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Mini-Review

Coronaviruses' sugar shields as vaccine candidates

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ABSTRACT

A successful global healthcare response relies on versatile vaccines and production of broadly virus-neutralizing antibodies by the immune system to protect us from emerging infectious diseases. The present 2019 severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic highlights the urgent need for development of anti-viral biodefense. Due to the genetic and proteomic diversities of viral pathogens, establishing versatile anti-viral vaccines or therapeutic agents is highly challenging. Carbohydrate antigens represent an important class of immunological targets for vaccine development and immunotherapy against microbial infections. In this mini review, some concepts and strategies for exploring the potential of immunogenic sugar moieties as CoV vaccine candidates are presented.

KEYWORDS: coronavirus, COVID-19, glyco-conjugate, lectin, N-glycans, oligomannoses, SARS, SARS-CoV-2, spike glycoprotein, vaccines, virus-neutralizing antibody.

INTRODUCTION

As the current worldwide COVID-19 pandemic rages, it is clear that an efficacious global healthcare response must include multipurpose vaccines that stimulate production of broadly virus-neutralizing antibodies (bnAbs) against emerging viral pathogens. Due to the genomic and proteomic diversities of viral pathogens, establishing such countermeasures against viral infections is difficult. The recent outbreaks of diseases caused by coronaviruses (CoVs), including the severe acute respiratory syndrome coronavirus (SARS-CoV) [1, 2], the Middle East respiratory syndrome coronavirus (MERS-CoV) [3, 4], and the 2019-nCoV in the present outbreak [5-9], pose new challenges to global efforts to combat infectious diseases.

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Protein targets

The 2019-nCoV has been phylogenetically mapped to the same Betacoronavirus clade as SARS-CoV [10, 11] and, accordingly, classified as SARS-CoV-2 [12]. However, the two viruses differ significantly in their amino acid sequences. As compared to the consensus sequences of SARS-CoV and SARS-like viruses, SARS-CoV-2 contains 380 amino acid substitutions in total and 27 substitutions in the spike glycoprotein (S) critical for viral entry and antibody-mediated virus-neutralization [10, 11]. Although the overall structure of SARS-CoV-2 resembles that of SARS-CoV, and shares the same functional host cell receptor—angiotensin-converting enzyme 2 (ACE2), the receptor-binding domain (RBD) in the SARS-CoV-2-S differs significantly from that of SARS-CoV-S, most notably in five of the six amino acid residues critical for binding to ACE2 [9]. Monoclonal antibodies (mAbs) specific for the RBD of SARS-CoV-S have no cross-reactivity to the SARS-CoV-2-S RBD [13]. Characteristic structural elements in SARS-CoV-2-S include a furin cleavage site at the boundary between the S1/S2 subunits and three adjacent

potential O-glycosylation sites not previously seen in lineage-B betacoronaviruses. Nevertheless, murine polyclonal anti-sera to SARS-CoV-S potently inhibited SARS-CoV-2-S-mediated entry into target cells [14], indicating the presence of shared antigenic structures—perhaps in the more conserved S2 region of the two CoVs. Much remains to be learned about the antigenic characteristics of the new CoV as compared to SARS-CoV and other human CoVs [15, 16].

Immunogenic carbohydrate moieties

Carbohydrates represent an important class of microbial antigens. These molecules are structurally diverse and prominently displayed on the surfaces of virtually every microbial pathogen [17, 18]. In 1917, Dochez and Avery [19] found that when *Pneumococci* were grown in fluid media, there was a substance in the culture fluid that precipitated specifically with antisera to the same *Pneumococcus*. Heidelberger and Avery [20] showed the substance recognized by the antibodies was a carbohydrate molecule and not a protein, as previously thought. It was later shown that almost every microorganism expresses such sugar signatures recognized by the host immune systems and that they are effective in stimulating specific antibody responses [17, 18]. Such immunogenic carbohydrate moieties often serve as key targets for development of vaccines against infectious diseases [21-25]. A large panel of pathogen-specific carbohydrate moieties has been identified; some have been successfully explored for use in vaccines or targeted immunotherapy against microbial infections [21-25]. A notable example is that introduction of carbohydrate-conjugate vaccines has virtually eradicated childhood meningitis and systemic lethal bacterial infection caused by *Haemophilus influenzae* B [23, 24, 26-28].

Like bacterial pathogens, viruses also decorate their outer surfaces with carbohydrate moieties. Unlike bacteria, which have evolved their own machineries for glycosylation and often produce unique sugar chain signatures, viruses depend on host cells for glycosylation and generally decorate their virions with the “self”-glycans of corresponding hosts. This “sugar shield” is thought to be one of the strategies viruses evolved to escape host immune rejection. For example, human immunodeficiency virus (HIV-1) [29], Lassa virus [30], hepatitis C virus [31], and Epstein–Barr virus [32] exhibit extensive N-linked glycans covering the exposed protein surfaces, including critical virus-neutralizing protein epitopes. Similarly, CoV S glycans mask the protein surface and consequently limit antibody access to protein-neutralizing epitopes [33].

Viral glycan shields as vaccine targets

New ideas and innovative strategies are urgently needed to establish multipurpose vaccines against the emergence or re-emergence of unexpected viral pathogens. Recently, carbohydrate researchers undertook an investigation to explore whether viruses of distinct phylogenetic origins, such as human cytomegalovirus (HCMV), HIV-1, and SARS-CoV, express conserved glyco-determinants that are suitable for broad-spectrum virus neutralization [34]. The assumption was that viruses depend on host glycosylation machinery for glycan synthesis and thereby may express the conserved viral carbohydrates. These studies led to the recognition of several glyco-antigens co-expressed by these viruses, including not only the known oligomannosyl antigens but also the previously less studied Tri/m-II, and Tri/m-Gn glyco-epitopes (Figure 1) [34]. Such glycan clusters belong to a class of N-glycan cryptic autoantigens with unique immunological properties. They are generally present intracellularly as glycosylation intermediates, but become overexpressed and/or surface-exposed by some viral pathogens [35-37] as well as tumor cells [38-40]. Thus, induction of immune responses to these targets is unlikely to be harmful to normal cells. Instead, antibodies or lectins targeting these cryptic intracellular antigens are likely essential for the clearance of autoantigens released from the aged or apoptotic cells *in vivo* [41, 42]. Interestingly, a broadly virus-neutralizing agent, Galanthus nivalis agglutinin (GNA), recognizes specific targets in the panel and effectively neutralizes many viruses [34, 43-46], including SARS-CoV [34, 43].

A common feature of CoVs is that their S glycoproteins are densely decorated by N-linked glycans protruding from the surfaces of the

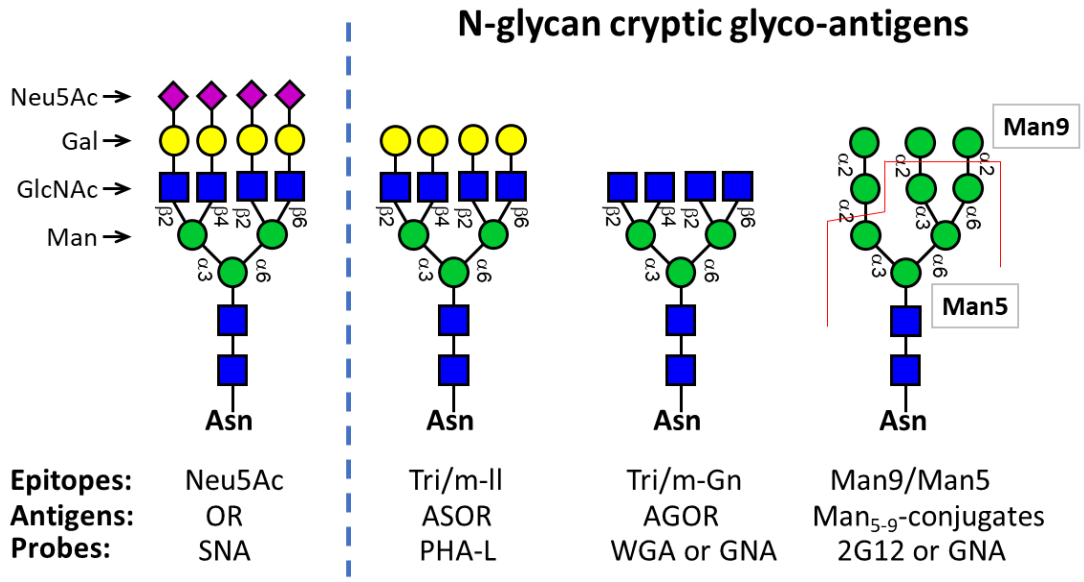

Figure 1. Schematic of a panel of *N*-glycan cryptic glyco-antigens. Asialo-orosomucoid (ASOR) and agalacto-OR (AGOR) were chemically prepared to expose cryptic glyco-epitopes, tri-antennary or multi-valent type II (Tri/m-II) and Tri/m-GlcNAc (Gn), respectively. Oligomannose moieties display a diverse panel of cryptic epitopes. As illustrated, these glyco-epitopes are recognized by specific virus-neutralizing agents: a) mannosyl-epitopes recognized by 2G12 or GNA; b) Tri/m-Gn epitopes stained by GNA or wheat germ agglutinin (WGA); and c) Tri/m-II epitopes that are highly reactive with PHA-L and SARS-CoV neutralization antibodies.

virions [33, 47-49]. The SARS-CoV-2-S comprises 22 N-linked glycosylation sites, and 16 of them were resolved in the cryo-electron microscopy (cryoEM) map as glycosylated. By comparison, SARS-CoV-S possesses 23 N-linked glycosylation sites with at least 19 of them confirmed to be glycosylated [47]. Twenty out of 22 SARS-CoV-2-S N-linked glycosylation sites are conserved in SARS-CoV-S. Specifically, 9 out of 13 sites in the S1 subunit and all 9 sites in the S2 subunit are conserved among SARS-CoV-2-S and SARS-CoV-S. CoVs may overexpress the high-mannose type since CoV virions are likely matured in and directly bud from the endoplasmic reticulum–Golgi intermediate compartment without further editing by the Golgi-residential glyco-enzymes [33, 50]. Thus, it is important to determine whether or not SARS-CoV-2 and other CoVs also express these GNA-positive glyco-determinants and other glycan-based virus-neutralizing epitopes.

Challenges and opportunities in CoV vaccine development

The current COVID-19 pandemic has ignited global efforts toward development of an effective SAR-CoV-2 vaccine [51-53]. One of the key targeted immunogens is SARS-CoV-2-S glycoprotein since it is crucial for receptor binding, membrane fusion *via* conformational changes, internalization of the virus, and host tissue tropism [54]. A novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine, mRNA-1273 (ModernaTX, Inc., Cambridge, MA), was designed to express a full-length, prefusion stabilized SARS-CoV-2-S protein. Since the human cells of each vaccinated person express the protein *in vivo*, the immunogen produced by mRNA-1273 can be viewed as a form of natural glycoconjugate with the sugar moieties displayed by the precisely translated S-protein carrier. This type of vaccine may induce T-dependent antibody responses to the glyco-determinants. Ideally, this RNA-based vaccine may trigger both anti-protein and anti-glycan antibody responses *in vivo* to enhance anti-SARS-CoV-2 immunity. Similarly, other vaccine platforms, such as virus-like particles, inactivated SARS-CoV-2, and DNA vaccines that produce S glycoprotein may also express carbohydrate epitopes. Thus, analyzing the vaccine responses may provide very useful data to evaluate potential

immunogenicity of vaccine components, including proteins and carbohydrates.

Carbohydrate microarrays have proven to be a powerful means for exploring the immunogenic sugar moieties recognized by host immune systems to mount antibody responses [22, 35, 55-58]. Unlike a conventional S glycoprotein immunoassay that detects the sum of anti-protein and anti-glycan antibodies, carbohydrate microarrays can be designed to present either pure carbohydrate moieties [22, 59] or glycoconjugates [46, 60] lacking S protein components and, thereby, can be used to decipher anti-glycan and anti-protein antibodies for a given immunogen or pathogen. Characterizing a SARS-CoV-2 vaccine response or COVID-19 patients' serological response using carbohydrate microarrays is, therefore, a practical approach to verify whether SARS-CoV-2 is also decorated with glyco-determinants that are promising immunological targets.

Due to variation in glycosylation patterns among different cell types, CoV virions produced by different cells may also carry unique glycan signatures. For example, bat cells carry many non-human glycans, such as non-human sialic acids [61], the Galili alpha-Gal epitopes [62], and, perhaps, bisecting GlcNAc moieties [63]. Whether the bat cell-produced CoVs express these highly immunogenic sugar moieties and if human infection caused by the first wave of bat-CoVs triggered hyperimmune responses to these non-human glycans and contributed to severity of the diseases remains to be seen. Characterizing cohorts of COVID-19 patients from different epi-centers—especially a comparative serological study of the early onset sample sets and the later human-human transmitted sample sets using carbohydrate microarrays and other glycan-specific immunoassays—may uncover important glyco-immunological information to guide development of glyco-conjugate vaccines and therapeutic antibodies to target the sugar shield of SARS-CoV-2 and perhaps other unexpected CoVs with human outbreak potential. The glyco-conjugate vaccines without any CoV protein component may have the unique advantage of avoiding undesired vaccine responses to the S-protein epitopes that were non-neutralizing but elicited the antibody-dependent enhancement of infectivity and severe Th2-type lung immunopathy observed during SARS-CoV vaccine development [53, 64-69].

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CONFLICT OF INTEREST STATEMENT

The author has no conflicts of interest to declare.

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