Regulation of and Regulation by CD44: A Paradigm Complex Regulatory Network

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Abstract—CD44 is expressed on the cell surface of lymphocytes and other hematopoietic and non-hematopoietic cells, where regulates cell-cell and environment-cell interactions by binding different components of extracellular matrix. CD44 is implicated in several other cellular processes, such as regulation of growth, survival, differentiation and motility, both under physiological and pathologic conditions. Studies on CD44-null or transgenic mice also established its involvement in diseases such as cancer, atherosclerosis and myocardial infarction. Its regulation involves several control mechanisms among which a fundamental role is played by alternative splicing of its pre-mRNA and by post-translational modifications. Here we review the mechanisms of regulation upstream and downstream of CD44.

Index Terms—CD44, CD44s, alternate splicing, ECM, HA, OPN, TLR

I. CD44

CD44 is a trans-membrane glycoprotein expressed on the cell surface of lymphocytes and other hematopoietic and non-hematopoietic cells [1]. It regulates the cell-cell and the environment-cell interactions by binding different components of extracellular matrix (ECM), including the basement membrane, which primarily comprised of collagens, heparin sulfate, laminin, fibronectin and proteoglycans. CD44 is implicated also in several other cellular processes, such as regulation of growth, survival, differentiation and motility, both under physiologic and pathologic conditions [2]. Hyaluronic acid (HA) represents its main ligand [3, 4]. Given its high expression on embryonic cells during the morphogenetic processes, CD44 is likely to have a role in embryogenesis [5-7]. CD44 is involved in hematopoiesis, immune response and inflammation; its expression is necessary in the early stage of maturation of B and T cells [3, 8-11]; it is upregulated after activation of the antigen receptor and after mitogenic stimuli [12-14] and is involved in cell trafficking during inflammation [15, 16], when the complex CD44/VLA-4 allows the binding of T cells to the endothelium and their migration [17]. Finally, activation of CD44 is associated with increase of the lytic activity of Natural Killer and cytotoxic T cells [18, 19].

Studies on CD44-null or transgenic mice also established its involvement in many diseases such as cancer, atherosclerosis and myocardial infarction [20, 21]. In particular, literature focused on the expression and transduction pathways of CD44 splicing variants in oncogenesis and metastasis, although the data are controversial; the upregulation of CD44 characterizes the early phases of colon carcinoma [22]; in many types of cancers high levels of CD44 have been correlated to unfavorable prognosis [23-25]; epigenetic hyper-methylation of its gene could be used as a diagnostic marker to distinguish mantle cells lymphoma from other lymphomas [26]. On the other hand, inactivation of CD44 is linked to the metastatic processes [27], but not to the early phases of oncogenesis, as shown in murine models [28]. CD44-null mice are resistant to S. Pyogenes [29] and monoclonal antibodies against CD44 in vitro help in controlling Shigella [30].

II. REGULATION OF CD44

A. The Gene Encoding for CD44

The CD44 proteins family is encoded by a highly conserved gene, on chromosome 11p13 (humans) and chromosome 2 (mice). The heterogeneity of CD44 isoforms derives from alternative splicing and post-transductional modifications and depends strictly on cellular type and growth conditions (see Figures 1 and 2). The CD44 pre-mRNA is encoded by 19 exons (e) in mice, and by 20e in humans. In mice, the alternative splicing involves only exons 6 to 15 (e6-e15, also named v1-10) and generates 6 isoforms. In humans there are 10 e undergoing alternative splicing control (e6a, e6b, e7-e14), but exons e6a (v1) contains a stop codon. Thus, 8 isoforms are described in...
humans. Both in humans and mice exons e1-e5 represent the constant domain of the extracellular region that primarily interacts with ligands; human e17 and mouse e17-e18 encode for the transmembrane region; the cytoplasmic tail in mice is encoded by e19; in humans e18 encodes for a long tail, while, alternatively, e19-e20 generate a short one. The constant regions are e1-e5 and e16-e19 in mice and e1-e5 and e15-e18 in humans.

The shortest isoform of CD44 (that lacks all the variant exons) is named “standard CD44” (CD44s), and it is ubiquitously expressed in vertebrates, mostly in quiescent cells. On the other hand the CD44 variants (CD44v) are expressed in a tissue-specific and cell cycle-specific manner (especially in the proliferative phases) [2, 31, 32]. The different expression of CD44s and CD44v is probably correlated to the fact that variant isoforms have specific function, most likely different from that of CD44s [32].

Quiescent T cells express mainly CD44s, but antigenic experience leads to alternative splicing of the primary transcripts as it occurs for other genes like CD45. Stimuli leading to differentiation or to mitosis can trigger signaling pathways, as Ras-MEK-ERK/Sam68, able to influence the spliceosome: thus, the over-expression of negative regulators hnRNP-A1 promotes the skipping of v5 and v6 exons [27, 31].

B. Alternative Splicing is the Main Mechanism Generating CD44 Isoforms

The alternative splicing represents the mechanism able to enlarge the variability of the proteome and occurs in many species through different mechanisms of regulation. It also provides a way to convert extracellular stimuli into changes in splice patterns resulting in physiological responses or disease [33]. Alternative splicing of CD44 pre-mRNA is particularly interesting because of the large variability derived from the choice of 10 variant exons. Exon skipping versus exon inclusion in different pre-mRNAs within the same cell suggests the existence of distinct factors that are able to recognize specifically pre-mRNA elements of different genes or sets of genes [27]. Highly conserved, pleiotropic signaling pathways link extracellular cues to splice regulation, providing an avenue for tissue-specific, developmental or pathology-associated splicing decisions.

It has been demonstrated that Ras–Raf–MEK–ERK signaling cascade induces the inclusion of CD44v5 sequences into mature mRNA, depending on signal-responsive exonic silencer [33]. In this study MEK–ERK pathway or JNK pathway is sufficient to inactivate splice silencing, while the p38 pathway does not affect CD44 splicing. Upregulation of CD44 variants has been reported in a large number of epithelial and hematopoietic tumors including high-grade malignant lymphomas and has been shown to be associated with poor prognosis [34].

The same extracellular signals have specific effects on exon usage of distinct proteins; thus, for instances, TCR stimulation results in changes of alternative splicing of both CD44 and CD45 pre-mRNAs, but TCR stimulation leads to inclusion of exons in CD44 mRNA, while exons are skipped in the case of CD45 [35, 36]. These latter studies proposed a post-translational modification such as signal-induced phosphorylation of regulatory splice factors, as the regulatory principle.

Since CD44 isoforms do not differ in their intra-cytoplasmic tail, the distinct activities must result from expression on cells and on interactions with distinct ligands. Thus, it has been demonstrated a correlation between CD44 isoforms and delayed-type hypersensitivity. Anti-CD44v7 (i.e. antibodies directed against variant exon 7) can prevent and cure a severe pancolitis, while anti-CD44v6 antibodies have no effect. Both these two human variants have been described for their involvement in lymphocytes activation and their presence during autoimmune diseases [37]. It has been demonstrated for these two variant, and it is hypothetical for the others, that each CD44 isoform is differently expressed on specific cellular subpopulations: for example CD44v7 is only on activated CD4 T cells, B cells and monocytes. This observation supports the idea that different isoforms have different activity and that splice variant molecules on a cell surface can be involved in distinct signaling pathways [38].

The role of Metalloproteinases and de-sialilation in modifying the functions of CD44 will be described below.

III. NOMENCLATURE OF CD44 ISOFORMS

The gene encoding for CD44 represents an example of the extremely fine biologic phenomenon of alternative splicing. From a highly conserved gene, various isoforms are generated and this variability can be ascribed not only to post-transudctional modifications as glycosylation, that depends from the cell type and the growth conditions, but above all to alternative splicing that involves principally the extracellular domain of the molecule [2].

The mouse (m)CD44s is also named isoform c (ENSMUST00000099673, NM_001039151.1, 365 AA encoded by 2848 bases) and is produced only from exons e1-e5 and e16-e19. The corresponding human (h)CD44s, also named isoform d (ENST0000263398, NM_000610.3), contains 361 AA.

The longest variants of CD44 are named “isoform a” both in humans and mice; they are also named mCD44v1-v10 (ENSMUST00000005218, NM_009851.2, 5641 bp and 780 AA) or hCD44v2-v10 (ENST00000428726, NM_001001391.1, 3046 bp and 742 AA), and are produced from the complete primary transcripts, without skipping any of the variant exons and by all the constant regions.

Apart the CD44s and isoform a, there are other variants of human and mice CD44 that share the same number of exons and similar length and structure. The murine “isoform d” or CD44v4-v10 (ENSMUST00000111198, NM_001177785.1, 1981 bp and 657 AA) contains the variant exons e9(v4)-e15(v10), like the human “isoform b” or CD44v3-v10 (ENST00000415148, NM_001001389.1, 2369 and 699 AA)
that comprises e7(v3)-e14(v10); the mouse “isoform e” or CD44v8-v10 (ENST00000011191, NM_001177786.1, 1533 bp and 498 AA) which skips e6(v1)-e12(v7) exons is similar to human “isoform c” or CD44v7-v10 (ENST000000013660, NM_010001390.1 with a pre-mRNA of 2292 bp and a protein of 493 AA); the mouse “isoform f” or CD44v9-10 (ENST00000011190, NM_001177787.1, 1412 bp and 464 AA) is encoded by the constant regions and e14(v9) and e15(v10), similarly to the human “isoform f” or CD44v10 (ENST000000433442, NM_001202555.1, 1634 bp and 429 AA).

Mouse “isoform h” or CD44v6-v10 (ENST000000060516, NM_001039150.1, 3495 bp and 139 AA) contains the variant exons from e11(v6) to e15 (v10) and is not similar to any human variant. On the other hand three more humans variants have been described that do not have a mouse correlate: “isoform g”, encoded only by e1-e5 and e16-e18 (ENST00000352818, NM_001202556.1, 1023 bp and 340 AA); “isoform h”, encoded from e1-e5 and e15-e17 (ENST000000442151, NM_001202557.1, 1825 bp 294 AA); and the smallest variant isoform, “isoform e” (ENST00000278386, NM_001001392.1, 484 bp and 139 AA) whose mRNA contains only the first two exons of extracellular constant region and two other exons named e19 and 20 and that generates a protein with a very short cytoplasmic tail.

IV. STRUCTURE OF CD44

The alternative splicing involves only exons coding for the extracellular domain of CD44.

The globular amino-terminal domain of the protein, encoded by five non-variable exons (e1-e5), contains the binding site(s) for extracellular matrix (ECM) ligands. It is composed by 90 aminoacids with a big homology with glycosaminoglicans (GAGs). This globular amino-terminal domain contains the binding motif for HA, one of the principal polysaccharidic constituent of the ECM, and a structural component of the connective tissue [39-43]. The HA is composed by D-glucuronic acid and N-acetyl-glucosamine linked β1-4 e β1-3 [39] and is involved in the regulation of inflammation [44-46]. However CD44 carries out also HA-independent functions [12, 47, 48], having several other possible ligands such as collagen, serglycin, mucosal addressin, MIB-1β [49-55], chondroitin sulfate [56], osteopontin [51], fibronectin [50] and heparin-binding growth factors [57]. The affinity for each ligand depends on the post-transductional modifications of the extracellular domain, as glycosilation [58], or on the expression of variant exons, as, e.g., v6 and v7 seem to be very important for binding to GAGs [55]. Three sulphur bridges confer stability to the amino-terminal domain, determining the correct folding necessary for the binding to HA [59].

The structural heterogeneity of CD44 isoforms not only determines its ligand repertoire, but also modulates its HA-binding ability [58]. It has been described that different grades of affinity in HA binding depend on the expression of CD44 isoforms during the cell cycle; lymphocytes in quiescent state, despite high expression of CD44s, bind HA with low affinity, while after activation through LPS [60], or through immune reaction in vivo [61] or through cytokines or CD3 cross-linking [62] improve their CD44-mediated HA binding properties. Inclusion of CD44v8-v10, for example, results in O-linked glycosylation that reduces HA binding in human melanoma [57].

The extracellular domain of CD44 includes the so-called proximal stem, that extends between aa 46 (CD44s) to aa 381 in humans and to aa 423 in mice (isoform a). In this case mitogen stimuli leading to Ras and to other oncogenes of the MAP-kinase pathway activation appear to regulate alternative splicing [33, 63]. In the proximal stem, exon v3 encodes for the binding site for heparan sulfate.

Twenty three hydrophobic AA and a cysteine (CYS286), that seems important for the oligomerization of CD44 [64], constitute the transmembrane domain that recruits CD44 into the membrane micro-domains named “lipid rafts” [65, 66].

The C-terminal domain regulates the interactions with many intracellular proteins involved in the organization of cytoskeleton and intracellular signaling. The most studied one is the interaction between CD44 and ankyrin that modulates cell mobility and adhesion through spectrin and actin, after binding of CD44 to HA [67, 68]. Another interaction that competes with ankyrin is that with proteins of the 4.1 band superfamiliy (that includes the ERM proteins as Exin, Radixin, and Moesin) [22], and that with Merlin leading to tumor suppression [2]. Protein kinases of the C family modify the affinity of CD44 to its intracellular partners; such action occurs directly on CD44, through the phosphorylation of Ser291 and Ser325 [69], but also at the level of the intracellular partners protein as Ezrin [70] and Merlin [71].

V. REGULATION BY CD44

There are many reports about physiological roles of CD44 and its activity during various diseases but we lack detailed explanation about most molecular mechanisms.

A. Regulation by CD44 as Receptor

The known functions of CD44 are mostly linked to its role as receptor for a variety of ligands, soluble macromolecules and components of the ECM environment (as briefly summarized above), modulating the binding of cells to, and the migration through, the matrix.

Its function as receptor seems to be under the regulation of the intracellular signals that modulate the formation and the expression of different variants, the alternative splicing, the glycosylation and the amount of the CD44 expressed on the cell surface. All these modifications influence the binding affinity, its capacity to form molecular clusters on the cells surface and other activity[2].

HA is the main ligand of CD44: its binding to CD44 is characterized by high affinity and regulated by intracellular signals able to modulate the expression on the surface of the cells. Changes of the glycosylation in the distal extracellular
domain have been implicated in the regulation of CD44-mediated cell binding of HA and probably also the phosphorylation of the serine residues in the intracellular domain [58].

proteins, GTP-binding proteins as Rac and Cdc42, involved in the cell migration through cytoskeletal remodeling [72]. It has been demonstrated that CD44 expressed on the cell surface is a platform for recruitment and binding of proteins involved in different cell processes, such as growth factors, the MMPs and others enzymes. In many cell lines of breast carcinoma and melanoma, CD44 recruits MMP9, which promotes the degradation of collagen and tumor invasiveness. The CD44-MMP9 binding site is distinct from the CD44-HA binding site [73]. MMP9 is also implicated in the conversion of TGFβ in its active form able to mediate neo-angiogenesis, and this occurs only through the interaction with CD44 [74].

Also MMP7 is expressed on the cell surface in a CD44-dependent mechanism. Its principal role is the degradation of heparin-binding epidermal growth factor (HBEGF), that co-localizes with MMP7 in heparan-sulfate binding site of CD44 (encoded by exon v3) and enhances cell survival through the activation of ERBB4 [74, 75].

![Fig. 1](http://www.researchpub.org/journal/iti/iti.html)

The proteolysis of CD44 in the extracellular domain through metalloproteinases (MMPs) not yet identified, represents another level of modulation of the affinity of the binding of HA. There are two different pathways that seem implicated in this mechanism; the first is activated by the egress of calcium and is dependent from the activation of the protein-kinase C (PKC); the second involves mitogenic stimuli, as in the neoplastic transformation, and also depends on the activation of PKC. Both pathways increase the proteolysis of CD44 and can be blocked by inhibitors of the MMPs. The mitogenic stimuli can regulate the proteolysis of CD44 also through the activation of the Rho proteins. In particular CD44 is linked to cytoskeleton by the interactions with the ERM proteins complex and the CD44-ERM binding is regulated by Rho proteins, through the activation of the Rho GTPases Rac and Cdc42 [72].

Fig. 1: Schematic representation of mouse CD44 mRNA coding for the various isoforms; e1-e17: Exons of CD44 gene. GREEN: constant exons encoding the globular amino-terminal extracellular domain; RED: variable exons encoding for the proximal stem; BLUE: constant exons encoding for the trans-membrane domain and for the intra-cytoplasmic tail.
The interactions between CD44 isoforms containing exons v6 and v7 and the phosphorylated form of osteopontin (OPN), a component of the extracellular matrix involved in inflammation, tissue remodeling and cell cycle, has been proposed as a mechanism of tissue integrity during inflammation [76]. Mice lacking of CD44-OPN interaction show low levels of inflammation [76].

Taken together these observations highlight the role of CD44 in the recruitment and concentration on the cell surface of many proteins with a very wide spectrum of activities that play a role in different mechanism as cell cycle, migration and cells death.

**B. Regulation by CD44 as a Co-Receptor**

It has been reported that CD44 can influence the activity of many cell surface receptor with tyrosine-kinase function; isoforms of CD44 containing exon v6 are the co-receptor of Met, binding protein of scatter factor/hepatocyte growth factor (SF/HGF) [77]; a similar role has been demonstrated also for the family of ERBB receptor, EGFR/HER1, HER2/neu, HER3 and ERBB4 [74, 78, 79]. In particular the interaction CD44-ERBB4 is involved in the activation of HBEGF by MMP7, as previously described, while the interaction with other members of this family seems fundamental for the self-activation of these receptors; CD44 mediates the heterodimerization between ERBB2 and ERBB3 in response to the neuregulin and this mechanism is necessary for differentiation, survival and proliferation in the peripheral nervous system of Schwann cells. [80].

![Diagram of Human CD44](image)

**Fig. 2.** Schematic representation of human CD44 mRNA coding for the various isoforms; e1-e20: Exons of CD44 gene. GREEN: constant exons encoding the globular amino-terminal extracellular domain; RED: variable exons encoding for the proximal stem; BLUE: constant exons encoding for the trans-membrane domain and for the intra-cytoplasmic tail.

in different mechanism as cell cycle, migration and cells death.

The role of CD44 isoforms as co-receptors has not been exhaustively clarified. The binding with CD44 can stabilize dimers, or can exclude from the interactions proteins that down-regulate kinase activity. This role of CD44 may be explained also by its ability to influence the pathways of signal transduction from the target receptor, even if CD44 lacks a catalytic site in its own intracellular domain. There are many proteins that interact with the intracellular domain of CD44,
such as the Src family (Lck and Fyn, in the signal transduction pathway of the TCR, Rho GTPase, Rho kinase, Rho GDP-dissociation inhibitor (GDI), T lymphoma invasion and metastasis-inducing protein 1 (TIAM1), VAV2, PKC and DBL [81-84].

C. Regulation by CD44 in T Lymphocytes

Isoform c (also known as standard isoform (CD44s)) is the main isoform of CD44 expressed on the surface of many immune cells, in particular T lymphocytes and hematopoietic cells [13, 85, 86]. The other variants can be expressed on T cells only after the activation by the antigen [13].

The expression of CD44 is finely regulated during the maturation and the activation of T cells, in particular its levels increase during the response to antigen. CD44 on T cells is also involved in lymphopoiesis [3, 8-11, 87], homing [87] and cell recruitment; combining its upregulation with LFA-1 expression leads to increase of adhesiveness of T cells [88]. During the activation of T cells following TCR/CD3 stimulation, CD44 acts as an important co-stimulator for cell proliferation and cytokine secretion [12-14, 89, 90], although the specific signal pathway involved has not yet been clarified.

During T cell activation, CD44 acts as a regulator of polarization, survival and differentiation by interacting with HA. Although the affinity of CD44-HA binding is relatively low (Kd=10-100 μ M), the highly repetitive structure of the ligand (one HA polymer contains more than 10000 di-saccharid units) allows the binding of more than 1000 molecules of CD44.

Inflammatory conditions can lead to HA degradation to molecules of 40-8 di-saccharid (with a molecular weight of 500 kDa). The small form of 8 di-saccarid of HA is able to bind CD44, but the binding is reversible [91]. In addition, CD44 is able to shift from a low affinity form to a high affinity form upon the enzymatic cut of a sialic acid residue [92].

During the formation of the immunological synapse, CD44 is localized near the TCR/CD3 complex and contributes to cell-cell interactions with the contribution of HA expressed on the surface of APC [93].

The activation of CD44 in quiescent T lymphocyte induces a rapid increase of phosphorylation of tyrosine residues of several intracellular proteins, suggesting an association between CD44 and protein-kinases involved in activation and proliferation of T cells in response to antigen. Studies of co-precipitation have demonstrated the interaction between CD44 and Lck that activate the tyrosine-kinase ZAP-70. Lck plays a key role in T cells development and signal transduction mediated by the TCR/CD3 complex. It has been suggested that direct or indirect recruitment of Lck makes CD44 available for interaction with the TCR; on the other side, the activation of ZAP-70 mediated by CD44 contributes to TCR signal. It has been reported that CD44 isoforms associate in different ways with Lck [94] and it is likely that the variant extracellular domains cause conformational changes able to modify the interaction with Lck even if the intra-cytoplasmic region is common.

CD44 expressed on peripheral T cell surface is physically associated with low-density plasma membrane fractions that represent specialized plasma membrane domains enriched in glycosphingolipids and glycosylphatidylinositol (GPI)-anchored proteins. CD44 and the GPI-anchored protein CD59 do not appear to interact directly, but 20 to 30% of the Src family protein tyrosine kinases, Lck and Fyn, are recovered from these fractions. CD44-associated protein kinase activity was selectively recovered from the low-density membrane fractions, corresponding to such microdomains [95].

Expression of CD44 plays a crucial role in Th differentiation, since a deletion of CD44 inhibits Th1/Th17 differentiation while simultaneously enhancing Th2/Treg cell differentiation. Accordingly, expression of CD44 promotes Th1/Th17 differentiation. [96]. However, it has been reported that CD44 is able to promote resistance to apoptosis only in the Th1 subpopulation [97].

In effector T cells, the interaction between low molecular weight HA and CD44 in vitro results in the production of high levels of IFN-γ, which is dependent on IL-12 and TCR stimulation [98]. In the murine model of Multiple Sclerosis, EAE, when osteopontin and HA were tested for their role in Th differentiation, osteopontin, but not HA, promoted Th1/Th17 differentiation. Furthermore, activation of CD44⁺ encephalitogenic T cells with myelin oligodendrocyte glycoprotein peptide leads to demethylation at the IFNγ /IL17a promoter region and to hypermethylation at the IL4/Foxp3 gene promoter. It has been demonstrated that CD44⁺ mice show a decrease in severity of EAE and a reduction of the encephalitogenic CD4⁺ T cells, which also secrete lower amounts of IFNγ and IL-17 compared to wild type mice [96].

Another important function of CD44 is the regulation of lymphocyte trafficking since, as said above, CD44 is involved in the migration of effector T cells in inflamed tissues. Migration of activated lymphocytes plays a crucial role in immune responses, and is the result of the integration of various signals from extracellular environment. The selective migration of leukocytes is linked not only to the site of inflammation, but also to duration and type of the requested immune response, and is associated to changes in the expression of several molecules on the surface of cells involved.

The principal mechanisms that allow the migration of effector lymphocytes in inflamed tissues are the interactions between lymphocytes and endothelium. In the target site, endothelial cells express E and P Selectins (main mediators of the rolling) in response to inflammatory cytokines. Recent evidence suggests that the signaling pathway(s) dependent on CD44 may be shared with those of other adhesion receptors in this phase. The presentation by endothelial cells of HA in the lumen can contribute to the binding of CD44 expressed on activated lymphocytes.

Studies in vitro demonstrated that cells from immunized mice [99] and patients affected by autoimmune diseases [100] are able to migrate on matrix in a CD44-HA dependent manner, even if it has not yet been proven clearly a direct implication of the CD44-HA complex in rolling and migration during inflammation in models in vivo [101].

T cells are dynamic and change their phenotype in terms of cell cycle and cytokine secretion. All these processes involve morphological transformation of cells during adhesion and...
migration and CD44 surely plays a key role in these phases, for example causing the transduction of signals that results in a cytoskeleton reorganization through the interaction with numerous proteins (ankyrin, actin and ERM family) and in stabilizing the interface between T cells and APC through the accumulation of actin [102].

Migration of effector lymphocytes is associated to LFA-1/ICAM-1 and VLA-4/VCAM-1 binding, but the evidence in the involvement of CD44 in T cell trafficking derives from a study of co-precipitation, showing that CD44 forms a bimolecular complex with VLA-4. In this model, CD44 was fundamental for the first mild adhesion to HA that allows subsequently a stable adhesion mediated by VLA4/VCAM1. The intra-cytoplasmic region of CD44 is necessary for this function of CD44 [17, 103].

The association between CD44 and recruitment of activated T lymphocytes during inflammation has been demonstrated also in the chronic-relapsing Experimental Autoimmune Encephalomyelitis. The wide lymphocytic infiltrate normally observed in the SNC is strongly reduced when antibodies against CD44 are used, possibly reducing the capability of leucocytes to migrate [16].

CD44 can directly share some pathway(s) of signal transduction with other adhesion molecules and this represents a fine mechanism for the extracellular environment to control the recruitment of activated T cells to the site of inflammation [103].

The movement of recruited T cells within the parenchyma in non-lymphoid tissue is controlled by chemotactic signals through the ECM, whose main component is high molecular weight HA. The basal membrane consists of laminin, collagen type IV, heparin sulfate, proteoglycans and entactin [104], all ligands of CD44. During migration in particular the cleavage of extracellular domain of CD44 is mediated by membrane type metalloproteinases on responding T cells [105] and a cytoskeletal rearrangement occurs [106, 107].

D. TLRs and CD44

The Toll-like receptors (TLRs) are widely expressed on the surface of immune cells and play a central role in regulation of host defense via recognition of specific pathogen-associated molecular patterns (PAMPs) on various microorganisms and in regulation of specific components of the extracellular matrix [108].

CD44 negatively regulates in vivo inflammation mediated by TLRs, interfering with NF-kappaB activation (which leads to proinflammatory cytokine production) directly associated with TLR2. The cytoplasmic domain of CD44 is crucial for its regulatory effect on TLR signaling [109]. This study indicates that CD44 plays a protective role in TLR-mediated inflammation and was the first to demonstrate a direct association between CD44 and a TLR.

TLR2 stimulation drives Jnk- and Erk-dependent TGFβ production that contributes to macrophage chemotaxis in HA-dependent manner [110], and we have previously shown that a polymorphism of TLR2 expressed by T cells modulates the mobility of encephalitogenic T cells in mice [111]. In addition, CD44 regulates TGFβ -RI expression in CD4 T cells, thereby modulating TGFβ signaling and Th17 differentiation [112]. It's still discussed if CD44 can act as a negative regulator of inflammation in EAE [96]. On the other hand, CD44 expression on blood brain barrier (BBB) is required to maintain endothelial cells junctions and BBB integrity. Post-translational modifications of CD44 (such as the action of MMPs or its de-sialilation) can result in loss of electrostatic charge repulsion between cells, resulting in increase or decrease of cell-cell adhesion.

Co-immunoprecipitation of CD44 and TLR4 suggested a close relationship between HA receptors and TLR signaling, hypothesizing a previously unknown mechanism for initiation of sterile inflammation that involves recognition of released HA fragments as an endogenous signal of tissue injury [113]. Liang et al. observed the up-regulation of TLR2 and TLR4 on macrophages with increased sensitivity to HA fragments, suggesting a pro-inflammatory mechanism by which persistence of HA fragments contributes to chronic inflammation [114]. Furthermore they also demonstrated that CD44−/− mice are more susceptible to LPS-induced shock, confirming that CD44 is required to clear HA during tissue injury, and that impaired clearance of HA results in unremitting inflammation.

These studies suggest a role for CD44 as an enhancer or stabilizer of the interactions between HA and TLRs. As outlined above, CD44 has several functions and furthermore complex alternatively spliced transcripts have been described as a consequence of particular cellular responses under both normal and pathological conditions [115, 116].

VI. CONCLUSIONS

In conclusion, CD44 appears to lie at the core of a regulatory network. Upstream of CD44, several pathways are involved in the regulation of its isoform expression and postranslational modifications thus modulating preferential binding to various ligands. Downstream, CD44 interacts with the ECM directly or by modifying other cell-surface proteins, thereby modulating cell trafficking, homing, survival and differentiation. Here we have summarized some of the results that have shed light on such a complex network.

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REFERENCES


Tertiet P, Banerji S, Noble M, Blundell CD, Wright AJ, Pickford AR, Lowe E, Mahoney DJ, Tammi MI, Kahmann JD, Campbell D,


Guo H, Nagarkatti PS, and Nagarkatti M. CD44 Reciprocally regulates the differentiation of encephalitogenic Th1/Th17 and Th2/regulatory T cells through epigenetic modulation involving DNA methylation of cytokine gene promoters, thereby controlling the development of experimental autoimmune encephalomyelitis. *Journal of immunology* 2011;186(12):6955-64.


