Chapter 2
Heparan Sulfate Proteoglycans in Infection

Allison H. Bartlett and Pyong Woo Park

Abstract To cause infections, microbial pathogens elaborate a multitude of factors that interact with host components. Using these host–pathogen interactions to their advantage, pathogens attach, invade, disseminate, and evade host defense mechanisms to promote their survival in the hostile host environment. Many viruses, bacteria, and parasites express adhesins that bind to cell surface heparan sulfate proteoglycans (HSPGs) to facilitate their initial attachment and subsequent cellular entry. Some pathogens also secrete virulence factors that modify HSPG expression. HSPGs are ubiquitously expressed on the cell surface of adherent cells and in the extracellular matrix. HSPGs are composed of one or several heparan sulfate (HS) glycosaminoglycan chains attached covalently to specific core proteins. For most intracellular pathogens, cell surface HSPGs serve as a scaffold that facilitates the interaction of microbes with secondary receptors that mediate host cell entry. Consistent with this mechanism, addition of HS or its pharmaceutical functional mimic, heparin, inhibits microbial attachment and entry into cultured host cells, and HS-binding pathogens can no longer attach or enter cultured host cells whose HS expression has been reduced by enzymatic treatment or chemical mutagenesis. In pathogens where the specific HS adhesin has been identified, mutant strains lacking HS adhesins are viable and show normal growth rates, suggesting that the capacity to interact with HSPGs is strictly a virulence activity. The goal of this chapter is to provide a mechanistic overview of our current understanding of how certain microbial pathogens subvert HSPGs to promote their infection, using specific HSPG–pathogen interactions as representative examples.

A.H. Bartlett
Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA

P.W. Park (*)
Division of Respiratory Diseases, Children’s Hospital, Harvard Medical School, 320 Longwood Avenue, Enders-461, Boston, MA 02115, USA
e-mail: pyong.park@childrens.harvard.edu

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2.1 Primer on HSPG Biology

The complex interplay between hosts and pathogens has many common themes. One of the first steps in infection is attachment to host tissues. The expression pattern of host cell surface proteins that serve as receptors for pathogen attachment has a significant role in determining the initiation, progression, outcome, and tissue tropism of infections. Among these, HSPGs are expressed ubiquitously on the surface of adherent cells and in the extracellular matrix (ECM), and many viral, bacterial, and parasitic pathogens have been described to interact with HSPGs (Rostand and Esko 1997; Bernfield et al. 1999; Spillmann 2001; Chen et al. 2008). HSPGs bind to and regulate growth factors, cytokines, and chemokines, and ECM components (Bernfield et al. 1999; Park et al. 2000; Bartlett et al. 2008) and are known to be involved in processes as diverse as wound healing (Fears and Woods 2006; Alexopoulou et al. 2007), angiogenesis (Stringer 2006), and neuronal development (Reizes et al. 2008). HSPGs are composed of a protein core to which one or more heparan sulfate (HS) glycosaminoglycan (GAG) chains are covalently attached. HS chains can be both N- and O-sulfated, and it is with these chains that most pathogens interact. The highly complex mechanisms of HSPG biosynthesis have been partially defined and are discussed in detail in other chapters of this book.

The major ECM HSPGs, perlecan and agrin, help to form the structure of the basement membrane (BM) and modulate growth factors to affect cell survival, motility, and tissue morphogenesis (Iozzo 2005). The major cell surface HSPGs are syndecans and glypicans (Bernfield et al. 1999). There are four syndecans in mammals (syndecan-1 through -4) with distinct extracellular domains to which HS chains are attached distally to the plasma membrane, and highly conserved transmembrane and cytoplasmic domains. Syndecan cytoplasmic domains contain one invariant serine and three invariant tyrosine residues as well as a C-terminal Glu–Phe–Tyr–Ala PDZ binding domain. Syndecan cytoplasmic domains can regulate interactions with host proteins such as c-Src (Kinnunen et al. 1998), cortactin (Kinnunen et al. 1998), syntenin (Grootjans et al. 1997), protein kinase A (Hayashida et al. 2006), CASK/LIN2A (Cohen et al. 1998; Hsueh et al. 1998), and protein kinase Cz (Kessler et al. 1997), among other signaling and scaffolding proteins.

There are six glypicans in mammals that differ from syndecans in several ways. Syndecan core proteins are type 1 transmembrane proteins, whereas glypicans are covalently linked to the cell surface by glycosylphosphatidylinositol (GPI) anchors (David et al. 1990). The extracellular core proteins of syndecans are rich in secondary structure-breaking proline residues and considered linear, whereas glypicans are thought to have a compact globular structure held in place by multiple disulfide bonds formed between several conserved cysteine residues (Chen and Lander 2001). Further, HS attachment sites in syndecans are distal to the plasma membrane, whereas those of glypicans are proximal (Chen and Lander 2001). In addition, syndecans and glypicans show distinct temporal and spatial expression patterns, which in part explain how these HSPGs may function specifically in vivo.
Cell surface HSPGs primarily function as coreceptors for HS-binding ligands, serving as a scaffold that localizes the ligands to the cell surface, enabling them to interact more efficiently with their respective signaling receptors. Cell surface HSPGs can also function as a primary receptor where they mediate the endocytosis of several HS-binding ligands via macropinocytosis and clathrin-dependent and clathrin-independent endocytosis (Poon and Gariepy 2007). Some of the examples include macropinocytotic uptake of FGF-2 (Tkachenko et al. 2004) and clathrin-independent endocytosis of unmodified lipoproteins through syndecans (Fuki et al. 1997). Cell surface HSPG-mediated endocytosis is thus an important mechanism that regulates growth factor signaling and lipoprotein degradation, though how cell surface HSPGs mediate outside-in signaling leading to ligand internalization is incompletely understood. As described later, certain microbial pathogens have adapted or evolved to subvert this mechanism to gain entry into host cells.

The syndecan family of cell surface HSPGs can be shed as intact, soluble HSPG ectodomains by proteolytic cleavage of the core protein. Soluble syndecan-1 and -4 ectodomains are detected in inflamed or infected body fluids, indicating a physiological role for shedding in diseases (Subramanian et al. 1997; Wang et al. 2008; Zvibel et al. 2009). Syndecan ectodomains are replete with all of their HS chains and are thought to maintain their ability to interact with the same ligands as the cell surface syndecans, and thus act as soluble autocrine or paracrine effectors. For example, syndecan-1 ectodomains regulate the proliferative response of cells in injured tissue to FGF-2 (Kato et al. 1998) and syndecan-1 ectodomains increase the invasiveness of a human breast cancer cell line in vitro (Nikolova et al. 2009). Syndecan-1 ectodomains also bind to neutrophil enzymes such as elastase and cathepsin G, protecting the enzymes from their physiological inhibitors and potentiating the enzymes’ activity (Kainulainen et al. 1998).

Our understanding of the mechanism whereby syndecan shedding occurs and is regulated is increasing. Syndecan ectodomains are shed by metalloproteinase sheds. For example, matrix metalloproteinase-7 (MMP-7, matrilysin) (Li et al. 2002; Ding et al. 2005), MMP-9 (gelatinase B) (Brule et al. 2006), MMP-14 (MT1-MMP) (Endo et al. 2003), and ADAM17 (TACE) (Pruessmeyer et al. 2010) can shed syndecan-1 ectodomains. Chemical inhibitor studies have shown that several kinases regulate syndecan shedding, such as MAP kinases ERK (extracellular signal-related kinase) and JNK (c-Jun NH₂-terminal kinase) (Fitzgerald et al. 2000), protein kinase C (PKC) (Subramanian et al. 1997; Fitzgerald et al. 2000), and protein tyrosine kinases (PTKs) (Fitzgerald et al. 2000; Park et al. 2000, 2004; Chung et al. 2006). The role of the highly conserved cytoplasmic domain of syndecan-1 in shedding regulation is becoming clear. The three invariant Tyr residues are required for agonist-induced syndecan-1 shedding, but Tyr phosphorylation of the cytoplasmic domain is not (Hayashida et al. 2008). Instead the cytoplasmic domain modulates syndecan-1 shedding by interacting with critical regulators of shedding. One such regulator identified recently is Rab5. Rab5 is a small GTP-binding protein, whose GDP-bound inactive form binds preferentially to the cytoplasmic domain of syndecan-1 (Hayashida et al. 2008). Stimulation of cells with syndecan-1 shedding agonists induces the activation of Rab5 and dissociation of activated GTP-bound Rab5 from
the syndecan-1 cytoplasmic domain, suggesting that Rab5 regulates syndecan-1 shedding by serving as an on–off switch of shedding through its alternation between GDP- and GTP-bound states (Hayashida et al. 2008).

Shed glypican ectodomains have been detected in cell culture media (Mertens et al. 1996; Fitzgerald et al. 2000), suggesting that glypicans are also shed. Indeed, the Drosophila Dally (glypican)-like protein Dlp can be cleaved from its GPI anchor and released by the protein Notum (a secreted member of the α/β-hydrolase family) altering the activity of the growth factor wingless (Wg) (Kreugera et al. 2004). Further, glypican-3 ectodomains have been detected in the serum of patients with hepatocellular carcinoma (Capurro et al. 2003; Hippo et al. 2004) and melanoma (Nakatsura et al. 2004), but whether this represents shedding or secretion remains uncertain. One study identified the soluble glypican-3 fragment in patients with hepatocellular carcinoma as the N-terminal end, with the core protein cleaved between Arg and Ser (Hippo et al. 2004), suggesting physiologic shedding of glypican-3 in the context of hepatocellular carcinoma. However, whether glypicans are shed upon infectious challenge remains to be determined.

2.2 Methods for HSPG Studies in Infectious Diseases

Several techniques have been applied to investigate the role of HSPGs in infections. One of the simplest methods to determine if HSPGs are involved in a process is to evaluate the ability of soluble HS or heparin to specifically inhibit the function being studied (e.g., attachment, internalization) (Rostand and Esko 1997). Comparing the ability of different GAGs to inhibit attachment also gives information on which proteoglycan(s) might be involved. When a specific HSPG, such as syndecan-1 is suspected, soluble forms of the purified ectodomain can also be used in addition to HS and heparin to probe the specific interaction. Further, various chemically desulfated heparin compounds have been used to determine the critical HS modification in HS–pathogen interactions. In addition, recent studies have generated means to produce structurally defined HS using specific HS biosynthetic enzymes in vitro (Chen et al. 2007a), which should further advance our understanding of critical HS modifications in future studies.

Additional information regarding precise GAG involvement can be obtained by treating cells in culture with a variety of GAG-digesting enzymes. There are three types of \textit{Flavobacterium} heparinases (I, II, and III) with different substrate specificities and three types of chondroitinases (ABC, AC, and C) that allow one to determine the relative contribution of different GAGs by selective removal of GAGs. For example, attachment of \textit{Toxoplasma gondii} to Vero cells is inhibited by heparinase I and II treatment, but unaffected by chondroitinase ABC treatment, suggesting that sulfated domains in HS mediate \textit{T. gondii} attachment. In contrast, the attachment of \textit{Neospora caninum} tachyzoites, a close relative of \textit{T. gondii}, to Vero cells is unaffected by heparinase treatment and inhibited by chondroitinase treatment.
(Naguleswaran et al. 2002), suggesting that *N. caninum* tachyzoites bind to CS to adhere to Vero cells.

Selective chemical inhibitors of HS biosynthesis, such as sodium chlorate and \(\beta\)-d-xylosides, have also been used to study the specificity and significance of HS–pathogen interactions. Growing cells in media that is deficient in inorganic sulfate and supplemented with the sulfation inhibitor sodium chlorate reduces the overall extent of sulfation of HS and other GAGs (Baeuerle and Huttner 1986). \(\beta\)-d-xylosides competitively inhibit the formation of the tetrasaccharide linkage region required for GAG attachment to proteoglycan core proteins, diverting the machinery and raw materials from building GAG chains on core proteins. The GAG chains built on the \(\beta\)-d-xylosides primers are secreted from cells, and the proteoglycans displayed on the cell surface are underglycanated (Esko and Montgomery 1995). However, caution must be taken when using sodium chlorate and \(\beta\)-d-xylosides because both can be toxic to cells when incubated at high concentrations or for long periods.

Cell lines deficient in certain HS biosynthetic enzymes have also been used as tools to study the relationship between the type and degree of sulfation in the context of microbial infection. Multiple mutant CHO K1 cell lines exist which produce different amounts and types of HS and CS: pgsA-745, which lacks xylosyltransferase produces no GAGs; pgsB-761, -618, and -650 have defects in galactosyltransferase I and produce ~5%, ~15%, or ~30% of wild type HS and CS; pgsD-677 has defects in \(N\)-acetylglucosaminyl transferase and glucuronosyltransferase and makes three times the wild type amount of CS but cannot make HS; pgsF-17 lacks functional 2-\(O\)-sulfotransferase thus produces 2-\(O\)-sulfate deficient HS but normal CS (Rostand and Esko 1997). Several specific sulfatases have also been identified, and these should also facilitate the determination of critical sulfate modifications in microbial pathogenesis. A method for synthesizing HS oligosaccharides with defined composition has been developed for studying structure–activity relations of specific HS oligosaccharides with HS-binding proteins (Arungundram et al. 2009). This method has not yet, to our knowledge, been used in the context of evaluating host–pathogen interactions, but remains an exciting tool likely to be used in the future to enhance our understanding of the molecular interactions between pathogens and HS. Lastly, recent studies have generated mutant mouse lines deficient in certain HS biosynthetic enzymes (Forsberg et al. 1999; Grobe et al. 2005; Pallerla et al. 2008) and these mice should allow investigators to better examine the physiological significance of HS modifications in infectious diseases.

2.3 HSPGs in Microbial Attachment and Internalization

Many viral, bacterial, and parasitic pathogens have been shown to subvert HSPGs in the course of infection (Table 2.1). In microbial pathogenesis, HSPGs function primarily as initial, low affinity coreceptors that concentrate pathogens on host cell surfaces, increasing binding to specific secondary receptors. For several pathogens
| Pathogen                          | Pathogen protein                  | HSPG          | Function/interaction     | Reference                                |
|----------------------------------|-----------------------------------|---------------|--------------------------|------------------------------------------|
| **Bacteria**                     |                                   |               |                          |                                          |
| Bacillus anthracis               | AnIB, AN1O, InhA, Npr599          | Syndecan-1    | Shedding                 | Chung et al. (2006), Popova et al. (2006) |
| Bacillus cereus                  | ClnA                              | Syndecan-1    | Shedding                 | Popova et al. (2006)                     |
| Borrelia burgdorferi             | 39 kDa protein                    | Unknown       | Attachment               | Isaacs (1994)                            |
| Bordetella pertussis             | Filamentous hemagglutinin         | Unknown       | Attachment               | Hannah et al. (1994)                     |
| Chlamydia pneumoniae             | OmcB                              | Unknown       | Attachment               | Moelleken and Hegemann (2008)            |
| Chlamydia trachomatis            | Unknown                           | Unknown       | Attachment, invasion     | Zhang and Stephens (1992), Chen and Stephens (1997) |
| Haemophilus influenzae, nontypable| High molecular weight protein     | Unknown       | Attachment               | Noel et al. (1994)                       |
| Helicobacter pylori              | Vacuolating cytotoxin (VacA)      | Unknown       | Toxin internalization    | Utt et al. (2001)                        |
| Listeria monocytogenes           | ActA                              | Syndecan-1    | Attachment, invasion     | Alvarez-Dominguez et al. (1997)          |
| Mycobacterium tuberculosis       | Hemagglutinin                     | Unknown       | Attachment               | Pethe et al. (2001)                      |
| Neisseria gonorrhoea             | Opa                               | Syndecan-1, -4| Attachment, invasion     | Freissler et al. (2000)                   |
| Neisseria meningitidis           | Opc                               | Unknown       | Attachment, invasion     | de Vries et al. (2002)                    |
| Neisseria meningitidis           | GNA2132 (Neisserial Heparin Binding Antigen (NHBA)) | Unknown | (presumptive) Attachment, resistance to serum killing | Serruto et al. (2010)                     |
| Orientia tsutsugamushi           | Unknown                           | Syndecan-4    | Attachment, invasion     | Kim et al. (2004)                        |
| Porphyromonas gingivalis         | LPS, gingipains                   | Syndecan-1    | Shedding                 | Andrian et al. (2005, 2006)              |
| Pseudomonas aeruginosa           | LasA                              | Syndecan-1    | Shedding                 | Park et al. (2000)                       |
| Staphylococcus aureus            | z-toxin, β-toxin                  | Syndecan-1    | Shedding                 | Park et al. (2004)                       |
| Streptococcus agalactiae         | Alpha C protein                   | Unknown       | Attachment, invasion     | Baron et al. (2004)                      |
| Streptococcus pyogenes           | M protein                         | Unknown       | Attachment               | Frick et al. (2003)                      |
| Streptococcus pneumoniae         | ZmpC                              | Syndecan-1    | Shedding                 | Chen et al. (2007b)                      |
| Yersinia enterocolitica          | LcrG                              | Unknown       | Attachment, invasion     | Boyd et al. (1998)                       |
| **Viruses**                      |                                   |               |                          |                                          |
| Adeno-associated virus type 2    | Capsid protein                    | Unknown       | Attachment               | Opie et al. (2003)                       |
| Adenovirus                       | Ad3 Fiber knob                    | Unknown       | Attachment               | Tuve et al. (2008)                       |
| Coronavirus                      | Spike protein                     | Unknown       | Attachment               | de Haan et al. (2008)                    |
| Coxsackievirus                   | Capsid protein                    | N- and 6-O-sulfated HSPGs | Attachment, endocytosis | Zautner et al. (2003, 2006)              |

(continued)
### Table 2.1 (continued)

| Pathogen                     | Pathogen protein | HSPG          | Function/interaction | Reference                                      |
|------------------------------|------------------|---------------|----------------------|------------------------------------------------|
| Cytomegalovirus              | gB               | Unknown       | Attachment           | Boyle and Compton (1998)                       |
| Dengue virus                 | E (envelope protein) | Unknown | Attachment, internalization | Chen et al. (1997), Hilgard and Stockert (2000) |
| FMDV                         | VP3              | Unknown       | Attachment           | Fry et al. (1999)                              |
| HSV-1 and -2                 | gB, gC, gD       | Syndecan-2    | Attachment           | Spear (2004), Cheshenko et al. (2007)          |
| Hepatitis B virus            | Large viral envelope protein | Unknown | Attachment           | Schulze et al. (2007)                          |
| Hepatitis C virus            | Envelope glycoprotein E2 | Unknown | Attachment           | Barth et al. (2006)                            |
| HHV-8 (KSHV)                 | gB, gpK8.1A      | Unknown       | Attachment           | Akula et al. (2001), Veeltil et al. (2006)     |
| HIV-1                        | Tat, Tat gp120 gp41 | Syndecan-3 Agrin | Attachment           | de Witte et al. (2007), Alfsen et al. (2005)  |
| HPV                          | L1 carboxy terminal | Syndecan-1, -3, -4, glypican-1 | Attachment           | Joyce et al. (1999), Shafti-Keramat et al. (2003), de Witte et al. (2008) |
| HTLV1                        | Surface glycoprotein gp46 | Unknown | Attachment           | Piñon et al. (2003)                            |
| Japanese encephalitis virus | Envelope (E) protein | Unknown | Attachment           | Lee et al. (2004)                              |
| Pseudorabies virus           | Glycoprotein C   | Unknown       | Attachment           | Trybala et al. (1998)                          |
| Respiratory syncytial virus | Fusion glycoprotein | Unknown | Attachment, infectivity | Crim et al. (2007)                             |
| Rhinovirus                   | VP1 E2 envelope glycoprotein | Unknown | Attachment           | Vlasak et al. (2005), Ryman et al. (2007)      |
| Sindbis virus                | Viral envelope protein A27L, VCP (Vaccinia virus complemet control protein) | Unknown | Fusion               | Hsiao et al. (1998)                            |
| Vaccinia virus               | Envelope (E) protein | Unknown | Attachment           | Lee et al. (2004)                              |
| Yellow fever virus           | Envelope (E) protein | Unknown | Attachment           | Nickells et al. (2008)                         |
| Giardia lamblia              | Alpha-1 giardin | Unknown       | Attachment           | Weiland et al. (2003)                          |
| Leishmania spp.              | Unknown          | Unknown       | Attachment           | Love et al. (1993)                             |
| Encephalitozoon spp.         | Spore wall protein EnP1 | Unknown | Attachment           | Southern et al. (2007)                         |

(continued)
and virulence factors, HSPGs can also function as a direct internalization receptor. Further, soluble HSPG ectodomains can enhance bacterial virulence in vivo by inhibiting innate host defense mechanisms. The following sections describe these major mechanisms of HSPG subversion using specific pathogens as examples. Other pathogens that use similar mechanisms for their pathogenesis are summarized in Table 2.1, and include *Bordetella pertussis*, *Mycobacterium tuberculosis*, cytomegalovirus, and *Giardia lamblia*, among many others. A prototype of a pathogen using HSPGs as an initial attachment receptor is shown in Fig. 2.1a.

### 2.3.1 Papillomaviruses

Papillomaviruses are nonenveloped, double-stranded DNA viruses that infect only skin and mucosal epithelial cells and manipulate the host cell cycle to create an environment ideal for viral replication. This alteration of the host cell cycle also
results in formation of squamous fibroepithelial tumors, condyloma, and malignant epithelial tumors. Over 100 types of human papillomaviruses (HPV) have been identified. HPVs are separated into two genuses: alpha, containing strains that preferentially infect the genital mucosa (including oncogenic strains 16 and 18 which cause cervical cancer) and beta, containing strains that cause skin infections. Papillomavirus capsids are made of a major capsid protein, L1, and a minor capsid protein, L2. Although papillomaviruses cannot be propagated in vitro, several substitute tools exist. Virus-like particles (VLPs) are generated by the synthesis of capsid proteins L1 and L2 (Kirnbauer et al. 1992; Rose et al. 1993; Volpers et al. 1994) and can bind to most cell lines tested (Qi et al. 1995; Volpers et al. 1995). Pseudovirions, VLPs with a marker plasmid inside, were created to facilitate the study of attachment and invasion (Unckell et al. 1997).

Research on HPV types 11 (Joyce et al. 1999), 16, 33, and 39 (Giroglou et al. 2001) showed a key role for HS in pseudoinfection. Heparin, but not dermatan sulfate or CS, blocked the attachment of pseudovirions to COS-7 cells (Giroglou et al. 2001). In agreement with this, treatment of cells with heparinase also blocked the ability of pseudovirions to attach (Giroglou et al. 2001). As is the case for other pathogens, HSPGs serve as low-affinity, but abundant, primary attachment receptors and a secondary receptor mediates internalization of HPVs. Christensen et al. (1995) found that neutralizing antibodies against VLPs were effective in preventing internalization up to 8 h after infection – without affecting the ability of the VLPs

Fig. 2.1 Mechanisms of microbial subversion of cell surface HSPGs. (a) A pathogen (P) binds to a cell surface HSPG which then facilitates binding of the pathogen to its specific receptor (*shown in pink*) leading to internalization of the pathogen–receptor complex. (b) HIV Tat protein binds to cell surface HSPGs and is internalized. Tat then travels to the nucleus where it transactivates transcription of host cell genes. (c) *P. falciparum* circumsporozoite protein (CSP) binds to HSPGs (*dark blue*). When it encounters a highly sulfated HSPG (*light blue*), as is found in hepatocytes, CSP is cleaved and the remaining molecule is internalized.
to attach to host cells (Christensen et al. 1995). Giroglou et al. (2001) extended the understanding of the timing of attachment and internalization: both neutralizing antibodies and heparin were able to decrease infectivity when added early after infection, but the ability of heparin to decrease infectivity diminished over a shorter time course. The authors propose that virions may initially bind to a single proteoglycan – from which they can easily be displaced by free heparin. Over time, more proteoglycan molecules are recruited and bind to each virion, making it less susceptible to competitive detachment by heparin, but still accessible to neutralizing antibodies which block attachment to a secondary receptor required for internalization (Giroglou et al. 2001). Studies comparing the ability of chemically modified heparins to inhibit infection have shown that VLP binding requires only O-sulfation of heparin, but pseudovirus infection requires both N- and O-sulfation (Selinka et al. 2003). The authors suggest this may be due to structural changes in the capsid proteins after DNA encapsidation (Selinka et al. 2003).

Continued studies are elucidating the specific roles for capsid proteins L1 and L2. HSPG-mediated attachment of L1 to a variety of cell types is an important first step in establishing infection. Following this initial binding, it is thought that the minor capsid protein L2 binds to a different receptor and mediates internalization. Consistent with this mechanism, it has been shown that virions with L1 and L2 have increased infectivity over virions with only L1 (Unckell et al. 1997; Yeager et al. 2000; Roden et al. 2001). In addition, neither anti-L2 neutralizing antibody (Roden et al. 1994a, b, 1995) nor deletion of either the N- or C-terminus of L2 can block virion binding to the host cell surface (Yang et al. 2003). In the case of HPV16, the L2 residues responsible for facilitating infection (aa residues 13–31) are not displayed on the capsid surface (Kawana et al. 1998, 1999), whereas a neighboring series of residues (aa residues 32–81) are present (Heino et al. 1995; Liu et al. 1997). This suggests a model in which HSPG binding of capsid protein L1 leads to a conformational change exposing residues on L2 that can then bind a secondary receptor to enhance infectivity. Richards et al. (2006) identified a host enzyme responsible for cleavage of the N-terminal residues, the proprotein convertase furin, which acts after the conformational change has occurred (Richards et al. 2006). The importance of this conformational change was highlighted by reports that L1-specific neutralizing antibodies induced by vaccination with HPV 16-VLP do not prevent attachment of mature virus to the host cell surface, but rather inhibit infection by preventing the conformational change of L2 (Day et al. 2008).

The secondary receptors that mediate papillomavirus internalization are not known. Using a related virus, bovine papillomavirus (BPV), Bossis et al. (2005) identified a series of conserved residues in the minor capsid protein L2 that are critical for association with the snare syntaxin 18 and subsequent trafficking to the endoplasmic reticulum (ER). The relevance of this interaction between capsid protein L2 and syntaxin 18 in endocytosis of HPV is unclear. Patterson et al. (2005) showed that HPV 31b infection of untransformed human keratinocytes in vitro did not require HS, but HS was required for HPV 31b infection of other cell lines, such as COS-7. The authors speculate that perhaps the secondary high-affinity receptor is so prevalent, that the primary, low-affinity HSPG interaction is
not required for infection in the natural host cell, the keratinocyte, and cast doubt upon the relevance of other in vitro findings as they relate to what truly happens in vivo (Patterson et al. 2005).

In vitro studies of papillomaviruses are important, but the ability of these findings to predict in vivo mechanisms are limited and the true role of HSPGs in HPV infections in vivo is not certain. Johnson et al. (2009) developed a murine model of cervicovaginal challenge and investigated the role for HSPGs in HPV 5, 16, and 31 attachment in vivo (Johnson et al. 2009). Heparin was able to inhibit adsorption of HPV 16 and 31 but not HPV 5 in vivo (Buck et al. 2006; Johnson et al. 2009). Together, these studies suggest that certain strains of HPV require HSPGs for fulminant infection in vivo, but this remains to be rigorously tested. Recent in vivo studies suggest that the L2 capsid protein cleavage event occurs while the virion is bound to HSPG in the BM, and the subsequent conformational change decreases the VLP’s affinity for HSPG, facilitating transfer to a non-HSPG receptor on the epithelial cell (Kines et al. 2009). The ECM produced by epithelial cells in vitro does not induce the same changes in the virion as BM in vivo, and can lead to underestimation of the importance of HSPG in HPV infection (Kines et al. 2009).

### 2.3.2 Dengue Virus

Dengue virus is a positive-sense, single-stranded RNA virus in the *Flavivirus* genus that is spread by mosquitoes. Each of the four serotypes can infect humans and cause diseases including dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. The envelope (E) protein of dengue virus, one of three structural proteins of the virion, is able to bind HS both as a purified protein (Chen et al. 1997) and as a virion component (Germi et al. 2002). Heparin is also able to inhibit viral penetration as well as attachment (Hung et al. 1999). Several other proteins have also been identified as part of a receptor complex for dengue virus. GRP78 (BiP) was identified as a key receptor for internalization of dengue in hepatocytes (Jindadamrongwech et al. 2004). Heat shock proteins (HSP) 70 and 90 have also been identified as coreceptors for dengue. Upon incubation with E protein, HSP70 and 90 on host cell membranes relocate into lipid rafts (Reyes-del Valle et al. 2005). The integrity of these lipid rafts is critical for infection, suggesting that these rafts facilitate clustering of the dengue virus receptor complex (Reyes-del Valle et al. 2005). The relationship between HSPGs and the other identified receptors for dengue is controversial. That λ-carrageenan, a sulfated galactan that presumably mimics HS can block dengue attachment and internalization but not replication events after internalization (Talarico and Damonte 2007), and heat shock of cells increases infectivity of dengue virus, but does not affect attachment (Chavez-Salinas et al. 2008) supports a role for HS in initial attachment of virus to host cells. Experiments with carrageenans also showed a potential role for HS in viral uncoating into the host cell as well (Talarico and Damonte 2007), illustrating the multiple ways by which dengue virus subverts HSPGs to cause infection.
2.3.3 *Listeria monocytogenes*

*L. monocytogenes* is a Gram-positive, intracellular bacterial pathogen. A foodborne pathogen, it crosses the intestinal mucosa and enters the systemic circulation where it can cause meningitis and sepsis in immunocompromised hosts, including pregnant women and fetuses. *L. monocytogenes* gains entry to cells via multiple mechanisms. Phagocytic cell uptake of *L. monocytogenes* occurs via the C3bi (Drevets and Campbell 1991) and C1q (Alvarez-Dominguez et al. 1993) complement receptors. Epithelial cell uptake occurs when internalin protein A (InlA) binds to E-cadherin (Mengaud et al. 1996) and the bacterium is endocytosed via caveolins or clathrin (Bonazzi et al. 2008). Hepatocyte uptake occurs when Internalin B (InlB) binds to the hepatocyte growth factor receptor Met, causing mono-ubiquitination of Met and subsequent clathrin-dependent endocytosis (Veiga and Cossart 2005). InlB also binds HSPGs and potentiates the activity of InlB-Met binding, possibly by clustering InlB and concentrating Met receptors (Jonquières et al. 2001) or by stabilizing the InlB–Met complex during invasion (Banerjee et al. 2004). Lastly, nonphagocytic cell uptake can also occur when InlB binds the C1q complement receptor (gC1qR) leading to phosphorylation of the adaptor proteins Gab1, Cbl, and Shc (Braun et al. 2000).

While InlA and InlB are clearly important in *L. monocytogenes* pathogenesis, InlA and B deletion mutant strains are still capable of invasion, suggesting other mechanisms are available (Dramsi et al. 1995, 1997; Lingnau et al. 1995). Indeed, the *L. monocytogenes* surface protein ActA has been shown to have a role in adherence and invasion (Alvarez-Dominguez et al. 1997), although its best-described function is to manipulate the host cell cytoskeleton to allow bacterial migration within and between host cells (Portnoy et al. 1992; Sheehan et al. 1994; Lasa and Cossart 1996). Alvarez-Dominguez et al. (1997) demonstrated that *L. monocytogenes* ActA, with three regions of positively charged residues, mediates attachment to cell surface HSPGs. ActA appears to mediate attachment to and invasion of epithelial cells, possibly through microvilli (Suarez et al. 2001). Consistent with this, coinjection of heparin during oral inoculation of mice with *L. monocytogenes* did not alter the bacterial load in the cecum, but did decrease extraintestinal dissemination, supporting the role of HSPGs in attachment and internalization of *L. monocytogenes* (Henry-Stanley et al. 2003). Although incompletely understood, the ability of ActA to interact with HSPGs and specifically mediate attachment of epithelial cells with microvilli may have important ramifications regarding how the bacterium crosses the intestinal epithelium.

2.4 HSPG as a Receptor for Virulence Factors

The HIV transcriptional activator protein (Tat) is, as its name suggests, the main transactivator of HIV (Gatignol and Jeang 2000). Tat is a small, cationic polypeptide that is released from HIV-infected cells (Noonan and Albini 2000) and can be
detected in the serum of HIV-infected individuals (Westendorp et al. 1995). Tat has important roles in biological effects of HIV on non-CD4 cells. Tat is thought to be a neurotoxin important for the pathogenesis of AIDS dementia (Dewhurst et al. 1996). Tat is also involved in tumorigenesis by inducing Kaposi Sarcoma Herpes Virus replication (Zeng et al. 2007). Four classes of receptors are known to interact with Tat: αvβ3 integrin (Urbinati et al. 2005); VEGF receptors Flt-1 and Flk-1/KDR (Albini et al. 1996); chemokine receptors CCR2, CCR3, and CXCR4 (Albini et al. 1998; Xiao et al. 2000); and HSPGs (Rusnati and Presta 2002).

Tat binds to HSPGs through a region of basic residues, and can be released intact and active from HSPGs by treatment with heparinase (Chang et al. 1997). Tat binding to cell surface HSPGs facilitates its internalization (Tyagi et al. 2001) and subsequent activation of transcription (Fig. 2.1b). Using a GFP-Tat fusion protein, Tyagi et al. (2001) demonstrated that Tat uptake requires cell surface HSPGs. The interaction between HSPGs and Tat depends on the size of HS and degree and type of sulfation (Rusnati et al. 1997, 1999). Overproduction of CS cannot compensate for a lack of HS, demonstrating the functional specificity of the Tat–HSPG interaction (Tyagi et al. 2001). As is seen in HSPG–growth factor interactions, HSPG binding leads to Tat oligomerization (Rusnati et al. 1999) which facilitates tyrosine-kinase receptor dimerization and signaling. Heparin–Tat binding also protects Tat from proteolytic degradation (Chang et al. 1997). Although this does not exclude the involvement of other types of HSPGs, studies have shown that the cell line WiDr, which lacks all HSPGs except perlecan, is permissive for Tat internalization (Argyris et al. 2004).

The HSPG–Tat interaction has also been shown to be important for lymphoid cell extravasation during HIV infection (Urbinati et al. 2009), which leads to viral dissemination and AIDS-associated leukemia/lymphoma (Chirivi et al. 1999). Tat accumulates on endothelial cell (EC) and B lymphoid cell surfaces by binding HSPGs (Urbinati et al. 2009). Tat bound to HSPGs on ECs or B lymphoid cells significantly increases adhesion to B lymphoid cells or ECs, respectively. Interaction between EC and B lymphoid cells mediated by HSPG requires Tat homodimerization and leads to B lymphoid cell transendothelial migration and extravasation.

The HIV protein gp120 is the envelope glycoprotein, which binds to the host cell receptor CD4. gp120 can also be found in a “free” form and, like Tat, can bind to HSPGs. This can lead to ECM accumulation of the free form of gp120 bound to HSPGs (Klasse and Moore 2004). HSPG binding of the envelope-embedded gp120 is thought to increase concentration of viruses on the cell surface facilitating interactions with HIV receptors such as CD4, CCR5, and CXCR4 (Clapham and McKnight 2001) thereby increasing infectivity. The use of polyanionic compounds to inhibit HIV binding mediated by both Tat and gp120 has been explored, but successful use of these compounds as a therapy remains to be established (Bugatti et al. 2007). Three of the four heparin binding domains identified in gp120 are in domains involved in coreceptor recognition and are located near each other and near the coreceptor binding site, making this an important potential target for drug development (Crublet et al. 2008). A compound has recently been developed that takes advantage of the ability of gp120 to bind HS and a coreceptor. A CD4-mimetic
peptide linked to a HS dodecasaccharide has been developed that binds to gp120, blocks the CD4 binding site, and opens the cryptic coreceptor binding domain to HS-mediated blocking (Baleux et al. 2009). In vitro, this compound effectively inhibits CCR5-, CXCR4-, and dual-tropic HIV-1, which is significant because currently there are no inhibitors available to block CXCR4 binding (Baleux et al. 2009).

The HIV–HS interaction is also implicated in the sexual transmission of HIV infection. HIV virions can bind to HS expressed by spermatozoa and thereby be transmitted in semen along with free virions and infected leukocytes (Ceballos et al. 2009). These spermatozoa-associated virions are then efficiently transmitted to dendritic cells (DCs), macrophages, and T-cells. Low pH (~6.5), similar to that of the vaginal mucosa after sexual intercourse, enhances transmission of virions to DCs, highlighting the potentially critical role in spermatozoa and DCs in HIV transmission in vivo (Ceballos et al. 2009). The source of HS on spermatozoa is most likely Sdc-3 and -4, as Sdc-1 and -2 are not expressed, and glypicans are not involved.

2.5 HSPGs in Tissue Tropism of Pathogens

*Plasmodium falciparum* sporozoites are the infectious agents that cause malaria, one of the most common infectious diseases worldwide. Infection occurs when an infected mosquito injects sporozoites into the skin of a mammalian host. The cycle that follows is complex, taking the sporozoite through the bloodstream to the liver where it invades hepatocytes and is transformed into extraerythrocytic forms. Eventually, each extraerythrocytic form releases thousands of merozoites. Merozoites enter the blood stream and infect erythrocytes, causing the symptoms of malarial disease, such as anemia, fever, arthralgia and in severe cases, coma and death.

The role of HSPGs in the *Plasmodium* life cycle is becoming clear and is unique among the pathogens discussed thus far (Fig. 2.1c). *Plasmodium* apparently uses the differential degree of sulfation of HSPGs in different tissues to complete its journey to the liver. The major surface protein of circumsporozoites, circumsporozoite protein (CSP), has been a target of many malaria vaccine efforts (Sharma and Pathak 2008). It attaches to hepatocytes through interaction with HSPGs, and this binding can be inhibited by heparinase treatment (Frevert et al. 1993). HSPGs are present on the surface of multiple cell types, but hepatocyte HSPGs are more sulfated than endothelial and dermal cell HSPGs (Lindblom and Fransson 1990; Lyon et al. 1994). Thus, it was hypothesized that the degree of sulfation, rather than simply the presence of HSPGs, was the key factor that allows sporozoites to migrate through multiple cell types and invade hepatocytes. Consistent with this, inhibition of sulfation decreased sporozoite migration (Humphries and Silbert 1988), and experiments of sporozoite migration in dermal fibroblasts, ECs, and hepatocytes showed that overall sulfation level of the cell type correlated inversely with migratory activity (Coppi et al. 2007).
Further study has shown that the HSPG–CSP interaction may regulate the proteolytic cleavage of CSP. CSP cleavage is associated with productive invasion (Coppi et al. 2005) and rapid cleavage is induced by highly sulfated HS chains (Coppi et al. 2007). Because CSP is not cleaved in dermal and ECs with less sulfated HSPGs, sporozoites migrate through these cell types. When they encounter highly sulfated HSPGs of hepatocytes, sporozoites are activated and productively invade these cells (Coppi et al. 2007). Subsequent studies showed that the plasmodium calcium-dependent protein kinase 6 (CDPK-6) is required for the cleavage event, and CDPK-6 deficient sporozoites migrate effectively through other cell types but are unable to productively invade hepatocytes (Coppi et al. 2007). However, how interaction with highly sulfated HSPGs regulates CDPK-6 is not understood.

Review of the complete malaria genome (Carlton et al. 2002; Gardner et al. 2002) has yielded information on several additional proteins involved in hepatocyte invasion. The transmembrane protein TRAP (thrombospondin-related anonymous protein) is present on the sporozoite surface, but its surface expression increases greatly on sporozoites after contact with hepatocytes suggesting a role for TRAP in invasion (Gantt et al. 2000). The extracellular portion of TRAP has two cell-adhesive regions: an integrin-like I-domain (A-domain) and a type 1 thrombospondin motif (TSR) (Menard 2000). Interestingly, TSR binds to HSPGs in the space of Disse (Muller et al. 1993; Robson et al. 1995) and the A-domain binds heparin (McCormick et al. 1999), but the exact role of the TRAP–HSPG interaction in malaria pathogenesis remains to be determined.

Pregnancy-associated malaria is a potentially severe disease leading to maternal anemia and low birth-weight infants because of sequestration of infected erythrocytes in the placenta. A variant of the P. falciparum Erythrocyte Membrane Protein 1 (PfEMP1) family named VAR2CSA is expressed only on infected erythrocytes in pregnant women (Salanti et al. 2003) and binds specifically to placental CSPG (Khunrae et al. 2010). In women, high antibody titers against VAR2CSA are protective against low birth-weight babies (Salanti et al. 2004) and VAR2CSA is a candidate antigen for a vaccine against pregnancy-associated malaria (Nielsen et al. 2009).

2.6 Multiple Roles of HSPG in the Pathogenesis of a Single Pathogen

T. gondii is a protozoan parasite able to infect all warm-blooded animals and is estimated to infect one third of humans (Kim and Weiss 2008). Common clinical syndromes associated with T. gondii infection include abortion and congenital infection as well as systemic and central nervous system infections in immunocompromised hosts (Kim and Weiss 2008). Infection is transmitted through ingestion of undercooked meats (especially pork) or contact with infected feces from the definitive hosts (felids), although water containing oocysts has been increasingly
identified as a source of outbreaks (Kim and Weiss 2008). As an obligate intracellular parasite, \textit{T. gondii} has a complicated life cycle requiring invasion of host cells and formation of a parasitophorous vacuole where the organism can evade host defenses and develop into tachyzoites. Tachyzoites differentiate into bradyzoites, which are contained in thick-walled cysts inside the parasitophorous vacuole and can persist indefinitely – or reactivate if the host becomes immunocompromised (Kim and Weiss 2008).

The first role identified for HSPGs in \textit{T. gondii} infection is its attachment to host cells (Carruthers et al. 2000). Because \textit{T. gondii} can infect a wide range of mammalian hosts and can invade nearly all mammalian and avian cell types, Carruthers et al. (2000) hypothesized that the \textit{T. gondii} receptor is an abundantly expressed and ubiquitous cell surface molecule, such as GAGs (Carruthers et al. 2000). As has been shown for other pathogens, incubation of target cells (human foreskin fibroblasts, in this case) with increasing concentrations of GAGs, including heparin, HS, and CS, inhibited parasite attachment (Carruthers et al. 2000). Parasite attachment to CHO cells with a mutation in xylose transferase, which lacks GAG expression, was significantly decreased supporting a role for HS and CS in attachment (Carruthers et al. 2000). Further, enzymatic removal of HS or CS from the cell surface demonstrated that HS was more important than CS in parasite attachment (Carruthers et al. 2000). Decreased attachment to CHO cells with a defect in NDST-1, an enzyme responsible for N-deacetylation/N-sulfation of GlcNAc in HS, was seen in vitro (Ortega-Barria and Boothroyd 1999), suggesting the importance of a specific HS modification in attachment. However, the importance of NDST-1 could not be confirmed in a mouse model of disseminated \textit{T. gondii} infection (Bishop et al. 2005), leaving its true contribution uncertain.

Subsequent studies confirmed the requirement for N-sulfation in parasite infectivity, but were unable to show that soluble HS could prevent attachment, and demonstrated that tachyzoites were able to attach to and invade \textit{Ndst1}−/− cells (Bishop et al. 2005). Surprisingly, the effect of NDST-1 deletion on tachyzoites was to decrease the rate of parasite replication in parasitophorous vacuoles (Bishop et al. 2005). A \textit{T. gondii} protein, SAG3, has been identified to bind HSPG and was suggested as the parasite protein, which attaches \textit{T. gondii} to host cells (Jacquet et al. 2001). However, with the role of HSPGs in attachment in doubt, some suggest SAG3 may actually function as an attachment coreceptor by binding to abundant sialic acid residues (Bishop and Esko 2005).

Recent studies into the relationship between \textit{T. gondii} infection and host cell apoptosis have revealed an additional interaction between \textit{T. gondii} and HSPGs. \textit{T. gondii}-infected cells were initially reported to be resistant to apoptosis, which benefits the parasite as it requires a living host cell for replication (Nash et al. 1998). However, several subsequent studies have demonstrated that \textit{T. gondii} infection can induce apoptosis, mostly in neighboring, uninfected host cells (Liesenfeld et al. 1997; Mordue et al. 2001; Nishikawa et al. 2007). Bannai et al. (2008) investigated a \textit{T. gondii} analog to the human protein Programmed Cell Death 5 (TgPDCD5) during apoptosis and during interactions with host cells. TgPDCD5 is a secreted peptide that contains an HSPG-binding motif and localizes mostly to the apical end.
of the parasite (Bannai et al. 2008). Although unable to induce apoptosis on its own, TgPDCD5 was able to enhance apoptosis in host cells treated with the topoisomerase II inhibitor etoposide and in cooperation with IFN-γ (Bannai et al. 2008). As for human PDCD5, the HSPG-binding motif is required for uptake of TgPDCD5 into host cells (Bannai et al. 2008). The exact role of HSPG binding remains to be fully elucidated, but it is tempting to speculate that HSPG-mediated endocytosis plays a role in TgDCD5 internalization into uninfected host cells where it can then enhance apoptosis.

2.7 HSPG Shedding in Bacterial Pathogenesis

Pathogens are known to enhance the ectodomain shedding of a variety of host cell surface molecules to modulate their environment and enhance virulence (Vollmer et al. 1996; Walev et al. 1996). It has been shown that certain pathogens can induce syndecan-1 shedding either by usurping the host cell machinery or through the pathogen’s own sheddase. Several pathogenic organisms have been evaluated for their ability to shed syndecan-1. *Staphylococcus aureus* (Park et al. 2004), *Pseudomonas aeruginosa* (Park et al. 2000), *Streptococcus pneumonia* (Chen et al. 2007b), and *Bacillus anthracis* (Popova et al. 2006) are able to induce shedding. However, several strains of other Gram-positive and Gram-negative bacteria, including *Staphylococcus saprophyticus*, *Staphylococcus xylosus*, *Salmonella enteritidis*, *Salmonella typhimurium*, and *Klebsiella pneumoniae* do not appear to enhance shedding (Park et al. 2000).

2.7.1 Pseudomonas aeruginosa

*P. aeruginosa* is a Gram-negative bacterium that is associated with infections of the skin, urinary tract, and lung. Particularly concerning is its role as a cause of ventilator-associated pneumonia in hospitalized patients, which complicates the courses of up to 25% of intubated patients, and can carry up to a 10-fold increased risk of mortality (Chastre and Fagon 2002). Culture supernatants of various strains of *P. aeruginosa* were observed to increase syndecan-1 shedding at least fourfold over baseline in multiple cell types (Park et al. 2000). The secreted protein responsible for inducing syndecan-1 shedding was identified as LasA (Kessler et al. 1997), which is a known virulence factor of *P. aeruginosa* in animal models of corneal (Preston et al. 1997) and lung infection (Woods et al. 1982; Blackwood et al. 1983). Although LasA has enzymatic activity (Kessler et al. 1997), it does not hydrolyze syndecan-1. Consistent with these data, studies using PTK inhibitors or metalloproteinase inhibitors (peptide hydroxamate) suggested that LasA induces syndecan-1 shedding via activation of the host cell’s shedding machinery (Park et al. 2000).
The physiological role of *P. aeruginosa*-induced syndecan-1 shedding was studied first in a mouse model of pneumonia. Seven-day-old syndecan-1 null mice (*Sdc1^−/−^) markedly resisted intranasal infection with *P. aeruginosa* as measured by lung and spleen bacterial burden, pneumonia and inflammation as seen on histopathology, as well as overall mortality (Park et al. 2001). As discussed earlier, for the majority of pathogens, HSPGs are used as attachment sites. However, *P. aeruginosa* did not bind to syndecan-1, and excess heparin had no effect on the adhesion of *P. aeruginosa* to lung epithelial cells in vitro (Park et al. 2001). Intranasal inoculation of *Sdc1^−/−^* mice with heparin or purified syndecan-1 ecto-domain at the time of intranasal infection with *P. aeruginosa* restored susceptibility to infection, but inoculation with CS or HS-free syndecan-1 core proteins had no effect (Park et al. 2001). Syndecan-1 ectodomains are detected in the bronchoalveolar lavage fluid after infection with *P. aeruginosa* or after instillation of LasA. Addition of the metalloproteinase inhibitor BB1101 was able to decrease the amount of syndecan-1, but not syndecan-4 ectodomains shed in vivo in response to *P. aeruginosa* infection or Las A instillation, consistent with in vitro results and supporting the specific shedding of syndecan-1 by LasA (Park et al. 2001). Together, these findings suggest that *P. aeruginosa* subverts syndecan-1 shedding to promote its pathogenesis by inhibiting host defense mechanisms in the airspace. 

*P. aeruginosa* is a key pathogen in sepsis following thermal injury (burn wounds). *Sdc1^−/−^* mice were found to be resistant to *P. aeruginosa* sepsis following thermal injury (Haynes et al. 2005). Absence of syndecan-1 had no effect on the ability of *P. aeruginosa* to colonize burned tissue locally, but led to significantly less systemic spread of infection – and this resistance to systemic spread could be overcome by local injection of HS (Haynes et al. 2005). Thus, syndecan-1 shedding may play a specific role in the dissemination of *P. aeruginosa* in the context of burn infection, but precisely how this is accomplished is incompletely understood.

The ability of *P. aeruginosa* to attach to polarized epithelial cells has been investigated recently. N-glycans, expressed at both the apical and basolateral surface of epithelial monolayers of renal or airway cells, mediate binding and entry of *P. aeruginosa* at the apical surface (Bucior et al. 2010). *P. aeruginosa* binding to the basolateral surface, in contrast, is mediated by HSPGs (Bucior et al. 2010). Tissue injury or epithelial damage increases apical surface expression of N-glycans leading to increased *P. aeruginosa* attachment. Using incompletely polarized cells as a model of injured tissue, it was shown that HSPGs are also upregulated at the apical surface further increasing *P. aeruginosa* binding and subsequent tissue damage (Bucior et al. 2010). The upregulation of expression of both N-glycans and HSPGs in the context of tissue damage and repair may explain the increased propensity for *P. aeruginosa* to cause infections in this context.

### 2.7.2 *Staphylococcus aureus*

*S. aureus* is an important Gram-positive bacterial pathogen of humans that causes a variety of diseases ranging from superficial skin and soft tissue infections to serious
invasive diseases such as pneumonia, osteomyelitis, endocarditis, and sepsis. *S. aureus* expresses several different classes of virulence factors including pore-forming toxins, such as α-toxin and Panton-Valentine leukocidin, secreted toxins such as β-toxin, toxic shock syndrome toxin-1 (TSST-1) and enterotoxins, cell-wall attached proteins such as protein A, and cell wall components such as peptidoglycan and lipoteichoic acid (Gordon and Lowy 2008).

*S. aureus* has been shown to induce syndecan-1 shedding through its cytotoxic virulence factors α-toxin and β-toxin. *S. aureus* α-toxin has been established or proposed as a virulence factor in many staphylococcal diseases, such as pneumonia (McElroy et al. 1999; Bartlett et al. 2008), sepsis (Buerke et al. 2002), endocarditis (Bayer et al. 1997), meningitis (Kielian et al. 2001; Maślińska et al. 2004), keratitis (Callegan et al. 1994; Girgis et al. 2005), dermatitis (Ezepchuk et al. 1996), septic arthritis (Nilsson et al. 1999), and mastitis (Bramley et al. 1989). Alpha-toxin is secreted as a soluble monomer and forms heptameric transmembrane pores in target cell membranes (Bhakdi and Tranum-Jensen 1991). Alpha-toxin has additional biological effects on host cells as it binds to a putative glycoprotein receptor (Bhakdi and Tranum-Jensen 1991) and activates intracellular signaling and modulates cellular processes (Bantel et al. 2001; Dragneva et al. 2001; Rose et al. 2002; Park et al. 2004; Haugwitz et al. 2006; Ratner et al. 2006; Liang and Ji 2007). However, the α-toxin receptor has been elusive, and precisely how the noncytolytic activities of α-toxin contribute to *S. aureus* virulence remains to be determined. To date, β1 integrin (Liang and Ji 2007), caveolin-1 (Vijayvargia et al. 2004), and band-3 (Maharaj and Fackrell 1980) have been proposed to be α-toxin receptors, but it remains to be determined if these indeed signal upon α-toxin binding.

*S. aureus* β-toxin has also been established or proposed as a virulence factor in several staphylococcal diseases, such as keratitis (O’Callaghan et al. 1997) and mastitis (Bramley et al. 1989), although it is one of the least-studied staphylococcal toxins and is found in few clinical isolates (Aarestrup et al. 1999). Beta-toxin is a Mg^{2+}-dependent sphingomyelinase that generates phosphorylcholine and the bioactive secondary messenger ceramide by hydrolyzing host cell membrane sphingomyelin (Doery et al. 1963; Marques et al. 1989; Vollmer et al. 1996). Beta-toxin does not lyse most cell types, but leaves them vulnerable to a number of other lytic agents. In fact, the cytotoxic effect of β-toxin is highly cell type- and species-specific, suggesting that its primary virulence activity is to modulate host processes that affect pathogenesis rather than to directly kill host cells.

Both α-toxin and β-toxin apparently induce syndecan-1 shedding by stimulating the host cell’s shedding mechanism. The metalloproteinase inhibitor GM6001 and PTK inhibitor Tyrphostin A25 inhibited toxin-induced syndecan-1 shedding when they were coincubated with toxins and host cells, but not when preincubated with toxins and removed prior to incubation with host cells (Park et al. 2004). These data suggest that both α-toxin and β-toxin activate a similar PTK-dependent, metalloproteinase-mediated shedding mechanism of host cells. The physiological significance of toxin-induced syndecan-1 shedding remains to be established. However, several data suggest that this is an important virulence activity. Instillation of β-toxin-positive (hlb+) *S. aureus* or purified β-toxin into mice induced syndecan-1
shedding from type II alveolar epithelial cells and caused pulmonary edema and inflammation, particularly neutrophilic influx into the airways (Hayashida et al. 2009). The capacity of β-toxin to induce syndecan-1 shedding is dependent on its sphingomyelinase activity because mutant proteins lacking this activity failed to trigger shedding and cause lung injury when inoculated intranasally (Hayashida et al. 2009). Consistent with these data, Sdc1−/− mice injected with purified β-toxin showed minimal inflammation, supporting the role of S. aureus β-toxin and its sphingomyelinase activity in enhancing syndecan-1 shedding leading to pulmonary inflammation and injury (Hayashida et al. 2009). These results suggest a model in which β-toxin acts as a virulence factor not through direct cytotoxicity, but rather through enhanced neutrophil infiltration which occurs in a syndecan-1 dependent manner (Hayashida et al. 2009) (Fig. 2.2).

The S. aureus hetero-oligomeric pore-forming toxin Panton-Valentine leukocidin (PVL) uses HS in a different manner. The signal peptide of the LukS component of PVL is released outside the cytosolic membrane after cleavage by the signal peptidase and the C-terminus can associate with the bacterial cell wall (Tristan et al. 2009). The positively charged N-terminus is then accessible to interact with negatively charged HS chains in the ECM, possibly forming a bridge between bacteria and ECM which explains the increased adherence to damaged epithelial cells seen in PVL+ strains of S. aureus (Tristan et al. 2009).

2.7.3 Streptococcus pneumoniae

S. pneumoniae is a Gram-positive bacterial pathogen causing diseases such as pneumonia, otitis media, sinusitis, and meningitis. Similar to S. aureus and P. aeruginosa,
S. pneumoniae induces syndecan-1 shedding from the surface of host cells in culture. However, available data indicate that S. pneumoniae directly sheds syndecan-1 ectodomains (Chen et al. 2007b). As such, the size of syndecan-1 ectodomains shed by S. pneumoniae is smaller than ectodomains produced by constitutive shedding. More importantly, ZmpC, one of the pneumococcal zinc metalloproteinases, has been shown to possess syndecan-1 sheddase activity in vitro (Chen et al. 2007b). These observations suggest that several major opportunistic bacterial pathogens use distinct mechanisms to induce syndecan-1 shedding, which can enhance bacterial virulence in vivo. However, it remains to be established that induction of syndecan-1 shedding is an important virulence activity of S. pneumoniae.

2.8 Future Perspectives

Studies during the last several decades have demonstrated that viral, bacterial, and parasitic pathogens elaborate factors that interact with host HSPGs. Some pathogens also express factors that modulate the expression pattern of HSPGs by inducing shedding. However, it is still not clear if these interactions benefit the host or the bacteria. Several in vivo studies suggest that induction of syndecan-1 shedding is a critical virulence activity of certain bacterial pathogens. However, the fact that the majority of intracellular pathogens exploit cell surface HSPGs for its attachment and cellular invasion also suggest that syndecan shedding is a host defense mechanism that rapidly and transiently downregulates microbial attachment sites. Perhaps only highly effective pathogens, such as S. aureus, have adapted or evolved to subvert this innate host mechanism. Future studies should be directed at testing the physiological significance of HSPG–pathogen interaction using animal models of infection that simulate human disease. In vivo studies using mice deficient in specific HS biosynthetic enzymes and mice deficient in certain HSPGs or HS enzymes in a cell-specific manner should also be attempted to define the molecular and cellular features of the HSPG–pathogen interaction. These studies should further define the underlying mechanisms and the physiological relevance of HSPG–pathogen interactions.

Although improvements in hygienic, prophylactic, and therapeutic interventions have significantly reduced the incidence of infections in the last century, infectious diseases continue to be a major public health threat. Further, the continuous emergence of drug-resistant strains is adding to this threat. Because available data suggest that the capacity to subvert HSPGs to promote infection is a pathogenic mechanism used by many pathogens, the HSPG–pathogen interaction is a potential candidate for novel antimicrobial therapy against a broad range of infectious diseases. For example, de Witte et al. (2008) showed that papillomavirus VLPs interact with HSPGs on the surface of DCs, as binding can be prevented by treatment of DCs with heparinase II. As DCs express syndecan-3 and play a critical role in immunity against HPVs, the HS moiety of syndecan-3 may be a target for anti-HPV therapy. Similarly, periodate-cleaved heparin fragments that lack
antithrombin-III binding activity and thus lack anticoagulant properties have been shown to retain several key functions that may have therapeutic benefits in the treatment of severe malaria; they can block merozoite invasion of erythrocytes, disrupt rosettes, inhibit endothelial binding of *Plasmodium* in vitro and reverse sequestration in vivo in rat and macaque models of malaria (Vogt et al. 2006). These data suggest that low molecular weight HS/heparin compounds that do not cause the unwanted side effects of heparin therapy, such as bleeding and thrombocytopenia, are potential candidates for antimalarial therapy. Deciphering the key mechanisms of HSPG–pathogen interactions should provide important insights into the design and development of novel HS-based antimicrobial strategies.

References

Aarestrup F, Larsen H et al (1999) Frequency of alpha- and beta-haemolysin in *Staphylococcus aureus* of bovine and human origin. A comparison between pheno- and genotype and variation in phenotypic expression. APMIS 107:425–430

Akula S, Pramod N et al (2001) Human herpesvirus 8 envelope-associated glycoprotein B interacts with heparan sulfate-like moieties. Virology 284:235–249

Albini A, Soldi R et al (1996) The angiogenesis induced by HIV-1 tat protein is mediated by the Flk-1/KDR receptor on vascular endothelial cells. Nat Med 2:1371–1375

Albini A, Ferrini S et al (1998) HIV-1 Tat protein mimicry of chemokines. Proc Natl Acad Sci USA 95:13153–13158

Alexopoulou A, Multhaupt H et al (2007) Syndecans in wound healing, inflammation and vascular biology. Int J Biochem Cell Biol 39:505–528

Alfsen A, Yu H et al (2005) HIV-1-infected blood mononuclear cells form an integrin- and agrin-dependent viral synapse to induce efficient HIV-1 transcytosis across epithelial cell monolayer. Mol Biol Cell 16:4267–4279

Alvarez-Dominguez C, Carrasco-Marin E et al (1993) Role of complement component C1q in phagocytosis of Listeria monocytogenes by murine macrophage-like cell lines. Infect Immum 61:3664–3672

Alvarez-Dominguez C, Vazquez-Boland J-A et al (1997) Host cell Heparan sulfate proteoglycans mediate attachment and entry of *Listeria monocytogenes*, and the listerial surface protein ActA is involved in heparan sulfate receptor recognition. Infect Immum 65(1):78–88

Andrian E, Grenier D et al (2005) *Porphyromonas gingivalis* lipopolysaccharide induces shedding of syndecan-1 expressed by gingival epithelial cells. J Cell Physiol 204:178–183

Andrian E, Grenier D et al (2006) *Porphyromonas gingivalis* gingipains mediate the shedding of syndecan-1 from the surface of gingival epithelial cells. Oral Microbiol Immunol 21:123–128

Argyris E, Kulkosky J et al (2004) The perlec an heparan sulfate proteoglycan mediates cellular uptake of HIV-1 Tat through a pathway responsible for biological activity. Virology 330:481–486

Arungundram S, Al-Mafraji K et al (2009) Modular synthesis of heparan sulfate oligosaccharides for structure-activity relationship studies. J Am Chem Soc 131(47):17394–17405

Baueerle P, Hutten W (1986) Chlorate – a potent inhibitor of protein sulfation in intact cells. Biochem Biophys Res Commun 141:870–877

Baleux F, Loureiro-Morais L et al (2009) A synthetic CD4-heparan sulfate glycoconjugate inhibits CCR5 and CXCR4 HIV-1 attachment and entry. Nat Chem Biol 5(10):743–748

Banerjee M, Copp J et al (2004) GW domains of the Listeria monocytogenes invasion protein InlB are required for potentiation of Met activation. Mol Microbiol 52:257–271
Bannai H, Nishikawa Y et al (2008) Programmed cell death 5 from Toxoplasma gondii: a secreted molecule that exerts a pro-apoptotic effect on host cells. Mol Biochem Parasitol 159:112–120
Bantel H, Sinha B et al (2001) alpha-Toxin is a mediator of Staphylococcus aureus-induced cell death and activates caspases via the intrinsic death pathway independently of death receptor signaling. J Cell Biol 155:637–648
Baron M, Bolduc G et al (2004) Alpha C protein of group B Streptococcus binds host cell surface glycosaminoglycan and enters cells by an actin-dependent mechanism. J Biol Chem 279:24714–24723
Barth H, Schnoere E et al (2006) Viral and cellular determinants of the hepatitis C virus envelope-heparan sulfate interaction. J Virol 80:10579–10590
Bartlett A, Foster T et al (2008) Alpha-toxin facilitates the generation of CXC chemokine gradients and stimulates neutrophil homing in Staphylococcus aureus pneumonia. J Infect Dis 198:1529–1535
Bayer A, Ramos M et al (1997) Hyperproduction of alpha-toxin by Staphylococcus aureus results in paradoxically reduced virulence in experimental endocarditis: a host defense role for platelet microbicidal proteins. Infect Immun 65:4652–4660
Bernfield M, Gotte M et al (1999) Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem 68:729–777
Bhakdi S, Tranum-Jensen J (1991) Alpha-toxin of Staphylococcus aureus. Microbiol Rev 55:733–751
Bishop J, Esko J (2005) The elusive role of heparan sulfate in Toxoplasma gondii infection. Mol Biochem Parasitol 139:267–269
Bishop J, Crawford B et al (2005) Cell surface heparan sulfate promotes replication of Toxoplasma gondii. Infect Immun 73:5395–5401
Blackwood L, Stone R et al (1983) Evaluation of Pseudomonas aeruginosa exotoxin A and elastase as virulence factors in acute lung infection. Infect Immun 39:198–201
Bonazzi M, Veiga E et al (2005) Successive post-translational modifications of E-cadherin are required for InlA-mediated internalization of Listeria monocytogenes. Cell Microbiol 10:2208–2222
Bossis I, Roden R et al (2005) Interaction of tSNARE syntaxin 18 with the papillomavirus minor capsid protein mediates infection. J Virol 79:6723–6731
Boyd A, Sory M et al (1998) Heparin interferes with translocation of Yop proteins into HeLa cells and binds to LcrG, a regulatory component of the Yersinia Yop apparatus. Mol Microbiol 27:425–436
Boyle K, Compton T (1998) Receptor-binding properties of a soluble form of human cytomegalovirus glycoprotein B. J Virol 72:1826–1833
Bramley A, Patel A et al (1989) Roles of alpha-toxin and beta-toxin in virulence of Staphylococcus aureus for the mouse mammary gland. Infect Immun 57:2489–2494
Braun L, Ghebrehiwet B et al (2000) gC1q-R/p32, a C1q-binding protein, is a receptor for the InlB invasion protein of Listeria monocytogenes. EMBO J 19:1458–1466
Brule S, Charnaux N et al (2006) The shedding of syndecan-4 and syndecan-1 from HeLa cells and human primary macrophages is accelerated by SDF-1/CXCL12 and mediated by the matrix metalloproteinase-9. Glycobiology 16:488–501
Bucior I, Mostov K et al (2010) Pseudomonas aeruginosa-mediated damage requires distinct receptors at the apical and basolateral surfaces of the polarized epithelium. Infect Immun 78 (3):939–953
Buck C, Thompson C et al (2006) Carrageenan is a potent inhibitor of papillomavirus infection. PLoS Pathog 2:e69
Buerke M, Sibeliu U et al (2002) Staphylococcus aureus alpha toxin mediates polymorphonuclear leukocyte-induced vasoconstriction and endothelial dysfunction. Shock 17:30–35
Bugatti A, Urbiniati C et al (2007) Heparin-mimicking sulfonic acid polymers as multitarget inhibitors of human immunodeficiency virus type 1 Tat and gp120 proteins. Antimicrob Agents Chemother 51:2337–2345
Callegan M, Engel L et al (1994) Corneal virulence of *Staphylococcus aureus*: roles of alpha-toxin and protein A in pathogenesis. Infect Immun 62:2478–2482

Capurro M, Wanless I et al (2003) Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology 125:89–97

Carlton J, Angiuoli S et al (2002) Genome sequence and comparative analysis of the model rodent malaria parasite Plasmodium yoelii yoelii. Nature 519:512–519

Carruthers V, Håkansson S et al (2000) *Toxoplasma gondii* uses sulfated proteoglycans for substrate and host cell attachment. Infect Immun 68:4005–4011

Ceballos A, Lenicov F et al (2009) Spermatozoa capture HIV-1 through heparan sulfate and efficiently transmit the virus to dendritic cells. J Exp Med 206(12):2717–2733

Chang H, Samaniego F et al (1997) HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. AIDS 11(12):1421–1431

Chastre J, Fagon J (2002) Ventilator-associated Pneumonia. Jean Chastre and Jean-Yves Fagon. Am J Respir Crit Care Med 165:867–903

Chavez-Salinas S, Ceballos-Olvera I et al (2008) Heat shock effect upon dengue virus replication into U937 cells. Virus Res 128:111–118

Chen R, Lander A (2001) Mechanisms underlying preferential assembly of heparan sulfate on glypican. J Biol Chem 276:7507–7517

Chen J, Stephens R (1997) Chlamydia trachomatis glycosaminoglycan-dependent and independent attachment to eukaryotic cells. Microb Pathog 22(1):23–30

Chen Y, Maguire T et al (1997) Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. Nat Med 3:866–871

Chen J, Jones C et al (2007a) Using an enzymatic combinatorial approach to identify anticoagulant heparan sulfate structures. Chem Biol 14:986–993

Chen Y, Hayashida A et al (2007b) Streptococcus pneumoniae sheds Syndecan-1 ECTODOMAINS through ZmpC, a metalloproteinase virulence factor. J Biol Chem 282:159–167

Chen Y, Götte M et al (2008) Microbial subversion of heparan sulfate proteoglycans. Mol Cells 26(5):415–426

Cheshenko N, Liu W et al (2007) Multiple receptor interactions trigger release of membrane and intracellular calcium stores critical for herpes simplex virus entry. Mol Biol Cell 18:3119–3130

Chirivi R, Taraboletti G et al (1999) Human immunodeficiency virus-1 (HIV-1)-Tat protein promotes migration of acquired immunodeficiency syndrome-related lymphoma cells and enhances their adhesion to endothelial cells. Blood 94:1747–1754

Christensen N, Cladel N et al (1995) Postattachment neutralization of papillomaviruses by monoclonal and polyclonal antibodies. Virology 207:136–142

Chung M, Popova T et al (2006) Secreted neutral metalloproteases of *Bacillus anthracis* as candidate pathogenic factors. J Biol Chem 281:31408–31418

Clapham P, McKnight A (2001) HIV-1 receptors and cell tropism. Br Med Bull 58:43–59

Cohen A, Wood D et al (1998) Human CASK/LIN-2 binds Syndecan-2 and Protein 4.1 and localizes to the basolateral membrane of epithelial cells. J Cell Biol 142:129–138

Coppi A, Pinzon-Ortiz C et al (2005) The Plasmodium circumsporozoite protein is proteolytically processed during cell invasion. J Exp Med 201(1):27–33

Coppi A, Tewari R et al (2007) Heparan sulfate proteoglycans provide a signal to Plasmodium sporozoites to stop migrating and productively invade cells. Cell Host Microbe 2(5):316–327

Crim R, Audet S et al (2007) Identification of linear heparin-binding peptides derived from human respiratory syncytial virus fusion glycoprotein that inhibit infectivity. J Virol 81:261–271

Crublet E, Andrieu J et al (2008) The HIV-1 envelope glycoprotein gp120 features four heparan sulfate binding domains, including the co-receptor binding site. J Biol Chem 283(22):15193–15200

David G, Lorie V et al (1990) Molecular cloning of a phosphatidylinositol-anchored membrane heparan sulfate proteoglycan from human lung fibroblasts. J Cell Biol 111:3165–3176
Day P, Gambhira R et al (2008) Mechanisms of human papillomavirus type 16 neutralization by 12 cross-neutralizing and 11 type-specific antibodies. J Virol 82(9):4638–4646

de Haan C, Haijema B et al (2008) Cleavage of group 1 coronavirus spike proteins: how furin cleavage is traded off against heparan sulfate binding upon cell culture adaptation. J Virol 82:6078–6083

de Vries F, Cole R et al (2002) Neisseria meningitidis producing the Opc adhesin binds epithelial cell proteoglycan receptors. Mol Microbiol 27:1203–1212

de Witte L, Bobardt M et al (2007) Syndecan-3 is a dendritic cell-specific attachment receptor for HIV-1. Proc Natl Acad Sci USA 104:19464–19469

de Witte L, Zoughlami Y et al (2008) Binding of human papilloma virus L1 virus-like particles to dendritic cells is mediated through heparan sulfates and induces immune activation. Immunobiology 212:679–691

Dewhurst S, Gelbard H et al (1996) Neuropathogenesis of AIDS. Mol Med Today 2(1):16–23

Ding K, Lopez-Burks M et al (2005) Growth factor–induced shedding of syndecan-1 confers glypic-an-1 dependence on mitogenic responses of cancer cells. J Cell Biol 171:729–738

Doery H, Magnusson B et al (1963) A phospholipase in staphylococcal toxin which hydrolyses sphingomyelin. Nature 198:1091–1092

Dragneva Y, Anuradha C et al (2001) Subcytocidal attack by staphylococcal alpha-toxin activates NF-kappaB and induces interleukin-8 production. Infect Immun 69:2630–2635

Dramsi S, Biswas I et al (1995) Entry of Listeria monocytogenes into hepatocytes requires the expression of InlB, a surface protein of the internalin multigene family. Mol Microbiol 16:251–261

Dramsi S, Dehoux P et al (1997) Identification of four new members of the internalin multigene family of Listeria monocytogenes EGD. Infect Immun 65:1615–1625

Drevets D, Campbell P (1991) Roles of complement receptor type 3 in phagocytosis of Listeria monocytogenes by inflammatory mouse peritoneal macrophages. Infect Immun 59:2645–2652

Endo K, Takino T et al (2003) Cleavage of Syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. J Biol Chem 278:40764–40770

Esko J, Montgomery R (1995) Synthetic glycosides as primers of oligosaccharide biosynthesis and inhibitors of glycoprotein and proteoglycan assembly. In: Ausubel F, Brent R, Kingston R et al. (eds) Current protocols in molecular biology. Greene and Wiley-Interscience, New York, pp 17.11.11–17.11.16

Ezepchuk Y, Leung D et al (1996) Staphylococcal toxins and protein A differentially induce cytotoxicity and release of tumor necrosis factor-alpha from human keratinocytes. J Invest Dermatol 107:603–609

Fears C, Woods A (2006) The role of syndecans in disease and wound healing. Matrix Biol 25:443–456

Fitzgerald M, Wang Z et al (2000) Shedding of Syndecan-1 and -4 ectodomains is regulated by multiple signaling pathways and mediated by a TIMP-3-sensitive metalloproteinase. J Cell Biol 148:811–824

Forsberg E, Pejler G et al (1999) Abnormal mast cells in mice deficient in a heparin-synthesizing enzyme. Nature 400:773–776

Freissler E, Meyer auf der Heyde A et al (2000) Syndecan-1 and syndecan-4 can mediate the invasion of OpaHSPG-expressing Neisseria gonorrhoeae into epithelial cells. Cell Microbiol 2:69–82

Frevert U, Sinnis P et al (1993) Malaria circumsporozoite protein binds to heparan sulfate proteoglycans associated with the surface membrane of hepatocytes. J Exp Med 177:1287–1298

Frick I, Schmidtchen A et al (2003) Interactions between M proteins of Streptococcus pyogenes and glycosaminoglycans promote bacterial adhesion to host cells. Eur J Biochem 270:2303–2311

Fry E, Lea S et al (1999) The structure and function of a foot-and-mouth disease virus-oligosaccharide receptor complex. EMBO J 18:543–554
Fuki I, Kuhn K et al (1997) The syndecan family of proteoglycans. Novel receptors mediating internalization of atherogenic lipoproteins in vitro. J Clin Invest 100:1611–1622
Gantt S, Persson C et al (2000) Antibodies against thrombospondin-related anonymous protein do not inhibit Plasmodium sporozoite infectivity in vivo. Infect Immun 68:3667–3673
Gardner M, Hall N et al (2002) Genome sequence of the human malaria parasite Plasmodium falciparum. Nature 419:498–511
Gatignol A, Jeang T (2000) Tat as a transcriptional activator and a potential therapeutic target for HIV-1. Adv Pharmacol 48:209–227
Germi R, Crane JM et al (2002) Heparan sulfate-mediated binding of infectious Dengue Virus Type 2 and Yellow Fever Virus. Virology 292:162–168
Girgis D, Sloop G et al (2005) Effects of toxin production in a murine model of Staphylococcus aureus keratitis. Invest Ophthalmol Vis Sci 46:2064–2070
Giroglou T, Florin L et al (2001) Human papillomavirus infection requires cell surface heparan sulfate. J Virol 75:1565–1570
Gordon R, Lowy F (2008) Pathogenesis of methicillin-resistant Staphylococcus aureus infection. Clin Infect Dis 46:S350–S359
Grobe K, Inatani M et al (2005) Cerebral hypoplasia and craniofacial defects in mice lacking heparan sulphate Ndst1 gene function. Development 132:3777–3786
Grootjans J, Zimmermann P et al (1997) Syndentin, a PDZ protein that binds syndecan cytoplasmic domains. Proc Natl Acad Sci USA 94:13683–13688
Hannah J, Menozzi F et al (1994) Sulfated glycoconjugate receptors for the Bordetella pertussis adhesin filamentous hemagglutinin (FHA) and mapping of the heparin-binding domain on FHA. Infect Immun 60:5010–5019
Haugwitz U, Bobkiewicz W et al (2006) Pore-forming Staphylococcus aureus alpha-toxin triggers epidermal growth factor receptor-dependent proliferation. Cell Microbiol 8:1591–1600
Hayashida K, Johnston D et al (2006) Syndecan-1 expression in epithelial cells is induced by transforming growth factor-beta through a PKA-dependent pathway. J Biol Chem 281:24365–24374
Hayashida K, Stahl P et al (2008) Syndecan-1 ectodomain shedding is regulated by the small GTPase Rab5. J Biol Chem 283:35435–35444
Hayashida A, Bartlett A et al (2009) Staphylococcus aureus beta-toxin induces lung injury through syndecan-1. Am J Pathol 174:509–518
Haynes A III, Ruda F et al (2005) Syndecan 1 shedding contributes to Pseudomonas aeruginosa sepsis. Infect Immun 73:7914–7921
Heino P, Skyldberg B et al (1995) Human papillomavirus type 16 capsids expose multiple type-restricted and type-common antigenic epitopes. J Gen Virol 76:1141–1153
Henry-Stanley M, Hess D et al (2003) Role of heparan sulfate in interactions of Listeria monocytogenes with enterocytes. Med Microbiol Immunol 192(2):107–115
Hilgard P, Stockert R (2000) Heparan sulfate proteoglycans initiate dengue virus infection of hepatocytes. Hepatology 32(5):1069–1077
Hippo Y, Watanabe K et al (2004) Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. Cancer Res 64:2418–2423
Horonchik L, Tzaban S et al (2005) Heparan sulfate is a cellular receptor for purified infectious prions. J Biol Chem 280:17062–17067
Hsiao J, Chung C et al (1998) Cell surface proteoglycans are necessary for A27L protein-mediated cell fusion: identification of the N-terminal region of A27L protein as the glycosaminoglycan-binding domain. J Virol 72:8374–8379
Hsueh Y, Yang F et al (1998) Direct interaction of CASK/LIN-2 and syndecan heparan sulfate proteoglycan and their overlapping distribution in neuronal synapses. J Cell Biol 142:139–151
Humphries D, Silbert J (1988) Chlorate: a reversible inhibitor of proteoglycan sulfation. Biochem Biophys Res Commun 154:365–371
Hung SL, Lee PL et al (1999) Analysis of the steps involved in dengue virus entry into host cells. Virology 257:156–167
Iozzo R (2005) Basement membrane proteoglycans: from cellar to ceiling. Nat Rev Mol Cell Biol 6:646–656
Isaacs R (1994) *Borrelia burgdorferi* bind to epithelial cell proteoglycans. J Clin Invest 93: 809–819
Jacquet A, Coulon L et al (2001) The surface antigen SAG3 mediates the attachment of *Toxoplasma gondii* to cell-surface proteoglycans. Mol Biochem Parasitol 116:35–44
Jindadamrongwech S, Thepparit C et al (2004) Identification of GRP 78 (BiP) as a liver cell expressed receptor element for dengue virus serotype 2. Arch Virol 149:915–927
Johnson K, Kines R et al (2009) The role of heparan sulfate in HPV attachment and infection of the murine female genital tract. J Virol 83(5):2067–2074
Jonquières R, Pizarro-Cerdá J et al (2001) Synergy between the N- and C-terminal domains of InlB for efficient invasion of non-phagocytic cells by Listeria monocytogenes. Mol Microbiol 42:955–965
Joyce J, Tung J et al (1999) The L1 major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. J Biol Chem 274:5810–5822
Kainulainen V, Wang H et al (1998) Syndecans, heparan sulfate proteoglycans, maintain the proteolytic balance of acute wound fluids. J Biol Chem 273:11563–11569
Kato M, Wang H et al (1998) Physiological degradation converts the soluble syndecan-1 ectodomain from an inhibitor to a potent activator of FGF-2. Nat Med 4:691–697
Kawana K, Matsumoto K et al (1998) A surface immunodeterminant of human papillomavirus type 16 minor capsid protein L2. Virology 245:353–359
Kawana K, Yoshikawa H et al (1999) Common neutralization epitope in minor capsid protein L2 of human papillomavirus types 16 and 6. J Virol 73:6188–6190
Kessler E, Safrin M et al (1997) Inhibitors and specificity of *Pseudomonas aeruginosa* LasA. J Biol Chem 272:9884–9889
Khunrae P, Dahlbäck M et al (2010) Full-length recombinant Plasmodium falciparum VAR2CSA binds specifically to CSPG and induces potent parasite adhesion-blocking antibodies. J Mol Biol 397(3):826–834
Kielian T, Cheung A et al (2001) Diminished virulence of an alpha-toxin mutant of *Staphylococcus aureus* in experimental brain abscesses. Infect Immun 69:6902–6911
Kim K, Weiss L (2008) Toxoplasma: the next 100 years. Microb Infect 10:978–984
Kim H, Choi M et al (2004) Role of Syndecan-4 in the cellular invasion of *Orientia tsutsugamushi*. Microb Pathog 36:219–225
Kines R, Thompson C et al (2009) The initial steps leading to papillomavirus infection occur on the basement membrane prior to cell surface binding. Proc Natl Acad Sci USA 106(48): 20458–20463
Kinnunen T, Kaksonen M et al (1998) Cortactin-Src kinase signaling pathway is involved in N-syndecan-dependent neurite outgrowth. J Biol Chem 273:10702–10708
Kirnbauer R, Booy F et al (1992) Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc Natl Acad Sci USA 89:12180–12184
Klasse P, Moore J (2004) Is there enough gp120 in the body fluids of HIV-1-infected individuals to have biologically significant effects? Virology 323:1–8
Kobayashi K, Kato K et al (2010) Plasmodium falciparum BAEBL binds to heparan sulfate proteoglycans on the human erythrocyte surface. J Biol Chem 285(3):1716–1725
Kreugera J, Pereza L et al (2004) Opposing activities of dally-like glypican at high and low levels of wingless morphogen activity. Dev Cell 7:503–512
Lasa I, Cossart P (1996) Actin-based bacterial motility: towards a definition of the minimal requirements. Trends Cell Biol 6:109–114
Lee E, Hall R et al (2004) Common E protein determinants for attenuation of glycosaminoglycan-binding variants of Japanese encephalitis and West Nile viruses. J Virol 78:8271–8280
Li Q, Park P et al (2002) Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. Cell 111:635–646
Liang X, Ji Y (2007) Involvement of alpha5beta1-integrin and TNF-alpha in Staphylococcus aureus alpha-toxin-induced death of epithelial cells. Cell Microbiol 9:1809–1821

Liesenfeld O, Kosek J et al (1997) Gamma interferon induces Fas-dependent apoptosis of Peyer’s patch T cells in mice following peroral infection with Toxoplasma gondii. Infect Immun 65:4682–4689

Lima A, Almeida P et al (2002) Heparan sulfate modulates kinin release by Trypanosoma cruzi through the activity of cruzipain. J Biol Chem 277:5875–5881

Lindblom A, Fransson L (1990) Endothelial heparan sulphate: compositional analysis and comparison of chains from different proteoglycan populations. Glycocon J 7(6):545–562

Lingnau A, Domann E et al (1995) Expression of Listeria monocytogenes EGD inlA and inlB genes, whose products mediate bacterial entry into tissue culture cell lines, by PrfA-dependent and -independent mechanisms. Infect Immun 64:1002–1006

Liu W, Gissmann L et al (1997) Sequence close to the N-terminus of L2 protein is displayed on the surface of bovine papillomavirus type 1 virions. Virology 227:474–483

Love D, Esko J et al (1993) A heparin-binding activity on Leishmania amastigotes which mediates adhesion to cellular proteoglycans. J Cell Biol 123:759–766

Lyon M, Deakin J et al (1994) Liver heparan sulfate structure. A novel molecular design. J Biol Chem 269(15):11208–11215

Maharaj I, Fackrell H (1980) Rabbit erythrocyte band 3: a receptor for staphylococcal alpha toxin. Can J Microbiol 26:524–527

Marques M, Weller P et al (1989) Phosphatidylinositol-specific phospholipase C, a possible virulence factor of Staphylococcus aureus. J Clin Microbiol 27:2451–2454

Maślińska D, Laure-Kamionowska M et al (2004) Toll-like receptors in rat brains injured by hypoxic-ischaemia or exposed to staphylococcal alpha-toxin. Folia Neuropathol 42:125–132

McCormick C, Tuckwell D et al (1999) Identification of heparin as a ligand for the A-domain of Plasmodium falciparum thrombospondin-related adhesion protein. Mol Biochem Parasitol 100:111–124

McElroy M, Harty H et al (1999) Alpha-toxin damages the air-blood barrier of the lung in a rat model of Staphylococcus aureus-induced pneumonia. Infect Immun 67:5541–5544

Menard R (2000) The journey of the malaria sporozoite through its hosts: two parasite proteins lead the way. Microb Infect 2:633–642

Mengaud J, Ohayon H et al (1996) E-cadherin is the receptor for internalin, a surface protein required for entry of L. monocytogenes into epithelial cells. Cell 84:923–932

Mertens G, Van der Schueren B et al (1996) Heparan sulfate expression in polarized epithelial cells: the apical sorting of glypican (GPI-anchored proteoglycan) is inversely related to its heparan sulfate content. J Cell Biol 132:487–497

Moelleken K, Hegemann J (2008) The Chlamydia outer membrane protein OmcB is required for adhesion and exhibits biovar-specific differences in glycosaminoglycan binding. Mol Microbiol 67:403–419

Mordue D, Monroy F et al (2001) Acute toxoplasmosis leads to lethal overproduction of Th1 cytokines. J Immunol 167:4574–4584

Muller H, Reckman I et al (1993) Thrombospondin related anonymous protein (TRAP) of Plasmodium falciparum binds specifically to sulfated glycoconjugates and to HepG2 hepatoma cells suggesting a role for this molecule in sporozoite invasion of hepatocytes. EMBO J 12: 2881–2889

Murthy K, Smith S et al (2001) Crystal structure of a complement control protein that regulates both pathways of complement activation and binds heparan sulfate proteoglycans. Cell 104:301–311

Naguleswaran A, Cannas A et al (2002) Vero cell surface proteoglycan interaction with the microneme protein NeMIC(3) mediates adhesion of Neospora caninum tachyzoites to host cells unlike that in Toxoplasma gondii. Int J Parasitol 32:695–704

Nakatsura T, Kageshita T et al (2004) Identification of Glypican-3 as a novel tumor marker for melanoma. Clin Cancer Res 10:6612–6621
Nash P, Purner M et al (1998) *Toxoplasma gondii*-infected cells are resistant to multiple inducers of apoptosis. J Immunol 160:1824–1830

Nickells J, Cannella M et al (2008) Neuroadapted yellow fever virus strain 17D: a charged locus in domain III of the E protein governs heparin binding activity and neuroinvasiveness in the SCID mouse model. J Virol 82(24):12510–12519

Nielsen M, Pinto V et al (2009) Induction of adhesion-inhibitory antibodies against placental *Plasmodium falciparum* parasites by using single domains of VAR2CSA. Infect Immun 77:2482–2487

Nikolova V, Koo C-Y et al (2009) Differential roles for membrane-bound and soluble syndecan-1 (CD138) in breast cancer progression. Carcinogenesis 30:397–407

Nilsson I, Hartford O et al (1999) Alpha-toxin and gamma-toxin jointly promote *Staphylococcus aureus* virulence in murine septic arthritis. Infect Immun 67:1045–1049

Nishikawa Y, Kawase O et al (2007) *Toxoplasma gondii* infection induces apoptosis in noninfected macrophages: role of nitric oxide and other soluble factors. Parasite Immunol 29:375–385

Noel G, Love D et al (1994) High-molecular-weight proteins of nontypeable *Haemophilus influenzae* mediate bacterial adhesion to cellular proteoglycans. Infect Immun 62:4028–4033

Noonan D, Albini A (2000) From the outside in: extracellular activities of HIV Tat. Adv Pharmacol 48:229–250

O’Callaghan R, Callegan M et al (1997) Specific roles of alpha-toxin and beta-toxin during *Staphylococcus aureus* corneal infection. Infect Immun 65:1571–1578

Oliveira FJ, Alves C et al (2008) *Trypanosoma cruzi* heparin-binding proteins and the nature of the host cell heparan sulfate-binding domain. Microb Pathog 44:329–338

Opie S, Warrington KJ et al (2003) Identification of amino acid residues in the capsid proteins of adenov-associated virus type 2 that contribute to heparan sulfate proteoglycan binding. J Virol 77:6995–7006

Ortega-Barria E, Boothroyd J (1999) A Toxoplasma lectin-like activity specific for sulfated polysaccharides is involved in host cell infection. J Biol Chem 274:1267–1276

Pallerla S, Lawrence R et al (2008) Altered heparan sulfate structure in mice with deleted NDST3 gene function. J Biol Chem 283:16885–16894

Park P, Pier G et al (2000) Syndecan-1 shedding is enhanced by LasA, a secreted virulence factor of *Pseudomonas aeruginosa*. J Biol Chem 275:3057–3064

Park P, Pier G et al (2001) Exploitation of syndecan-1 shedding by *Pseudomonas aeruginosa* enhances virulence. Nature 411:98–102

Park P, Foster T et al (2004) Activation of Syndecan-1 ectodomain shedding by Staphylococcus aureus z-toxin and β-toxin. J Biol Chem 279:251–258

Patterson N, Smith J et al (2005) Human papillomavirus type 31b infection of human keratinocytes does not require heparan sulfate. J Virol 79:6838–6847

Pethe K, Alonso S et al (2001) The heparin-binding haemagglutinin of *M. tuberculosis* is required for extrapulmonary dissemination. Nature 412:190–194

Piñon J, Klasse P et al (2003) Human T-cell leukemia virus type 1 envelope glycoprotein gp46 interacts with cell surface heparan sulfate proteoglycans. J Virol 77:9922–9930

Poon G, Gariepy J (2007) Cell-surface proteoglycans as molecular portals for cationic peptide and polymer entry into cells. Biochem Soc Trans 35:788–793

Popova T, Millis B et al (2006) Acceleration of epithelial cell syndecan-1 shedding by anthrax hemolytic virulence factors. BMC Microbiol 6:8

Portnoy D, Chakraborty T et al (1992) Molecular determinants of *Listeria monocytogenes* pathogenesis. Infect Immum 60:1263–1267

Preston M, Seed P et al (1997) Contribution of proteases and LasR to the virulence of *Pseudomonas aeruginosa* during corneal infections. Infect Immun 65:3086–3090

Pruessmeyer J, Martin C et al (2010) A disintegrin and metalloproteinase 17 (ADAM17) mediates inflammation-induced shedding of syndecan-1 and -4 by lung epithelial cells. J Biol Chem 285 (1):555–564
Qi Y, Peng S et al (1995) Epithelial cells display separate receptors for papillomavirus VLPs and for soluble L1 capsid protein. Virology 216:35–45
Ratner A, Hippe K et al (2006) Epithelial cells are sensitive detectors of bacterial pore-forming toxins. J Biol Chem 281:12994–12998
Reizes O, Benoit S et al (2008) The role of syndecans in the regulation of body weight and synaptic plasticity. Int J Biochem Cell Biol 40:28–45
Reyes-del Valle J, Chávez-Salinas S et al (2005) Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. J Virol 79(8):4557–4567
Richards R, Lowy D et al (2006) Cleavage of the papillomavirus minor capsid protein, L2, at a furin consensus site is necessary for infection. Proc Natl Acad Sci USA 103(5):1522–1527
Robson K, Frevert U et al (1995) Thrombospondin-related adhesive protein (TRAP) of Plasmodium falciparum: expression during sporozoite ontogeny and binding to human hepatocytes. EMBO J 14:3883–3894
Roden R, Kirnbauer R et al (1994a) Interaction of papillomaviruses with the cell surface. J Virol 68:7260–7266
Roden R, Weissinger E et al (1994b) Neutralization of bovine papillomavirus by antibodies to L1 and L2 capsid proteins. J Virol 68:7570–7574
Roden R, Hubbert N et al (1995) Papillomavirus L1 capsids agglutinate mouse erythrocytes through a proteaceous receptor. J Virol 69:5147–5151
Roden R, Day P et al (2001) Positively charged termini of the L2 minor capsid protein are necessary for papillomavirus infection. J Virol 75:10493–10497
Rose R, Bonnez W et al (1993) Expression of human papillomavirus type 11 L1 protein in insect cells: in vivo and in vitro assembly of viruslike particles. J Virol 67:1936–1944
Rose F, Dahlem G et al (2002) Mediator generation and signaling events in alveolar epithelial cells attacked by S. aureus alpha-toxin. Am J Physiol Lung Cell Mol Physiol 282:L207–L214
Rostand K, Esko J (1997) Microbial adherence to and invasion through proteoglycans. Infect Immun 65:1–8
Rusnati M, Presta M (2002) HIV-1 Tat protein: a target for the development of anti-AIDS therapies. Drug Fut 27:481–493
Rusnati M, Coltini D et al (1997) Interaction of HIV-1 Tat protein with heparin. Role of the backbone structure, sulfation, and size. J Biol Chem 272:11313–11320
Rusnati M, Tulipano G et al (1999) Multiple interactions of HIV-I Tat protein with size-defined heparin oligosaccharides. J Biol Chem 274:28198–28205
Ryman K, Gardner C et al (2007) Heparan sulfate binding can contribute to the neuroviroence of neuroadapted and nonneuroadapted Sindbis viruses. J Virol 81:3563–3573
Salanti A, Staaslooe T et al (2003) Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering Plasmodium falciparum involved in pregnancy-associated malaria. Mol Microbiol 49:179–191
Salanti A, Dahlback M et al (2004) Evidence for the involvement of VAR2CSA in pregnancy-associated malaria. J Exp Med 200:1197–1203
Schulze A, Gripon P et al (2007) Hepatitis B virus infection initiates with a large surface protein-dependent binding to heparan sulfate proteoglycans. Hepatology 46:1759–1768
Selinka H, Giorgioli T et al (2003) Further evidence that papillomavirus capsids exist in two distinct conformations. J Virol 77:12961–12967
Serruto D, Spadafina T et al (2010) Neisseria meningitidis GNA2132, a heparin-binding protein that induces protective immunity in humans. Proc Natl Acad Sci USA 107(8):3770–3775
Shafti-Keramat S, Handisurya A et al (2003) Different heparan sulfate proteoglycans serve as cellular receptors for human papillomaviruses. J Virol 77:13125–13135
Sharma S, Pathak S (2008) Malaria vaccine: a current perspective. J Vector Borne Dis 45:1–20
Sheehan B, Kocks C et al (1994) Molecular and genetic determinants of the Listeria monocytogenes infectious process. Curr Top Microbiol Immunol 192:187–216
Southern T, Jolly C et al (2007) EnP1, a microsporidian spore wall protein that enables spores to adhere to and infect host cells in vitro. Eukaryot Cell 6:1354–1362
Spear P (2004) Herpes simplex virus: receptors and ligands for cell entry. Cell Microbiol 6:401–410
Spillmann D (2001) Heparan sulfate: anchor for viral intruders? Biochimie 83(8):811–817
Stringer S (2006) The role of heparan sulphate proteoglycans in angiogenesis. Biochem Soc Trans 34:451–453
Suarez M, Gonzalez-Zorn B et al (2001) A role for ActA in epithelial cell invasion by Listeria monocytogenes. Cell Microbiol 3:853–864
Subramanian S, Fitzgerald M et al (1997) Regulated shedding of syndecan-1 and -4 ectodomains by thrombin and growth factor receptor activation. J Biol Chem 272:14713–14720
Talarico L, Damonte E (2007) Interference in dengue virus adsorption and uncoating by carrageenans. Virology 363(2):473–485
Taylor D, Whitehouse I et al (2009) Glypican-1 mediates both prion protein lipid raft association and disease isoform formation. PLoS Pathog 5(11):e1000666
Tkachenko E, Lutgens E et al (2004) Fibroblast growth factor 2 endocytosis in endothelial cells proceed via syndecan-4-dependent activation of Rac1 and a Cdc42-dependent macropinocytic pathway. J Cell Sci 117:3189–3199
Tristan A, Benito Y et al (2009) The signal peptide of Staphylococcus aureus panton valentine leukocidin LukS component mediates increased adhesion to heparan sulfates. PLoS One 4(4):e5042
Trybala E, Bergstrom T et al (1998) Interaction between pseudorabiesvirus and heparin/heparan sulfate. Pseudorabies virus mutants differ in their interaction with heparin/heparan sulfate when altered for specific glycoprotein C heparin-binding domain. J Biol Chem 273:5047–5052
Tuve S, Wang H et al (2008) Role of Cellular Heparan Sulfate Proteoglycans in Infection of Human Adenovirus Serotype 3 and 35. PLoS Pathog 4:e1000189
Tyagi M, Rusnati M et al (2001) Internalization of HIV-1 Tat requires cell surface heparan sulfate proteoglycans. J Biol Chem 276(5):3254–3261
Unckell F, Streeck R et al (1997) Generation and neutralization of pseudovirions of human papillomavirus type 33. J Virol 71:2934–2939
Urbinati C, Bugatti A et al (2005) α(v)β3-integrin-dependent activation of focal adhesion kinase mediates NF-B activation and motogenic activity by HIV-1 Tat in endothelial cells. J Cell Sci 118:3949–3958
Urbinati C, Nicoli S et al (2009) HIV-1 Tat and heparan sulfate proteoglycan interaction: a novel mechanism of lymphocyte adhesion and migration across the endothelium. Blood 115(15):3335–3342
Utt M, Danielsson B et al (2001) *Helicobacter pylori* vacuolating cytotoxin binding to a putative cell surface receptor, heparan sulfate, studied by surface plasmon resonance. FEMS Immunol Med Microbiol 30:109–113
Veeltil M, Sharma-Walia N et al (2006) RhoA-GTPase facilitates entry of Kaposi’s sarcoma-associated herpesvirus into adherent target cells in a Src-dependent manner. J Virol 80:11432–11446
Veiga E, Cossart P (2005) Listeria hijacks the clathrin-dependent endocytic machinery to invade mammalian cells. Nat Cell Biol 7:894–900
Vijayvargia R, Suresh C et al (2004) Functional form of Caveolin-1 is necessary for the assembly of alpha-hemolysin. Biochem Biophys Res Commun 324:1130–1136
Vlasak M, Goesler I et al (2005) Human rhinovirus type 89 variants use heparan sulfate proteoglycan for cell attachment. J Virol 79:5963–5980
Vogt AM, Pettersson F et al (2006) Release of sequestered Malaria parasites upon injection of a glycosaminoglycan. PLoS Pathog 2(9):e100
Vollmer P, Walev I et al (1996) Novel pathogenic mechanism of microbial metalloproteinases: liberation of membrane-anchored molecules in biologically active form exemplified by studies with the human interleukin-6 receptor. Infect Immun 64:3646–3651
Volpers C, Schirmacher P et al (1994) Assembly of the major and the minor capsid protein of human papillomavirus type 33 into virus-like particles and tubular structures in insect cells. Virology 200:504–512
Volpers C, Unckell F et al (1995) Binding and internalization of human papillomavirus type 33 virus-like particles by eukaryotic cells. J Virol 69:3258–3264

Walev I, Vollmer P et al (1996) Pore-forming toxins trigger shedding of receptors for interleukin 6 and lipopolysaccharide. Proc Natl Acad Sci USA 93:7882–7887

Wang J, Tian C et al (2008) Increased levels of soluble syndecan-1 in the subretinal fluid and the vitreous of eyes with rhegmatogenous retinal detachment. Curr Eye Res 33:101–107

Weiland M, Palm J et al (2003) Characterisation of alpha-1 giardin: an immunodominant Giardia lamblia annexin with glycosaminoglycan-binding activity. Int J Parasitol 33:1341–1351

Westendorp M, Frank R et al (1995) Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 375:497–500

Woods D, Cryz S et al (1982) Contribution of toxin A and elastase to virulence of Pseudomonas aeruginosa in chronic lung infections of rats. Infect Immun 36:1223–1228

Xiao H, Neuveut C et al (2000) Selective CXCR4 antagonism by Tat: implications for in vivo expansion of coreceptor use by HIV-1. Proc Natl Acad Sci USA 97:11466–11471

Yang R, Day P et al (2003) Cell surface-binding motifs of L2 that facilitate papillomavirus infection. J Virol 77:3531–3541

Yeager M, Aste-Amezaga M et al (2000) Neutralization of human papillomavirus (HPV) pseudovirions: a novel and efficient approach to detect and characterize HPV neutralizing antibodies. Virology 278:570–577

Zautner A, Komer U et al (2003) Heparan sulfates and coxsackievirus-adenovirus receptor; each one mediates coxsackievirus B3 PD infection. J Virol 77:10071–10077

Zautner A, Jahn B et al (2006) N- and 6-O-sulfated heparan sulfates mediate internalization of Coxsackievirus B3 variant PD into CHO-K1 cells. J Virol 80:6629–6636

Zeng Y, Zhang X et al (2007) Intracellular Tat of human immunodeficiency virus type 1 activates lytic cycle replication of Kaposi’s sarcoma-associated herpesvirus: role of JAK/STAT signaling. J Virol 81:2401–2417

Zhang J, Stephens R (1992) Mechanism of C. trachomatis attachment to eukaryotic host cells. Cell 69:861–869

Zvibel I, Halfon P et al (2009) Syndecan 1 (CD138) serum levels: a novel biomarker in predicting liver fibrosis stage in patients with hepatitis C. Liver Int 29:208–212