The role of heparan sulphate in inflammation

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Abstract | The polysaccharide heparan sulphate is ubiquitously expressed as a proteoglycan in extracellular matrices and on cell surfaces. Heparan sulphate has marked sequence diversity that allows it to specifically interact with many proteins. This Review focuses on the multiple roles of heparan sulphate in inflammatory responses and, in particular, on its participation in almost every stage of leukocyte transmigration through the blood-vessel wall. Heparan sulphate is involved in the initial adhesion of leukocytes to the inflamed endothelium, the subsequent chemokine-mediated transmigration through the vessel wall and the establishment of both acute and chronic inflammatory reactions.

G-protein-coupled receptor (GPCR). A receptor that is composed of seven membrane-spanning helical segments. These receptors associate with G-proteins, which are a family of trimeric intracellular-signalling proteins with common β- and γ-chains, and one of several α-chains. The α-chain determines the nature of the signal that is transmitted from a ligand-occupied GPCR to downstream effector systems.

Tight junction
A ring of proteins that seals apical epithelium. Tight junction proteins include the integral membrane proteins occludin and claudin, in association with cytoplasmic zona occludens proteins.

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An essential feature of any inflammatory response is the rapid recruitment of leukocytes from the blood to the site of inflammation, usually through post-capillary venules[1,2]. This recruitment process requires leukocytes to migrate through the blood-vessel wall and enter tissues by a multistep mechanism known as extravasation or diapedesis (FIG. 1). Key steps during extravasation are as follows: the initial attachment and rolling of leukocytes on the inflamed endothelium; activation of leukocytes by endothelial-cell-bound chemokines; stable adherence of the activated leukocytes to the endothelium; degradation of the subendothelial basement membrane; and migration of leukocytes, possibly along chemokine gradients, into the target tissue. Once leukocytes have entered the target tissue they can perform various immune activities, such as pathogen elimination and tissue repair. Remarkably, this extravasation process occurs with little or no evidence of damage to the endothelium.

Leukocyte recruitment to inflammatory sites is a complex process, with many molecules involved. Initially the tethering to and rolling of leukocytes on the inflamed endothelium involves up to three calcium-dependent (C-type) lectins, namely lymphocyte (L)-, platelet (P)- and endothelial-cell (E)-selectin, and their counter ligands[3]. L-selectin is constitutively expressed by leukocytes, and P- and E-selectin are expressed on endothelial cells activated by various pro-inflammatory cytokines[3].

Initial adhesion to the endothelium is probably mediated by P-selectin. L-selectin subsequently reduces the rolling velocity of leukocytes and allows the cells to interact, through high-affinity G-protein-coupled receptors, with chemokines that are bound to the inflamed endothelium[4]. Chemokine binding activates leukocyte integrins, a process that enables the leukocytes to form a highly stable interaction with endothelial cells[1]. Once arrested on the endothelial-cell surface, the activated leukocytes traverse the endothelial-cell layer[1,5], usually between the endothelial cells, and passage through the subendothelial basement membrane by deploying various degradative enzymes, such as matrix metalloproteinases and heparanase[2,6]. *In vivo* studies indicate that the complete transmigration process takes 15–45 minutes, although *in vitro* transendothelial migration is reported to occur within 2 minutes[2]. In fact, endothelial cells of post-capillary venules have weak tight junctions, a factor that would favour the rapid passage of leukocytes through this endothelial-cell barrier[7].

Several oligosaccharides and glycosaminoglycans (such as hyaluronan, dermatan sulphate and heparan sulphate)[8] have been implicated in leukocyte transmigration. One glycosaminoglycan that is believed to have multiple roles in the extravasation process is heparan sulphate. This Review focuses on heparan sulphate and, in particular, highlights recent studies that indicate that heparan sulphate is a key player in the regulation of leukocyte transmigration.

HSPGs in the blood-vessel wall

Proteoglycans are proteins to which one or more glycosaminoglycan chains are covalently attached. Heparan sulphate proteoglycans (HSPGs) have the polysaccharide (glycosaminoglycan) heparan sulphate covalently attached as their carbohydrate component. HSPGs are ubiquitously expressed, with different types of HSPG occurring on cell surfaces and throughout the extracellular matrix of all mammalian tissues (BOX 1).

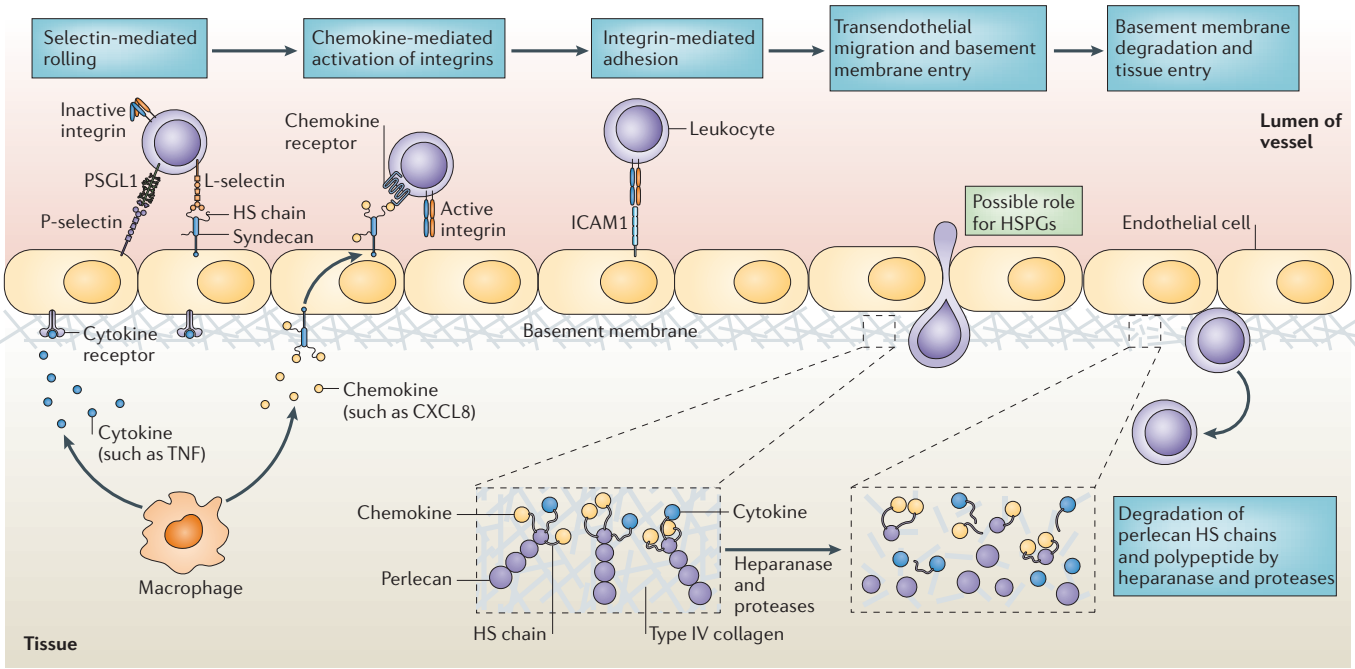

Figure 1 | **The importance of heparan sulphate at different stages during the entry of leukocytes into sites of inflammation.** Following an inflammatory stimulus, macrophages that are resident in tissues produce cytokines, such as tumour-necrosis factor (TNF), that induce the rapid induction of expression of pre-formed platelet (P)-selectin on the surface of endothelial cells. Endothelial-cell (E)-selectin expression can also be induced on the surface of endothelial cells by cytokines (not shown). The interaction between P-selectin and ligands, such as P-selectin glycoprotein ligand 1 (PSGL1), on leukocytes probably initiates rolling on the endothelium. Rolling is further stabilized by lymphocyte (L)-selectin binding to endothelial-cell heparan sulphate (which is depicted here as the heparan sulphate proteoglycan (HSPG) syndecan but other HSPGs might also be involved; see BOX 1). Heparan sulphate also presents chemokines, such as CXC-chemokine ligand 8 (CXCL8) produced by tissue-resident macrophages, to chemokine receptors on leukocytes. This process activates leukocyte integrins and results in more stable leukocyte adhesion. The transcytosis of chemokines across the endothelial-cell barrier is also heparan-sulphate dependent. Once leukocytes have completed transendothelial migration, they interact with chemokines in the underlying basement membrane, the chemokines being presented by HSPGs such as perlecan (depicted), agrin and type VIII collagen. Endothelial-cell heparan sulphate might also assist transendothelial migration by binding proteins, such as kininogen, that regulate vascular permeability[12], although the molecular details of this process are not included here. In addition, for leukocytes to traverse the subendothelial basement membrane, they need to solubilize the basement membrane components by deploying various proteases and the heparan-sulphate-degrading enzyme heparanase. During basement membrane solubilization, heparanase releases growth factors associated with basement membrane HSPGs. These growth factors eventually contribute to a chronic inflammatory response by inducing angiogenesis and tissue remodelling. HS, heparan sulphate; ICAM1, intercellular adhesion molecule 1.

The heparan sulphate chains of HSPGs can have extraordinary structural diversity, owing to differences in the position of the sulphate groups along the polysaccharide chain and epimerization of glucuronic acid residues to iduronic acid[9–11] (FIG. 2). Based on these different structural modifications, it has been estimated that up to 48 different disaccharides could occur in heparan sulphate, although so far only 23 have been detected *in vivo*[9]. Nevertheless, the known heparan sulphate structures have a remarkable diversity that far exceeds that of nucleic acids. This structural heterogeneity, which is usually concentrated in areas of high sulphation along the heparan sulphate chain (FIG. 2), allows heparan sulphate to interact with a wide range of functionally diverse proteins, such as growth factors, cytokines, chemokines, proteases, lipases and cell-adhesion molecules.

There is also evidence that unique structural motifs in heparan sulphate bind to different heparan-sulphate-binding proteins[9–13] despite the polyanionic nature of the molecule, which initially led to the suggestion that it would bind to a wide range of proteins through non-specific electrostatic interactions. The conclusion that heparan-sulphate binding is specific implies that cells and tissues, by modifying the structure of the heparan sulphate chains they express, can change their ability to interact with different heparan-sulphate-binding proteins. The functional consequences of such changes could be considerable.

As with many other tissues, different HSPGs are expressed throughout the blood-vessel wall (TABLE 1). For example, syndecans-1–4 and glypican-1 have been reported to be expressed by various endothelial-cell preparations[12,14–17], but it is probable that different

Polyanionic
A molecule that has many negatively charged chemical entities attached, the most common being negatively charged carboxyl, sulphate and phosphate groups.

Box 1 | Heparan sulphate proteoglycans

There are only five classes of heparan sulphate proteoglycans (HSPGs) that always carry heparan sulphate chains and, consequently, are called 'full-time' HSPGs. These molecules are encoded by 13 genes and account for most (>95%) of the heparan-sulphate-bearing molecules expressed on mammalian cell surfaces and in basement membranes and extracellular matrices[14,37]. There are also several HSPGs that carry heparan sulphate chains only under specific circumstances and, therefore, are referred to as 'part-time' HSPGs. Such molecules include CD44, betaglycan and testican[14,37,126].

The full-time HSPGs that are expressed on cell surfaces consist of four syndecans, which are integral membrane proteins, and six glypicans, which are attached to the cell surface by a glycosylphosphatidylinositol (GPI) anchor. The three extracellular matrix HSPGs are perlecan, agrin and type XVIII collagen. Splice variants of these three HSPGs have also been reported. There is considerable diversity in the size and complexity of the polypeptide core of the various HSPG types; the size varies from 20 kDa for syndecan-4 to ~470 kDa for perlecan. Similarly, the linear heparan sulphate chains vary in size from ~5 to ~70 kDa and when attached to the protein core at multiple sites can dramatically increase the size of the proteoglycan[37]. The heparan sulphate chains are composed of alternating glucuronic acid (GlcA) and *N*-acetylglucosamine (GlcNAc) residues that can carry a range of modifications such as *O*-sulphation at various positions, *N*-deacetylation and *N*-sulphation of GlcNAc residues as well as C5 epimerization of GlcA to iduronic acid[9–11] (FIG. 2).

As a result of these modifications, heparan sulphate has remarkable structural heterogeneity. The modified residues are concentrated in hot spots along the molecular backbone and separated by flexible spacers of low sulphation (FIG. 2). The well-known anticoagulant heparin is a highly sulphated version of heparan sulphate that has a much more restricted cellular distribution, being mainly present in mast-cell granules[117]. Finally, it should be noted that some HSPGs, such as syndecan-1 (REF. 127) and syndecan-4 (REF. 128), also carry chondroitin sulphate chains and this glycosaminoglycan might contribute to the biological activities of the syndecan molecules[31].

vascular beds can specifically express different syndecan and glypican isoforms. Furthermore, it is known that various pro-inflammatory cytokines can upregulate the surface expression of specific HSPGs on endothelial cells, such as syndecan-1 (REF. 12). Leukocytes have a similar diversity of HSPG expression to endothelial cells, although peripheral-blood lymphocytes, monocytes and neutrophils express only very low amounts of cell-surface heparan sulphate, predominantly as syndecan-4 (REFS 18–22).

It is only following activation of leukocytes by various cytokines or the induction of proliferation or differentiation that high HSPG expression is observed (TABLE 1). For example, human monocytes exposed to interleukin-1 (IL-1) rapidly and specifically express syndecan-2 (REF. 18), whereas the differentiation of monocytes into macrophages results in high expression of syndecan-1, -2 and -4 (REF. 21). Similarly, following antigen-driven activation, $CD4^+$ T cells upregulate their expression of both syndecans and glypicans[22]. However, B cells, when they differentiate into plasma cells, selectively produce high levels of syndecan-1 (REFS 15,23).

Further complexity is introduced by the 'part-time' HSPGs (BOX 1), which include: the type III transforming growth factor-β (TGFβ) receptor, betaglycan, which is expressed by endothelial cells[24] and macrophages[21]; and the v3 isoform of CD44 (REF. 25), which has been reported to be expressed by macrophages[26], cytokine- and lipopolysaccharide-activated monocytes[26] and endothelial cells[27]. Furthermore, the part-time HSPG testican is produced by endothelial cells, although it is probably a secreted proteoglycan rather than being expressed at the cell surface[28,29].

It is conceivable that changes in the HSPG polypeptide chain alter the structure of the cell-surface heparan sulphate displayed by endothelial cells and leukocytes. Similarly, the extracellular polypeptide domains of each of the syndecan-family members might be of functional importance as they are highly divergent in sequence[30], with some having chondroitin and/or dermatan sulphate chains attached. In fact, some growth factors bind to syndecans through both chondroitin and heparan sulphate chains[31], whereas certain chemokines and growth factors interact with the heparan sulphate chains and polypeptide core of syndecans[31,32].

The subendothelial basement membrane, which can be 20–100-nm thick[2] and represents a formidable barrier to leukocyte extravasation, is also rich in extracellular HSPGs (FIG. 2). An important feature of this structure is that pericytes, which are associated with the abluminal side of all post-capillary venules, probably contribute to the synthesis of venular basement membrane HSPGs and other components[33]. Basement membranes represent a complex network of interacting molecules, the main constituents being laminin, type IV collagen, entactin (also known as nidogen-1) and extracellular HSPGs[34]. Laminin, of which there are multiple isoforms[35], assembles to form the initial polymeric scaffold to which the other basement membrane components are attached[36].

Perlecan, agrin and type XVIII collagen are ubiquitously expressed HSPGs that are found in subendothelial basement membranes[14,37] and can potentially function in several ways to regulate leukocyte extravasation. First, as with other basement membranes, it can have an essential structural role by interacting with other membrane components and forming a stable scaffold[36]. Also, heparan sulphate is highly hydrated and therefore has a key space filling and molecular sieving role in basement membranes[36]. Second, because of its structural properties, it can function as an important physical barrier to leukocyte transmigration[6]. Third, it can bind and sequester numerous heparan-sulphate-binding proteins that regulate leukocyte migration and wound healing, such as growth factors[38], cytokines[39] and chemokines[40]. Last, it can provide, in combination with the proteinaceous components of the basement membrane, adhesion ligands for migrating leukocytes[41,42]. In the following sections, I consider in more detail the evidence supporting the role of heparan sulphate in various stages of leukocyte extravasation.

Heparan sulphate as a vascular adhesion ligand

It has been known for several years that L-selectin and P-selectin can interact with heparin and heparan sulphate[12]; however, because of structural differences between heparin and heparan sulphate, experimental results obtained with heparin are often difficult to interpret. An early study showed that heparan sulphate is a ligand expressed by endothelial cells for L-selectin[43]. Further reports showed that heparin and heparan sulphate can directly interact with L- and P-selectin

Pericytes
Cells embedded in the vascular basement membrane of microvessels that are thought to be derived from the vascular smooth muscle lineage. They make close cellular contact with endothelial cells and this interaction is essential for the maintenance of vessel function, as well as for the regulation of angiogenesis and vascular remodelling.

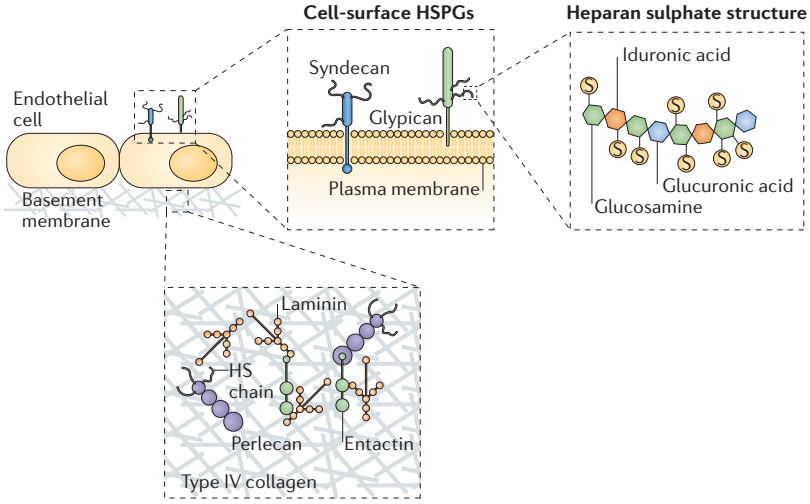

Figure 2 | **The structure of heparan sulphate and its location in the blood-vessel wall.** In the blood-vessel wall, heparan sulphate can be expressed as follows: in the plasma membrane of endothelial cells as the heparan sulphate proteoglycans (HSPGs) syndecan and glypican; or in the subendothelial basement membrane as perlecan, agrin (not shown) and type XVIII collagen (not shown), which associate with the other main basement membrane components, laminin, type IV collagen and entactin. Heparan sulphate is a linear polysaccharide consisting of alternating *N*-acetylglucosamine and glucuronic acid residues that in certain 'hotspots' are *O*- and *N*-sulphated at various positions. Combined with the epimerization of glucuronic acid to iduronic acid, this results in a region with great structural diversity. The iduronic acid residues also add rotational flexibility, which results in the polysaccharide adopting a helical conformation. Because of their great structural diversity, a wide range of proteins, such as chemokines and growth factors, can bind to the hot spots of heparan sulphate chains, with many proteins binding to unique saccharide sequences. HS, heparan sulphate.

Intravital microscopy
This is used for examination of biological processes, such as leukocyte–endothelial-cell interactions, in living tissue. In general, translucent tissues are used, such as the mesentery or cremaster muscle, which can be exposed and mounted for microscopic observation.

Sialylated
A molecule that has the monosaccharide sialic acid (also known as neuraminic acid) covalently attached. Most of the sialic-acid residues are attached to the non-reducing termini of oligosaccharide chains linked to proteins or lipids.

Fucosylated
A molecule that has the monosaccharide L-fucose covalently attached, which is normally a terminal rather than an intra-chain residue in carbohydrates. L-Fucose is usually associated with oligosaccharide chains linked to proteins or lipids.

in vitro[44,45] and interfere with selectin-mediated leukocyte rolling[45]. Furthermore, removal of cell-surface heparan sulphate from aortic endothelial cells by bacterial heparitinase digestion inhibited L-selectin-mediated binding of monocytes to the endothelial cells *in vitro*[45].

Further studies have shown that heparin and heparin-derived oligosaccharides have anti-inflammatory activity *in vivo* and function by blocking the action of L- and P-selectin[46–48], an effect that is independent of the anti-coagulant activity of heparin[48]. Furthermore, by using intravital microscopy, it has been possible to show that low-molecular-weight heparin can substantially block leukocyte rolling, adhesion and extravasation through tumour-necrosis factor (TNF)-activated endothelium[49]. By contrast, there is little evidence to indicate that heparan sulphate is a ligand for E-selectin [44], although there is a report that heparin- and syndecan-1-associated heparan sulphate can inhibit the binding of recombinant E-selectin to bovine capillary endothelial cells[50].

Although these studies show that heparan sulphate can interact with L- and P-selectin, in most cases they do not prove that heparan sulphate is the physiological ligand. In fact, there is a large body of evidence indicating that all three selectins interact *in vivo* with mucins, rather than heparan sulphate, expressed by the endothelium. For example, P- and E-selectin expressed by endothelial cells can recognize sialylated, fucosylated mucins carried by leukocytes, with *O*-linked sialyl Lewis X and adjacent acidic amino acids, and sulphated tyrosine residues in the polypeptide portion of the leukocyte surface molecule PSGL1 (P-selectin glycoprotein ligand 1) being the dominant ligands[51] (FIG. 1). Similarly, L-selectin expressed by leukocytes can interact with sulphated, sialylated and fucosylated mucins on endothelial cells. It should be noted, however, that L-selectin has the important dual function of both participating in the interaction of leukocytes with the inflamed endothelium and mediating the selective binding of lymphocytes to high endothelial venules (HEVs)[52]. Recent studies have unequivocally shown that 6-sulpho sialyl Lewis X, which is usually attached to CD34, podocalyxin and endoglycan, is the dominant L-selectin ligand expressed by HEVs[53,54].

At sites of chronic inflammation, the microvasculature gains an HEV-like appearance and can bind the monoclonal antibody MECA-79, which is specific for 6-sulpho sialyl Lewis X[51,52]. Therefore, it seems highly probable that during a chronic inflammatory response the dominant L-selectin ligand is not heparan sulphate but a sulphated, sialylated and fucosylated mucin, namely 6-sulpho sialyl Lewis X. The same cannot be said during an acute inflammatory response, when post-capillary venules lack the HEV ligand for L-selectin.

In fact, a recent report has provided strong evidence that heparan sulphate is the dominant L-selectin ligand on the inflamed endothelium during acute inflammatory responses[55]. In this study[55], *N*-acetyl glucosamine *N*-deacetylase-*N*-sulphotransferase, an enzyme that attaches *N*-sulphate groups to heparan sulphate, was selectively inactivated in either endothelial cells or leukocytes. This genetic manipulation resulted in partially sulphated heparan sulphate being produced by either endothelial cells or leukocytes. The endothelial-cell-specific knockout mice were found to have an impaired influx of neutrophils into tissues in several inflammatory models, with this effect being partially explained by a reduction in the ability of L-selectin to bind to heparan-sulphate-defective endothelial cells. By contrast, it was found that, when leukocytes expressed partially sulphated heparan sulphate, their entry into inflammatory sites was unimpaired.

Collectively, these data indicate that during an acute inflammatory response endothelial-cell heparan sulphate might be an important L-selectin ligand but, as previously proposed, P-selectin (and presumably E-selectin) probably interact with mucinous ligands on leukocytes[3]. Indeed, the early observation that P-selectin has a lower affinity for heparin and heparan sulphate than L-selectin supports the view that, *in vivo*, heparan sulphate is an L-selectin rather than a P-selectin ligand[46].

It remains to be determined, however, whether 'full-time' HSPGs, such as the syndecans and glypicans, or part-time HSPGs, such as betaglycan and CD44, display the heparan sulphate ligand for L-selectin on inflamed endothelium. The most probable candidate is the syndecans (FIG. 2) but, paradoxically, syndecan-1-deficient mice have increased inflammatory responses and increased leukocyte–endothelial-cell interactions, leading to the intriguing conclusion that syndecan-1 is a negative regulator of leukocyte–endothelial-cell adhesion[56,57].

Table 1 | **Different full-time heparan sulphate proteoglycans involved in inflammation**

HSPG	Location	Cellular expression pattern	Function of heparan sulphate chains in inflammation
Syndecan-1–4	Cell surface, integral membrane proteins Shed syndecan at inflammatory sites	• Resting lymphocytes, monocytes, neutrophils, eosinophils and platelets express low levels of syndecan-4 (REFS 18–22,129,130) • Macrophages express syndecan-1, -2 and -4 (REFS 21,131) • Endothelial cells can express syndecan-1–4 (REFS 15,17) • Endothelial-cell and monocyte expression of syndecans is upregulated by cytokines[14,18,32,132,133] • Proliferating and/or activated T cells upregulate expression of syndecans[22] • Pre-B cells and plasma cells express high levels of syndecan-1 (REFS 15,23)	• Ligand for L-selectin[55] • Cell-surface receptor for chemokines[67] • Mediator of chemokine transcytosis[67,75,81] • Mediator of adhesion to basement membrane components[30] • Modulator of angiogenic growth factor activity[10] • Internalization receptor for heparanase?[92] • Signalling molecule[30]
Glypican-1–6	Cell surface, glycosylphosphatidyl-inositol-linked proteins	• Vascular endothelial cells express glypican-1 (REF. 16) • Macrophages in inflammatory sites express glypicans[134] • Proliferating and/or activated $CD4^+$ T cells upregulate expression of glypicans[22]	• Functions possibly similar to syndecans[104]
Perlecan	Basement membrane and/or extracellular matrix	• Subendothelial basement membrane expresses perlecan[14,37]	• Barrier to leukocyte migration[37] • Stabilizes chemokine gradients[67] • Acts as a reservoir of heparan-sulphate-binding growth factors and/or cytokines[38,39]
Type XVIII collagen	Basement membrane and/or extracellular matrix	• Subendothelial basement membrane expresses type XVIII collagen[14,37]	• Functions similar to perlecan • Ligand for L-selectin[41,42]
Agrin	Basement membrane and/or extracellular matrix	• Subendothelial basement membrane expresses agrin[14,37]	• Functions similar to perlecan

HSPG, heparan sulphate proteoglycan; L-selectin, lymphocyte selectin.

One possible explanation for this result is that the ectodomain of syndecan-1 is shed by epithelial cells in inflammatory sites and competes for pro-inflammatory chemokines that are destined for the endothelium[58]. It is conceivable, however, that some of the effects observed in syndecan-1-deficient mice might be a result of the lack of the polypeptide portion of the HSPG rather than the heparan sulphate chains of the proteoglycan. For example, syndecans have conserved cytoplasmic domains that interact with kinases and cytoskeletal components and could therefore deliver intracellular signals[59,60]. In addition, the extracellular polypeptide domain of syndecan-1 could interact with other cell-surface molecules and modify leukocyte adhesion[30].

The possibility still remains that other heparan-sulphate-binding molecules might also participate in the adhesion of leukocytes to the inflamed endothelium. It has been shown that the leukocyte integrin CD11b–CD18 (also known as Mac-1) binds to heparan sulphate and heparin[61,62], with a binding affinity that is comparable to that for other integrin ligands[62]. In fact, under conditions of flow, this interaction can stabilize adhesion initiated by E-selectin[62]. Furthermore, when it is enzymatically inactive owing to an inappropriate pH[63], mutational modification[64,65] or being in pro-heparanase form[66], the heparan-sulphate-degrading enzyme heparanase can interact with T-cell heparan sulphate and promote the adhesion of these cells to extracellular matrix components under shear flow conditions through a β_1-integrin- or CD44-dependent mechanism. The physiological importance of this interaction remains to be determined but it could be important not only during leukocyte–endothelial-cell adhesion but also during the transmigration of leukocytes through basement membranes (see later).

High endothelial venules (HEVs). HEVs are specialized post-capillary venules in lymphoid organs through which lymphocytes enter. L-selectin plays an essential part in this entry process by mediating the initial interaction of lymphocytes with HEVs.

Modulation of chemokines by heparan sulphate

So far, 45 chemokines have been identified, many of which have a crucial role in recruiting different leukocyte subsets across the endothelium during inflammatory responses[4]. Of particular relevance to this article, however, is that all chemokines seem to have the ability to interact with heparan sulphate on the surface of cells and in the extracellular matrix[67]. In fact, structural studies have shown that chemokines have four different modes of binding heparan sulphate, based on the way they oligomerize and fold a carboxy-terminal stretch of positively charged amino acids[40]. These different heparan-sulphate-binding sites, which are separate from the G-protein-coupled-receptor-binding domain of the molecule, explain how the various chemokines can interact with unique heparan sulphate sequences[40,68].

There are at least three ways in which heparan sulphate can control the function of chemokines, with

mounting evidence indicating that chemokines are unable to carry out their full range of functional activities in inflammatory sites unless they can bind to heparan sulphate. First, at the physicochemical level, the interaction of chemokines with heparan sulphate can protect them from proteolysis[69,70] and induce them to oligomerize; oligomerized chemokines are thought to be more active than their monomeric counterparts[71–73]. The functional relevance of oligomerization has been questioned, however, as many chemokines readily form dimers and tetramers in the absence of heparan sulphate[40], although others have suggested that higher order oligomers induced by heparan sulphate are required for maximal chemokine activity[74]. In fact, CC-chemokine ligand 5 (CCL5; also known as RANTES) that has been mutated to remove its heparan-sulphate-binding site can function as a dominant-negative inhibitor by forming non-functional heterodimers with wild-type CCL5; the resultant heterodimers are unable to produce heparan-sulphate-induced higher order oligomers[74].

Second, heparan sulphate plays an essential role in immobilizing chemokines on the luminal surface of endothelial cells[55,75] and might establish chemokine gradients on the vascular endothelium[75,76]. Without chemokines being displayed in this way leukocytes are unable to form stable, integrin-mediated interactions with the endothelium and cannot migrate in a directional manner through the blood-vessel wall. A recent study highlighted this point by showing that endothelium-bound chemokines, but not soluble chemokines, induced a rapid extension of bent (inactive) lymphocyte function-associated antigen 1 (LFA1). The extended form of this integrin can then interact with endothelial-cell intercellular adhesion molecule 1 (ICAM1) and trigger stable cell adhesion[77]. Whether leukocytes follow chemokine gradients during transmigration, however, is controversial, with shear forces[78] and chemorepulsion[79,80] possibly providing alternative directional cues.

Third, active transport of chemokines across the endothelial-cell layer, a process known as transcytosis, seems to be dependent on heparan sulphate. During an inflammatory response, perivascular leukocytes, such as tissue-resident macrophages and mast cells, release chemokines into the surrounding environment. For these chemokines to be displayed on the luminal surface of vascular endothelial cells, usually bound to heparan sulphate, they need to be transported across the endothelial-cell barrier. Theoretically this can be achieved by the diffusion of the chemokines through gaps between endothelial cells or by transcytosis.

Until recently the evidence for transcytosis relied on immuno-electron microscopy studies that followed the transport of chemokines across the endothelial-cell barrier[75,81]. These studies indicated that transcytosis does occur *in vivo*; for example, transported CXC-chemokine ligand 8 (CXCL8; also known as IL-8) is presented to leukocytes on the tips of endothelial-cell microvilli. Furthermore, endothelial transcytosis of CXCL8 depends on the heparin-binding domain of the chemokine, indicating that the process is heparan-sulphate dependent[75,81]. A recent study has confirmed this model by showing that endothelial cells deficient in highly sulphated heparan sulphate are much less efficient at mediating chemokine (CXCL8) transcytosis[55].

Transcytosis
The process of transport of material across a cell layer by uptake on one side of the cell into a coated vesicle. The vesicle might then be sorted through the trans-Golgi network and transported to the opposite side of the cell.

Immuno-electron microscopy
A procedure that uses antibodies, usually coupled to electron-dense particles such as colloidal gold, to determine the location of molecules on the cell surface and in cells at the electron-microscopic level.

Basement membrane heparan sulphate

Once leukocytes have traversed the endothelial-cell layer of the blood-vessel wall, the subendothelial basement membrane represents an important physical barrier to further migration. The basement membrane HSPGs perlecan, type XVIII collagen and agrin are key constituents of the structure (TABLE 1) and, consequently, make a considerable contribution to the barrier function of basement membranes. To pass through this barrier, leukocytes deploy a range of degradative enzymes.

Although many proteases have been described that can participate in basement membrane disassembly[82,83], the endo-β-glucuronidase heparanase, which can cleave the heparan sulphate chains of HSPGs, is known to be an essential participant in this process[84–87] (FIG. 3). Indeed, degradation of basement membrane heparan sulphate by heparanase not only facilitates the passage of leukocytes through the basement membrane barrier but also seems to be a principal contributor to the increased vascular permeability observed at inflammatory sites[88]. It should be noted, however, that processes other than heparanase-mediated HSPG degradation could disrupt the integrity of basement membrane heparan sulphate and aid cell invasion, although the importance of these alternative mechanisms remains to be proven. For example, reactive oxygen species that are produced by infiltrating leukocytes could depolymerize heparan sulphate[89]. Also, sulphatases that are synthesized by endothelial cells and monocytes have been shown to desulphate extracellular-matrix-bound heparan sulphate, a modification that is likely to destroy the barrier function of HSPGs[90].

There are several potential sources of heparanase during an inflammatory response. Endothelial cells and various leukocyte populations have been reported to produce heparanase, particularly following exposure to pro-inflammatory cytokines, and platelets contain high levels of heparanase, which is released when they degranulate[6,85,90]. In fact, it has been proposed that degradation of the basement membrane is a collaborative process between endothelial cells, leukocytes and platelets, with the different enzymes required for basement membrane solubilization being donated by the various cell types[6]. A recent study supports this view: it was reported that endothelial-cell-derived, rather than leukocyte-derived, heparanase aided leukocyte extravasation in a delayed-type hypersensitivity model[88]. Under such circumstances, it is conceivable that heparanase released by platelets or endothelial cells is taken up by leukocytes, particularly as cell-surface receptors for heparanase have been postulated[64,87,91,92] and the cell-surface expression of degradative enzymes is known to aid the migration of invading cells in a directional manner[93].

Heparanase not only aids leukocyte invasion of the basement membrane but also regulates inflammatory responses in several other ways (FIG. 3). First, many growth factors and cytokines interact with heparan sulphate and,

consequently, are stored in the basement membrane bound to HSPGs[38,39]. These stores can be liberated by heparanase and made available for tissue remodelling and angiogenesis[38,39,86,87], with heparan sulphate on the surface of target cells also being important for signalling through growth-factor receptors[10]. Such processes are crucial for establishing a chronic inflammatory response. Second, as mentioned earlier, enzymatically inactive pro-heparanase can bind to T cells and aid the adhesion of these cells to extracellular matrix components[66], a process that presumably aids T-cell migration through the basement membrane. Third, heparanase not only cleaves heparan sulphate chains that are associated with basement membrane HSPGs, such as perlecan[94], but also can potentially degrade heparan sulphate that is expressed on the surface of migrating leukocytes[94]. The effect this has on leukocyte migration is unclear, although, based on tumour metastasis studies, it might aid rather than inhibit cell invasion[94], possibly by preventing the interaction of the heparan sulphate chains of syndecan with basement membrane components[30]. Fourth, heparanase might inhibit an ongoing inflammatory response by releasing a trisulphated disaccharide from basement membrane HSPGs that inhibits TNF production by T cells and macrophages[95].

Last, it should be noted that although the sub-endothelial basement membrane acts as a general physical barrier to leukocyte migration, in some cases the basement membrane heparan sulphate chains can directly aid leukocyte extravasation (TABLE 1). Therefore, based on their ability to interact with chemokines, basement membrane heparan sulphate molecules can form stable chemokine gradients that may facilitate leukocyte transmigration[67]. Similarly, the heparan sulphate chains attached to type XVIII collagen, and not other basement membrane HSPGs, might assist leukocyte migration by acting as L-selectin ligands, particularly under pathophysiological conditions[41,42]. Theoretically, it is possible that degradation of basement membrane HSPGs by heparanase could eventually destroy these chemokine gradients and L-selectin ligands, but little is known about this possibility.

Vascular HSPGs as regulators of inflammation

It is clear that HSPGs and the heparan sulphate chains they carry have an important role in many aspects of an inflammatory response, from the initial adhesion of leukocytes to the endothelium to the subsequent leukocyte extravasation and establishment of a chronic inflammatory response. It seems highly probable, therefore, that vascular beds can modulate inflammatory responses by changing the chemical structure of the heparan sulphate chains they express. Indeed, there is good evidence that endothelial cells in both normal and inflamed synovium express syndecan-3, but heparan sulphate chains on syndecan-3 bind CXCL8 only under inflammatory conditions[96].

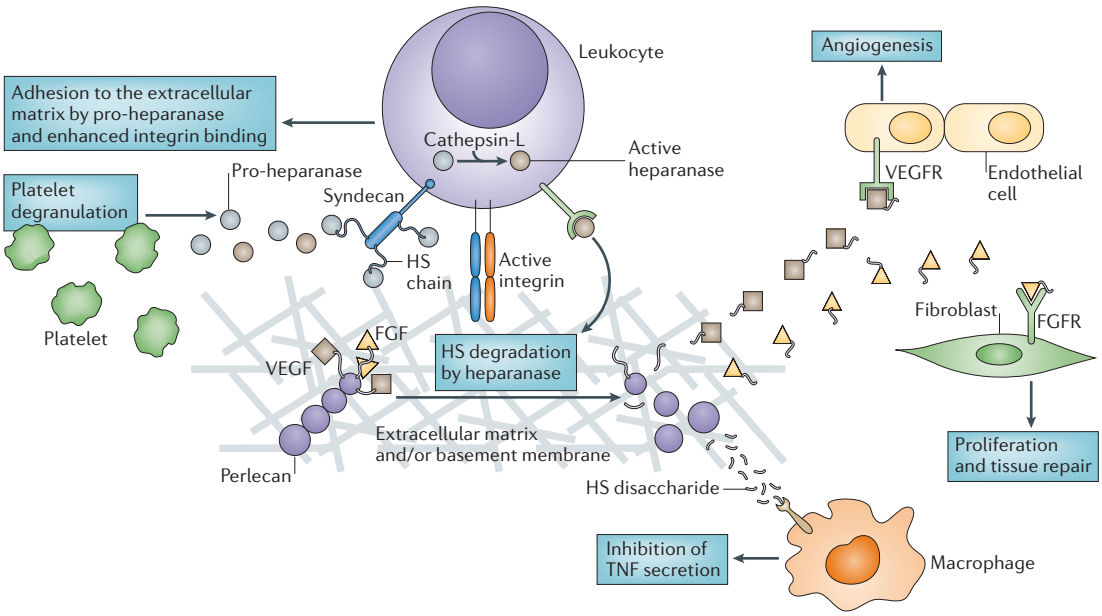

Figure 3 | **The multiple roles of the endoglycosidase heparanase in leukocyte migration through the vessel wall.** Heparanase occurs as an enzymatically inactive pro-heparanase that can be converted to an active enzyme following intracellular processing by proteases, such as cathepsin-L. Pro-heparanase can aid adhesion of leukocytes to extracellular matrices and the basement membrane by binding to components of the extracellular matrix and basement membrane. This interaction further increases leukocyte adhesion by activating extracellular-matrix-binding integrins. During inflammation, heparanase derived from degranulating platelets or directly synthesized by leukocytes or endothelial cells, has multiple roles. It can aid cell invasion by degrading heparan sulphate proteoglycans (HSPGs) and can release heparan-sulphate-bound growth factors that initiate angiogenesis (such as vascular endothelial growth factor, VEGF) or activate tissue repair (such as fibroblast growth factors, FGFs). A heparanase-liberated sulphated disaccharide also inhibits tumour-necrosis factor (TNF) production by macrophages and, therefore, acts as a negative regulator of inflammation. FGFR, FGF receptor; HS, heparan sulphate; VEGFR, VEGF receptor.

Similarly, the heparan sulphate chains carried by syndecan-2 have binding sites for CXCL8 when the HSPG is expressed by human umbilical vein endothelial cells[32] but not when it is produced by human monocyte-derived macrophages[18]. Furthermore, various growth factors have been shown to bind to different saccharide motifs in heparan sulphate, with cells and tissues changing their expression of such motifs during differentiation and development[10]. In the context of inflammation, one could predict that these changes in growth-factor binding would be important for the angiogenesis and tissue remodelling that is associated with chronic inflammatory responses[56,97–100].

Further regulation of heparan-sulphate function can occur by changing the accessibility of the polysaccharide at sites of inflammation. For example, protease-mediated shedding of HSPG ectodomains from cell surfaces has been reported[101], with plasmin[102] and certain matrix metalloproteinases[103–105] being implicated in this process. This shedding not only would result in the loss from the endothelium of adhesion ligands and binding sites for chemokines, but also the shed ectodomains and their associated heparan sulphate chains could compete with their cell-surface counterparts for various ligands. In fact, shed CXCL8–heparan-sulphate–syndecan-1 complexes have been shown to inhibit CXCL8-mediated transendothelial migration of neutrophils[102]. Conversely, shed chemokine–syndecan-1 complexes can produce chemokine gradients that aid neutrophil migration[76].

Furthermore, heparan-sulphate-binding proteins have been described that could interact with heparan sulphate and mask ligand-binding sites; two important binding proteins are platelet factor 4 (PF4) and histidine-rich glycoprotein (HRG). Both PF4 and HRG can bind to heparan sulphate and mask growth-factor-binding sites[106,107], but HRG can also block heparanase cleavage sites[108]. PF4 is released by degranulating platelets and consequently should often be present during inflammatory responses. HRG is an abundant plasma protein that has the interesting property of binding strongly to heparan sulphate under conditions of acidosis and elevated concentrations of Zn^{2+} (zinc ions), an environment that is associated with tissue injury and chronic inflammation[109]. Based on these data, PF4 and HRG would be expected to be important negative regulators of an ongoing inflammatory response.

Therapeutic implications

Because heparan sulphate is associated with many aspects of inflammation, molecules that interfere with heparan-sulphate function have considerable potential as anti-inflammatory agents. In this regard, it has been known for many years that heparin has anti-inflammatory activity[110–115], most probably by interfering with heparan-sulphate-dependent stages of leukocyte extravasation[13,47,111,115–117]. A principal difficulty with heparin, however, is that it is structurally diverse and therefore has many effects *in vivo*, some of which are undesirable. As there is considerable evidence that different heparan-sulphate-binding proteins interact with unique saccharide sequences in heparan sulphate[9–11], it should be possible to design small heparan-sulphate mimetics that specifically disrupt various stages of leukocyte transmigration[116]. In fact, several mimetics have already been developed that have potential anti-inflammatory activity by blocking heparanase activity[118–123], and by disrupting the interaction of chemokines[122] and growth factors[118,119,122,123] with heparan sulphate. Of particular interest was the finding that the binding specificity of the mimetics is crucially dependent on the relative position of negatively charged sulphate groups in the molecules[122]. This finding supports the view that heparan-sulphate mimetics can be synthesized that specifically target certain heparan-sulphate–protein interactions.

An alternative to the use of heparan-sulphate mimetics is the synthesis of small peptides that resemble the heparan-sulphate-binding sites of cytokines, chemokines and adhesion molecules. One peptide of this type has already been produced that corresponds to the heparan-sulphate-binding region of interferon-γ. It interferes with the binding of interferon-γ and some chemokines to glycosaminoglycans and is effective in delaying the rejection of skin allografts in rats[124]. Another approach is to manufacture chemokine variants that have a defective heparan-sulphate-binding region. Such molecules interfere with chemokine function by preventing heparan-sulphate-dependent oligomerization of chemokines[73,74].

Future prospects

There is now ample evidence that the glycosaminoglycan heparan sulphate participates in many aspects of inflammation. Nevertheless, much still remains to be understood. For example, there are indications that vascular beds can regulate inflammatory responses by changing the HSPGs they produce[96], but far more experimental evidence is required to verify this attractive hypothesis. Conversely, there is little evidence that heparan sulphate on the surface of leukocytes participates in leukocyte adhesion to the endothelium[55]; however, this view seems overly simplistic as numerous stimuli can dramatically upregulate HSPG expression by leukocytes (TABLE 1). The source of heparanase used to solubilize HSPGs in the subendothelial basement membrane is also controversial. Is it mainly endothelial-cell derived, as recently reported[88], or does it come from other sources? Certainly, activated T cells produce large amounts of heparanase[85,90,125] and this might be expected to aid T-cell extravasation. In addition, one might predict that platelets, which release substantial quantities of pre-formed heparanase on degranulation[85,90], could also participate in basement membrane degradation. The issue will probably only be resolved when mutant mouse strains are available that have cell-specific deletion of heparanase activity.

Furthermore, limited studies with heparan-sulphate mimetics and related compounds indicate that specifically disrupting heparan-sulphate–protein interactions represents a viable approach for the design of new anti-inflammatory drugs. Clearly, analysis of the participation of heparan sulphate in inflammatory responses has reached an interesting stage with many future avenues of research now possible.

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