Chapter 6
Anti-Carbohydrate HIV Vaccine Design

Lai-Xi Wang, Paolo Costantino, Yu Geng, and Hong Zhang

Abstract HIV has evolved a number of strong defense mechanisms, including extensive glycosylation of its surface envelope glycoprotein, to evade host immune responses. Nevertheless, the discovery of a series of glycan-dependent broadly neutralizing antibodies (bNAbs) from HIV-infected individuals suggests that HIV's "glycan shield" also represents a possible Achilles' heel and thus an attractive target for vaccine design. In this chapter, we review current understanding of the glycan-associated epitopes of these bNAbs and discuss how this insight may translate into the engineering of a carbohydrate-based HIV vaccine.

Keywords HIV • Vaccine • Carbohydrate antigen • Epitope • Neutralizing antibody • Glycoprotein • Glycopeptide • Oligosaccharide

6.1 Introduction

An effective HIV-1 vaccine capable of eliciting broadly neutralizing antibodies (bNAbs) is still elusive despite tremendous progress in our understanding of the mechanism and pathogenesis of HIV-1 infection and substantial investments in vaccine clinical trials (Nabel 2001; Burton et al. 2004, 2012; Zolla-Pazner 2004; Stamatatos et al. 2009; Kim et al. 2010; Vaccari et al. 2010; Kwong et al. 2011, 2012).

L.-X. Wang (✉)
Institute of Human Virology and Department of Biochemistry & Molecular Biology,
University of Maryland School of Medicine,
725 West Lombard Street, Baltimore, MD 21201, USA
e-mail: LWang@som.umaryland.edu

P. Costantino
Novartis Vaccine and Diagnostics Research Center, Via Fiorentina 1, 53100 Siena, Italy

Y. Geng • H. Zhang
ProSci, Inc., 12170 Flint Place, Poway, CA 92064, USA

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Although HIV-1 has evolved a number of defense mechanisms to evade host NAb recognition (Wyatt and Sodroski 1998; Nabel 2001; Burton 2002; Calarota and Weiner 2003), the discovery of several classes of bNAbs that target distinct and accessible neutralizing epitopes on the HIV-1 envelope glycoprotein, the target of NAb responses, provides optimism that a vaccine can be designed that will elicit similar types of antibodies. Extensive glycosylation of the viral spike constitutes one of the mechanisms evolved by HIV-1 to evade immune responses. However, there is also good reason to consider viral glycans as a target for vaccine design: they are accessible to immune recognition at the viral surface and some form highly conserved antigenic structures. This conservation was first made apparent upon identification of the bNAb 2G12, which binds an oligomannose cluster comprising several N-glycans on HIV-1 envelope glycoprotein (Trkola et al. 1996; Sanders et al. 2002; Scanlan et al. 2002; Calarese et al. 2003). For over a decade, 2G12 was the only bNAb that is specific for HIV-1 carbohydrate antigens. However, this situation has changed recently with the discovery of more than a dozen potent and broadly cross-reactive NAbs (e.g., PG9 and PG16, PGT121–128, PGT130–137, and PGT141–145), the specificities of which are highly glycan-dependent (Walker et al. 2009, 2011b). X-ray crystallography studies show that these antibodies target conserved glycopeptide epitopes (McLellan et al. 2011; Pejchal et al. 2011). In addition, high-mannose glycan-specific NAbs have been detected during natural HIV-1 infection in humans (Walker et al. 2010; Lavine et al. 2012; Moore et al. 2012) and one study found that a chimeric simian-human immunodeficiency virus (SHIV)-infected macaque developed particularly potent cross-clade NAbs that specifically bound to high-mannose glycan epitopes on the HIV spike (Walker et al. 2011a). Equally encouraging, passive immunization with anti-glycan NAbs has shown to provide protection against SHIV mucosal infection in macaque models (Mascola et al. 2000; Hessell et al. 2009; Moldt et al. 2012). Taken together, these studies attest to the potential and feasibility of exploring the glycans that decorate the surface of the HIV envelope spike as targets for vaccine design. This chapter highlights current understanding of the carbohydrate epitopes of several glycan-dependent bNAbs with implications for exploring these epitopes as templates for vaccine design. In particular, the design, synthesis, and immunological studies of 2G12 epitope-based immunogens are described in detail.

6.2 Structure and Function of HIV Glycosylation

6.2.1 Structural Features of Glycosylation of HIV-1 Gp120

The HIV-1 envelope glycoprotein spike consists of the subunits gp120 and gp41. Gp120 is heavily glycosylated, with the carbohydrates comprising half of the total molecular mass. N-glycosylation is the predominant form of gp120 glycosylation, with a typical gp120 possessing 24–25 conserved N-glycosylation sites (NXS/T;

where X is any amino acid except proline). O-glycosylation of gp120 is rarely reported but one recent report suggests the presence of an O-linked glycan at Thr-499 (HXB2 numbering) of some HIV-1 gp120 glycoproteins (Go et al. 2013). This result is consistent with early immunochemical studies implicating the presence of O-linked glycans on gp120 (Hansen et al. 1990, 1991, 1996). The trans-membrane envelope glycoprotein gp41 has four conserved N-glycosylation sites that are normally occupied (Lee et al. 1992a; Perrin et al. 1998).

A notable feature of HIV-1 glycosylation is the tremendous heterogeneity in both the frequency with which a glycosylation site is occupied and the nature of the particular glycan attached at a given site (Geyer et al. 1988; Mizuochi et al. 1988, 1990; Leonard et al. 1990; Shilatifard et al. 1993; Yeh et al. 1993; Bolmstedt et al. 1997; Butters et al. 1998; Zhu et al. 2000; Go et al. 2008, 2009; Doores et al. 2010a; Raska et al. 2010; Bonomelli et al. 2011). For example, analysis of the glycosylation pattern of a recombinant HIV-1_{IIIB} gp120 expressed in Chinese hamster ovary (CHO) cells showed that all 24 potential N-glycosylation sites in the gp120 analyzed were utilized. Among them 13 sites were occupied by complex-type N-glycans and 11 sites by high-mannose- or hybrid-type N-glycans (Leonard et al. 1990). However, subsequent studies have demonstrated that glycosylation profiles, including the ratio of high-mannose- to complex-type, the decorations (e.g., sialylation and core fucosylation) and branching of the N-glycans, can vary significantly, depending on whether the gp120 is virus-associated or recombinant, the particular virus strain from which the gp120 is derived and, in the case of recombinant gp120, the specific cells used for producing the gp120 (Geyer et al. 1988; Mizuochi et al. 1988, 1990; Leonard et al. 1990; Shilatifard et al. 1993; Yeh et al. 1993; Bolmstedt et al. 1997; Butters et al. 1998; Zhu et al. 2000; Go et al. 2008, 2009; Doores et al. 2010a; Raska et al. 2010; Bonomelli et al. 2011).

As discussed in more detail in Chap. 1, a particularly striking feature of HIV-1 envelope spike glycosylation is the high number of high-mannose type oligosaccharides in comparison to typical mammalian glycoproteins. For example, an early study found that the high-mannose type structures ($Man_{7-9}GlcNAc_2$) accounted for 80 % of the total N-glycans on gp120 recovered from lysates of infected T cells in culture, and more than 50 % of those on gp120 from cell-free culture supernatant (Geyer et al. 1988). More recently, the glycans of virion-associated envelope glycoprotein gp120 from primary isolates of HIV-1 (clades A, B, and C) as well as a simian immunodeficiency virus (SIV) were found to be predominantly high-mannose type N-glycans, independent of production system or viral clades (Doores et al. 2010a; Bonomelli et al. 2011). This was in stark contrast to recombinant monomeric gp120 which, as discovered early on (Leonard et al. 1990), bears more complex-type glycans than high-mannose glycans.

Early modeling of N-glycans on the crystal structure of the gp120 core (Kwong et al. 1998; Wyatt et al. 1998) revealed that some form clusters on gp120, in which large sections of the protein surface are covered up by carbohydrate structures (Kwong et al. 1998; Wyatt et al. 1998; Wyatt and Sodroski 1998). Since the glycans are produced by the host glycosylation machinery and therefore would be expected

to appear as "self" to the immune system, the carbohydrates provide a strong defense against host immune surveillance and limit NAb access to neighboring and underlying sequence-conserved segments of the protein backbone.

6.2.2 *Biological Functions of HIV Glycosylation*

Glycosylation plays critical roles in the folding, processing, and maturation of the HIV-1 envelope glycoprotein (Pal et al. 1989; Li et al. 1993). Appropriate N-glycosylation of gp120 is also important for HIV-1 infectivity (Lee et al. 1992b) and exerts a profound effect on the antigenicity and immunogenicity of the viral envelope glycoprotein. As demonstrated in an early study in rhesus monkeys, SIV mutants lacking N-glycans in the V1 region of gp120 were much more immunogenic than wild-type SIV (Reitter et al. 1998). In other studies, it was shown that deleting specific N-glycosylation sites on the HIV-1 envelope glycoprotein, such as the V1/V2 domains of HIV-1 gp120, rendered the underlying protein domains more vulnerable to antibody binding and dramatically increased sensitivity of the virus to antibody neutralizations (Wyatt et al. 1995; Cao et al. 1997; Kolchinsky et al. 2001a, b; Li et al. 2001, 2008). Thus glycosylation provides a strong defense to protect the virus from immune recognition and limit the effectiveness of antibody neutralization.

Comprehensive analyses of escape mutations in the HIV-1 envelope glycoprotein during natural infection have highlighted the "dynamic glycosylation" mechanism by which HIV can evade host NAb responses (Wei et al. 2003). It was shown that a high frequency of mutations occurred at consensus N-glycosylation sites (NXS/T) but, interestingly, the mutations usually resulted in a reposition or shifting of the conserved N-glycosylation sites, keeping the global glycosylation and the total numbers of N-glycans relatively constant. This "evolving glycan shield" represents an elegant mechanism for HIV-1 to maintain replication under the persistent pressure of host immune surveillance.

In addition to the role of glycosylation in evading immune responses, HIV-1 glycans also play an active role in promoting HIV-1 infection and transmission, via their interactions with various glycan-binding proteins on the host cells. As discussed in more extensive detail in Chap. 2, high-mannose type on HIV-1 gp120 can serve as ligands for DC-SIGN, a dendritic cell-specific C-type lectin, to mediate HIV-1 transmission from the mucosal infection sites to secondary lymphoid organs, where the virus effectively infects T cells (Geijtenbeek et al. 2000; Feinberg et al. 2001, 2007; Mitchell et al. 2001; Hong et al. 2002; Su et al. 2004; Snyder et al. 2005). High-mannose glycans on gp120 can also be bound by mannose receptors such as expressed on macrophages, facilitating viral infection of macrophages (Stahl et al. 1978; Shepherd et al. 1981; Larkin et al. 1989; Ji et al. 2005). However, some nonmammalian lectins and other glycan-binding compounds exhibit potent antiviral activities (Barrientos and Gronenborn 2005; Balzarini 2006, 2007; Anderluh et al. 2012) and, as reviewed in Chap. 7, are being developed as potential HIV microbicides.

6.3 Glycan-Dependent Epitopes of Broadly Neutralizing Antibodies

For many years, 2G12 was the only NAb described that binds carbohydrates on HIV-1 gp120. However, concerted efforts to understand the unusually broad humoral immune response in some HIV-1-infected individuals have led to the identification of additional glycan-dependent bNAbs in the past several years. These include the somatic relevant bNAbs PG9 and PG16, as well as the PGT series antibodies, such as PGT121–123 and PGT125–128. These newly discovered bNAbs represent a new class of bNAbs; unlike 2G12 these new antibodies target glycopeptide epitopes in the variable domains of gp120 and are able to neutralize with remarkable breadth and potency. Here we describe briefly what we know about the structural features of the neutralizing epitopes of these glycan-dependent antibodies, which forms the basis for HIV-1 vaccine design. For a more extensive review of the different binding modes of these glycan-dependent antibodies, the reader is referred to Chap. 5.

6.3.1 The Epitope of Broadly Neutralizing Antibody 2G12

Human monoclonal antibody 2G12 is one of the first bNAbs identified from HIV-infected individuals (Trkola et al. 1996). Initial epitope mapping suggested that 2G12 was largely dependent on glycans at several conserved N-glycosylation sites including N295, N332, N386, N392, and N448 (Trkola et al. 1996). Since previous glycan profiling analysis indicated that all these sites were occupied by high-mannose type N-glycans (Leonard et al. 1990), these results suggested that 2G12 would most likely target a unique cluster of high-mannose N-glycans on gp120. Two subsequent studies, which used alanine scanning mutagenesis and specific enzymatic glycan trimming coupled with 2G12-binding analysis, concluded that Manα1,2Man-linked residues were required for gp120 interaction with 2G12, and that the epitope of 2G12 might consist of several Manα1,2Man moieties contributed principally by high-mannose type N-glycans at N295 and N332, with glycans at neighboring sites (e.g., at N339, N386, and N392) playing an indirect role (Sanders et al. 2002; Scanlan et al. 2002).

Further characterization of the 2G12 epitope came from the X-ray structural study of 2G12 Fab in complex with synthetic disaccharide (Manα1,2Man) and a natural high-mannose N-glycan, $Man_9GlcNAc_2$ (Calarese et al. 2003). This structural study revealed an unusual Fab domain-swapped structure that created extended multivalent binding sites to accommodate several N-glycans. A total of four $Man_9GlcNAc_2$ moieties are bound to each Fab dimer: two correspond to the normal antibody combining sites and interact with D1 arm while two additional sites within the V_H/V'_H interact with the D2 arm of the $Man_9GlcNAc_2$ oligosaccharide. The main contact comes from interactions between 2G12 Fab and the D1 arm tetrasaccharide of $Man_9GlcNAc2$ (Fig. 6.1).

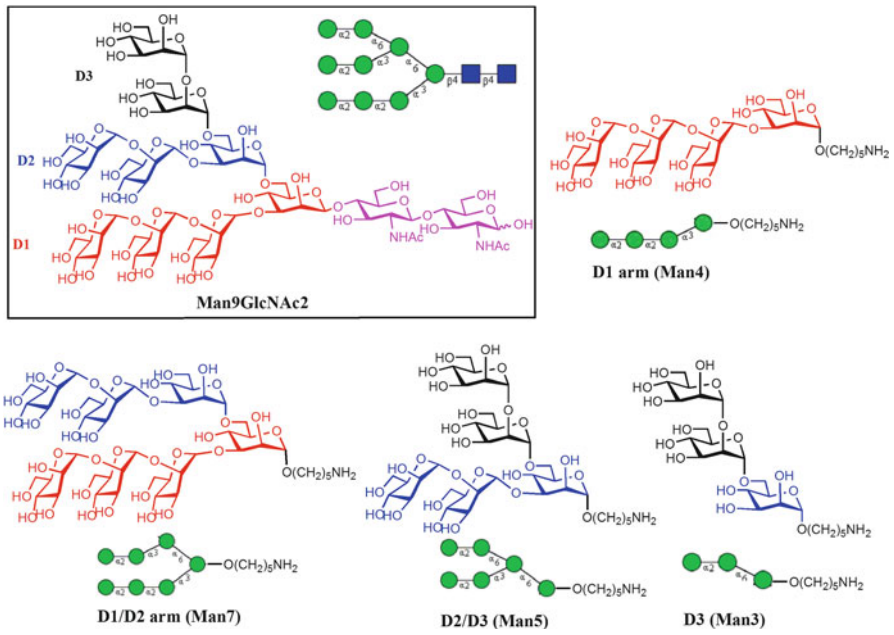

Fig. 6.1 Chemical structures of natural and synthetic high-mannose type glycans used for structural and binding studies. The various structures are colored so as to highlight similarities (chitobiose/$GlcNAc_2$ core: *magenta*; D1 arm: *red*; D2 arm: *blue*; D3 arm: *black*). A schematic showing the nature of the glycosidic linkage between sugar residues is shown next to each chemical structure

While the enzymatic trimming analysis using α-1,2-mannosidase indicated that the Manα1,2Man unit was required for 2G12 binding (Sanders et al. 2002; Scanlan et al. 2002) and the X-ray structure revealed how $Man_9GlcNAc_2$ was recognized by 2G12 Fab, it was still not clear whether other high-mannose and/or hybrid N-glycans also contributed to 2G12 recognition. Several groups have applied a synthetic approach coupled with 2G12-binding analysis to address this question (Adams et al. 2004; Lee et al. 2004; Wang et al. 2004). In one study, Wong and co-workers synthesized a series of high-mannose oligosaccharides corresponding to $Man_9GlcNAc_2$ and tested their ability to inhibit the interaction between 2G12 and immobilized gp120 in an enzyme-linked immunosorbent assay (ELISA) (Lee et al. 2004). It was found that the D1 arm tetrasaccharide and the D2D3 pentasaccharide was almost equally efficient to inhibit 2G12 binding to gp120 as the natural full-size N-glycan $Man_9GlcNAc_2$, while the Manα1,2Manα1,6Man trisaccharide corresponding to the D3 arm was fivefold less active than the D1 arm oligosaccharide or the natural $Man_9GlcNAc_2$. Interestingly, the heptasaccharide that combined the D1 and D2 arm did not show further enhanced affinity for 2G12 in comparison with the D1 arm alone (Fig. 6.1). These glycan-binding specificities were further confirmed by additional X-ray crystallographic analysis of the complexes of 2G12 Fab and the synthetic high-mannose type oligosaccharides (Calarese et al. 2005). Independently,

Wang and co-workers used a set of pure natural high-mannose oligosaccharides ($Man_5GlcNAc$, $Man_6GlcNAc$, and $Man_9GlcNAc$) to probe the 2G12 binding in a competitive ELISA (Wang et al. 2004), while Seeberger and co-workers utilized a series of synthetic oligosaccharides in a glycan microarray format to analyze the binding of different oligosaccharides to 2G12 (Adams et al. 2004). Both aforementioned studies confirmed the requirement of a Manα1,2Man subunit for 2G12 binding. In another independent study, Danishefsky and co-workers performed chemical synthesis of several glycopeptides carrying $Man_9GlcNAc_2$ and hybrid oligosaccharides (Dudkin et al. 2004; Geng et al. 2004; Mandal et al. 2004). Surface plasmon resonance (SPR) analyses showed that 2G12 had significantly higher affinity for a dimerized glycopeptide carrying a $Man_9GlcNAc_2$ glycan than for the corresponding monomer glycopeptide, indicating the importance of multivalency for adequate 2G12 recognition (Dudkin et al. 2004). The lack of 2G12 binding to glycopeptides containing hybrid-type N-glycans also confirmed that hybrid type N-glycans on HIV-1 gp120 are not part of the 2G12 epitope (Dudkin et al. 2004). Taken together, these mutational, biochemical, structural, and synthetic studies show that the epitope of antibody 2G12 is a terminal Manα1,2Man subunit, contributed by two main high-mannose type glycans (most likely at sites of N295 and N332 or N392) located within a unique oligomannose cluster on gp120 of vicinal glycans that maintain epitope conformation. These studies have provided the basis for the design and synthesis of oligomannose clusters as mimics of 2G12 epitope, which may be used as immunogens to raise 2G12-like antibodies (vide infra).

6.3.2 The Putative Epitopes of Broadly Neutralizing Antibodies PG9 and PG16

In 2009, over a decade after 2G12 was first described, two new glycan-dependent NAbs, PG9 and PG16, were reported (Walker et al. 2009). These antibodies were isolated from an HIV subtype A-infected individual whose serum demonstrated significantly broad and potent neutralizing activity against primary HIV-1 strains (Walker et al. 2009). PG9 and the somatically related PG16 were shown to neutralize 70–80 % of circulating HIV-1 isolates and, most strikingly, their neutralizing potency was up to tenfold higher than that of the previously reported bNAbs including 2G12 (Walker et al. 2009). Initial studies suggested that PG9 and PG16 only bind membrane-anchored trimeric forms of the HIV envelope glycoprotein (Walker et al. 2009), although later studies have shown that both antibodies can bind select monomeric gp120s and trimeric gp140s (Davenport et al. 2011; Hoffenberg et al. 2013). Site-directed mutagenesis analysis mapped the epitopes to a V1V2 domain peptide, with particular dependence on N-glycans located at two conserved N-glycosylation sites, N160 and N156 (HXB2 numbering) (Walker et al. 2009; Doores and Burton 2010), suggesting that conserved glycopeptide sequence located in the V1V2 domain of gp120 most likely constitute the epitopes of PG9 and PG16. Kwong and co-workers subsequently reported the crystal structures of PG9 Fab in

complex with scaffolded V1V2 domains derived from two HIV-1 strains, the CAP45 and ZM109 strains (McLellan et al. 2011). The study revealed a novel antigen recognition mode for PG9 and showed that a $Man_5GlcNAc_2$ N-glycan at position N160 was a major contact site for the antibody, while additional contributions were made by the interactions with another N-glycan at N156 (CAP45 strain) or N173 site (ZM109 strain) as well as mostly main-chain contacts with a strand of the V1V2-derived peptide. Interestingly, it was found that the N-glycans at the N156 (CAP45 strain) and the N173 (ZM109 strain) sites were spatially equivalent to each other in the three-dimensional structures for recognition with PG9. This novel recognition mode partially explains why PG9 neutralizes diverse HIV-1 primary strains.

While the aforementioned mutational and structural studies suggest that conserved V1V2 glycopeptides likely constitute the epitope of PG9 (and also likely PG16), the nature of the N-glycans attached at the two conserved sites still need to be further characterized. A $Man_5GlcNAc_2$ glycan at the N160 site seems required as the structures of the Fab/V1V2 domain complexes clearly show the intensive interactions of PG9 Fab with most of the residues in the $Man_5GlcNAc_2$ moiety. However, the nature of the N-glycan at the secondary site (N156 or N173) was not well defined in the crystal structures. Preliminary studies with a series of synthetic V1V2 glycopeptides carrying defined N-glycans at the N156 and N160 sites suggest that a $Man_5GlcNAc_2$ glycan at the N160 site is indeed essential for the recognition by PG9 and PG16 (Lai-Xi Wang et al., unpublished results). Moreover, a sialylated complex type N-glycan at the secondary glycosylation site seems critical for high-affinity interaction (Lai-Xi Wang et al., unpublished results). A recent study suggests that PG9, in addition to interacting with the N156 and N160 glycans, makes secondary interactions with an N160 glycan from an adjacent gp120 protomer in the antibody-trimer complex (Julien et al. 2013). Clearly, further fine characterization of the epitopes of PG9 and PG16 will provide important insight for HIV vaccine design.

6.3.3 The Putative Epitopes of the PGT Series of Broadly Neutralizing Antibodies

Soon after report of the PG9 and PG16 antibodies, a plethora of additional glycan-dependent bNAbs was reported (Walker et al. 2011b). The PGT series of bNAbs, such as PGT121–123 and PGT125–128, were isolated also from HIV-infected individuals whose sera exhibits exceptionally broad and potent HIV-neutralizing activity. Impressively, many of these new antibodies are more potent (up to tenfold) than the PG9 and PG16 antibodies, and thus up to 100-fold more potent than bNAbs such as 2G12 that were discovered earlier. Mutational and biochemical analysis indicated that the HIV neutralization by these antibodies is dependent on the V3 domain of gp120 as well as on the presence of N-glycans at the conserved N332 and/or N301 sites. In addition, these antibodies could bind efficiently to monomeric gp120. Glycan specificity analysis suggested that most of these antibodies recognized high-mannose type N-glycans in the context of the peptide domain, implicating novel V3

glycopeptides as the epitopes of these bNAbs (Walker et al. 2011b). This notion was reinforced by the X-ray crystal structures of complexes of PGT128 with $Man_9GlcNAc_2$ glycan as well as a glycosylated gp120 mini outer domain (Pejchal et al. 2011). This study, led by Wilson and Burton, revealed that the antibody was able to penetrate the glycan shield to recognize two conserved N-glycans (at N332 and N301 sites, respectively) and a short β-strand peptide segment at the stem of the gp120 V3 loop. More recently, Bjorkman and co-workers solved the crystal structure of another PGT antibody, PGT121, and analyzed the glycan specificity of PGT121 by glycan microarray technology (Mouquet et al. 2012). The crystal structural study indicated that PGT121 can recognize complex type N-glycans. An extended glycan microarray analysis confirmed that PGT121 could bind complex type N-glycans (sialylated or non-sialylated), but it did not show affinity for free high-mannose type N-glycans. However, further analysis indicated that PGT121 was able to bind the gp120 glycoform that carries only high-mannose type N-glycans. The nature of the epitope of PGT121 remains to be characterized. As the neutralization by PGT121 is dependent on the glycosylation at N332 and also likely on N301, further characterization of the nature of glycans attached at the N332 and N301 sites will provide novel glycopeptide templates valuable for immunogen design.

6.4 Synthetic Carbohydrate Antigens as Mimics of 2G12 Epitope

Previous mutational and biochemical studies suggest that a unique oligomannose cluster on gp120 constitutes the neutralizing epitope of antibody 2G12. To design an immunogen capable of raising 2G12-like antibodies, research has been focused on the design and synthesis of novel oligomannose clusters as mimics of 2G12 epitope and their application as components of immunogens.

6.4.1 Synthetic Oligomannose Clusters Based on Small-Molecule Scaffolds

The first attempt to mimic the 2G12 epitope by designing oligomannose clusters was described by Wang et al. (2004). After having determined by competitive ELISA that $Man_9GlcNAc_2$ was a better subunit than $Man_6GlcNAc_2$ or $Man_5GlcNAc_2$ for 2G12 recognition, further confirming the structural importance of terminal Manα1,2Man linkages in 2G12 epitope, they selected $Man_9GlcNAc_2$ as the oligomannose subunit and used galactose as the scaffold to present 2–4 copies of the $Man_9GlcNAc_2$ subunit. The choice of a galactose scaffold was based on the consideration that the oligomannose subunits being installed at the C-3, 4, and 6 positions of the galactopyranose ring would all face one side of the ring to form a cluster,

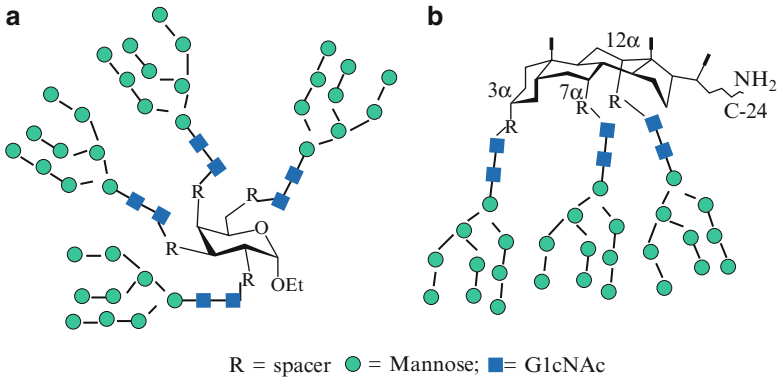

Fig. 6.2 Structures of small-molecule-scaffolded oligomannose clusters. (**a**) Oligomannose cluster using galactopyranoside as a scaffold; (**b**) oligomannose cluster using cholic acid as a scaffold. Particulars of the synthesis of these structures are reported elsewhere (Wang et al. 2004; Li and Wang 2004)

while the oligomannose sugar chain installed at position C-2 was likely to be located on the flank of the cluster. This design was hypothesized to form oligomannose clusters that mimic the 2G12 epitope on gp120. For this purpose, a galactopyranoside was functionalized with 2–4 maleimide groups and the $Man_9GlcNAc_2Asn$ was modified to install a thiol group. Chemoselective ligation between the thiol-tagged $Man_9GlcNAc_2Asn$ and the maleimide-functionalized scaffolds then led to the synthesis of bi-, tri-, and tetravalent galactose-based oligomannose clusters (Wang et al. 2004). The structure of a tetravalent $Man_9GlcNAc_2$ cluster is shown in Fig. 6.2a. The affinity of the synthetic clusters was evaluated by a competitive ELISA in which 2G12 binding to the immobilized HIV-1 gp120 was competed with the oligomannose clusters. The IC_{50} values decreased from 0.95 mM for $Man_9GlcNAc_2Asn$ to 0.013 mM for the tetravalent $Man_9GlcNAc_2$ cluster, clearly showing that 2G12 binds better to multivalently displayed oligomannose.

In an attempt to more precisely mimic the spatial configuration of the 2G12–gp120 interaction proposed by Wilson and co-workers, where the three $Man_9GlcNAc_2Asn$ glycans located at positions N332, N339, and N392 were proposed to interact with the domain-exchanged configuration of antibody 2G12 (Calarese et al. 2003), Wang and co-workers explored cholic acid as a new scaffold to present the oligomannose subunits (Li and Wang 2004). They derivatized the hydroxyl groups in position 3α, 7α, and 12α of cholic acid with a maleimide-functionalized spacer in such a way that the oligosaccharide attaching sites are at a distance comparable (between 12 and 20 Å) to that of the side chains of N332, N339, and N392. Coupling the thiol-tagged $Man_9GlcNAc_2Asn$ to the maleimide scaffold provided a novel trivalent oligomannose cluster (Fig. 6.2b). The affinity of the trivalent oligomannose cluster for 2G12 was then evaluated by measuring the inhibition of the 2G12–gp120 binding by competitive ELISA. The IC_{50} of the cluster was found to be ~20 μM, indicating a roughly 50-fold higher affinity than that of

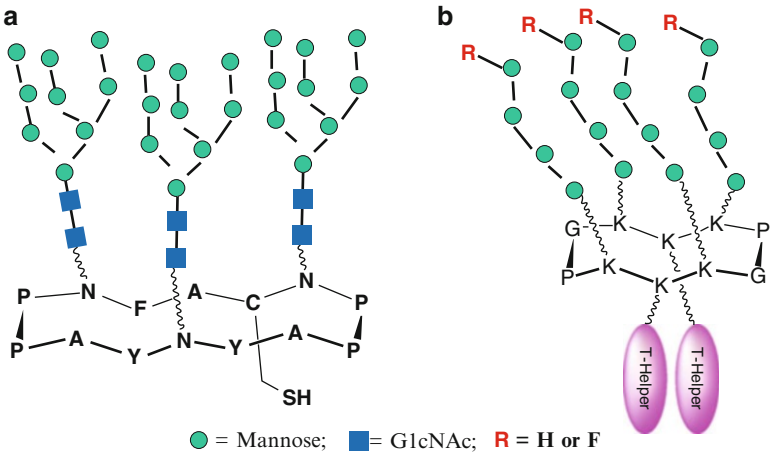

Fig. 6.3 Structures of cyclic peptide-based oligomannose clusters. (**a**) Synthetic $Man_9GlcNAc_2$ cluster (Krauss et al. 2007); (**b**) selectively fluorinated Man_4 cluster (Wang et al. 2007)

the $Man_9GlcNAc_2Asn$ subunit. Again, a clear clustering effect in 2G12 binding was observed for the synthetic mimic of 2G12 epitope. Despite the significant affinity enhancement of the tri- and tetravalent $Man_9GlcNAc_2$ glycan clusters in comparison to the oligomannose subunit, the affinity of the best synthetic epitope mimics for 2G12 was in the micromolar range, which was still distant from the nanomolar affinity between HIV-1 gp120 and 2G12. Thus, further work was necessary in order to achieve a better mimic of the 2G12 epitope, such as by optimizing the rigidity of the scaffold configuration.

6.4.2 *Oligomannose Clusters Based on Cyclic Peptide Scaffolds*

In order to design an effective structural mimic of 2G12 epitope, Danishefsky and co-workers used a cyclic peptide scaffold with built-in flexibility to present synthetic oligomannose glycans in a predetermined orientation, number, and distance (Krauss et al. 2007). They prepared two cyclic peptide scaffolds with handles for two or three $Man_9GlcNAc_2$ oligosaccharides, respectively, and one carrier protein (Fig. 6.3a). Using SPR, they studied the binding affinity for 2G12 of the mono-, bi-, and trivalent oligomannoses. They found that while the monovalent construct did not show a measurable response, the bi- and trivalent glycopeptides exhibited apparent binding to 2G12, suggesting the importance of multivalency for 2G12–glycopeptide interaction. Based on this observation, they conjugated the bivalent $Man_9GlcNAc_2$-cyclic peptide to the Outer Membrane Protein Complex (OMPC) of *Neisseria meningitidis* for immunization studies (Joyce et al. 2008).

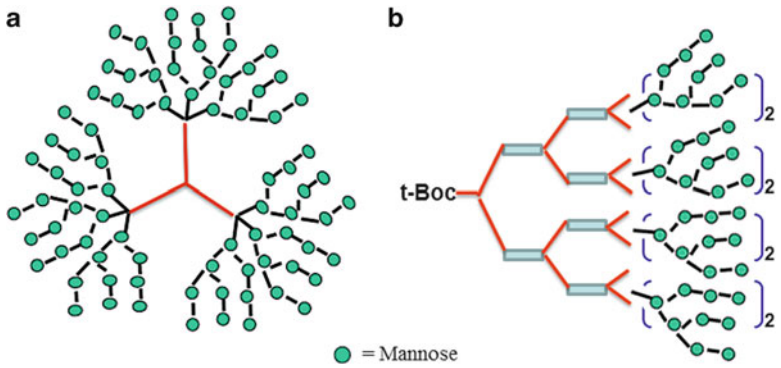

Fig. 6.4 Structures of dendron-based oligomannose clusters. (**a**) Glycodendrimer based on an AB3 type dendrimeric skeleton (Wang et al. 2008); (**b**) glycodendrimer based on a polyamidoamine (PAMAM) scaffold (Kabanova et al. 2010)

Independently, Wang and co-workers synthesized another cyclic peptide template, the regioselectively addressable functionalized template (RAFT), as a scaffold to construct clusters of the D1 arm of $Man_9GlcNAc_2$, as mimic of the 2G12 epitope. Using copper(I)-catalyzed 1,3-dipolar azide–alkyne cycloaddition (CuAAC), they coupled four α-linked Man_4 to one face of the cyclic peptide and two copies of a T-cell peptide to the other face (Wang et al. 2007) (Fig. 6.3b). In one construct the C6 position of the nonreducing terminal mannose of D1 was fluorinated in the hope of enhancing the immunogenicity if the construct moves on for immunization studies in animals. The binding affinity of the synthetic oligomannose clusters for 2G12 was evaluated by SPR analysis. As expected, the cyclic peptide-based oligomannose clusters displayed significant binding for 2G12 while the D1 arm oligomannose alone did not show any measurable binding. In addition, it was shown that the introduction of two T-helper peptides on the other face of the template did not affect the recognition of the oligomannose clusters by 2G12.

6.4.3 Synthetic Oligomannose Clusters Based on Dendron Scaffolds

To achieve high-affinity binding for 2G12, Wong and co-workers designed and synthesized oligomannose dendrons that display multivalent oligomannoses in high density (Wang et al. 2008). They used an AB3 type dendrimeric skeleton as the scaffold and applied the CuAAC reaction to attach 3 (first generation), 9 (second generation), and 27 (third generation) copies of synthetic D1 arm tetrasaccharide (Man_4) or Man_9 oligosaccharide on the dendrimeric scaffold. Competitive binding analysis showed that the dendrimers carrying 9–25 copies of the D1 arm Man_4 or the Man_9 subunits could bind to 2G12 very efficiently. In particular, a second generation (9-valent) Man_9 dendrimer (Fig. 6.4a) showed optimized affinity for 2G12

and the dendritic cell-specific lectin DC-SIGN (with an IC_{50} in the nanomolar range). Thus the synthetic glyco-dendrimers are comparable to HIV-1 gp120 in their binding to 2G12 and DC-SIGN, implicating their efficient mimicking of HIV-1 gp120 in terms of the presentation of the dense oligomannose clusters. These results suggest that the synthetic glyco-dendrimers may be further developed as candidate vaccines or as HIV microbicides for blocking virus transmission.

In another study, Costantino and co-workers utilized a polyamidoamine (PAMAM) scaffold to generate four- and eight-valent oligomannose clusters of HIV-1-related oligomannose antigens Man_4, Man_6, and Man_9 (Fig. 6.4b) (Kabanova et al. 2010). The ability of the different constructs to inhibit the binding of gp140 to 2G12 was measured by a competitive SPR assay. Multivalent presentation of oligomannoses increased the binding ability of Man_4 and Man_9 to 2G12. Disappointingly, the highest affinity 8-valent cluster, with eight copies of the Man_9 subunit, binds to 2G12 with an IC_{50} in only the micromolar range, which is comparable to the aforementioned small-molecule scaffold (galactose, cholic acid, and cyclic peptide)-based oligomannose clusters.

6.4.4 Oligomannose Clusters Based on Viral Capsids and Gold Nanoparticles

Finn, Burton and co-workers explored virus-like particles (VLPs) as scaffolds to present multiple copies of oligomannose in a rigid and highly repetitive fashion, aiming to mimic the high-density presentation of high-mannose glycans on gp120 (Astronomo et al. 2010). CuAAC chemistry was used for conjugation, which was achieved by chemically replacing surface-exposed lysine residues on the viral scaffolds with alkynyl groups, followed by reaction with azido derivatives of the oligomannosides (Fig. 6.5). To investigate the importance of the number and geometry of glycan conjugation points for recreating features of the HIV glycan shield and thus eliciting 2G12-like antibodies, oligomannosides were coupled to the surface of the icosahedral capsids of wild-type bacteriophage Qβ and cowpea mosaic virus (CPMV) which, although similar in size, differ in the geometric arrangement of surface-exposed lysines. Two Qβ mutants, dubbed QβK16M and QβHPG, were also explored. In mutant QβK16M, the lysine that is most exposed on wild-type bacteriophage Qβ is replaced by methionine, thus reducing the relative number of glycan conjugation sites on the capsid protein. In mutant QβHPG, the most-exposed lysine is replaced by an alkyne-containing unnatural amino acid (“HPG”), which enables attachment of different glycan types at different locations on the viral particle scaffold (Fig. 6.5). The oligomannose compounds Man_4, Man_8, and Man_9 were used for conjugation. The ability of the different capsid glycoconjugates to interact with 2G12 was analyzed by two different methods: (a) a conventional ELISA where serial dilutions of 2G12 IgG were allowed to bind antigens coated onto ELISA plates and (b) a 2G12 sandwich ELISA where glycoconjugates were captured onto microtiter plates coated with serial concentrations of 2G12 and the captured

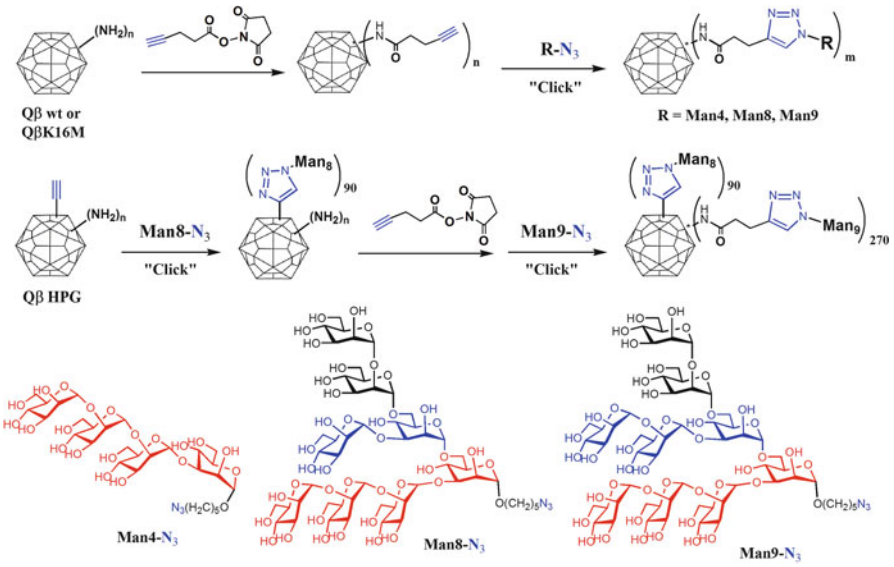

Fig. 6.5 Synthesis of the Qβ-based oligomannose clusters. *Top panel*: Synthesis of wild-type (wt) Qβ and mutant QβK16M glycoconjugates. *Middle panel*: Synthesis of QβHPG glycoconjugates QβHPG-Man_8 and QβHPGMan_8/Man_9. *Bottom panel*: Chemical structure of the azido-derived oligomannosides (Man_4, Man_8, Man_9) for conjugation to Qβwt and QβK16M. Details of the synthesis of these conjugates are reported elsewhere (Astronomo et al. 2010). Coloring of the chemical structures is the same as in Fig. 6.1

conjugates then detected with biotinylated 2G12. Although the Qβ (wild-type) and QβK16M conjugates carrying Man_4 and Man_9 showed nanomolar apparent affinities for 2G12, this was still 50–100-fold weaker than 2G12's apparent affinity for its cognate antigen gp120. Surprisingly, none of the CPMV conjugates were recognized by 2G12. Moreover, a bovine serum albumin (BSA)-$(Man_4)_{14}$ glycoconjugate also failed to interact with 2G12 in the aforementioned modified sandwich ELISA, suggesting that uncontrolled multimerization of oligomannose on a protein carrier might not be sufficient to correctly represent the 2G12 epitope.

In an approach similar to that by Finn and Burton described above, Davis and co-workers conjugated a non-self D1 tetrasaccharide (Man_4) derivative containing a C-6 methylated mannose residue at the nonreducing terminus to Qβ (Doores et al. 2010b). They found that the resulting VLP-based glycoconjugate was able to bind 2G12 even better than the D1 Qβ-Man_4 conjugate.

Penadés and co-workers instead explored gold nanoparticles (GNPs) as scaffolds to display oligomannose in a high-density three-dimensional arrangement of carbohydrate antigens mimicking the 2G12 epitope (Marradi et al. 2011). A tetra-mannoside-coated GNP with an average substitution degree of 7 showed the inhibition of the gp120–2G12 interaction, yet at a micromolar level of affinity, which is comparable to the GNP coated with an average of 56 copies of tetra-mannoside. In vitro assays demonstrated that the Man_4-GNPs conjugates could block the 2G12-mediated

neutralization of a replication-competent virus. An advantage of using GNPs as a scaffold is that additional components such as T-helper and adjuvants could be inserted into the scaffold to improve the immunogen design.

6.5 Immunogen Design and Immunization Studies

Carbohydrates are in general poorly immunogenic due to the lack of helper T cell epitopes and thus the inability to engage T cells. This has traditionally been overcome by conjugating carbohydrates to carrier proteins that provide T cell epitopes. The hapten-carrier concept has been known since 1929 when Avery and Goebel demonstrated that simple derivatives of glucose and galactose were able to induce specific antibodies in rabbits when conjugated to proteins, otherwise non-immunogenic (Avery and Goebel 1929). Glycoconjugate vaccines are among the safest and most efficacious vaccines developed to date. The first protein–polysaccharide conjugate vaccine was developed against *Haemophilus influenzae* type b (Hib), which was based on pioneering studies of John Robbins's team (Schneerson et al. 1980) and was licensed between 1987 and 1990. Research on glycoconjugate vaccines has been extended to target other life-threatening pathogens, such as *Neisseria meningitidis*, *Streptococcus pneumoniae,* and group B *Streptococcus* (Astronomo and Burton 2010), and nowadays conjugate vaccines against 13 most common pneumococcal serotypes and meningococcal serogroups A, C, W-135, and Y are commercially available. The conjugation approach is now being applied to the development of vaccines against fungal diseases, malaria, and noninfectious diseases, such as cancer, Alzheimer's, hypertension, autoimmunity, and drug abuse (Costantino et al. 2011).

The main aspects of adaptive immune responses to polysaccharide and glycoconjugate antigens have been covered by a number of excellent reviews (Kelly et al. 2005; Pollard et al. 2009; Astronomo and Burton 2010; Avci and Kasper 2010). Chemical conjugation of polysaccharides to protein carriers allows uptake and processing of the protein carrier by antigen-presenting cells (APCs) including dendritic cells, macrophages, and polysaccharide-specific B cells and presentation, on their surface, of the resulting peptides or glycopeptides in association with MHC class II. Further interaction with carrier-specific T cells then induces polysaccharide-specific B-cell differentiation, leading to a higher quality response characterized by immunological memory and boosting by repeated vaccine doses. However, despite the long history of glycoconjugate vaccines and their track records in term of safety and efficacy, their mechanisms of action are still not completely understood. In particular, the function of the sugar moiety during antigen presentation in association with MHCII complex and subsequent interaction with T cell receptor are still unclear. Knowing that carbohydrate antigens are T-independent antigens and that most of the carbohydrate antigens are not able to bind MCHII after APC processing, the elicitation of T-cell help by glycoconjugates has been attributed to the peptide moiety derived from protein processing. However, another mechanism has been

proposed by Kasper and co-workers (Avci et al. 2011). In their model, there are T-cell populations that recognize carbohydrate epitopes derived by APC processing of conjugate vaccines. When presented in association with MHCII, these epitopes recruit T-cell help for the induction of carbohydrate-specific antibodies. It is conceivable that more than one mechanism of interaction with T cells exists and contributes to the activation and differentiation of carbohydrate-specific B cells.

After identification of the 2G12 epitope on HIV gp120 and application of synthetic strategies to reproduce it, several research groups have moved on to formulating immunogens aimed at eliciting 2G12-like antibodies that recognize the same epitope on the surface of gp120 and have biological properties comparable to 2G12. Dealing with a carbohydrate epitope with a propensity of poor immunogenicity, the basic concepts and lessons learned from bacterial polysaccharide vaccine development have been applied to date in developing a carbohydrate-based HIV vaccine. Accordingly, most if not all of the synthetic constructs prepared to mimic the 2G12 epitope have been designed with the built-in functionality to link the selected oligomannose and/or its clusters to protein carriers or T cell epitope peptides. Major immunogens designed so far and tested in animals are summarized in Fig. 6.6.

6.5.1 Immunogens Based on Conjugation of Oligomannose Clusters and a Carrier Protein

Wang and co-workers conjugated the tetravalent $Man_9GlcNAc_2$ clusters presented on a galactose scaffold (Man_9 cluster) to the tetanus toxoid-derived T cell epitope peptide CGQYIKANSKFIGITEL or to KLH as carrier protein and tested these constructs (Fig. 6.6a) for their immunogenicity in rabbits (Ni et al. 2006). While moderate anti-carbohydrate antibodies were raised that recognized HIV-1 gp120, the majority of immune responses raised were against the maleimido linker used for the conjugation. The authors concluded that the selection of the linker in raising antibodies against particularly weak carbohydrate haptens seems critical. This is in line with other reports which indicated that constrained spacers such as cyclohexyl maleimide elicited a significant amount of undesirable antibodies (Peeters et al. 1989; Phalipon et al. 2009).

To test the efficacy of cyclic peptide-templated glycopeptides, Joyce et al. (2008) conjugated a bivalent glycopeptide to the powerful immune-stimulating OMPC of *Neisseria meningitidis* (Fig. 6.6b). Roughly 2,000 copies of the glycopeptide were conjugated to the OMPC. The glycoconjugates, adsorbed onto aluminum hydroxyphosphate and formulated with QS21 adjuvant, elicited high levels of carbohydrate-specific antibodies in guinea pigs and rhesus macaques (Joyce et al. 2008). However, these antibodies poorly recognized recombinant gp160 and did not prevent HIV infection of target cells in vitro. To examine whether antibodies produced during natural infection could recognize the mimetics, the authors used the glycoconjugates to screen a panel of HIV-1-positive and HIV-1-negative sera (Joyce et al. 2008).

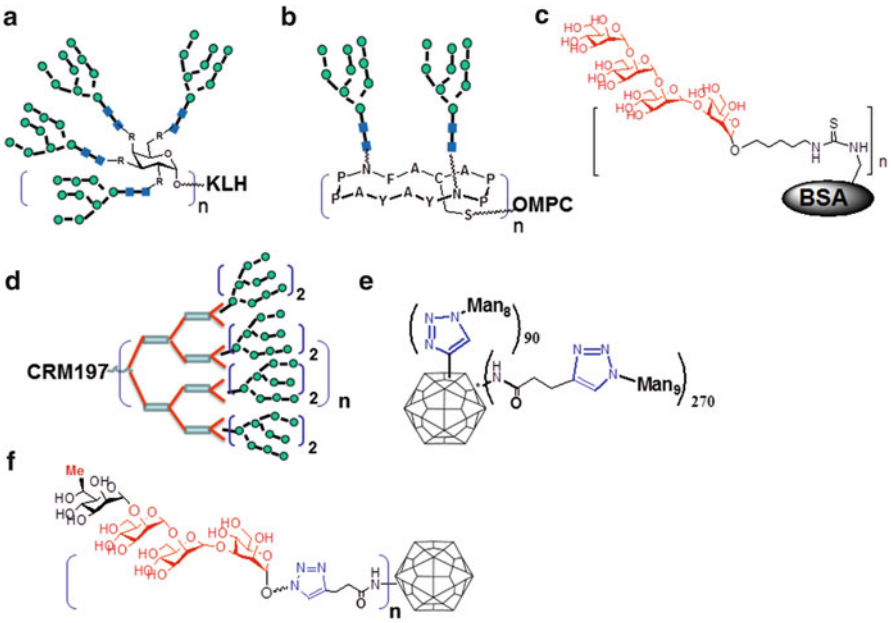

Fig. 6.6 Overview of synthetic glycoconjugate immunogens used for immunizations. Coloring of the chemical structures is the same as in Fig. 6.1. *Green circles* represent mannose residues and *blue squares* represent GlcNAc residues. (**a**) $Man_9GlcNAc_2$-cluster/KLH conjugate (Ni et al. 2006). The carbohydrate content of the cluster is ~15 %; (**b**) Glycoconjugate of a synthetic cyclic peptide-based bivalent $Man_9GlcNAc_2$ and the OMPC of *Neisseria meningitidis* (Joyce et al. 2008). The number (*n*) of glycopeptide molecules per molecule of OMPC ranges from ~2,000 to 3,000; (**c**) Man_4-BSA glycoconjugate (Astronomo et al. 2008). The number (*n*) of Man_4 molecules per BSA molecule is 14; (**d**) Glycoconjugate comprises a $Man_9(PAMAM)_8$ dendron-based oligomannose cluster and the carrier protein CRM197 (Kabanova et al. 2010). The average molar loading of glycan onto the protein is 2 for $Man_9(PAMAM)_8$ clusters; (**e**) bacteriophage QβK16M-based conjugates carrying Man_4 or Man_9 (Astronomo et al. 2010). In each case, the number (*m*) of glycans per phage molecule is 450 ± 50; (**f**) Qβ-based conjugates carrying nonself C6-methylated mannose moiety (Doores et al. 2010b). The average number (*n*) of glycans per Qβ particle is ~300

Strikingly, they observed no significant recognition of the glycopeptides even though some sera contained antibodies that competed with 2G12 for binding to recombinant gp120. These results illustrate the difficulty of mimicking the antigenic presentation of high-mannose sugars on gp120 and show that despite the apparent immunogenicity of tightly clustered oligomannose sugars in natural HIV infection (Walker et al. 2010; Lavine et al. 2012; Moore et al. 2012), immunizing with tightly clustering oligomannose sugars is insufficient to yield anti-carbohydrate antibodies that neutralize HIV. One possible explanation, as noted by the authors (Joyce et al. 2008), could be conformational flexibility of the oligomannose arms which might cause a dilution of the immune response to carbohydrate structures that resemble those found on gp120.

To examine the immunogenicity of the D1 arm (Man_4) oligosaccharide, Burton and co-workers generated a BSA glycoconjugate (BSA-$(Man_4)_{14}$) by coupling an activated ester of the synthetic Man_4 to lysine residues on BSA (Fig. 6.6c) (Astronomo et al. 2008). Immunization of rabbits with BSA-$(Man_4)_{14}$ elicited significant titers of antibodies specific for Man_4. However, these antibodies were unable to bind HIV-1 gp120. Further analysis with glycan microarray technology revealed that the serum antibodies were able to bind a variety of unbranched and, to a lesser extent, branched Man_9 derivatives, but were unable to recognize natural oligomannose that contains the chitobiose $GlcNAc_2$ core. These results suggest potential differences in the presentation of Man_4 on neoglycoconjugates versus natural glycoproteins in which the N-glycans are linked to the protein through a chitobiose core. This difference poses a challenge for eliciting anti-oligomannose antibodies capable of cross-reacting with gp120 and neutralizing HIV-1. However, it is worth noting that glycoconjugates incorporating the chitobiose core have also not elicited the desired antibodies, suggesting that the issue is more complex.

In another study, Costantino and co-workers conjugated PAMAM dendron-based Man_4 and Man_9 clusters to the protein carrier CRM197 (Fig. 6.6d) (Kabanova et al. 2010). CRM197 is a nontoxic mutant of diphtheria toxin (Giannini et al. 1984) for which the crystal structure has been solved recently (Malito et al. 2012) and which has been used successfully as a carrier for pneumococcal, *Haemophilus influenza* type b and meningococcal glycoconjugate vaccines. The average molar loading onto the protein was 10 and 6 for $Man_4(PAMAM)_4$ and $Man_9(PAMAM)_4$, respectively, and 4 and 2 for the $Man_4(PAMAM)_8$ and $Man_9(PAMAM)_8$ clusters, respectively. The conjugates were formulated with MF59, a well-known adjuvant for human use and in particular commonly used for seasonal flu vaccination, and tested for their immunogenicity in rabbits and mice with a three-injection immunization schedule. All the conjugates induced Man_9-specific IgG antibodies in both rabbits and mice; however, the sera again failed to recognize recombinant HIV-1 gp120 proteins. Inappropriate spacing of oligomannose antennae in the synthesized clusters, too much flexibility of the linker region between the oligomannoses and the PAMAM core or insufficient density of the cluster molecules on the carrier protein surface were considered as possible issues for future investigation.

6.5.2 Immunogens Based on Virus-Like Particles

In addition to the aforementioned BSA-Man_4 conjugates, Burton and co-workers also investigated the immunogenicity of the QβK16M-Man_4 and QβK16M-Man_9 glycoconjugates in rabbits (Astronomo et al. 2010) (Fig. 6.6e). The two conjugates were able to elicit antibodies specific for different synthetic oligomannoses. Disappointingly however, none of the sera bound recombinant gp120s corresponding to different HIV-1 isolates or exhibit HIV-neutralizing activities in vitro. Glycan microarray analysis of the serum antibody specificities confirmed that the sera were unable to recognize natural high mannose glycans.

Hypothesizing that the difficulty to induce 2G12-like antibodies may be due to the use of conjugates containing the natural D-mannose sugar moiety, Davis and co-workers designed a strategy based on the observation (Calarese et al. 2003) that the nonself sugar D-fructose binds 2G12 more tightly than D-mannose (Doores et al. 2010b). Informed by the crystal structure of the D-fructose/2G12 Fab complex and by docking studies of D-mannose derivatives in complex with 2G12 Fab, they considered that nonself modifications with alkyl groups at C3, C5, and C6 on the nonreducing terminal mannose of the D1 arm could result in glycans still capable of being efficiently recognized by 2G12. Their binding studies suggested that a methyl substitution at C6 of D-mannose generated a more potent inhibitor of the 2G12–gp120 binding than D-fructose, and that the D1 arm tetrasaccharide derivative with a nonself methyl substitution at C6 of the nonreducing terminal mannose was a stronger binder to 2G12 than D1. Encouraged by these findings, the authors conjugated the C6 methyl D1 derivative and native D1 to Qβ VLPs (Fig. 6.6f). Rabbits immunized with the Qβ-nonself D1 conjugate elicited higher levels antibodies to anti-Man_4 and anti-Man_9 in comparison to rabbits immunized with the Qβ-native D1 glycoconjugate, supporting the hypothesis that nonself modification improved immunogenicity. However, despite this improvement, the sera were unable to bind gp120 and failed to show any HIV-neutralizing activity in pseudovirus neutralization assays.

As can be concluded from the sections above, no glycoconjugate immunogen thus far reported has been able to induce 2G12-like antibodies capable of neutralizing HIV-1. Notably, most of the anti-oligomannose antibodies raised by the synthetic oligomannose-containing glycoconjugates were unable to bind to HIV-1 gp120, even though HIV-1 gp120 carries a large number of high-mannose type N-glycans. This observation raises questions about the particular presentation and accessibility of the high-mannose N-glycans on the gp120 surface of infectious virions. Given that glycoconjugates containing the full-size N-glycan with two GlcNAc moieties, such as $Man_9GlcNAc_2$, could raise antibodies that at least moderately bound gp120 or gp160 (Ni et al. 2006; Joyce et al. 2008), the findings implicate that the innermost two GlcNAc moieties in the N-glycan might play an important role in determining the appropriate orientation of the N-glycans in the synthetic glycoconjugates or in HIV-1 envelope glycoproteins. This factor should be taken into account in future immunogen design. Recent immunization studies with yeast mutants expressing the natural $Man_8GlcNAc_2$ N-glycans, performed by Geng and co-workers, further highlighted this notion (Luallen et al. 2008, 2010; Agrawal-Gamse et al. 2011). Their studies demonstrated that a yeast mutant expressing exclusively $Man_8GlcNAc_2$ N-glycans was able to elicit carbohydrate-specific IgG antibodies that were cross-reactive with HIV-1 gp120, and that the yeast-elicited antibodies could efficiently neutralize virions expressing exclusively high-mannose N-linked glycans (vide infra) (Agrawal-Gamse et al. 2011).

Another key challenge to eliciting 2G12-like bNAbs is the design of immunogens capable of promoting the development of domain-exchanged antibodies. As discussed by Burton and co-workers (Doores et al. 2013), a successful immunization strategy may require 2G12 germ line B-cell activation to generate, following

somatic mutations, a B-cell population displaying a portion of domain-swapped B-cell receptors (BCR) with high affinity for clustered mannose antigens, and this could lead to the preferential selection of those B cells expressing a domain-exchanged BCR instead of those expressing a conventional one. Taken together, these studies suggest that both the nature of the HIV-1 high-mannose N-glycans and the context where they are present, including the nature of linkages between the glycans and proteins, are likely critical for raising anti-glycan NAbs that can neutralize HIV-1.

6.6 Natural Carbohydrate Antigens as Mimics of 2G12 Epitope

Given that the glycan shield on HIV is well exposed on the viral spike and that the cluster(s) of high-mannose glycans are a viable target, the key questions would be how to design immunogens that can best mimic the conformational epitopes recognized by glycan-specific bNAbs and how to elicit such types of bNAbs through immunization. Considerable efforts have been made to recapitulate the 2G12 epitope in a variety of contexts using chemically synthesized oligomannose-containing glycoconjugates as described in above sections and previous reviews (Wang 2006; Astronomo and Burton 2010). In this section, we will focus on recent advances in the development of natural or biological carbohydrate antigens as mimics of the 2G12 epitope, which mainly includes three approaches, i.e., manipulation of the N-glycan-processing pathway in mammalian cells, genetic engineering of yeast strains, and identification of natural oligosaccharides in bacteria.

To artificially create the 2G12 epitope, Scanlan and co-workers treated human 293T cells expressing a selection of non-antigenic self proteins with kifunensine, a plant alkaloid that potently inhibits α-mannosidase I activity in the endoplasmic reticulum (ER), which prevents further trimming of mannose residues on $Man_9GlcNAc_2$ in the Golgi apparatus and therefore enriches the expression of high-mannose glycans, mainly non-processed $Man_9GlcNAc_2$. As a result, multiple 2G12 epitopes were created on the surface of the otherwise non-antigenic proteins (Scanlan et al. 2007). Among the proteins of particular note was CEACAM1, a highly glycosylated protein containing 21 potential N-linked glycosylation sites (PNGS) in its 492 amino acids of mature form.

Recently, Pantophlet and co-workers reported a naturally occurring 2G12 epitope mimic derived from a bacterial lipooligosaccharide (LOS) of the soil bacterium *Rhizobium radiobacter* Rv3 (Clark et al. 2012). The carbohydrate backbone of Rv3 LOS consists of a unique tetramannose segment that is an analog to the D1 arm of high-mannose glycan (De Castro et al. 2008). 2G12 bound to the purified Rv3 LOS and Rv3 bacterial cells, and immune sera raised with heat-killed whole bacteria bound to BSA-Man_4 and monomeric gp120 but failed to neutralize the virus. Among the three approaches, genetically manipulated yeast systems have been more extensively studied and the progress in this area is summarized below.

6.6.1 *A Functional System Versus a Specific Target*

Considering the facts that a safe and effective vaccine with cheap production in large scale is the best hope to stop the epidemic of HIV/AIDS, Geng and co-workers have searched for an expression system aiming to identify heterologous glycoproteins as mimics of 2G12 epitope. They initially screened several species, including plants, insects, fungi, and mammals, in hopes of identifying a species in which the N-glycan-processing machinery can be genetically manipulated and glycoproteins can be produced cost-effectively. Among these species, *Saccharomyces cerevisiae* stood out based upon the following characteristics: (a) its N-glycosylation pathway is relatively simple without obviously recognized homologs of the key enzymes that process $Man_8GlcNAc_2$ in the Golgi apparatus (Dean 1999; Herscovics 1999), which makes it possible to produce exclusive $Man_8GlcNAc_2$ type of N-glycans, the major form of glycans in the 2G12 epitope, without concerns for functional replacement of the mutated genes by their homologs; (b) recombinant proteins produced in this system have been used clinically as therapeutic drugs and prophylactic vaccines for humans (Walsh and Jefferis 2006); (c) it is easy to grow, producing glycoproteins inexpensively in large scale (Walsh and Jefferis 2006); and (d) its genome sequence information has been well annotated and can be used for identification and production of target glycoproteins (Cherry et al. 1997; Winzeler et al. 1999; Giaever et al. 2002). Thus, the baking yeast *S. cerevisiae* offers multiple advantages over other species and was selected as a practical system to develop genetic scaffolds as mimics of the 2G12 epitope from not only *S. cerevisiae* but also other species.

6.6.2 *Genetically Engineered* S. cerevisiae *Mutant Strains*

The key to replicate the 2G12 epitope is to produce glycoproteins with dense clusters comprising several high-mannose glycans, specifically the D1 arm with a structure of Manα1,2Manα1,2Manα1,3Man tetrasaccharides on $Man_9GlcNAc_2$, $Man_8GlcNAc_2$, or an isoform of $Man_7GlcNAc_2$, collectively referred to as $Man_{7-9}GlcNAc_2$. In the ER, the N-linked glycan-processing pathway in yeast and mammalian cells is basically identical, resulting in proteins containing the $Man_8GlcNAc_2$ type of high-mannose glycan with the terminal α1,2-linked mannose on D2 arm being trimmed off. In mammalian cells, $Man_8GlcNAc_2$ is further trimmed by mannosidases to $Man_5GlcNAc_2$ or $Man_3GlcNAc_2$ in the Golgi apparatus followed by adding other sugar residues to form hybrid- or complex-types of N-glycans. In yeast cells, however, mannose residues are directly added to the core $Man_8GlcNAc_2$ structure without further trimming in the Golgi apparatus (Dean 1999; Herscovics 1999). Three mannosyltransferases encoded by the *och1*, *mnn1,* and *mnn4* genes, respectively, in the *S. cerevisiae* Golgi apparatus are the essential enzymes responsible for the initiation of a polymannose oligosaccharide, with a side chain being initiated by Och1p, and the addition of terminal α1,3-linked mannose and

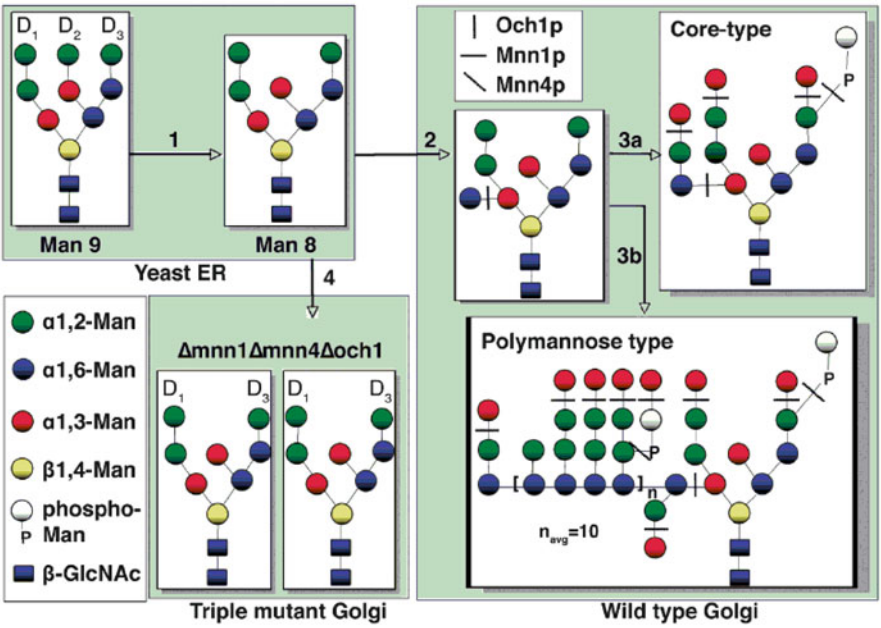

Fig. 6.7 N-linked glycosylation pathway in wild-type *S. cerevisiae* and *S. cerevisiae* triple-mutant (Δ*och1*Δ*mnn1*Δ*mnn4*). In the ER, after *en bloc* transfer of $Glc_3Man_9GlcNAc_2$ to nascent polyproteins, the three glucose residues are first cleaved, and then the terminal mannose residue on D2 arm of $Man_9GlcNAc_2$ is trimmed by mannosidase Mns1p (step 1). In the Golgi apparatus of wild-type yeast, N-glycans are processed as follows. In step 2, the first α1,6-linked-mannose backbone residue is added by Och1p in the *cis*-Golgi to initiate the polymannose side chain. In step 3a, a few mannose residues are added by Mnn1p and Mnn4p on the core $Man_8GlcNAc_2$, which cap the terminal α1,2-Man on D1 and D3 arms. In step 3b, numerous mannose residues, with an average of 160, are added by different mannosyltransferases to elongate the polymannose side chain (Dean 1999). Mannose sugars added by Och1p, Mnn1p, and Mnn4p in the Golgi apparatus of wild-type yeast are depicted with vertical, horizontal, and diagonal lines, respectively. In the TM yeast, there is neither addition of mannose residues on $Man_8GlcNAc_2$ nor initiation of polymannose side chain due to the absence of Och1p, Mnn1p, and Mnn4p in Golgi apparatus, which results in a majority of N-glycans being $Man_8GlcNAc_2$ type with minor amounts of untrimmed $Man_9GlcNAc_2$ (step 4)

phosphomannose residues by Mnn1p and Mnn4p, respectively, on the D1 and D3 arms of the core $Man_8GlcNAc_2$ structure (Fig. 6.7). To produce glycoproteins with only a $Man_8GlcNAc_2$ structure, an *S. cerevisiae* triple mutant (TM) was generated by eliminating the three aforementioned genes (*och1*, *mnn1,* and *mnn4*) as illustrated in Fig. 6.7 (Luallen et al. 2008). 2G12 bound well to the whole yeast cells of the TM strain, but not to the wild-type, as indicated by immunostaining and whole cell ELISA. Glycan profiling of the carbohydrates released from the whole yeast cells indicated that N-glycans from this mutant were almost exclusively $Man_8GlcNAc_2$, the major form of the glycans that constitute the 2G12 epitope. Immunization of rabbits with heat-killed whole TM yeast cells induced antibodies

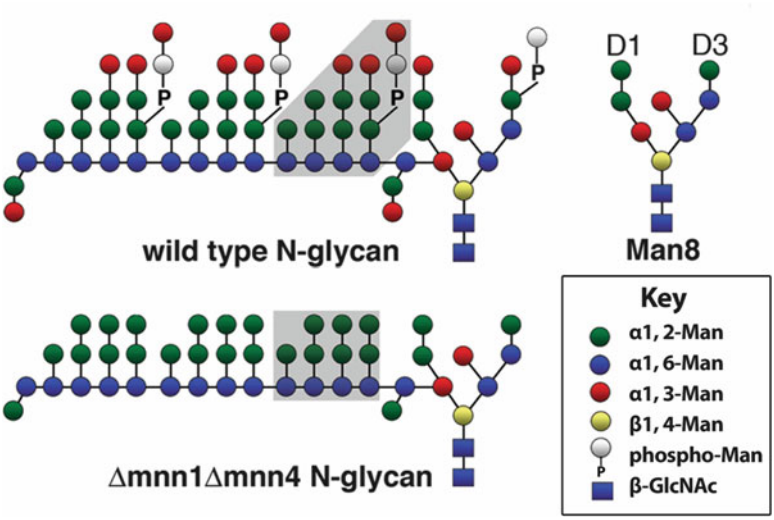

Fig. 6.8 Depiction of expected structures of polymannose-type N-glycans produced in wild-type and Δ*mnn1*Δ*mnn4* yeast strains, with the indicated type and linkage of each monosaccharide. The *shaded boxes* represent repeated oligosaccharide units, with an average of ten units per N-glycan. Expected structures of N-glycans from wild-type and Δ*mnn1*Δ*mnn4* strains are based on previous studies of wild-type yeast and the Δ*mnn1* mutant (Ballou 1990; Dean 1999). $Man_8GlcNAc_2$, an important N-glycan in the 2G12 epitope, is depicted with the D1 and D3 arms

that were able to cross-react with the carbohydrates on HIV-1 gp120 as described in detail below.

Since the Manα1,2Manα1,2Man structure on the D1 arm of high-mannose glycans is the key component of 2G12 epitope, Geng and co-workers also created a double mutant strain, Δ*mnn1*Δ*mnn4*, to expose the Manα1,2-Manα1,2-Man trisaccharide structures not only on the D1 arm of $Man_8GlcNAc_2$ but also on the polymannose oligosaccharides of side chain as illustrated in Fig. 6.8 (Luallen et al. 2010). While neither the whole yeast cells of the Δ*mnn1*Δ*mnn4* strain nor the glycoproteins expressed in this strain demonstrated efficient binding to 2G12, the immune sera raised with heat-killed whole yeast of the Δ*mnn1*Δ*mnn4* strain recognized gp120 from multiple HIV-1 and SIV strains in a mannose glycan-dependent manner and bound specifically to the glycans that contain Manα1,2Manα1,2Man trisaccharide structures as detected by glycan microarray (Luallen et al. 2010).

A similar approach was pursued by Scanlan and co-workers, in which an *S. cerevisiae* mutant being deficient in only α1-3 mannosyltransferase gene (Δ*mnn1*) was used to immunize rabbits. The resulting antibodies exhibited carbohydrate specificity to Manα1,2Man motif, similar to 2G12, but demonstrated extremely weak neutralization of HIV (Dunlop et al. 2010).

6.6.3 Discovery and Characterization of 2G12-Reactive Heterologous Glycoproteins

While all three mutant strains of *S. cerevisiae* described above are capable of inducing mannose-specific HIV gp120-binding antibodies, the TM (Δ*och1*Δ*mnn1*Δ*mnn4*) yeast system offers additional advantages, such as the expression of endogenous glycoproteins that are recognized by 2G12. In an attempt to identify glycoproteins that could present the high-mannose structures in a manner that could recapitulate the 2G12 epitope, Geng and co-workers searched for endogenous proteins from TM yeast and identified four candidates (Ecm33, Gas1, Gp38, and YJL171c) that bound to 2G12 efficiently (Luallen et al. 2008). These endogenous yeast glycoproteins, similar to gp120, contain a large number (10–15) and high density of PNGS, and the carbohydrates account for greater than 50 % of the molecular mass of these proteins. In addition, the same group also identified another 2G12-reactive glycoprotein, Pst1, in the mutant yeast strain Δ*mnn1*Δ*pmr1*. The later component, Δ*pmr1*, affects the function of Mn^{2+}-dependent mannosyltransferases in the Golgi apparatus, which results in inefficient transfer of mannose residues to the core $Man_8GlcNAc_2$ structure. Pst1 is a highly glycosylated yeast cell wall protein that contains 15 PNGS. When expressed in the TM yeast, Pst1 demonstrated high affinity to 2G12, efficiently inhibited gp120 interactions with 2G12 or DC-SIGN, and also blocked 2G12-mediated neutralization of HIV-1 pseudoviruses (Luallen et al. 2009).

To compare the antigenicity and to determine the lead candidates for immunization studies, all five 2G12-reactive yeast glycoproteins (Ecm33, Gas1, Gp38, Pst1, and YJL171c) were expressed in TM yeast after cloning their genes into an expression vector and purified using a combination of several approaches. Comparison of the five yeast glycoproteins for their binding to 2G12 in ELISA, inhibition of gp120-2G12 and gp120-DC-SIGN interactions, and competitive inhibition of 2G12 neutralization of pseudoviruses demonstrated that Pst1 and Gp38 have similar antigenicity and the highest binding affinity for 2G12, followed by ECM33, YJL171c, and Gas1. Significantly, all five 2G12-reactive yeast glycoproteins supported strong binding to the most potent glycan-dependent bNAbs (PGT125–128, PGT130, and PGT135), with a similar pattern to 2G12 (Yu Geng et al., unpublished data). Moreover, three yeast proteins Pst1, Gp38, and Ecm33 together could absorb approximately 80 % of HIV gp120 cross-reactive antibodies elicited by TM yeast (Agrawal-Gamse et al. 2011). A preliminary immunization study using the top three candidates Pst1, Gp38, and Ecm33 showed that Pst1 and Gp38 induced stronger gp120 cross-reactive antibody responses than Ecm33 (Yu Geng et al., unpublished data).

Collectively, these results highlight the potential of these heterologous glycoproteins of TM yeast as genetic scaffolds to recapitulate 2G12 and PGT-like bNab epitopes, and identify Pst1 and Gp38 as lead candidates to potentially elicit antibodies with 2G12- or PGT-like specificity (Luallen et al. 2008, 2009; Yu Geng et al., unpublished data).

6.6.4 *Elicitation of High-Mannose-Specific HIV Cross-Reactive Antibody Responses with Whole Yeast Cells and 2G12-Reactive Yeast Glycoproteins*

Immunization of rabbits with TM (Δ*och1*Δ*mnn1*Δ*mnn4*) yeast, double mutant (Δ*mnn1*Δ*mnn4*) yeast, or single 2G12-reactive yeast glycoproteins elicited antibodies that not only specifically recognized synthetic mannose-containing glycans, but also recognized monomeric gp120 proteins from virtually all HIV strains tested (Luallen et al. 2008, 2010; Agrawal-Gamse et al. 2011; Yu Geng et al., unpublished data). The interaction of these immune sera with HIV gp120 is high-mannose carbohydrate-dependent since treatment of gp120 with α-mannosidases abolished gp120 binding by the immune sera. Furthermore, the antibodies preferentially bound to the glycans containing terminal Manα1,2Manα1,2Man trisaccharides, a structure on the D1 arm of $Man_{8-9}GlcNAc_2$ high-mannose glycans, regardless of the number of mannose residues or branches on a particular glycan. While the immune sera elicited by TM yeast glycoproteins bound well to gp120, and some also bound to envelope glycoprotein trimers, they only exhibited efficient neutralizing activity when the virus was produced in the presence of kifunensine, which forces retention of high-mannose glycans at all PNGS on gp120 (Agrawal-Gamse et al. 2011; Yu Geng et al., unpublished data).

These features of the immune sera raise an important question—why do they bind well to HIV gp120 but do not efficiently neutralize the virus. To address this, the mannose-specific IgG in the immune sera were purified with 2G12-reactive proteins (Pst1, Gp38, and Ecm33) and compared with 2G12 for binding affinity toward gp120. The purified antibodies (IgG) bound to gp120s from clade B viruses with roughly 100–1,000-fold lower affinities than that of 2G12 and bound trimeric envelope glycoproteins poorly. However, these antibodies bound to a broader spectrum of gp120s from different HIV-1 subtypes than 2G12 and neutralized a genetically diverse panel of HIV-1 strains, albeit only when the viruses were produced in the presence of kifunensine, implying that the antibodies elicited with TM yeast recognize diverse glycan epitopes with configurations or conformations that may differ from the cluster recognized by 2G12 (Luallen et al. 2008; Agrawal-Gamse et al. 2011). These results further highlight the challenge of eliciting high-affinity glycan-specific antibodies that can recognize the cluster of oligomannose neutralizing epitopes on gp120.

6.6.5 *Challenges and Future Studies with the Yeast Genetic Approaches*

Tremendous challenges have been experienced during the past decades in developing an effective HIV/AIDS vaccine. This certainly holds true for attempts to elicit NAbs targeting the HIV glycan shield due to its antigenic complexity. Fortunately, high-mannose glycan clusters on individual 2G12-reactive yeast glycoproteins could

be much less diversified than those on the whole TM yeast cells, and the glycan clusters can be easily manipulated and optimized to best recapitulate 2G12 or PGT bNAb epitopes. In addition, using this TM yeast system, any highly glycosylated protein from diverse organisms, such as fungi, insects, worms, plants, and mammals, can be easily produced, which will provide additional opportunities to identify alternative lead candidates to elicit 2G12- and PGT-like bNAb specificity.

The key challenges experienced in HIV-1 glycoconjugate vaccine design using natural carbohydrate approach are to induce high-affinity antibodies against the carbohydrate antigens and to focus the immune responses to neutralizing epitopes on the native envelope spike. Given the fact that carbohydrate antigens are in general poorly immunogenic and usually induce low affinity antibodies (Astronomo and Burton 2010), future studies may need to explore different immunization strategies, such as including more potent adjuvants and extending immunization schedules, to potentiate immune responses to the glycan antigens and promote antibody affinity maturation. In the meantime, further optimization of the high-mannose glycan clusters on lead candidates using genetic approaches to best recapitulate 2G12 and PGT bNAb epitopes and focus the immune responses to the neutralizing determinants are also needed. As many of the recent glycan-dependent bNAbs engage glycans as well as protein backbone in their epitopes, an immunization regimen employing yeast glycoprotein to elicit mannose-specific antibodies followed by boosting with HIV envelope glycoprotein trimers to direct the immune response toward the glycan-specific or glycan-dependent neutralizing epitopes on the native virus may be required for the development of an effective carbohydrate-based HIV-1 vaccine.

6.7 Conclusion

Among the few targets for the design of an effective immunogen to induce HIV-1 NAbs, the glycan shield on the viral envelope glycoprotein has drawn particular attention recently as a legitimate target for HIV-1 vaccine design. Particularly, concerted efforts have been made to recapitulate the 2G12 epitope in a variety of contexts. These attempts have focused on the multivalent display of chemically synthesized oligomannose-containing glycoconjugates and the identification of heterologous glycoproteins with natural high-mannose glycans that support 2G12 binding through genetic manipulation of N-glycosylation pathway. These approaches have provided insightful information on the structural requirement for 2G12 recognition and propelled the development of diverse scaffolds to mimic the antibody epitope. Some of these scaffolds have shown high affinity binding to antibody 2G12. In particular, the scaffold derived from a triple mutant yeast strain can elicit mannose-specific antibodies that are able to cross-react with the HIV envelope glycoprotein and efficiently neutralize genetic diversity of viruses when all viral N-glycans are forced to retain high-mannose type N-glycans. Nevertheless, these strategies have had limited success in eliciting robust levels of glycan-specific antibodies capable of neutralizing HIV-1. Future studies should be directed toward optimization of

the immunogens by presenting the identified oligosaccharide or glycopeptide epitopes in the right context to best recapitulate the presentation of the glycan-specific or glycopeptide-dependent epitopes on the viral envelope, and dissection of the discrepancy between gp120 binding and virus neutralization of the antibodies elicited with the yeast genetic scaffolds. In addition, it is also important to explore the immunization conditions, including the testing of potent adjuvants and prime-boost strategies, to potentiate the glycan-specific immune response and direct the immune response toward neutralizing epitopes on the glycan shield of HIV-1.

References

Adams EW, Ratner DM, Bokesch HR, McMahon JB, O'Keefe BR, Seeberger PH (2004) Oligosaccharide and glycoprotein microarrays as tools in HIV glycobiology; glycan-dependent gp120/protein interactions. Chem Biol 11:875–881

Agrawal-Gamse C, Luallen RJ, Liu B, Fu H, Lee FH, Geng Y, Doms RW (2011) Yeast-elicited cross-reactive antibodies to HIV Env glycans efficiently neutralize virions expressing exclusively high-mannose N-linked glycans. J Virol 85:470–480

Anderluh M, Jug G, Svajger U, Obermajer N (2012) DC-SIGN antagonists, a potential new class of anti-infectives. Curr Med Chem 19:992–1007

Astronomo RD, Burton DR (2010) Carbohydrate vaccines: developing sweet solutions to sticky situations? Nat Rev Drug Discov 9:308–324

Astronomo RD, Lee HK, Scanlan CN, Pantophlet R, Huang CY, Wilson IA, Blixt O, Dwek RA, Wong CH, Burton DR (2008) A glycoconjugate antigen based on the recognition motif of a broadly neutralizing human immunodeficiency virus antibody, 2G12, is immunogenic but elicits antibodies unable to bind to the self glycans of gp120. J Virol 82:6359–6368

Astronomo RD, Kaltgrad E, Udit AK, Wang SK, Doores KJ, Huang CY, Pantophlet R, Paulson JC, Wong CH, Finn MG, Burton DR (2010) Defining criteria for oligomannose immunogens for HIV using icosahedral virus capsid scaffolds. Chem Biol 17:357–370

Avci FY, Kasper DL (2010) How bacterial carbohydrates influence the adaptive immune system. Annu Rev Immunol 28:107–130

Avci FY, Li X, Tsuji M, Kasper DL (2011) A mechanism for glycoconjugate vaccine activation of the adaptive immune system and its implications for vaccine design. Nat Med 17:1602–1609

Avery OT, Goebel WF (1929) Chemo-immunological studies on conjugated carbohydrate-proteins: I. Immunological specificity of synthetic sugar-protein antigens. J Exp Med 50: 533–550

Ballou CE (1990) Isolation, characterization, and properties of Saccharomyces cerevisiae mnn mutants with nonconditional protein glycosylation defects. Methods Enzymol 185:440–470

Balzarini J (2006) Inhibition of HIV entry by carbohydrate-binding proteins. Antiviral Res 71:237–247

Balzarini J (2007) Carbohydrate-binding agents: a potential future cornerstone for the chemotherapy of enveloped viruses? Antivir Chem Chemother 18:1–11

Barrientos LG, Gronenborn AM (2005) The highly specific carbohydrate-binding protein cyanovirin-N: structure, anti-HIV/Ebola activity and possibilities for therapy. Mini Rev Med Chem 5:21–31

Bolmstedt A, Biller M, Hansen JE, Moore JP, Olofsson S (1997) Demonstration of peripheral fucose units in N-linked glycans of human immunodeficiency virus type 1 gp 120: effects on glycoprotein conformation. Arch Virol 142:2465–2481

Bonomelli C, Doores KJ, Dunlop DC, Thaney V, Dwek RA, Burton DR, Crispin M, Scanlan CN (2011) The glycan shield of HIV is predominantly oligomannose independently of production system or viral clade. PLoS One 6:e23521

Burton DR (2002) Antibodies, viruses and vaccines. Nat Rev Immunol 2:706–713
Burton DR, Desrosiers RC, Doms RW, Koff WC, Kwong PD, Moore JP, Nabel GJ, Sodroski J, Wilson IA, Wyatt RT (2004) HIV vaccine design and the neutralizing antibody problem. Nat Immunol 5:233–236
Burton DR, Ahmed R, Barouch DH, Butera ST, Crotty S, Godzik A, Kaufmann DE, McElrath MJ, Nussenzweig MC, Pulendran B, Scanlan CN, Schief WR, Silvestri G, Streeck H, Walker BD, Walker LM, Ward AB, Wilson IA, Wyatt R (2012) A blueprint for HIV vaccine discovery. Cell Host Microbe 12:396–407
Butters TD, Yudkin B, Jacob GS, Jones IM (1998) Structural characterization of the N-linked oligosaccharides derived from HIVgp120 expressed in lepidopteran cells. Glycoconj J 15:83–88
Calarese DA, Scanlan CN, Zwick MB, Deechongkit S, Mimura Y, Kunert R, Zhu P, Wormald MR, Stanfield RL, Roux KH, Kelly JW, Rudd PM, Dwek RA, Katinger H, Burton DR, Wilson IA (2003) Antibody domain exchange is an immunological solution to carbohydrate cluster recognition. Science 300:2065–2071
Calarese DA, Lee HK, Huang CY, Best MD, Astronomo RD, Stanfield RL, Katinger H, Burton DR, Wong CH, Wilson IA (2005) Dissection of the carbohydrate specificity of the broadly neutralizing anti-HIV-1 antibody 2G12. Proc Natl Acad Sci U S A 102:13372–13377
Calarota SA, Weiner DB (2003) Present status of human HIV vaccine development. AIDS 17(suppl 4):S73–S84
Cao J, Sullivan N, Desjardin E, Parolin C, Robinson J, Wyatt R, Sodroski J (1997) Replication and neutralization of human immunodeficiency virus type 1 lacking the V1 and V2 variable loops of the gp120 envelope glycoprotein. J Virol 71:9808–9812
Cherry JM, Ball C, Weng S, Juvik G, Schmidt R, Adler C, Dunn B, Dwight S, Riles L, Mortimer RK, Botstein D (1997) Genetic and physical maps of Saccharomyces cerevisiae. Nature 387:67–73
Clark BE, Auyeung K, Fregolino E, Parrilli M, Lanzetta R, De Castro C, Pantophlet R (2012) A bacterial lipooligosaccharide that naturally mimics the epitope of the HIV-neutralizing antibody 2G12 as a template for vaccine design. Chem Biol 19:254–263
Costantino P, Rappuoli R, Berti F (2011) The design of semi-synthetic and synthetic glycoconjugate vaccines. Expert Opin Drug Discov 6:1045–1066
Davenport TM, Friend D, Ellingson K, Xu H, Caldwell Z, Sellhorn G, Kraft Z, Strong RK, Stamatatos L (2011) Binding interactions between soluble HIV envelope glycoproteins and quaternary-structure-specific monoclonal antibodies PG9 and PG16. J Virol 85:7095–7107
De Castro C, Molinaro A, Lanzetta R, Silipo A, Parrilli M (2008) Lipopolysaccharide structures from Agrobacterium and *Rhizobiaceae* species. Carbohydr Res 343:1924–1933
Dean N (1999) Asparagine-linked glycosylation in the yeast Golgi. Biochim Biophys Acta 1426:309–322
Doores KJ, Burton DR (2010) Variable loop glycan dependency of the broad and potent HIV-1-neutralizing antibodies PG9 and PG16. J Virol 84:10510–10521
Doores KJ, Bonomelli C, Harvey DJ, Vasiljevic S, Dwek RA, Burton DR, Crispin M, Scanlan CN (2010a) Envelope glycans of immunodeficiency virions are almost entirely oligomannose antigens. Proc Natl Acad Sci U S A 107:13800–13805
Doores KJ, Fulton Z, Hong V, Patel MK, Scanlan CN, Wormald MR, Finn MG, Burton DR, Wilson IA, Davis BG (2010b) A nonself sugar mimic of the HIV glycan shield shows enhanced antigenicity. Proc Natl Acad Sci U S A 107:17107–17112
Doores KJ, Huber M, Le KM, Wang SK, Doyle-Cooper C, Cooper A, Pantophlet R, Wong CH, Nemazee D, Burton DR (2013) 2G12-expressing B cell lines may aid in HIV carbohydrate vaccine design strategies. J Virol 87:2234–2241
Dudkin VY, Orlova M, Geng X, Mandal M, Olson WC, Danishefsky SJ (2004) Toward fully synthetic carbohydrate-based HIV antigen design: on the critical role of bivalency. J Am Chem Soc 126:9560–9562
Dunlop DC, Bonomelli C, Mansab F, Vasiljevic S, Doores KJ, Wormald MR, Palma AS, Feizi T, Harvey DJ, Dwek RA, Crispin M, Scanlan CN (2010) Polysaccharide mimicry of the epitope

of the broadly neutralizing anti-HIV antibody, 2G12, induces enhanced antibody responses to self oligomannose glycans. Glycobiology 20:812–823
Feinberg H, Mitchell DA, Drickamer K, Weis WI (2001) Structural basis for selective recognition of oligosaccharides by DC- SIGN and DC-SIGNR. Science 294:2163–2166
Feinberg H, Castelli R, Drickamer K, Seeberger PH, Weis WI (2007) Multiple modes of binding enhance the affinity of DC-SIGN for high mannose N-linked glycans found on viral glycoproteins. J Biol Chem 282:4202–4209
Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, Middel J, Cornelissen IL, Nottet HS, KewalRamani VN, Littman DR, Figdor CG, van Kooyk Y (2000) DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 100: 587–597
Geng X, Dudkin VY, Mandal M, Danishefsky SJ (2004) In pursuit of carbohydrate-based HIV vaccines, Part 2: the total synthesis of high-mannose-type gp120 fragments-evaluation of strategies directed to maximal convergence. Angew Chem Int Ed 43:2562–2565
Geyer H, Holschbach C, Hunsmann G, Schneider J (1988) Carbohydrates of human immunodeficiency virus. Structures of oligosaccharides linked to the envelope glycoprotein 120. J Biol Chem 263:11760–11767
Giaever G et al (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418:387–391
Giannini G, Rappuoli R, Ratti G (1984) The amino-acid sequence of two non-toxic mutants of diphtheria toxin: CRM45 and CRM197. Nucleic Acids Res 12:4063–4069
Go EP, Irungu J, Zhang Y, Dalpathado DS, Liao HX, Sutherland LL, Alam SM, Haynes BF, Desaire H (2008) Glycosylation site-specific analysis of HIV envelope proteins (JR-FL and CON-S) reveals major differences in glycosylation site occupancy, glycoform profiles, and antigenic epitopes' accessibility. J Proteome Res 7:1660–1674
Go EP, Chang Q, Liao HX, Sutherland LL, Alam SM, Haynes BF, Desaire H (2009) Glycosylation site-specific analysis of clade C HIV-1 envelope proteins. J Proteome Res 8:4231–4242
Go EP, Liao HX, Alam SM, Hua D, Haynes BF, Desaire H (2013) Characterization of host-cell line specific glycosylation profiles of early transmitted/founder HIV-1 gp120 envelope proteins. J Proteome Res 12:1223–1234
Hansen JE, Clausen H, Nielsen C, Teglbjaerg LS, Hansen LL, Nielsen CM, Dabelsteen E, Mathiesen L, Hakomori SI, Nielsen JO (1990) Inhibition of human immunodeficiency virus (HIV) infection in vitro by anticarbohydrate monoclonal antibodies: peripheral glycosylation of HIV envelope glycoprotein gp120 may be a target for virus neutralization. J Virol 64: 2833–2840
Hansen JE, Nielsen C, Arendrup M, Olofsson S, Mathiesen L, Nielsen JO, Clausen H (1991) Broadly neutralizing antibodies targeted to mucin-type carbohydrate epitopes of human immunodeficiency virus. J Virol 65:6461–6467
Hansen JE, Jansson B, Gram GJ, Clausen H, Nielsen JO, Olofsson S (1996) Sensitivity of HIV-1 to neutralization by antibodies against O-linked carbohydrate epitopes despite deletion of O-glycosylation signals in the V3 loop. Arch Virol 141:291–300
Herscovics A (1999) Processing glycosidases of *Saccharomyces cerevisiae*. Biochim Biophys Acta 1426:275–285
Hessell AJ, Rakasz EG, Poignard P, Hangartner L, Landucci G, Forthal DN, Koff WC, Watkins DI, Burton DR (2009) Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Pathog 5:e1000433
Hoffenberg S, Powell R, Carpov A, Wagner D, Wilson A, Kosakovsky Pond S, Lindsay R, Arendt H, Destefano J, Phogat S, Poignard P, Fling SP, Simek M, Labranche C, Montefiori D, Wrin T, Phung P, Burton D, Koff W, King CR, Parks CL, Caulfield MJ (2013) Identification of an HIV-1 clade A envelope that exhibits broad antigenicity and neutralization sensitivity and elicits antibodies targeting three distinct epitopes. J Virol 87:5372–5383
Hong PW, Flummerfelt KB, de Parseval A, Gurney K, Elder JH, Lee B (2002) Human immunodeficiency virus envelope (gp120) binding to DC-SIGN and primary dendritic cells is carbohy-

drate dependent but does not involve 2G12 or cyanovirin binding sites: implications for structural analyses of gp120-DC-SIGN binding. J Virol 76:12855–12865

Ji X, Gewurz H, Spear GT (2005) Mannose binding lectin (MBL) and HIV. Mol Immunol 42:145–152

Joyce JG, Krauss IJ, Song HC, Opalka DW, Grimm KM, Nahas DD, Esser MT, Hrin R, Feng M, Dudkin VY, Chastain M, Shiver JW, Danishefsky SJ (2008) An oligosaccharide-based HIV-1 2G12 mimotope vaccine induces carbohydrate-specific antibodies that fail to neutralize HIV-1 virions. Proc Natl Acad Sci U S A 105:15684–15689

Julien JP, Lee JH, Cupo A, Murin CD, Derking R, Hoffenberg S, Caulfield MJ, King CR, Marozsan AJ, Klasse PJ, Sanders RW, Moore JP, Wilson IA, Ward AB (2013) Asymmetric recognition of the HIV-1 trimer by broadly neutralizing antibody PG9. Proc Natl Acad Sci U S A 110: 4351–4356

Kabanova A, Adamo R, Proietti D, Berti F, Tontini M, Rappuoli R, Costantino P (2010) Preparation, characterization and immunogenicity of HIV-1 related high-mannose oligosaccharides-CRM197 glycoconjugates. Glycoconj J 27:501–513

Kelly DF, Pollard AJ, Moxon ER (2005) Immunological memory: the role of B cells in long-term protection against invasive bacterial pathogens. JAMA 294:3019–3023

Kim JH, Rerks-Ngarm S, Excler JL, Michael NL (2010) HIV vaccines: lessons learned and the way forward. Curr Opin HIV AIDS 5:428–434

Kolchinsky P, Kiprilov E, Sodroski J (2001a) Increased neutralization sensitivity of CD4-independent human immunodeficiency virus variants. J Virol 75:2041–2050

Kolchinsky P, Kiprilov E, Bartley P, Rubinstein R, Sodroski J (2001b) Loss of a single N-linked glycan allows CD4-independent human immunodeficiency virus type 1 infection by altering the position of the gp120 V1/V2 variable loops. J Virol 75:3435–3443

Krauss IJ, Joyce JG, Finnefrock AC, Song HC, Dudkin VY, Geng X, Warren JD, Chastain M, Shiver JW, Danishefsky SJ (2007) Fully synthetic carbohydrate HIV antigens designed on the logic of the 2G12 antibody. J Am Chem Soc 129:11042–11044

Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA (1998) Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393:648–659

Kwong PD, Mascola JR, Nabel GJ (2011) Rational design of vaccines to elicit broadly neutralizing antibodies to HIV-1. Cold Spring Harb Perspect Biol 3:a007278

Kwong PD, Mascola JR, Nabel GJ (2012) The changing face of HIV vaccine research. J Int AIDS Soc 15:17407

Larkin M, Childs RA, Matthews TJ, Thiel S, Mizuochi T, Lawson AM, Savill JS, Haslett C, Diaz R, Feizi T (1989) Oligosaccharide-mediated interactions of the envelope glycoprotein gp120 of HIV-1 that are independent of CD4 recognition. AIDS 3:793–798

Lavine CL, Lao S, Montefiori DC, Haynes BF, Sodroski JG, Yang X (2012) High-mannose glycan-dependent epitopes are frequently targeted in broad neutralizing antibody responses during human immunodeficiency virus type 1 infection. J Virol 86:2153–2164

Lee WR, Yu XF, Syu WJ, Essex M, Lee TH (1992a) Mutational analysis of conserved N-linked glycosylation sites of human immunodeficiency virus type 1 gp41. J Virol 66:1799–1803

Lee WR, Syu WJ, Du B, Matsuda M, Tan S, Wolf A, Essex M, Lee TH (1992b) Nonrandom distribution of gp120 N-linked glycosylation sites important for infectivity of human immunodeficiency virus type 1. Proc Natl Acad Sci U S A 89:2213–2217

Lee HK, Scanlan CN, Huang CY, Chang AY, Calarese DA, Dwek RA, Rudd PM, Burton DR, Wilson IA, Wong CH (2004) Reactivity-based one-pot synthesis of oligomannoses: defining antigens recognized by 2G12, a broadly neutralizing anti-HIV-1 antibody. Angew Chem Int Ed 43:1000–1003

Leonard CK, Spellman MW, Riddle L, Harris RJ, Thomas JN, Gregory TJ (1990) Assignment of intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1 recombinant human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells. J Biol Chem 265:10373–10382

Li H, Wang LX (2004) Design and synthesis of a template-assembled oligomannose cluster as an epitope mimic for human HIV-neutralizing antibody 2G12. Org Biomol Chem 2:483–488
Li Y, Luo L, Rasool N, Kang CY (1993) Glycosylation is necessary for the correct folding of human immunodeficiency virus gp120 in CD4 binding. J Virol 67:584–588
Li Y, Rey-Cuille MA, Hu SL (2001) N-linked glycosylation in the V3 region of HIV type 1 surface antigen modulates coreceptor usage in viral infection. AIDS Res Hum Retroviruses 17: 1473–1479
Li Y, Cleveland B, Klots I, Travis B, Richardson BA, Anderson D, Montefiori D, Polacino P, Hu SL (2008) Removal of a single N-linked glycan in human immunodeficiency virus type 1 gp120 results in an enhanced ability to induce neutralizing antibody responses. J Virol 82: 638–651
Luallen RJ, Lin J, Fu H, Cai KK, Agrawal C, Mboudjeka I, Lee FH, Montefiori D, Smith DF, Doms RW, Geng Y (2008) An engineered Saccharomyces cerevisiae strain binds the broadly neutralizing human immunodeficiency virus type 1 antibody 2G12 and elicits mannose-specific gp120-binding antibodies. J Virol 82:6447–6457
Luallen RJ, Fu H, Agrawal-Gamse C, Mboudjeka I, Huang W, Lee FH, Wang LX, Doms RW, Geng Y (2009) A yeast glycoprotein shows high-affinity binding to the broadly neutralizing human immunodeficiency virus antibody 2G12 and inhibits gp120 interactions with 2G12 and DC-SIGN. J Virol 83:4861–4870
Luallen RJ, Agrawal-Gamse C, Fu H, Smith DF, Doms RW, Geng Y (2010) Antibodies against Manalpha1,2-Manalpha1,2-Man oligosaccharide structures recognize envelope glycoproteins from HIV-1 and SIV strains. Glycobiology 20:280–286
Malito E, Bursulaya B, Chen C, Lo Surdo P, Picchianti M, Balducci E, Biancucci M, Brock A, Berti F, Bottomley MJ, Nissum M, Costantino P, Rappuoli R, Spraggon G (2012) Structural basis for lack of toxicity of the diphtheria toxin mutant CRM197. Proc Natl Acad Sci U S A 109:5229–5234
Mandal M, Dudkin VY, Geng X, Danishefsky SJ (2004) In pursuit of carbohydrate-based HIV vaccines, Part 1: the total synthesis of hybrid-type gp120 fragments. Angew Chem Int Ed 43: 2557–2561
Marradi M, Di Gianvincenzo P, Enriquez-Navas PM, Martinez-Avila OM, Chiodo F, Yuste E, Angulo J, Penades S (2011) Gold nanoparticles coated with oligomannosides of HIV-1 glycoprotein gp120 mimic the carbohydrate epitope of antibody 2G12. J Mol Biol 410:798–810
Mascola JR, Stiegler G, VanCott TC, Katinger H, Carpenter CB, Hanson CE, Beary H, Hayes D, Frankel SS, Birx DL, Lewis MG (2000) Protection of macaques against vaginal transmission of a pathogenic HIV- 1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 6:207–210
McLellan JS et al (2011) Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature 480:336–343
Mitchell DA, Fadden AJ, Drickamer K (2001) A novel mechanism of carbohydrate recognition by the C-type lectins DC- SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. J Biol Chem 276:28939–28945
Mizuochi T, Spellman MW, Larkin M, Solomon J, Basa LJ, Feizi T (1988) Carbohydrate structures of the human-immunodeficiency-virus (HIV) recombinant envelope glycoprotein gp120 produced in Chinese-hamster ovary cells. Biochem J 254:599–603
Mizuochi T, Matthews TJ, Kato M, Hamako J, Titani K, Solomon J, Feizi T (1990) Diversity of oligosaccharide structures on the envelope glycoprotein gp 120 of human immunodeficiency virus 1 from the lymphoblastoid cell line H9. Presence of complex-type oligosaccharides with bisecting N- acetylglucosamine residues. J Biol Chem 265:8519–8524
Moldt B, Rakasz EG, Schultz N, Chan-Hui PY, Swiderek K, Weisgrau KL, Piaskowski SM, Bergman Z, Watkins DI, Poignard P, Burton DR (2012) Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc Natl Acad Sci U S A 109:18921–18925
Moore PL, Gray ES, Wibmer CK, Bhiman JN, Nonyane M, Sheward DJ, Hermanus T, Bajimaya S, Tumba NL, Abrahams MR, Lambson BE, Ranchobe N, Ping L, Ngandu N, Abdool Karim Q,

Abdool Karim SS, Swanstrom RI, Seaman MS, Williamson C, Morris L (2012) Evolution of an HIV glycan-dependent broadly neutralizing antibody epitope through immune escape. Nat Med 18:1688–1692

Mouquet H, Scharf L, Euler Z, Liu Y, Eden C, Scheid JF, Halper-Stromberg A, Gnanapragasam PN, Spencer DI, Seaman MS, Schuitemaker H, Feizi T, Nussenzweig MC, Bjorkman PJ (2012) Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. Proc Natl Acad Sci U S A 109:E3268–E3277

Nabel GJ (2001) Challenges and opportunities for development of an AIDS vaccine. Nature 410:1002–1007

Ni J, Song H, Wang Y, Stamatos NM, Wang LX (2006) Toward a carbohydrate-based HIV-1 vaccine: synthesis and immunological studies of oligomannose-containing glycoconjugates. Bioconjug Chem 17:493–500

Pal R, Hoke GM, Sarngadharan MG (1989) Role of oligosaccharides in the processing and maturation of envelope glycoproteins of human immunodeficiency virus type 1. Proc Natl Acad Sci U S A 86:3384–3388

Peeters JM, Hazendonk TG, Beuvery EC, Tesser GI (1989) Comparison of four bifunctional reagents for coupling peptides to proteins and the effect of the three moieties on the immunogenicity of the conjugates. J Immunol Methods 120:133–143

Pejchal R et al (2011) A potent and broad neutralizing antibody recognizes and penetrates the HIV glycan shield. Science 334:1097–1103

Perrin C, Fenouillet E, Jones IM (1998) Role of gp41 glycosylation sites in the biological activity of human immunodeficiency virus type 1 envelope glycoprotein. Virology 242:338–345

Phalipon A, Tanguy M, Grandjean C, Guerreiro C, Belot F, Cohen D, Sansonetti PJ, Mulard LA (2009) A synthetic carbohydrate-protein conjugate vaccine candidate against Shigella flexneri 2a infection. J Immunol 182:2241–2247

Pollard AJ, Perrett KP, Beverley PC (2009) Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. Nat Rev Immunol 9:213–220

Raska M, Takahashi K, Czernekova L, Zachova K, Hall S, Moldoveanu Z, Elliott MC, Wilson L, Brown R, Jancova D, Barnes S, Vrbkova J, Tomana M, Smith PD, Mestecky J, Renfrow MB, Novak J (2010) Glycosylation patterns of HIV-1 gp120 depend on the type of expressing cells and affect antibody recognition. J Biol Chem 285:20860–20869

Reitter JN, Means RE, Desrosiers RC (1998) A role for carbohydrates in immune evasion in AIDS. Nat Med 4:679–684

Sanders RW, Venturi M, Schiffner L, Kalyanaraman R, Katinger H, Lloyd KO, Kwong PD, Moore JP (2002) The mannose-dependent epitope for neutralizing antibody 2G12 on human immunodeficiency virus type 1 glycoprotein gp120. J Virol 76:7293–7305

Scanlan CN, Pantophlet R, Wormald MR, Ollmann Saphire E, Stanfield R, Wilson IA, Katinger H, Dwek RA, Rudd PM, Burton DR (2002) The broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2G12 recognizes a cluster of alpha1→2 mannose residues on the outer face of gp120. J Virol 76:7306–7321

Scanlan CN, Ritchie GE, Baruah K, Crispin M, Harvey DJ, Singer BB, Lucka L, Wormald MR, Wentworth P Jr, Zitzmann N, Rudd PM, Burton DR, Dwek RA (2007) Inhibition of mammalian glycan biosynthesis produces non-self antigens for a broadly neutralising, HIV-1 specific antibody. J Mol Biol 372:16–22

Schneerson R, Barrera O, Sutton A, Robbins JB (1980) Preparation, characterization, and immunogenicity of Haemophilus influenzae type b polysaccharide-protein conjugates. J Exp Med 152:361–376

Shepherd VL, Lee YC, Schlesinger PH, Stahl PD (1981) L-Fucose-terminated glycoconjugates are recognized by pinocytosis receptors on macrophages. Proc Natl Acad Sci U S A 78: 1019–1022

Shilatifard A, Merkle RK, Helland DE, Welles JL, Haseltine WA, Cummings RD (1993) Complex-type N-linked oligosaccharides of gp120 from human immunodeficiency virus type 1 contain sulfated N-acetylglucosamine. J Virol 67:943–952

Snyder GA, Ford J, Torabi-Parizi P, Arthos JA, Schuck P, Colonna M, Sun PD (2005) Characterization of DC-SIGN/R interaction with human immunodeficiency virus type 1 gp120 and ICAM molecules favors the receptor's role as an antigen-capturing rather than an adhesion receptor. J Virol 79:4589–4598

Stahl PD, Rodman JS, Miller MJ, Schlesinger PH (1978) Evidence for receptor-mediated binding of glycoproteins, glycoconjugates, and lysosomal glycosidases by alveolar macrophages. Proc Natl Acad Sci U S A 75:1399–1403

Stamatatos L, Morris L, Burton DR, Mascola JR (2009) Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? Nat Med 15:866–870

Su SV, Hong P, Baik S, Negrete OA, Gurney KB, Lee B (2004) DC-SIGN binds to HIV-1 glycoprotein 120 in a distinct but overlapping fashion compared with ICAM-2 and ICAM-3. J Biol Chem 279:19122–19132

Trkola A, Purtscher M, Muster T, Ballaun C, Buchacher A, Sullivan N, Srinivasan K, Sodroski J, Moore JP, Katinger H (1996) Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. J Virol 70:1100–1108

Vaccari M, Poonam P, Franchini G (2010) Phase III HIV vaccine trial in Thailand: a step toward a protective vaccine for HIV. Expert Rev Vaccines 9:997–1005

Walker LM et al (2009) Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science 326:285–289

Walker LM, Simek MD, Priddy F, Gach JS, Wagner D, Zwick MB, Phogat SK, Poignard P, Burton DR (2010) A limited number of antibody specificities mediate broad and potent serum neutralization in selected HIV-1 infected individuals. PLoS Pathog 6:e1001028

Walker LM, Sok D, Nishimura Y, Donau O, Sadjadpour R, Gautam R, Shingai M, Pejchal R, Ramos A, Simek MD, Geng Y, Wilson IA, Poignard P, Martin MA, Burton DR (2011a) Rapid development of glycan-specific, broad, and potent anti-HIV-1 gp120 neutralizing antibodies in an R5 SIV/HIV chimeric virus infected macaque. Proc Natl Acad Sci U S A 108: 20125–20129

Walker LM et al (2011b) Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature 477:466–470

Walsh G, Jefferis R (2006) Post-translational modifications in the context of therapeutic proteins. Nat Biotechnol 24:1241–1252

Wang LX (2006) Toward oligosaccharide- and glycopeptide-based HIV vaccines. Curr Opin Drug Discov Devel 9:194–206

Wang LX, Ni J, Singh S, Li H (2004) Binding of high-mannose-type oligosaccharides and synthetic oligomannose clusters to human antibody 2G12: implications for HIV-1 vaccine design. Chem Biol 11:127–134

Wang J, Li H, Zou G, Wang LX (2007) Novel template-assembled oligosaccharide clusters as epitope mimics for HIV-neutralizing antibody 2G12. Design, synthesis, and antibody binding study. Org Biomol Chem 5:1529–1540

Wang SK, Liang PH, Astronomo RD, Hsu TL, Hsieh SL, Burton DR, Wong CH (2008) Targeting the carbohydrates on HIV-1: interaction of oligomannose dendrons with human monoclonal antibody 2G12 and DC-SIGN. Proc Natl Acad Sci U S A 105:3690–3695

Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, Salazar-Gonzalez JF, Salazar MG, Kilby JM, Saag MS, Komarova NL, Nowak MA, Hahn BH, Kwong PD, Shaw GM (2003) Antibody neutralization and escape by HIV-1. Nature 422:307–312

Winzeler EA et al (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285:901–906

Wyatt R, Sodroski J (1998) The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 280:1884–1888

Wyatt R, Moore J, Accola M, Desjardin E, Robinson J, Sodroski J (1995) Involvement of the V1/V2 variable loop structure in the exposure of human immunodeficiency virus type 1 gp120 epitopes induced by receptor binding. J Virol 69:5723–5733

Wyatt R, Kwong PD, Desjardins E, Sweet RW, Robinson J, Hendrickson WA, Sodroski JG (1998) The antigenic structure of the HIV gp120 envelope glycoprotein. Nature 393:705–711

Yeh JC, Seals JR, Murphy CI, van Halbeek H, Cummings RD (1993) Site-specific N-glycosylation and oligosaccharide structures of recombinant HIV-1 gp120 derived from a baculovirus expression system. Biochemistry 32:11087–11099

Zhu X, Borchers C, Bienstock RJ, Tomer KB (2000) Mass spectrometric characterization of the glycosylation pattern of HIV- gp120 expressed in CHO cells. Biochemistry 39:11194–11204

Zolla-Pazner S (2004) Identifying epitopes of HIV-1 that induce protective antibodies. Nat Rev Immunol 4:199–210