

Glyco-Forum section

Working group report: the roles of glycans in hemostasis, inflammation and vascular biology

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Context

The first major collaborative effort on glycomics funded by the US National Institutes of Health was the Consortium for Functional Glycomics, begun in 2001. The focus of the consortium, which was funded by the National Institute of General Medical Science (NIGMS), includes cataloging structures of glycans in selected cell types and organs using mass spectrometry, creating and characterizing the phenotypes of mice engineered with deficiencies in glyco-related genes, and creating specialized microarrays to study gene expression and glycan binding specificity. New approaches will be needed to capitalize on this and other emerging glycomics knowledge to develop new diagnostics and treatments. With this in mind, the Division of Blood Diseases and resources of the National Heart, Lung, and Blood Institute (NHLBI) convened a working group of scientific investigators on February 25–26, 2008, in Bethesda, MD, to identify scientific opportunities and priorities emerging from the recent explosion of technological and biological advances in the glycosciences. Aligned with the NHLBI Strategic Plan Goals (<http://apps.nhlbi.nih.gov/strategicplan/>), the focus of this group was on “The Roles of Glycans in Hemostasis, Inflammation, and Vascular Biology.” Dr. Susan Shurin, Deputy Director,

NHLBI, in her welcome message to the group noted that the full potential of the genomic and proteomic tools and knowledge bases recently generated with NHLBI funding will not be realized without an equally strong emphasis on glycomics. The working group was charged to identify key research areas as well as to prioritize the most fruitful areas for basic, preclinical, and clinical research related to heart, lung, and blood diseases. The meeting was well attended by NHLBI program officials in addition to intramural scientists from NHLBI and the National Cancer Institute (NCI). Scientific review officers from the Center for Scientific Review and extramural program officials from NCI, NIGMS, and the National Institute for Allergy and Infectious Diseases (NIAID) also attended.

Preamble

Glycosylation is among the most common and abundant post-translational modifications of proteins and lipids. Glycans affect most major developmental, biological, and pathological processes. The working group met at the request of the NHLBI to discuss the importance of glycans to the missions of the institute, with a specific emphasis on their roles in hemostasis, inflammation, and vascular biology. Members of the working group (the authors of this report listed on top) first gave scientific presentations, which highlighted some unexpected and provocative new discoveries on the biological roles of glycans. Areas represented in these presentations included:

- Chronic inflammation due to evolutionary loss of Siglec expression on human leukocytes.
- Incorporation of the non-human sialic acid Neu5Gc into human endothelia from dietary sources, with the potential for vascular inflammation caused by anti-Neu5Gc antibodies.
- Identification of heparan sulfate as the long-sought receptor for plasma lipoprotein clearance.
- Heparan sulfate-mediated chemokine transcytosis and presentation during inflammation and leukocyte trafficking.
- Heparan sulfate dependence for angiogenesis.
- Chemoenzymatic synthesis of pharmaceutical heparins.
- Carbohydrate-based nanotechnology.
- Nonthrombogenic heparinized biomaterials.
- Biochemical and genetic regulation of protein glycosylation in blood leukocytes and endothelial cells.
- Roles of glycosylation in hematopoiesis and leukocyte trafficking during inflammation and thrombosis.
- Roles of the Cosmc molecular chaperone in regulating O-glycosylation, and its role in disorders such as Tn polyagglutination syndrome, and IgA nephropathy.
- Recognition and signaling in blood cells through glycan recognition by the galectin, siglec, and selectin families of glycan-binding proteins.

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- Roles of the *O*-GlcNAc nuclear-cytosolic protein modification in diabetes-associated vascular disease and glucose toxicity.
- Role of *O*-GlcNAc in myocardial protection following ischemic injury.
- Control of T-cell development by *O*-linked fucosylation of Notch.
- Modulation of myelopoiesis by *O*-linked fucosylation of Notch.
- Control of leukocyte trafficking by sialylated, fucosylated glycans.
- Regulation of monocyte integrin function by variant sialylation.
- Role of BACE1 secretase in macrophage adhesion.
- Galectin regulation of dendritic cell migration into sites of inflammation.
- Galectins as “alarmins” to initiate sterile inflammation.
- Glycans as receptors for viral vectors for gene transfer in hematopoietic stem cells.
- Role of glycans in hematopoietic stem cell homing and engraftment.

These and other contemporary examples presented by working group members exemplify a recent dramatic enhancement of our understanding of the cellular and molecular biology of glycans in nature and their roles in evolution, development, physiology, and pathology. Despite these exciting and unanticipated developments, there remains an enormous gulf between this new knowledge and its applications to the biomedical sciences. One major reason for this anomaly is that the methodologies for glycan analysis have been more difficult to develop than those for DNA, RNA, and proteins. A second reason is that there are currently a relatively small number of investigators studying glycans. These and other factors have contributed to the slow pace of discovery relative to other molecules of biomedical importance, such as DNA, RNA, and proteins. However, recent advances and breakthroughs in the technologies and knowledge base for studying the structure and function of glycans now allow “Glycobiology” to become an integral part of the genomic and postgenomic revolution.

Rationale

Despite recent advances in understanding the role of glycosylation in themes relevant to the NHLBI, most active biomedical scientists have a limited knowledge and expertise in glycosciences. Thus, although the great majority of current NHLBI-funded projects involve molecules that contain and/or recognize glycans, most investigators typically do not address this key aspect of their systems. Consequently, at the same time when many major advances in understanding the roles of glycans in blood and vascular diseases are possible, there is a dearth of investigators pursuing these opportunities. Clearly, there is a need for funding mechanisms that will bring together the existing experts in the study of glycans with investigators in blood and vascular diseases, to foster fruitful collaborations that will bridge the existing gulf. Importantly, this frontier area is also ripe for translational research and drug development. Also of note, the situation is different in many European and Asian countries, e.g., Sweden, Australia, and Japan, where there are

major nationwide commitments to, and interest in the study of glycans. Thus, the USA lags behind the rest of the world in taking advantage of these opportunities.

The study of glycans in blood and vascular disease aligns with the strategic goals of the NHLBI, i.e., “increasing our understanding of the molecular and physiological basis of health and disease”; “improving understanding of the clinical mechanisms of disease”; and “translation of research into the practice of medicine.” In this report, we focus on three specific areas of relevance to these NHLBI goals: the roles of glycans in hemostasis, inflammation, and vascular biology. Some classic examples are the interactions of antithrombin with the anticoagulant heparin and the roles of selectins in initiating leukocyte trafficking. In addition to the short-term goal of fostering collaborative interdisciplinary research in such areas of special interest to NHLBI, this report addresses the long-term need to educate the next generation of investigators, who must effectively integrate the study of glycans into their explorations of physiology and disease. Such measures will undoubtedly facilitate the overall NHLBI goal, “to improve the diagnosis, treatment, and prevention of disease.”

Research opportunities and priorities

In discussions that followed the scientific presentations, many additional research opportunities and priorities were identified regarding the impact of glycans in hemostasis, inflammation, and vascular biology. Some of the more remarkable areas are mentioned below, along with specific examples.

- Chronic inflammation, e.g., lectin-dependent activation of macrophages and dendritic cells.
- Vascular remodeling and angiogenesis, e.g., the critical role of *O*-glycosylation in endothelial cell development.
- Thrombotic disorders, e.g., effects of glycosylation on coagulation factor and platelet half-life and clearance.
- Sickle cell disease, e.g., role of P-selectin in mediating erythrocyte adhesion in vasoocclusive crises.
- Reperfusion injury, e.g., the potential therapeutic value of nonanticoagulant glycosaminoglycans.
- Atherosclerosis, e.g., multiple roles of glycans in the initiation and progression of coronary artery disease, stroke, and peripheral vascular disease.
- Glycan-related therapeutics, e.g., PSGL-1 to improve graft recovery following transplantation.
- Cancer-associated coagulopathies, e.g., role of mucins and heparin in Trousseau’s syndrome.
- Glycan-based targeting, e.g., use of glycan-coated particles for gene therapy, drug delivery, or imaging.
- Hematopoietic stem cells, e.g., use of in vitro glycan modification to optimize homing to the marrow.
- Hematopoietic disorders, e.g., the deficiency of glycopospholipid anchors in paroxysmal nocturnal hemoglobinuria.
- Restenosis, e.g., use of glycan-coatings as a means to reduce severity.
- Tissue engineering, e.g., use of glycan-based biodegradable scaffolds.
- Glycans as potential biomarkers, e.g., serum levels of mannose-binding protein as predictors of autoimmune vasculitis.

- Transfusion medicine, e.g., the key role of glycans as blood group antigens; the use of glycosidases to generate “universal donor” cells; and the use of galactosyltransferase to increase the shelf-life of donor platelets

It is important to emphasize that opportunities for major breakthroughs are not limited to the examples given above. Indeed, many areas of potential importance remain completely unexplored, e.g., effects of chronic hypertension on glycans that could affect vascular wall resistance and potential polymorphisms in the enzymes, critical glycosylation sites, or glycan-binding sites of proteins that might correlate with propensity to disease. Thus, this initiative should catalyze advances in our knowledge far beyond those listed above.

Existing challenges

As discussed above, there are numerous exciting opportunities, but there are also some challenges. The current generation of established researchers is largely unfamiliar with glycan structure and biology, and the related methodologies and terminology. Consequently, many opportunities for discovery are missed, and there is limited potential for the younger generation of biomedical scientists to become exposed to this field. There is also a paucity of grant proposal reviewers with expertise in glycans and awareness of their important biological roles. Given these and other issues, the group feels that the most acute need is to expand the pool of investigators who routinely incorporate the study of glycans into their research programs.