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Recent advances in the regulation of CD44 expression and its role in inflammation and autoimmune diseases

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Summary

Interaction of CD44, an adhesion molecule, with its extracellular matrix ligand, hyaluronan (HA), has been suggested to play a critical role in a number of biological manifestations, including cell migration, tumorigenesis, metastasis, and regulation of immune responses. CD44 comprises a large family of transmembrane glycoproteins that exhibit extensive molecular heterogeneity. This heterogeneity in size is generated by alternative RNA splicing of variable exons as well as by post-translational modifications. Most cell types express CD44 but do not bind HA. The biological functions of CD44, including the regulation of lymphocyte recruitment to the sites of inflammation, have been attributed to the generation of a functionally active, HA-adhesive phenotype. The molecular mechanisms underlying the regulation of CD44 expression and the generation of a functionally active HA-binding phenotype are not well understood. Recently, CD44-HA interactions have been reported to play a critical role in a number of autoimmune diseases in humans and experimental animal models. Initial studies have taken advantage of anti-CD44 antibodies which specifically block CD44-HA interactions. Administration of these antibodies in several experimental murine models of autoimmune diseases resulted in alleviation of inflammatory reactions. In addition, the generation of CD44-deficient animals has facilitated our understanding of the involvement of CD44 in inflammation and autoimmune diseases. This review will focus on the recent advances in the molecular mechanisms regulating CD44 expression, ligand binding, as well as the contribution of CD44 to the development of inflammation and autoimmune disorders.

Key words: CD44 • hyaluronan • inflammation • autoimmunity • cytokines

Abbreviations: aa - amino acid, **AP-1** - activation protein-1 transcription factor, **B-LCL** - EBV-transformed lymphoblastoid B cells, **CD44 H** - the standard or hematopoietic CD44 isoform, **CD44 R1** - CD44 isoform containing variable exon 8-10, **CD44 R2** - CD44 isoform containing variable exon 10, **CD44-HA** - CD44-mediated hyaluronan-binding, **CTL** - cytotoxic T lymphocyte, **EBV** - Epstein-Barr virus, **ECM** - extracellular matrix, **EGF** - epidermal growth factor, **Egr-1** - early growth response gene, **HA** - hyaluronan, **IBD** - inflammatory bowel disease, **IFN- γ** - interferon γ , **IL-1 β** - interleukin 1 β , **JNK** - c-Jun-N-terminal kinase, **LPL** - lamina propria lymphocytes, **LPS** - lipopolysaccharide, **MAPK** - mitogen-activated protein kinase, **NK** - natural killer cells, **PKC** - protein kinase C, **RA** - rheumatoid arthritis, **RASF** - RA synovial fibroblast, **SLE** - systemic lupus erythematosus, **TCR** - T cell receptor, **TGF- β** - transforming growth factor β , **TNF- α** - tumor necrosis factor- α , **V1-V10** - variable exons 1-10, **VLS** - vascular leak syndrome.

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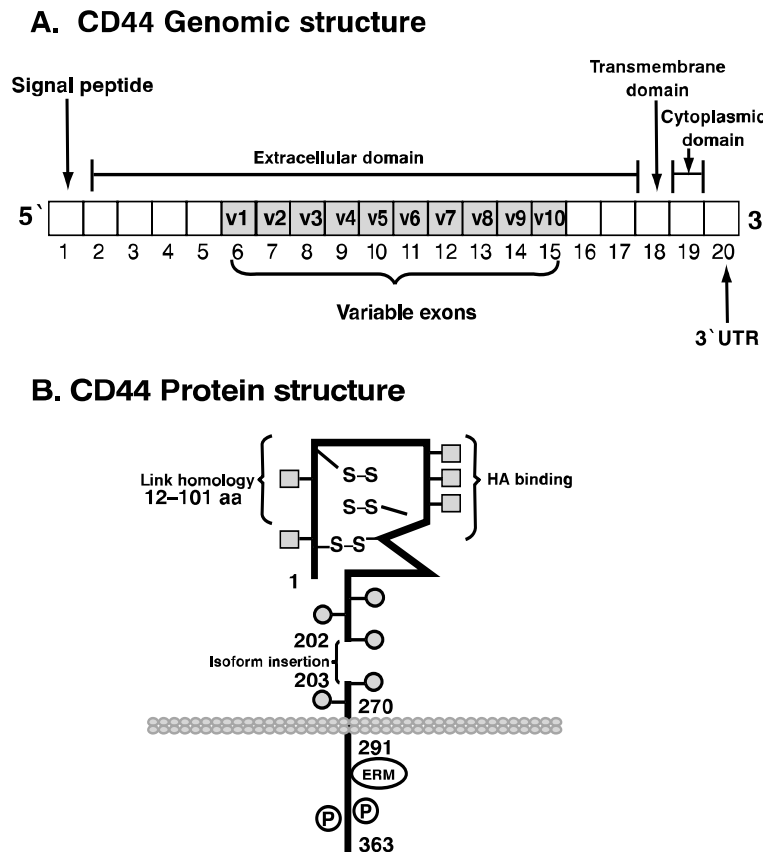


Figure 1. CD44 structure and genomic organization. **A** – genomic organization of CD44. Exons are represented by the numbered boxes. The 10 variable exons (V1–10) are as indicated by the shading. The three protein domains of CD44 are as indicated. **B** – structure of the CD44 protein. The CD44 protein exists as a transmembrane glycoprotein. The link homology and hyaluronan (HA)-binding domains are indicated by the brackets. Sites of either N- or O-linked glycosylation are represented by the shaded squares or circles, respectively. The site of alternative isoform insertion is as indicated. Amino acids 268 and 290 flank the transmembrane domain as indicated. The two serines with the potential for phosphorylation are indicated by the encircled “P”, and the binding site for members of the ezrin-radixin-moesin (ERM) family is indicated by the encircled “ERM”.

INTRODUCTION

Cell-cell and cell-extracellular matrix (ECM) interactions play a critical role in many biological processes, including the development of an immune response, inflammation, tumor formation and metastasis. CD44 is among a plethora of adhesion molecules which participate in these processes. It comprises a family of 85–200 kDa transmembrane glycoproteins that are expressed in a variety of cell types, including leukocytes, fibroblasts and cells of mesodermal and neuroectodermal origin. CD44 binds to high endothelial venules and the ECM via its interaction with its principle ligand, hyaluronan (HA)^{88, 109}. CD44-HA interactions have been implicated mainly in cell-cell and cell-matrix adhesion and hence have been suggested to play a key role in a variety of physiological processes, including cell migration, lymphocyte homing, cell activation, and hema-topoiesis. In

keeping with these biological functions, CD44 has been implicated in a number of diseases, including chronic inflammatory and autoimmune disorders as well as tumor metastasis^{4, 9, 31, 43, 88, 97, 109, 116, 122}.

STRUCTURE OF CD44

Gene

The CD44 gene is located on the short arm of chromosome 11 in humans⁴² and chromosome 2 in the mouse¹⁵. It spans approximately 50 kb of human DNA and genomic analysis has revealed a total of 20 exons, 12 of which participate in alternative splicing^{52, 94, 105} (Fig. 1). Exons 1–5 encode part of the constant region of the extracellular domain (constant exons) and are invariably transcribed. Exons 6–15 comprise the variable region of the extracellular domain (variable exons V1–V10) as

they can participate in alternative splicing. These exons are included in different combinations into nascent mRNAs, which explains, at least in part, the extensive CD44 molecular heterogeneity. Exons 16 and 17 are constant and encode the membrane proximal region of the extracellular domain. The constant exon 18 encodes the hydrophobic transmembrane region as well as the first 3 amino acids (aa) of the cytoplasmic tail of CD44. Exons 19 and 20 are also subject to alternative splicing. Inclusion of exon 19, containing an A+T rich untranslated region, is believed to confer instability on the mRNA and results in the generation of a short cytoplasmic tail of only 3 aa. Exon 20 contributes to the long (70 aa) cytoplasmic tail of CD44 and is found in greater abundance than is the short version^{40, 94}.

CD44 isoforms

At least 20 different isoforms generated by alternative RNA splicing have been described^{69, 88}. Cells are capable of expressing numerous isoforms simultaneously and it has become clear that their expression is regulated in a tissue- and cell-differentiation stage-dependent manner^{69, 88}. The standard or hematopoietic (CD44 H) isoform is the smallest, most commonly expressed isoform and does not contain any variable exons. Northern blot analysis reveals three major transcripts of 4.8, 2.2, and 1.6 kb^{40, 112}, which are believed to arise by the use of different polyadenylation signals^{45, 108}. All blood cells, including granulocytes, T and B cells, natural killer (NK) cells, and cells of the monocyte/macrophage lineage, express CD44 H^{40, 112, 145}. In addition to the CD44 H isoform, several other isoforms have been described with unique functions. For example, CD44 E, containing exons V8–10, refers to the epithelial variant, which is weakly expressed in normal epithelium but is present in abundance in many carcinomas. Northern analysis revealed slightly larger transcripts, in comparison with CD44 H, of 2.0, 2.6, and 5.4 kb¹¹³. Two similar isoforms, CD44 R1 (V8–10) and CD44 R2 (V10), cloned by other investigators from human myelomonocytic cell lines, have been described^{16, 22}. CD44 R1 and R2 expression was detected in normal peripheral blood mononuclear cells, granulocytes, and certain leukemias^{16, 22}. CD44 V4–7 and CD44 V6–7 expression has been associated with the metastasis of rat pancreatic carcinoma and mammary adenocarcinoma⁴³. Interestingly, V6-containing isoforms have been associated with increased aggressiveness in human non-Hodgkin's lymphoma⁶⁰. These isoforms are generally not detected in normal lymphocytes but are upregulated upon mitogenic or antigenic activation of human lymphocytes^{1, 60}. Some of the other identified CD44 variants include CD44 V3,8–10, CD44 V6–10, CD44 V7–10, and V3–10^{48, 51}.

Protein

CD44 exists as a type I membrane glycoprotein. The most abundant CD44 H isoform, containing 363 aa, acquires a molecular weight of 85 kDa following post-translational modifications. It is subdivided into three regions, namely, a 72 aa c-terminal cytoplasmic domain, a 21 aa transmembrane domain, and a 270 aa extracellular domain⁶⁹. The extracellular domain can be subdivided into the amino-terminal, variable, and membrane-proximal regions¹⁴⁵. The amino-terminal region contains a hydrophobic stretch of residues (aa 12–101) which is well conserved between species (80–90% sequence similarity). This stretch exhibits a 30% homology with the cartilage link and proteoglycan core proteins, which are known to make up ECMs in association with the disaccharide polymer hyaluronan^{41, 69, 112}. There are also 6 conserved cys residues within the amino-terminal region, which are thought to function in the generation of a globular structure via disulfide bridging (Fig. 1B)⁴¹. The membrane-proximal region is less well conserved, exhibiting only 35–45% sequence similarity between species. N-linked, O-linked glycosylation sites, and ser/gly motifs (ser-gly-X-gly, where X is any aa) capable of being chondroitin sulfate or glycosaminoglycan-modified, are found within the amino-terminal and membrane-proximal regions of the extracellular domain^{6, 105}. The variable region, consisting of different combinations of variable exons (V1–V10), is inserted between aa 202 and 203 of the extracellular domain (Fig. 1B)⁶⁹. Unlike in the mouse, V1 is not expressed in humans as it contains a stop codon; exons V2–V10 contribute a total of 381 aa, showing a 64% interspecies homology¹⁰⁵. This region contains an additional 4 N-linked and a large number of O-linked glycosylation sites. The transmembrane and cytoplasmic domains are highly conserved regions of CD44, and exhibit 80–90% inter-species homology. The transmembrane domain contains 2 cys residues at the interface between the transmembrane and the cytoplasmic domains, which can potentially be modified with palmitic acid^{7, 69, 74, 88}. The cytoplasmic domain contains 6 potential phosphorylation sites, out of which ser 303 and 305 are constitutively phosphorylated^{69, 88, 96}.

REGULATION OF CD44 EXPRESSION AND ITS BINDING TO HA

CD44-HA-binding

Hyaluronan, the principle CD44 ligand, is a high-molecular-weight, negatively charged polysaccharide composed of linear repeating units of the disaccharide D-glucuronic acid (1-β-3) N-acetyl-D-glucosamine and is found surrounding proliferating and migrating cells in

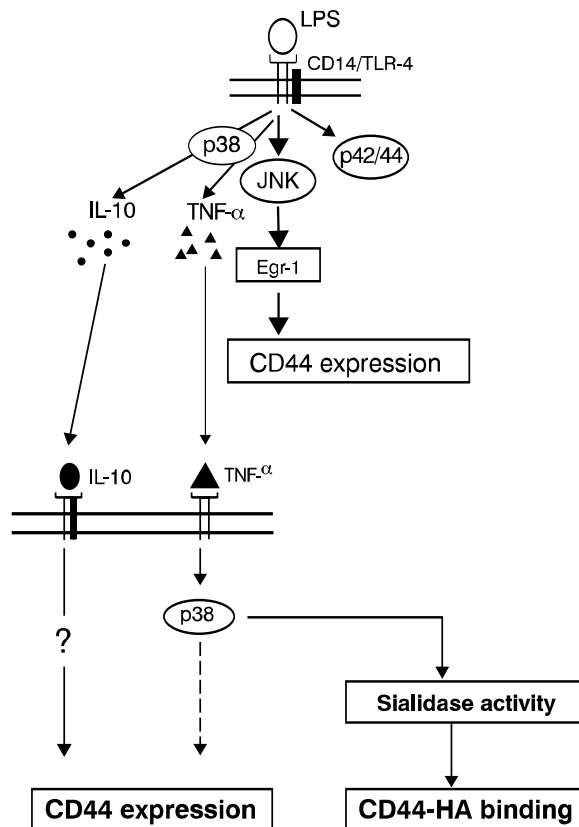


Figure 2. Overview of the signaling pathways responsible for the induction of CD44 during cell migration at an inflammatory site (adapted from⁵⁴). Chemoattractants and cytokines induced by the inflammatory agent activate the endothelial cells and thus affect receptor expression on the endothelial cell surface as well as on the passing lymphocytes. Activation of the cells induces the expression of the necessary adhesion receptors and ligands to initiate lymphocyte homing. The proteins responsible for the interactions of the leukocyte with the endothelial cells are depicted above the diagram. Initially cells “roll” along the activated endothelial vessel wall (indicated by the dark squares). The selectins are responsible for the initiation of rolling along the activated endothelial cell surface. Cells undergo firm adhesion mediated by the integrins followed by diapedesis towards the inflammatory agent. The small circles represent inflammatory agents such as chemokines and cytokines that direct and activate the leukocytes as well as the endothelial cells.

connective and lymphoid tissues¹²¹. Other CD44 ligands include fibronectin, collagen, and serglycin^{53, 89, 123, 124, 131, 133}; however, interactions between CD44 and HA are the best characterized. Although most cells express some form of CD44, not all cells constitutively bind HA^{61, 62, 66}. For initiation of CD44-mediated HA-binding, CD44 must first be “activated”. Generation of the HA-binding phenotype of CD44 appears to be a tightly regulated event and is highly dependent on the cell type, state of cell activation, and differentiation^{61, 62, 66}. The HA-binding capacity of CD44 appears to be influenced by multiple factors that include structural variations in the CD44 extracellular domain, oligomerization of CD44 on the cell membrane, and phosphorylation of its cytoplasmic tail^{67, 73, 88, 119}. It is also well documented that alterations

in the N- and O-linked glycosylation pattern of CD44 play a vital role in the regulation of its binding to HA^{3, 67, 119}. Increased complexity of the N-linked glycosylation of CD44 is associated with a decrease in CD44-HA-binding capacity in cell model systems such as oncostatin-M- and transforming growth factor β (TGF- β)-treated lung epithelial cells¹⁴ and in tumor necrosis factor α (TNF- α)-stimulated monocytic cells⁷¹.

Recently, an inducible lysosomal sialidase was shown to influence the CD44-HA-binding of lipopolysaccharide (LPS)-stimulated human monocytic cells⁵⁵. Specifically, sialidation of glycosyl moieties has been shown to inhibit CD44-HA interactions, whereas removal of sialyl residues by sialidases results in enhanced HA-binding⁵⁵.

Sulfation of CD44 has also been shown to play a role in mediating CD44-HA interactions^{10, 20, 54, 76}. For example, treatment of monocytic cells with TNF- α , interferon γ (IFN- γ), LPS, or interleukin 1 β (IL-1 β) increased the sulfation of CD44, concurrent with induction of CD44-HA-binding¹⁰. However, CD44-HA-binding induced following stimulation of T cells through the CD3-T cell receptor (TCR) complex did not involve sulfation of the CD44 molecule, suggesting that the mechanism involved is dependent on the stimulus as well as the cell type¹⁰.

Regulation of CD44 expression and the HA-adhesive phenotype

Since CD44 plays a role in cell migration, modulation of CD44-HA-binding by endotoxins and inflammatory cytokines may have profound effects on the migration of immune cells to sites of inflammation and on the development of immune responses. The effect of cytokines on CD44 expression and binding capacity has been investigated in different cell types. IL-5, a key mediator of asthma-related eosinophilic inflammation, has been shown to enhance CD44 expression in human eosinophils¹⁷ and in murine B cells⁸⁶. In human hematopoietic progenitor cells, IL-3 and granulocyte macrophage colony-stimulating factor, cytokines which direct leukocyte development, induce CD44-HA-binding⁶⁵. In addition, IL-12 and IL-18, the proinflammatory cytokines, were found to modulate CD44-HA interactions in normal human T cells². However, in rat epithelial cell lines, IFN- γ was shown to upregulate CD44 expression, but not HA-binding¹⁰⁰.

Results generated in our laboratory have indicated that in normal human B cells, CD44 expression is enhanced by stimulation with PMA, whereas IFN- γ and IL-4 inhibited PMA-induced CD44-HA-binding⁶¹. However, we have also shown that IL-4 increases CD44 expression and HA-binding in a human Epstein-Barr virus (EBV)⁺ Burkitt's lymphoma B cell line, but not in EBV-transformed normal B cells (B-LCL)³⁹. In contrast, IL-13, a cytokine with biological effects similar to those of IL-4, failed to induce CD44 expression or binding in either BL cells or B-LCLs. This was attributed to the loss of IL-13 receptor expression in these cells³⁹. However, the molecular mechanism by which EBV transformation of normal human B cells abrogates IL-4-induced CD44 expression and HA-binding is not understood at present. We and others have also demonstrated that LPS is a potent inducer of CD44 expression and HA-binding in monocytic cells^{37, 38, 70, 71}. Furthermore, TNF- α and IL-10, immunoregulatory cytokines produced endogenously following LPS stimulation, regulate LPS-induced CD44 expression and CD44-mediated HA-binding in monocytic cells^{20, 37, 38, 71, 76}.

SIGNALING PROTEINS AND TRANSCRIPTION FACTORS INVOLVED IN THE CONTROL OF CD44 EXPRESSION AND THE GENERATION OF THE HA-BINDING PHENOTYPE

The signal transduction events involved in CD44 regulation are not well understood. CD44 expression has been shown to be regulated via calmodulin/Ca²⁺ signaling pathways in PMA-stimulated T lymphoma cells¹¹⁰, whereas PI3 kinase and protein kinase C (PKC) were shown to regulate CD44 expression in neuroblastoma cells²⁶. We have investigated the molecular mechanism, in particular the role of mitogen-activated protein kinases (MAPK), in the regulation of LPS-induced CD44 expression in human monocytic cells (Fig. 2). Our results show a distinct involvement of c-Jun-N-terminal kinase (JNK) MAPKs in LPS-induced, but not in TNF- α -induced, CD44 expression in monocytic cells³⁸. In contrast, IL-10-induced CD44 expression did not involve activation of any of the MAPKs³⁸. Recently, MAPK have also been implicated in the regulation of CD44 alternative splicing activity. For example, in a murine T cell line, the extracellular signal-regulated kinase signaling cascade was necessary for the inclusion of the variable exon 5 into CD44 mRNA¹³⁵.

We have also examined the signal transduction pathways involved in the regulation of CD44-HA adhesion. Studies designed to delineate the role of MAPKs revealed that p38 activation was required for LPS- and TNF- α -induced expression of HA-adhesive CD44 in LPS-stimulated normal human monocytic cells. Our results also showed that sialidase activation is required for the acquisition of HA-binding capacity under these conditions. In addition, TNF- α -induced sialidase activity and CD44-HA-binding was found to be regulated by p38 MAPK activation (Fig. 2)³⁷. Taken together, our results have clearly demonstrated that the induction of CD44 expression and the generation of a CD44 capable of binding HA are two independent events that are regulated by distinct MAPK signaling pathways. Induction of CD44 expression may involve the activation of JNK MAPK, whereas activation of p38 MAPK may be required for the generation of HA-adhesive CD44 in LPS-stimulated monocytic cells. However, none of the MAPKs were found to be involved in IL-10-induced CD44-HA-binding³⁷. Examination of the promoter region of CD44 revealed the role of the early growth response gene (Egr-1) transcription factor in the regulation of CD44 expression in the murine B cell line WEHI-231 following B cell receptor stimulation⁷⁷ and in the human endothelial cell line ECV304 following IL-1 stimulation²⁹. In addition to Egr-1, the activation protein 1 (AP-1) transcription factor has been shown to regulate CD44 expression in Fos- and epidermal growth factor (EGF)-transformed cells⁶⁴, IL-

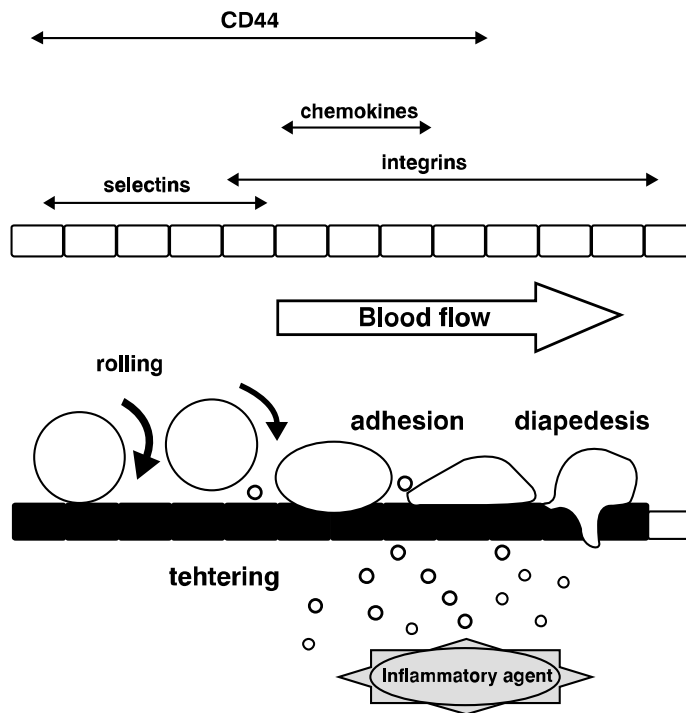


Figure 3. Overview of the general leukocyte endothelial interactions expression and CD44-HA-binding in monocytic cells. Treatment of monocytic cells with LPS induces the expression of CD44 and CD44-HA-binding in addition to the endogenous cytokines, IL-10 and TNF- α . These two cytokines are also capable of inducing CD44 expression and HA-binding. The signaling pathway responsible for the LPS-induced expression of CD44 involves the activation of JNK MAPK. However, the LPS-induced expression of TNF- α and IL-10 involves that activation of p38 MAPK. TNF- α -induced CD44 expression partially involves p38 MAPK activation as indicated by the dashed line, whereas the IL-10-induced CD44 expression does not involve any of the three main MAPK members. Regulation of CD44-HA-binding induced by LPS is via the endogenously expressed TNF- α . TNF- α induces CD44-HA interactions via the activation of sialidase which occurs through the activation of p38 MAPK.

1 β -induced rat aortic smooth muscle cells³⁰, and transformed fibroblast cells¹¹⁴. AP-1 has also been shown to regulate cell motility and invasion of transformed fibroblast cells in response to EGF. Furthermore, a PKC θ isoform was identified as a potential suppressor of the invasion program in these cells by down-regulating AP-1 activity¹¹⁴.

The role of signaling proteins responsible for the activation of the transcription factors critical to CD44 expression is not well understood. Transformation of cells using v-src or v-ras has been shown to enhance CD44 expression⁶³. Ladedda et al.⁶³ showed that the activation of the G protein Ral A plays a role in the induction of CD44 in these oncogenically transformed cells. We have demonstrated that LPS-induced CD44 expression may be regulated by Egr-1 through JNK MAPK activation in human monocytic cells³⁸. However, the transcription factors involved in TNF- α -induced regulation of CD44 expression remain to be determined.

Chromatin remodeling

Chromatin remodeling is an important event in any cellular response, particularly in inflammation. Changes in the chromatin structure allow for the formation or disbandment of transcription factor complexes that serve to regulate gene expression. Recently the BRG-1 and BRM subunits of the chromatin-remodeling complex SWI/SNF have been implicated in the regulation of CD44 expression¹¹⁷. It was demonstrated that C33A, the cervical cancer cell line, which is negative for BRG-1 expression, did not express CD44; however, fusion with the BRG-1⁺ cells SAOS-2 restored CD44 expression, suggesting the involvement of the BRG-1 subunit in CD44 induction¹¹⁷. On the other hand, mice that are BRM⁻ but BRG-1⁺ still lack CD44 expression¹⁰¹. These somewhat conflicting results may be due to the combinatorial effects of other members of the SWI/SNF complex that as yet are either unidentified or have not been implicated in the regulation of CD44 expression in the cell types investigated.

Methylation of the CD44 promoter

CD44 has been suggested to play a major role in tumor growth and metastasis. Upregulation and down-regulation of both the standard and the variant CD44 proteins have been correlated with the metastatic behavior of various human cancers^{27, 43, 81, 138}. It has been suggested that the expression of CD44 isoforms, for example CD44 V6, is upregulated in benign colorectal adenomas and well-differentiated colorectal carcinomas; however, its expression is down-regulated in poorly differentiated cancers^{27, 134}. Because the functional expression of CD44 is associated with decreased tumorigenic properties in advanced human cancers, in particular the prostate and colorectal carcinomas and the neuroblastomas^{47, 75, 103, 111, 143}, CD44 has been designated as a tumor suppressor gene. Such studies add to the multitude of factors that are known to regulate CD44 on a molecular basis. The molecular mechanism for the down-regulation of CD44 isoforms in advanced stages of cancers is not well understood. This down-regulation has been suggested to be via methylation of the CpG islands in the promoter region of CD44^{57, 75, 103, 111, 126, 127, 143}. In studies using human prostate cancer cell lines and tissue specimens, it was demonstrated that the methylation of CD44 correlated with decreased CD44 expression, increased tumor grade, and metastasis^{57, 75, 127}. One study has shown that in murine CD44⁻ AKR-1 and CD44⁺ BW5147 T cell lymphoma cell lines, the CpGs islands were heavily methylated. However, upon activation of CD44 by transient transfection of c-Jun, a subunit of the AP-1 transcription factor, into an AKR-1 subline containing the polyoma large T antigen, changes in the methylation state of CpG islands were not observed⁴⁹. This suggests that although methylation of the CD44 gene can contribute in some systems to the regulation of CD44 expression, it is likely not the only factor involved. Whether cytokines, such as TNF- α , IL-10, or IL-4, and mitogens, such as LPS, regulate CD44 expression by influencing the methylation status of the CD44 promoter remains to be investigated.

ROLE OF CD44 IN INFLAMMATION AND AUTOIMMUNE DISEASES

Inflammation is a rapid and temporary regulatory response to harmful stimuli, including infection, trauma, and chemical injury. The acute inflammatory response involves lymphocyte homing or recruitment to inflammatory sites, elimination of foreign substances and eventually the recruited cells, and finally the healing process. However, in autoimmune diseases a chronic response can develop in which activated inflammatory cells continue to accumulate in extra-vascular connective tissue and cause tissue destruction.

Lymphocyte homing under normal and inflammatory conditions requires the activation and expression of many cell surface adhesion proteins, including L-selectin and CD44 (Fig. 3). It has been shown that T cell activation through the TCR-CD3 complex and/or cytokines induces the HA-binding form of CD44¹⁸. Additionally, inflammatory agents, such as LPS, or cytokines, such as TNF- α and IL-1 β , have also been shown to induce HA expression on endothelial cells⁸⁴. It has been suggested that CD44 may not be involved in the homing of lymphocytes to lymphoid tissues during physiological cell trafficking, but it appears to participate in the homing of cells to sites of injury or inflammation^{18, 19, 25, 33, 68, 84, 115, 130}. L-selectin is a key adhesion molecule, expressed at high levels in naive T cells, which mediates their homing to lymphoid tissues under physiological conditions^{11, 130}. However, in the presence of proinflammatory agents or cytokines, L-selectin expression is down-regulated, whereas that of CD44 and its HA-binding capacity are enhanced. Furthermore, the recruitment of activated cells was found to be CD44-HA-dependent and L-selectin-independent under *in vivo* and *ex vivo* inflammatory conditions^{25, 33, 84, 116}. Since autoimmune inflammatory disorders are characterized by high numbers of lymphocytes bearing the activated, HA-binding form of CD44^{25, 68}, CD44 has been suggested to play a critical role in the maintenance and propagation of autoimmune-induced inflammation.

Animal models

The role of CD44 in inflammatory and autoimmune disorders has been investigated using anti-CD44 antibodies capable of blocking the binding of CD44 to HA. In a murine model of proteoglycan-induced arthritis, treatment of mice with anti-CD44 antibodies decreased tissue edema, leukocyte extravasation into the inflamed joints, and rheumatoid arthritis (RA)-related inflammation^{82, 83}. In murine experimental allergic encephalomyelitis, a model for multiple sclerosis, the administration of anti-CD44 antibodies reduced T cell entry as well as lesion formation in the brain⁹. Similar treatments with anti-CD44 antibodies were found to confer resistance to the onset of diabetes in a non-obese murine model of autoimmune diabetes¹³⁶. However, antibody administration did not prevent extravasation of autopathogenic T cells in experimental autoimmune thyroiditis⁹¹. These observations clearly point towards a significant role for CD44 in several murine models of autoimmune disease.

Although the antibodies used may indeed block CD44-HA-binding, these antibodies may involve a more complex mechanism, complicated by delivering signals through the CD44 molecule resulting in, for example, the expression of proinflammatory cytokines and co-

stimulatory adhesion molecules^{34–36, 79, 80, 93, 132}. Expression of these molecules may in turn alter CD44 expression and its binding capacity. Hence, decreased incidence and disease severity observed following administration of anti-CD44 antibodies may not be entirely due to the blockage of CD44-HA interactions. CD44 knock-out mice provide an alternate model to study the *in vivo* involvement of CD44 in migration patterns and inflammatory responses observed in autoimmune diseases. Despite the fact that CD44 was thought to be essential for tissue morphogenesis, CD44 knock-out mice did not show any gross developmental defects^{95, 104}. However, these animals have been successfully used to investigate the role of CD44 in inflammation in a number of murine models of autoimmune diseases.

In the last few years, results from several studies employing CD44 knock-out mice have clearly suggested that CD44 plays a critical role at many stages of inflammation. The most obvious mechanism for CD44 would be in the regulation of infiltrating lymphocyte extravasation to the inflammatory site. This has been amply demonstrated by using CD44-deficient animals in murine models of collagen-induced arthritis. These mice exhibited a decrease in incidence and severity of disease^{115, 116}. This decrease in disease severity was attributed to the delayed entry of CD44-deficient donor lymphocytes into the arthritic joints of recipient mice^{115, 116}. Similar results were obtained when migration patterns of lymphocytes were studied in wild type and CD44-deficient mice with collagen-induced arthritis^{115, 116} and superantigen-induced peritonitis^{18, 115}. While lymphocytes from CD44 knock-out mice preferentially homed to lymph nodes, their entry into inflamed synovial joints was considerably delayed¹¹⁵.

In a model of inflammatory bowel disease (IBD), Wittig et al.^{139, 141, 142} demonstrated that the expression of CD44 V7-containing isoforms on hematopoietic cells is critical in the maintenance of colonic inflammation. In CD44 V7-deficient animals, inflammatory cells present in the lesions exhibited a higher rate of apoptosis. The presence of CD44 V7 isoforms on inflammatory cells was suggested to promote the survival of effector lymphocytes and the maintenance of inflammation^{141, 142}.

Inflammatory diseases are also characterized by endothelial cell damage that gets accentuated by the release of a number of cytokines, such as IL-2, during inflammation^{5, 102, 128}. It has been suggested that immune cells, particularly cytotoxic T lymphocytes (CTL), play a role in causing endothelial cell injury^{5, 21, 102, 125}. Using CD44-deficient mice, CD44 was shown to be critical in mediating endothelial cell damage during the process of

vascular leak syndrome (VLS) induced following intraperitoneal administration of IL-2^{98, 99}. The reduction of VLS and endothelial cell death observed in CD44 knock-out mice was apparently not due to the inability of lymphocytes to home to the inflammatory sites, as perivascular lymphocyte infiltration to the lungs and liver was not affected by the absence of CD44. The mechanism of endothelial cell injury involved a reduced lytic capacity of the CD44⁺ effector CTLs, NK and lymphokine-activated killer cells. These *in vivo* studies are supported by *in vitro* experiments demonstrating the role of CD44 in inducing NK activity⁹⁸.

CD44 was shown to be pivotal in determining the normal progression from an inflammatory to a healing response in a murine model of lung injury and inflammation^{122, 129}. Teder et al.¹²² reported that wild-type mice recovered fully when treated with bleomycin, a model for lung injury and inflammation. Interestingly, CD44-deficient mice succumbed to an unremitting inflammation. The persisting lung inflammation observed in these mice was related to extended chemokine expression, the accumulation of low-molecular-weight HA fragments, reduced clearance of apoptotic polymorphonuclear leukocytes, and impaired induction of TGF- β_1 . This inflammation was partially reduced by re-introduction of CD44^{122, 129}. The above studies suggest the critical involvement of CD44 in a number of inflammatory conditions. However, it appears that the exact role of CD44 may vary depending on the model system used in the disease process in question due to the multiple functions exerted by CD44 (cell migration, survival, cytotoxicity, etc.).

Human studies

The compelling evidence for the role of CD44 in murine models of inflammation has led several investigators to study CD44 in the context of human inflammatory and autoimmune disorders, including RA, IBD, systemic lupus erythematosus (SLE), multiple sclerosis, and Sjogren's syndrome^{12, 72, 87, 118}. Most of these studies have been limited to determining the expression levels of CD44 or its isoforms on different cell types present in the inflammatory lesions, their ability to bind HA, the presence of soluble CD44 in serum and other body fluids, and the relationship of these parameters with disease severity.

CD44 expression has been extensively studied in patients with RA^{8, 24, 44, 46, 56, 58, 59, 87, 118}. The level of CD44 expression on monocytic cells in the synovial fluid from patients with RA has been shown to be elevated^{8, 24, 44, 46, 56, 58, 59}. This increase in CD44 expression was positively correlated with the degree of synovial inflammation in RA⁴⁶. In

addition, increased concentrations of HA in synovial fluid and tissue were observed^{23,78,107,120}. The increased HA expression serves to trap water, resulting in increased edema, facilitates CD44-mediated cell migration, and induces the production of proinflammatory cytokines at the inflammatory site³². These results were further supported in a study wherein RA synovial fibroblasts (RASf) exhibited enhanced destruction of cartilage and chondrocytes. This RASf-induced destruction was exacerbated by the proinflammatory cytokines IL-1 β and TNF- α produced following interaction of RASf with HA. Furthermore, antibodies to IL-1 β and CD44 reversed this process⁹⁰. Alterations in CD44 isoform expression have also been shown to play a role in RA. A number of soluble CD44 variant proteins, including CD44 V5, V6 and V10, have been detected in the synovial fluids and serum of arthritic patients^{24, 44, 58}. CD44 V3 and CD44 V6 splice variant expression was also found to be elevated in fibroblast-like synoviocytes isolated from patients with RA¹³⁷. Furthermore, cells expressing these isoforms were more invasive compared with those expressed on normal cells, suggesting that expression of CD44 V3 and CD44 V6 variants may be associated with increased disease severity¹³⁷. In the same study, the expression of CD44 V7–8 isoforms was also found to be associated with increased proliferation of fibroblast-like synoviocytes isolated from patients with RA¹³⁷.

Cell-mediated immunity and T cell activation are important features of chronic IBD, such as Crohn's diseases and ulcerative colitis. CD44 isoforms, in particular CD44 V6 variants, are not detected on resting naive T cells, but are induced only following activation. Recently, CD44 isoforms have been suggested to be involved in the pathogenesis of IBD^{141, 142}. This is evidenced by the constitutive expression of CD44 V6 variants on non-activated peripheral blood lymphocytes (PBLs) in patients with IBD. Furthermore, CD44 V6 expression was shown to be correlated with disease severity^{13, 50, 92, 140, 142}. There is growing evidence that chronic inflammatory bowel diseases are associated with dysregulation of intestinal lymphocyte activation, mainly of CD4⁺ T cells^{28, 144}. The CD44 V6 isoform was induced following activation of intestinal lamina propria lymphocytes (LPLs). However, CD44 V6 expression was found to be decreased on LPLs of IBD patients⁹². The significance of differential CD44 V6 expression on PBLs and LPLs and their association with development of disease is not clear at present.

In contrast to the enhanced CD44 expression observed in RA and IBD patients, CD44 expression is reduced on monocytes and neutrophils of SLE patients. SLE patients show increased numbers of apoptotic neutrophils and impaired monocyte/macrophage clearance¹². Therefore, the decreased CD44 expression may

be related to the reduced clearance of apoptotic neutrophils. However, this study was unable to correlate CD44 expression on monocytes and neutrophils with SLE disease severity. It has also been shown that the CD44-dependent primary adhesion or rolling of T cells on vascular endothelial cells is increased in symptomatic SLE patients compared with patients with inactive disease²⁵. The presence of elevated numbers of HA-binding T cells in SLE patients may provide a reliable marker for existing autoimmune activity. Furthermore, the manifestation of increased CD44-HA interactions in this autoimmune disease may have significant therapeutic implications. CD44 isoform expression has also been implicated in autoimmune reactions of the skin observed in SLE¹⁰⁶. CD44 V3 expression on T cells and vascular endothelial cells was increased in SLE patient skin biopsies, whereas that of CD44 V10 was increased only on monocytic cells. The biological significance of this differential expression with respect to disease activity remains to be investigated.

PERSPECTIVES

One of the pertinent questions in CD44 biology deals with the cellular and molecular mechanisms involved in the regulation of CD44 expression and conversion from a non-functional, protein into the functionally active, high-affinity HA-binding state of the receptor. For example, the cellular and biochemical mechanisms by which cytokines such as TNF- α , IL-10, or IL-4 modulate CD44 expression and ligand binding in immune cells remains largely unknown^{37,39,71,76}. Understanding the molecular mechanisms governing these processes may lead to the development of novel strategies for the treatment of inflammatory diseases. It is of paramount importance to determine if modulation of CD44-HA binding *in vivo* can in fact modulate disease progression. This may be addressed by employing recently described small inhibitory molecules such as HA-binding peptides⁸⁵ that may inhibit CD44-HA interactions in animal models of inflammation. The studies conducted in our laboratory have also established the role of p38 MAPKs and of lysosomal sialidases in the generation of the functionally active HA-binding phenotype of CD44 in monocytic cells³⁷. It will be interesting to determine whether the inhibition of p38 MAPK or lysosomal sialidases could prevent CD44 activation *in vivo*. Therefore, by inhibiting CD44 activation *in vivo*, it may be possible to modulate the development or progression of inflammatory response in experimental animals and eventually benefit patients with inflammatory or autoimmune disease.

Although several studies have suggested a key role for CD44 in inflammation and auto-immune diseases^{88, 94, 97, 109, 115, 116, 122}, the important question regarding the net impact of

CD44 at distinct stages during the progression of inflammation *in vivo* needs further investigation. In addition, the precise role of CD44-mediated HA interactions in the development of immune effector cells and immune responses is not clear, keeping in view that CD44 can act as a signaling molecule and interaction of CD44 with HA can induce the expression of a number of immunoregulatory cytokines^{34-36,79,80,132}. The unraveling of the mechanisms by which CD44 influences the outcome of inflammatory reactions and understanding the contribution of CD44 with respect to resolution or persistence of inflammation is critical in exploiting this molecule as a drug target.

The increased levels of soluble CD44 in serum/body fluids have been correlated with disease severity in a number of inflammatory and autoimmune diseases^{25,72}. However, the cellular and molecular mechanisms involved in the generation of soluble CD44 molecules, their impact on disease progression, and the development of immune responses are not known.

Most cell types express the standard CD44 H in addition to a host of other CD44 isoforms. However, the relative contribution of CD44 isoforms *vis-a-vis*

CD44H expressed on hematopoietic and epithelial cells in determining the CD44-mediated inflammatory responses is not clear. It is likely that some CD44 isoforms may have distinct biological functions. For example, Wittig et al. have discovered that CD44 isoforms containing exons V6 or V7 block apoptosis^{141,142}. It is likely that antibodies specific for V6 or V7 isoforms may be able to induce apoptosis and could be potentially useful in experimental models of multiple sclerosis and arthritis by blocking cell activation and inflammatory responses.

Although correlative studies with respect to the levels of soluble CD44 or the expression levels of CD44 on various cell types with respect to disease severity in humans have been made^{12,72,87,118}, the precise impact of CD44-HA interactions in human diseases remains unknown. At present, it may not be possible to perform mechanistic studies of the role of CD44-HA interactions in the context of disease progression in humans. We believe that clinical studies involving the use of inhibitors of CD44-HA interactions, at least under certain conditions involving localized autoimmune arthritic conditions, may prove therapeutically beneficial and could provide additional insights into the role of CD44 in human disease.

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