Published in final edited form as:

Curr Opin Virol. 2015 April ; 11: 63–69. doi:10.1016/j.coviro.2015.02.002.

Targeting host-derived glycans on enveloped viruses for antibody-based vaccine design

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Abstract

The surface of enveloped viruses can be extensively glycosylated. Unlike the glycans coating pathogens such as bacteria and fungi, glycans on viruses are added and processed by the host-cell during biosynthesis. Glycoproteins are typically subjected to α -mannosidase processing and Golgi-mediated glycosyltransferase extension to form complex-type glycans. In envelope viruses, exceptions to this default pathway are common and lead to the presence of oligomannose-type glycan structures on the virion surface. In one extreme example, HIV-1 utilizes a high density of glycans to limit host antibody recognition of protein. However, the high density limits glycan processing and the resulting oligomannose structures can be recognised by broadly neutralising antibodies isolated from HIV-1 infected patients. Here we discuss how divergence from host-cell glycosylation can be targeted for vaccine design.

Introduction

The glycan structures coating the surface of bacteria, fungi, parasites and viruses are critical for disease transmission through interaction with host receptors, in particular lectins, and in shielding pathogens from the immune system. Since the discovery that conjugation of polysaccharides to carrier proteins can lead to successful T cell dependent immune responses to carbohydrates, there has been significant success in the development of polysaccharide conjugate vaccines that protect against bacterial infections including *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis* and *Streptococcus pneumoniae* [1]. However, there are currently no carbohydrate-based vaccines that protect against viral infection. In this review we explore the scope and potential for targeting the glycan structures on viruses for vaccine design with particular reference to HIV-1 where, in

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some patients, glycan-binding broadly neutralizing antibodies (bnAbs) are elicited during HIV-1 infection.

Viral glycosylation

Upon entry into a mammalian cell, a virus must replicate and produce new viral particles to sustain and spread infection. Viruses hijack the protein synthesis, glycosylation machinery and folding pathway of the host cell to produce the necessary proteins and glycoproteins required for virion production. In the endoplasmic reticulum (ER) Glc₃Man₉GlcNAc₂ is transferred to Asn residues within the glycosylation sequence Asn-X-Thr/Ser (where X can be any amino acid except Pro). Typically glycoproteins are next subjected to a very ordered pathway of glycosidase and glycosyltransferase enzymes that first see the glycan trimmed to Man₅GlcNAc₂. Diversification to complex-type glycans begins with addition of a β 1,2-linked GlcNAc residue to Man₅GlcNAc₂ in the medial Golgi apparatus. Further trimming and processing in the medial and late Golgi apparatus leads to a wide array of hybrid- and complex-type glycans and these structures are often dependent on the producer cell type [2].

Challenges for developing vaccines targeting viral glycan epitopes

Generation of antibodies to glycans has several challenges [3]. Firstly, due to the inherent weakness of carbohydrate-protein interactions binding affinities must be enhanced through avidity effects. Lectins for example are able to overcome this by using multiple carbohydrate binding domains to interact with arrays of glycan ligands. Secondly, glycoproteins usually always exist as a number of different glycoforms where the same protein backbone is glycosylated with different glycan structures [4]. This microheterogeneity weakens the antigenic response to the individual glycan structures. Further, these glycans are often dynamic and multiple conformations may be presented to the immune system further weakening the response. Thirdly, as glycosylation is ubiquitous to all mammalian cells, the host may display tolerance towards these sugars. Combined, these effects result in glycans being poorly immunogenic. The major concern, and potential limitation of generating antibodies against self-glycan structures, is their potential auto-reactivity and negative selection in vivo.

Envelope glycosylation exhibits features of self and non-self

Cases in which the viral glycosylation diverges from the typical pathway may present opportunities for exploiting viral glycosylation for vaccine design. The producer cell dependence of the Golgi processing phase gives rise to the capacity for viruses to exhibit antigenic shift both during inter- and intra-species transmission and this can be pronounced in inter-species transmission of enveloped viruses. At one extreme, in the initial infection of a human host by arthropod-borne arboviruses the virus displays insect-derived glycans. These are typically dominated by paucimannose structures but shift to human complex-type glycosylation as soon as viral production is established in the new host. An illustration of this antigenic shift has been revealed by the mass spectrometric analysis of Semliki Forest virus glycans derived from mammalian and insect cells [5]. Similarly, Dengue virus (DENV) is transmitted to humans via mosquitoes and therefore DENV Env produced in insect cells contains mostly oligomannose and paucimannose structures whereas virus Env produced in

primary dendritic cells contains complex sugars [6, 7]. These differences in glycan structures impact on binding to the viral entry factors DC-SIGN and L-SIGN and subsequently cell tropism [6].

A similar but subtler effect can even be detected during viral transmission between humans and derives from glycan modifications of the ABO blood group system. The carbohydrate epitopes have been detected on the surface of HIV-1 particles and anti-A and anti-B-group antibodies can infer some immunity to virus derived from A-positive and B-positive carriers, respectively [8]. It may be expected that antibodies to other non-human carbohydrate epitopes, such as alpha-Gal epitopes, may similarly impede intra-species viral transmission. The antiviral properties of naturally occurring anti-glycan antibodies may point to a universal vaccine strategy against viruses from particular non-human vectors.

Virion assembly and secretion pathway leave an imprint on the glycome

While cell-origin can influence the glycosylation of enveloped viruses, the viral structure and assembly pathway can also have a significant impact. A number of viruses display oligomannose-type glycans on their envelope glycoprotein and the mechanisms by which these oligomannose-type glycans are retained differ depending on the viral structure and secretion pathway. For example, unlike many enveloped viruses that bud from the infected cell membrane, HCV buds from the ER and as such the E1/E2 proteins that are incorporated into the virion contain almost entirely under-processed Man₈₋₉GlcNAc₂ sugars [9]. In the above example of Semliki Forest virus, a significant population of oligomannose-type glycans were retained even when derived from mammalian cells. Similarly, the Gc glycoprotein of Uukuniemi virus (UUKV), the model virus for the phlebovirus genus, is dominated by oligomannose-type glycans. These glycans provide the ligands for the attachment receptor, DC-SIGN [10]. The abundance of complex glycans on UUKV Gn together with a trace population of *N*-acetylpolylactosamines is consistent with viral budding from the medial-Golgi apparatus and a steric mechanism that shields Gc glycans from α -mannosidase processing [11].

Ebola virus envelope trimer has two highly glycosylated domains; the glycan cap and the mucin like domain [12]. Due to the inherent difficulties of working with this virus the glycosylation of GP1/GP2 has only been studied using recombinant proteins. The glycans released from GP consist of oligomannose, hybrid, bi-, tri- and tetra-antennary compounds [13, 14]. The high degree of glycan processing implies that the glycans are largely accessible to glycosidase and glycosyltransferases. Similar studies with recombinant attachment glycoproteins from Nipah, Hendra and Machupo viruses (the latter being the causative agent of Bolivian haemorrhagic fever) revealed similar highly processed structures typical of secreted and cell surface glycoproteins [15–17]. In this way, the self-glycans can be seen as an immunologically silent cloak covering the viral protein.

Dense clustering of glycans on HIV-1 leads to divergence from self

The extremely high density of glycan on the HIV-1 viral spike is unusual amongst enveloped viruses. The HIV-1 envelope glycoprotein consists of a trimer of a gp120 and gp41 heterodimer. Each gp120 subunit has a median of 25 N-linked glycosylation sites [18] and

approximately 50% of its mass consists of carbohydrate making it one of the most heavily glycosylated proteins known. Although the glycans are added by the host-cell as described above, analysis of the glycans released from recombinantly expressed gp120 has revealed an unusual and conserved population of Man₈₋₉GlcNAc₂ structures [19–23]. It has been shown that the tight clustering of glycans on Env prevent the ER α -mannosidase I enzyme from accessing its substrate and glycans are trapped as Man₈₋₉GlcNAc₂ [20]. These oligomannose-type glycans form a cluster on the envelope surface, often referred to as ‘the mannose patch’ or ‘intrinsic mannose patch’ (IMP), that is present across all viral clades (Figure 1) [19, 20, 24]. The recent crystal and cryo-EM structures of a stabilised Env trimer mimic further demonstrated the close proximity of the N-linked glycans on HIV-1 [25–27]. Additional studies have shown that the abundance of oligomannose-type glycans is further increased in the context of the intact trimer [28] and on the virion surface [19, 20] leading to a ‘trimer-associated mannose-patch’ (TAMP). This increase likely arises from further restriction of the glycan processing enzymes due to additional protein-glycan and glycan-glycan interactions occurring at the interface of monomers within the trimer. Therefore although HIV-1 has used the host cell machinery for glycosylation of Env, the clustering of glycan sites creates a non-self glycan motif on gp120.

HIV-1 bnAbs target Envelope glycans

Despite the challenges described above some HIV-1 infected individuals develop antibodies capable of binding the glycans on HIV-1 Env. Approximately 10–30% of HIV-1 infected individuals develop broadly neutralizing serum after 3 years of infection [29]. Over the last 5–10 years there has been a considerable effort to identify sites of vulnerability on the HIV-1 envelope glycoprotein that can be targeted for vaccine design. Several groups have isolated bnAbs from such individuals. These bnAbs are extremely broad and potent [30–35], e.g. PGT128 can neutralize 72% of circulating HIV-1 strains with median IC₅₀ of 0.02 μ g/mL [30], and have been shown to protect against SHIV infection when passively administered to macaques at low serum concentrations [36, 37]. Therefore immunogens capable of eliciting these bnAbs would likely form an important component of an effective HIV-1 vaccine [38].

Until relatively recently, the glycosylation of HIV-1 Env has traditionally been referred to as the HIV-1 ‘glycan-shield’ or ‘silent-face’ due to the poor immunogenicity of carbohydrates and their ‘self’ nature. However, combined biochemical and structural analyses have revealed that a significant proportion of the isolated HIV-1 bnAbs bind to glycan structures on HIV-1 Env [29]. These bnAbs target three main regions on Env; i) the N332 glycan (e.g. 2G12, 10–1074, PGT121, PGT128, PGT135 [30, 39, 40]), ii) the N160 glycan (e.g. PG9, PGT145, CH04 [30, 33, 41]) and iii) the glycans at the gp120-gp41 interface (e.g. PGT151 [42] and 35O22 [43]) (Figure 2). 2G12 overcomes the weak carbohydrate-protein interactions by using a unique domain-exchanged structure in which the two variable heavy chain domains cross-over to form a multivalent binding surface [44, 45]. The remaining N332 bnAbs use long CDRH3 loops to penetrate through the glycan shield and interact with the V3/V4 loops, the N332 glycan and at least one other neighbouring glycan [25, 46, 47]. Similarly, the long CDRH3 loops of N160 binding bnAbs penetrate through the glycan shield and interact with the V1 loop, the N160 glycan and the N156 glycan [48–50]. The PGT151-Env interaction, although less characterised, appears to include either tri- and tetra-

antennary glycans on gp41 and protein residues in gp120 [42]. The common feature of the three HIV bnAb classes described is that the weak protein-carbohydrate interactions are overcome through multivalent binding to multiple glycans and protein residues within one Fab. Interestingly, crystal structures of Abs binding to other glycan antigens show whole saccharide units binding either within a pocket or groove [51, 52]. However, the majority of HIV glycan-binding bnAbs, with the exception of 2G12, bind across the face of the glycan [53]. Overall, unlike previously thought, isolation of glycan-binding bnAbs from HIV-1 infected patients strongly highlights the glycan structures on HIV-1 Env as a feasible target for HIV-1 vaccine design.

Perspective: Strategies for eliciting carbohydrate-binding antibodies

There are significant challenges to overcome in the development of vaccines that target viral glycan antigens. In the case of HIV-1 the immune system has overcome these challenges and several HIV-1 infected individuals have developed glycan-binding HIV-1 bnAbs. These bnAbs typically target the non-self cluster of oligomannose-type glycans on gp120. Attempts to elicit 2G12-like bnAbs exploiting the antigenic mimicry of yeast carbohydrates [54–56] or using synthetic derived oligomannose clusters has had very limited success [57] presumably due to the requirement of domain-exchange for neutralization and challenges associated with eliciting antibodies with this unusual structure [58]. The challenge now is to develop glycan-based immunogens that have a glycan-protein signature similar to that on HIV-1 virions [19, 20, 59] and that elicit bnAbs capable of binding multiple glycans and protein surfaces. Recent studies have revealed that some glycan-binding bnAbs, e.g. PGT121 and PGT128, must also actively avoid or accommodate additional glycans to bind potently [60, 61]. In these cases high levels of somatic hypermutation and insertions and deletions are critical for neutralization [47, 62, 63]. These findings highlight the need for immunogens to not only display the glycans important for binding but carefully positioned glycans that the antibody must evolve to avoid for potent neutralizing activity. Further, as it can take up to three years for these bnAbs to develop efforts in the HIV-1 vaccine field are also currently focussed on studying the evolution of glycan-binding bnAbs longitudinally to gain insight into how these bnAbs evolved during infection [62, 64, 65] and using this information for immunogen design.

It is clear that to generate vaccines against viral sugar antigens one must exploit their non-self features. What is not yet known is whether the glycan structures and arrangements on viruses other than HIV-1 will allow for development of potent neutralizing antibodies. Although an HCV and an influenza A neutralizing antibody (nAbs) have been identified that make minor contacts with glycans on E2 and hemagglutinin respectively [66, 67], the neutralization dependency on Env glycosylation was minimal. Lectins however have been shown to have anti-viral activity against HCV, Ebola, and DENV [68–71]. For example, cyanovirin (CVN) and scytovirin (SVN) that have specificity for oligomannose-type glycans inhibit entry of Ebola into Vero cells [68, 69]. Therefore if carbohydrate-binding antibodies could be generated against such viruses they could inhibit viral entry. Without antibody templates from which immunogens can be designed, the prospect of vaccines against the viruses discussed above might be more challenging. However with the advancements in glycan microarray technologies [72] and antibody isolation techniques [73] it may be

possible to screen for such neutralizing antibodies from serum of infected patients to identify such templates.

Acknowledgments

K.J.D. is supported by an MRC Career Development Fellowship (MR/K024426/1) and M.C. is supported by the Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery Grant (UM1AI100663) and the International AIDS Vaccine Initiative through the Neutralizing Antibody Consortium and Bill and Melinda Gates Center for Vaccine Discovery.

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Highlights

- Glycosylation of enveloped viruses exhibit features of self and non-self
- HIV envelope displays a conserved cluster of oligomannose glycans
- Divergence of HIV-1 envelope glycosylation from self forms a target for bnAbs
- HIV-1 glycan-binding bnAbs are elicited during natural infection

Figure 1. The HIV glycan shield

HIV-1 Env glycosylation contains a population of oligomannose-type glycans that can be ascribed to the intrinsic mannose patch (IMP) and trimer-associated mannose patch (TAMP). Monomeric gp120 expressed outside of the context of the trimer only contains the IMP. The Env protein surface (grey) is derived from the crystal structure [25] and the glycans have been modelled in green (Pritchard et al., unpublished). The glycan structure is $\text{Man}_9\text{GlcNAc}_2$.

Figure 2. Regions of immune vulnerability on HIV-1 Env

HIV-1 bnAbs target three main glycan sites; i) the N332 glycan (e.g. 2G12, 10–1074, PGT121, PGT128, PGT135 [30, 39, 40]), ii) the N160 glycan (e.g. PG9, PGT145, CH04 [30, 33, 41]) and iii) the glycans at the gp120-gp41 interface (e.g. PGT151 [42] and 35O22 [43]). The Env protein surface (grey) is derived from the crystal structure [25] and the glycans have been modelled in green (Pritchard et al., unpublished). Regions of vulnerability to bnAbs are indicated with only one copy per trimer shown for simplicity.