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Glycosaminoglycans in infectious disease

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ABSTRACT

Glycosaminoglycans (GAGs) are complex carbohydrates that are ubiquitously present on the cell surface and in the extracellular matrix. Interactions between GAGs and pathogens represent the first line of contact between pathogen and host cell and are crucial to a pathogen's invasive potential. Their complexity and structural diversity allow GAGs to control a wide array of biological interactions influencing many physiological and pathological processes, including adhesion, cell-to-cell communication, biochemical cascades, and the immune response. In recent years, increasing evidence indicates an extraordinary role for GAGs in the pathogenesis of viruses, bacteria and parasites. Herein, we examine the interface between GAGs and different pathogens, and address the divergent biological functions of GAGs in infectious disease. We consider approaches to use this understanding to design novel therapeutic strategies addressing new challenges in the treatment of infectious diseases.

Key words: glycosamimoglycans, infectious disease, virus, bacteria, parasites, protein-carbohydrate interactions.

CONTENTS

I. INTRODUCTION

One of the world's greatest health challenges is the battle against infectious disease. According to the World Health Organization (WHO) infectious diseases account for more than 8 million deaths per year (WHO, 2011), and in 2010 infectious diseases caused 58% of deaths (4.4 million worldwide) in children under 5 years of age (Global Health Observatory (GHO), 2012). Although the majority of these deaths occur in developing countries, the re-emergence of 'old' pathogens that were once considered eradicated, drug-resistant strains and new pathogens, along with globalised mass transportation, resulted in infectious diseases that threaten both developing and developed countries. Pathogens, whether they are viruses, bacteria or parasites, must overcome a complex and sophisticated defence network

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protecting their host targets. One important interface between pathogens and host is the glycosaminoglycans (GAGs). The past few decades have provided evidence of the importance of GAGs in the processes of adhesion and invasion, which are critical for successful pathogen invasion.

GAGs are polysaccharides expressed ubiquitously on the cell surface, in intracellular compartments, and in the extracellular matrix (ECM). Most GAGs are complex, linear, heterogeneous, polyanionic, and polydisperse with a structural diversity that enables these biological macromolecules to play a critical role in myriad biological processes including cell adhesion, cell migration, tissue repair, ECM assembly, cell signalling, and the immune response (Linhardt & Toida, 2004; Aquino, Lee & Park, 2010). Proteoglycans (PGs) consist of a core protein and one or more types of GAG chains, which generally comprise over 50% of their total molecular mass. These GAG chains bind to many classes of proteins including chemokines, cytokines, growth factors, enzymes, ECM, and adhesion molecules (Yamada & Sugahara, 2008). Cell surface GAGs can serve not only as primary receptors for some ligands but also as co-receptors that capture ligands and facilitate their encounter with appropriate, protein-based, signalling receptors (Bartlett & Park, 2010). These characteristics of GAGs and PGs facilitate the specific interaction between host and pathogen during infection by bacteria, viruses and parasites (Wadstrom & Ljungh, 1999). Many pathogens attack host cells by binding to their GAGs, and interactions among GAGs and pathogen proteins are often the decisive factor in the initial stages of infection. Thus, manipulating these interactions might lead to effective therapeutic approaches to defend host cells from infection. We focus this review on the role of GAGs in the interaction between pathogen and host.

II. STRUCTURE AND FUNCTION OF GAGS

GAGs are linear, anionic polysaccharides, composed of repeating disaccharide units. They are polydisperse and range in molecular weight from 15 to over 100 kDa. Despite their simple backbone sequence, most GAGs are complex mixtures of molecules characterised by a heterogeneous primary structure resulting from the variable modification of their repeating disaccharide units, including the presence and variable positioning of sulpho groups (Table 1). GAGs are generally divided into four families based on their chemical structure: hyaluronan (HA), heparin (HP)/heparan sulphate (HS), chondroitin sulphate (CS)/dermatan sulphate (DS) and keratan sulphate (KS).

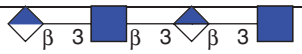
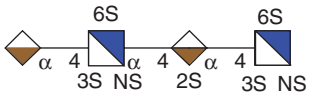
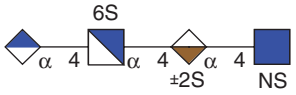
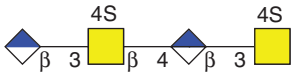
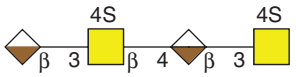
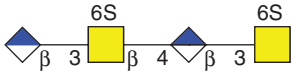
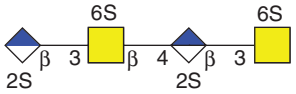
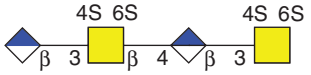
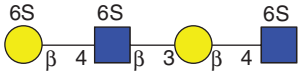
Hyaluronan (HA), the simplest GAG, is primarily found in connective tissue and is a prominent component of the ECM, it is also found in epithelial and neural tissues. Unlike the other GAGs, its biosynthesis is carried out in the plasma membrane instead of in the Golgi. The structure of HA is based on a single repeating unit, (1–3) β-*N*-acetyl-D-glucosamine (1–4) β-D-glucuronic acid. HA is generally larger than other GAGs (100–1000 kDa), it has no sulpho groups and is not biosynthesised as a PG linked to a core protein.

Heparin and heparan sulphate are probably the most complex and best studied of the GAGs. They are found on the cell surface, within the cell, or secreted into the ECM. Although HP is best known for its anticoagulant properties (Linhardt & Claude, 2003), both HP and HS have been recognised as key players in cell signalling and growth, cell adhesion, migration and differentiation as well as in angiogenesis and host-pathogen interactions. The molecular weight of HP and HS range from 15 to 20 kDa (Zhang *et al.*, 2011). HP is biosynthesised as a PG, *O*-linked to serglycin, while HS is *O*-linked to a number of different core proteins. Their structure is based on L-iduronic acid or D-glucuronic acid linked 1–4 to *N*-sulpho or *N*-acetyl D-glucosamine. Both HP and HS have highly variable repeating units with HP being more highly sulphated than HS. The major repeating unit in heparin is a trisulphated disaccharide unit of the structure $\rightarrow$ 4)-*N*-sulpho 6-*O*-sulpho-α-D-glucosamine (1 $\rightarrow$ 4) -2-*O*-sulpho α-L-iduronic acid (1 $\rightarrow$. The major repeating unit in HS is monosulphated, $\rightarrow$ 4)-α-D-*N*-acetyl or *N*-sulpho glucosamine (1 $\rightarrow$ 4)-β-D-glucuronic (1 $\rightarrow$, with 0 to 1 *O*-sulpho groups present in each disaccharide.

Chondroitin sulphate has been shown to be required for an array of biological processes including morphogenesis (Mizuguchi *et al.*, 2003; Nandini & Sugahara, 2006), central nervous system (CNS) development (Sugahara & Mikami, 2007), and signal transduction (Sato *et al.*, 2008; Yamada & Sugahara, 2008, and references therein). CS is found on cell surfaces and in the ECM. It is particularly abundant in bone, tendons, blood vessels, nerve tissue, and cartilage (Nandini & Sugahara, 2006). Naturally occurring CS, which is widely distributed in animal tissues, has been reported to have an average molecular weight of 20–30 kDa. CS is biosynthesised as a PG *O*-linked to a number of different core proteins. The CS family of GAGs has a basic repeating disaccharide unit of alternating glucuronic or iduronic acid and *N*-acetylgalactosamine. Differences among the CS GAGs (CSA-E) result from differential modification of the disaccharide units, including sulphation level and position, and isomerization of the C5-position of the glucuronic acid residue. CSA has a β-D-glucuronic acid 1 $\rightarrow$ 3 *N*-acetyl, 4-*O*-sulpho β-D-galactosamine 1 $\rightarrow$ 4 repeating unit. CSB [also known as dermatan sulphate (DS)] has the same structure as CSA except for the replacement of the β-D-glucuronic acid with α-L-iduronic acid. CSC has the same structure as CSA except the 4-*O*-sulpho group on the β-D-galactosamine residue is replaced by a 6-*O*-sulpho group. CSD is similar to CSC but has a second sulpho group on the 2-position of the β-D-glucuronic acid residue. CSE has a structure similar to both CSA and CSC with a *N*-acetyl, 4,6-di-*O*-sulpho-β-D-galactosamine residue.

There are two forms of keratan sulphate: KSI, originally isolated from corneal tissue is *N*-linked to various core proteins and KSII, originally found in cartilage and bone, is *O*-linked to various core proteins (Funderburgh, 2000).

Table 1. Compilation of general chemical properties of glycosaminoglycans (GAGs)

GAG	Molecular properties Mw Disaccharide Mw Polydispersity (PD)	Average chain length in disaccharides	Sulpho groups (per disaccharide unit)	Structure
Hyaluronan (HA)	~ 100–1000 kDa 379.1 Da	260–2600	0	
Heparin (HP)	~ 15 kDa> 476.9 Da PD – 1.4	~ 30	~ 2.7	
Heparan sulphate (HS)	~ 20 kDa 459.1 Da	~ 40	~ 1	
Chondroitin sulphate –A (CSA)	~ 20 kDa 469 Da PD – 1.2	~ 40	≤ 1	
Chondroitin sulphate-B (CSB or DS)	~ 30 kDa 459.1 Da	~ 60	≤ 1	
Chondroitin sulphate-C (CSC)	~ 20 kDa 459.1 Da	~ 40	≤ 1	
Chondroitin sulphate-D (CSD)	~ 20 kDa 539 Da	~ 40	~ 2	
Chondroitin sulphate-E (CSE)	~ 20 kDa 539 Da	~ 40	~ 2	
Keratan sulphate-I (KS I)	~ 14 kDa 500 Da	~ 35 ~ 10	~ 1.5	
Keratan sulphate-II (KS II)	~ 5 kDa 500 Da			

Glucuronic acid (GlcA) Glucosamine (GlcN) N-Acetylglucosamine (GlcNAc) Iduronic acid (IdoA) Galactose (Gal) N-Acetylgalactosamine (GalNAc)

Structure of the major disaccharide units are represented based on the consortium for functional glycomics nomenclature. The abbreviations 2S, 4S or 6S indicate 2-*O*, 4-*O* or 6-*O*-sulpho, respectively. Mw, molecular weight.

Corneal KS is believed to function as a dynamic buffer promoting corneal hydration while cartilage KS GAGs form part of large hydrated PGs that serve as shock absorbers in cartilage. The repeating disaccharide units of KS are → 3)-β-D-galactose (1 → 4) β-D-*N*-acetyl glucosamine (1 →, where the 6-positions on either residue can be modified with *O*-sulpho groups. Other rare modifications are possible, such as fucosylation of the repeating unit or capping of the GAG chain with *N*-acetyl neuraminic acid (sialic acid).

There are a number of polysaccharides and glycans that, while not GAGs, can mimic some GAG functions and compete with or substitute for GAGs in protein interactions. These GAG-like glycans include acidic microbial polysaccharides, such as alginate comprised of guluronic and manuronic acids (Silva, Mano & Reis, 2010), or acidic glycoconjugates such as acidic mucins containing sulphated sugars and sialic acid (Nystrom *et al.*, 2010). In addition there are synthetic and semi-synthetic GAG analogues, such as dextran sulfate (DexS) (Zhang, Yoder & Linhardt, 2004).

III. BIOSYNTHESIS OF GAGS

PGs can be found at high concentrations in animal cells, 10^5–10^6 copies per cell and at mg/ml concentration in the ECM (Zhang, 2010), and thus represent a substantial portion of the glycocalyx. The secondary structures of these PG GAG chains consist of extended helices as a result of their high negative charge, and these polysaccharides can reach a length of 260–2600 disaccharide units. Chain length as well as sulphation pattern are regulated in a spatio-temporal manner during the diverse stages of development. These structural features dictate differential interactions with protein targets, impacting a range of physiological processes (Capila &

Linhardt, 2002; Pichert *et al.*, 2011; Pickford *et al.*, 2011). GAGs can be found as free entities (e.g. HA), however, they are usually attached to a core protein in the form of PGs. Following translation of the core protein in the endoplasmic reticulum, the protein follows the secretory pathway with all GAGs except HA undergoing biosynthesis in the Golgi apparatus. The synthesis of *O*-linked GAGs starts with the generation of a common linkage region. First, a xylose (Xyl) is attached to a specific serine (Ser) residue of the core protein; this Ser residue is known as the gagylation site. While there is no known consensus sequence (Blom *et al.*, 2004) for *O*-glycosylation, a recent publication suggests that a set of rules rather than a simple consensus sequence governs the selection of a site for gagylation (Hamby & Hirst, 2008). Following the addition of a Xyl, two residues of galactose (Gal) and one residue of glucuronic acid (GlcA) are added resulting in a tetrasaccharide (GlcA-Gal-Gal-Xyl) GAG-protein linkage region. At this point the addition of N-acetyl-D-glucosamine will commit PG synthesis towards HS, while addition of N-acetylgalactosamine will result in synthesis of a CS. Next, the disaccharide chain is extended stepwise with sugar residues added incrementally; the core proteins are believed to determine the number of GAG chains, type, and ultimate cellular (or extracellular) destination of the resulting PG.

GAGs are subjected to many modifications; HS can be both *N*- and *O*-sulphated at the 3- and 6-positions of the glucosamine and/or 2-position of iduronic acid formed through the epimerisation of glucuronic acid. CS can be modified by *O*-sulphation at the 4- and 6-positions of the *N*-acetyl galactosamine residue and position 2- of the iduronic acid residue formed through the epimerization of glucuronic acid. The HS and CS GAG disaccharides can undergo five or six different modifications resulting in a variety of different isomers. Moreover, the modifications tend to occur in clusters along the chain, with regions devoid of sulphate affording domain structures (Nandini & Sugahara, 2006; Ly *et al.*, 2011). In general, sulphation of the different GAGs does not proceed to completion, resulting in tremendous heterogeneity within the modified regions of both HS and CS GAGs. This regulated pattern of modification has been referred to as the 'sulphation code' (Gama *et al.*, 2006). It is hypothesised that the position of the different modifications results in spatial/sequence information that can be recognised by the corresponding protein thus enabling fine-tuned recognition processes. It is not yet clear how the enzymes in the GAG biosynthetic pathway are regulated to achieve tissue-specific expression of ligand-binding sequences.

IV. BIOLOGY: CELL-CELL INTERACTION AND INFECTIOUS DISEASE

(1) Pathogen-host interaction

Evidence that specific protein receptors recognise specific carbohydrate structures has been mounting over the past few decades. The carbohydrate-binding receptors can be divided into two major subgroups, GAG-binding receptors and lectins (Mulloy & Linhardt, 2001; Esko & Selleck, 2002; Drickamer & Taylor, 2003; Varki & Angata, 2006). The lectins can be further subdivided into three major classes: galectins, siglecs and selectins (Schnaar, 2004; Linnartz, Bodea & Neumann, 2012). There is extensive evidence that GAGs promote microbial pathogenesis and invasion (Rostand & Esko, 1997) by interacting with bacteria (e.g. *Helicobacter pylori*, *Bordetella pertussis*, *Mycobacterium tuberculosis* and *Chlamydia trachomatis*), viruses (e.g. herpes simplex and dengue virus) and protozoa (e.g. *Plasmodium* spp. and *Leishmania* spp.). These interactions result from pathogen surface proteins capable of binding with host cell surface HS and CS, and these interactions appear to mediate infection (Rostand & Esko, 1997). GAGs are believed to be responsible for cell-cell or host-pathogen interactions because most eukaryotic cells as well as many bacteria express cell surface GAGs. GAGs bind directly to various biological ligands such as growth factors, proteinases, and antimicrobial factors in the ECM or receptors in the host cells. Protein-glycan interactions play a major role during the physical interaction between parasites and host cells (van Kooyk & Rabinovich, 2008). Rapid modifications of glycans can take place by either microbial or host glycosyl transferases or glycosidases or transglycosidases, sulphotransferases or sulphatases, increasing the complexity of host-microorganism interactions (Sharon, 2007; Marth & Grewal, 2008; van Kooyk & Rabinovich, 2008).

Creating a well-defined map of the network of GAG–protein interactions (interactome) (Polanska, Fernig & Kinnunen, 2009) is hindered by the fact that only a few methods exist for the rapid identification of such interactions. In an attempt to address this challenge surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), carbohydrate microarrays, and computational modeling (Rogers *et al.*, 2011) have been used to study protein–GAG interactions (Nelson *et al.*, 1995; Capila & Linhardt, 2002). These studies generally indicate that weak (micromolar) individual binding-site interactions are often enhanced by multivalency, resulting in high (nanomolar) affinity binding (Ascencio *et al.*, 1993), and that these interactions are generally rapid (seconds to minutes) as a result of a fast on-rate (Zhang *et al.*, 2012).

The complement system is a first-line defence mechanism against infection from parasites, bacteria or viruses. Because it is deeply connected with the immune system, complement deficiencies are relevant to most infectious diseases including microbial infections, such as meningococcal disease, viral infections, such as HIV/AIDS (Ram, Lewis & Rice, 2011), and parasitic infections. There are three different complement pathways: classical, the lectin and the alternative pathway. Mannan-binding lectin binds in a Ca^{2+}-dependent manner to the target *via* its C-type lectin domains, which recognise neutral sugars (preferentially mannose, *N*-acetylglucosamine and fucose) on the surfaces of a range of microorganisms, such as *Neisseria* and *Leishmania* species

(Rooijakkers & van Strijp, 2007). Polyanions, such as GAGs are known to regulate the activation of complement in both the classical and alternative pathways (Edens, Linhardt & Weiler, 1993). Both direct and indirect inhibition of complement esterases by highly sulphated GAGs, such as heparin, is believed to be responsible for this regulation.

The complement system is an essential part of the innate and acquired immune systems. It can rapidly recognise bacteria for phagocytosis by professional phagocytes or kill bacterial pathogens directly by membrane perturbations (Brown, Joiner & Frank, 1983). A number of bacterial pathogens evade complement attack by sequestration of human regulators to the bacterial surface. Bacterial surface proteins can bind human complement component 4-binding protein (C4BP) and factor H (FH)/factor H-1 (FHL-1). These regulators are captured when they interact with complement component 3 (C3) convertases and function as cofactors in factor I cleavage of complement component 3b (C3b)/complement component 4b (C4b). In addition, deficiencies of the terminal complement pathway can occur, for example infection by *Neisseria meningitides* subverts the immune response by recruiting the host complement regulator FH (Schneider *et al.*, 2009).

Parasites entering the bloodstream and causing infections need to avoid destruction by the complement system. Several specific strategies of complement resistance have been reported and these findings can help in the development of new therapeutic and preventive approaches to control parasitic diseases (Jokiranta, Jokipii & Meri, 1995). Both classical and alternative complement pathways can be activated by parasites. The classical pathway nearly always requires the involvement of antibodies whereas the alternative pathway is activated directly by products released by the parasites or present in their outer membranes. Activation of complement through the alternative pathway may also be a prerequisite for cellular adherence to parasites, which results in parasite destruction (Santoro, Bernal & Capron, 1979).

Viruses use a variety of strategies to evade the host's immune response (Bartlett & Park, 2010; Garcia-Mayoral *et al.*, 2010). A number of viruses not only avoid inactivation and destruction by complement but also use complement receptors to initiate infection. Murine gamma herpesvirus 68 (MHV-68), a regulator of complement activation (RCA) protein, inhibits complement activation at the level of C3 (Kapadia *et al.*, 1999), playing a critical role in viral evasion of the complement system in the acute, persistent and latent phase of infection (Kapadia *et al.*, 2002). Furthermore, MHV-68 is a major GAG-binding protein (Gillet, Adler & Stevenson, 2007), promoting the initial contact of virions with host cell GAGs (Steer *et al.*, 2010).

(2) Viruses

The underlying nature of viruses as obligatory intracellular parasites requires the internalization of the viral particle into the host cell. Since cells are enclosed in a glycocalyx, a virus must negotiate its way through a landscape of GAGs. It has been known for over two decades that some viruses utilise GAGs in their initial step of entering a host cell (Lycke *et al.*, 1991; Roderiquez *et al.*, 1995) (Table 2). Furthermore, viruses take advantage of GAG properties in different steps in their invasion of the host cell, from the initial process of cell recognition to the final step of endocytosis of the viral particle into the host cell. Viruses are able to recognise GAGs as co-receptors on the surface of host cells much in the same way as bacteria. The adeno-associated virus (AAV), a member of the *Dependovirus* genus, represents a well-documented example of such interactions between virus and GAG. AAV is a non-enveloped parvovirus that was first discovered in tissues of children infected with adenovirus (Salo & Mayor, 1979). AAV-2 (serotype 2) is considered a promising vector for gene therapy, because the virus elicits a low immune response, is non-pathogenic, infects dividing as well as non-dividing cells, integrates at a specific genomic site, and has a wide range of target cells. The wide cell tropism stems from interaction of AAV-2 with HS-PG as a primary low-affinity receptor, followed by binding to a co-receptor for infection (Summerford & Samulski, 1998). Analysis of the crystal structure of AAV-2 suggested that the HS binding site might be a region of positive charge on surface protrusions where four arginine residues and a lysine are arranged together (Xie *et al.*, 2002). This prediction was later verified by three independent mutagenesis studies indicating that the basic residues on the capsid surface were crucial for HS-PG binding (Kern *et al.*, 2003; Opie *et al.*, 2003; Lochrie *et al.*, 2006). Twelve AAV serotypes have been identified (Schmidt *et al.*, 2008). In spite of the high sequence identity of their genomes (53–93%), these AAV serotypes are antigenically distinct and use a range of different receptors for cell binding. In a recent publication, the interaction of AAV serotype 3b with HP was analysed using X-ray diffraction. While the binding between the capsid and HP is similar for AAV-2 and AAV-3b (in both cases positive charges are present in the binding site), the amino acids that participate in these interactions are different, suggesting a mechanistic explanation for the antigenic differences between these serotypes (Lerch & Chapman, 2012).

Vaccinia virus is a large enveloped DNA virus that belongs to the *Orthopoxvirus* genus. *Vaccinia* virus envelope proteins A27 and H3 bind to the cell surface HS (Chung *et al.*, 1998; Lin *et al.*, 2000), while the D8 envelope protein binds to CS (Hsiao, Chung & Chang, 1999). By introducing a soluble form of the D8 protein, as well as through the genetic manipulation of the D8 protein, adsorption of the virion to the host cell could be inhibited. Thus, a single virus has distinct proteins capable of recognizing either HS or CS. This enables flexible and robust recognition and binding of virus to host cells that depends on the presence of either HS or CS GAGs as recognition moieties. Similar virus-GAG interactions have been demonstrated in *Flavivirus* infection. The interaction between the *Dengue* virus (a positive single-stranded RNA virus of the *Flavivirus* genus) envelope proteins could be antagonised by competing HP with a minimum chain length of a decasaccharide. This interaction could be

Table 2. Virus-glycosaminoglycan (GAG) interactions

Pathogen	Disease	GAG	References
Adeno-associated virus (AAV)	Leading candidate vector for human gene therapy	HS	Lerch & Chapman (2012) and O'Donnell *et al.* (2009)
Cytomegalovirus (CMV)	Kaposi sarcoma	HS	Boyle & Compton (1998)
Dengue	Dengue fever	HS	Chen *et al.* (1997), Kato *et al.* (2010) and Prestwood *et al.* (2008)
Epstein–Barr virus (EBV)	Burkitt's lymphoma	HS	Ianelli *et al.* (1998)
Foot and mouth disease viruses	—	HS	Hayman *et al.* (2005)
Hepatitis B virus (HBV) Hepatitis C virus (HCV) Hepatitis E virus (HEV)	Hepatitis	HP	Leistner *et al.* (2008), Germi *et al.* (2002), Barth *et al.* (2003, 2006) and Kalia *et al.* (2009)
Human immunodeficiency virus (HIV)	AIDS	HS	Patel *et al.* (1993) and Roderiquez *et al.* (1995)
Herpes simplex virus (HSV)	Herpes	CSB, Heparin, LMWH, HS	Jenssen *et al.* (2008), Copeland *et al.* (2008), Nyberg *et al.* (2004) and Spear *et al.* (1992)
Human papillomavirus	Cervical cancer	HS	Giroglou *et al.* (2001)
Japanese encephalitis virus	Encephalitis	HS	Chien *et al.* (2008)
Lentivirus/retrovirus	—	CSC	Landazuri & Le Doux (2004)
Respiratory syncytial virus (RSV)	Lower respiratory tract infections	HS, CS	Klenk & Roberts (2002)
Vaccinia virus	Smallpox	HS	Chung *et al.* (1998), Lin *et al.* (2000) and Hsiao *et al.* (1999)
Varicella zoster	Chicken pox	HS	Jacquet *et al.* (1998)

CSB and CSC, chondroitin sulphates B and C; HP, heparin; HS, heparan sulphate; LMWH, low molecular weight heparin.

antagonised by a highly sulphated form of HS (derived from liver) while HS (derived from testis) with lower sulphation showed much lower potency (Chen *et al.*, 1997).

In a recent publication, CSE showed antiviral activity as an entry inhibitor, targeting envelope (E) protein located on the lipid bilayer envelope of the virus in all four serotypes of *Dengue* virus (Kato *et al.*, 2010). Viral infection is initiated by the interaction between E protein and protein, lipid, or carbohydrate host cell receptors (Aoki *et al.*, 2006). Okamoto *et al.* (2011) further extended our understanding of this interaction. Using a cloned K562 cell line, in conjunction with a specific *Dengue* virus strain model [DEN2 16681 (Wu-Hsieh, Yen & Chen, 2009)] they showed that the *Dengue* virus interacts with the PG, syndecan-2. Syndecans are transmembrane HS-PG receptors linked to the actin cytoskeleton (Choi *et al.*, 2010). Syndecan-2 can have 1 to 4 GAG chains linked to specific serine residues (at positions: 41, 53, 55 and 57). Mutagenesis of Ser41 (one of these gagylation sites) to alanine (Ala) inhibits viral infection while the triple Ser to Ala mutant, at positions 53, 55 and 57, has no effect on viral infectivity (Fig. 1A) (Okamoto *et al.*, 2011).

Viral attachment to the cell surface initiates a cascade of events resulting in the infection of the cell. It was recently observed in at least in two unrelated viruses (retroviruses and papillomavirus) that following virion attachment to the filopodia, the virion migrates and travels along these extensions to reach the cell body for infection. This phenomenon, coined 'viral surfing', was first reported in *Herpes simplex* virus 1 (HSV1) and was shown to depend upon the underlying actin cytoskeleton and functional myosin II (Lehmann *et al.*, 2005; Schelhaas *et al.*, 2008). HSV1 undergoes initial attachment to the target cell through an interaction between HSV1 glycoproteins gB, gC and gD and HS (Spear *et al.*, 1992; Shukla & Spear, 2001; Liu *et al.*, 2002; Sharma-Walia *et al.*, 2004). Viral entry requires a specialised cell surface HS, known as 3-*O*-sulphated HS, that interacts with gD. HSV1 infection was inhibited by challenging the virus with a synthetic defined 3-*O*-sulphated octasaccharide sequence (Copeland *et al.*, 2008). Using live cell imaging a directed 'surfing' movement of the HSV1 toward the cell body was recorded (Fig. 1B). This movement depended on the interaction of gB glycoprotein with HS; removal of either HS (by heparinase treatment) or gB glycoprotein (by genetic manipulation) results in the loss of this surfing movement, indicating a role for HS as a mediator of this viral transport phenomenon (Oh *et al.*, 2010).

Virus particles can enter a cell through a highly regulated process that first requires binding to the host cell. There are many different internalization processes; in enveloped viruses this process requires the fusion of the viral outer membrane with the cytoplasmic membrane of the cell, resulting in release of the viral particle into the cytoplasm of the host cell. However, a virus with two membranes needs to shed both membranes to reach the host cell cytoplasm as a free particle. A mechanism to achieve membrane shedding is exhibited by the *Vaccinia* virus. Using immuno-labeling coupled with electronmicroscopic imaging of *Vaccinia* virus infection, the outer membrane of the virus was shown to be disrupted and shed, leaving the inner membrane free to fuse with the cytoplasmic membrane of the host cell (Law *et al.*, 2006) (Fig. 1C). Outer membrane shedding depends on viral glycoproteins A34 and B5, and actual membrane disruption

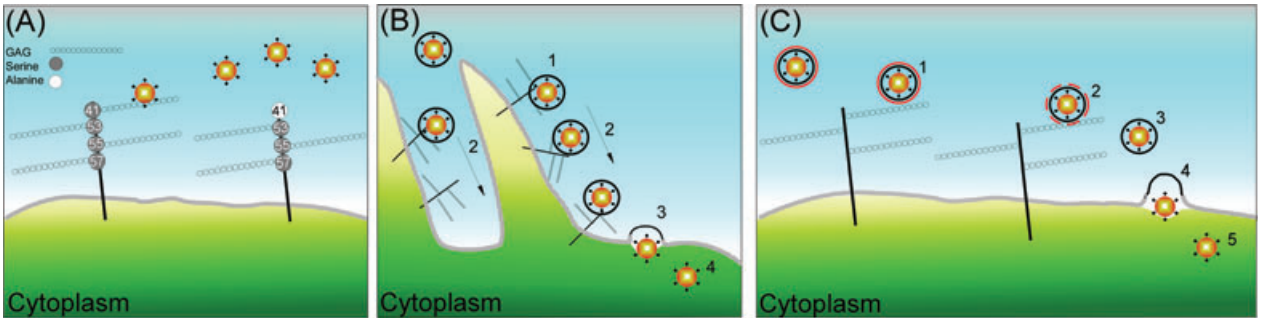


Fig. 1. Viral-glycosaminoglycan (GAG) interactions. (A) The *Dengue* virus interacts specifically with heparan sulphate (HS) linked to syndecan-2 at Ser41. (B) *Herpes simplex* virus 1 (HSV1) interacts with an HS-proteoglycan (PG) through the gB/gC viral envelope protein (1). This interaction allows the virus to 'surf' on extruding filopodia to the body of the host cell (2). Viral host cell interaction results in fusion of the viral membrane with the host cytoplasmic membrane (3) and the release of the HSV capsid into the cytoplasm of the host (4). (C) *Vaccinia* virus must shed its outer membrane before it is able to fuse with the host cell cytoplasmic membrane. The viral glycoproteins A34 and B5 interact with HS or chondroitin sulphate (CSB) GAG (1); this results in disruption and shedding of the outer viral membrane (2, 3). The virion is now able to fuse its inner membrane with the host-cell cytoplasmic membrane (4) and invade the cytoplasm of the host cell (5).

is attributed to HS and CSB GAG interaction. Both the high negative charge and the specific carbohydrate structure of GAGs are required for efficient *Vaccinia* virus membrane rupture, as was confirmed by challenging the virus with different polyanions (Law *et al.*, 2006; Roberts *et al.*, 2009). Blocking of viral attachment or entry into host cells is an effective strategy to control viral infection (Altmeyer, 2004) and hence this interaction may provide an important target for new drug design.

(3) Bacteria

GAGs are involved in multiple aspects of the bacteria-host cell interaction, and as a consequence they play an important role in bacterial pathogenesis (Duensing, Wing & van Putten, 1999; Yamada & Sugahara, 2008) (Table 3). Bacteria usually must overcome mechanical protection by the host organism, including peristaltic intestinal motion, airway reflexes, and shear stress. A pathogen lacking the ability to attach efficiently to the host is often readily eliminated. The human respiratory system epithelial cell surface contains a glycocalyx, consisting of mucins and GAGs. Immune cells and commensal bacteria are also heavily glycosylated with GAGs. During infection, a bacterium can attach to host cells reversibly or irreversibly, modifying glycan structure to establish and maintain colonization. Bacterial modification of host glycans can contribute to revealing receptors for adherence, the modification of host defence, the progression of the bacteria through the glycocalyx, interspecies competition, biofilm formation, and the release of carbohydrates for growth (Sauer *et al.*, 2002; Stoodley *et al.*, 2002; King, 2010).

An important feature of bacteria-GAG binding is the high on-rate and off-rate exhibited by these interactions. GAGs serve as co-receptors for bacteria and represent the first interface for host-pathogen interactions. This interface is then extended to more stable interactions that usually involve protein-protein interactions. Most publications in this area suggest that GAGs serve predominantly as co-receptors (Chen *et al.*, 1995; Fagan, Lambert & Smith, 2008) (Fig. 2A). While most GAG-bacteria interaction studies have focused on HP and HS (Liang *et al.*, 1992; Fleckenstein, Holland & Hasty, 2002; Gu *et al.*, 2008), HA and CS have also been shown to be involved in such interactions (Hafez *et al.*, 2008). For example, treatment of patients suffering from recurring cases of urinary tract infections (UTIs) with HA and CS resulted in decreased recurrence of the UTI. While the study did not focus on a specific bacterium, in 76% of cases the causative pathogen was *Escherichia coli*. The tendency to develop a UTI was attributed to a damaged GAG layer lining the transitional epithelium of the human bladder that led to direct exposure of epithelial cells to urine components, increasing the possibility of bacterial adherence and infection (Damiano *et al.*, 2011).

Recent studies show that polysaccharides can serve as the primary binding entity between pathogen and host. Carbohydrate-containing molecules rather than protein ligands have been shown to mediate Gram-positive *Enterococcus faecalis* adhesion to eukaryotic cells by using proteinases and sodium periodate oxidation of carbohydrates to modify the bacterial surface (Sava *et al.*, 2009). While most evidence does not identify a specific mechanism for bacterial-host interaction, these studies suggest a potential role for GAGs in bacterial infection beyond simply serving as co-receptors. Moreover, GAGs have also been implicated in processes of bacterial internalization by a host. Host cell HS has been identified as a receptor for alpha C protein (ACP), a surface protein in Group B *Streptococcus* bacteria (GBS). ACP was shown to bind to HS-PG and to facilitate GBS internalization into host cells through a mechanism requiring rho GTPase-mediated actin polymerization (Baron *et al.*, 2004, 2009). A recent publication provided evidence that GAG binding facilitates entry of a bacterial pathogen across the blood–brain barrier (BBB) into the central nervous system (CNS) of *Drosophila melanogaster*. In this study a

Table 3. Bacteria-glycosaminoglycan (GAG) interactions

Pathogen	Disease	GAG	References
Borrelia burgdorferi (−)	Lyme disease	HP	Leong *et al.* (1998)
Brodetella pertussis (−)	Pertussis	HS	Hannah *et al.* (1994) and Menozzi *et al.* (2002)
Chlamydia pneumoniae (−)	Pneumonia	HP	Yan *et al.* (2006)
Chlamydia spp. (−)	Pneumonia, atherosclerosis and Alzheimer's disease	HS	Derre *et al.* (2007)
Chlamydia trachomatis (−)	Chlamydia	HS	Fadel & Eley (2008)
Enterococcus faecalis (+)	Nosocomial infections (root-canal)	HP, HS, CSA	Baldassarri *et al.* (2005) and Sava *et al.* (2009)
Escherichia coli (+)	Gastrointestinal infections, neonatal meningitis	HP, HS	Fagan *et al.* (2008), Gu *et al.* (2008), Chena *et al.* (2012) and Fleckenstein *et al.* (2002)
Haemophilus influenzae (−)	Meningitis, pneumonia	HP, HS	Noel *et al.* (1994)
Helicobacter pylori (−)	Ulcer	HS	Ascencio *et al.* (1993)
Legionella pneumophila (−)	Severe respiratory illness	CS, HS, HP	Duncan *et al.* (2011)
Leptospira interrogans (−)	Leptospirosis	CSB	Frick *et al.* (2003)
Listeria monocytogenes (+)	Listeriosis	HP	Hayman *et al.* (2005)
Listeria spp. (+)	Listeriosis	HS	Alvarez-Dominguez *et al.* (1997)
Mycobacterium spp.	—	HP	Hayman *et al.* (2005)
Mycobacterium tuberculosis	Tuberculosis	HP	Dupres *et al.* (2005)
Neisseria gonorrhoeae (−)	Gonorrhoea	HP, HS	Chen *et al.* (1995)
Staphylococcus aureus (+)	Staphylococcal scalded-skin-syndrome, MRSA	HP, HS	Fallgren *et al.* (2001) and Hess *et al.* (2006)
Staphylococcus epidermis (+)	Catheter-associated infection	CS, HP	Krevvata *et al.* (2011) and Arciola *et al.* (2003)
Staphylococcus haemolyticus (−)	Catheter-associated infections	HP, HS	Fallgren *et al.* (2001)
Staphylococcus hominis (+)	Nosocomial infections	HP, HS	Fallgren *et al.* (2001)
Streptococcus agalactiae (+)	Bacterial septicemia, neonatal meningitis	HS	Baron *et al.* (2004, 2009), Chang *et al.* (2011) and Wang *et al.* (2010)
Streptococcus gordonii	Endocarditis	HP	Vacca-Smith *et al.* (1994)
Streptococcus mutans (+)	Tooth decay, cardio vascular disease	HP > HS > CSA	Choi & Stinson (1989) and Nakano *et al.* (2006)
Streptococcus pneumoniae (+)	Bacterial meningitis	HS, CSA	Rigden & Jedrzejas (2003) and Tonnaer *et al.* (2006)
Streptococcus pyogenes (+)	Throat infection (pharyngitis, tonsillitis) and skin infection	HP	Frick *et al.* (2003)

+/− in parentheses indicate Gram positive/negative bacteria, respectively.
CSA, CSB and CSC, chondroitin sulphates A, B and C; HP, heparin; HS, heparan sulphate.

mutation in the ACP of GBS resulted in significantly reduced GAG binding without affecting overall ACP structure. As a result the mutated GBS showed a reduced ability to transport to the head of the fly. Moreover, three different HS-PG mutant fly strains, deficient in membrane HS-PG core proteins, HS polymerases, or *N*-deacetylase-*N*-sulphotransferase were studied. After infection with wild-type GBS, all three HS-PG mutant fly strains exhibited lower head/total body colony forming unit (CFU) ratios relative to wild-type control flies. The long chain length and high negative charge of GAGs are both critical in providing the binding force needed for ACP interaction (Fig. 2B) (Chang *et al.*, 2011).

While GAGs are required in many instances to mediate the interaction between the bacteria and the host, they can sometimes also antagonise binding, acting as antibacterial molecules. GAG-binding proteins such as the inflammatory mediator azurocidin may even play a role in the morbidity associated with sepsis in bacterial infections (Linder, Soehnlein & Akesson, 2010). Baranska-Rybak *et al.* (2006) showed that GAGs found in wound fluid can inhibit the bactericidal activity of the antibacterial peptide, LL-37. They suggested that specific peptide–GAG interactions result in competition for bacterial binding, explaining the loss of *in vitro* antibacterial activity of LL-37 in GAG-rich wound fluids. This property of GAGs can be utilised by bacteria; *Pseudomonas aeruginosa* is able to release GAGs from connective tissues to block the bactericidal actions of LL-37 (Schmidtchen *et al.*, 2003) (Fig. 2C). The interaction between GAGs and this antimicrobial peptide may have a significant impact on cystic fibrosis (CF) patients. Physiological concentrations of LL-37 had minimal potency in bronchoalveolar lavage fluid. This reduced potency of LL-37 does not stem from degradation because it is protected from proteolysis by interactions with GAGs; however this interaction results directly in inactivation of LL-37 antimicrobial activity. Digestion of GAGs present in CF lavage fluid releases LL-37 restoring its antimicrobial properties. This inhibitory interaction between GAGs and LL-37 was also seen in the sputum of CF patients. The

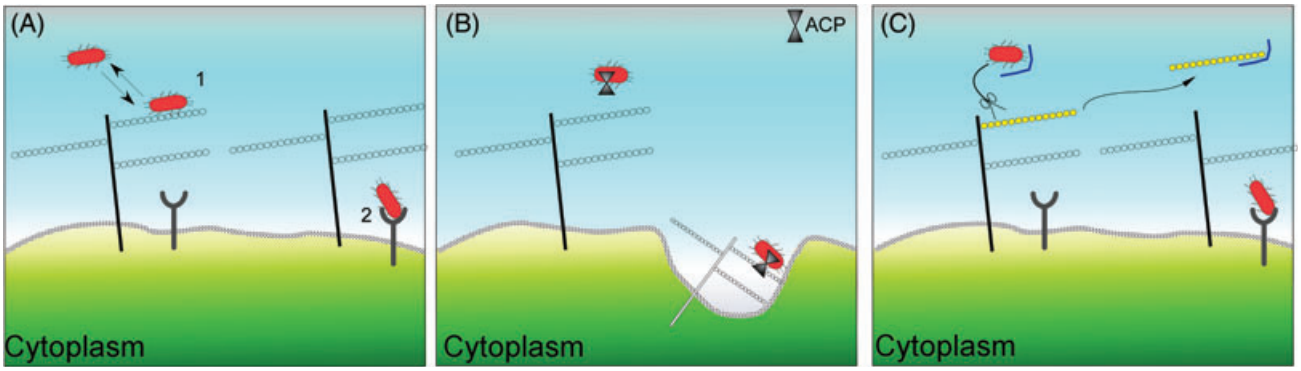


Fig. 2. Bacterial-glycosaminoglycan (GAG) interactions. (A) In many cases bacteria first interact with a GAG. GAGs often serve as co-receptors, taking advantage of GAG-protein interaction properties (fast on/off-rates). The bacteria undergo an initial 'weak' interaction with the GAG (1) before establishing a more stable interaction with a cell surface protein receptor (2). (B) Alpha C protein (ACP – hourglass shape) on the cell surface of Group B *Streptococcus* bacteria (GBS) binds directly with heparan sulphate proteoglycan (HS-PG). The direct interaction between the GBS and HS-PG results in invasion of the cytoplasm by the internalization of the bacteria. (C) *Pseudomonas aeruginosa* induces the release of GAG from its PG carrier. The released GAG can then interact with the antimicrobial peptide LL-37 (blue line). This interaction frees the bacteria to attack the host cell while the antibacterial peptide is engaged with the released GAG.

antibacterial peptide regained its potency after the lungs were washed with nebulised hypertonic saline (7% NaCl) resulting in the release of LL-37 from the bound GAG (Bergsson *et al.*, 2009).

Recent evidence has expanded our understanding of the mechanisms underlying the involvement of GAGs in host-pathogen interactions. Both Gram-negative and Gram-positive bacteria contain polysaccharide capsules that mimic the structure of GAGs. These are typically found in the form of an extracellular coating known as a capsule (Vann *et al.*, 1981; Jann & Jann, 1992; DeAngelis, 2002; DeAngelis *et al.*, 2002). Microbial GAGs are apparently used for one or more bacterial defence strategies including molecular mimicry, hijacking biological pathways, or altering host defences (Wessels & Bronze, 1994; Dale *et al.*, 1996; Chang *et al.*, 2011). These capsules can serve as molecular camouflage for commensal populations of pathogenic bacteria allowing these microorganisms to evade the host immune response (DeAngelis *et al.*, 2002).

(4) Parasites

GAG-binding proteins in parasites are important for recognition, attachment and invasion of host cells (Wadstrom & Ljungh, 1999). Binding of PGs to parasites requires specific GAG chains in a variety of human-pathogen adherence mechanisms (Oliveira *et al.*, 2008). Pathogens use surface GAGs as adhesion receptors to attach to the host cell (Ortega-Barria & Boothroyd, 1999; Tonnaer *et al.*, 2006; Sinnis *et al.*, 2007). Many studies present evidence that cell surface GAGs play a role as receptors for various parasitic pathogens such as the genus *Toxoplasma* (Harper, Hoff & Carruthers, 2004; Gong *et al.*, 2012), the genus *Plasmodium* (Rathore *et al.*, 2003; Cowman & Crabb, 2006), and the genus *Trypanosoma* (Ortega-Barria & Pereira, 1991) (Table 4). Mast cells, which protect host organisms against parasitic infections, are particularly rich in highly sulphated GAGs, such as HP and CSE, which are released during mast cell degranulation in response to parasites (Linhardt, 2003). Parasites such as schistosomes can either synthesise or acquire GAGs from their host to modulate the host immune response (Cummings & Nyame, 1999).

At the onset of malaria infection of the human host, the circumsporozoite protein expressed on the surface of *Plasmodium* species sporozoites plays a critical role in recognizing and binding to HS in hepatocytes (Rathore *et al.*, 2003). Pregnancy-associated malaria (PAM) results from the ability of *P. falciparum*-infected red blood cells (IRBCs) to bind to CSA on the human placental epithelium (Clausen *et al.*, 2012). VAR2CSA, a parasite protein present on IRBC, is the most characterised *P. falciparum* erythrocyte membrane protein (PfEMP1). The extracellular portion of PfEMP1 consists of six Duffy-binding-like (DBL) domains and there are several reports that domains such as DBL3X, DBL1X-6ε and DBL2X have binding-specificity to CSA (Fig. 3A) (Singh *et al.*, 2008; Srivastava *et al.*, 2010; Dahlback *et al.*, 2011). In the insect host, mosquito salivary gland HS binds to the circumsporozoite protein of *Plasmodium* and this binding is involved in the transmission of *Plasmodium* (Sinnis *et al.*, 2007). CSA in mosquito midgut binds to *Plasmodium falciparum* PfEMP1 proteins during infection (Dinglasan *et al.*, 2007).

Another example of direct binding between host GAGs and a parasite can be seen in *Toxoplasma gondii*. The protein *T. gondii* micronoe 4 (TgMIC4), one of the PAN/apple domain proteins, binds to CS and one specific surface antigen on host cells. SAG3 another surface protein in *T. gondii* interacts with HS on the membrane of Chinese hamster ovary cells (Jacquet *et al.*, 2001). These proteins have been suggested as possible therapeutic targets for the development of blocking peptides or vaccines (Clausen *et al.*, 2012).

The glycan-binding proteins in parasites (Scharfstein, 2006) are important for attachment to the host cell glycans

Table 4. Parasite-glycosaminoglycan (GAG) interactions

Parasite	Disease	GAG	Protein target	References
Ascaris lumbricoides (giant roundworm)	Ascariasis, pneumonia	HP	Cell-surface glycoprotein encoded by the CD44 gene (CD44)	Ponce-Leon *et al.* (2009)
Encephalitozoon intestinalis	Microsporidia microsporidiosis	CSA, CSB, HP	Recognises the terminal galactose/*N*-acetyl-D-galactosamine	Hayman *et al.* (2005)
Enterocytozoon cuniculi (obligate intracellular parasite)	Acute diarrhoea	HS	Endospore protein 1 (EnP1), a microsporidian adherence protein	Sandfort *et al.* (1994) and Southern *et al.* (2007)
Fasciola hepatica (liver fluke)	Fascioliasis (also fasciolosis)	HP analogue DexS	Cathepsin B-like enzyme (FhcatB1)	Beckham *et al.* (2006)
Helminth	Asthma	HP	Eosinophil cationic protein (ECP)	Hayman *et al.* (2005)
Leishmania viannia braziliensis (protozoan)	Leishmaniasis	HP	HP-binding protein	de Castro Cortes *et al.* (2010)
Neospora caninum (coccidian)	Spontaneous abortion in infected livestock	CSA	N. caninum microneme protein 4 (NcMIC4)	Keller *et al.* (2004)
Plasmodium falciparum (protozoan)	Pregnancy-associated malaria (PAM)	HP, HS, CSA	*Plasmodium falciparum* erythrocyte membrane protein (PfEMP1)	Maier *et al.* (2009)
Plasmodium sp.	—	HS	—	Hayman *et al.* (2005)
Schistosoma mansoni (trematode)	Schistosomiasis (bilharzia)	HP	*Schistosoma mansoni* cathepsin B1 (SmCB1)	Horn *et al.* (2011)
Toxoplasma gondii (protozoan)	Toxoplasmosis	CS	Protein of 104 kDa (P104)	Gong *et al.* (2012)
		HP	Microneme-2 (MIC2) integrin-like A-domain	Harper *et al.* (2004)
Trypanosoma cruzi	Chagas disease	HS	High molecular weightz kininogen	Scharfstein (2006)
		CS		

CS chondroitin sulphate; CSA and CSB chondroitin sulphates A and B; HP, heparin; HS, heparan sulphate; DexS, dextran sulphate.

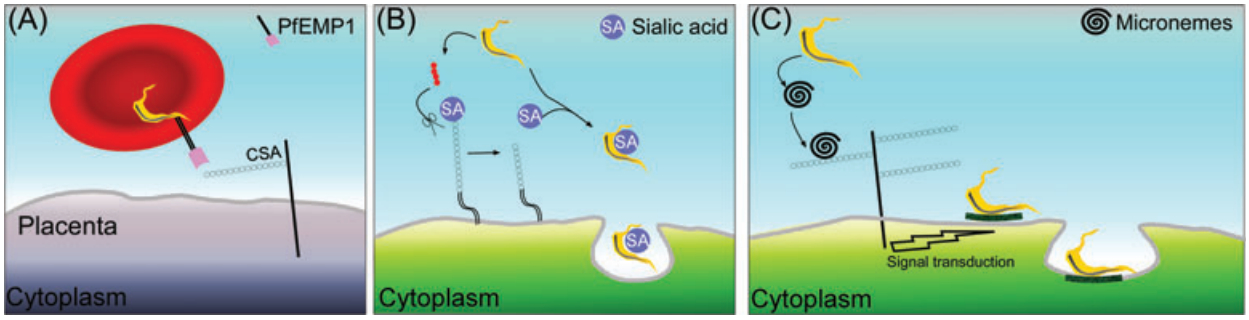


Fig. 3. Parasite-glycosaminoglycan (GAG) interactions. (A) Pregnancy-associated malaria results from the ability of *Plasmodium falciparum*-infected red blood cells to bind to human placental epithelium in pregnant women. The *P. falciparum* erythrocyte membrane protein (PfEMP) VAR2CSA is exposed on the outer membrane of infected red blood cells and specifically binds to chondroitin sulphate (CSA) on the placental epithelium. (B) *Trypanosoma cruzi* expresses a unique enzyme called trans-sialidase (TcTS) which is important for the survival of the parasite in the mammalian or insect host. TcTS transfers sialic acid from the host glycoconjugate to a mucin on the parasite surface and among host macromolecules. The transferred sialic acid plays a role in the recognition and invasion of host cells. (C) *Toxoplasma gondii* and *Neospora caninum* can release secretory proteins called micronemes. They bind to GAGs on the host cell surface and induce a signalling cascade resulting in adhesion of the parasite to the cell membrane and penetration of the host cell membrane by endocytosis or invasion.

and subsequent invasion (Keller *et al.*, 2004; Li *et al.*, 2009; Horn *et al.*, 2011). Some parasites express a trans-sialidase (TcTS), which is a glycosylphosphatidylinositol anchored protein with two active sites: a sialic binding site and a galactose binding site (Buschiazzo *et al.*, 2002). Parasites can express active and inactive trans-sialidases involved in host cell invasion and immunomodulation (Harper *et al.*, 2004; Gong *et al.*, 2012). *Trypanosoma cruzi* utilises TcTS to transfer sialic acid from host glycoconjugates to mucin (a GAG-like biopolymer) of the parasite. The transferred sialic acid plays a role in host cell invasion and protection of the parasite against complement. Thus, TcTS increases the pathogenicity and virulence of the parasite (Fig. 3B) (Harper *et al.*, 2004; Giorgi *et al.*, 2010). *R. prolixus* is the main vector of Chagas disease caused by *T. cruzi* in Central America and its midgut digestive enzymes play

a critical role in the survival and infection of *T. cruzi* (Lopez-Ordonez, Rodriguez & Hernandez-Hernandez, 2001). The incubation of *T. cruzi* epimastigotes with sialic acid and mannose inhibits their attachment to the midgut epithelium of the triatomid bug *Rhodnius prolixus* and defers epimastigote growth and differentiation into trypomastigotes (Nogueira *et al.*, 2007). This shows that both negatively charged specific carbohydrates in the midgut and carbohydrate binding proteins on the *T. cruzi* surface are involved in the attachment process (Alves *et al.*, 2007; Nogueira *et al.*, 2007).

HP-binding proteins from *T. cruzi* epimastigotes have been characterised recently and found to be capable of recognizing not only HP but also HS and CS in the midgut of *R. prolixus* (Oliveira *et al.*, 2008, 2012). This suggests that heparin-binding proteins have crucial roles in adhesion to and invasion of the host during infection. *In vitro* binding assays, using mouse cardiomyocytes, demonstrate that *T. cruzi* trypomastigotes selectively bind to HS GAGs (Calvet *et al.*, 2003) while *T. cruzi* epimastigotes show a less selective profile by binding to both HS and CS GAGs. This suggests the presence of different GAG-binding sites in the two forms of the parasite. It also indicates that GAGs are one of the determinants of parasite infection in the insect vector and that the recognition mechanisms involved might depend upon the physicochemical nature of both GAGs in the insect midgut and carbohydrate binding proteins on the surface of *T. cruzi* (Gonzalez *et al.*, 2011).

Studies show that a variety of glycans can inhibit spores from attaching to host cells, suggesting that the ability of microsporidia to infect a wide range of hosts and tissues may correlate with their ability to utilise multiple GAGs for adherence. Elucidation of the mechanisms of spore adherence may lead to the development of a new class of therapeutics (Hayman, Southern & Nash, 2005).

Some parasites employ host cell invasion as a key initiator of their life cycle. *Neospora caninum* is an apicomplexan parasite that must enter host cells to survive and proliferate (Keller *et al.*, 2004). During this process, the parasite sequentially releases micronemes, rhoptries, and dense granules from secretory organelles and interacts physically with the host cell. At the onset of recognition, microneme proteins containing some adhesive domains are released by exocytosis from the apical end of the parasites and promote invasion onto the host cell membrane. Among the known microneme proteins, NcMIC1 and NcMIC4 are known to bind to CS (Fig. 3C) (Keller *et al.*, 2002, 2004). Another micronemal protein MIC2 from *T. gondii* can bind to HP, resulting in a multimerised MIC2. This multimer may form multivalent adhesive junctions during parasite entry (Harper *et al.*, 2004).

V. SPECIFICITY IN GAG BINDING AND THE DESIGN OF NEW THERAPIES

GAG-protein interactions depend on ionic, hydrogen-bonding and hydrophobic interactions and generally exhibit fast on-rates and off-rates. Despite a good understanding of the mechanism of GAG-protein interaction, the conformational flexibility of GAGs and our incomplete knowledge of GAG primary structure make it difficult to establish the specificity of such interactions. Thus, the nature of the interaction between pathogen surface proteins and different GAGs might be argued to be either specific or promiscuous.

GAG binding might be promiscuous, and binding between pathogen surface proteins and GAGs on the surface of the target cell might simply be based on electrostatic and relatively non-specific interactions of negatively charged GAGs with positively charged pathogen surface proteins. In this model, the pathogen seeks a 'landing patch' on the surface of the cell and the kinetic properties of GAG interaction, mainly high on/off-rates, provides an appealing platform. This aligns well with the current view of surface GAGs serving primarily as co-receptors. Once the pathogen binds on this 'landing patch' a more specific and stronger binding then follows with a protein-based receptor, resulting in a cascade of events leading to infection or an immune response. This may benefit the pathogen by providing a highly flexible interface for the pathogen to interact with a variety of hosts and an array of molecules and hence to increase its infective potential (Duensing *et al.*, 1999).

Alternatively, GAG-pathogen interactions may be highly specific. Variations in the sulphation pattern of HS and CS chains (Nandini & Sugahara, 2006), together with different lengths and patterns of disaccharide inclusion provide an immense informational landscape, as can be seen when comparing GAGs purified from different origins (Warda *et al.*, 2006; Zhang *et al.*, 2011). GAG structure varies across cell type, tissue type, and organism even within a given GAG family (Shi & Zaia, 2009). Such differences in structure of GAG chains can have discrete biological effects. For example, only the HS chain bound to Ser-41 is important in the *Dengue* virus infection while the presence of HS on other positions of the syndecan-2 protein is not sufficient to facilitate viral attachment (Okamoto *et al.*, 2011).

The GAG-pathogen interaction can be quite strong with dissociation constant (K_d) values in the nanomolar range; $K_d = 61$ nM for CSA in the CSA – VAR2CSA interaction in the malaria parasite *Plasmodium falciparum* (Srivastava *et al.*, 2010) and $K_d = 9$ nM for *Helicobacter pylori* binding to HS (Ascencio, Fransson & Wadstrom, 1993). Such strong interactions suggest high specificity. It is noteworthy that CSE and HP show common structural properties such as the presence of two *O*-sulpho groups at a specific distance on the same side of a sugar, that are believed to underlie their anti-viral properties (Kato *et al.*, 2010). Hence, the distribution and positioning of sulphate groups may have a critical role in the recognition event between pathogen and GAG; this is emphasised by the fact that different levels of sulphation do not necessarily result in loss of interaction (Ghosh *et al.*, 2009). Gama *et al.* (2006) introduced a 'sulphation code' to describe the information encoded in a sequence-specific manner in GAGs, in an analogous way to proteins and

nucleic acids. The presence of specific interactions between GAGs and pathogens does not exclude the possibility of cross talk between different proteins and a specific GAG (or *vice versa*), a well-known phenomenon in proteins. However, the 'sulphation code' together with other unique structural features such uronic acid epimers suggest that a mechanism exists for specific interaction between GAGs and proteins.

It is still not possible to resolve the issue of specificity of GAG-protein interactions primarily due to technical challenges. GAG biosynthesis is not template-driven, and it is hard to envision a mechanism that can result in the generation of a specific GAG sequence that would allow the storage of spatial/temporal information with the same exquisite accuracy seen in nucleic acids or proteins. Also unlike proteins and nucleic acids it is still a considerable challenge to sequence a GAG. Recent successes in top-down sequencing of a GAG chain by Fourier-transformation mass spectrometry suggest that some simple GAGs might indeed have defined sequences (Ly *et al.*, 2011). Much work remains before we can sequence more complex GAGs such as HS. Sequence information is critical as it will provide us with the 'GAGome' at high resolution and facilitate our understanding of the information encoded in GAGs and, hence, their functional biological roles.

The indiscriminate implementation of anti-viral/antibiotic/antiparasitic drugs poses a major problem in the development of resistance and the destruction of beneficial commensal microbiota. Improved ability to sequence GAGs might reveal unique recognition elements in their structure that will enable the development of target-specific drug treatments improving selectivity and decreasing problems associated with drug resistance.

The interaction between pathogen and host cell through GAGs is a critical step in pathogen attachment, invasion/transmission/internalization and evasion of host defence mechanisms. As yet there are no therapeutic agents based solely on GAGs that target viruses/bacteria/parasites. However, the multiple roles of GAGs in the interaction between pathogen and host cell represent an appealing target for the design of new drugs. Identification and characterization of specific GAG sequences involved in such interactions will allow synthesis of competitive antagonists to inhibit the ability of a pathogen to interact with endogenous GAGs (Rusnati & Urbinati, 2009). One example is the off-label use of suramin (a relatively toxic synthetic HP analog only approved for the treatment of African trypanosomiasis) for the treatment of dengue fever (Chen *et al.*, 1997; Marks *et al.*, 2001). One advantage of using GAGs in such an approach is that their toxicity level is very low, thus, allowing for the administration of high doses. As an alternative approach, it is conceivable to design proteins or small molecule analogs (Schuksz *et al.*, 2008) to bind to the GAG sites targeted by the pathogen, and thus competitively inhibit the pathogen's ability to bind to its GAG target. The shedding of GAGs from the surface of a cell that is under attack by a pathogen might also result in protection from pathogens. The development of an agent that could 'trim' away the GAG, preferably in a selective manner, to disconnect a specific PG from the cell surface could conceivably confer protection to the host cell. As we increase our understanding of the exact mechanisms of pathogen-GAG interactions, it may be possible to identify specific modifications to GAGs that can then be targeted. For example, the loss of specific sulpho residues may have a dramatic effect on the potential binding ability of a pathogen. While these suggestions remain hypothetical, they all represent valid and potentially potent approaches that could allow the utilization of GAG-pathogen interactions as a new frontier to combat pathological conditions.

VI. CONCLUSIONS

(1) GAGs represent a physiologically important group of molecules involved in a variety of biological functions such as cell proliferation, cell-to-cell communication, wound healing, coagulation, morphogenesis and pathogenesis.

(2) Advances in analytical techniques will enable higher resolution studies of GAG structure. Such new techniques may reveal a new layer of information encoded in GAGs (the sulphation code) that will facilitate understanding the specificity that underlies GAG-pathogen interactions.

(3) The classical view of the role of GAGs in pathogenesis is that they function as co-receptors. However, accumulating evidence suggests a more complex role in the pathogen life cycle, facilitating specific recognition and interactions between the pathogen and host cell.

(4) (GAGs present an intriguing target for the design of new approaches for diagnostic and therapeutic agents against various infectious diseases.

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