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REVIEW

Heparan sulfate as a regulator of inflammation and immunity

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Abstract

Heparan sulfate is found on the surface of most cell types, as well as in basement membranes and extracellular matrices. Its strong anionic properties and highly variable structure enable this glycosaminoglycan to provide binding sites for numerous protein ligands, including many soluble mediators of the immune system, and may promote or inhibit their activity. The formation of ligand binding sites on heparan sulfate (HS) occurs in a tissue- and context-specific fashion through the action of several families of enzymes, most of which have multiple isoforms with subtly different specificities. Changes in the expression levels of these biosynthetic enzymes occur in response to inflammatory stimuli, resulting in structurally different HS and acquisition or loss of binding sites for immune mediators. In this review, we discuss the multiple roles for HS in regulating immune responses, and the evidence for inflammation-associated changes to HS structure.

KEYWORDS

chemokines, cytokines, heparan sulfate, inflammation, leukocyte

1 | INTRODUCTION

Heparan sulfate (HS) is a ubiquitous but highly structurally variable polysaccharide found on cell surfaces and in extracellular matrices (ECMs), conjugated to one of several types of core protein. Due to its strong anionic properties and the numerous possible combinations of modifications, HS can bind to over 400 soluble protein mediators and cell-surface receptors,[1] with disparate effects on their activity.

HS plays well-established roles in the formation of chemokine gradients on the vascular endothelium and in regulating the activity of chemokines, cytokines, and growth factors through physical sequestration in the matrix and protection from enzymatic proteolysis (recently reviewed in Ref. 2). Here, we briefly outline our current knowledge of the role of HS in these processes, then discuss other currently less appreciated roles for HS in immune regulation, including direct signaling through TLR4, involvement in phagocytosis, and regulating the interaction of inflammatory mediators with their receptors. HS additionally functions in self versus non-self discrimination by the innate immune system through its role as a regulator of the alternative pathway of complement activation, which is reviewed in a number of excellent publications.[3–5] Here again, it is the structural variability of HS that enables it to perform various functions in a tissue- and context-specific manner, acting either as an inhibitor of the complement cascade through binding factor H on self surfaces and thus accelerating C3b inactivation, or as an activator through binding the stabilizing factor properdin on apoptotic cells.

HS is found covalently attached to one of several types of core protein, thus forming proteoglycans. The two most abundant types of cell-surface HS core protein are the trans-membrane syndecans and the GPI-anchored glypicans, but others such as the secreted proteins perlecan and agrin are also decorated with HS. In addition, some proteins, such as isoform 3 of the hylaruonan receptor (CD44), and betaglycan (TGFβR3) are "part-time HSPGs" in that they bear HS chains in certain tissues or under certain conditions. HS biosynthesis occurs in the Golgi network via the combined actions of more than 25 enzymes, which has been recently and comprehensively reviewed.[6] Briefly, biosynthesis begins with the assembly of a tetrasaccharide linkage region at glycosaminoglycan (GAG) attachment sites on the core protein, then the linear HS polysaccharide backbone is elongated by the sequential addition of alternating glucuronic acid and N-acetylglycosamine residues, catalyzed by enzymes of the exostosin (EXT) family. Concurrently, HS chains undergo extensive enzymatic modification beginning with N-de-acetylation/N-sulfation, and epimerization of some glucuronic acid residues to iduronic acid by the D-glycuronyl C5-epimerase (GLCE). Sequence complexity is further increased by sulfation at various

Abbreviations: A1AT, alpha-1 anti-trypsin; APC, antigen-presenting cell; DAMP, damage-associated molecular pattern; DC, dendritic cell; ECM, extracellular matrix; EXT, exostosin; EXTL, exostosin-like; FGF-2, fibroblast growth factor 2; GAG, glycosaminoglycan; HEV, high endothelial venule; HS, heparan sulfate; HS2S, heparan sulfate 2-O-sulfotransferase; HS3ST, heparan sulfate 3-O-sulfotransferase; HS6ST, heparan sulfate 6-O-sulfotransferase; HSPG, heparan sulfate proteoglycan; NDST, N-deacetylase/N-sulfotransferase; PMN, polymorphonuclear neutrophil; SLE, systemic lupus erythratomatus; TGFβR3, transforming growth factor beta receptor 3; WT, wild-type.

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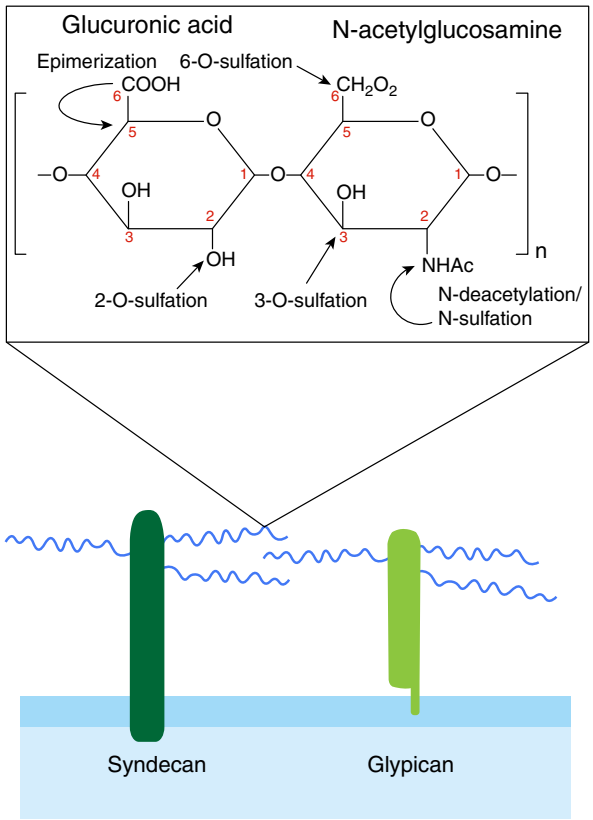

FIGURE 1 Heparan sulfate chains are extensively modified. Biosynthesis of heparan sulfate begins by attachment of a tetrasaccharide linkage region to the core protein (e.g., syndecan and glypican) and elongation of the polysaccharide backbone by alternate additions of glucuronic acid and N-acetylglucosamine residues by the EXT and EXTL enzymes. This is followed by N-deacetylation and N-sulfation of N-acetylglucosamine residues by the NDST enzymes, and epimerization of some glucuronic acid residues to iduronic acid by the D-glucuronyl C5-epimerase GLCE. Sulfotransferase enzymes (HS2ST, HS3STs, and HS6STs) then add sulfate groups at the 2-O-, 3-O-, and 6-O- positions of the sugar ring

positions by 2-O-, 3-O-, and 6-O-sulfotransferases that act in a template-independent manner, producing a varied and heterogeneous structure (Fig. 1). The requirement for previous modifications to form the substrate for subsequent reactions results in highly sulfated domains flanked by less sulfated transition zones, separated by regions of little or no modification.

How HS structure is regulated is poorly understood, but as most of the modifying enzymes have multiple isoforms with different substrate specificities,[7] it is thought that cells may regulate the structure of their HS by altering the relative expression levels of the modifying enzymes.[8]

2 | HS IN THE MICROENVIRONMENT REGULATES THE ACTIVITIES OF IMMUNE CELLS

HS is an important component of basement membranes and the ECM, where it immobilizes a variety of ligands. This can have a range of functional consequences including local retention, regulation of receptor interactions, and protection from the actions of proteases, as discussed below and illustrated in Fig. 2. In addition, HS can itself be a ligand for certain receptors involved in leukocyte recruitment to inflammatory sites.

2.1 | HS immobilizes chemokines on vascular and lymphatic endothelium

Circulating blood leukocytes such as monocytes and neutrophils are stimulated to crawl along the endothelium towards inflammatory sites by an immobilized gradient of chemokines, a process called haptotaxis or chemotaxis. More than 40 chemokines have been identified, all of which have been shown or are predicted to bind heparin or HS.[1] It is thought that a major role of endothelial HS is to form a concentration gradient that directs the migration of leukocytes by immobilizing chemokines and preventing them being washed away by the blood flow (Fig. 2). This hypothesis is supported by observations that mutant chemokines lacking the ability to bind HS fail to recruit leukocytes in vitro and in vivo.[9–11] Moreover, heparanase-overexpressing mice, which have very short HS chains, show impaired neutrophil crawling in response to wild-type (WT) chemokines,[12] further demonstrating the importance of the chemokine–GAG interaction.

It has also been observed that many chemokines oligomerize on HS and that the ability to form oligomers is required to achieve maximal local concentrations.[13] Oligomerization, as well as ability to bind HS, appears to be critical for the activity of certain chemokines, as monomeric mutants of RANTES, MIP-1β and MCP-1 have severely reduced chemotactic activity in vivo when injected IP into mice despite unchanged affinity for their receptors.[10]

The variation of HS structure in different tissues may be relevant to chemokine binding; for example, the HS from aortic and venous endothelial cells differs in degree of sulfation[14] which results in the formation of binding sites for chemokines only at post-capillary venules and small veins, where leukocyte emigration occurs, and not in capillaries and arteries.[15,16] Chemokine binding to HS also has some tissue specificity, as demonstrated by the retention of IL-8 in the lungs but not the skin.[13] These findings suggest that HS-mediated control of chemokine binding might determine to which sites leukocytes are recruited. Furthermore, it is possible that dynamic regulation of HS during inflammation modulates chemokine gradients and thus chemotaxis. In support of this, staining for HS in high endothelial venules (HEVs) of the lymph node revealed more HS on the basolateral side than the luminal side, and increased deposition of HS on only the basolateral side after stimulation with Total Freund's Adjuvant.[17] Thus HS may facilitate formation of a trans-epithelial chemokine gradient that becomes more pronounced during inflammation.

2.2 | Endothelial HS as a ligand for leukocyte selectins

In addition to their role in the presentation of chemokines, HS proteoglycans are more directly involved in leukocyte recruitment through their capacity to act as ligands for selectins (Fig. 2), thus

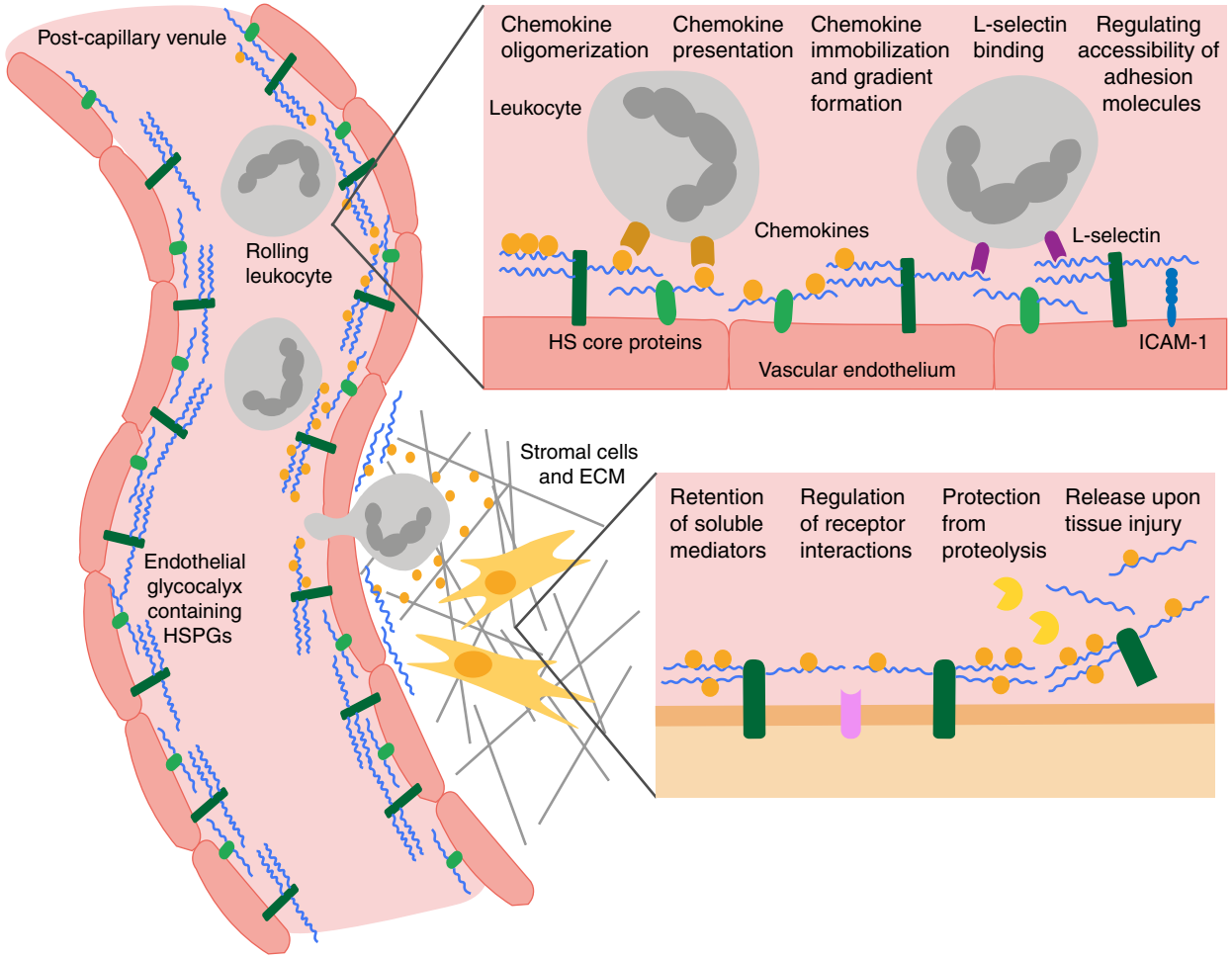

FIGURE 2 **Heparan sulfate plays several roles in local acute inflammation**. HS on the vascular endothelium binds chemokines and thus facilitates their oligomerization and presentation to circulating leukocytes, prevents them being washed away by the blood flow, and forms the chemokine gradient necessary for directional migration of leukocytes. Endothelial HS may also directly regulate the recruitment of leukocytes by acting as a ligand for leukocyte L-selectin, or by masking other adhesion molecules on the vascular endothelium to make them less accessible to leukocyte integrins. HS also functions in the extracellular matrix (ECM) to retain cytokines and growth factors. This interaction with HS can regulate local signaling by promoting or inhibiting receptor–ligand interactions, and can protect cytokines from inactivation by proteases. The core protein of the HSPG can be cleaved by the proteases released during an inflammatory response to release the ectodomain and its HS chains from the cell surface. Heparanase can digest the HS chains to release small active fragments that can act as danger-associated molecular patterns (DAMPs), or continue to regulate the activity of HS-bound cytokines through acting as a coreceptor (promotes signaling) or a decoy receptor (inhibits signaling)

facilitating leukocyte rolling and adhesion (reviewed in Ref. 18). The nature of this interaction has been difficult to unravel since HS is present on both endothelial cells and leukocytes, and selectins are also expressed by both cell types: leukocytes constitutively express L-selectin, whereas endothelial cells upregulate P-selectin upon activation.

In vitro studies using endothelial monolayers in flow chambers to mimic the physiological shear stress of blood vessels have provided evidence of a role for HS in leukocyte recruitment. For example, digesting HS from endothelial monolayers with heparinase III, or adding heparin or soluble HS to the medium, significantly reduces the number of rolling and firmly adhering granulocytes,[19] and the total HS deficiency resulting from knockdown of Ext1 in HUVECs prevents normal neutrophil arrest and transendothelial migration.[20] Similarly, genetic inactivation of Ndst1 in endothelial cells inhibits granulocyte adhesion to endothelial monolayers and is also associated with decreased binding of recombinant L-selectin[21] suggesting that HS is a physiological ligand for this adhesion molecule. Further supporting this hypothesis, pretreatment of monocytes with an L-selectin-blocking antibody inhibits their adhesion to endothelial monolayers,[22] while the binding of recombinant L-selectin to HEV in tissue sections from various organs is sensitive to heparinase digestion.[23]

In vivo studies also imply a major role for the HS-L-selectin interaction in leukocyte recruitment in various inflammatory models. Heparin infusion dramatically inhibited neutrophil infiltration into the peritoneal cavity following thioglycollate injection, and reduced ear thickness and inflammatory cell infiltrate in a contact dermatitis model.[24] Importantly, L- and P-selectin doubly deficient mice showed no further decrease in leukocyte recruitment following heparin treatment,

confirming that heparin inhibits inflammation primarily through these two selectins.[24] Similarly, the reduced HS sulfation achieved by conditional deletion of Ndst1 from only endothelial cells and leukocytes results in reduced leukocyte recruitment in models of peritonitis,[25] allergen-induced airway remodeling,[26,27] and diabetic[28] and experimental[21] glomerulonephritis. Of particular interest was the observation that chimeric WT mice with Ndst1-null bone marrow do not show any defect in thioglycollate-induced lymphocyte homing, whereas KO mice given WT bone marrow show the same reduction in cell infiltrate seen in the mice lacking both endothelial and leukocyte Ndst1.[25] Conversely, the compensatory increase in N- and 6-O-sulfation resulting from endothelial Hs2st deletion leads to increased leukocyte recruitment that is returned to control levels by an L-selectin-blocking antibody.[29] These results imply that the crucial interaction is that between the sulfated motifs of endothelial HS and leukocyte L-selectin, whereas leukocyte HS and P-selectin have little effect in this context.

However, while most data indicate that endothelial HS promotes leukocyte adhesion through L-selectin, some studies suggest that HS may have an inhibitory effect on leukocyte attachment. For example, heparinase digestion of microperfused murine venules results in more adherent leukocytes[30] and increased binding of fluorescently-labeled microspheres coated with anti-ICAM-1 antibody,[31] implying that the HS layer might inhibit leukocyte binding by masking endothelial adhesion molecules and making them less accessible to leukocyte integrins (Fig. 2). In support of this, intravital microscopy showed that the loss of endothelial glycocalyx following LPS infusion led to increased adhesion of GFP^+ neutrophils and of anti-ICAM-1 microspheres in WT but not heparanase-null mice.[32] These results suggest that heparanase-mediated shedding of endothelial HS in response to a potent inflammatory stimulus may expose more adhesion molecules such as ICAM-1. Thus HS may play two separate roles in leukocyte recruitment: directly by facilitating selectin-mediated leukocyte attachment and rolling, and indirectly through regulating accessibility of other adhesion ligands such as ICAM-1. Further studies are thus required to determine the relative contributions made by HS as a selectin ligand, and as a barrier to ICAM-1 accessibility, over time during the phases of initiation and resolution of an immune response.

Given the multi-faceted role for endothelial HS in leukocyte recruitment, one might expect the structure of HS to be altered in response to inflammatory stimuli or tissue damage in order to regulate immune cell infiltration, and there is evidence that this is indeed the case. For example, endothelial HS sulfation is increased by in vitro stimulation with TGF-β,[33] IFN-γ,[34] IL-1β,[35] or TNF[36] with associated upregulation of Ndst1, 2, Hs6st1 and 2,[36] and increased ability to bind L-selectin[22] and support the arrest and adherence of leukocytes.[19] In vivo, renal ischemia/reperfusion stimulates the formation of binding sites for L-selectin in the interstitial capillaries that are not present in the contralateral control kidney.[37] Experimentally induced nephritis is associated with increased expression of highly sulfated HS domains,[38] which are also seen in kidney sections from systemic lupus erythratomatus (SLE) patients and MRL/*lupr* mice[39] but not healthy controls. Thus, it seems likely that dynamically regulating the structure of endothelial HS may be a mechanism to fine-tune cell recruitment during an immune response.

2.3 | HS in the ECM regulates activity of cytokines

Cytokine activity is determined by a combination of factors including localization close to the target cell, susceptibility to inactivation by proteolytic cleavage, and ability to interact with high affinity receptors. Numerous cytokines have been demonstrated to bind heparin or HS in vitro[1] but the biological consequences of this interaction for cytokine activity have been explored for only a few cytokines. The available data indicate that HS exerts diverse effects on different cytokines, which is hardly surprising given the structural heterogeneity in this functional class of molecules.

Although often described as soluble mediators, cytokines exert their effects locally not systemically and binding to ECM HS has been proposed as a mechanism for retaining cytokines close to their site of production and action. For example, endogenous IL-2 is detectable in murine spleen, liver sinusoids, and kidney glomeruli, and staining is absent after treating the sections with heparitinase, but not with chondroitinase.[40] Similarly, IFN-γ injected into rats rapidly disappears from the circulation and accumulates in specific tissues including the liver, spleen, and kidney, which is inhibited by coinjection with heparin.[41] Thus, HS immobilizes certain cytokines in the extracellular space, forming localized reservoirs that may facilitate paracrine signaling (Fig. 2).

Whether HS-immobilized chemokines are able to interact with their receptor depends partly on whether the heparan- and receptor-binding sites overlap. Surface plasmon resonance experiments demonstrated that the binding of IFN-γ to its receptor or to HS are mutually exclusive,[42] which would imply that immobilized IFN-γ in the matrix cannot signal. However, endothelial monolayers pulsed with IFN-γ stimulate MHC-II upregulation by coincubated target cells which is abolished by heparinase II treatment,[43] suggesting that cell-surface HS might present IFN-γ in trans in a signaling-competent manner. Whether HS-bound IFN-γ must dissociate from the matrix to bind its high-affinity receptor, or can bind both simultaneously, remains unresolved. It was recently demonstrated for IL-2 that reservoirs on endothelial and smooth muscle cells of blood vessels are liberated by heparanase in a biologically active form that is competent to stimulate target cell proliferation.[44] Thus, changes to the matrix that occur during inflammation may release cytokines from ECM reservoirs and enable them to signal.

In addition to regulating availability of soluble cytokine, HS may also influence cytokine activity by controlling their accessibility to proteases (Fig. 2). For example, proteolytic cleavage of the C-terminal portion of IFN-γ increases receptor binding if fewer than ten terminal amino acids are removed,[42] but binding is decreased by larger deletions.[45] Heparin and highly sulfated HS bind IFN-γ at its C-terminus,[46] and coinjection of heparin protects IFN-γ from proteolytic degradation in vivo.[47] Heparin has also been shown to prevent degradation of other cytokines including IL-6[48] and IL-7[49] in vitro. Thus binding to matrix HS may serve to protect the active form of the cytokine, as well as forming local reservoirs.

Soluble HS fragments released in inflammation may also regulate signaling of some cytokines by promoting their interaction with receptors on target cells. It has been demonstrated that addition of soluble HS to cells that lack detectable levels of this GAG on their surface augments IL-5-induced proliferation[50] and IL-12-stimulated IFN-γ production.[51] The mechanism by which soluble HS promotes signaling of these cytokines has not been explored, and the relevance of these observations to inflammatory processes in vivo is not yet clear.

3 | SOLUBLE HS DIRECTLY STIMULATES IMMUNE CELLS, ACTING ON APCS TO CONTROL IMMUNE RESPONSES

HS fragments liberated from the ECM by enzymes released during inflammation can promote immune activation by signaling through TLR4 and activating APCs.

3.1 | HS is degraded in inflammation to become a potent TLR4 ligand

It has become evident that soluble fragments of HS can signal through TLR4, an innate pattern recognition receptor for which the prototypic ligand is bacterial LPS. Stimulation of dendritic cells (DCs) with HS fragments induces upregulation of costimulatory molecules including CD86 and CD40 and secretion of proinflammatory cytokines,[52] which is inhibited by TLR4 mutation or the TLR4 antagonist Rs-DPLA.[53] These findings suggest that HS is a non-classical ligand for TLR4 that is capable of signaling to trigger immune activation (Fig. 3). Similarly, cardiac fibroblasts stimulated with HS upregulate V-CAM1 and I-CAM1, leading to increased adhesion of polymorphonuclear neutrophils (PMNs) and smooth muscle cells, which is completely abolished by the TLR4 inhibitor TAK-242 or by blocking the downstream signaling molecules NFkB or PI3K/Akt.[54]

Proliferation of T cells after coincubation with allogeneic DCs is augmented by soluble HS, but not when the DCs come from mice deficient in TLR4 or its adaptor protein MyD88. However, knockout of these molecules in T cells had no effect,[55] and HS treatment of purified T cells stimulated with anti-CD3 in the absence of DCs did not augment proliferation,[56] demonstrating that HS indirectly controls lymphocyte activity through regulating APC maturation. Furthermore, murine peritoneal macrophages stimulated with HS upregulate IL-1, IL-6, TNF, and IL-12, and show increased cytotoxicity toward a leukemia cell line.[57] In vivo evidence for the role of the HS-TLR4 axis in inflammation comes from infusion of soluble HS into the pancreas of mice, which results in neutrophil infiltration and increased myeloperoxidase activity in WT but not TLR4-null mice.[58]

It is thought that the elevated activity of metalloproteases,[59] heparanase,[60] and other enzymes that occurs in inflammation liberates HS fragments from cell-surface and ECM HSPGs. These soluble HS fragments could then signal through TLR4 to alert the immune system to tissue damage, thus acting as a damage-associated molecular pattern (DAMP). In support of this hypothesis, endothelial cells with metabolically labeled sulfated GAGs show a decrease in cell-surface HS and an increase in smaller HS fragments in the conditioned medium upon LPS stimulation[61] or exposure to activated neutrophils, which can be reduced by inhibitors of serine proteases and elastase.[62] Furthermore, heparanase treatment of human PBMCs resulted in a reduction of cell-surface HS and an upregulation of proinflammatory cytokines similar to that seen by addition of HS fragments, which was abrogated by inhibitors of heparanase or by MyD88 or TLR4 deficiency.[63]

In vivo administration of the serum protease inhibitor alpha-1 antitrypsin (A1AT) to recipients of allogeneic bone marrow transplants increased survival and lowered serum HS and histological scores. Furthermore, while TLR4 gene deletion conferred some protection from graft-versus-host disease, no further benefit was seen with A1AT treatment,[55] indicating that the protective effects of A1AT are mediated through the HS-TLR4 axis. Similarly, injection of elastase into WT mice induced loss of HS from blood vessels, an increase in serum TNF and ultimately death of the mice, whereas TLR4-null mice showed improved survival.[64] Collectively, these results suggest a pathway in which fragments of HS produced enzymatically during inflammation signal through TLR4 to further activate the immune system, which may have beneficial or detrimental effects depending on the context.

There is evidence to suggest that the immune system is able to distinguish between endogenous stimuli indicative of tissue damage and exogenous stimuli present as a result of infection, enabling a context-appropriate response to be mounted. For example, macrophages stimulated with LPS or the matrix glycoprotein tenacin-C, which is produced upon tissue injury and also signals through TLR4, resulted in different transcriptional responses and two distinct macrophage phenotypes.[65] LPS-stimulated macrophages had greater collagen-degrading ability and inflammatory cytokine production, whereas tenacin-C treatment induced macrophages to synthesize collagens and other proteins associated with tissue repair. One might thus predict that HS, another endogenous DAMP that signals through TLR4, also stimulates an immune response different to that induced by LPS. In support of this, the kinetics of TLR4 signaling in response to LPS or HS are different, with LPS inducing much more rapid nuclear translocation of NFkB than HS[53] and HS triggering a slower and more sustained increase in intracellular calcium.[66] Further studies are required to define how TLR4 signaling induced by endogenous DAMPs such as HS is different to that induced by infection-associated stimuli such as LPS, and whether this results in context-appropriate responses from the immune cell.

4 | HS ON LEUKOCYTE SURFACE REGULATES THEIR IMMUNE AND INFLAMMATORY FUNCTIONS

Although frequently considered a matrix molecule, HS is expressed on the surface of most cells, including immune cells, in the form of proteoglycans. In addition to regulating receptor interactions both cell autonomously and through ligand presentation to other cells, leukocyte cell-surface HS appears to contribute to specific functions such

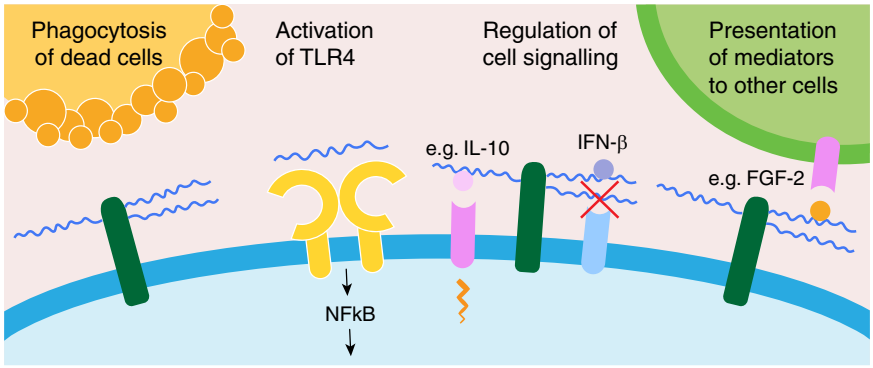

FIGURE 3 Leukocyte HS plays many roles in immune regulation. Heparan sulfate proteoglycans facilitate uptake of late apoptotic and necrotic cells, although the mechanism remains unclear. Soluble fragments of HS released during tissue inflammation activate cells through TLR4. HS chains on leukocyte membrane proteins can promote or inhibit receptor-ligand interactions for cytokines and growth factors. Cell-surface proteoglycans can also regulate signaling in trans by presenting growth factors such as FGF-2 to target cells

as phagocytosis. This may be a physiologically relevant mechanism of immune regulation, as the structure of leukocyte HS is altered in response to various inflammatory stimuli.

4.1 | Leukocyte HS promotes phagocytic activity

Cell-surface HS is thought to be involved in recognition and uptake of dead cells by phagocytes through exposure of HS binding sites on late apoptotic and necrotic cells that have lost membrane integrity (Fig. 3). For example, FITC-labeled heparin was found to bind to the surface of apoptotic and necrotic cells, but not live cells, and this interaction was inhibitable by heparin but not dermatan or chondroitin sulfates.[67] Beads coated with anti-HS antibodies were bound and internalized by epithelial cell lines, but not if the cells had been pretreated with heparinase III or in the presence of inhibitors of PKC or actin polymerization,[68] suggesting that ligation of cell-surface HS is sufficient to stimulate actin-mediated endocytosis in non-professional phagocytes. Similarly, CHO cells and human skin fibroblasts bind and internalize latex beads, and this is strongly reduced by the addition of heparin or soluble HS fragments or by pretreatment of the cells with heparinase III.[69] Additionally, CHO mutant cells lacking the ability to synthesize HS showed defective binding and internalization of the beads, adding further support to an HS-mediated mechanism of phagocytosis. These studies were performed in largely artificial systems with non-professional phagocytes, but there is some evidence that an HS-dependent mechanism of phagocytosis is conserved in a relevant immune setting: human PMNs more efficiently bind and internalize a strain of *Helicobacter pylori* that can bind HS than a strain without HS-binding ability, and preincubation of the bacteria with heparin inhibited uptake of the HS-binding strain but did not further reduce uptake of the non-HS-binding strain.[70] However, further studies are required to determine the relative contribution that HS makes as an endocytic receptor in the presence of canonical phagocytic receptors, antibodies and other opsonins that have an established role in phagocytosis in vivo.[71]

4.2 | Leukocyte HS regulates responses to inflammatory mediators

HS on the surface of leukocytes appears to play a role in regulating signaling of certain inflammatory mediators through their cell-surface receptors, either by promoting ligand interaction with its receptor, thus acting as a coreceptor, or by inhibiting receptor–ligand interactions, depending on the ligand (Fig. 3). For example, the B cell activating factor APRIL, which is essential for IgA class switching in response to mucosal antigens,[72] requires HS on the B cell surface for optimal receptor activation and downstream signaling. Heparinase III digestion of B cells strongly reduced APRIL-induced proliferation, IgA production, and NFkB translocation.[73] APRIL can be competed off the B cell surface by heparin, and mutant forms of APRIL without HS-binding ability fail to stimulate IgA production by B cells.[74] APRIL does not bind to the surface of $Glce^{-/-}$ B cells, which lack iduronic acid residues, and cannot promote their survival in vitro.[75] This may be due to altered downstream modifications by the 2-O- and 6-O-sulfotransferases, whose activity is affected by epimerase inactivation.[76]

Another B cell factor, IL-7, also binds heparin and HS, and IL-7-dependent proliferation is inhibited by the addition of heparin,[49] suggesting that binding to cell-surface HS promotes IL-7 interacting with its receptor. In support of this, heparitinase treatment of primary B cell precursors diminished surface binding of IL-7 and reduced IL-7-driven proliferation.[77]

IL-10 binds both heparin and HS, and the addition of soluble fragments of HS inhibits the ability of IL-10 to upregulate CD64 and CD16 on monocytes.[78] Similarly, IL-10 induced expression of these markers is inhibited by treatment of monocytes and macrophages with sodium chlorate, which prevents sulfation of GAGs,[78] indicating that cell-surface HS promotes the interaction of IL-10 with its receptor. The crystal structure for IL-10 bound to IL-10R1 shows no overlap with the GAG-binding site,[79] but there is currently no crystal structure of the ternary complex composed of IL-10 and both receptor subunits. Therefore, it remains unclear whether HS acts directly as a coreceptor in the formation of productive IL-10–IL-10R complexes, or simply facilitates IL-10 signaling by preventing diffusion of the cytokine away from the cell surface.

Cells transfected with the splice variant of the hyaluronan receptor (CD44) containing HS attachment sites retain hepatocyte growth factor (HGF) on their cell surface, whereas no binding is seen on cells expressing the isoform of CD44 lacking the HS attachment region.[80] The same study showed that ability to bind HGF on the surface resulted in phosphorylation of the HGF receptor tyrosine kinase c-Met, and of ERK-1 and ERK-2. Surface binding and signaling were abolished by heparitinase (but not chondroitinase ABC) treatment or by mutation of the HS-binding domain of HGF, suggesting that HS-mediated capture of HGF on the cell surface facilitates receptor

binding and activation. This appears to be a relevant mechanism in B cells since human tonsillar B cells activated by coincident ligation of CD40 and the BCR express a higher molecular weight form of CD44, with concurrent acquisition of HGF-binding ability. Downstream c-Met phosphorylation was abolished by heparitinase treatment of activated B cells,[81] indicating that CD44 is decorated with HS following proinflammatory stimulation.

However, leukocyte HS may not always promote ligand-receptor interactions, as for certain ligands it acts instead as a barrier and inhibits signaling. Macrophages deficient in HS sulfation through deletion of Ndst1 show increased responsiveness to IFN-β stimulation and elevated production of proinflammatory cytokines and chemokines,[82] suggesting that the role of HSPGs in IFN-β signaling is to sequester it away from its receptor and thus maintain macrophages in a quiescent state.

4.3 | Leukocyte HS regulates cell activation in trans

In addition to these cell-autonomous functions, leukocyte cell-surface HS can also be involved in presentation of growth factors or inflammatory mediators produced by leukocytes to their target cells, as has been demonstrated for fibroblast growth factor 2 (FGF-2) (Fig. 3). During monocyte-to-macrophage maturation, cells aquire the capacity to bind FGF-2, which is inhibitable by heparin and further augmented by stimulation with IL-1 or LPS.[83] The same study showed that activated macrophages augmented the proliferative response of HS-deficient BaF32 cells to low concentrations of FGF-2, which was abolished by heparinase III treatment, indicating that macrophage HS enables trans-presentation of FGF-2 to target cells in a signaling-competent manner. Similarly, activated macrophages increase expression of the CD44v3 isoform of the hyaluronan receptor that contains HS chain attachment sites, and cell lines transfected with this HS-sufficient form of CD44, but not those with the HS-deficient variant, augmented FGF-2-induced proliferation of BaF32 cells.[84] Interestingly, both studies found that T cells do not increase expression of CD44v3 or their total HS levels upon mitogenic stimulation, and T cells actually decrease HS expression and lose the ability to bind FGF-2 during consecutive rounds of proliferation.[85] These observations suggest that HS-mediated trans-presentation of growth factors may be a mechanism unique to those cells producing such growth factors upon activation. It remains to be determined whether modulation of HS expression regulates the activity of other HS-binding inflammatory mediators through surface presentation.

4.4 | Leukocyte HS structure is altered during inflammation

For leukocyte HS to be considered an active regulator of cell responses, it must itself be altered during the course of an immune response to facilitate changes in inflammatory processes, and there is increasing evidence that such dynamic regulation of HS does occur. B cells from virus-infected mice, or stimulated in vitro with the IFN-I-inducer Poly I:C or IFN-β, show increased surface expression of HS and augmented APRIL-induced production of IgA.[86] Similarly, stimulation with PMA or ligation of CD40 results in an increase in HS on the B cell surface and a switch to the higher molecular weight form of CD44 that has HS chains.[81] HS structure may also be regulated during B cell development, as ligation of the pre-BCR induces upregulation of numerous HS-associated genes including Hs3st1.[87] Furthermore, microarray analyses of different B cell subsets show greater expression of HS biosynthetic enzymes in plasma cells than memory B cells,[88] suggesting activation is associated with an increase in cell-surface HS.

Differential expression of HS is also a feature of macrophage subsets, as human monocyte-derived macrophages polarized to an anti-inflammatory reparative phenotype display greater upregulation of sulfotransferases than proinflammatory macrophages, resulting in more cell-surface HS with a higher degree of 2-O-sulfation.[89] Importantly, this differential expression of HS was shown to have functional consequences: reparative macrophages bound more FGF-2 and augmented the FGF-2-dependent proliferation of a target cell line, consistent with the requirement of 2-O-sulfation for FGF-2 binding.[90] This suggests that the expression of more highly sulfated HS in reparative macrophages may be important for their role in the resolution of inflammation. Various other stimuli have been reported to affect HS structure on macrophages, such as LPS and TNF which induce upregulation of HS3ST3B and consequently an increase in 3-O-sulfation,[91] and hypoxia which significantly reduces expression of biosynthetic enzymes and total HS content.[92]

Interestingly, macrophages from the synovial fluid of RA and SLE patients show greater FGF-2 binding than blood monocytes,[83] and CD44-HS splice variants were highly expressed in RA but not OA joints and colocalized with staining for macrophage markers and FGF-2.[84] These findings suggest that excessive growth factor activity seen during chronic inflammation may be partly due to aberrant regulation of leukocyte HS sulfation.

5 | CONCLUSIONS

It is becoming increasingly apparent that HS has multiple roles in immune regulation and signaling during inflammation, both in the microenvironment and on the cell-surface of leukocytes. Soluble HS fragments also have signaling properties and may serve as indicators of tissue damage. Further studies are required for a better understanding of the changes in HS expression that occur during inflammation and the structural requirements for binding and regulation of immune mediators. Understanding these structure–function relationships will be essential for the development of therapeutics that interfere these processes to combat immunopathology, which might take the form of synthetic HS oligosaccharides or HS-specific antibodies.

AUTHORSHIP

L.E.C. conceived the idea and wrote the manuscript. L.T. edited the paper.

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DISCLOSURES

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional information may be found online in the Supporting Information section at the end of the article.

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