MINI REVIEW

Dermatan sulfate: new functions from an old glycosaminoglycan

Janet M. Trowbridge and Richard L. Gallo

Division of Dermatology, Department of Medicine, University of California
San Diego and VA San Diego Healthcare Center, 3350 La Jolla Village Drive,
San Diego, CA 92161, USA

Accepted on May 3, 2002

Glycosaminoglycans constitute a considerable fraction of the glycoconjugates found on cellular membranes and in the extracellular matrix of virtually all mammalian tissues. Their ability to bind and alter protein–protein interactions or enzymatic activity has identified them as important determinants of cellular responsiveness in development, homeostasis, and disease. Although heparan sulfate tends to be emphasized as the most biologically active glycosaminoglycan, dermatan sulfate is a particularly attractive subject for further study because it is expressed in many mammalian tissues and it is the predominant glycan present in skin. Dermatan and dermatan sulfate proteoglycans have also been implicated in cardiovascular disease, tumorigenesis, infection, wound repair, and fibrosis. Growing evidence suggests that this glycosaminoglycan, like the better studied heparin and heparan sulfate, is an important cofactor in a variety of cell behaviors.

Key words: dermatan sulfate/extracellular matrix/glycosaminoglycan/proteoglycans/skin

Introduction

The study of glycosaminoglycan (GAG) and proteoglycan (PG) structure and function has significantly influenced our understanding of a wide variety of biological processes, including cellular proliferation, differentiation, and wound healing. Historically, these cell surface and extracellular molecules were generically designated “ground substances” or “mucopolysaccharides,” and, due to their carbohydrate content and heterogeneity, were extremely difficult to study relative to proteins. The advent of the field of glycobiology with tools and techniques for studying GAGs has done much toward furthering appreciation for how carbohydrate moieties can affect cellular responses to a variety of stimuli.

GAGs such as heparin, heparan sulfate (HS), and dermatan sulfate (DS) serve as key biological response modifiers by acting as (1) stabilizers, cofactors, and/or coreceptors for growth factors, cytokines, and chemokines; (2) regulators of enzyme activity; (3) signaling molecules in response to cellular damage, such as wounding, infection, and tumorigenesis; and (4) targets for bacterial, viral, and parasitic virulence factors for attachment, invasion, and immune system evasion (Rostand and Esko, 1997; Schmidtchen et al., 2001).

A thorough analysis of PG and GAG biology is well beyond the scope of this review. Several sources exist for interested readers (Esko and Lindahl, 2001; Gallo, 1999; Iozzo, 2001; Tumova et al., 2000b; Turnbull et al., 2001). We will focus on what is known and postulated about a single GAG, DS. DS has been the topic of limited studies in comparison to the more commonly studied GAGs (HS, heparin, and hyaluronic acid). That DS is the predominant GAG expressed in the skin and is released at high concentrations during wound repair makes it a particularly interesting topic for evaluation (Penca et al., 1998). Emerging evidence outlined later suggests that DS, like heparin and HS, serves a variety of roles. DS adds flexibility to many normal and pathological responses, such as development, growth, wound repair, infection, and tumorigenesis.

Dermatan sulfate structure

DS, also known as chondroitin sulfate B (CS-B), is composed of linear polysaccharides assembled as disaccharide units containing a hexosamine, N-acetyl galactosamine (GalNAc) or glucuronic acid (GlcA) joined by β 1,4 or 1,3 linkages respectively (Figure 1). DS is defined as a chondroitin sulfate by the presence of GalNAc. The presence of iduronic acid (IdoA) in DS distinguishes it from chondroitin sulfates-A (4-O-sulfated) and -C (6-0-sulfated) and likens it to heparin and HS, which also contain this residue. IdoA appears to play a key role in binding site specificity for GAG-binding proteins, as will be discussed later. A clue to the importance of IdoA residues for biological effects has been suggested by data showing that GAG chains containing high amounts of IdoA inhibit the proliferation of normal fibroblasts more than GAGs with high GlcA content (Westergren-Thorsson et al., 1991) and by several studies to be discussed later that find functional similarities between DS and HS.

The variable total length of the DS polysaccharide chain, variable placement of IdoA, variable sulfation, and multiple alternatives for core proteins dictate the level of complexity of DS and DS-containing PGs. As will be discussed, there is growing evidence that this variable DS chain length, disaccharide composition, and sulfation determine binding affinity and control functional interactions with potential protein partners. DS is modified by sulfation at C4 and C6 of the hexosamine

1To whom correspondence should be addressed; E-mail: rgallo@vapop.ucsd.edu
Unlike other Chondroitins
Dermatan contains Iduronic acids

DERMATAN

Variable epimerization of uronic acids and Variable sulfation


Specific Structural Sequence Information

Fig. 1. Disaccharide comparison of dermatan sulfate and example of potential dermatan sulfate oligosaccharide structures.

(DSPGs)

DSPGs

DS is attached covalently via an O-xylose linkage to serine residues of core proteins to form DSPGs. Table I presents a protein in specific developmental and physiologic conditions (Bernfield and Sanderson, 1990; Kim et al., 1994; Litwack et al., 1998). Therefore, to understand the functional significance of DS one must consider both the GAG structure and the core protein that makes up a dermatan sulfate proteoglycan (DSPG).
Table I. Partial list of dermatan sulfate–containing proteoglycans

<table>
<thead>
<tr>
<th>Core protein</th>
<th>Cell/tissue expression</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decorin</td>
<td>Fibroblasts/skin, cornea, cartilage</td>
<td>Day et al., 1986</td>
</tr>
<tr>
<td>Biglycan</td>
<td>Bone, cartilage, skin</td>
<td>Fisher et al., 1989</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>Endothelia/cartilage, skin, cornea</td>
<td>Preissner et al., 1990</td>
</tr>
<tr>
<td>Endocan</td>
<td>Endothelia</td>
<td>Bechard et al., 2001</td>
</tr>
<tr>
<td>Epiphycan</td>
<td>Cartilage</td>
<td>Johnson et al., 1997</td>
</tr>
<tr>
<td>Versican</td>
<td>Fibroblasts and keratinocytes/connective tissue, skin</td>
<td>Zimmermann and Ruoslahti, 1989; Westergren-Thorsson et al., 1992</td>
</tr>
</tbody>
</table>

partial list of core proteins that have been found to contain DS. The two best studied DSPGs are the small leucine-rich PGs decorin and biglycan. Both contain small protein cores, and both are secreted matrix proteins. Unlike larger PGs, such as versican and aggrecan, that contain many GAG chains, decorin and biglycan have 1–2 DS chains, respectively. Depending on cellular context, other PGs traditionally thought to contain HS exclusively may also occasionally contain other GAGs, such as DS. For example, syndecans, well-characterized cell surface HS-containing PGs, are altered by the presence of transforming growth factor-β (TGF-β) to include more CS chains (Rapraeger, 1989). In corneal fibroblasts, both calf serum (10%) and TGF-β affect PG synthesis; decorin synthesis is decreased by 10% serum and TGF-β, whereas perlecan-bearing CS chains was increased with TGF-β but decreased in serum (Brown et al., 1999). Fibroblast growth factor-2 (FGF-2) has also been reported to alter the expression and structure of PGs in corneal fibroblasts (Schmidt et al., 1995). Therefore, although several PGs, such as decorin, biglycan, epiphycan, versican, and endocan, have been shown to contain DS, it is not correct to conclude these PGs are always DSPGs or that other PGs cannot exist with DS.

**DS binding partners: methods for study**

DSPGs bind a diverse range of molecules including (but not limited to) matrix molecules, growth factors, protease inhibitors, cytokines, chemokines, and pathogen virulence factors (Table II). The specific binding site has not been identified for many of these proteins, nor has the binding affinity for different structural variants of DS been thoroughly investigated. Multiple approaches have been taken to identify or quantify the ability of DS to associate with proteins. A common approach to investigating putative binding interactions has relied on affinity chromatography. The GAG chain of interest is immobilized and exposed to a potential binding protein that is then eluted with increasing salt concentrations. Alternatively, a GAG binding protein can serve as the stationary phase, and different GAGs can be passed over the column and then eluted (Lyon et al., 1998; Proudfoot et al., 2001). The latter approach has made possible the characterization of different GAG subspecies that differ by chain length and charge/mass ratio. Other methods for demonstrating GAG–protein binding (such as affinity coelectrophoresis, filter binding assays, and solid phase or cell surface binding assays on tissue culture plates) are also used either independently or in conjunction with chromatography (Desnoyers et al., 2001; Hirose et al., 2001; Mongiat et al., 2000; Penc et al., 1998; Proudfoot et al., 2001). However, chromatography allows the investigator to gauge binding affinity because the salt concentration required for elution is generally proportional to the K_d of a given interaction (Esko, 1999b). Once an interaction is identified via chromatography, other methods become indispensable, in that physical interactions per se do not always correspond to physiologically relevant interactions that occur in vitro or, more importantly, in vivo.

Table II lists a number of DS-binding proteins. Although many of these interactions were first identified using other GAGs, notably heparin and HS, it has been appreciated that many proteins can interact with more than one type of GAG chain albeit with differing affinities. The characterization of GAG/PG–protein interactions is complicated by the fact that many interactions are between the PG protein core and the binding protein rather than the GAG. For example, decorin binds collagen via its protein core (Iozzo, 1997), whereas decorin interacts via its DS side chain with tenascin-X, another extracellular matrix molecule (Elefteriou et al., 2001). It is conceivable that certain GAG/PG–protein binding interactions involve both the GAG side chain(s) and the PG protein core.

One limitation to the study of DS–protein binding interactions lies in the structural complexity of the GAG molecule. As mentioned, the final composition of a DS chain depends not only on chain length but also on postsynthetic modifications, such as epimerization and sulfation. Given the heterogeneous nature of DS, it is technically difficult to obtain standardized reagents with which to work. Many binding studies done on DS and other GAGs have used material that is believed to be heterogeneous in both size and charge. Even commercially available GAGs are assumed to be of mixed structure and may contain more than one GAG species given that the tissues used for their preparation contain multiple GAGs. Fortunately, there are methods available to separate both DS from contaminating GAGs and DS subspecies based on size and charge, including nitrous acid degradation and enzymatic treatment with heparinases that can be used to specifically remove contaminating heparin and HS. Advances in GAG structural analysis have been made primarily with HS (Merry et al., 1999; Turnbull et al., 1999; Vives et al., 1999; Rhomberg et al., 1998), but these techniques will undoubtedly contribute in the near future to a better understanding of DS structural characteristics required for specific binding interactions.
Table II. Binding interactions of dermatan sulfate and dermatan sulfate proteoglycans

<table>
<thead>
<tr>
<th>Binding protein</th>
<th>GAGs bound</th>
<th>Binding sequence</th>
<th>Physiologic effect</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin cofactor II</td>
<td>DS, Hep</td>
<td>IdoA(2-OSO3)-GalNAc(4-OSO3) hexasaccharide</td>
<td>Enzymatic inactivation of thrombin</td>
<td>Maimone and Tollefsen, 1990; Mascellini et al., 1993; Liaw et al., 2001; Scully et al., 1988</td>
</tr>
<tr>
<td>Thrombin</td>
<td>DS, Hep</td>
<td>ND</td>
<td>Anticoagulation</td>
<td>Liaw et al., 2001</td>
</tr>
<tr>
<td>Activated protein C</td>
<td>DS, Hep</td>
<td>ND</td>
<td>Anticoagulation</td>
<td>Fernandez et al., 1999</td>
</tr>
<tr>
<td>Protein C inhibitor</td>
<td>DS, HS, Hep</td>
<td>ND</td>
<td>Stimulates serpin activity</td>
<td>Priglinger et al., 1994</td>
</tr>
<tr>
<td>Platelet factor 4</td>
<td>DS, Hep</td>
<td>ND</td>
<td>unknown</td>
<td>Cella et al., 1992; Maione et al., 1990</td>
</tr>
<tr>
<td>Collagen</td>
<td>DS</td>
<td>Binds protein core</td>
<td>Extracellular matrix stability</td>
<td>Walker and Gallagher, 1996; Schmidt et al., 1987; Tunova et al., 2000a</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>DS, HS</td>
<td>Binds protein core</td>
<td>Extracellular matrix stability</td>
<td>Iozzo, 1997</td>
</tr>
<tr>
<td>Tenascin-X</td>
<td>DS, HS</td>
<td>Binds GAG chain</td>
<td>Collagen matrix stability</td>
<td>Elefteriou et al., 2001</td>
</tr>
<tr>
<td>Borrelia burgdorferi adhesions</td>
<td>DS</td>
<td>ND</td>
<td>Increased infectivity</td>
<td>Brown et al., 2001</td>
</tr>
<tr>
<td>α-defensin</td>
<td>DS</td>
<td>ND</td>
<td>Increased infectivity</td>
<td>Schmittchen et al., 2001</td>
</tr>
<tr>
<td>RANTES</td>
<td>DS, Hep, CS, HS</td>
<td>ND</td>
<td>Modulation of inflammatory response</td>
<td>Proudfoot et al., 2001; Kuschert et al., 1999</td>
</tr>
<tr>
<td>Interferon gamma</td>
<td>Hep, HS, DS</td>
<td>ND</td>
<td>Receptor for INF-γ</td>
<td>Brooks et al., 2000</td>
</tr>
<tr>
<td>Transforming growth factor-beta</td>
<td>DS</td>
<td>Binds protein core</td>
<td>Growth regulation</td>
<td>Hildebrand et al., 1994; Yamaguchi et al., 1990</td>
</tr>
<tr>
<td>Fibroblast growth factors 1 and 2</td>
<td>DS, Hep, HS</td>
<td>ND</td>
<td>Cellular proliferation via tyrosine kinase activation</td>
<td>Tunova et al., 2000b; Kreuger et al., 1999; Penc et al., 1998</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>DS</td>
<td>ND</td>
<td>Atherosclerotic plaque stabilization ?</td>
<td>Kovanen and Pentikainen, 1999; Pentikainen et al., 1997</td>
</tr>
</tbody>
</table>

ND, not done.

**DS and the coagulation cascade**

One particularly well-studied DS binding interaction occurs with heparin cofactor II (HCII). This serpin homolog of antithrombin III acts by inhibiting the procoagulative effect of thrombin. This effect is enhanced 1000-fold in the presence of DS or heparin. It is postulated that the GAG forms a stable ternary complex between the serpin and the protease (Liaw et al., 2001). DS fragments reported to bind HCII include octasaccharides and hexasaccharides with the highest-affinity fraction bearing a sulfate on IdoA C2 and on the C4 of GalNAc (Maimone and Tollefsen, 1990; Tollefsen et al., 1986). Interestingly, this species represents only about 5% of intact porcine skin DS. Despite its high binding affinity, the purified fraction was estimated to be 20 times less active than unfractionated DS in a thrombin inhibition assay (Maimone and Tollefsen, 1990; Tollefsen et al., 1986). With a different source of DS (bovine mucosa), the total charge of the DS disaccharide subunits appear to matter more than IdoA content per se. Two disaccharide species (IdoA 2-OSO3-GalNAc-4-OSO3 and GlcA-GalNAc-4,6 diOSO3) were primarily identified in DS that activated HCII (Denti et al., 1995).

Heparin has been shown to have a fivefold greater affinity for HCII than DS, but the complex formed between heparin, fibrin, and thrombin is different than the DS, fibrin, thrombin complex (Liaw et al., 2001). These authors postulate that heparin binds both thrombin and fibrin, thereby forming a ternary complex and causing a change in the HCII “active site,” of thrombin such that it is no longer sensitive to inhibition. DS binds directly to thrombin only, not to fibrin, and the bound thrombin retains sensitivity toward the serpin. This suggests that DS might be a useful clinical tool for specifically treating or preventing thrombosis. In fact, one study has shown DS to be more effective than heparin in preventing postoperative thrombosis in cancer surgery patients (Carlo, V, et al., 1999). Given that DS is more effective against fibrin-bound thrombin than heparin, it is not surprising that it has become the subject of interest in the development of new anticoagulant agents. The overlapping activities of DS and heparin might make for a useful combined strategy in developing new drugs. A potential problem with this approach is that both GAGs may compete for binding to HCII. Indeed, in the presence of heparin, the DS–HCII complex rate of thrombin inhibition was decreased (Liaw et al., 2001).

DS may also influence coagulation by enhancing the effects of activated protein C (APC), an endogenous inhibitor of the clotting cascade. Heparin enhances the anticoagulative effects of APC against factor V (Petaja et al., 1997). Other GAGs (HS, CS-A, CS-C, and DS) also enhance APC activity (Fernandez et al., 1999). At a physiologically relevant concentration, DS enhanced APC activity more than the other GAGs tested. Moreover, when fractions of DS separated based on charge density were tested, the fraction containing the highest charge density enhanced APC activity more than unfractionated
Decorin and other PGs may also play important roles in cardiovascular disease. Changes in PG expression, structure, and function have been reported to occur within atherosclerotic plaques, under conditions of inflammation-associated angiogenesis and arterial mechanical strain (Lee et al., 2001; Niemarzka et al., 2001; Shirik et al., 2000). The DS chain of decorin has also been shown to bind to low-density lipoprotein in the setting of atherosclerotic plaques (Kovanen and Pentikainen, 1999; Pentikainen et al., 1997). Whether this represents a cause or outcome of plaque formation is not yet clear, but the implications for diagnosis and potential treatment design are substantial.

**DS and the extracellular matrix**

Decorin, a member of the small leucine-rich proteoglycans, is a secreted DSPG that binds collagen fibrils and is believed to be involved in extracellular matrix assembly. Decorin “decorates” connective tissue matrices and is believed to participate in intercellular signaling as well as structural integrity. The protein core of decorin binds to collagen fibrils. The single DS chain of decorin binds to tenascin-X, another extracellular matrix protein that colocalizes with collagen fibrils in connective tissues (Eleferiou et al., 2001). Interestingly, patients deficient in tenascin-X and mice deficient in decorin both have increased skin fragility (Burch et al., 1997; Danielson et al., 1997). These phenotypic similarities suggest that the association between tenascin-X and collagen is mediated by the DS of decorin and that this bridging action is critical to establishing the normal tensile strength of the skin.

Decorin also binds to fibronectin (Schmidt et al., 1987), thrombospondin (Winnemoller et al., 1992), the complement protein Clq (Krundieck et al., 1992), TGF-β (Hildebrand et al., 1994; Yamaguchi et al., 1990), low-density lipoprotein (Kovanen and Pentikainen, 1999; Pentikainen et al., 1997), and the epidermal growth factor receptor (Iozzo et al., 1999). The physiological relevance of these interactions remains unclear but may include effects on fibrosis, and control of proliferation. Binding of the decorin core protein to the epidermal growth factor results in sustained activation of the mitogen-activated protein kinase (MAPK) pathway, inactivation and down-regulation of ErbB2 (an oncogenic member of the EGF tyrosine kinase receptor family associated with poor prognosis in tumors of the breast, ovary, and prostate), and the induction of the cell cycle inhibitor p21 (Luca, A. et al., 1996; Moscatello et al., 1998; Santra et al., 1997, 2000). This leads to G1 cell cycle arrest and growth suppression in several tumor cell lines.

The involvement of GAGs/PGs in oncogenesis is particularly intriguing since changes in matrix composition of these molecules are a hallmark of several tumors and tumor-derived cell lines (Iozzo, 1997). A decrease in endothelial and melanoma proliferation and invasion has been shown following treatment of cell lines with chondroitinases A+C and B. Furthermore, following chondroitinase B treatment, human dermal fibroblasts have decreased growth responsiveness to FGF-2 (Denholm et al., 2000, 2001). These observations, whose origins lie in understanding the interactions of DS with the extracellular matrix, focus attention on the important interactions that have been described between DS and growth factors.

**DS and growth factors**

The most intensively studied growth factor–GAG interactions are those involving the FGF family. This family of proteins bind to heparin, HS, and DS (Ornitz and Itoh, 2001; Penc et al., 1998). To date, the focus of most studies has been on the interactions of heparin and HS with FGF-1 (acidic FGF) and FGF-2 (basic FGF). This likely reflects the earlier discovery of these interactions and greater availability of these growth factors as recombinant proteins. Of the FGF family, only FGF-2 has thus far been shown to bind and be activated by DS. Heparin- and HS–FGF interactions have been associated with biologically meaningful responses, including cellular adhesion, migration, proliferation, and differentiation (Tumova et al., 2000b), and the interaction between DS and FGF-2 has only been studied with respect to cellular proliferation (Penc et al., 1998). In this study however, DS exceeded HS for its capacity to stimulate cell growth in vitro.

The preferred heparin oligosaccharide sequences bound by FGF-1 and -2 have been investigated using structural biology techniques. The crystal structure of FGF-1 (DiGabriele et al., 1998) and FGF-2 (Faham et al., 1996) bound to heparin have both been solved, providing new insights into the topology of GAG–protein binding surface. The ternary FGF–heparin–FGFR complex structure has been described (Schlessinger et al., 2000). For FGF-1, a highly sulfated heparin decasaccharide capable of activating neurite outgrowth in vitro was shown to facilitate the dimerization of two FGF-1 monomers (DiGabriele et al., 1998). Notably, the structures obtained agree with those reported using a tetrasaccharide and hexasaccharide bound to FGF-2 (Faham et al., 1996). No crystallographic data are yet available for DS or for naturally occurring HS bound to FGFs.

DS has also been shown to bind and activate hepatocyte growth factor/scatter factor (HGF/SF), a paracrine growth factor whose receptor, c-met (previously characterized as a protooncogene), is also a transmembrane tyrosine kinase. HGF/SF is produced by fibroblasts, nonparenchymal liver cells and vascular smooth muscle cells. It acts on both epithelial and endothelial cells. Although HGF/SF was initially isolated from hepatocytes as a potent mitogen for regeneration, it has become clear that this growth factor plays a role in morphogenesis, differentiation, motility, and angiogenesis in a variety of cell types (Matsumoto and Nakamura, 1996). Aberrant expression of either the growth factor or its receptor has been implicated in tumorigenesis and metastasis. HGF/SF binds both HS and DS, with the latter interaction being a 10- to 100-fold weaker (Lyon et al., 1994, 1998). The minimal HS size required for binding is an octasaccharide, but higher-affinity binding is found with a decasaccharide or dodecasaccharide (Ashikari et al., 1995; Lyon et al., 1994). The minimal DS binding oligosaccharide has been identified as an octasaccharide with unsulfated IdoA residues in combination with a 4-O-sulfated
GalNAc, Id-GalNAc(4-OSO3) (Lyon et al., 1998). As has been reported for HS, dodecasaccharides bound with a higher affinity.

The mechanism of action for DS- and HS-HGF/SF binding has not been precisely characterized. Although it is clear that the GAGs bind the ligand in a manner reminiscent of FGF signaling, it is not clear whether the HGF/SF receptor is also bound by either the GAG chain or the protein core. The stoichiometry of GAG/PG binding to HGF/SF has not yet been addressed. Unlike the proliferation and signaling data reported on for HS (Sergeant et al., 2000), no comparable work has been published detailing the physiological significance of DS chain binding to this pleiotropic factor. It will be fascinating to see whether DSPGs support cellular proliferation and MAPK signaling and whether DS from varying sources differentially affects these outcomes.

**DS and Infection**

DS and DSPGs have been associated with an ability to modify resistance to infectious disease. Decorin has been identified as a binding target for *Borrelia burgdorferi*, the etiologic agent of Lyme disease. The organism expresses two decorin-binding proteins. Decorin-deficient mice show an increased resistance to *B. burgdorferi* infection, particularly in the joint space (Brown et al., 2001). The actual binding site has not yet been identified, and decorin is not exclusively responsible for the pathogenesis of this disease. HS PGs also serve as targets for a variety of pathogens, including Dengue virus, herpes simplex virus 1 and six other herpes viridae, malarial sporozoites, and *Neisseria gonorrhoea* (Rostand and Esko, 1997; Sawitzky, 1996). Given the similarities in binding observed for peptide growth factors, it is quite possible that DS may also be involved in binding by these microbes. By understanding the structural motifs of GAGs favored by these and other pathogens, novel strategies for vaccine development might be possible.

DS also appears to be involved in infection through mechanisms independent of microbial adherence. Pathogenesis of *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Streptococcus pyogenes* involves release of proteinases that degrade DS-containing PGs (Schmidtchen et al., 2001). The free DS binds to and inactivates the neutrophil-derived cationic antimicrobial peptide α-defensin. Defensins, as well as cathelicidins, are small cationic peptides essential for resistance to infection (Nizet et al., 2001). Inactivation of these peptides through binding to DS would be an effective strategy to increase pathogenesis. A similar association between *P. aeruginosa* infection and PG expression has also been described in newborn mice deficient in the PG syndecan-1 (Park et al., 2001). These mice are relatively resistant to *P. aeruginosa* compared to littersmates expressing syndecan-1. Administration of soluble syndecan-1 restores pathogenicity, an observation that argues against action as an adherence molecule and favors a process similar to antimicrobial peptide inactivation. The dramatic differences in infections seen in these animal models of PG deficiency suggest that further involvement of DSPGs in infectious processes are forthcoming.

**DS and chemokines and cytokines**

Chemokines selectively recruit immune cells and participate in the mediation of the immune response. Approximately 50 different chemokines have been identified so far. Although these molecules have traditionally been categorized as mediators of leukocyte trafficking, their influence on immune surveillance and regulation of acute and chronic immune reactions has also been recognized. Several chemokines, including IL-8, MIP-1α and β (macrophage inflammatory peptides), RANTES (regulated on activation normal T cell expressed and secreted), and MCP-1 (monocyte chemoattractant protein-1) are able to bind GAGs. RANTES exhibits the most variability, binding GAGs in the following order of preference: heparin > DS > HS = CS (Kuschert et al., 1999; Proudfoot et al., 2001). IdoA is likely to be an important player in this interaction because similarly sulfated DS and CS showed very different IC₅₀ values, 22 versus 1200 µg/ml, respectively (Kuschert et al., 1999). Using a different model system, the binding affinities of RANTES were different: heparin > HS = CS-C > DS > CS-A (Martin et al., 2001). These authors found no absolute requirement for GAG binding to initiate RANTES receptor binding. It is unclear whether these conflicting findings represent bona fide cellular or technical differences.

Interferon gamma (IFN-γ) also binds DS (Brooks et al., 2000). Mouse peritoneal mast cells obtained from IFN-γ receptor knockout mice bound to IFN-γ as well as wild-type mast cells and were able to induce nitric oxide production in macrophages. The mast cell INF-γ binding element was identified as DS by using bacterial lyase digestion and confirmed by an inhibition enzyme-linked immunosorbent assay (Brooks et al., 2000).

The importance of GAG–chemokine/cytokine interactions likely lies in the establishment of gradients along the extra-cellular matrix and the facilitation of the binding of the ligands to their receptors. Changes in the local production of chemokines, cytokines, and GAGs in response to tissue injury makes for a versatile signaling system. Membrane-bound and soluble GAGs exhibit opposite effects on chemokine/cytokine receptor binding and activation. This phenomenon has been seen with both RANTES and IFN-γ signaling (Brooks et al., 2000; Kuschert et al., 1999) and may represent a method of modulation. Further study is required to determine the preferred GAG structure bound by the different chemokines and cytokines.

**DS in wound repair**

Skin is organized in two primary layers, the epidermis and dermis. DS derives its name from the dermis, its primary source. As mentioned earlier, DS is a major constituent of the skin and makes up as much as 0.3% of its dry weight. Considering its location and activity in coagulation, cell growth, and immune defense, the function of DS in wound repair is an active area of investigation.

The study of DS in wound repair has involved both descriptive observations of the expression of DSPGs and functional studies of wound DS activity. Following skin injury, the synthesis of several PGs is rapidly induced in a cell-specific pattern. Syndecan-1 increases severalfold on endothelial cells in the wound, decreases on keratinocytes at the advancing edge of
chains differ during successive stages of development and under conditions of disease versus health? Does DS expression and/or structure change during the normal aging process (as has been suggested for HS [Feyzi et al., 1998] and some DSPGs [Carrino et al., 2000])? Does DS play a permissive or preventive role in cellular transformation? Do these extracellular events signal to the cell’s interior? Finally, how is DS release and degradation controlled? The effects of soluble DS on FGF activity and the function of innate antimicrobial peptides make this process particularly important.

It may be premature to discuss the clinical relevance of understanding DS structure and function. Wound healing is certainly a relevant avenue of inquiry. Do chronic wounds lack normal DSPG regulatory mechanisms? Biologically active DS has been isolated from acute surgical wounds; might not later-stage healing wounds demonstrate quantitative or qualitative differences in DS? Studies to answer these questions might also address the question of DS involvement in regulating infection. By better understanding the role of DS in wound healing, new diagnostic and therapeutic strategies for wound repair could be developed. In addition, the importance of DS in systemic disease is also of great clinical interest. Does cardiovascular disease correlate with changes in the vascular endothelial composition of DS, and is this a prerequisite or an outcome of the disease process itself? Is decorin binding of LDL a potential target for intervention in hypercholesterolemia or merely a marker of significant disease progression? Again, the diagnostic and therapeutic implications are significant. Overall, it is likely that studies of DS will continue to expand our understanding of the biological functions of this GAG.

Acknowledgments
R.L.G. is supported by NIH Grant AR45676 and a VA Merit Award. J.M.T. is supported by NIH Grant 5T32CA81211.

Abbreviations
APC, activated protein C; CS, chondroitin sulfate; DS, dermatan sulfate; DSPG, dermatan sulfate proteoglycan; FGF(R), fibroblast growth factor (receptor); GAG, glycosaminoglycan; HCII, heparin cofactor II; HGF/SF, hepatocyte growth factor/scatter factor, HS, heparan sulfate; IdoA, iduronic acid; IFN-γ, interferon-gamma; MAPK, mitogen-activated protein kinase; MIP, macrophage inflammatory peptide; PG, proteoglycan; RANTES, regulated on activation normal T cell expressed and secreted; TGF; transforming growth factor.

References
Dermatan sulfate


