrin molecules. Chemokine stimulation up-regulates integrin function, which then can mediate firm adhesion to the HEV. Upon attachment, signals are sent to the lymphocyte to facilitate its migration through the endothelial layer (Fig. 4 and reviewed in Refs. 7 and 79).

During an inflammatory immune response, neutrophils, monocytes, and activated T lymphocytes leave the circulation and enter tissues. Inflammatory agents released at the inflammatory site cause the nearby microvascular endothelium to become activated. This results in the increased expression or activation of several cell adhesion molecules, including ICAM-1 and E- and P-selectin. These molecules then recruit the activated leukocytes to the inflamed microvasculature by mediating leukocyte rolling, adhesion, arrest, and diapedesis (Fig. 4). While many similarities exist between extravasation at an inflammatory site and in lymph nodes, important differences also must exist, as the cells that extravasate at inflammatory sites are distinct from the naive recirculating T and B cells that bind to lymph node HEV. In this respect, activated $CD4^+$ T cells have a different array of cell adhesion molecules on their cell surface than the naive $CD4^+$ T cells. Naive T cells express high levels of L-selectin and low levels of CD44 and $\alpha 2$ and $\beta 2$ integrins, whereas the activated/memory $CD4^+$ T cell population express low levels of L-selectin and higher levels of CD44 and $\beta 1$, 2, and 7 integrins [80].

A ROLE FOR CD44 AND HA IN LEUKOCYTE–ENDOTHELIAL CELL INTERACTIONS

Initial *in vitro* studies to identify a lymphocyte homing molecule in humans resulted in the generation of the Hermes-3 mAb, an anti-CD44 mAb that blocked leukocyte adhesion to frozen mucosal lymph node sections [81]. However, other CD44 antibodies did not block adhesion in either the human or mouse system. In addition, Hermes-3 did not block the HA binding ability of CD44 [82], which had been shown to be a factor in the binding of cell lines and soluble CD44-Ig fusion proteins to endothelial cells [16, 27, 83]. With these apparent anomalies and the subsequent identification of the L-selectin homolog in humans, attention was focused on the selectin molecules.

In 1993, an interesting *in vivo* observation was made in a

murine model of delayed-type hypersensitivity where the administration of CD44 antibodies (which resulted in the loss of CD44 from the cell surface) delayed leukocyte infiltration at the cutaneous site, but had no effect on lymphocyte recirculation [84]. This suggested that CD44 may play a role *in vivo* in the initial phase of the delayed-type hypersensitivity response, possibly by facilitating extravasation into extralymphoid inflammatory sites. More recently, *in vitro* studies looking at leukocyte rolling under flow conditions have identified an interaction between CD44 on activated lymphocytes, T and B cell lines, and HA on an endothelial cell line (SVEC4–10) [61]. CD44 also has been implicated in adhesion under flow between tonsillar lymphocytes or SKW3 T lymphoma cells and human tonsil stromal cells, but not with TNFα-stimulated HUVEC [85]. In both examples, the lymphocyte rolling could be blocked by CD44 antibodies or exogenous hyaluronan. Further experiments by Siegelman's group have indicated that CD44 binding to HA could be induced transiently in T cells upon *in vivo* superantigen stimulation. These cells exhibited CD44–HA-dependent rolling and CD44–HA-dependent extravasation at an inflammatory site [62, 65]. This is the strongest evidence to date for a role of CD44 and HA in T cell extravasation at an inflammatory site.

Although a role for selectins and integrins is well established in leukocyte rolling and attachment, a role for CD44 is not. Leukocyte extravasation to inflammatory sites is thought to occur primarily in the microvasculature, and it is these cells that can up-regulate HA expression in response to inflammatory cytokines *in vitro*. Likewise, proinflammatory cytokines can induce the HA binding ability of CD44 on monocytes, cells that are known to extravasate at these sites. The *in vivo* data obtained by treatment of leukocytes with an anti-CD44 mAb suggest that CD44 may play a role in leukocyte extravasation at inflammatory sites, although down-regulation of CD44 expression by CD44 mAb treatment also may cause other effects. Antigen-induced T cell activation can transiently induce the HA binding ability of CD44, which would allow these cells to bind to HA expressed on the activated endothelial cells. Inhibition of T cell extravasation by treatment with CD44 mAb or injection of HA supports this idea [65]. These data are consistent with a role for CD44 and HA in the inducible adhesion at inflammatory sites, but not in the binding of naive lymphocytes to lymph node HEV. However, arguing against an important role for CD44 in inflammation are the data from the CD44 gene knockout mice, which indicate no significant change in the inflammatory response in the skin after induction of delayed-type contact hypersensitivity [24].

SULFATION AS A POTENTIAL REGULATOR OF CD44–HA BINDING

CD44 has been shown to be modified by CS, HS, and keratan sulfate. In examples where sulfated GAG side chains are added, a specific role for sulfation has not been defined. Sulfation of the standard 85-kDa form of CD44 was observed in peripheral blood leukocytes in 1988 [86], and sulfation of the CD44v4–7 isoform transfected into a rat pancreatic carcinoma cell line was found to occur on a tyrosine residue present in exon v5 [87]. However, the significance of the sulfation of CD44 in either case was not addressed. CD44 was sulfated in a myeloid cell line, in response to TNFα stimulation [77]. In this example, the sulfation of CD44 was required for the binding of HA and for adhesion to the SVEC4–10 endothelial cell line. The CD44 present on the SVEC4–10 could bind HA, indicating that it was present in its active form. Both hyaluronidase and CD44 mAbs blocked leukocyte adhesion to the endothelial cell line, suggesting that this was a CD44–CD44-mediated interaction, bridged by HA. However, this may not necessarily be so, as the adhesion could not be inhibited by the addition of exogenous HA. Sulfation was shown to be an important mediator of the leukocyte–endothelial cell interaction, as inhibition of sulfation blocked the ability of the leukocyte to bind HA and the endothelial monolayer. The sulfated moiety of CD44 is not yet known, nor is the mechanism of how sulfation induces HA binding, although perhaps it may alter either the aggregation state or the conformation of CD44. This model system suggests that sulfation may be an inducible post-translational modification that can regulate the HA binding ability of CD44 in monocytes in response to TNFα stimulation. While it is known that TNFα and other inflammatory cytokines can induce HA binding in monocytes, it is not yet known whether this also occurs by inducible sulfation of CD44. Whether inducible sulfation also occurs to transiently induce HA binding in T cells after antigen stimulation also remains to be determined.

SULFATION AS A MEDIATOR OF LEUKOCYTE–ENDOTHELIAL CELL INTERACTIONS

The best example of a role for sulfation in mediating cell adhesion in eukaryotes is the interaction of selectins with their sulfated ligands [88–91]. Selectins play a major role in mediating leukocyte–endothelial cell interactions in the lymph node and at inflammatory sites [7–9, 92]. The interaction between selectins and their ligands can mediate leukocyte rolling on specialized endothelial cells present in lymph nodes or induced at inflammatory sites. It was first noted in the early 1980s that lymph node HEV incorporated large amounts of sulfate [93] and constitutively expressed sulfated glycoproteins on the cell surface. It was subsequently shown that L-selectin recognizes sulfated O-linked carbohydrates expressed on HEV-derived sialomucins, GlyCAM-1, CD34, and MAdCAM-1 (reviewed in Refs. 91 and 94). The sulfation of these L-selectin ligands is crucial for L-selectin binding, as treatment with chlorate abolished binding [95]. In addition, MECA-79, a mAb that blocks HEV specific lymphocyte adhesion, recognizes a sulfated carbohydrate epitope on HEV [96]. These findings

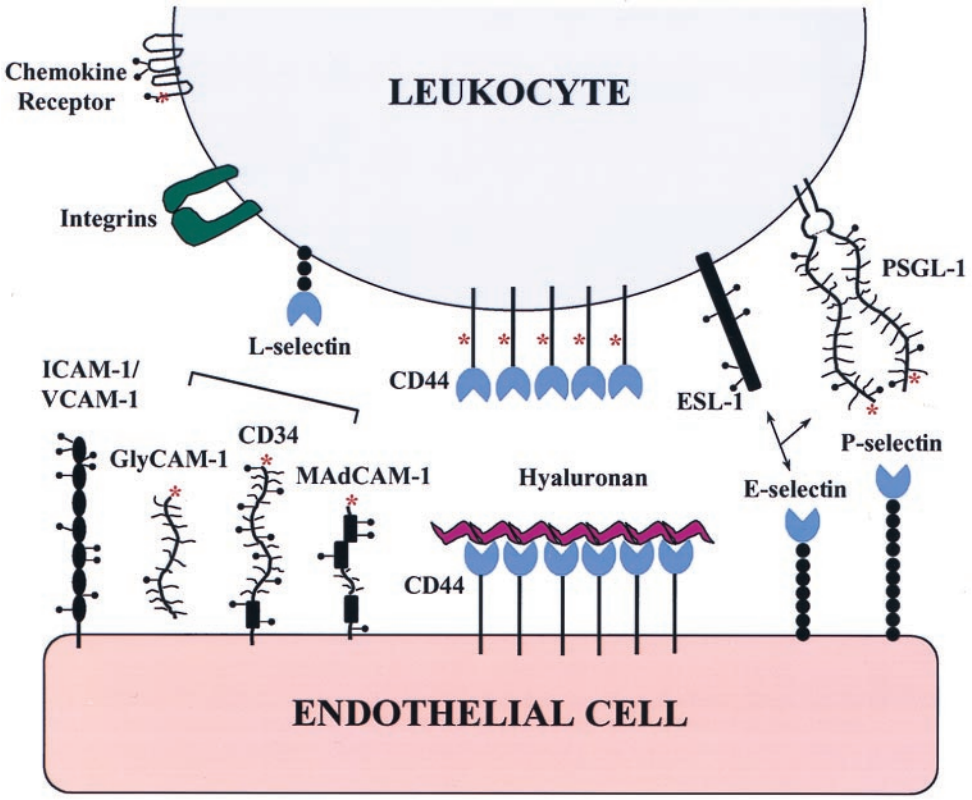

FIG. 5. Illustration of the molecules implicated in leukocyte adhesion to the endothelium in the lymph node or at inflammatory sites. N-linked glycosylation is indicated by (●), O-linked glycosylation by (∼∼), and molecules that are known to be sulfated are indicated with (✻).

strongly suggest that the interaction between naive lymphocytes, which express high levels of L-selectin, and lymph node HEV depends upon the presence of sulfated ligands on HEV.

P- and E-selectin are induced on activated endothelium at inflammatory sites and bind carbohydrate ligands on activated leukocytes. PSGL-1 is a sialomucin identified as a P-selectin ligand on neutrophils [97]. PSGL-1 is tyrosine sulfated, and this, together with recognition of a sialyl Lewis X-like epitope, is required for P-selectin binding [98–100]. Thus, both L- and P-selectins recognize sulfated ligands, although in each case the sulfated moiety is different. In addition, L- and P-, but not E-selectin, can bind sulfated molecules such as heparin and sulfatides ([101] and reviewed in Refs. 89 and 102), and L-selectin can bind CS and HS proteoglycans [103]. Modified PSGL-1 and ESL-1 have been identified as E-selectin ligands [104, 105], yet unlike L- and P-selectin ligands, the tyrosine sulfation of PSGL-1 is not required for E-selectin binding [98, 99, 106], and ESL-1 possesses primarily N-linked carbohydrates and is not a sialomucin. Thus E-selectin recognition of its physiological ligands differs from L- and P-selectin ligand interactions and does not appear to involve sulfation. Sulfation, therefore, is important for recognition and binding by L- and P-selectins and occurs constitutively in lymph node HEV to facilitate lymphocyte recirculation. It is possible that sulfation may be induced in cells at inflammatory sites to facilitate the initial interactions between leukocytes and endothelial cells. A summary of the molecules implicated in leukocyte–endothelial cell adhesion and their sulfation status is shown in Fig. 5.

PARALLELS BETWEEN THE CD44–HA INTERACTION AND SELECTIN–LIGAND INTERACTIONS

The interaction between CD44 and HA has many similarities to the interactions of the selectins with their carbohydrate ligands. First, both N-terminal regions of CD44 and the selectins are likely to be structurally related; CD44 contains a Link module, which is structurally similar to the C-type lectin domain expressed by all selectins. Both CD44 and the selectins use this lectin-like domain to bind carbohydrate moieties. This lectin–carbohydrate interaction can mediate *in vitro* leukocyte–endothelial cell interactions and leukocyte rolling under flow conditions. Both are cell adhesion molecules that can be proteolytically shed from the cell surface as a potential means to regulate transient adhesion. Both molecules can bind to CS-modified proteins. Sulfate has been implicated in the regulation of HA binding by CD44 and is also required for high affinity selectin interactions. However, L- and P-selectin bind to sulfated ligands, whereas sulfation occurs on the CD44 lectin molecule itself, which then can influence its

interaction with a non-sulfated ligand. Whether CD44 itself can bind the sulfated moiety and induce HA binding, either by aggregating or by causing a conformational change in the lectin binding domain, remains to be determined.

SULFATION OF OTHER LEUKOCYTE CELL SURFACE MOLECULES

Sulfated glycoconjugates have been found in several species, from bacteria to mammals, and have been implicated in regulating cellular interactions and adhesion (reviewed in Ref. 107). In PHA-stimulated peripheral blood lymphocytes, CD43 and CD45 were found to be sulfated. Although the nature of the sulfation is not known, sulfation was implicated in modulating homotypic aggregation after CD43 mAb treatment [108]. Recently, tyrosine sulfation of the chemokine receptor CCR5 was identified and shown to contribute to the binding of the chemokines MIP-1α and β and to the binding and entry of HIV [109]. The chemokine receptor CXCR4 also is sulfated. Sulfated proteoglycans also facilitate attachment of other enveloped viruses such as herpes simplex virus 1 [110]. Sulfation, therefore, may occur on leukocyte cell surface proteins to regulate cell, viral, or chemokine interactions. Given the potential association of sulfation with regulation of cell adhesion, it is perhaps worthwhile to re-examine the extent and type of sulfation occurring at the leukocyte cell surface.

FUTURE PROSPECTS

Sulfation is a post-translational modification that can occur on eukaryotic cell surface molecules to regulate cell adhesion. As phosphorylation has become a major regulator in intracellular protein interactions, perhaps sulfation will become a major regulator of extracellular interactions. The state of sulfation, like phosphorylation, may be controlled by the balance of the function of sulfotransferases and sulfatases. While the involvement of CD44, HA, and inducible sulfation in leukocyte–endothelial cell interactions and extravasation at inflammatory sites is currently tantalizing, further work has to be done to establish their roles in this process. If this is done, then exciting new prospects will exist for therapeutic intervention. One potential attraction of inhibitors of the CD44–HA interaction is that this interaction may be restricted to inflammatory sites. If inducible sulfation is shown to be a factor for leukocyte recruitment to inflammatory sites, then inhibitors of the signaling pathway leading to the induction and activation of sulfotransferases also will be of pharmacological interest, as will sulfotransferase inhibitors. Given the dependence of L- and P-selectin interactions on sulfation, inhibitors of specific sulfotransferases are already of interest in the development of new anti-inflammatory drugs. Presently, very little is known about the sulfotransferases responsible for sulfating the selectin ligands or CD44. Specific sulfotransferase activity has been identified recently in vascular endothelial cells [111], and glycosyl sulfotransferases are now being cloned [112, 113]. The sulfotransferases responsible for sulfating the selectin ligands are now being identified and characterized and the factors that regulate their activity will soon be determined. This new information, no doubt, will provide a fertile area for the development of new anti-inflammatory drugs. With such incentives, this will continue to be an active area of research for the foreseeable future, and we look forward to the exciting new information that will emerge.

We would like to thank G. J. Dougherty, C.M. Isacke, and H. Yarwood for helpful discussions. We also acknowledge support from the National Science and Engineering Research Council of Canada and the Heart and Stroke Foundation of British Columbia and the Yukon.

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