Role of heparan sulfate in sexually transmitted infections

Introduction

Sexually transmitted infections (STIs) are a major public health problem and also a significant financial burden on the economy. STIs cost the U.S. healthcare system $17 billion annually and cost individuals even more in immediate and lifelong health consequences (Centers for Disease Control and Prevention 2006). There are more than 30 different sexually transmissible bacteria, viruses, and parasites. In 2004, the Centers for Disease Control and Prevention (CDC) estimated that 19 million new STIs occur each year in the United States (Weinstock et al. 2004; CDC 2007). Of the new cases, half occur in patients aged 15–24 years. Although there have been significant advances in prevention, diagnosis, treatment and education, the occurrence of STIs continues to increase (Rompalo 2011). Common etiologic agents of STIs include Chlamydia trachomatis, herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), human papillomavirus (HPV), Neisseria gonorrhoeae, Treponema pallidum, hepatitis B virus (HBV), and human immunodeficiency virus (HIV). Any common link that connects the major STIs can be of an immense therapeutic or prophylactic value and, as a result, the developments of new topical microbicides that reduce the transmission of one or more STIs are currently a worldwide priority.

One such common link is represented by heparan sulfate (HS) glycosaminoglycans (GAGs). GAGs are expressed widely in the human body. They are terminal carbohydrate structures in the extracellular matrix that are in turn linked to protein cores buried in cell membranes. A number of bacterial, viral and parasitic pathogens, such as HSV, HIV, HPV and C. trachomatis (Shukla and Spear 2001; Moelleken and Hegemann 2008; Johnson et al. 2009; Sapp and Bienkowska-Haba 2009; De Francesco et al. 2011), have been shown to express surface proteins that interact with HS to mediate the adhesions of microbes to eukaryotic cells as a primary mechanism during mucosal infections (Wadström and Ljungh 1999; Chen et al. 2008; Aquino et al. 2010; Bartlett and Park 2010). In addition, roles for HS in trafficking of pathogens (e.g. surfing), uptake, spread and virulence have also been proposed (Gallay 2004; Clement et al. 2006; Ceballos et al. 2009; Kerur et al. 2010; Oh et al. 2010; Gardner et al. 2011; Imamura et al. 2011; Karasneh et al. 2011; Teng et al. 2012). HS has also been identified as a pathological chaperone in the HIV Tat-mediated biological response in target cells (Urbinati et al. 2009), and HS proteoglycans (HSPGs) have been shown to act as Tat receptors that...
promote HIV-associated neuroinflammation (Hui et al. 2006). Additionally, a reduced ability of dengue virus to bind HS was shown to cause severe illness in mice (Prestwood et al. 2008). Therefore, it is clear that HS can play multiple important functions in microbial invasion of human hosts, and hence, it can be considered a crucial common link that many pathogens modulate to infect human hosts and cause diseases including those originating from the STIs.

**HS and its biosynthesis**

HS is a negatively charged linear carbohydrate polymer composed of repeating uronic acid [D-glucuronic acid (GlcA) or L-iduronic acid (IdoA)] and D-glucosamine (GlcN) disaccharide units (Figure 1). HS is ubiquitously expressed on the cell surface and in the extracellular matrix of almost all cell types as HSPGs (Esko and Lindahl 2001). HSPGs are composed of HS polysaccharide side chains covalently linked to a protein core via a tetrasaccharide link. HS plays a role in multiple biological processes involved in maintaining homeostasis including blood coagulation, lipid metabolism, regulation of embryonic development, neurogenesis, angiogenesis, axon guidance and cytokine/growth factor interaction (Iozzo and San Antonio 2001; Parish 2006; Stringer 2006; Gorsi and Stringer 2007). Apart from these important physiological functions, HS can also serve as a receptor for many viruses and bacteria (Wadström and Ljungh 1999; Chen et al. 2008; Aquino et al. 2010; Bartlett and Park 2010) and is therefore an ideal target for blocking some common viral and bacterial infections. As shown in Table I and Figure 2, cell surface HS serves as a receptor for a variety of pathogens from many different families, including some important STIs, such as HSV, HPV, HBV, HIV and C. trachomatis, many of which are discussed in this review.

One of the reasons that HS interacts with a number of pathogens relates to the structural and functional diversity of HS originating from extensive modifications during its biosynthesis. The biosynthesis of HS is a sequential, multistep process that occurs in the Golgi apparatus. Synthesis begins with the assembly of the tetrasaccharide linker region (GlcA-Gal-Gal-Xyl) on serine residues of the protein core (Esko and Selleck 2002). After the initial addition of an N-acetylated GlcN (GlcNAc) residue to start the HS chain, polymerization proceeds by the addition of alternating GlcA and GlcNAc residues. The chain extension is also

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**Fig. 1.** Molecular diversity within HS chains. (A) Unmodified HS is a representative of a disaccharide unit consisting of a GlcA and a GlcNAc residue. (B) Modified HS. Biosynthesis and modification of HS add diversity in the disaccharide chain. The disaccharide unit consisting of a GlcA and GlcNAc residue undergoes modifications in the following order: (I) N-deacetylation and N-sulfation of GlcNAc occurs, converting it to GlcNS, (II) C5 epimerization of GlcA to IdoA, (III) 2-O-sulfation of IdoA and GlcA, (IV) 6-O-sulfation and (V) 3-O-sulfation of GlcN residues. The last step in the modification in HS chain, i.e. 3-O-sulfation, can be catalyzed by multiple isoforms of 3-OST.
accompanied by a series of modifications. The modifying enzymes include glycosyltransferases, an epimerase and sulfotransferases. The modifications are known to follow a sequential order. During the first step, N-deacetylation and N-sulfation of GlcNAc occurs, converting it to N-sulfo-GlcN (GlcNS). Next is the C5 epimerization of GlcA to IdoA followed by O-sulfation, which is performed by 2-O-sulfotransferases (2-OSTs), 6-OST or 3-OST in the following order: Initially, 2-O-sulfation of IdoA and GlcA occurs followed by 6-O-sulfation of GlcNAc and GlcNS units and, finally, 3-O-sulfation of GlcN residues (Esko and Lindahl 2001). Various arrangements of these modified residues create distinct binding motifs on the HS chain that are believed to regulate its functional specificity in distinct biological processes within the host. Concurrently, modifications of HS residues allow for distinct functions in pathogen-host interactions including viral membrane fusion and penetration (Shukla and Spear 2001; Liu and Thorp 2002).

3-O-sulfation of HS

As mentioned, the final modification step during the biosynthesis of HS (summarized in Figure 1) is the 3-O-sulfation of HS. Sulfate from adenosine 3′-phosphate 5′-phosphosulphate can be transferred to the 3-OH position of GlcN residues to form 3-OS HS (Xu et al. 2005). 3-O-sulfation is a relatively rare modification of HS. It is carried out by the members of the 3-OST family. Currently, seven members of the family have been identified: 3-OST-1, -2, -3A, -3B, -4, -5, -6 (O’Donnell et al. 2010). The 3-OSTs consist of a divergent N-terminal domain and a C-terminal sulfotransferase domain that is conserved among all isoforms (Shworak et al. 1999). The sulfotransferase domain determines the sequence specificity of each isoform (Shworak et al. 1999; Yabe et al. 2001). The isoforms, 3-OST-3A and 3-OST-3B, have nearly identical amino acid sequences in the sulfotransferase domain and generate the same 3-O-sulfated (3-OS) disaccharides (Liu et al. 1999; Shworak et al. 1999). It has been suggested that each of the 3-OST isoforms are able to recognize unique saccharide sequences around the modification site (Liu et al. 1999; Shworak et al. 1999). This site-specific function of each isoform allows them to generate their own distinct 3-OS motifs. Thus, each isoform is able to produce a unique 3-OS HS chain with its own distinct function. For example, HS modified by 3-OST-1 contains high-affinity binding sites for anti-thrombin, which are absent in the HS modified by 3-OST-2, 3-OST-3, 3-OST-4 and 3-OST-6. The latter are also predicted to have unique specificities for their binding proteins (Shworak et al. 1999). Quite interestingly, HSV-1 glycoprotein D (gD) can also bind to and utilize 3-OS HS generated by all 3-OSTs as an entry receptor with the exception of 3-OST-1, which fails to generate gD-binding sites (Shukla et al. 1999; Tiwari et al. 2005; Xu et al. 2005; O’Donnell et al. 2006). It is also noteworthy that HS modified by 3-OST-5 has both anticoagulant activity and binds HSV-1 gD (Xia et al. 2002). Unique substrate specificity of these enzymes, along with their distinct expression patterns in cells and tissue, suggests some key roles in the regulation of HS functions (Shworak et al. 1999).

**Table I. Role of cell surface HS in STIs**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>GAG ligand</th>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
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<tr>
<td>HSV-1</td>
<td>HS gB/C/D</td>
<td>Cold sore, genital herpes</td>
<td>Shukla and Spear (2001)</td>
</tr>
<tr>
<td>HSV-2</td>
<td>HS gB/C</td>
<td>Genital herpes</td>
<td>Cheshenko and Herold (2002)</td>
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<tr>
<td>Human cytomegalovirus</td>
<td>HS gB</td>
<td>CMV-retinitis</td>
<td>Compton et al. (1993)</td>
</tr>
<tr>
<td>Human papilloma virus</td>
<td>HS-VLP</td>
<td>Cervical cancer, genital warts</td>
<td>Johnson et al. (2009)</td>
</tr>
<tr>
<td>HIV</td>
<td>HS-gp120; gp41</td>
<td>AIDS</td>
<td>Cladera et al. (2001) and Ceballos et al. (2009)</td>
</tr>
<tr>
<td>HBV</td>
<td>HS-L-viral envelope protein</td>
<td>Hepatitis B</td>
<td>Schulze et al. (2007)</td>
</tr>
<tr>
<td>Molluscum contagiosum</td>
<td>HS-MC54L protein</td>
<td>Molluscum lesions</td>
<td>Xiang and Moss (2003)</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
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<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>HS-Omp</td>
<td>Chlamydia</td>
<td>Fadel and Eley (2008)</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoea</em></td>
<td>HS-OpA</td>
<td>Gonorrhea</td>
<td>Freissler et al. (2000)</td>
</tr>
<tr>
<td><em>Treponema pallidum</em></td>
<td>HS-fibronectin</td>
<td>Syphilis</td>
<td>Alderete and Baseman (1989)</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
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<tr>
<td><em>Candida albicans</em></td>
<td>HS-SGSP1/ECM-proteins</td>
<td>Candidiasis</td>
<td>Klotz and Smith (1992) and Hoffman and Haidaris (1994)</td>
</tr>
</tbody>
</table>

GAG, glycosaminoglycan; HS, heparan sulfate; VLP, virus like particles; Omp, outer membrane protein; OpA, opacity protein; DsrA, *H ducreyi* serum resistance antigen A; SGSP1, submandibular gland secreted proteoglycan 1.
infection, pathogenesis itself is a multistep process which also depends on cellular functions, such as the capacity of the host to develop a proper immune response, the ability of virus to replicate efficiently within cells of various tissue origins, and the spread of infection within and between organs. Indeed, interactions with HS often form the basis of many host and/or microbe-dependent key events that affect pathogenicity and enhance disease progression (Spillmann 2001; Vivès et al. 2006). Cell surface HS is involved in viral infection and pathogenesis through its uses as a receptor by a number of viruses (Liu and Throp 2002). The major characteristics that confer cellular HS the ability to bind diverse pathogens are as follows. First, HS is a linear carbohydrate polymer with repeating pockets of negative charges (Wadström and Ljungh 1999). Most protein–HS interactions are mediated by the electrostatic interaction between clusters of basic amino acids arranged in a three-dimensional array on the ligand and a concentrated negative charge on the sulfated polysaccharide chain. Second, clustering of these modifications along the HS chain yields highly N-sulfated domains (NS domains) of ~12–20 residues that alternate with typically larger sized, relatively unmodified, N-acetyl-rich domains (NA domains). The NS domains can assume several different conformations and, thus, influence the orientation of the sulfate residues in space. This domain organization places relatively flexible NA domains adjacent to relatively rigid NS domains, thus facilitating protein interactions with the sulfate residues. Finally, the newly identified micro-sequence diversity, presumably the result of the cell type-specific repertoires of HS chain-modifying enzymes, can provide unique HS–protein interaction sites (Esko and Lindahl 2001). Due to many of the features discussed above, the binding affinity between HS and viral proteins can be relatively strong. For example, the $K_D$ for the interaction between gB of human cytomegalovirus and HS is estimated to be in the range of 0.04–0.3 μM (Boyle and Compton 1998). It is also very interesting that modifications within HS may change with an infection (e.g. HSV-2), which may alter its ability to interact with viral or non-viral ligands (Ali et al. 2012).

### Role of HS in HSV-2 infection

HSV-2, the serotype responsible for the majority of genital herpes infections, is one of the most prevalent STIs worldwide. It is the most common cause of genital ulcers (Schomogyi et al. 1998; Sacks et al. 2004; Keller et al. 2005) and a well-recognized co-factor in the acquisition of HIV (Corey et al. 2004; Keller et al. 2005; Wilson et al. 2009). HSV-2 interacts with cell surface HSPGs during virus attachment and viral spread (Table 1 and Figure 3). gB of HSV-2 mediates the interaction between the virion and cell surface HSPGs (Cheshenko and Herold 2002). This conclusion is supported by the finding that a gB2-deficient virus is significantly impaired in HS binding and that binding is restored when the virus is repaired by growth on gB complementing

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**Fig. 2.** Microbes involved in STIs utilize HS for cell attachment, internalization and spread. (1) *Chlamydia trachomatis* uses HS to bind to mucosal cells via outer membrane protein (OmcB). Mutant Chinese hamster ovary (CHO) cell lines that were defective in HS biosynthesis were less susceptible to *Chlamydia* infection than was the wild-type CHO-K1 cell line (a and b). In addition, the enzymatic removal of HS moieties from the host cell surface led to a marked decrease in *Chlamydia* infectivity (c). (2) *Neisseria gonorrhoeae* (Ngo) expressing the outer membrane protein (Opa) can adhere to and invade epithelial cells via binding to HSPG receptors (a). Syndecan-1 and syndecan-4 can both mediate Ngo uptake into epithelial cells, and their intracellular domains play a crucial role in this process, perhaps by mediating signal transduction or anchorage to the cytoskeleton (b). (3) *Treponemes* coat themselves with GAGs during syphilis infection (a). These layers of GAGs act as a matrix for binding specific lipoproteins and also protect *Treponemes* from host defense and the immune response (b). (4) Binding of mucosotropic human papilloma virus (HPV) L1 VLPs to host cells through HS triggers immune response (a and b). The interaction between the basic amino residue of L1 and L2 viral capsid protein and the negatively charged groups of HS is critical for both vaccine development and targeted microbicide. (5) Spermatozoa expressing HS capture HIV promoting the sexual transmission of HIV infection via transfer to DCs, macrophages and CD4+ cells (a and b).
The degree of sulfation of the GAG is also an important determinant for recognition by gB2. Heparin is more highly sulfated than HS and was a better competitive inhibitor of gB2 binding to immobilized heparin. Interestingly, it has been proposed that serotype differences between HSV-1 and HSV-2 lie in their ability to bind specific HS moieties (Trybala et al. 2000). For instance, HS structures rich in OS IdOα are more permissive for HSV-1 infection than cells expressing few OS HS structures. In the case of HSV-2, 6-O-desulfated and 2-O-desulfated and 3-O-desulfated heparins make cells more susceptible to HSV-2 than to HSV-1 (Gerber et al. 1995; Herold et al. 1996). These results suggest that there may be differences in the structural sequences of HS with which HSV-1 and HSV-2 preferentially bind and differences in initial interactions with cell surface HS. These differences, coupled with serotype differences in the roles of gC and gB in viral binding, may contribute to the observed differences in epidemiology and cell tropism (Herold et al. 1996). The shared features, on the other hand, should pave the way for a broad spectrum anti-viral agent that blocks host-HSV interactions. In fact, recent animal studies suggest that blocking HS can protect animals from getting infected with HSV-1 and HSV-2 (Tiwari et al. 2011; Ali et al. 2012).

Role of HS in HIV infection

A growing body of evidence suggests that HSPGs possess the capacity to modulate HIV-1 pathogenesis and can be exploited for blocking HIV-1 pathogenesis. Initially, it was shown that HIV-1 exploits the long anionic HS chains of HSPGs to attach to and enter into several T-cell lines (Patel et al. 1993). Enzymatic removal of cell surface HS chains drastically impaired the capacity of HIV-1 to infect these cells (Patel et al. 1993; Roderiquez et al. 1995). These findings were confirmed by many investigators by showing that HSPGs on the surface of specific cell types may greatly influence HIV infection (Ohshiro et al. 1996; Ibrahim et al. 1999; Saphire et al. 2001; Guibinga et al. 2002; Zhang et al. 2002). Primary human endothelial cells richly express HSPGs both in vitro and in vivo, and these HSPGs efficiently capture HIV-1 particles on the surfaces of the cells (Bobardt et al. 2004). Their abundance on the surface of primary endothelial cells and their high capacity to capture HIV-1 make HSPGs prime candidates for facilitating the invasion of the brain by HIV-1. HS expressed by microvasculature, endothelial cells of the blood brain barrier capture HIV and contribute to HIV brain invasion (Bobardt et al. 2004). It is considered an ancillary attachment factor for HIV-1 in dendritic cells (DCs), macrophages and epithelial and endothelial cells (Saphire et al. 2001; Argyris et al. 2003; Wu et al. 2003; Gallay 2004; de Witte et al. 2007). Recently, it has been shown that HS expressed on spermatozoa plays a key role in HIV transmission to DCs, macrophages and T cells (Ceballos et al. 2009). At the mucosal surface, the main point of entry for the virus into the host, HS sequesters viral particles and is involved in their translocation across epithelial barriers (Crublet et al. 2008). The ability of HS to recognize HIV-1 appears to depend on the HS-binding domains identified in the V2 and V3 loops, in the C-terminal domain and within the CD4-induced bridging sheet of the gp120 (de Parseval et al. 2001).
Role of HS in HPV infection

HPVs, belonging to the alpha genus, preferentially infect the genital mucosa, and a subset of this genus include the types (e.g., HPV16, −18, −31, −33, and −45) that are the causative agents of cervical carcinoma (Trottier and Franco 2006; Trottier and Burchell 2009). These viruses do not grow well in vitro and, hence, investigators have used recombinant HPV virus-like particles (VLPs) to study initial viral interactions with host cells. Several lines of evidence suggest that HS plays a critical role in the binding and entry of HPV-VLPs to the host cell (Sapp and Bienkowska-Haba 2009; Horvath et al. 2010; Letian and Tianyu 2010). First, enzymatic removal of HS on keratinocytes with heparinase or heparitinase resulted in an 80–90% reduction of HPV-11 VLPs binding (Joyce et al. 1999). Second, pseudoinfection of HPV-16 and -33 was inhibited by heparin, reduced with a decline in the level of surface sulfation, and abolished via a heparinase treatment (Giroglou et al. 2001). Third, HPV VLPs-mediated gene transfer was inhibited when the pseudovirions were preincubated with heparin (Combita et al. 2001). Using the murine cervicovaginal challenge model, Johnson et al. provided the evidence that in vivo HPV-31 infection was dependent on HS-dependent attachment. In these organisms, it appears that HS is located on the surface of the bacterium and that it functions as a bridge between a chlamydial adhesin and an undetermined ligand on the host cell. Thus, a trimolecular mechanism of GAG-dependent cell attachment was proposed (Zhang and Stephens 1992). However, subsequent studies revealed that GAGs are being used for attachment by many chlamydial species in various ways (Fadel and Eley 2008). Chlamydial ligand cysteine-rich outer membrane protein has also been proposed to bind HSPG (Fadel and Eley 2008). Though this remains a somewhat unexplored area in chlamydial pathobiology, it is safe to say that chlamydia-HS interactions are likely to be important in initial attachment of this pathogen to host cells and anti-HS agents may protect the hosts from the infection.

Role of HS in Gonococcal infections

Gonorrhea is an STI of worldwide importance (Bradley and Satterwhite 2012). Neisseria gonorrhoea invasion of human mucosal cells is considered to be a primary event in the pathogenesis of a gonococcal infection (Edwards and Apicella 2004). Binding of a particular opacity outer membrane protein A (Opa) of N. gonorrhoeae to cell surface HSPG of epithelial cells results in tight bacterial adherence (Dehio et al. 1998). It has been shown that syndecan-1 and syndecan-4 play important roles during uptake of N. gonorrhoea by epithelial cells and interfering with adhesion is likely to yield strong therapeutic results (Freissler et al. 2000). In fact, sulfated carbohydrates such as pentosan polysulfate, dextran sulfate and soluble heparin have been shown to efficiently block cellular
adherence of *N. gonorrhoeae* in a human fallopian tube organ culture model (Herold et al. 1997).

**Role of HS in Syphilis infection**

Syphilis is an STI caused by the bacterium *T. pallidum* (Ho and Lukehart 2011). Very limited information is available on the molecular basis of the bacteria’s ability to adhere to human cells. GAGs are known to accumulate and coat treponemes shortly after and during infection of host cells in vitro and in rabbit testes in vivo (Alderete and Baseman 1989). HS is also known to inhibit Fibronectin-primed treponemal attachment to extra cellular matrix. While it is likely that HS has a role in infection and anti-HS agents may have some therapeutic value in treating Syphilis, more information is still required to firmly establish a role for GAGs in Syphilis infection.

**Therapeutic implications of HS-based antimicrobial agents**

The development of new microbicides targeting HS to garner protection against common STIs may represent a unique approach to antimicrobial therapy. Synthetic as well as naturally-existing compounds that offer strong competition for binding to the HS chain can provide broad-spectrum microbical benefits. Synthetic inhibitors can be structured to either prevent the first and initial step of microbial binding/attachment to the host cell or intercellular spread. The major advantages of any putative HS-mimetic is their potential for effectiveness, ease in designing specific modifications to enhance anti-microbial potency and, perhaps most importantly, the potential to develop multi-strain broad-spectrum formulas against many STIs. Recent studies have established the proof-of-principle that the compounds that mimic HS block microbial infections. HS-mimetics and similar anti-microbial compounds are beginning to gain popularity (Nyberg et al. 2004; Balzarini 2007; Urbinati et al. 2008; Rusnati and Urbinati 2009; Gandhi and Mancera 2010). For instance, synthetic-CD4 HS conjugate inhibits CCR5 and CXCR4 mediated HIV-1 attachment and entry (Baleux et al. 2009). Similarly, several sulfated seaweed polysaccharides show high antiviral activity against HIV and HSV (Witvrouw and De Clercq 1997). Along the same lines, the role of sulfated carbohydrate compounds to prevent microbial adherence by HSV-2, *C. trachomatis*, *N. gonorrhoea* and HIV has been well documented (Herold et al. 1997; Scordi-Bello et al. 2005). In addition, a heterogeneous, chemically synthesized sulfated polymer lignin sulfate that mimics HS was shown to inhibit HSV-1, HSV-2 and HIV entry in vitro (Raghuraman et al. 2007). Similarly carrageen, a type of sulfated polysaccharide extracted from red algae, which resembles HS, is an extremely potent infection inhibitor for HPV, HIV and HSV (González et al. 1987; Kilmarx et al. 2008). Likewise, a sugar binding protein, cyanovirin-N, acts as a potent inhibitor for multiple viruses that cause STIs (Tiwari et al. 2009; Xiong et al. 2010). Furthermore, *E. coli* capsular polysaccharide K5, whose structure is the same as the heparin/HS biosynthetic precursor, N-acetyl heparosan, has been shown to have a broad-spectrum activity against HIV, HSV-2 and HPV (Rusnati et al. 2009). In parallel, two new, non-cytotoxic attachment blocking microbicides have reached clinical trials. These include polystyrene sulfonate (T-PSS) and cellulose sulfate (Ushercell). Ushercell is a long-chain sulfated polysaccharide (≏1900 kDa) and T-PSS is a long-chain sulfonated polymer (≏751 kDa). Both have broad-spectrum microbialic activity (Anderson et al. 2002) and are known to inhibit HIV, HPV, HSV-2, *C. trachomatis* and *N. gonorrhoeae* in vitro (Herold et al. 2000; Christensen et al. 2001; Anderson et al. 2002; Zaneveld et al. 2002). It is important to note that these compounds do not inhibit *Lactobacillus*, an important protective normal vaginal flora, in vitro, and have been proven to be safe in a variety of animal studies. Recently, they were demonstrated to inhibit *Gardnerella vaginalis* and anaerobes commonly associated with bacterial vaginosis (Simoes et al. 2002). Along the same lines, a 12-mer arginine-rich peptide was shown to act as a microbicide against genital herpes infection in female mice (Ali et al. 2012). The peptide binds 3-OH HS and show strong efficacy against HSV-1, HSV-2, CMV and HHV-8 (Tiwari et al. 2011). Thus, targeting HS using sulfated carbohydrates, heparan mimetics, anti-HS peptides and other small molecule inhibitors of microbial interactions with HS, exhibits strong promise for development as broad spectrum vaginal microbicides. Such HS-based microbicides are also expected to reduce HIV adherence to the cells of the genital mucosa.

**Future prospects and challenges**

The versatility of HS and HSPG lies in their ability to interact with diverse proteins including the microbial surface proteins involved in attachment and/or entry into the host. They are a largely untapped source of novel chemical entities and, therefore, offer exciting new opportunities for the development of novel antimicrobial molecules. Development of a common microbicide that will disrupt the sexual transmission of multiple pathogens including HIV, HSV and HPV now seems possible (Madan et al. 2006; Nikolic and Piguet 2010). Polyanionic heparin-like compounds are fast emerging as ideal multitarget antiviral drugs that may soon be used to stop the transmission of many STIs (Rusnati et al. 2009). The future challenge is to decode the structural complexity of HS especially in the light of a new intriguing possibility that structural modifications within HS change upon infection (Ali et al. 2012). Structural elucidation of HS, especially in context of its role in STIs, has remained an under-investigated area. Despite the existence of good knowledge on the basic structure and composition of HS chains and their expression as HSPGs on the cell surface, the issues of specificity of microbial interactions and corresponding alterations in HS structure have remained poorly understood. At present, two broad possibilities exist. First is that HS may have universal binding sites, which are non-specifically exploited by a diverse group of pathogens. If that is true then any given pathogen, whether it is HSV, HIV, HPV or *Chlamydia*, will bind to the common region within the HS chain and thereby rationalize the future development of universal inhibitors against the STIs. However, the second possibility is that if a given pathogen...
binds to unique sites that are uncommon to others then it would lead to the development of pathogen-specific inhibitors. In that case, a cocktail of inhibitors may be needed to prevent a myriad of STIs. It is also possible that HS serves as a key element in dictating or guiding pathogen tropism and this situation will promote the development of cell and tissue specific inhibitors to the pathogen. We have already learned about the latter possibilities in the case of HSV (O’Donnell and Shukla 2008; O’Donnell et al. 2010), which is further supported by the fact that the type of modified HS expression varies in cells and tissues (Tiwari et al. 2006). Thus, we and others have reasons to believe that HS could be a controlling factor that determines host susceptibility to any one or multiple STIs (Herold et al. 1997; Aquino et al. 2010). It is more than likely that the expression of HS-modified HS or HSPG makes the host more susceptible to co-infections and results in disease development and that targeting HS by new strategies can provide initial control over the onset of infection.

Overall, the mapping of HS-regions that are used by sexually transmitted pathogens and the genes governing these host cell structures are needed to fully understand their relationship to infection and disease development. The goal of such studies will be to develop interventional therapeutic and/or prophylactic modalities against pathogens. Due to the common utilization of HS or HS-like structures for host interactions, sexually transmitted pathogens are among the likeliest targets of such modalities. This will require continuing collaborative efforts from multiple disciplines of biochemistry, structural biology and infectious diseases as well as the challenges of clinical trials. Given the significance of HS in cell signaling the HS mimics may pose a negative effect on the proper functioning of the immune system, which is a real possibility that will need to be addressed as well. If we can address the challenges adequately, certainly we will have novel HS-based therapies capable of targeting STIs and improving human health.

References
Ceballos A, Remes Lenicov F, Sabatté J, Rodríguez Rodríques C, Cabrini M, Cianci C, Rainero R, Nuccitelli J, Marín-Briggler C, et al. 2009. Spermatozoa capture HIV-1 through HS expression and Shukla D. 2008; O’Donnell et al. 2010), which is further evaluated HS expression varies in cells and tissues (Tiwari et al. 2006). Thus, we and others have reasons to believe that HS could be a controlling factor that determines host susceptibility to any one or multiple STIs (Herold et al. 1997; Aquino et al. 2010). It is more than likely that the expression of HS-modified HS or HSPG makes the host more susceptible to co-infections and results in disease development and that targeting HS by new strategies can provide initial control over the onset of infection.

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Conflict of interest
None declared.

Abbreviation
CDC, Centers for Disease Control and Prevention; DC, dendritic cell; GAG, Glycosaminoglycan; GlcA, glucuronic acid; GlcN, d-glucosamine; GlcNAc, N-acetylated GlcN; GlcNS, N-sulfo-GlcN; HPV, human papillomavirus; HS, heparan sulfate; HSPG, HS proteoglycans; HSV-1, herpes simplex virus type 1; IdoA, iduronic acid; NA domain, N-acetyl-rich domain; NS domain, N-sulfated domain; Opa, opacity protein A; OS, O-sulfated; OST, O-sulfotransferase; STI, sexually transmitted infection; VLP, virus-like particle.


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