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Syndecan-1 as a Mediator of Bacteria-Enterocyte Interactions

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Normal enteric bacteria frequently cause complicating infections in immunosuppressed and postsurgical patients, as well as patients with shock and trauma. Many nosocomial infections have an undefined focus and appear to be caused by translocating normal enteric bacteria that somehow penetrate the intestinal epithelium and enter otherwise sterile extraintestinal tissues[1,2,3]. Associated mortality is often high (20–40%) despite appropriate antimicrobial therapy[2,4]. Thus, clarification of the mechanisms by which normal enteric flora penetrate the intestinal epithelium is important because antibiotic therapy is often ineffective and because new knowledge may suggest novel prophylactic and therapeutic agents to decrease the costly morbidity associated with translocating enteric flora.

A variety of clinical conditions are associated with increased passage of normal enteric bacteria across the intestinal epithelial barrier. These conditions include enteric microbial overgrowth, gut atrophy, liquid diet, gut stasis, ischemia-reperfusion injury, immunosuppression, surgery, burn wounds, shock, trauma, and increased circulating endotoxin. These diverse conditions are associated with increased intestinal epithelial permeability, facilitating exposure of basolateral enterocyte surfaces, normally joined by tight junctions that prevent the paracellular passage of microbes and unwanted macromolecules (reviewed in [1]). Based on evidence that internalization of a number of bacteria is favored at the basolateral (as opposed to apical) surface of polarized epithelial cells[5,6], we recently tested the hypothesis that syndecan-1, a heparan sulfate (HS) proteoglycan (PG) prominently expressed on the basolateral epithelial surface[7,8], may be involved in interactions of normal enteric bacteria with host enterocytes[9].

The syndecans are a family of four (syndecan-1, -2, -3, -4) widely expressed transmembrane PGs. Each syndecan core protein has a transmembrane portion, a relatively short intracytoplasmic region, and an extracellular domain decorated with three to five glycosaminoglycan (GAG) chains[10,11]. Ectodomains of syndecan-1 (Fig. 1), -3, and -4 may bear both HS and chondroitin sulfate (CS) GAG chains, while syndecan-2 appears to express only HS[10,12]. Syndecan-1 is mainly/predominantly expressed on epithelia and may be found in the mesenchyme during development. Syndecan-2 is prominent in mesenchymal tissues, and on liver and neuronal cells. Syndecan-3 is associated with neural tissues and syndecan-4 is widely distributed in many cell types[10,13]. Syndecans can bind growth factors, chemokines, cytokines, extracellular matrix constituents, components of the coagulation cascade, and various microbes[14,15], and a number of elegant reviews describe aspects of syndecan structure and function[10,11,14,16].

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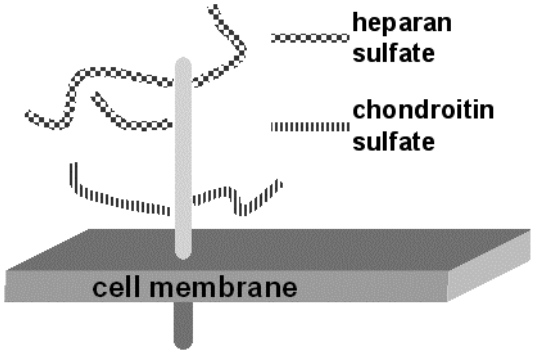

FIGURE 1. Diagrammatic representation of the structure of syndecan-1, a transmembrane HSPG with a comparatively short cytoplasmic domain and an ectodomain decorated with heparan sulfate and chondroitin sulfate.

Binding to host tissues is generally considered prerequisite for successful infection by a microbial pathogen. Using microbial HS-binding proteins, a variety of microbes apparently bind heparin/HS, including protozoa (*Leishmania* spp., *Plasmodium* spp., *Trypanosoma cruzi*), viruses (cytomegalovirus, herpes simplex virus, human immunodeficiency virus, pseudorabies virus), gram-negative bacteria (*Borrelia burgdorferi*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Hemophilus influenza*, *Helicobacter pylori*, *Bordetella pertussis*), gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus* spp.), and acid-fast *Mycobacterium* spp.[8,15,17]. In some instances, a microbe appears to use HS as a coreceptor. For example, initial binding of herpes simplex virus to host cell HS is mediated by viral glycoprotein C, followed by fusion of virions with the host cell membrane using interactions between other viral glycoproteins and other host determinants[18,19]. Thus, HS participates in interactions of a variety of frankly pathogenic microbes with mammalian cells, but the ability of endogenous intestinal bacteria to interact with HSPGs, including syndecan-1 has received little attention.

Although there are hundreds of microbial species in the human intestine, comparatively few (e.g., *Enterococcus faecalis*, *Escherichia coli*, and other members of the family Enterobacteriaceae) are typically associated with systemic infection in high-risk patients[1,2,3,4]. To investigate the role of syndecan-1 in interactions of normal enteric flora with intestinal epithelium, we selected bacteria that might use the enterocyte as a portal of entry for systemic infection. Two enteric pathogens, *L. monocytogenes* and *Salmonella typhimurium*, known to penetrate the intestinal tract of otherwise healthy individuals, were also used as positive controls for bacterial invasion of enterocytes. Normal enteric flora included *E. faecalis*, *E. coli*, and other members of the family Enterobacteriaceae (*Klebsiella pneumonia*, *Citrobacter freundii*, *Morganella morganii*, *Enterobacter cloacae*, *Proteus mirabilis*). Other species included *Streptococcus bovis*, *S. agalactiae*, *S. pyogenes*, and *S. mitis*, as well as *Staphylococcus aureus* and *S. epidermidis*. *Streptococcus bovis* bacteremia and endocarditis have been associated with gastrointestinal disease, primarily colon cancer[20]. *S. agalactiae* carriage is monitored using vaginal and anorectal swabs[21]. *S. mitis* is associated with bacteremia in patients with chemotherapy-induced mucosal injury of the alimentary tract[22]. Intestinal *S. aureus*[23] and *S. epidermidis*[24] have been implicated in systemic infection in immunosuppressed patients, and *S. aureus* can translocate out of the intestinal tract of orally inoculated mice[25]. As a caveat, only one or two strains of each species were used in the study highlighted here[9], so resulting data cannot be extrapolated to define the properties of any particular species. Nonetheless, these data provide useful information about the potential scope of the interactions of syndecan-1 with normal enteric bacteria.

Of the above bacterial species, only *L. monocytogenes*, *S. bovis*, *S. agalactiae*, *S. pyogenes*, *S. mitis*, *S. aureus*, and *S. epidermidis* showed demonstrable binding of heparin-albumin-biotin, indicating expression of bacterial heparin-binding proteins. With exception of *S. mitis*, these species (with heparin-

binding proteins) showed increased adherence to ARH-77 cells that normally have little detergent extractable HS and were transfected to express syndecan-1[26]. In this system, lack of detectable reactivity with *S. mitis* could have been due to the need for a syndecan-1 coreceptor, but this investigation was beyond the scope of the study. Fig. 2 shows an example of comparative binding of *S. pyogenes* to vector control and syndecan-1 transfected ARH-77 cells. Two polarized enterocyte cells lines were subsequently used to determine if heparin, an HS analog, could inhibit bacterial internalization by cultured enterocytes. Caco-2 enterocytes have relatively low syndecan-1 expression[8], and heparin had no noticeable effect bacterial internalization. However, using HT-29 enterocytes with prominent syndecan-1 expression[8], heparin inhibited internalization of *S. bovis*, *S. agalactiae*, *S. pyogenes*, and *S. aureus*. (Again, lack of inhibition with *S. epidermidis* and *S. mitis* could have been due to the need for a syndecan-1 coreceptor.) Results from a companion study[27] confirmed that HSPGs played an important role in internalization of *S. aureus* by HT-29 enterocytes, and this mechanism appeared different from a previously described model employing fibronectin as a mediator of *S. aureus* binding to eucaryotic cells[17,28]. Data from experiments with mutant Chinese hamster ovary cells with altered GAG expression indicated that both HS and CS (GAGs on the syndecan-1 ectodomain) participated in interactions of *L. monocytogenes*, *S. bovis*, *S. agalactiae*, *S. pyogenes*, *S. mitis*, *S. aureus*, and *S. epidermidis* with mammalian cells. These observations are summarized in Table 1.

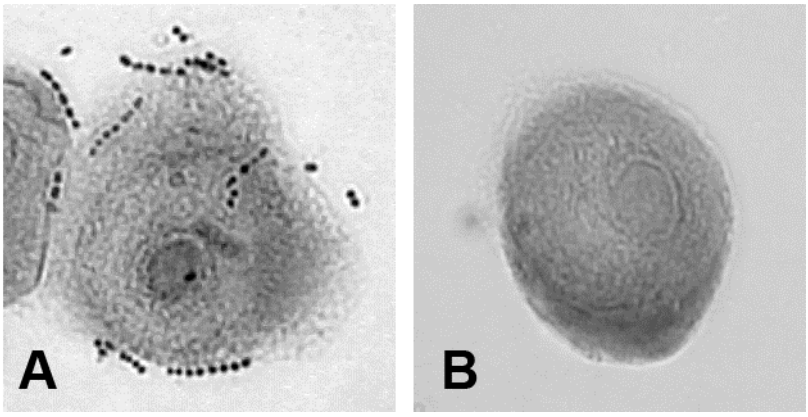

FIGURE 2. Phase contrast images showing adherence of many *S. pyogenes* (chains of cocci) to ARH-77 cells transfected to express syndecan-1 (A), compared to the lack of *S. pyogenes* adherence to vector control ARH-77 cell (B).

The functional assay with HT-29 enterocytes (Table 1) tested the ability of cell-bound HS to interact with enteric bacteria. Interactions with CS were not clarified, but it seems reasonable to speculate that bacterial interactions with CS-bearing syndecan-1 may be sterically hindered due to the proximity of CS to the cell membrane (Fig. 1). Interestingly, physiologically active syndecan ectodomains are constitutively shed at low levels into the extracellular milieu, shedding appears increased in wounds and in epithelium over inflamed intestinal mucosa, and shed ectodomains can compete for the same ligands as their cell surface counterparts[7,16,29]. Little is known about bacterial interactions with shed syndecan-1, and with CS itself, but there is evidence that CS interacts with *S. uberis*[30] and *B. pertussis*[31], and this is likely a fertile area for future investigation.

We originally hypothesized that intestinal syndecan-1 might provide a portal of entry for normal intestinal bacteria that cause systemic infection in high-risk patients. Although our results to date indicate that *E. faecalis, E. coli,* and other gram-negative enterobacteria do not interact with syndecan-1, this HSPG may mediate enterocyte interactions with some staphylococci and streptococci. Such interactions could have great importance and could affect our views regarding the use of GAG derivatives as prophylactic or therapeutic agents in specific populations of high-risk patients.

TABLE 1
Summarized Results[9] of Assays Designed to Detect Bacterial Expression of Heparin-Binding Proteins and to Quantify the Ability of GAG Expression by Mutant Chinese Hamster Ovary (CHO) Cells to Modulate Bacterial Internalization, the Ability of Bacteria to Show Increased Adherence to ARH-77 Cells Transfected to Express Syndecan-1, and the Ability of Exogenous Heparin to Inhibit Bacterial Internalization by HT-29 Enterocytes (a Cell Line with Prominent Syndecan-1 Expression). *E. coli, P. vulgaris, P. mirabilis, K. pneumoniae, C. freundii, E. cloacae, S. typhimurium,* and *E. faecalis* (not included below) Gave Negative Results in All Assays.

Enteric Bacterial Species	Expression of Heparin-Binding Proteins	GAGs Modulate Internalization by Mutant CHO Cell Lines	Adherence to Syndecan-1-Expressing ARH-77 Cells	Heparin-Induced Inhibition of Internalization by HT-29 Cells
L. monocytogenes	+	+	+	+
S. bovis	+	+	+	+
S. agalactiae	+	+	+	+
S. pyogenes	+	+	+	+
S. aureus	+	+	+	+*
S. epidermidis	+	+	+	–
S. mitis	+	+	-	–

*Two of three *S. aureus* strains showed heparin-induced inhibition[9,26].

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BIOSKETCHES

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