Chapter 2
Heparan Sulfate Proteoglycans in Infection

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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.

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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 Cα (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 sheddases. 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 NH_2-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 ^{358}Arg and ^{359}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 *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 *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 *T. gondii* attachment. In contrast, the attachment of *Neospora caninum* tachyzoites, a close relative of *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 β-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). β-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 β-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 β-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

Table 2.1 HSPG–pathogen interactions

Pathogen	Pathogen protein	HSPG	Function/ interaction	Reference
Bacteria				
Bacillus anthracis	AnlB, ANlO, 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 (HMW)	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 gonorrhoaea	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	α-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 VP3	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 VP1	N- and 6-0-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	Perlecan	Tat internalization	Argyris et al. (2004)
	Tat	Unknown	Lymphoid cell extravasation	Urbinati et al. (2009)
	gp120	Syndecan-3	Attachment	de Witte et al. (2007)
	gp41	Agrin	Attachment	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	Unknown	Attachment	Vlasak et al. (2005)
Sindbis virus	E2 envelope glycoprotein	Unknown	Attachment	Ryman et al. (2007)
Vaccinia virus	Viral envelope protein A27L	Unknown	Fusion	Hsiao et al. (1998)
	VCP (Vaccinia virus complement control protein)	Unknown	Anchoring of RCA (regulators of complement activation) to cell membrane	Murthy et al. (2001)
West Nile virus	Envelope (E) protein	Unknown	Attachment	Lee et al. (2004)
Yellow fever virus	Envelope (E) protein	Unknown	Attachment	Nickells et al. (2008)
Parasites				
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*)

Table 2.1 (continued)

Pathogen	Pathogen protein	HSPG	Function/ interaction	Reference
Neospora caninum	Microneme protein $NcMIC_3$	Unknown (CS)	Attachment	Naguleswaran et al. (2002)
Plasmodium spp.	Circumsporozoite protein (CSP)	Multiple	CSP cleavage, productive invasion	Coppi et al. (2007)
	Thrombospondin-related anonymous protein (TRAP)	Unknown	Invasion	McCormick et al. (1999)
	BAEBL (EBA-140)	Multiple (HS)	Invasion	Kobayashi et al. (2010)
	VAR2CSA	Chondroitin Sulfate A (CSA)	Attachment (to placenta)	Khunrae et al. (2010)
Toxoplasma gondii	Surface antigen 3	Unknown	Attachment	Jacquet et al. (2001)
	Unknown	Unknown (*N*-sulfation required)	Replication in parasitophorous vesicle	Bishop et al. (2005)
	Programmed cell death 5 (TgPDCD5)		Protein internalization and enhanced apoptotic activity	Bannai et al. (2008)
Trypanosoma cruzi	Cruzipain	HSPG	Enhanced enzymatic activity	Lima et al. (2002)
	Heparin-binding protein (HBP-Tc)	Unknown	Attachment	Oliveira et al. (2008)
Other				
Prion	PrP^C	Glypican-1	Lipid raft association, conversion to PrP^{Sc}	Horonchik et al. (2005), Taylor et al. (2009)

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

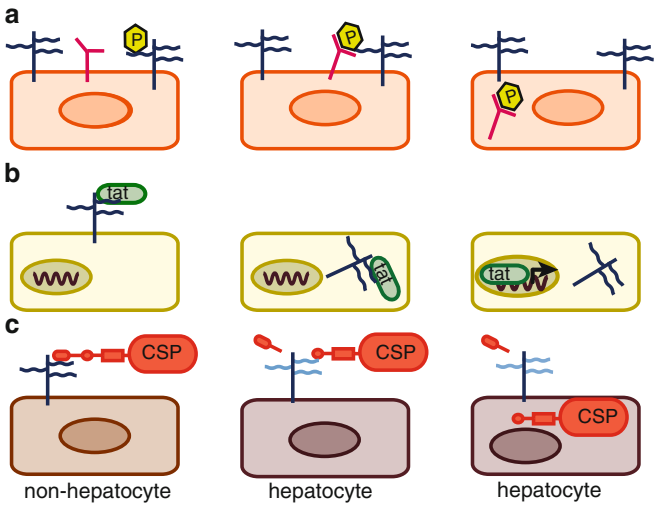

Fig. 2.1 Mechanisms of microbial subversion of cell surface HSPGs. (**a**) A pathogen (P) binds to a cell surface HSPG which then facilitates binding of the pathogen to its specific receptor (*shown in pink*) leading to internalization of the pathogen–receptor complex. (**b**) HIV Tat protein binds to 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

results in formation of squamous fibroepitheilial 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

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 food-borne 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 caveolin 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, coinstillation 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, *T. gondii* has a complicated life cycle requiring invasion of host cells and formation of a parasitophorus 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 *T. gondii* infection is its attachment to host cells (Carruthers et al. 2000). Because *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 *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 *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 *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 *T. gondii* protein, SAG3, has been identified to bind HSPG and was suggested as the parasite protein, which attaches *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 *T. gondii* infection and host cell apoptosis have revealed an additional interaction between *T. gondii* and HSPGs. *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 *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 *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 ectodomain 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*,

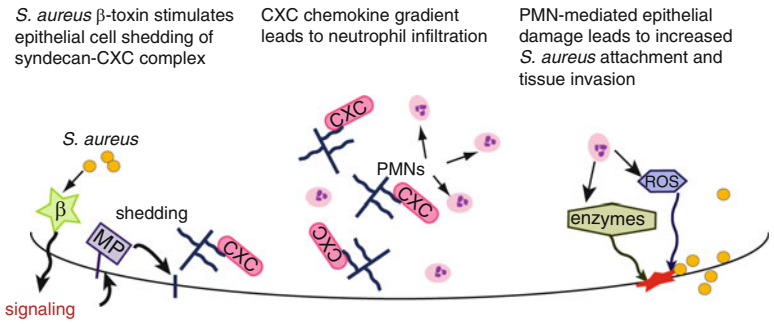

Fig. 2.2 Mechanisms of microbial subversion of HSPG shedding. *S. aureus* β-toxin induces shedding of syndecan-1, which is shown here bound to the cytokine IL-8. *S. aureus* β-toxin stimulates an intracellular signaling pathway through its sphingomyelinase activity, which leads to acceleration of the metalloproteinase-mediated cleavage of syndecan-1 ectodomain-IL-8 complex at the cell surface. Shedding of the syndecan-1-IL-8 complex creates a chemotactic gradient guiding neutrophils to migrate into the airspace leading to inflammation and tissue damage

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.

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