Role of Glycosaminoglycans in Cellular Communication

ROBERT J. LINHARDT*,† AND TOSHIHIKO TOIDA‡

Departments of Chemistry and Chemical Biology, Biology, and Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York 12210, and Graduate School of Pharmaceutical Sciences, Chiba University, Japan

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ABSTRACT

Glycosaminoglycans are of critical importance in intercellular communication in organisms. This ubiquitous class of linear polyanions interacts with a wide variety of proteins, including growth factors and chemokines, which regulate important physiological processes. The presence of glycosaminoglycans on cell membranes and in the extracellular matrix also has resulted in their exploitation by infectious pathogens to gain access and entry into animal cells. This Account examines the structural and physical characteristics of these molecules responsible for their interaction with proteins important in cell−cell communication.

Introduction

This Account focuses on the role of glycosaminoglycans (GAGs) in cellular communication important in the physiology and pathophysiology of multicellular organisms. Glycosaminoglycans are linear, highly charged, acidic polysaccharides commonly found linked to core proteins of glycoconjugates called proteoglycans (PGs).[1] There are several major classes of GAGs, including heparan sulfate (HS)/heparin, chondroitin sulfate/dermatan sulfate, hyaluronan, and keratan sulfate families (Figure 1). GAGs covalently linked to core proteins (PGs) reside on the membrane of cells (i.e., syndecan, decorin, glypican) or within the extracellular matrix (ECM). These PGs form a canopy of negative charge on the upper layer the glycocalyx, which coats virtually all animal cells and acts as the glue holding together the ECM. Because of their extracellular location and conserved structure across virtually all animal species, they appear to perform a vital role in cell signaling and cell−cell communication. Heparin is a specialized HSPG[1] uniquely found within the granules of certain cells and linked to the core protein serglycin.[1,2] Because heparin is released on degranulation in an allergic response, it appears to have a unique and possibly defensive role in response to infectious and parasitic disease. Hyaluronan, a second unique, high molecular weight, viscose GAG, orders water in the extracellular environment giving both structure and flexibility to tissue.

PG biosynthesis begins with the assembly of core protein in the endoplasmic reticulum. Saccharides are added through the action of glycosyl transferases in the Golgi and are subsequently modified through the action of deacetylases, epimerases, and sulfo-transferases.[4] Multiple isoforms of these enzymes are differentially expressed in a temporal and spatial fashion.[5] The control of this biosynthetic process is not well understood, but the result is the appropriate synthesis of unique and specific saccharide sequences capable of a variety of biological functions. As with other carbohydrates, the role of GAGs is primarily mediated through their interactions with proteins.[6]

Biochemical Cascades

Intracellular cascades (such as signal transduction) primarily rely on protein−protein interactions. Multicellular organisms, however, must regulate physiological and pathophysiological processes intercellularly. Moreover, higher animals must regulate biochemistry in a dynamic fluid-filled cardiovascular system. Here, shear forces and flow favors weak, multivalent, fast on-rate binding associated with protein−carbohydrate interactions, as opposed to the strong, monovalent, slow on-rate binding characterizing protein−protein interactions.[7] Two important extracellular biochemical cascades are regulated by GAGs, the coagulation[8] and complement[9] cascades.

Coagulation Cascade. The regulation of hemostasis involves both cell- and plasma-based processes. Cell-based regulation begins at the endothelial surface that is lined with HSPG containing a specific saccharide sequence capable of preventing blood from clotting at the uninjured endothelial surface. Platelets, also involved in blood coagulation, form aggregates at the site of an injury and release procoagulants neutralizing the anticoagulant activity of HS.[10] The plasma-based blood coagulation cascade is regulated by endogenous anticoagulant HS or exogenously administered heparin.

The coagulation cascade (Figure 2a) is activated by intrinsic factors within the blood or extrinsic factors coming from tissues at the site of injury.[8] Once activated, the extrinsic and intrinsic systems cascade through the action of serine proteases on their inactive apoenzyme substrates until the pathways converge with their activation of apoenzyme factor X to serine protease factor Xa. Factor Xa then activates apoenzyme prothrombin (factor II) to serine protease thrombin (factor IIa). Thrombin acts on fibrinogen to convert it into insoluble fibrin that then forms a clot. This cascade is highly regulated by the endogenous HS found on the lumen of the endothelium

Robert J. Linhardt obtained his Ph.D. in Organic Chemistry from the Johns Hopkins University and did his postdoctoral training at MIT. On the faculty of the University of Iowa for 21 years and since joining RPI in 2003, he has worked on glycosaminoglycans. He is the recipient of the 1999 Volwiler Award in Pharmaceutical Research and the 2003 Hudson Award from the American Chemical Society.

Toshihiko Toida obtained his Ph.D. in Analytical Chemistry from Chiba University in 1983. In 1989, he was appointed Associate Professor in Chiba University, Faculty of Pharmaceutical Sciences, becoming a Professor in 2003. He was on sabbatical in Professor Linhardt's laboratory in 1996. His research interests are focused on the structure and biological function of sulfated polysaccharides from the natural products.

* Corresponding author. E-mail: linhar@rpi.edu.
† Rensselaer Polytechnic Institute.
‡ Chiba University.

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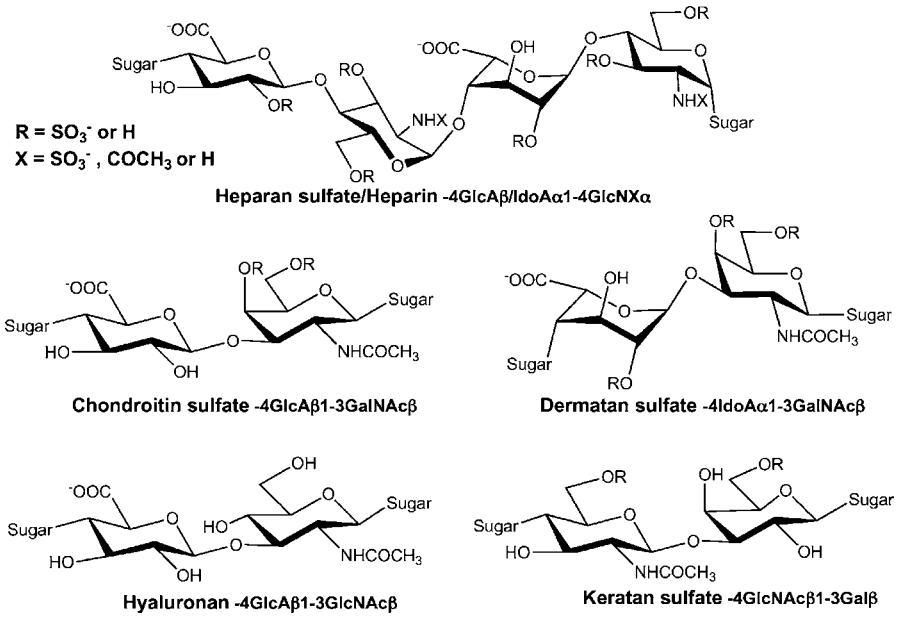

FIGURE 1. Structure of GAGs.

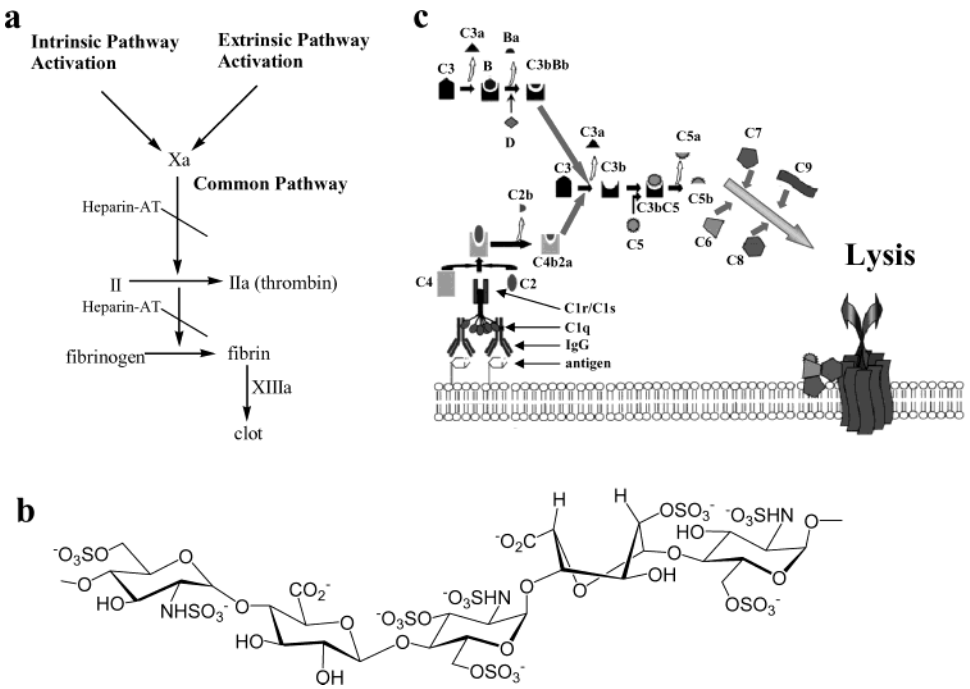

FIGURE 2. Biochemical cascades: (a) coagulation cascade activation of serine proteases leading to a clot; (b) AT-binding pentasaccharide sequence; (c) complement cascade activation of complement esterases (C) leading to terminal attack complex and cell lysis.

as well as the exogenously administered drug heparin. These GAGs bind to the plasma protein antithrombin (AT) through a specific pentasaccharide sequence (Figure 2b) resulting in the extrusion of a loop in AT, making it an excellent substrate and a potent mechanism-based inhibitor of thrombin and several other serine proteases in the coagulation cascade.

Based on this biochemistry, heparin has been widely used as an intravenously administered anticoagulant.[1] About a decade ago, low molecular weight heparins (LMWHs), selectively inhibiting factor Xa to a greater extent than thrombin, were introduced. This is because factor Xa can bind directly to the AT−heparin pentasaccharide but thrombin binds to heparin adjacent to bound AT and thus requires an additional extended heparin sequence to form this ternary complex. The rationale for introducing LMWHs was that their selectivity for factor Xa was believed to result in a reduction of hemorrhagic complications. The success of LMWHs, however, is primarily attributed to their subcutaneous bioavailability resulting in a decrease in cost to administer.

The regulation of the coagulation cascade relies on HS−protein interactions. The site of these interactions is spatially restricted to the undamaged endothelium. The rates of carbohydrate−protein interactions are very fast making these effective in a dynamic environment. HS

anticoagulant activity can be controlled at the biosynthetic level by failure to insert the AT pentasaccharide sequence and can be rapidly neutralized by the cellular release of procoagulant proteins, that is platelet factor 4.

Complement Cascade. The complement cascade (Figure 2c) is a plasma-based system consisting of two modes of activation, the classical (activated by an immune complex Ab·Ag) and alternative pathways.[9] As in the coagulation cascade, complement proteins can be activated by cleavage with both pathways converging to a common pathway with the cleavage of C3. Ultimately, complement activation results in formation of an attack complex able to lyse cells, providing an important defense against pathogens. Endogenous HS regulates this cascade at a number of sites in a number of ways. As in the coagulation pathway, HS/heparin is able to bind to C1INH and inhibit C1 activity in the classical pathway. In the alternative pathway, HS/heparin interaction with factor B can prevent hemolysis, such as that caused by cobra venom factor.[11] HS/heparin also prevents common pathway assembly of the terminal complex involved in cell lysis.

Complement activation is important in infectious disease but can pose problems in the form of autoimmune diseases[12] and in extracorporeal therapy where complement is inappropriately or continuously activated. Thus, the application of exogenous GAG represents a potentially important avenue of therapeutic intervention.

Cell Adhesion

Interaction of ECM Components. ECM resides between different cell types as both a barrier and a scaffold on which tissues are built. ECM is composed of GAGs and proteins with which they interact. These proteins include adhesion proteins, fibronectin, vitronectin, laminin, tenascin, and collagen. The GAGs found in ECM include chondroitin/dermatan sulfates, HS, and hyaluronan. Cellular adhesion is particularly important for anchorage-dependent cells. Adhesion proteins, such as fibronectin in the ECM, can interact through its heparin-binding domain with the HS of cell membrane PGs, such as syndecan, promoting initial adhesion and acting synergistically with internal membrane proteins such as integrins that bind to the RGD sequences in fibronectin.

In adhesion−anchorage processes, the initial binding usually involves the fast on-rate, low-affinity protein−GAG interaction, which is then followed by the slow on-rate, high-affinity protein−protein interaction. A classic example of the role of carbohydrates in the initial events of cell adhesion is in neutrophil extravascularization.

Neutrophils circulating in the bloodstream initially interact with the endothelium through the weak multivalent carbohydrate−protein interaction, resulting in rolling along the endothelium (Figure 3). This interaction is the result of selectin and its carbohydrate ligand, either sLe^x or HS.[13] When the rolling neutrophil stops, it anchors to the endothelium through integrin-based, strong protein−protein interactions. The anchored neutrophil is now in a position to follow a chemotactic signal through the endothelium to the underlying tissue site of inflammation.

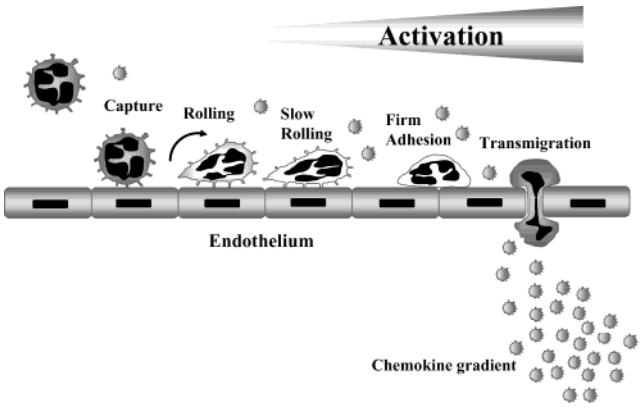

FIGURE 3. Neutrophil migration and extravascularization following a cheomkine gradient.

The sites within adhesion proteins at which specific GAG sequences bind are the subject of intensive investigation.[14] Interference or promotion of such interactions may offer important therapeutic opportunities. The complex interplay between protein−GAG and protein−protein interactions in cell adhesion requires further investigation.

Chemokine Signaling

Chemokine/Cytokine Function. Chemokines are chemotactic cytokines and are divided into multiple structural families based on their conserved cysteine-containing motifs.[15] Some chemokines, for example, are released at the site or an injury, infection, or inflammation resulting in a concentration gradient. As chemokines diffuse outward from their origin, they can interact with protein-based chemokine receptors on neighboring cells signaling a response, such as cell movement toward the origin (Figure 3). The well-studied role of chemokines in neutrophil migration from the blood to a tissue site of inflammation exemplifies this process.[16]

Many chemokines interact with GAGs in the ECM or on cell membranes.[17,18] These interactions spatially fix the soluble chemokine gradient giving it a larger duration of chemotactic signaling.[19] The fixed chemokine gradient might be influenced by the extracellular distribution of GAGs and specific chemokine binding domains within GAGs.[20] GAG and chemokine catabolism might be linked. The presence of GAG binding proteins (either endogenous proteins or microbial proteins) in the ECM might occupy chemokine GAG-binding sites or displace chemokines, reducing their ability to effectively signal.

Inflammation/Immunity. The roles of GAGs in inflammation and immunity are often linked to chemokines, suggesting the possibility of therapeutic intervention. Recently, the widespread application of chondroitin as a nutraceutical has been driven by its touted antiinflammatory and antiarthritic properties.[21] Chondroitin upregulates the antigen-specific Th1 immune response in sensitized splenocytes, suppressing antigen-specific (IgE) allergic responses. This response is the result of secretion of Th1 chemokines, including interleukin (IL)-2 and IL-

12, and suppression of Th2 chemokines, including IL-5 and IL-10. The promotion of Th1 and inhibition of Th2 splenocyte activity is due to the interaction of a specific disulfated chondroitin disaccharide sequence with L-selectin on the T cell.[22]

HS displays a multifactoral affect on the immune response.[23] In the early events, HS is involved in the complement cascade and the containment and walling off of infection through interaction with complement and platelets. In the intermediate stage, the role of HS in inflammation involves the regulation of the formation of chemotactic complement proteins triggering neutrophils to respond to chemokines and release proteases and oxidants and endothelial expression of P-selectin promoting neutrophil adhesion. This proinflammatory endothelial response results in its production of chemokines, including IL-8, major capsid protein (MCP)-1, and regulated on activation normal T cell expressed and secreted (RANTES), resulting in the influx of neutrophils to the site of injury. In the final (adaptive) stages of the immune response, HS and complement activate antigen-presenting cells activating macrophages and leading to enhanced expression of major histocompatibility complex (MHC) class II, CD54, and CD86 proteins.

One of the most important roles of HS in the immune response is in the regional containment of infection through regulation of thrombosis, platelet function, complement activation, and chemokines. Thus, the location, distribution, concentration, and precise chemical structure of GAGs within tissues remains an important factor in controlling the immune response.

Signal Transduction

Cell−cell communication takes place in the extracellular environment when a secreted chemical from a signaling cell interacts with a receptor on the membrane of a second (signal receiving) cell. This membrane receptor on the second cell acts as an antennae transducing the signal, moving the signal (not the signaling molecule) across the membrane into the cell. The membrane-bound protein receptor often makes use of a GAG to assemble an active signaling complex.

FGF Signal Transduction. Fibroblast growth factor (FGF) and the tyrosine kinase integral membrane protein FGF receptor (FGFR) rely on a HSPG coreceptor to transduce the signal across the membrane (Figure 4). An ordered assembly[24] of a $FGF_2/FGFR_2/HSPG_2$ complex[25] tranduces a signal for gene transcription.

There are 23 members of the FGF family with four FGFR subtypes. The interaction of both FGF and FGFR subtypes with HS is also dependent on its saccharide sequence. Furthermore, in this complex[25] the nonreducing terminus of HS is involved, requiring the appropriate display of a suitable sequence.[26] These multiple signaling components can transduce many different types of signals resulting in a wide array of cellular responses. There also

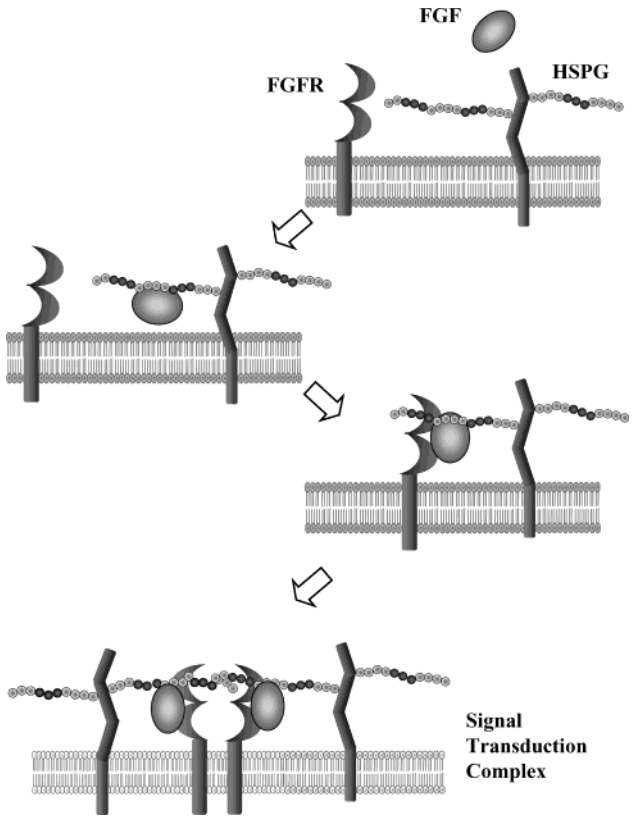

FIGURE 4. Assembly of a $FGF_2/FGFR_2/HSPG_2$ signal transduction complex on the outer surface of a cell signaling cell division.

appears to be temporal control of the activation of this pathway important in dictating the nature of the cellular response.[27]

One therapeutic intervention in signal transduction would be the stable formation of an incomplete (non-signaling) complex to block cell replication for the treatment of cancer.[28,29]

Insulin Signaling. Obesity and diabetes is a growing health problem in the developing world. Insulin is responsible for regulating the cellular uptake of glucose and its regulation is important in the pathophysiology of this disease. A number of insulin binding proteins also bind HS and regulate the interaction of insulin with its protein-based cellular receptors.[30] HSPG is also linked to human obesity in Simpson−Golabi−Behmel overgrowth syndrome[31] and excessive weight gain in genetically engineered mice.[32]

Knowledge of the role of GAGs in insulin signaling will be important in treating diabetes and understanding obesity. Small molecule based up-regulation and down-regulation of this signaling might have therapeutic importance in the treatment of insulin-related diseases.

Development. During development cells replicate and differentiate forming a variety of tissues that eventually comprise the multiple organ systems of an animal at birth. The process of cell replication is controlled by heparin-binding growth factors. Furthermore, morphogens, such as chemokines, carry information (or signals) to specific sites in a developing embryo that determine the dif-

ferentiation that the cells receiving this information will undergo. While morphogen gradients arise by diffusion, PGs appear to exert an additional level of control on gradient formation and gradient stability.[20]

Much of what is known of animal development is learned from organisms defective in components essential in embryogenesis. Mutants with genetic defects in GAG biosynthesis can be arrested in the early stages of development, born dead, born with severe (or mild) phenotypes or display no obvious phenotype. The sequencing of the human and mouse genomes, the identification of all the enzymes involved in GAG biosynthesis and the successful production of various knock-out mice have led to an improved but incomplete understanding of the importance of GAGs in developmental biology. Removal of chondroitin from the worm *Caenorhabditis elegans,* arrests embryogenesis preventing the transition from four cells to eight cells.[33] Removal of a single isoform of *N*-deacetylase/*N*-sulfotransferase results in lethality in mice at birth.[34] A failure of syndecan for a brief time in embryogenesis results in the absence of digits in the limbs of mice. Surprisingly, however, the knockout mice missing the isoform of 3-*O*-ST, critical in the biosynthesis of the AT-binding pentasaccharide sequence, show no obvious coagulation abnormalities. A redundancy of isoforms of the GAG biosynthetic enzymes involved in critical functions such as blood coagulation may protect against the catastrophic loss of these individual genes.[35] Moreover, compensatory mechanisms, in which different GAGs or sequences within a class of GAGs can substitute for others, may also be an evolutionary protection. Redundancy in GAG binding partners, such as a multiplicity of FGFs/FGFRs, may also play a protective role in defective GAG biosynthesis.

The application of intermediate- and high-through-put screening methods for protein−GAG interactions such as glycan chips offers new ways to diagnose, analyze, and identify genetic diseases relating to GAG structure and might result in improved approaches for their prevention, treatment, or both.

Cancer. There are many different types of cancer, but each contains similar elements of uncontrolled cell replication, abnormal differentiation, inappropriate cell migration leading to metastasis, and angiogenesis. GAGs play a role in each of these processes. Growth factors control cell replication and cell differentiation through the signal transduction pathway. In cells with aberrant differentiation, neonatal carbohydrate antigens, including GAGs, can be produced.[36] Cell migration involves chemokine signaling and cancer cell movement through the GAG-rich ECM. Indeed, many metastatic tumor cells produce heparanase, which acts to break down HS in the ECM facilitating their migration.[37] Metastatic tumor cells use the same selectin-mediated pathway as neutrophils (Figure 3) to enter and exit the vasculature in establishing a secondary tumor. In angiogenesis, tumor cells release chemokines that recruit blood vessels from the surrounding tissues and utilize neovascularization to provide oxygen necessary for rapid tumor growth.[38]

GAGs have a number of roles in carcinogenesis and their derivatives or antagonists might be used to interfere with processes such as the assembly of signal transduction complex, blocking cell replication, cell differentiation or both.[36,39] GAGs with abnormal structure may serve as markers for cancer and might be useful in diagnosis or evaluation of metastatic forms of cancer.[40] GAGs can be used to modify chemokine signaling.[41] Heparanase inhibitors can inhibit the ability of tumor cells to migrate to secondary sites.[42] GAG-derived oligosaccharides have also demonstrated antiangiogenic activity.[43,44] Finally, a retrospective study of a large number of patients treated for cardiovascular diseases with LMW heparins demonstrated a decrease in cancer deaths.[45] A major reason for heparin's chemoprotective effect may be through blocking of platelets that cloak circulating metastatic cancer cells by interfering with selectin-mediated interaction between platelets and neonatal mucin carbohydrates expressed by the cancer cells.[46]

Wound Healing and Repair Processes. Many of the pathophysiological processes assisting the growth and spread of cancers are physiological processes, considered in a positive light in wound healing. In wound healing, tissue regrowth is required, necessitating enhanced cell replication, cell migration, neovascularization,[47] and cell differentiation. Wounds in a fetus or newborn, expressing neonatal carbohydrate antigens, heal faster than those in adult animals in the absence of scarring, and GAG content and structure appears to play a role.[48]

GAGs, such as heparin, have been used in the treatment of wounds including ulcers, stroke, diseased heart muscle, and atherogenesis.[47] Despite a fear of bleeding associated with the use of heparin in wound healing applications, it appears that the anticoagulant activity is separable from wound healing activity. Furthermore, wound fluid is particularly rich in HS oligosaccharides, suggesting that these molecules are an integral part of the normal physiological process of wound healing.[47]

Pathogen Recognition

Since pathogens coevolved with their hosts, they developed a means to subvert the use of host extracellular receptors for infection.[49,50] GAGs are among the most prominent of these receptors acting like a canopy covering the glycocalyx of animal cells. The low-affinity, multivalent, fast on-rate binding associated with protein−carbohydrate interactions is ideally suited to the binding of cell−cell, cell−virus, cell−bacteria, and cell−parasite in a dynamic environment. Moreover, host proteins that recognize and bind to GAGs, such as growth factors and chemokines, are acquired and incorporated into the genome of infectious pathogens because they provide an advantage of allowing the infectious agent to bind to the host cell and gain access to its surface proteins that can act as receptors facilitating infection.

In addition to acquiring host proteins to localize and infect animal cells, microbial pathogens also subvert GAG-mediated processes, including chemokine signaling.[51]

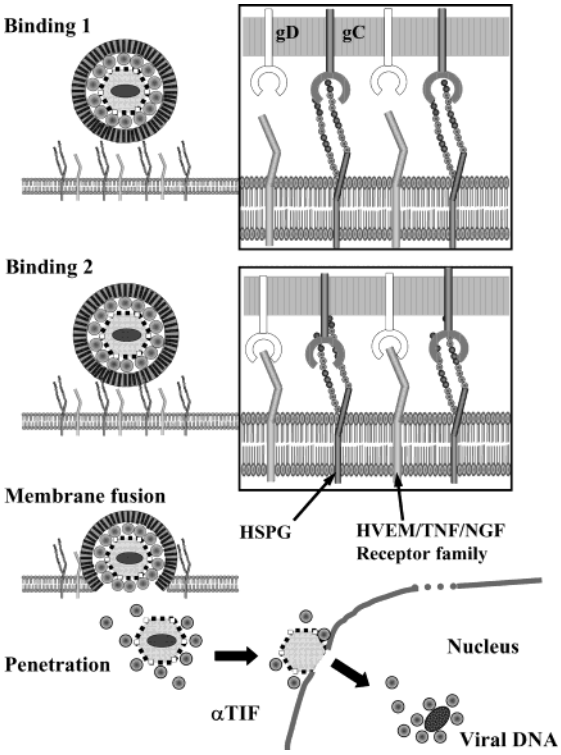

FIGURE 5. Binding of HSV surface glycoprotein gC to target cell HSPG (binding 1) followed by gD and gC to both protein and HSPG receptors (binding 2) resulting in membrane fusion, penetration, and infection.

Microbes do this by producing proteins with chemokine or chemokine receptor activity, altering host expression of these proteins, producing GAGs or analogues, or producing enzymes capable of degrading chemokines or chemokine receptors of GAGs. These microbial strategies act to dysregulate neutrophil chemotaxis (Figure 3) circumventing host response and helping the microbe to evade the host inflammatory response and immune system.

Viruses. Viruses are obligate intracellular parasites that take over the machinery of their host cell acquiring genes that give advantage in infection and replication. It is clear that GAGs play a role in infection by herpes simplex virus (HSV) and human immunodeficiency virus (HIV) based on the inhibitory effect of heparin and synthetic polyanions on viral infection.[52,53] Heparin-binding motifs in animal proteins, such as growth factors,[54] are also found in viral envelope proteins, suggesting that virus infection might be linked to viral binding to sites on HSPGs involved in growth factor signaling. Furthermore, a study of the specific sequences in HS that bound growth factors and the tissue/organ distribution of HS with these sequences have helped explain the tropism of viral diseases including Dengue[55] virus and hepatitis C virus (HPCV).[56] A highly sulfated decasaccharide sequence binding Dengue envelop protein was effectively mimicked with a currently used polyanionic drug, suramin, suggesting a new antiviral application for this agent.[57,58] In the future, chemokines might also be examined to determine whether viruses are mimicking their heparin-binding domains.

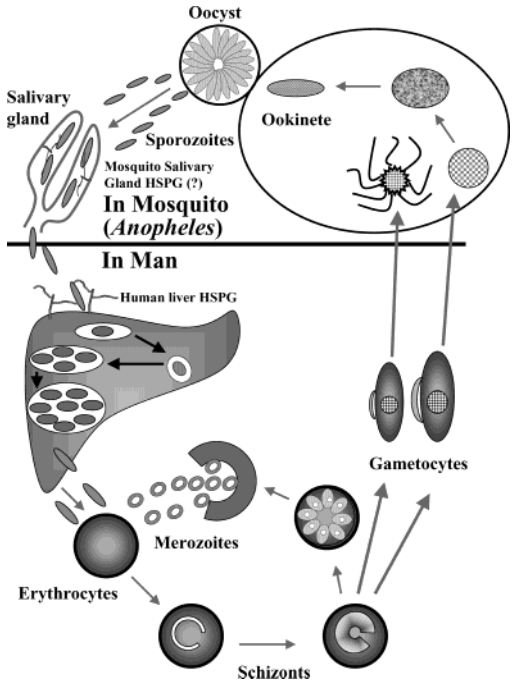

FIGURE 6. Life cycle of the malaria parasite being transmitted from mosquito to man; transfer of circumsporozoite from a putative HSPG in the mosquito salivary gland to HSPG receptor in human liver.

Recent studies on HSV suggest that a specific isoform of 3-*O*-sulfotransferase is capable of introducing a sulfo group critical for both binding and entry of HSV into its host cell[53,58] (Figure 5). Furthermore, the distribution of this isoform offers an explanation of the susceptibility of brain to HSV infection.

Bacteria. Bacteria express GAG-binding proteins that are important in adhesion and invasion, both critical elements in pathogenesis. Diverse bacteria including, *Bordetella pertussis*, *Borrelia burgdorferi*, *Chlamydia trachomatis*, *Helicobacter pylori*, *Listeria monocytogenes*, *Neisseria gonorrheae*, and *Streptococcus pyogenes* bind to HS on the surface of cells.[59] *Chlamydia* is a particularly unusual example because it is an obligate intercellular bacteria involved in a variety of diseases in man. The attachment of *Chlamydia* to host cell is mediated by GAG bridges between protein receptors on both bacteria and host cells and binding can be disrupted with heparin or HS.[50] HS mediates *Listeria* and *Neisseria* invasion of host cells.[60,61] The major surface-expressed virulence protein in *Streptococcus pyogenes*, M-protein, binds dermatan facilitating is adhesion and penetration through skin.[59] Bacteria also can produce enzymes capable of breaking down GAGs. *Proteus vulgaris*, for example, uses its broad-specificity chondroitin ABC lyase to penetrate the skin barrier to infect its human host.[59] Additional pathogenic bacteria need to be studied for their interaction with HS and other GAGs because the interference of this interaction might represent a potentially useful new antibiotic approach.

Parasites. Leshmania are intracellular protozoan parasites that cause a variety of illnesses in man. Heparin interferes with adhesion of these protozoans. *Trypanosoma cruzi*, the parasite that causes Chagas' disease,

multiplies in the gut of insects before they are transmitted to man. Their binding to human host cells is interfered with heparin. The most studied and certainly the most important parasitic infection is malaria, which kills more humans than any other infectious disease. *Plasmodium falciparum* sporozoites carried by the *Anopholes* mosquito are injected into humans where they rapidly infect the liver (Figure 6). Hepatocytes are invaded through the interaction of circumsporozoite protein with a highly sulfated liver HSPG. This HSPG is apparently the apoE receptor responsible for lipid metabolism in the liver.[62,63] Different *Plasmodium* species infect different animals (i.e., humans, nonhuman primates, rodent, avian, etc.). The liver HS of these species differ and may be partially responsible for this species specificity.[64] Insects are known to produce HS. The structure of HS from the midgut and salivary glands of *Anopholes* may be similar to that of human liver HS. Small sulfated molecules are potential agents for prevention or treatment of malaria infection in mosquito or human hosts.

Conclusions

GAGs are important in intercellular communication in animals. Their prominent extracellular location and their ubiquitous presence in all animals have ensured their importance in evolution. The high negative charge associated with GAGs facilitates their interaction with a large array of extracellular proteins.[6] The linear structures of GAGs restrict movement of bound proteins to one dimension in three-dimensional space, facilitating intercellular communication over these molecular wires.[7] The fast on-rates and multivalency of protein−GAG binding make these interactions particularly important in dynamic systems. GAGs also act to facilitate molecular encounters between proteins in the assembly of multicomponent complexes. An improved understanding of the role that GAGs play in cellular communications should facilitate the development of new therapeutic strategies for the treatment of a wide variety of disease states.

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