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# GLYCOSAMINOGLYCAN AND SIALIC ACID BINDING MICROBIAL PROTEINS IN GUT TISSUE ADHESION AND INVASION

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## SUMMARY

Glycosaminoglycans (GAGs), heparin, heparan sulphate (HS) and other sulphated molecules and hyaluronic acid, form part of the extracellular matrix (ECM), mediate cell-ECM adhesion, cell migration and growth, and bind growth factors and growth factor-binding proteins. Bacterial pathogens, like *Helicobacter pylori*, *Staphylococcus aureus* and *Streptococcus pyogenes*, and parasites such as *Trypanosoma cruzi* and *Leishmania* were shown to express cell surface proteins binding specific HS molecules on macrophages, triggering cell uptake and adhesion to fibronectin and other molecules involved in the phagocytic process. So, in addition to acting as a mechanism of tissue adhesion GAG binding may interfere with phagocytosis. It is tempting to speculate that GAG binding may play an important role in intracellular survival in macrophages. Several microbial cell surface proteins interact with highly negatively charged sialic acid-containing glycoconjugates, e.g. fimbriae of *Escherichia coli* and *Plasmodium falciparum*, recognising glycophorin on erythrocytes. *Yersiniae* cells can utilise HS binding for gut translocation, and *Listeria monocytogenes* cell entry is mediated by HS binding. Heparin was shown to mediate the erythrocyte invasion by *P falciparum* merozoites. *H pylori* invades through tight junctions which may be enhanced by expression of plasminogen binding. Heparin binding may interfere with vitronectin binding and complement activation. GAG binding proteins of *Borrelia* sp. are vaccine candidates for prevention and treatment of infections. Likewise, with *H pylori* a similar anti-adhesion approach is promising. Heparin binding microbes may interfere with the effect normally exerted by heparin binding growth factors, like wound healing and tissue integration. Heparin was shown to inhibit the mucosal inflammation and enhance tissue healing in mice infected by *H pylori*. Likewise, in patients with ulcerative colitis, heparin was shown to enhance the healing process. Before anti-adhesion treatment directed against GAG- and sialic acid binding proteins is developed effects on the normal intestinal microbial flora have to be elucidated.

## INTRODUCTION

Glycosaminoglycans (GAGs), i.e., heparan sulphate (HS), dermatan sulphate (DS), chondroitin sulphate (CS), keratin sulphate, heparin and other sul-

**Table 1:** Some examples of glycosaminoglycan microbial binding,

| Organism | Mol.wt (kDa) | GAG recognised |
|---|---|---|
| *Bordetella pertussis* (FHA) | | |
| *Borrelia burgdorferi* | 20 and 22 | decorin |
| *Helicobacter pylori* | 55-60 | heparin, HS, DS |
| *Listeria monocytogenes* (ActA) | | heparin, HS |
| *Mycobacterium tuberculosis* | 28 | heparin |
| *Mycoplasma pneumoniae* | | DS |
| *Neisseria gonorrhoeae* (Opa) | | heparin |
| *Staphylococcus aureus* | 60 | heparin, HS |
| *Streptococcus pyogenes* | | |
| *Yersinia enterocolitica* (LcrG) | | heparin, DS |
| *Plasmodium falciparum* | | CSA |
| *Toxoplasma gondii* | - | heparin, HS, CSA, CSC |
| *Herpes simplex* glycoprotein C, B | | HS |
| *Cytomegalovirus* | 30, 34 | HS |
| *Candida albicans* | | GAG |

phated molecules, and unsulphated hyaluronic acid form a major part of the extracellular matrix (ECM). Cell surface GAG molecules mediate many cellular processes: cell-ECM adhesion, cell migration and growth, and bind growth factors and growth factor-binding proteins (*Wadström* and *Ljungh*, 1999; *Lane* and *Lindahl*, 1989). Many pathogenic bacteria, viruses, fungi, and parasites use cell surface glycolipids, glycoproteins and GAGs as receptor molecules for cell and ECM attachment, as well as for eukaryotic cell invasion and intercellular migration processes. These interactions usually involve specific surface proteins of micro-organisms, named adhesins (*Rostand* and *Esko*, 1997). Proteins, interacting with specific carbohydrate structures of glycoconjugates, are defined as microbial surface lectins (*Wadström* and *Trust*, 1984).

Microbial binding to glycolipids and glycoproteins by specific surface lectins, often with haemagglutinating (HA) activity, has been extensively studied. In contrast, binding to cell surface ECM proteins and GAGs has been studied less (Table 1) (*Ljungh* and *Wadström*, 1995; *Wadström* and *Ljungh*, 1999; *Ljungh* et al., 1996; *Conrad*, 1998). More recently, bacterial pathogens such as *Helicobacter pylori* (*Ascencio* et al, 1993a; *Ascencio* et al, 1995; *Utt* and *Wadström*, 1997), *Staphylococcus aureus* (*Liang* et al., 1992), and *Streptococcus pyogenes* (group A streptococci) (*Bergey* and *Stinson*, 1988), and parasites like *Trypanosoma cruzi* (*Prioli* et al., 1987) and *Leishmania* (*Love* et al., 1993) were shown to express cell surface proteins that bind specific HS molecules on macrophages which triggers cell uptake and adhesion to specific ECM molecules involved in phagocytic processes, such as fibronectin (*Chmiela* et al., 1995a; *Duensing* et al., 1999). *Mycobacterium tuberculosis* binds HS by specific surface molecules, and binding to this GAG molecule on macrophages and other cells may trigger cellular uptake (*Menozzi* et al., 1996).

**Table 2:** Intracellular pathogens using heparan sulphate-like molecules in lectinophagocytosis

| Organism | Reference |
|---|---|
| *Borrelia burgdorferi** | *Guo* et al. (1995) |
| *Listeria monocytogenes* | *Alvarez-Dominguez* et al. (1997) |
| *Mycobacterium tuberculosis* | *Menozzi* et al. (1996) |
| *Neisseria gonorrhoeae* | *Chen* et al. (1995) |
| *Helicobacter pylori** | *Chmiela* et al. (1995) |
| *Yersinia enterocolitica* | *Boyd* et al. (1998) |
| *Leishmania donovani* | *Love* et al. (1993) |
| *Plasmodium falciparum* | *Chen* et al. (1997) |
| *Toxoplasma gondii* | *Franklin* et al. (1996) |
| *Trypanosoma cruzi* | *Ortega-Barria* and *Pereira* (1991) |

*Possibly not intracellular.

Interestingly, *H. pylori* and *Neisseria gonorrhoeae* (*Chen* et al., 1995) bind heparin and HS, like some pathogens commonly defined as extracellular microbes, i.e. *Borrelia spp.* and *Treponema pallidum* (Table 2) (*Guo* et al., 1995; *Alderete* and *Baseman*, 1989). These pathogens may adapt to multiply in the ECM of various body tissues without triggering uptake by macrophages and other phagocytes. Initial cell binding is usually followed by binding to other cell surface glycoconjugates, such as cell surface glycolipids, like with cytomegalovirus (CMV) and dengue virus (*Compton* et al., 1993; *Chen* et al., 1997). Prions may also use HS or other similar cell surface and ECM molecules, such as laminin, in cellular interactions in the brain and other body tissues.

## GLYCOSAMINOGLYCAN CHEMISTRY

Cell membrane proteoglycans have single transmembrane-spanning domains in a type I orientation, with syndecan-1 as the prototype. Each of these molecules has conserved attachment sites for 3 to 5 GAG chains (*Linhardt* et al., 1992; *Lane* and *Lindahl*, 1989). The other major family of proteoglycans is named glypicans, which appear to contain only HS chains. Interestingly, expression of these proteoglycans occurs in a tissue-specific manner. It is noteworthy that heparin is cleaved from a core protein in mast cells. Free heparin chains form complexes with basic proteases and peptidases which are packed in secretory granules. Upon degranulation, these are released and dissociate. In contrast, HS is secreted intact from cells (*Kusche* et al., 1991).

Proteoglycans exhibit an enormous structural heterogeneity caused by great variations in glycosylation patterns, variations in glycan chain length of GAG chains, and variation in the extent and pattern of sulphation (*Lane* and *Lindahl*, 1989; *Heremans* et al., 1989). HS chains usually contain 0.8-1.4 sulphate groups/disaccharide per unit, while heparin, synthesised in intracellular granulae of mast cells, contain ≥2.4 sulphate groups/unit. Various modifications occur to a greater extent in heparin than in HS (e.g., >80% of glycosamine residues are N-sulphated compared to <60% of HS).

## GAG BINDING IN MICROBIAL ADHESION TO MUCOSAL SURFACES

Intramolecular variations in the GAG chain, chain length and degree of sulphatation define how these molecules interact with specific ECM proteins - fibronectin, collagen type I, laminin, vitronectin, and various microbes (*Bober-Barkalov* and *Schwarzbauer*, 1991; *Casu*, 1994; *Conrad*, 1998; *Hayashi* et al., 1980; *Keller*, 1994; *Murphy-Ullrich* et al., 1993; *Zou* et al., 1992). Heparin-dependent growth factors, the acidic and basic growth factors (aFGF, bFGF) and platelet-derived growth factor (PDGF) bind to distinct pentasaccharide units with specifically positioned 3-0 sulphated glucosamine residues, shown also to bind to antithrombin III (*Baird* and *Klagsbrun*, 1991; *Dowd* et al., 1999; *Hileman* et al., 1998; *Kinsella* et al., 1998; *Kost* et al., 1992; *Ljungh* and *Wadström*, 1995; *Schlessinger* et al., 1995). The HS-binding peptide from *Plasmodium falciparum* is also a CS-binding protein like the N-terminal region of an actin-binding protein, ActA, of *Listeria monocytogenes* (*Pouvelle* et al., 1997; *Alvarez-Dominguez* et al., 1997).

Several microbial cell-surface proteins interact with highly negatively charged sialic acid-containing glycoconjugates (S fimbriae, K88, K99, CFAI and CFA II surface lectins of *Escherichia coli*) (*Ascencio* et al., 1993b; *Lindahl* et al., 1988; *Sun* et al., 2000; *Virkola* et al., 1993; *Wadström*, 1993; *Wadström* and *Trust*, 1984). Similarly, *P. falciparum* recognises sialic acid and glycophorins on erythrocytes (*Templeton et al*, 1998). Heparin and some other GAG molecules also express a negatively charged surface *in vivo*

The precise mechanism by which heparin blocks cell adhesion of microbes has been difficult to elucidate because of lack of commercially available purified heparins and heparin-derived fragments, now in clinical use in drugs like Fragmin® (KABI-Pharmacia, Stockholm, Sweden). Such fragments of heparin and other GAG chains, as well as those from various natural sulphated carbohydrate polymers like fucoidan and some carrageenans, often called 'heparinoids', are used as adhesion inhibitors to define the specificity of sulphate interactions in the binding of viruses, bacteria and parasites to specific tissue culture cells and ECM derived from cells grown on various biosurfaces (*Duensing* et al., 1999; *Hoffman*, 1993; *Kinsella* et al., 1998; *Pascu* et al., 1995; *Yahi* et al., 1994). Bacteria may also produce heparin and HS lyases with certain specificities (*Nader* et al., 1999).

Moreover, a number of specific glycosidases, such as heparinase, heparitinases and chondroitin sulphatases were used as 'receptor-destroying enzymes' in an analogy to the use of sialidases (neuraminidases) to destroy sialoglycoconjugate cell receptors for influenza virus and *T. cruzi* (*Prioli* et al., 1987).

Examination of the binding properties of $^{125}$I-HS with sialoglycoconjugates, and HA assays with fetuin, glycophorins and hyaluronic acid in combination with a great number of 'heparinoids' as potential HA inhibitors showed that heparin-inhibitable $^{125}$I-HS binding occurs with all strains of *H. pylori* tested (so called class I, compared to class II strains, or *cagA* pathogenicity 'island' positive) (*Utt* and *Wadström*, 1997). Likewise, expression of sialic acid haemagglutinin and other sialic acid-binding surface lectins (SALs; present in ~50% of strains of mainly class I strains; *T. Wadström* et al., unpublished results) did not influ-

ence binding of heparin. Interestingly, *Bordetella pertussis* expresses a filamentous haemagglutinin (FHA) that binds heparin and other GAG molecules by its C-terminal, whereas the pertussis toxin binds specific sialoglycoconjugates (*Geuijen* et al., 1998; *Menozzi* et al., 1994; *van ´t Wout* et al., 1992). Other *Helicobacter* spp. isolated from animals, such as *H. felis* and *H. mustelae*, commonly express GAG-binding surface molecules but not SALs (*T. Wadström* et al., unpublished results).

Further analyses of the molecular properties of the first *H. pylori* heparin binding protein or HEBP (57 kDa) (*Utt* and *Wadström*, 1997) will reveal if this protein belongs to a new class of *microbial* GAGBPs (see below). Interestingly, HEBP of *M. tuberculosis* and *M. bovis* is a haemagglutinin that agglutinates rabbit erythrocytes (*Menozzi* et al., 1996).

With *Mycoplasma pneumoniae*, DS has been shown to inhibit adhesion to pulmonary epithelium (*Krivan* et al., 1989). DS and other GAG molecules bind primarily to heparin-binding ECM molecules like fibronectin and, in so doing, cover or mask potential binding ligands in ECM, e.g. for *Candida albicans* (*Klotz* and *Smith*, 1992; *Hileman* et al., 1998). DS, which is more negatively charged than heparin, did not show such an effect indicating that specific interactions occur between heparin or HS and sub-endothelial ECM.

For the recently described GAGBPs of *M. tuberculosis* and *L. monocytogenes* (*Menozzi* et al., 1996; *Alvarez-Dominguez* et al., 1997), similar studies on expression of these proteins *in vivo* are lacking. GAG molecules have been shown to block *Chlamydia* infection *in vitro*, but this infection was not blocked in a mouse model of infection. Specific biovariants (of the trachoma-*Lymphogranuloma venerum* (LGV) group) also express neuraminidase-sensitive eukaryotic cell receptors. However, as heparin inhibits cell binding of all *Chlamydiae*, expression of GAG binding is likely to be common in the genus, including *Chlamydia pneumoniae* (*Stephens*, 1994).

## POSSIBLE ROLE OF GAG BINDING IN INTRACELLULAR SURVIVAL

Survival and multiplication of intracellular micro-organisms in macrophages and other eukaryotic cells is vital in the pathogenesis of infections caused by such microbes. It has been suggested that GAG-binding surface molecules confer the resistance to phagocytosis seen with *L. monocytogenes*, *H. pylori* and *N. gonorrhoeae* (Table 2) (*Chen* et al., 1995; *Chmiela* et al., 1995a,b; *Chmiela* et al., 1996). It is tempting to speculate that GAG binding may play an important role in intracellular survival in specific cells, such as macrophages, known to express mannose-sialoglycoconjugates and HA on the surface.

However, more *in vitro* studies in serum-containing and serum-free systems with macrophages are necessary to define a possible interplay of GAG molecules with individual pathogens. Such studies will form a basis for deciding if GAG molecules such as heparin and various ‘heparinoids’ can be used in combination with other glycoconjugates to block uptake into macrophages, other professional phagocytes and other target cells in pathogenesis, including the human gastric epithelium for studies of *H. pylori*.

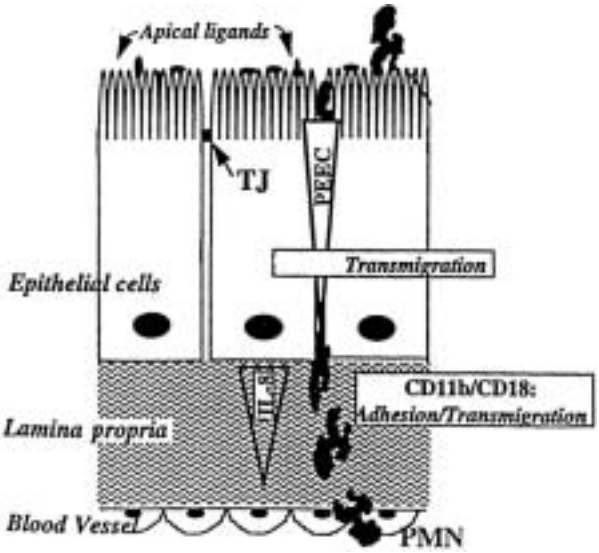


**Figure 1:** Model of gastro-intestinal epithelium. *Salmonella* sp. invades epithelial cells directly or through tight junctions (TJ) whereas *H. pylori* invades probably only via TJ.

## GAG BINDING IN INVASION AND TRANSLOCATION

*Yersiniae* cells can utilise HS exposed on eukaryotic cells for gut translocation (*Boyd* et al., 1998; *Cornelis* and *Wolf-Watz*, 1997; *Pusztai* and *Bardocz*, 1995), and *L. monocytogenes* cell entry is mediated by HS binding (*Menozzi* et al., 1996). GAG-binding is responsible for the gliding motility by *Toxoplasma gondii*, and GAG-deficient mutant host cells could not be invaded by this parasite (*Carruthers* et al., 2000). Likewise, heparin binding is involved in erythrocyte invasion by *P. falciparum* merozoites (*Kulane* et al., 1992). HS binding by Type 1 glycoprotein B and C of *Herpes simplex* was shown to be involved in attachment, cell-to-cell spread and invasion of eukaryotic cells but in different ways (*Laquerre* et al., 1998). Whether or not gastro-intestinal (GI) pathogens, like *Salmonella* and *H. pylori* invade epithelial cells by GAG binding ability has not been elucidated. *H. pylori* invades through tight junctions (Figure 1). Possibly, expression of plasminogen binding may enhance gut translocation and tissue invasion, as described for several other invasive pathogens (*Pantzar* et al., 1998; *Ljungh*, 2000; *Lähtenmääki* et al., 2000). Moreover, heparin binding may interfere with complement and vitronectin binding, and modulate microbe-phagocyte interactions as well as binding to other cells, as shown for *C. albicans* and staphylococci (*Calderone* et al., 1988; *Duensing* et al., 1999; *Ljungh* and *Wadström*, 1995; *Lundberg* et al., 1997). Epithelial cells undergo rapid apoptosis and loss of contact with the underlying matrix. As several ECM proteins have heparin-binding domains, it is possible that expression of heparin binding by microbes may enhance apoptosis by interfering with the matrix-epithelium contact (*Duensing* et al., 1999; *Wadström* and *Ljungh*, 1999).

At least three growth factors require heparin for activation. These are aFGF, bFGF and PDGF (*Baird* and *Klags-*

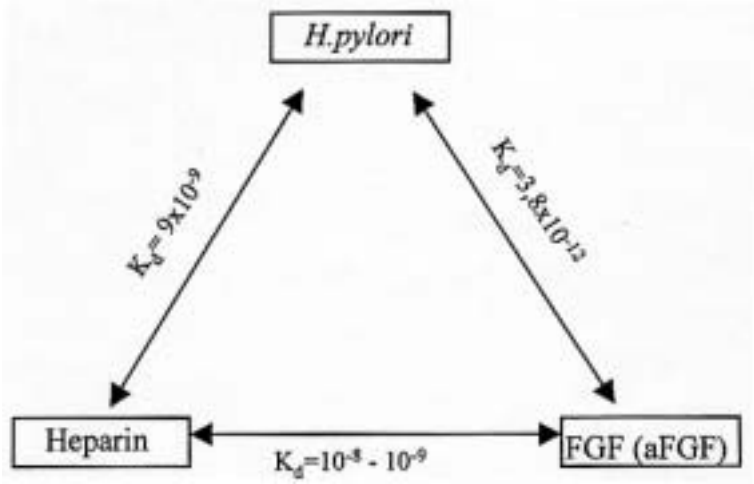


**Figure 2:** Interactions between *H. pylori*, heparin and heparan sulphate, and heparin-dependent growth factors.

*brun*, 1991). *H. pylori* binds bFGF and PDGF specifically which may indicate that binding of heparin or growth factors can interfere with wound healing (Figure 2) (*Ascencio* et al., 1995). In addition, it has been suggested that such heparin depending growth factor interactions target *Pseudomonas aeruginosa* exotoxin to susceptible cells (*Mesri* et al., 1993).

Surface domains may express proteins rich in charged and hydrophobic domains ('hydrophobins') of importance for the pathogen to evade a strong immune response. Thus, studies on *Borrelia sp.*, *H. pylori* and other GAG-binding pathogens seem to be of great importance because GAGBPs, as mucosal epithelium adhesins, are vaccine antigen candidates and for targeting molecules (beside other surface lectins and ECM-binding proteins) for an anti-adhesion approach to prevent and treat infections by a 'non-antibiotic' strategy (*Breithaupt*, 1999; *Feng* et al., 1998; *Hanson* et al., 1998; *Ruiz-Bustos* et al., 2000). Preliminary studies involving the use of modified heparin molecules and specific sialoglycoconjugates to block *H. pylori* GAGBPs and SALs in a BALB/c mouse and a primate model have been promising (see below).

Standard commercial heparin and low molecular weight or LMW heparin (Fragmin) were shown to disrupt rosette formation between *P. falciparum* and both infected and non-infected red blood cells from patients with cerebral malaria. Other investigators have confirmed this proposal and shown that chondrotin-4-sulphate is the natural receptor for *Plasmodium* spp. in the placenta, and is involved in transmission of the parasite from the mother to the foetus (*Gysin* et al., 1999; *Reeder* et al., 2000).

Altogether, these findings suggest that modified LMW heparin and other GAG molecules, or heparinoids, are indeed candidates for future development of antibacterial, antiviral and antiparasitic drugs.

## MICROBIAL AND PLANT LECTINS IN THE GASTRO-INTESTINAL TRACT

We know from pioneer studies by *Pusztai* and *Bardocz* (1995) that feeding plant lectins to young animals such as rats and guinea pigs affect the maturation process of the GI mucosa. More recently, it has been debated that gene-modified plants with high lectin contents may enhance these effects and maybe predispose to overproliferation of the gut mucosa.

Pioneer studies in gnotobiology a few decades ago showed that maturation of the gut mucosa and lymphoid GALT system is much delayed in germfree (GF) compared to conventional rats, and is more associated with specific species of the indigenous gut microflora such as *Enterococcus faecalis.* Despite these observations we have still a very poor knowledge of how microbial lectins with various carbohydrate specificities and plant lectins (fucose, galactose and mannose specific lectins) are involved in such growth stimulation of mucosal epithelium and cells associated with the GALT lymphoid system of the gut (*Pusztai* and *Bardocz*, 1995; *Gabius* and *Gabius*, 1993).

However, the situation is different for some bacterial lectins, such as SALs and heparin or GAG binding proteins. These latter proteins are not often referred to as lectins for the simple fact that the carbohydrate binding studies often involved several units unlike plant lectins mostly recognising one or two units (*Gabius* and *Gabius*, 1993). Moreover, modification of sulphate groups can drastically affect binding of heparin, HS and other GAG molecules such as CS.

We know that in the evolution of pathogenic microbes of the GI mucosa, SALs are common lectins among enteropathogenic *E. coli* and *H. pylori*, and less well characterised microbes such as *Campylobacter jejuni* and other campylobacter species (*Wadström* and *Trust*, 1984; *Gabius* and *Gabius*, 1993).

GAG-binding proteins are common among various tissue invading pathogens like staphyloccoci, streptococci, *L. monocytogenes* and various parasites (Table 1). For some of these organisms such as gonococci and *M. pneumoniae* GAGBP seem crucial to trigger later stages of glycolipid cell receptor binding and uptake in gut mucosa cells as well as professional phagocytes (*Krivan* et al., 1989).

On the contrary, our knowledge on putative GAGBPs and SALs of members of the indigenous microbial microflora on various mucosal surfaces is nearly non-existing. Such studies are needed since tissue trauma of the gut mucosa exposes ECM and GAG molecules. Based on studies of GAGBPs of *H. pylori* binding to GAGs on cell surfaces and in the ECM in experimental studies, we propose that other GAG binding pathogens and maybe also members of the indigenous microflora can colonise tissue wounds further down the GI tract. Studies on various new Helicobacters colonising the GI mucosa of rodents, dogs and cats as well as primates are now underway. Recent reports on how such organisms may cause chronic colitis as well as hepatitis ("gut-liver link") as defined in the pathogenesis of primary sclerosing cholangitis and chronic ulcerative colitis (UC) will encourage further studies (*Kirsner*, 2000; *Franklin* et al., 1996). Furthermore, early observations of ECM binding *E. coli* colonising lesions in patients with UC (*Ljungh* and *Wadström*, 1988; *Ljungh* et al., 1988; *Ljungh*, 1992) suggest that studies on GAG binding properties of other GI

**Table 3:** History of anti-peptic ulcer drugs*

| | | |
|---|---|---|
| 1907 | Deklug | Theory of mucosal protection by gastric mucus |
| 1931 | Fogelson | Clinical application of gastric mucin |
| 1932 | Babkin and Komarov | Discovery of antipeptic effect of gastric mucus |
| 1954 | Levey and Sheinfeld | Antiulcerogenic effect of chondroitin sulphate |
| 1959 | Anderson | Antiulcerogenic effect of carageenan |
| 1967 | Cayer | Clinical application of amylopectin sulphate |
| | Hino Nao | Clinical application of aluminum sucrose sulphate (sucralphate) |
| 1968 | Ishimori | Clinical application of sorbitol sulphate, dextran sulphate, amylopectin sulphate and aluminum dextran sulphate |

* Modified from A. Ishimori (*Hollander* and *Tytgat*, 1995)

inhabitants like *Bacteroides* and lactic acid bacteria (LAB) now are needed. We have earlier characterised binding of collagen by *Lactobacillus reuteri*, and ECM binding by various anaerobic species (*Aleljung* et al., 1994; *Szöke* et al., 1996). Interestingly, *Bacteroides* species were recently shown to produce glycosaminoglycan-degrading enzymes (*Ahn* et al., 1998).

## PEPTIC ULCERS DISEASE (PUD) OF THE STOMACH AS A MODEL FOR ULCERATIVE COLITIS AND OTHER CHRONIC GUT-BARRIER DESTROYING DISEASES

Already studies in the 1960's in Japan showed that sulphated natural carbohydrates often called 'heparinoids' enhance the wound healing process in chronic PUD (Table 3) (*Hollander* and *Tytgat*, 1995). These studies stimulated the development of sucralphate by the Chugai Company as a drug to enhance PUD healing and as a mucosal protecting agent. Later studies have proposed that this drug accumulates in mucosal lesions, and protects various heparin dependant growth factors against rapid breakdown by tissue and microbial proteases such as ECM associated metalloproteases, also proposed to play a key role in the pathogenesis of UC and maybe of Mb Crohn (*Keusch* et al., 1880; *Kirsner*, 2000; *Parks* and *Mecham*, 1999). Interestingly, recent studies in mouse models for *H. pylori* gastritis showed that heparin inhibits mucosal inflammation and enhances tissue healing (*Wang* et al., 2001). Moreover, studies in patients with UC have shown that various forms of heparin such as low molecular weight heparin enhance the healing process of the colonic mucosa (*Gaffney* et al., 1995; *Korzenik* et al., 1998; *Törkvist* et al., 1999). We should then remember that early studies on sucralphate in colonic enemas showed a mucosal healing effect in patients with UC (*Hollander* and *Tytgat*, 1995). These observations will stimulate more studies on the effect on heparin and other GAG-models in inflammations and ulcer healing in various parts of the GI tract.

## HEPARIN GROWTH FACTORS AND CYTOKINES

A first study on how *H. pylori* GAG-binding surface factors can attenuate growth suggests that this pathogen may deplete growth factors from mucosal lesions, and thus delay the natural healing process in type B gastritis and PUD (*Ascencio* et al., 1995). Recently, hepatocyte growth factor and other heparin dependant growth factors (aFGF and bFGF) were shown to be upregulated in mucosal lesions in rats (*T. Watanabe*, personal communication), and oral treatment trials with synthetic stable acid growth factors enhanced the healing process in a proton pump based therapy in PUD, and might also enhance the healing in treatment with antibiotics (*I. Kondo*, personal communication).

However, recent findings that heparin also affects tissue and ECM binding of various growth factors such as interleukin 6 (IL-6) and IL-8 suggest that heparin or other GAGs may wash out a surplus of cytokines in mucosal lesions (*Dobosz* et al., 1996). On the contrary, other sulphated carbohydrates such as DS and carrageenan under experimental conditions enhance the mucosal inflammation in rats. Thus, more research is needed to study how various 'heparinoids' and GAG binding molecules trigger mucosal inflammation in some situations, and other molecules suppress tissue inflammation mediated, probably by heparin-binding growth factors and mucosa associated cytokines (*Korszenik* et al., 1998; *Kuschert* et al., 1999). Most likely such compounds are candidates for a novel therapy of various inflammatory and infectious processes in the GI tract. However, possible side effects on the indigenous flora have to be studied to rule out that these compounds cause side effects as broad spectrum antibiotics to destroy the normal GI indigenous microflora (*Breithaupt*, 1999).

During chronic tissue inflammation processes such as transplant rejection, there is a selective loss of HS. Soluble GAG molecules can reduce the inflammation in experimental animal models and a low dose of heparin inhibits delayed type hypersensitivity reactions, adjuvant arthritis, allergen induced eosinophilic infiltration maybe interfere with both heparin binding cytokines growth factors as well as L and P selectins (*Kuschert* et al., 1999). Moreover, heparin may inhibit mast cell granulation and complement activation, which may also decrease the inflammation process.

Thus, GAG binding microbes involved in chronic infections such as *H. pylori* and *M. tuberculosis* may modulate and affect tissue inflammation in a complex fashion by modulating GAG/heparin binding surface proteins and indirectly also affect GAG binding tissue molecules such as heparin binding growth factors and cytokines. This may indirectly affect multiple chemoattractants in control of leukocyte homing into inflammation target tissues such as the gut mucosa (*Naef* et al., 1996; *Kuschert* et al., 1999).

It has been postulated that heparin and DS bind to similar tissue substrates but have opposite effects on the same pathway (*Korzenik* et al., 1998). Experimental models for chronic colitis in rats and mice have shown that DS can induce a chronic colitis in conventional but not in Germ-free animal models (*T. Midtvedt*, personal communication). It seems likely that DS as well as other sulphated glycoconjugate molecules such as carrageenans, can modulate the binding of growth factors to ECM like bFGF-β1 and PDGF. Heparin and

other GAG molecules act as antagonists for DS in these experimental models and probably also in patients with inflammatory bowel disease (IBD) (*Korzenik* et al., 1998). Heparin has also been shown to decrease the cell concentration of the IL-6 cytokine in tissue inflammation in UC and have a beneficial role on the microcirculation and to inhibit nitric oxide synthase (*Dobosz* et al., 1996). Thus, heparin and GAGs are interesting candidates for therapy of UC and other forms of IBD. If mast cells play a key role in the first step of this inflammation process like for the *H. pylori* induced type B gastritis should be further studied.

Early studies on enterotoxigenic *E. coli* (ETEC) infecting young pigs, calves and lambs revealed that galactose and sialic acid-specific lectin associated with fimbriae and fibrillar surface structures determine colonisation of the mucus layer and the intestinal epithelium (*Lindahl* et al., 1988; *Sun* et al., 2000). Interestingly, studies of ETEC causing diarrhoea in young children and adult travellers revealed that ETEC fimbriae-associated HA's or lectins, such as CFAI and CFAII (CFA, colonisation factor antigen) which recognise terminal sialic acid in $\alpha$-2,3, $\alpha$-2,6, and also $\alpha$-2,8 linkages (*Wadström* and *Trust*, 1984). More recent studies showed that *H. pylori* SAL also recognise $\alpha$-2,3 linkage (*Hirmo* et al., 1996). An experimental therapy based on polysialyllactose was shown to inhibit the gastritis of monkeys experimentally infected by *H. pylori* (*Mysore* et al., 1999). Promising studies have recently shown that sialic acid rich glycoconjugates from bovine milk inhibit *H. pylori* induced gastritis in a mouse model (*Wang* et al., 2001). However, since *H. pylori* also produces Lewis B blood group fucose specific lectin and GAGBP, an optimal therapy has to be designed (*Guruge* et al., 1998; *Hileman* et al., 1998).

## FUTURE PERSPECTIVES

With the rapidly increasing knowledge on microbial infections in the last two decades it is most encouraging to enhance development of an anti-adhesin therapy to combat *H. pylori* and other GI infections. A non-antibiotic approach seems attractive to eradicate or suppress *H. pylori* infection to avoid an overuse of antibiotics and antibiotic resistance development in *H. pylori* and in the indigenous oral and GI flora (*Breithaupt*, 1999). That HS and DS inhibit *H. pylori* infection in the gastric mucosa but have opposite effects on infections in the large intestine may indicate different modulating effect of members of the normal intestinal microflora (*Utt* and *Wadström*, 1997). Hence, future studies have to include an analysis on possible effects of these glycoconjugates on the normal GI microflora. Since a number of glycosialidases are produced by the complex GI microflora, studies on break-down of sialoglycoconjugates, GAGs and heparin-like molecules should be studied especially among strict GI anaerobes, such as various species of *Bacteroides* and *Eubacteria*, and among LAB and *Bifidobacteria* (*Ahn* et al., 1998; *Nader* et al., 1999).

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## LITERATURE

Ahn, M.Y., Shin, K.H., Kim, D.-H., Jung, E.-A., Toida, T., Linhardt, R. and Kim, Y.S.: Characterization of a *Bacteroides* species from human intestine that degrades glycosaminoglycans. Can. J. Microbiol. 44, 423-429 (1998).

Alderete, J.F. and Baseman, J.B.: Serum lipoprotein binding by *Treponema pallidum*: Possible role for proteoglycans. Genitourin. Med. 65, 177-182 (1989).

Aleljung, P., Shen, W., Rozalska, B., Hellman, U., Ljungh, Å., and Wadström, T.: Purification of collagen binding proteins of *Lactobacillus reuteri*. Curr.Microbiol. 28, 231-236 (1994).

Alvarez-Dominguez, C., Vazquez-Boland, J.-A., Carrasco-Marín, E., López-Mato, P., and Leyva-Cobian, F.: Host cell heparan sulphate proteoglycans mediate attachment and entry of *Listeria monocytogenes*, and the listerial surface protein ActA is involved in heparan sulphate receptor recognition. Infect. Immun. 65, 78-88 (1997).

Ascencio, F., Hansson, H.-A., Larm, O., and Wadström, T.: *Helicobacter pylori* interacts with heparin and heparin-dependent growth factors. FEMS Immunol. Med. Microbiol. 12, 265-272 (1995).

Ascencio, F., Liang, O.D., Fransson, L.-Å., and Wadström, T.: Affinity of the gastric pathogen *Helicobacter pylori* for the N-sulphated glycosaminoglycan heparan sulphate. J. Med. Microbiol. 38, 240-244 (1993a).

Ascencio, F., Liang, O.D., Schmidt, K.H., Fransson, L.-Å. and Wadström, T.: Comparative studies on heparin- and heparan sulphate-binding bacterial lectins. In: Lectins Textop, Hellerup. 235-242 (1993b).

Baird, A. and Klagsbrun, M.: The fibroblast growth factor family. NYAS, New York (1991).

Bergey, E.J. and Stinson, M.W.: Heparin-inhibitable basement membrane-binding protein of *Streptococcus pyogenes*. Infect. Immun. 56, 1715-1721 (1988).

Bober Barkalov, F.J. and Schwarzbauer, J.E.: Localization of the major heparin-binding site in fibronectin. J. Biol. Chem. 266, 7812-7818 (1991).

Boyd, A.P., Sory, M.-P., Iriarte, M., and Cornelis, G.R.: Heparin interferes with translocation of Yop proteins into HeLa cells and binds to LcrG, a regulatory component of the *Yersinia* Yop apparatus. Molec. Microbiol. 27, 425-436 (1998).

Breithaupt, H.: The new antibiotics. Nature Biotechnology 17, 1165-1170 (1999).

Calderone, R.A., Linehan, L., Wadsworth, E., and Sandberg, A.L.: Identification of C3d receptors on *Candida albicans*. Infect. Immun. 56, 252-258 (1988).

Carruthers, V.B., Håkansson, S., Giddings, O.K., and Sibley, L.D.: *Toxoplasma gondii* uses sulphated proteoglycans for substrate and host cell attachment. Infect. Immun. 68, 4005-4011 (2000).

Casu, B.: Protein-binding domains of heparin and other sulphated glycosaminoglycans. Carbohydrates in Europe 18-21 (1994).

Chen, T., Swanson, J., Wilson, J., and Belland, R.J.: Heparin protects Opa+ *Neisseria gonorrhoeae* from the bactericidal action of normal human serum. Infect. Immun. 63, 1790-1795 (1995).

Chen, Y., Maguire, T., Hileman, R.E., Fromm, J.R., Esko, J.D., Linhardt, R.J., and Marks, R.M.: Dengue virus infectivity depends on envelope protein binding to target cell heparan sulphate. Nature Medicine 3, 866-871(1997).

Chmiela, M., Paziak-Domanska, B., Ljungh, Å., Wadström, T., and Rudnicka, W.: The proliferation of human T-lymphocytes stimulated by *Helicobacter pylori* antigens. Immunobiology 195, 199-208 (1996).

Chmiela, M., Paziak-Domanska, B., Rudnicka, W., and Wadström, T.: The role of heparan sulphate-binding activity of *Helicobacter pylori* bacteria in their adhesion to murine macrophages. APMIS 103, 469-474 (1995a).

Chmiela, M., Paziak-Domanska, B., and Wadström, T.: Attachment, ingestion and intracellular killing of *Helicobacter pylori* by human peripheral blood mononuclear leukocytes and mouse peritoneal inflammatory macrophages. FEMS Immunol. Med. Microbiol. 10, 307-316 (1995b).

Compton, T.D., Nowlin, D.M.,, and Cooper, N.R.: Initiation of human cytomegalovirus infection requires initial interaction with cell surface heparan sulphate. Virology 193, 834-841(1993).

Conrad, H.E.: Heparin-bidning proteins. Academic Press, San Diego (1998).

Cornelis, G.R. and Wolf-Watz, H.; The *Yersinia* Yop virulon: A bacterial system for subverting eukaryotic cells. Molec. Microbiol. 23, 861-867 (1997).

Dobosz, M., Mionskowska, L., Dobrowolski, S., Dymecki, D., Makarewicz, W., Hrabowska, M., and Wajda, Z.: Is nitric oxide and heparin treatment justified in inflammatory bowel disease? An experimental study. Scand. J. Clin. Lab. Investig. 56, 657-663 (1996).

Dowd, C.J., Cooney, C.L., and Nugent, M.A.: Heparan sulphate mediates bFGF transport through basement membrane by diffusion with rapid reversible binding. J. Biol. Chem. 274, 5236-5244 (1999).

Duensing, T.D., Wing, J.S., and van Putten, J.P.M.: Sulphated polysaccharide-directed recruitment of mammalian host proteins: A novel strategy in microbial pathogenesis. Infect. Immun. 67, 4463-4468 (1999).

Feng, S., Hodzic, E., Stevenson, B., and Barthold, S.W.: Humoral immunity to *Borrelia burgdorferi* N40 decorin binding proteins during infection of laboratory mice. Infect. Immun. 66, 2827-2835 (1998).

Franklin, C.L., Beckwith, C.S., Livingston, R.S., Riley, L.K., Gibson, S.V., Besch-Williford, C.L., and Hook Jr., R.R.: Isolation of a novel *Helicobacter* species, *Helicobacter cholecystus* sp. nov., from the gallbladders of Syrian hamsters with cholangiofibrosis and centrilobar pancreatitis. J. Clin. Microbiol. 34, 2952-2958 (1996).

Gabius, H.-J. and Gabius, S.: Modulation lectins and glycobiology. Springer Verlag, Berlin, Heidelberg (1993).

Gaffney, P.R., Doyle, C.T., Gaffney, A., Hogan, J., Hayes, D.P., and Annis, P.: Paradoxical response to heparin in 10 patients with ulcerative colitis. Am. J. Gastroenterol. 90, 220-223 (1995).

Geuijen, C.A.W., Willems, R.J., Hoogerhout, P., Pujik, W.C., Meloen, R.H., and Mooi, F.R.: Identification and characterization of heparin binding regions of the Fim2 subunit of *Bordetella pertussis*. Infect. Immun. 66, 2256-2263 (1998).

Guo, B.P., Norris, S.J., Rosenberg, L.C., and Höök, M.: Adherence of *Borrelia burgdorferi* to the proteoglycan decorin. Infect. Immun. 63, 3467-3472 (1995).

Guruge, J.L., Falk, P.G., Lorenz, R.G., Dans, M., Wirth, H.-P., Blaser, M.J., Berg, D.E., and Gordon, J.I.: Epithelial attachment alters the outcome of *Helicobacter pylori* infection. Proc. Nat. Acad. Sci. USA 95, 3925-3930 (1998).

Gysin, J., Pouvelle, B., Fievet, N., Scherf, A., and Lépolard, C.: *Ex vivo* desequestration of *Plasmodium falciparum*-infected erythrocytes from human placenta by chondroitin sulphate A. Infect. Immun. 67, 6596-6602 (1999).

Hanson, M.S., Cassatt, D.R., Guo, B.P., Patel, N.K., McCarthy, M.P., Dorward, D.W., and Höök, M.: Active and passive immunity against *Borrelia burgdorferi* decorin binding protein A (DbpA) protects against infection. Infect. Immun. 66, 2143-2153 (1998).

Hayashi, M., Schlesinger, D.H., Kennedy, D.W., and Yamada, K.M.: Isolation and characterization of a heparin-binding domain of cellular fibronectin. J. Biol. Chem. 255, 10017-10020 (1980).

Heremans, A., van der Schueren, B., De Cock, B., Paulsson, M., Cassiman, J.-J., van den Berghe, H., and David, G.: Matrix-associated heparan sulphate proteoglycan: core protein-specific monoclonal antibodies decorate the pericellular matrix of connective tissue cells and the stromal side of basement membranes. J. Cell Biol. 109, 3199-3211 (1989).

Hileman, R.E., Fromm, J.R.W.J.M., and Linhardt, R.J.: Glycosaminoglycan-protein interactions: Definition of consensus sites in

glycosaminoglycan binding proteins. BioEssays 20, 156-167 (1998).

Hirmo, S., Kelm, S., Schauer, R., Nilsson, B., and Wadström, T.: Adhesion of *Helicobacter pylori* strains to _-2,3-linked sialic acids. Glycoconjugate J. 13, 1005-1011 (1996).

Hoffman, R.: Carrageenans inhibit growth-factor binding. Biochem. J. 289, 331-334. (1993).

Hollander, D. and Tytgat, G.N.J.: Sucralphate. From basic science to bedside. Plenum Medical Book Co., New York (1995).

Keller, J.M.: Specificity in heparin/heparan sulphate-protein interactions. Glycobiology 4, 1-4 (1994).

Keusch, G.T., Cerami, A., and Takaku, F.: Cytokines and metabolism. NYAS, New York (1990).

Kinsella, L., Chen, H.-L., Smith, J.A., Rudland, P.S., and Fernig, D.G.: Interactions of putative heparin-binding domains of basic fibroblast growth factor and its receptor, FGFR-1, with heparin using synthetic peptides. Glycoconjugate J. 15, 419-422. (1998).

Kirsner, J.B.: Inflammatory Bowel Disease. W.B. Saunders Co., Philadelphia (2000).

Klotz, S.A. and Smith, R.L.: Glycosaminoglycans inhibit *Candida albicans* adherence to extracellular matrix proteins. FEMS Microbiol.Lett. 99, 205-208 (1992).

Korzenik, J.R., Hsu, A., and Robert, M.E.: Effect of heparin on dextran sulphate sodium-induced colitis. Dig. Dis. Sci. 43, 1800-1805 (1998).

Kost, C., Stüber, W., Ehrlich, H.J., Pannekoeck, H., and Preissner, K.T.: Mapping of binding sites for heparin, plasminogen activator inhibitor-1, and plasminogen to vitronectin's heparin-binding region reveals a novel vitronectin-dependent feedback mechanism for the control of plasmin formation. J. Biol. Chem. 267, 12098-12105 (1992).

Krivan, H.C., Olson, L.D., Barile, M.F., Ginsburg, V., and Roberts, D.D.: Adhesion of *Mycoplasma pneumoniae* to sulphated glycolipids and inhibition by dextran sulphate. J. Biol. Chem. 264, 9283-9288 (1989).

Kulane, A., Ekre, H.-P., Perlmann, P., Rombo, L., Wahlgren, M., and Wahlin, B.: Effect of different fractions of heparin on *Plasmodium falciparum* merozoite invasion of red blood cells *in vitro*. Am. J. Trop. Med. Hyg. 48, 589-594 (1992).

Kusche, M., Hannesson, H.H., and Lindahl, U.: Biosynthesis of heparin. Biochem. J. 275, 151-158 (1991).

Kuschert, G.S.V., Coulin, F., Power, C.A., Proudfott, A.E.I., Hubbard, R.E., Hoogewerf, A.J., and Wells, T.N.C.: Glycosaminoglycans interact selectively with chemokines and modulate receptor binding and cellular responses. Biochem. 274, 5236-5244 (1999)

Lane, D.A. and Lindahl, U.: Heparin. Edward-Arnold, London (1989).

Laquerre, S., Argnani, R., Anderson, D.B., Zucchini, S., Manservigi, R., and Glorioso, J.C.: Heparan sulphate proteoglycan binding by *Herpes simplex* virus Type 1 glycoproteins B and C, which differ in their contributions to virus attachment, penetration, and cell-to-cell spread. J. Virol. 72, 6119-6130 (1998).

Liang, O.D., Ascencio, F., Fransson, L.-Å., and Wadström, T.: Binding of heparan sulphate to *Staphylococcus aureus*. Infect. Immun. 60, 899-906 (1992).

Lindahl, M., Brossmer, R., and Wadström, T.: Sialic acid and N-acetylgalactosamine specific bacterial lectins of enterotoxigenic *Escherichia coli* (ETEC). Adv. Exp. Med. Biol. 228, 123-152 (1988).

Linhardt, R.J., Ampofo, S.A., Fareed, J., Hoppensteadt, D., Mulliken, J.B., and Folkman, J.: Isolation and characterization of human heparin. Biochem. 31, 12441-12445 (1992).

Ljungh, Å.: Microbiological aspects of inflammatory bowel disease. In: Inflammatory bowel disease (Eds.: Järnerot, G., Lennard-Jones, J., and Truelove, S.). Corona Press, Malmö, 73-89 (1992).

Ljungh, Å.: *Helicobacter pylori* interactions with plasminogen. Methods 21, 151-157 (2000).

Ljungh, Å., Eriksson, M., Eriksson, Ö., Henter, J.-I., and Wadström, T.: Shiga-like toxin production and connective tisuue protein binding of *Escherichia coli* isolated from a patient with ulcerative colitis. Scand. J. Infect. Dis. 20, 443-446 (1988).

Ljungh, Å., Moran, A.P., and Wadström, T.: Interactions of bacterial adhesins with extracellular matrix and plasma proteins:

Pathogenic implications and therapeutic possibilities. FEMS Immunol. Med. Microbiol. 16, 117-126 (1996).

Ljungh, Å. and Wadström, T.: Subepithelial connective tissue protein binding *of Escherichia coli* isolated from patients with ulcerative colitis. In: Inflammatory bowel disease: Current status and future approach (Ed.: McDermott, R.P.). Elsevier Science Publ., Amsterdam, 571-575 (1988).

Ljungh, Å. and Wadström, T.: Binding of extracellular matrix proteins by microbes. In: Methods of Enzymology. Microbial adhesion (Eds.: Doyle, R.J. And Ofek, I.). Academic Press, New York, 501-514 (1995).

Love, D.C., Esko, J.D., and Moser, D.M.: A heparin-binding actin *on Leishmania amastigotes* which mediates adhesion to cellular proteoglycans. J. Cell. Biol. 123, 759-766 (1993).

Lundberg, F., Lea, T., and Ljungh, Å.: Vitronectin-binding staphylococci enhance surface-associated complement activation. Infect. Immun. 65, 897-902 (1997).

Lähteenmäki, K., Kuusela, P., and Korhonen, T.K.: Plasminogen activation in degradation and penetration of extracellular matrices and basement membranes by invasive bacteria. Methods 21, 125-132 (2000).

Menozzi, F.D., Mutombo, R., Renauld, G., Gantiez, C., Hannah, J.H., Leininger, E., Brennan, M.J., and Locht, C.: Heparin-inhibitable lectin activity of the filamentous hemagglutinin adhesin of *Bordetella pertussis*. Infect. Immun. 62, 769-778 (1994).

Menozzi, F.D., Rouse, J.H., Alavi, M., Laude-Sharp, M., Muller, J., Bischoff, R., Brennan, M.J., and Locht, C.: Identification of a heparin-binding hemagglutinin present in mycobacteria. J. Exp. Med. 184, 993-1001 (1996).

Mesri, E.A., Kreitman, R.J., Fu, Y.-M., Epstein, S.E., and Pastan, I.: Heparin-binding transforming growth factor _-*Pseudomonas* exotoxin A. J. Biol. Chem. 268, 4853-4862 (1993).

Murphy-Ullrich, J.E., Gurusiddappa, S., Frazier, W.A., and Höök, M.: Heparin-binding peptides fom thrombospondins 1 and 2 contain focal adhesion-labilizing activity. J. Biol. Chem. 268, 26784-26789 (1993).

Mysore, J.V., Wiggington, T., Simon, P.M., Zopf, D., Heman-Ackah, L.M., and Dubois, A.: Treatment of *Helicobacter pylori* infection in rhesus monkeys using a novel antiadhesion compound. Gastroenterol. 117, 1316-1325 (1999).

Nader, H.B., Kobayashi, E.Y., Chavante, S.F., Tersariol, I.L.S., Castro, R.A.B., Shinjo, S.K., Naggi, A., Torri, G., Casu, B., and Dietrich, C.P.: New insights on the specificity of heparin and heparan sulphate lyases from *Flavobacterium heparinum* revealed by the use of synthetic derivatives of K5 polysaccharide from *E.coli* and 2-O-desulphated heparin. Glycoconj. J. 16, 265-270 (1999).

Naef, M., Yokoyama, M., Friess, H., Buchler, M.W., and Korc, M.: O-expression of heparin-binding EGF-like growth factor and related peptides in human gastric carcinoma. Int. J. Cancer 66, 315-321 (1996).

Ortega-Barria, E. and Pereira, M.E.A.: A novel *T. cruzi* heparin-binding protein promotes fibroblast adhesion and penetration of engineered bacteria and trypanosomes into mammalian cells. Cell 67, 411-421 (1991).

Pantzar, M., Ljungh, Å., and Wadström, T.: Plasminogen binding and activation at the surface of *Helicobacter pylori* CCUG 17874. Infect. Immun. 66, 4976-4980 (1998).

Parks, W.C. and Mecham, R.P.: Matrix metalloproteinases. Academic Press, San Diego, London, Boston (1999).

Pascu, C., Hirmo, S., Ljungh, Å., and Wadström, T.: Inhibition of extracellular matrix and serum protein binding to *Staphylococcus aureus* by heparin and various polysulphated agents. Med. Microbiol. Lett. 4, 397-405 (1995).

Pouvelle, B., Meyer, P., Robert, C., Bardel, L., and Gysin J.: Chondroitin-4-sulphate impairs *in vitro* and *in vivo* cytoadherence of *Plasmodium falciparum* infected erythrocytes. Molecular Medicine 3, 508-518 (1997).

Prioli, R.P., Roenberg, I., and Pereira, M.E.A.: Specific inhibition of *Trypanosoma cruzi* neuraminidase by the human plasma glycoprotein "cruzin". Proc. Nat. Acad. Sci. USA 84, 3097-3101 (1987).

Pusztai, A. and Bardocz, S.: Lectins: Biomedical perspectives. Taylor and Francis, London (1995).

Reeder, J.C., Hodder, A.N., Beeson, J.G., and Brown, G.V.: Identification of glycosaminoglycan binding domains in *Plasmodium falciparum* erythrocyte membrane protein 1

of a chondroitin sulphate A-adherent parasite. Infect. Immun. 68, 3923-3926 (2000).

Rostand, K.S. and Esko, J.D.: Microbial adherence to an invasion through proteoglycans. Infect. Immun. 65, 1-8 (1997).

Ruiz-Bustos, E., Sierra-Beltran, A., Romero, M.J., Rodriguez-Jaramilo, C., and Ascencio, F.: Protection of Balb/c mice against experimental *Helicobacter pylori* infection by oral immunization with *H. pylori* heparan sulphate-binding protein coupled to cholera toxin B subunit. J. Med. Microbiol. 49, 535-542 (2000).

Schlessinger, J., Lax, I., and Lemmon, M.: Regulation of growth factor activation by proteoglycans: What is the role of the low affinity receptors? Cell 83, 357-360 (1995).

Stephens, R.S.: Molecular mimicry and *Chlamydia trachomatis* infection of eukaryotic cells. Trends Microbiol. 2, 99-101 (1994).

Sun, R., Anderson, T.J., Erickson, A.K., Nelson, E.A., and Francis, D.H.: Inhibition of adhesion of *Escherichia coli* K88ac fimbriae to its receptor, intestinal mucin-type glycoproteins, by a monoclonal antibody directed against a variable domain of the fimbria. Infect. Immun. 68, 3509-3515 (2000).

Szöke, I., Pascu, C., Nagy, E., Ljungh, Å., and Wadström, T.: Binding of extracellular matrix proteins to the surface of anaerobic bacteria. J. Med. Microbiol. 45, 338-343 (1996).

Templeton, T.J., Keister, D.B., Muratova, O., Procter, J.L., and Kaslow, D.C.: Adherence of erythrocytes during exflagellation of *Plasmodium falciparum* microgametes is dependent on erythrocyte surface sialic acid and glycophorins. J. Exp. Med. 187, 1599-1609 (1998).

Törkvist, L., Thorlacius, H.S.U., Bohman, L., Lapidus, A., Flood, L., Ågren, B., Raud, J., and Löfberg, R.: Low molecular weight heparin as adjuvant therapy in active ulcerative colitis. Aliment. Pharmacol. Ther. 13, 1323-1328 (1999).

Utt, M. and Wadström, T.: Identification of heparan sulphate binding surface proteins of *Helicobacter pylori*: Inhibition of heparan sulphate binding with sulphated carbohydrate polymers. J. Med. Microbiol. 46, 541-546 (1997).

van 't Wout, J., Burnette, W.N., Mar, V.L., Rozdzinski, E., Wright, S.D., and Tuomanen, E.J.: Role of carbohydrate recognition domains of pertussis toxin in adherence of *Bordetella pertussis* to human macrophages. Infect. Immun. 60, 3303-3308 (1992).

Virkola, R., Parkkinen, J., Hacker, J., and Korhonen, T.K.: Sialyloligosaccharide chains of laminin as an extracellular matrix target for S fimbriæ of *Escherichia coli*. Infect. Immun. 61, 4480-4484 (1993).

Wadström, T.: Sialic-acid specific bacterial lectins of enterotoxigenic *Escherichia coli* (ETEC) and *Campylobacter pylori*. In: Lectins (Eds.: Driessche, E., Franz, H., Beeckmans, S., Pfuller, U., Kallikorm, A., and Bog-Hansen, T.C.). Textop, Hellerup, 229-234 (1993).

Wadström, T. and Ljungh, Å.: Glycosaminoglycan binding microbial proteins in tissue adhesion and invasion: Key events in microbial pathogenicity. J. Med. Microbiol. 48, 1-11 (1999).

Wadström, T. and Trust, T.J.: Bacterial lectins. In: Medical microbiology IV (Eds.: Easmon, C.S.F. and Jeljaswicz, J.). Academic Press, London, 287-334 (1984).

Wang, X., Hirmo, S., Willén, R., and Wadström, T.: Inhibition of *Helicobacter pylori* infection by bovine milk glycoconjugates in a mouse model. J. Med. Microbiol. 50, 430-435 (2001).

Yahi, N., Sabatier, J.-M., Nickel, P., Mabrouk, K., Gonzalez-Scarano, F., and Fantini, J.: Suramin inhibits binding of the V3 region of HIV-1 envelope glycoprotein gp120 to galactosylceramide, the receptor for HIV-1 gp120 on human colon epithelial cells. J. Biol. Chem. 269, 24349-24353 (1994).

Zou, S., Magura, C.E., and Hurley, W.L.: Heparin-binding properties of lactoferrin and lysozyme. Comp. Biochem. Physiol. 103B, 889-895 (1992).