GUEST EDITORIAL

The Cell “Coat of Many Colors”

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This issue of *The American Journal of Pathology* features a series of review articles that highlight the glycocalyx, a carbohydrate-rich layer on the surface of plasma membranes of many mammalian cells. The term was coined by Bennett¹ in 1963, but first electron microscopy visualization of this structure was deferred to 1966, when Rambourg et al,² using methenamine-stained tissue sections, and Luft,³ using ruthenium red staining, codiscovered it. Glycocalyx performs diverse and vitally important functions, such as regulation of proliferation, differentiation, adhesion, and motility, among others. In endothelial cells, where glycocalyx is better studied, it is also responsible for the regulation of traffic of circulating proinflammatory cells, vascular permeability, mechanotransduction, and signaling to adjust vessel diameter to the blood flow rate, and to harbor distinct growth factors, regulators of coagulation, and extracellular antioxidant defense. Glycocalyx is continuously renewed via the balanced and rather intense processes of degradation and synthesis. In the course of diverse diseases, this balance is shifted toward degradation, leading to the net loss of glycocalyx. With it are lost or perturbed the many functions for which it is responsible. Depending on the disease, the rate with which the loss of glycocalyx occurs differs, and this may be of critical importance for the developing clinical sequelae and the therapeutics required for its restoration. Among diseases with precipitous degradation of glycocalyx and exigent requirement for its restitution are acute ischemia-reperfusion conditions, sepsis, surgical stress, burns, aortic clamping for cardiovascular bypass, and infectious diseases caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or anthrax, in which shedding of syndecans is induced by Las A (metalloproteinase), sphingomyelinase β -toxin, pore-forming α -toxin, or lethal toxin.⁴ This group may also include dengue fever and, possibly, but not proved, thrombotic microangiopathy and sickle cell crisis. The second large group of diseases is associated with the gradually accruing imbalance between synthesis and

degradation, leading to defective glycocalyx. It is represented by diabetes mellitus, chronic cardiovascular diseases, chronic kidney diseases, tumors, chronic inflammatory processes, and aging, to mention a few. Some of these conditions will be reviewed in depth by a group of experts.

Specifically, Cosgun et al⁵ open the series with an overview of chemical composition and nanomechanical properties of glycocalyx as a key to understanding its functional properties. This is followed by Butler and colleagues,⁶ who describe the regulation of glycocalyx permeability to macromolecules. Abassi and colleagues⁷ summarize data on the degradation of glycocalyx during ischemia-reperfusion injury. Dogné and Flamion⁸ focus on impaired hyaluronan metabolism in disease and its properties as a marker of glycocalyx degradation. This is followed by Wang et al,⁹ providing evidence and consequences of glycocalyx degradation in diabetes. Finally, Sun and Goligorsky¹⁰ shall argue that severity of sepsis is to a significant degree determined by the loss of endothelial glycocalyx.

Pondering on the degradation of glycocalyx, it is essential to grasp its mechanisms. On the basis of the composition of glycocalyx-proteoglycan core proteins (ie, single membrane-spanning syndecans 1-4 and glycosylphosphatidylinositol-anchored glypicans 1-6), as well as covalently attached to them through serine-glycine repeats, by the action of xylosyltransferases, glycosaminoglycans, such as heparan sulfate (HS), chondroitin sulfate, dermatan sulfate, as well as the intertwined within this bushy

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structure hyaluronic acid chains, it is obvious that the paths to their enzymatic degradation involve concerted actions of metalloproteinases, heparanase-1, and hyaluronidases. In the group of metalloproteinases, metzincins play a predominant role. These Zn^{2+} -containing endopeptidases belong to a large group of matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs) and ADAMs with a thrombospondin motif, and several bacterial proteases.¹¹ MMPs and ADAMs are particularly important in shedding proteoglycan core proteins, followed by glycocalyx degradation. They contain a propeptide sequence that shields the Zn -containing catalytic domain via interaction with a conserved cysteine residue and Zn^{2+} ion (the cysteine switch that maintains them in the form of inactive zymogen). Removal of the prodomain by furin or plasmin inactivates cysteine switch and converts MMPs to the active form. In addition, reactive oxygen species acting on cysteine disable cysteine switch mechanism and liberate the catalytic activity by leading to allosteric dislodgement of the prodomain. The preservation of the active conformation requires also activation (cell stress induced) of scramblases bidirectionally translocating phospholipids between the outer and inner leaflets of the plasma membrane and exposing phosphatidylserine residues at the outer leaflet. In the case of ADAM17, the negatively charged phosphatidylserine residue groups engage the membrane-proximal domain of ADAM, thus positioning the catalytic domain in apposition to its target, the syndecan-4, and rendering it a functional sheddase.¹² Shedding of syndecan 1, on the other hand, appears to be accomplished by the membrane-type MMP1. In addition, MMPs 9 and 13, regulated by the occupancy of the sphingosine-1-phosphate receptor by its cognate ligand sphingosine-1-phosphate, are also involved in syndecan-1 shedding.¹³ Notably, sphingosine-1-phosphate is attached to albumin, which electrostatically interacts with glycocalyx under normal conditions. It follows that when the electrostatic interaction is perturbed, either by dissipation of the negative charges on glycosaminoglycans or by depletion of albumin–sphingosine-1-phosphate, glycocalyx shedding is activated. It is possible that this mechanism is most relevant to the maintenance phase of glycocalyx degradation. All syndecans are cleaved within the 15–amino acid juxtamembrane region, thus explaining the requirement for sheddases to acquire the membrane-proximal conformation of the catalytic center. Shedding of ectodomains is followed by the intramembrane proteolysis by γ -secretase of the remaining fragment of syndecans. Intriguingly, this remaining fragment serves signaling functions, inhibiting migration, invasivity, and tumorigenicity of A549 lung cancer cells in mice.¹⁴ In contrast, the stromal ectodomain of syndecan 1, in the presence of HS and growth factors, can mediate proliferation of breast cancer cells.¹⁵ In leukocytes, the lack of syndecan-1 facilitates recruitment to inflammatory sites. In contrast, the lack of HS on endothelial cells interferes with leukocyte recruitment.¹⁶ This phenomenon is at hand

in sepsis, where ADAM17 inactivation improves leukocyte recruitment and bacterial clearance.^{17,18}

Heparanases, a focus of review by Abassi and colleagues,⁷ are a family of endo- β (1-4)-D-glucuronidases, with heparanase-1 being the unique mammalian enzyme that cleaves the glycoside bond in HS and HS-proteoglycans, thus yielding 5- to 10-kDa fragments. By degrading HS chains, heparanase-1 is involved in multiple functions, such as release of growth factors and cytokines bound to HS, interaction with heparanase-binding proteins and activating kinases, such as Src, Akt, and p38 mitogen-activated protein kinase, modulation of the activity of fibroblast growth factor-2 and transforming growth factor- β signaling pathways, and activation of fibroblast growth factor-2 signaling.¹⁹ In this manner, heparanase may be involved in tissue fibrosis. Regarding glomerular protein passage, HS and heparanase, according to some accounts, do not appear to play a dominant role,²⁰ but removal of HS prevents neuraminidase-induced albuminuria. Heparanase knockout mice exhibit resistance to sepsis-induced degradation of pulmonary endothelial glycocalyx and endothelial hyperpermeability.²¹ On the other hand, transgenic mice overexpressing heparanase exhibit reduced leukocyte crawling, adhesion, and infiltration of inflamed sites, reportedly due to the defective ability of truncated HS chains in glycocalyx to ligate chemokines, as well as to the resultant increase in heparin-resembling HS fragments endowed with anti-inflammatory properties.²²

Hyaluronidase involvement in regulating structure and function of endothelial glycocalyx has been revealed by Dogné et al²³ and discussed by Dogné and Flamion⁸ in this issue. Their investigative group has demonstrated that hyaluronidase 1 knockout prevents diabetes-induced glomerular endothelial barrier dysfunction, increases glycocalyx thickness, and maintains its structure, all otherwise impaired in wild-type mice with streptozotocin-induced diabetes. Remarkably, hyaluronidase 2–deficient mice develop chronic anemia and thrombocytopenia, 10-fold increased level of circulating hyaluronan, endothelial damage, and microvascular fibrin deposition, all features of chronic thrombotic microangiopathy with hemolytic anemia. Notably, the products of hyaluronidase activity—low-molecular-weight fragments of hyaluronic acid chains are cognate ligands of the toll-like receptors, thus capable of inducing systemic inflammatory response.²⁴

The interactome of HS, the spectrum of HS-binding proteins, is broad, including >300 interacting partners,²⁵ such as chemokines and cytokines, growth factors and morphogens, coagulation factors, components of complement, extracellular matrix proteins, signaling receptors, cell adhesion proteins, lipid-binding and amyloid proteins, and proteins expressed by pathogenic organisms to facilitate cell invasion. Hence, serving as a platform for protein-protein interaction, HS chains facilitate oligomerization and alter activities of these proteins. HS ability to interact with integrins is essential for orchestrating cell adhesion and

motility, whereas HS interaction with fibroblast growth factors and their cognate receptors facilitates the presentation of these growth factors to and activation of the receptor, thus regulating cell cycle.²⁶

While shedding of glycocalyx occurs constitutively, diverse noxious stimuli lead to the burst of shedding and glycocalyx degradation. These are triggered by cell stress, chemokines, bacterial virulence factors, growth factors, trypsin, and bacterial toxin, as discussed above.^{4,15,27,28} Not surprisingly, shedding of glycocalyx reduces cell-matrix adhesion and increases cell motility.²⁶ Shed ectodomains compete with the intact core proteins and their cell-specific glycosaminoglycans for growth factors, cytokines, and extracellular matrix proteins, and thereby modulate an array of cell functions. Of note, degradation of glycocalyx can be induced by physical forces, as has been shown for the interaction between endothelial cells and erythrocytes, either one with degraded glycocalyx, which leaves fingerprints on the glycocalyx of the second partner.²⁹ This would imply that any circulating formed element that has lost its lubricating glycocalyx surface can adversely affect endothelial glycocalyx and vice versa. The profound differences between membrane-bound and shed ectodomains of syndecan-1 have been emphasized by Nikolova et al³⁰ by contrasting MCF-7 breast cancer cells expressing constitutively membrane-bound or constitutively shed syndecan-1. Investigators showed that the membrane-bound syndecan-1 inhibited invasiveness, whereas constitutively shed syndecan-1 promoted cell invasiveness. Microarray analysis revealed that the soluble syndecan-1 repressed tissue inhibitor of metalloproteinase 1 (TIMP-1), furin, and urokinase-type plasminogen activator receptor genes, further implicating syndecan ectodomains in modifying cellular phenotype.

There exists a remarkable cross talk between various components of glycocalyx and glycocalyx-degrading enzymes. For instance, heparan sulfate proteoglycans have been shown to activate pro-MMP-7 by binding the polyanionic heparin to the basic residues of the zymogen, thus leading to the allosteric activation of proteolytic function.³¹ Syndecan-1 has been found to be critical for heparanase internalization and endosomal-lysosomal processing, leading to its activation.³² Specifically, conserved domain 2 (with interacting syntein) and variable regions of the cytoplasmic tail, as well as α -actinin, are involved in the process. In turn, heparanase modifies syndecan for exosome production.³³ Collectively, interactions between syndecan, heparanase, and syntein establish a pathway for exosomal production, which is particularly important for tumor progression. Heparan sulfate has been shown to regulate shedding of syndecans: heparin lyase-induced removal of HS chains results in increased levels of syndecan-1 in the conditioned medium³⁴; and so does elevated heparanase activity. Furthermore, syndecan oligomerization can be modified by heparan and chondroitin sulfates,²⁶ whereas HS removal accelerates shedding of syndecans.

Circulating fragments of glycocalyx are emerging as diagnostic and prognostic tools. The risk of sepsis after

trauma has been examined in the framework of the Pragmatic, Randomized Optimal Platelet and Plasma Ratios trial, and it was shown that elevated levels of syndecan-1 at 4 hours after admission were significantly associated with an increased likelihood of developing sepsis.³⁵ In patients with sepsis, circulating levels of syndecan-1 predicted development of disseminated intravascular coagulation.³⁶ It is also a predictor of respiratory failure and survival in septic patients.^{37,38} In patients with multiple myeloma, circulating levels of syndecan-1 ectodomain are indicative of tumor burden and have prognostic value.³⁹ Furthermore, in patients with chronic kidney disease, the circulating components of glycocalyx, hyaluronic acid, and syndecan-1 have been found to correlate with the markers of endothelial dysfunction, such as angiopoietin-2, von Willebrand factor, soluble vascular cell adhesion molecule-1, and soluble Fms-related tyrosine kinase 1 (Flt-1).⁴⁰ This is in accord with the concept of a vicious circle between the state of endothelial dysfunction and glycocalyx degradation.⁴¹ In 135 donors and 50 recipients of lung transplants, elevated levels of hyaluronic acid fragments in donors predicted acceptability of the graft, whereas high syndecan-1 blood levels in donors or in recipients were associated with primary graft dysfunction.⁴²

These general principles underlie the search for therapeutic interventions to prevent degradation and/or restore glycocalyx, as well as reduce the level of shed components of glycocalyx, some of which are endowed with diverse unwarranted functions. The detailed analysis of efforts to prevent degradation and restore glycocalyx integrity is discussed in depth by the constellation of renowned expert reviews assembled herein.

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