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Collaborative Directions:
Common Goals for Two Canadian
Research Networks
Workshop Report
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GlycoNet—CNTRP Collaborative Directions:
Common Goals for Two Canadian Research Networks

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Executive summary:

A joint workshop held in May 2016 between two national research networks in Canada, GlycoNet and the Canadian National Transplant Research Program (CNTRP), is summarized. The **Canadian Glycomics Network (GlycoNet)** is a Network of Centres of Excellence linking over 110 research groups in Canada, harnessing the power and promise of glycomics to deliver health solutions. The **Canadian National Transplant Research Program (CNTRP)** is a network of basic and clinical scientists working with chemists, biomedical engineers, health economists, legal and ethics researchers, and policy experts at 30 sites across Canada. Researchers in these two networks presented current work and discussed future areas of potential collaboration and enhanced synergy. Topics included the use of enzymes to generate universal tissue and blood products, the role of antibody glycan structures in inflammation, glycosylation of exosomes and microvesicles, cryopreservation technologies, mass cytometry analysis, microbiome glycans, and xenotransplantation. Technologies available for routine analysis of glycoprotein and glycolipid samples were discussed. Finally, strategies for future funding and collaboration between the networks were discussed.

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1 Introduction / Background

Solid organ transplantation and hematopoietic cell transplantation are well-established therapies for incurable acute and chronic medical conditions that affect tens of thousands of Canadians. However, critical barriers prevent donation and transplantation from being fully effective and Canada needs to develop new knowledge and healthcare practices to overcome these barriers. Currently, over 4,500 Canadians with end-stage organ failure and hematologic malignancies are waiting for life-saving transplants, and too few donors are identified and utilized in our current medical system. As wait-lists continue to grow in every province across Canada, patients suffer prolonged illness at high health care costs, and many die before suitable donors are found. The growing imbalance between organ supply and demand means that Canadians needing transplants face a 30–40% lifetime probability of never receiving one. To address these problems, the Canadian Institutes of Health Research (CIHR), the national funding agency for health research in Canada, presented a novel challenge: to bring communities across the country together into a research network that would improve the health of Canadians. It was recognized that only a new type of innovative team structure would support and nurture the emergence of synergies and important evidence required to transform the field. The transplant community responded to this challenge in 2013 by building a program that is not only unique in Canada but also in the world. The Canadian National Transplant Research Program (CNTRP) is a coalition of more than 300 scientists, students, collaborators, patient partners and knowledge-users at 30 sites throughout Canada actively engaged in interdisciplinary and trans-sectorial research in the fields of solid organ transplant, hematopoietic cell transplant and organ/tissue donation.(1)

Carbohydrates, despite being well known as a source of biochemical energy, also play critical roles in a broad range of biological processes. Oligosaccharides (often referred to as “glycans”) are found decorated on proteins and lipids as long polymer chains, and form the backbone of DNA polymers. The structural complexity of glycans continues to make their characterization more challenging than proteins and DNA; however, substantial progress in recent decades has brought this class of biomolecules to the frontiers of biotechnology.(2,3) Modern synthetic, analytical, and biochemical methods directed at understanding the roles of glycans in biological systems have enabled major advances.(4) The study of the composition and function of glycans within biological systems is often referred to as “Glycomics.”(5)

The Canadian Glycomics Network, or GlycoNet, is a Network Centre of Excellence of Canada launched in 2015. The network brings together over 110 researchers across Canada, working in research themes including antimicrobial drugs, genetic diseases, diabetes, obesity, chronic disease, and vaccine development. Part of the Network’s mission is to bring together biomedical scientists and researchers with industry partners. The application of glycomics to problems in the field of transplantation has a long history. Predicting and classifying the compatibility of solid tissue grafts and blood products is an

essential first step in transplant medicine. Perhaps the best illustration of this is the use of the ABO-blood group system, first introduced by Landsteiner in 1900.(6) This system, which relies on the detection of serum antibodies that recognize carbohydrate antigens, is the first step in evaluating donor and recipient compatibility. Incompatible transplantation is possible in certain settings, but is most often associated with catastrophic failure – such as hyperacute antibody mediated rejection (AMR).(7) Moreover, the major obstacles in the field of xenotransplantation, a potential solution to the critical shortage of organ/tissue donors, are related to cross-species glycan incompatibilities. Therefore, the identification of important glycan antigens, their classification and detection, as well as diagnostics remain an active area of research.(8)

In May 2016, these two major Canadian research networks held a joint workshop in Banff, Alberta to identify strategies to combine efforts on current problems in transplantation. Representatives from the CNTRP and GlycoNet met to present current areas of research and to discuss future areas of collaboration. Topics of discussion are outlined below, and a discussion of potential strategies for the two networks to work together follows.

2 Areas for collaborative research

2.1 Glycan antigen removal with enzymes

The ability to cross the ABO barrier safely between transplant recipients and donors would considerably expand the current potential donor pool, increasing access of Canadians to life-saving transplants. (9,10) However, ABO antigen incompatibility in transplantation can trigger deleterious immune responses. There is a need to develop innovative tools to modify these glycans. In principle, the glycan structure of ABO antigens on grafts can be modified by enzymatic treatment to convert them to compatible, or even universally compatible products. Glycosidases that effectively cleave A/B antigens (converting the tissue to a universal O) have been identified for producing universal red blood cells (11) and solid organs.(12) Kobayashi *et al.* employed a recombinant endo-bgalactosidase enzyme (ABase) to cleave A/B antigens from within an excised baboon kidney (using biopsy and immunohistochemistry (IHC)).(12) Several groups within GlycoNet have expertise in the identification and optimization of new glycosidase enzymes that could be applied to problems in transplantation. A new and improved enzyme was discussed that cleaves both A and B antigens from more than one subtype,(13) and high throughput methods to screen for new enzymes.(14) Applications in *ex vivo* organ perfusion were discussed, given the important opportunity allowed by this rapidly evolving technology, and in xenotransplantation (see below).

2.2 Antibody glycan signatures

Acute and chronic antibody-mediated rejection (AMR) are playing an increasingly critical role in allograft loss and are considered among the most important barriers that limit long-term transplant outcomes (15). Until now, research has focused on the antigen recognized by the Fab portion of these antibodies and less so on their general structure. Circulating antibodies are glycoproteins, which typically feature a complex N-linked glycan in the Fc domain. The specific glycan structure can have an impact on the function of antibodies.(16) For example, the amount of sialic acid found in the glycan affects interactions with Fc-receptor and inflammation.(17,18) Hence antibody glycosylation state has the potential to modulate the severity of vascular injury or AMR in transplantation. This was recognized as an area that could be best explored with collaborations between clinical and glycoscience groups to identify changes in antibody glycosylation status relevant to transplantation. Discussion centered around a need to obtain clinical samples for analysis of antibody glycosylation state in patients at risk of AMR. Methods and services to characterize glycoproteins are identified in Sec. 2.8.2, and are also relevant to Sec 2.3.

2.3 Glycans of microvesicles & exosomes

2.3.1 Interactions of extracellular vesicles & glycans in the immune system/ inflammation; pathophysiology of rejection and GVHD

Over the past decade, there has been rapid growth in studies of secreted membrane vesicles, collectively called extracellular vesicles (EVs). Publications in high-impact journals have proposed exciting functional roles of EVs, and increasing evidence indicates that EVs contribute to the pathogenesis of various human diseases (19,20). *Éric Boilard* (Univ Laval) described the work of his team that characterized platelet-derived microparticles as important components in the dissemination of inflammatory signals (21-23). *Mélanie Dieudé* and the *Hébert* team identified and characterized a novel type of membrane vesicle (apoptotic exosome-like vesicles) released by apoptotic endothelial cells as accelerators of vascular rejection and graft-*vs*-host disease (GVHD) in animal models (24). The glycome of these vesicles undoubtedly contains vital clues essential to understanding the biogenesis and function of vesicles that will help delineate further the pathophysiology of rejection and chronic GVHD. Discussion centered around approaches that could be undertaken to perform glycomic analysis of these platelet and endothelial cell-derived vesicles to catalog the surface oligosaccharide and polysaccharide structures and, in addition, the carbohydrate-binding proteins found on and inside EVs.

2.3.2 Implications as potential biomarkers of inflammation/injury in transplantation: glycan detection in extracellular vesicles isolated from biofluids

Tremendous interest has been generated in microvesicles/exosomes following a number of recent, high-profile reports describing their potential utility, particularly in diagnostic and prognostic roles (19). It is increasingly appreciated that the cargo (protein/RNA) and glycan signature of EVs can reflect different biologic states (19,20). Discussion focused on determination of the glycan signature of EVs isolated from biofluids such as serum, plasma, urine (and possibly bronchoalveolar lavage fluid). The signatures reflecting different disease states and healthy controls should be compared to define disease state glycan-specific changes. Samples from individuals before and after solid organ or bone marrow transplantation, from healthy controls as well as donors should be studied. These glycan signatures might constitute novel tools of potential clinical utility in assessing risk of alloimmune activation and transplant dysfunction in patients. Glycan signatures from EVs released during *ex vivo* organ perfusion would also be of great interest to monitor organ injury and predict allograft function and risk of rejection.

2.4 Cryopreservation technologies

The clinical use of blood products and tissue often involves cryopreservation. However, many challenges hamper effective use of cryopreservation at temperatures below the freezing point of water due to cell destruction. At these temperatures, damage to cells occurs due to the formation of ice crystals that disrupt cellular structures. *Robert Ben* (Univ Ottawa) presented work on the development of ice recrystallization inhibitors (IRI) that can improve the survival of cells in cryopreservation.(25,26) There was discussion of the application of these findings to tissue cryopreservation. Further interactions between clinicians working on *ex vivo* organ perfusion and development of IRI inhibitors are recommended.

2.5 Multiparameter mass cytometry analysis

Flow cytometry is a common laboratory method for the analysis of cells. Typical flow cytometry instruments are able to use multiple lasers to allow detection of 1–5 possible markers simultaneously. *Mark Nitz* (Univ Toronto) discussed work being done in collaboration with a Canadian instrument company (Fluidigm, Markham, ON) to develop mass cytometry tags that offer a far greater range of multiplex detection. Applications for detecting changes in cellular oxygenation levels were presented.(27,28) Potential applications in transplantation may include analysis of a panel of HLA and ABO antigens in a single assay for personalized management of transplant patients.

2.6 Microbiome glycans

Research on the influence of commensal microbiota on human health is a rapidly growing field.(29-31) Interactions between the gut microbiome and the immune system are known to be important, and likely have an impact on immune responses that contribute to post-transplant outcomes. Recently, animal and human studies have shown that gut microbial populations and diversity are altered after allogeneic solid organ and bone marrow transplantation (32). Heavy and frequent use of anti-microbial prophylaxis in transplant patients may additionally contribute to increased risk of the emergence of anti-microbial resistance. Moreover, when complications such as infection, rejection and GVHD occur, gut microbial populations and diversity present a significant dysbiosis (32). Microbiota metabolize glycans in the gut and may have an influence on metabolism of the host.(33) Workshop participants discussed exploring the contributions of gut microbiota and glycan processing to transplant rejection and cGVHD. Another area of interest is investigating the precise role of the gut microbiome as the presumed stimulus for the production of “natural” antibodies to non-self ABO (discussed above) and xenoantigens (discussed below). Connections between gut microbiota and transplantation are just emerging,(34) and this was recommended as an area for future research.

2.7 Xenotransplantation

Xenotransplantation – the transplantation of organs and tissues between animal species – could supply an unlimited number of organs to overcome the shortage of organs from deceased or living human donors. However, non-human glycan antigens are major targets for human "natural" (preformed) anti-xenograft antibodies. The binding of human IgM and IgG antibodies to these glycan antigens initiates a process of hyperacute antibody-mediated rejection, resulting in destruction of the xenograft within minutes or hours. The prototypic example is the use of porcine organs, which display a substantial amount of the Gal-a-(1 →3)-Gal epitope (often referred to as the “a-Gal” or “Galili” epitope).(35) Similar to natural ABO antibodies, humans produce abundant circulating antibodies that recognize this glycan epitope, preventing use of, for example, porcine organs for transplantation. A number of groups have worked on genetically engineering pigs to remove expression of this and related carbohydrate epitopes that would lead to AMR. *Matt Tector* (Univ Alabama at Birmingham) presented work on the newest generation of engineered pigs in which the a-(1 →3)-GalT (GGTA1), CMAH, and b4GalNT2 genes were eliminated using CRISPR technology.(36) This field, which had substantially declined in the last decade due to concerns of risk of transmission of porcine endogenous retroviruses through xenotransplantation, is of renewed interest to transplant researchers and to industry,(37)(38) and was seen as an area of potential opportunity for Canadian researchers.

2.8 Topics for future development

Topics for future discussion and development include:

- Identification of glycan structures as possible immune targets in transplantation. Immune responses to glycan epitopes could be the cause of some instances of acute or chronic rejection or GVHD. The identification of unknown antigens would therefore be important in defining these immune responses in transplantation. Examples may include chronic lung bronchiolitis obliterans syndrome (BOS).(52,53)
- Detection of the glycan signature of EVs in biofluids may be important in terms of identifying biomarkers of injury or inflammation in transplantation.
- Cryptic antigens that may be revealed due to changes in glycan biosynthesis or processing, or that are unmasked due to physiologic conditions such as ischemia/re-perfusion injury, may play a role in AMR.
- A number of researchers in GlycoNet are developing new strategies for antibiotic therapies (*Eric Brown, Sachiko Sato, Donald Sheppard, Lynne Howell, Alasdair Boraston, Joseph Lam, Chris Whitfield, Andrew Bennet*). Targets of specific interest in transplantation medicine, including antimicrobial resistance and the role of the microbiome, could be a productive area of collaboration between the two networks.
- Characterization of important protein-carbohydrate interactions, such as ABO-antigen–antibody interactions, was identified as an important mechanistic target.

2.9 Resources available for researchers in glycoscience

Participants were made aware of existing resources that can enable discovery in glycoscience. Several examples are summarized below.

2.9.1 Glycan microarrays

James Paulson (Scripps) communicated that access to the Consortium for Functional Glycomics (CFG) glycan array is available for a nominal fee ($250 USD.) The CFG glycan array currently has over 600 glycans.(39) Commercial microarrays are also available, along with screening as a fee-for-service.(40),(41)

2.9.2 Mass spectrometry reagents and service

Commercial sources of kits and reagents for the analysis of glycopeptides and released glycans are available from multiple companies, including Prozyme(42) and Waters.(43),(44),(45) Multiple glycomics research institutes provide fee-for-service analysis of glycans and glycoproteins, including the Alberta Glycomics Centre/GlycoNet Core Services,(46) the Complex Carbohydrate Research Centre (CCRC),(47) and others.(48) Training courses are offered annually by the CCRC.(49)

2.9.3 Carbohydrate synthesis

The Alberta Glycomics Centre/GlycoNet Synthetic Core Services offers fee-for-service custom synthesis of oligosaccharides.(50) Custom manufacturers in Canada, such as Sussex Research,(51) are also available. Access to unique or rare oligosaccharide samples may be critical for the validation of new biomarkers and for the development of new antibody reagents for their study in biological systems.

3 Barriers and Challenges

Major obstacles to advancing research in the areas discussed above include the high barrier to development of glycomics technologies. The analysis of glycoproteins and glycan structures requires dedicated expertise and instrumentation. The continued support of core services from the Alberta Glycomics Centre/GlycoNet and the continued encouragement of non-GlycoNet researchers to become GlycoNet Network Investigators are recommended.

Although the workshop highlighted a large number of common interests between the two research networks, there is a need for increased interaction between these communities. The development of regular symposia or workshops to highlight specific areas is recommended. Additionally, satellite meetings (such as the GlycoNet–CNTRP Workshop) may be an excellent and cost-effective mechanism to increase interactions.

The topics highlighted above are compelling due to their bridging of fundamental research with clinical applications. Projects that may emerge from the workshop are likely to bring together broad and multi-disciplinary teams. While this is an essential component to making progress on these problems, it must be recognized that the evaluation and logistics of truly multi-disciplinary projects present a major challenge.(54) The first of these is the development of strategies for fair evaluation of projects that require diverse expertise. Although researchers are often encouraged to engage in inter-disciplinary work,(55) research has found these projects do not fare well in peer review systems. Systematic studies have found that inter-disciplinary grant proposals have a lower probability of being funded than straightforward single disciplinary applications.(56)(57) Therefore, it is recommended that any funding strategies aimed at innovative cross-network collaborations attempt to mitigate these issues to identify projects with the best likelihood of success.

4 Future Directions & Funding strategies

Funding strategies for future projects were discussed at the workshop. It was pointed out that GlycoNet has a “Catalyst” funding stream targeted at initiating new projects. This may be a good starting point for new inter-network projects. Future strategies could include specific calls in priority areas. Although current network investigators are likely applicants, we would recommend the continuation of keeping the barrier low for entry

into one or both networks if this will be a requirement for applicants. This strategy should help to expand the community of researchers working to forge compelling new projects at the intersection of glycomics and medicine. An expanded form of the GlycoNet catalyst grant that focuses on applications in transplantation could be a joint initiative of the two networks. Partnership with national funding organizations, including charities (e.g., Canadian Liver Foundation), may also be worth pursuing.

5 Conclusions

The workshop highlighted a number of important areas of common research interest and the potential for substantial translational outcomes. There were several examples where new technologies were highlighted that could be brought to clinical application, including glycan microarrays, engineered glycosidases, improved flow cytometry methods, cryopreservation using glycoconjugates and transgenic animals. Increased discussions between the two networks will enable new, currently unforeseen, clinical applications of new technologies in glycomics. Additionally, the increased accessibility of more routine technologies such as synthesis, microarray screening, and mass spectrometry analysis should enable a broader base of researchers to address questions that relate to glycomics.

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