**The role of heparan sulphate in host macrophage infection by *Leishmania* species**

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Abbreviations list: HS, heparan sulphate; HBPs, heparain binding proteins; HIV, human immunodeficiency virus; *spp*., species; NDST, N-deactylase/N-sulphotransferase; EXT, exotoses; Sulf, sulphatase GlcNS,N-sulphoglucosamine; IdoA, iduronic acid; GlcA, glucuronic acid; GlcNAc, N-acetylglucosamine

**Abstract**

The leishmaniases are a group of neglected tropical diseases caused by parasites from the *Leishmania* genus. More than twenty *Leishmania* species are responsible for human disease, causing a broad spectrum of symptoms ranging from cutaneous lesions to a fatal visceral infection. There is no single safe and effective approach to treat these diseases and resistance to current anti-leishmanial drugs is emerging. New drug targets need to be identified and validated to generate novel treatments. Host heparan sulphates are abundant, heterogenous polysaccharides displayed on proteoglycans that bind various ligands, including cell surface proteins expressed on *Leishmania* promastigote and amastigote parasites. The fine chemical structure of heparan sulphate is formed by a plethora of specific enzymes during biosynthesis, with various positions (N-, 2-O-, 6-O-, and 3-O-) on the carbon sugar backbone modified with sulphate groups. Post-biosynthesis mechanisms can further modify the sulphation pattern or size of the polysaccharide, altering ligand affinity to moderate biological functions. Chemically-modified heparins used to mimic the heterogenous nature of heparan sulphate influence the affinity of different *Leishmania* species, demonstrating the importance of specific heparan sulphate chemical sequences in parasite interaction. However, the endogenous structures of host heparan sulphates that might interact with *Leishmania* parasites during host invasion have not been elucidated, nor has the role of heparan sulphates in host-parasite biology. Decoding the structure of heparan sulphates on target host cells will increase understanding of heparan sulphate/parasite interactions in leishmaniasis, potentiating identification of new opportunities for the development of novel treatments.

**Leishmaniases**

The protozoan *Leishmania spp*. are the causative agents of a group of tropical diseases, collectively known as Leishmaniases. The parasites are transferred to the host through the bite of the female phlebotomine sandfly. Approximately 1 billion people around the world are at risk of infection, with an estimated 1 million new cases annually (1, 2). There are more than twenty *Leishmania*species that infect humans, manifesting in three main forms of pathology: visceral (kala-azar), cutaneous and mucocutaneous leishmaniasis. Cutaneous leishmaniasis is the most common form of the disease, with many different *Leishmania* species responsible for symptoms from both the Old World (*L. major, L. tropica, L. aethiopica*) and New World (*L****.*** *mexicana, L. amazonesis, L. braziliensis, L. panamensis, L. guanensis*). Cutaneous leishmaniasis is characterised predominantly by dermal lesions, which may persist for months or even years. The lesions often ulcerate and secondary infections may develop, causing pain and delayed healing. Atrophic scars form during the healing process that can be stigmatizing. In cases of mucocutaneous leishmaniasis, the mucosal tissues are also affected as well as the skin, which can lead to severe facial disfigurement. Visceral leishmaniasis, the most severe form of disease, causes around 30,000 deaths per annum (1). It is usually caused by *L. infantum* or *L. donovani*, affecting internal organs, particularly the spleen, liver and bone marrow. Symptoms include fever, weight loss, pancytopenia and hepatosplenomegaly. Some patients develop post kala-azar dermal leishmaniasis, which can appear at intervals after therapy for visceral leishmaniasis. Additionally, leishmaniasis patients may also present at the clinic with co-infections of other major diseases including tuberculosis (3), HIV (4), malaria (5), filarial nematodes (6), and schistosomiasis (7-9), as well as opportunistic bacterial secondary infections (10). These can cause complications for diagnosis, treatment (drug interactions, toxicity) and recovery for the patient.

*Leishmania* *spp.* have two distinct morphological forms: intracellular amastigotes and flagellated extracellular promastigotes. The principle form of *Leishmania* in humans is the amastigote stage, although less is known about this obligate intracellular stage than the promastigote stage. In the sandfly vector, procyclic promastigotes transform into host-infective virulent metacyclic promastigotes. When the sandfly takes a blood meal, metacyclic promastigotes in the saliva of the sandfly are injected into the skin of the host, exposing parasites to phagocytic target host cells. The parasites predominantly reside inside macrophages, but parasite infection of neutrophils (11) and dendritic cells (9) have also been described. Once intracellular, parasites reside inside a parasitophorous vacuole. Here, they differentiate into non-motile amastigotes that replicate and proceed to infect other mononuclear phagocytic cells. Both parasite and host factors determine the type of disease manifestation, although how this occurs this is not fully understood.

Current treatments for leishmaniasis involve administration of pentavalent antimonials, miltefosine, amphotericin B, or paromomycin. However, there is no universal treatment for leishmaniasis. There is associated drug toxicity, administration is difficult and resistant strains of the parasite are emerging. Thus, there is a pressing need for alternative strategies and novel drug targets to combat disease (reviewed in (12)). Research is currently focused on drug targets in the parasite and parasite-host interactions, which are also important for disease, have received less attention. Host polysaccharides, such as heparan sulphate (HS), are known to interact with both promastigotes and amastigotes *in vitro* (13, 14). Mechanisms of invasion by infectious agents involving HS proteoglycans have been described for other parasites (*Trypanosoma cruzi, Toxoplasma gondii*)(15-18), bacteria (19-21), and viruses (22-27), suggesting that interaction of *Leishmania* parasites with host HS may be purposeful. To infect host cells, *Leishmania* must successfully navigate both the host HS extracellular matrix and penetrate the HS glycocalyx surrounding host cells before entry into the host cell. During this process, specific epitopes (oligosaccharide sequences present within the HS chain) in host HS glycocalyx may be recognised and utilised by the parasites. Decoding these information-rich oligosaccharides and identifying the HS proteoglycans displaying these sequences may offer new strategies for fighting disease.

**HS proteoglycans**

HS molecules are linear, negatively charged heterogeneous polysaccharides synthesised on protein cores in the Golgi, forming HS proteoglycans. These biomolecular glycoconjugates have diverse roles in biology with the HS chains modulating proteoglycan interactions with a multitude of ligands, including: growth factors, cytokines, extracellular matrix proteins and infectious agents (for reviews see (28, 29)). HS proteoglycans are present in numerous locations within the host. Many are membrane-bound at the cell surface (syndecans, glypicans, CD44v3, betaglycan, neuropilin-1) or are incorporated within transport vesicles for extracellular release (serglycin). Other HS proteoglycans are secreted and interpolated into the extracellular matrix (perlecan, collagen XVIII, agrin). One or more HS chains may be attached to the proteoglycan, with some HS proteoglycans intermittently displaying HS chains (termed part-time HS proteoglycans) (30). Cell surface proteoglycans often display multiple HS chains that are involved in cell-cell and cell-matrix interactions, modulate cellular signalling or act as endocytic receptors (31) for recycling, clearance of bound ligands or cell crosstalk (exosome uptake) (32). HS proteoglycans present in basement membranes and other extracellular matrices define host extracellular structure and provide guidance for cell migration, as well as establishing morphogen gradients or serve as reservoirs for ligands (30).

Human macrophages, the predominant host reservoir for *Leishmania* parasites, display all four members of the syndecan family (33, 34), glypican-1, and glypican-4 (35), although it is possible that the other glypicans (-2, -3, -5 and -6) may be expressed in different stimulatory conditions. Parasites have been shown to bind the ectodomain of syndecan-2 and glypican-2, suggesting that some strains may interact with more than just the HS part of proteoglycans (14).Other *Leishmania*-targeted host cells such as dendritic cells (36) and neutrophils (37) also express various membrane-bound HS proteoglycans, including CD44v3 (38-40).

**The biosynthesis and modification of HS**

The HS portion of the proteoglycan contributes largely to its function, with the chemical structure of HS underpinning HS/ligand affinity (41). The main structure of HS is formed during its biosynthesis, but post-production modifications alter the fine structure and modulte HS bioactivity. HS biosynthesis occurs in tandem with protein translation in the endoplasmic reticulum/Golgi. The polysaccharide chain is built on a tetrasaccharide linker (xylose-galactose-galactose-glucuronic acid- (GlcA)) that tethers the HS to the protein structure at serine or threonine residues. The exotoses (EXT) co-polymerase complex (EXT1/2) elongates the backbone of HS by the sequential addition of N-acetylglucosamine (GlcNAc) and GlcA residues, to form repeating disaccharide units along the chain (reviewed in (41), (42) and (43)). During elongation, the backbone is extensively modified by an array of HS-specific enzymes to produce the fine chemical structure of the polymer (Figure 1). The N-deacetylase/sulphotransferase (NDST) family of enzymes replaces the acetyl group of some of the GlcNAc residues with sulphate groups, to form N-sulphoglucosamine (GlcNS). Production of GlcNS residues usually precedes further chain modifications that include: conversion of GlcA to iduronic acid (IdoA) by C5-epimerase and the transfer of sulphur groups conducted by sulphotransferase enzymes to different positions (2-O-, 6-O- and rarely 3-O-) on the nascent chain. These modifications often occur in an incomplete, and inter-dependent manner to produce the fine chemical patterning of HS chains. During this process, HS domain structure is formed, consisting of regions of high sulphation (NS domains) flanked by sections of intermediate sulphation (NS/NA domain) that interject unmodified regions (NA domain) (41, 42).

After translocation of HS proteoglycans to the cell surface, further modification of the chemical HS structure can also occur (Fig. 1.). Sulphatases (Sulf-1, Sulf-2) may remove 6-sulphate groups from the mature structure of the polysaccharide (44, 45). Additionally, the parent polysaccharide can be partially cleaved into fragments (oligosaccharides), where the glycosidic bond is broken by heparanase at specific sites within the HS chain (46, 47). This results in proteoglycans with shortened HS sequences and free oligosaccharides. Liberation of these oligosaccharide species (both on the proteoglycan and released HS sequences) may then modify the availability of binding epitopes for HS ligands, orchestrating their mechanistic activity through potential changes in binding partners, their localisation and therefore function.

Empirical, HS compositional analysis has been conducted on monocytes (20) and polarized macrophages (48), where the HS chain is first isolated, then depolymerised into disaccharides using bacterial heparinases and the percentage of each disaccharide is calculated. Unfortunately, in-depth detail of HS chain length, domain structure or proteoglycan source remains elusive. Similarly, HS analysis of *Leishmania*-infected macrophageshas not yet been completed, although there is evidence to suggest that distinct HS binding epitopes may influence host infection for individual *Leishmania* species (14). A recent study showed that live non-dividing promastigote parasites from a range of different species representing cutaneous, mucocutaneous and visceral forms of the disease all bind HS and heparin *in vitro*, suggesting a common interaction mechanism between *Leishmania* species. The interaction depended largely on the *Leishmania* species and each one tested differed in its sensitivity to chemically-modified heparins. Overall, reduction of sulphation resulted in decreased *Leishmania*/heparin binding, with uniform removal of 6-O-sulphation reducing binding across all the species tested (14). In contrast, complete removal of 2-O-sulphation of the chain, increased binding for three of the six species investigated. Equally, uniform removal of N-sulphation abolished binding for *L. major,* but had varying degrees of effect for *L. tropica (*~55% decreased)and *L. guyanensis,* (~25% decreased) and de-N-sulphated/re-acetylated heparin abolished binding to *L. donovani* but rescued binding for *L. major*. Together, these findings suggest that the chemical structure of the HS chain is functionally important in *Leishmania-*host HS interactions. Thus, detailing the structure and bioactivity of endogenous host HS material will be a crucial next step for the role of HS in *Leishmania* infection to be deciphered.

***Leishmania* heparin-binding proteins (HBPs)**

Although information on host HS-parasite interactions in leishmaniasis is in its infancy, expression of heparin-binding proteins in *Leishmania* *spp*. has been detailed more extensively, with the majority of research focusing on promastigotes (Table 1). Two major heparin-binding proteins (55 kDa and 65 kDa) localised in the flagellum and cell plasma membrane of promastigotes (*L. braziliensis*) have been described (49, 50). Flagellum HBPs form stable complexes with heparin (49) and heparin binding to cell surface HBPs has been shown to decrease promastigote protein phosphorylation (51). These events may trigger changes in the parasite that consequently influence survival of the parasite in the host environment. Metacyclic non-dividing promastigotes bind heparin more avidly than non-infective (log phase) promastigotes, with this increased capacity for heparin-binding coinciding with parasite differentiation into the infective metacyclic form (52). Interestingly, amastigotes have a higher affinity for HS/heparin than either procyclic or metacyclic promastigote stages (13), suggesting that this obligate intracellular stage may also utilise HS ligands in the host. However, potential amastigote HBP candidates have not yet been reported.

In addition to the host, HBPs isolated from promastigotes (*L. braziliensis*) bind multiple ligands extracted from sandfly intestinal epithelium in both *Lutzomyia intermedia* and *Lutzomyia whitmani* species, (53) as well as adhering to Lulo cells derived from *Lutzomyia longipalpis* (49).Therefore, this suggests that *Leishmania* interaction with HS may also be important in the vector phase of the parasite lifecycle.

Evidence suggests that HS-parasite interactions could be important at multiple points during the *Leishmania* life cycle, with specificity in the interactions between different HS structures (14) relevant for different parasite species and life cycle stages (13, 14, 49, 52) that may be both temporally and spatially regulated (i.e. extracellular matrix, cell surface proteoglycans). This provides a number of potential opportunities for interference of *Leishmania* recognition and binding to host HS, offering potential targets for the development of competitive inhibitors to prevent or reduce infection. Future investigation of potential changes in the host HS present within the glycocalyx during and after parasite invasion, may uncover unique signatures specific for infected host cells or host cell susceptibility for parasite invasion. Knowledge of these underpinning structure:function relationships will assist in the development of novel HS-targeted therapeutics with reduced toxicity and side effects, whilst expanding our knowledge of the role of host HS in *Leishmania* infection.

**Summary**

It is clear that *Leishmania* parasites possess cell surface ligands with HS/heparin affinity and that the chemical structure of the HS/heparin affects its binding capacity. There is evidence to support the idea that ligand interaction occurs in both the host and vector stages of the parasite life cycle. Currently, much less is known about the amastigote stage of the parasite and the transfer of infection from host cell to host cell, or back to the vector, and whether HS plays a role in these events remains unexplored. Although, parasite binding studies utilising chemically modified heparins have highlighted the requirement of specific types of HS sulphation, there is little information about the structure of HS produced by target host cells, such as neutrophils, macrophages and dendritic cells. In order to further elucidate the role of HS in host-parasite biology, the composition of HS and its domain structure from host target cells needs to be elucidated. Insight into the mechanisms of parasite-host HS biology could elicit new targets for the development of anti-leishmanial drugs.

**Declarations of interest**

The authors declare no conflict of interest.

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**Author contribution statement**

MLMH conceived, wrote and edited the review. HP and MAS edited the manuscript.

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Fig. 1. HS chemical structure and modification. HPSE, heparanase. SULF, sulphatase.HS graphic revised (2).tiff