Advances in glycoscience to understand viral infection and colonization

Interactions between carbohydrates and the proteins that bind them (lectins) are often some of the first between a host cell and a viral invader. With its highly glycosylated spike protein, SARS-CoV-2 is no exception. Interrogating glycosylation is vital to understand viral infection, yet it has been a challenge. Improvement in methods ranging from mass spectrometry to glycan arrays and modeling simulations are yielding atomic-level information about the glycans that decorate viruses and host cells alike.

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Human evolution has been guided by the viruses that take refuge inside us; this ancient relationship is recorded in our genomes[1]. Most human viruses have been studied in the context of infection (pathogens) rather than colonization (commensals); however, we are beginning to appreciate the ways that the latter promotes fundamental aspects of our survival[2,3]. Regardless of its classification, the goal of each virus is universally the same: to transfer its genetic material into host cells and create more viruses. This seemingly straightforward goal is replete with challenges; namely, most viruses are not on the guestlist for admission to the exclusive party in our body. Undeterred, these con artists have evolved clever strategies to disguise themselves as friends and to forge their own invitations. A prime example — the ongoing SARS-CoV-2 pandemic — has illuminated how adept viruses can be at sweet-talking their way in.

All cells wear a carbohydrate coat. Glycans (glycoproteins, glycolipids and polysaccharides) make up this coat, radiating from the cell surface and facilitating communication and engagement with the surrounding environment. Although glycans are present across all domains of life, we are still adding to their already known functions in health and disease[4]. These enigmatic modifications are installed onto lipids or proteins by a suite of enzymes that act in concert to synthesize, modify and attach cognate glycans to biomolecules in and on our cells[5]. As cells divide and differentiate during human development, unique glycan signatures on cell surfaces help distinguish cell types from one another. Our bodies are trained to monitor these extracellular glycans and, importantly, to read glycan signatures to distinguish self from non-self. In short, if viruses want to gain entry to our bodies and stay there, they must adhere to our dress code.

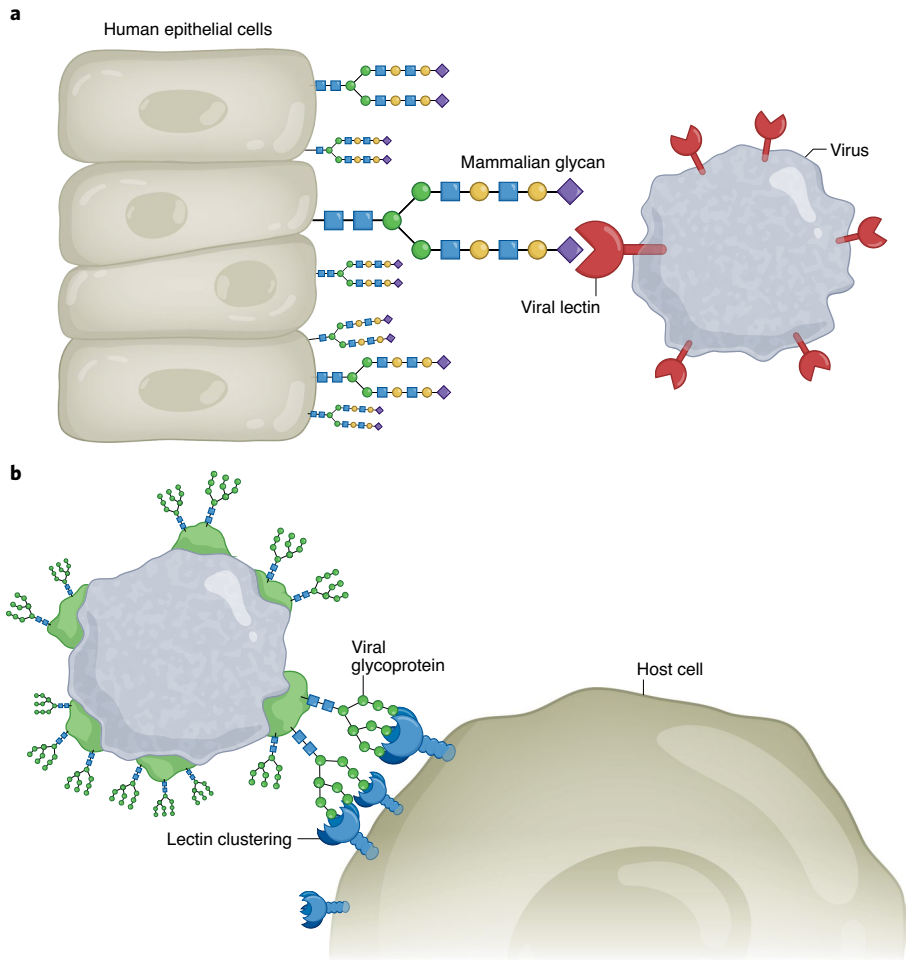

Fig. 1 | Viruses and mammalian cells both express lectins. a, Viral lectins or adhesins (red) recognize mammalian glycans to facilitate viral entry. **b**, Lectins (blue) on host cells can recognize viral glycans, leading to clearance or viral uptake. The glycans are depicted using the standard code.

Masters of disguise

Viruses can cleverly cloak their surfaces with a glycan coat that mimics the sugar chains found on host cells[6]. Enveloped viruses (for example, influenza A, measles, human immunodeficiency virus (HIV) and coronaviruses) are encapsulated in lipid envelopes decorated with densely glycosylated proteins. This capsule masks immunogenic viral components from the host immune system and facilitates viral attachment and entry into host cells

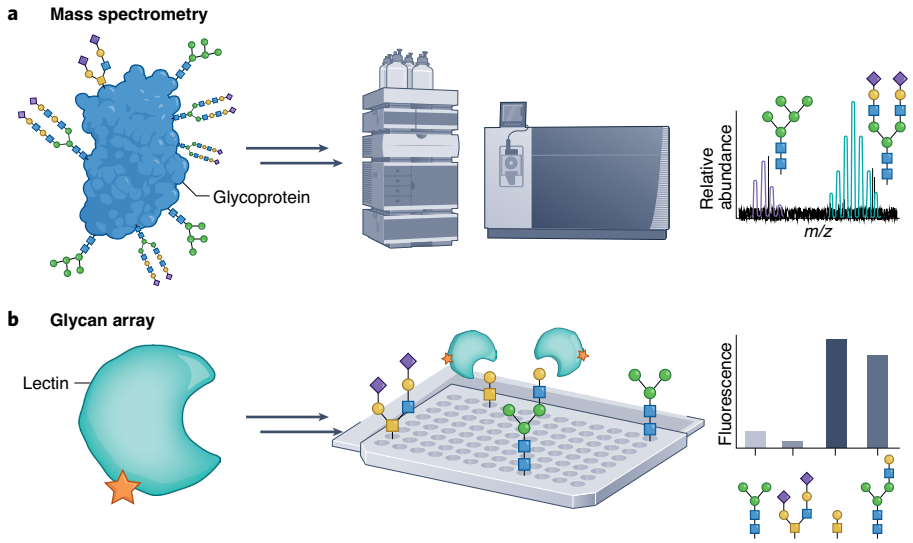

Fig. 2 | Elucidating viral glycosylation. a, MS yields information regarding glycan structures on viral (or host) proteins. Glycosylation is determined through mass-to-charge ratio (*m/z*), and the connectivity is typically inferred from known host biosynthetic pathways. **b**, Glycan arrays help determine the carbohydrate binding specificity of rapidly evolving or strain-specific viral adhesins.

(Fig. 1). Once inside, they hijack host protein-folding and glycosylation machinery to assemble new viruses disguised with host glycans. Non-enveloped, or naked, viruses (for example, hepatitis A, rotavirus and poliovirus) lack an attached lipid envelope, yet recent studies suggest that many package themselves into glycan-containing host membrane vesicles, or exosomes, to achieve the same effect[7,8].

Viruses are notorious for rapid acquisition of mutations and corresponding changes in virulence. As new strains of virus emerge, we are readily able to decode amino-acid mutations from sequencing data, yet the effects on viral glycosylation are far more challenging to predict. The glycoproteins embedded in enveloped viruses often serve as antigens to direct humoral responses. How does viral glycosylation change as viruses evolve and what are the consequences for host recognition and immunity? Changes in the location and composition of viral glycans may have dramatic effects on tissue tropism, immune activation and the efficacy of circulating antibodies and vaccination efforts. Thus, we need tools to address these questions. Methods for the analysis of viral glycosylation and its consequences are critical for understanding infection, immunity and immune evasion.

Analytical and computational approaches are essential components of glycan analysis (Fig. 2). Advances in mass spectrometry (MS) have been instrumental in characterizing the location and extent of viral glycosylation in emergent pathogens such as SARS-CoV-2, but certain caveats still remain[9–11]. To manufacture enough product for analysis while limiting exposure to infectious virions, MS analysis is routinely performed on recombinantly expressed viral proteins. As such, the cell type chosen for expression (for example, ovarian versus epithelial cells), as well as the protein expression system (bacterial, insect or mammalian), can result in dramatic differences in the observed glycosylation patterns on the viral protein under investigation. MS analyses can deconvolute glycan sequences and structures, but the connectivity of the sugars is inferred; that is, the data are based on assumptions about the type of glycan being analyzed (for example, *N*-glycosylation) and biosynthetic pathways present in the host[12]. In most cases, the method does not directly report on anomeric position and polysaccharide branching. Although these assumptions are generally warranted, they may break down in cells under stress, for example, during infection or in altered states of proteostasis[13,14].

However, MS studies do not only provide information on viral glycan identity, but also insight into how glycosylation may alter the conformational dynamics of glycoproteins. For example, MS identification of site-specific glycans on the SARS-CoV-2 spike protein helped guide molecular dynamic simulations to examine glycoprotein dynamics. These computational approaches, in combination with genetic and structural studies, afforded models that shed light on how glycan structures and dynamics contribute to host–virus interactions[15–17]. As these approaches evolve and improve, the field will be poised to predict how viruses and their variants employ glycan masks in cell attachment, infection or colonization. Looking forward, the development of complementary approaches that more rapidly fingerprint changes in viral glycosylation would be a welcomed addition to monitor emergent strains in real-time.

Getting past security: lectins as bouncers

The innate immune system is at the frontline of our response to microbial colonization and infection. A central component of innate immunity are lectins, or carbohydrate-recognition proteins, that help our bodies differentiate self from non-self (Fig. 1). Lectins involved in microbial recognition are characterized by their monosaccharide specificity and their structural architecture[18]. Most lectins oligomerize into higher-order structures. In this way, they achieve high avidity for the multivalent glycans found on host cells and viral envelopes.

Lectins are used by both the host and viruses. Viral lectins are used to adhere to host tissues, as in the case of influenza virus, which binds to sialic-acid residues that coat our airways[19]. Influenza is just one of the many viruses that encode lectins and adhesion proteins to latch onto glycans and glycoproteins on host cells and thereby force viral entry[20–22]. As another example, the proteoglycan heparan sulfate is a popular target for viral adhesion proteins, including those found on SARS-CoV-2, HIV, papillomaviruses, dengue viruses and hepatitis C, among others[23–25]. However, a comprehensive understanding of other host cell glycan targets for viruses is lacking. Clustered regularly interspaced short palindromic repeats (CRISPR)-based strategies have been used recently to reveal host factors that are involved in coronavirus infections[26], but genetic modifications can have deleterious and pleiotropic phenotypes that may be challenging to parse.

Chemical biology approaches complement genetic efforts and provide powerful tools in glycomics research. A prime example is live cell proximity-based tagging, which offers an impressive route to elucidate the carbohydrate targets of glycan-binding proteins[27–29]. Applying this approach to viral adhesins could reveal a comprehensive portrait of adhesin–carbohydrate interactions. Similarly, synthesis and incorporation of non-natural sugars into the host glycome can help address this question. In this

approach, synthetic monosaccharides functionalized with covalent capture and label transfer chemical handles are metabolically incorporated or appended via glycosyltransferases into the host extracellular glycocalyx[30,31]. Activation of these non-natural sugars in the presence of a binding partner facilitates capture of proteins that recognize these monosaccharides. One could easily envision how the development and application of chemical approaches could advance our understanding of viral pathogenesis.

Viral entry can also be mediated by viral glycans interacting with host lectins. Which lectins are targeted by viruses during infection? This question is central to viral pathogenesis. Lectins exist as secreted or cell-bound receptors, and their expression is tissue specific. Elucidating lectin–virus interactions can thus help reveal the tissue-specific tropism of human viruses. Of particular interest are viruses can that enter through one cell type and become infectious in another cell type. For example, the lectin DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) facilitates the uptake of HIV into dendritic cells. These antigen-presenting cells then introduce HIV to T cells, which are the major cell type for infection[22]. Similarly, SARS-CoV-2 begins its infection in the respiratory tract, yet pleiotropic effects have been observed in the neurological, cardiovascular and gastrointestinal systems. Elucidating the molecular underpinnings of these observations is an area of active investigation; we suspect changes in glycan recognition contribute to these phenotypes.

Customized camouflage: tailoring of viral glycans

Viruses can alter their glycan shield by perturbing host glycosylation and proteostasis pathways, and by encoding their own glycosidases and glycosyltransferases that prune and elaborate glycans. By modifying their sugary coat, viruses can tune their attachment to host lectins and, consequently, either promote infection (lectin-mediated uptake of viruses) or evade lectin detection and clearance. One way to explore viral glycosylation is lectin arrays[32]. Lectin arrays serve as excellent platforms to characterize altered glycosylation states during infection and reveal the human lectins that engage viruses[33]. In this approach, glycosylated biomolecules, cells or viruses are applied to an array of immobilized lectins with varying monosaccharide specificities[32]. Bound biomolecules are detected via fluorescence, thus revealing the lectins that attach to their glycans. Expansion of mammalian lectins on these arrays can help identify lectins that may target viruses for uptake or expulsion. Toward this goal, efforts to produce sufficient quantities of properly folded and functional recombinant human lectins should be prioritized.

Recently, lectin arrays revealed how perturbations to proteostasis and glycosylation are intimately linked[14]. Accordingly, many viruses have mechanisms to control unfolded protein responses in the cell, which gives insight into additional mechanisms by which viruses may alter their patterns of glycosylation to manipulate lectin–virus interactions and promote infection[33]. The relationship between proteostasis and glycosylation is supported by previous observations documenting changes in cellular glycosylation during infection[13]. Alterations to host glycosylation can affect cell signaling, adhesion and differentiation, among others. Aberrations in cellular glycosylation have been mapped in tumor biopsies using MS imaging — a technique that has revealed differences in the patterns of glycosylation in the context of cancer progression[34]. Application of this approach to viral infected tissues could therefore reveal changes in the mammalian glycome in the context of infection and colonization. With this information, we could gain insight into how viral infection leads to downstream effects in cell signaling and recognition (Fig. 2).

Moreover, recent advances in glycan visualization offer opportunities to track how cellular glycosylation patterns change over the course of infection. In one approach, non-natural sugars are metabolically incorporated into extracellular mammalian glycans[35]. These carbohydrates are functionalized with bio-orthogonal conjugation handles that facilitate visualization of cell glycans following a reaction with a 'clickable' fluorophore. More recently, biosynthetic incorporation of non-natural carbohydrates offers another glycan visualization approach that bypasses the metabolic pathways previously required for incorporation[36]. Although pioneered in bacterial systems, its expansion to mammalian glycomes could report on glycan dynamics during viral infection.

Many questions remain in viral pathogenesis and mutualism. For example, what are the consequences of mounting an immune response against viruses that display self-glycans? A correlation between viral infection and humoral autoimmune responses has been documented, but the contribution of glycans is unclear[37,38]. Glycan arrays may offer insight into the carbohydrate-binding specificity antibodies from convalescent patients. A recent study using glycan arrays revealed that patients convalescent for SARS-CoV-2 possessed antibodies capable of recognizing self-glycans, including gangliosides, *N*-glycans, *N*-acetyllactosamine (LacNAc) and sialyl Lewis^X (ref. [39]). Autoimmune targeting of these epitopes may in part explain the symptoms observed in tissues distal to the primary infection site in SARS-CoV-2 patients. Furthermore, how do mutations in viral adhesins influence glycosylation and carbohydrate recognition? With regard to the former, changes in spike protein glycosylation influence viral neutralization[40]. What will be the glycan specificity for the next viral pathogen? Understanding the structure–function relationships in both host and viral glycan recognition is critical.

Conclusion

As the SARS-CoV-2 pandemic appears to relent in its severity, we reflect on the many unknowns left in its wake. Will new emerging variants, with altered glycan shields, be able to thwart global vaccination efforts? What are the short- and long-term effects of SARS-CoV-2 infection on host glycosylation, especially in tissues with low capacity for cellular renewal (for example, brain and heart tissue)? We need means to address these questions, including methods to assess alterations in the glycosylation of infected cells, to profile viral interactions with human lectins and image changes in viral glycosylation, and to evaluate the effects of viral glycosylation on antibody neutralization. New tools and research programs that address these needs will be critical to combat and manage the next sweet-talking virus that walks into the room. ❐

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References

1. Griffiths, D. J. *Genome Biol.* **2**, reviews1017.1–reviews1017.5 (2001).
2. Adiliaghdam, F. & Jeffrey, K. L. *Genome Med.* **12**, 66 (2020).
3. Magiorkinis, G., Katzourakis, A. & Lagiou, P. *Trends Microbiol.* **25**, 876–877 (2017).
4. Ohtsubo, K. & Marth, J. D. *Cell* **126**, 855–867 (2006).
5. Schjoldager, K. T. et al. *Nat. Rev. Mol. Cell Biol.* **21**, 729–749 (2020).
6. Van Breedam, W. et al. *FEMS Microbiol. Rev.* **38**, 598–632 (2014).

7. Feng, Z. et al. *Nature* **496**, 367–371 (2013).
8. Chen, Y.-H. et al. *Cell* **160**, 619–630 (2015).
9. Watanabe, Y. et al. *Science* **369**, 330–333 (2020).
10. Shajahan, A. et al. *Glycobiology* **30**, 981–988 (2020).
11. Hargett, A. A. & Renfrow, M. B. *Curr. Opin. Virol.* **36**, 56–66 (2019).
12. Leymarie, N. & Zaia, J. *Anal. Chem.* **84**, 3040–3048 (2012).
13. Kreisman, L. S. & Cobb, B. A. *Glycobiology* **22**, 1019–1030 (2012).
14. Wong, M. Y. et al. *Proc. Natl Acad. Sci. USA* **115**, E10089–E10098 (2018).
15. Grant, O. C. et al. *Sci. Rep.* **10**, 14991 (2020).
16. Yang, Q. et al. *eLife* **9**, e61552 (2020).
17. Casalino, L. et al. *ACS Cent. Sci.* **6**, 1722–1734 (2020).
18. Wesener, D. A., Dugan, A. & Kiessling, L. L. *Curr. Opin. Struct. Biol.* **44**, 168–178 (2017).
19. Shinya, K. et al. *Nature* **440**, 435–436 (2006).
20. Koehler, M. et al. *Annu. Rev. Virol.* **7**, 143–165 (2020).
21. Li, Y. et al. *Front. Immunol.* **12**, 638573 (2021).
22. Dimitrov, D. S. *Nat. Rev. Microbiol.* **2**, 109–122 (2004).
23. Clausen, T. M. et al. *Cell* **183**, 1043–1057 (2020).
24. Liu, L. et al. *ACS Cent. Sci.* **7**, 1009–1018 (2021).
25. De Pasquale, V. et al. *Int. J. Mol. Sci.* **22**, 6574 (2021).
26. Wang, R. et al. *Cell* **184**, 106–119 (2021).
27. Joeh, E. et al. *Curr. Protoc.* **1**, e104 (2021).
28. Chang, L. et al. *J. Proteome Res.* **16**, 3929–3941 (2017).
29. Wu, G., Nagala, M. & Crocker, P. R. *Glycobiology* **27**, 800–805 (2017).
30. Tanaka, Y. & Kohler, J. J. *J. Am. Chem. Soc.* **130**, 3278–3279 (2008).
31. Liu, Z. et al. *Cell* **183**, 1117–1133 (2020).
32. Bojar, D. et al. *ACS Chem. Biol.* https://doi.org/10.1021/acschembio.1c00689 (2022).
33. Heindel, D. W. et al. *Proc. Natl Acad. Sci. USA* **117**, 26926–26935 (2020).
34. Drake, R. R. et al. *Adv. Cancer Res.* **134**, 85–116 (2017).
35. Laughlin, S. T. & Bertozzi, C. R. *Proc. Natl Acad. Sci. USA* **106**, 12–17 (2009).
36. Marando, V. M. et al. *J. Am. Chem. Soc.* **143**, 16337–16342 (2021).
37. Ahmed, S. S. et al. *Sci. Transl. Med.* **7**, 294ra105 (2015).
38. Cutillo, G., Saariaho, A.-H. & Meri, S. *Cell. Mol. Immunol.* **17**, 313–322 (2020).
39. Butler, D. L. & Gildersleeve, J. C. Preprint at *bioRxiv* https://doi.org/10.1101/2020.10.15.341479 (2020).
40. Nabel, K. G. et al. *Science* **375**, eabl6251 (2022).

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Competing interests
The authors declare no competing interests.

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Response under pressure: deploying emerging technologies to understand B-cell-mediated immunity in COVID-19

Critical technological advances have enabled the rapid investigations into the immune responses elicited by SARS-CoV-2, the pathogen responsible for the COVID-19 pandemic. We discuss the cutting-edge methods used to deconvolve the B-cell responses against this virus and the impact they have had in the ongoing public health crisis.

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The COVID-19 pandemic is extracting an enormous toll on human populations worldwide. As overwhelming evidence has accumulated to indicate the participation of inflammatory and autoimmune responses in adverse outcomes, there is a major need to understand the immunological underpinnings of protective and pathogenic responses to a life-threatening virus for which there was little, if any, preceding immunological memory. The confluence of public health need, scientific opportunity and unparalleled technological and computational tools has provided a unique opportunity to understand the underpinnings and broad heterogeneity of the human immune response in general, and particularly in the context of primary immune responses. The study of B-cell responses in this context, responsible for antibody production in both vaccination and infection, has been a critical point of focus throughout the pandemic in understanding natural immunity development against SARS-CoV-2, vaccine longevity and memory durability against emergence viral variants.

The rapid development of technology around immunologic investigation generally, and B-cell response monitoring specifically, has resulted in a robust experimental toolset capable of extracting significant data down to the single-cell level (Fig. 1). The emergence of these tools, and their application to critical areas of human health such as vaccination[1], infection and autoimmunity[2], has allowed for the creation of a framework for B-cell response classification and development. Advances in surface phenotyping have led to an increased depth of B-cell subset identification and correlated function[3]. Next-generation sequencing has provided understanding of developmental B-cell programs[4], with single-cell technology promising to push those efforts even further. Broad antigen-specific screening technologies combined with robust monoclonal antibody (mAb) production pipelines have enabled us to understand emerging antigen-specific responses and rapidly evaluate potential therapeutics[5].

With the new technological advances, B-cell immunologists are now perfectly poised to rapidly understand mechanisms of viral clearance, disease pathogenesis and immune protection in both infection and vaccination. Furthermore, some of these novel tools have been successfully deployed to develop mAb therapeutics against SARS-CoV-2 in weeks rather than years[5]. The result has been an explosion of understanding around humoral immune development in human viral infection. Although certainly not a comprehensive list, it is important to document how these technologies have contributed to our collective investigations in dissecting the immune responses surrounding COVID-19.

High-dimensional cytometry

Since its inception, flow cytometry has served as a cornerstone technology in the identification and classification of leukocytes into increasingly refined subpopulations[6]. As such, its ability to provide increased breadth or depth of cellular characterization is a direct reflection of the number of cellular markers that can be simultaneously