Potential of Carbohydrate-Binding Agents as Therapeutics Against Enveloped Viruses

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Abstract: Twenty-seven years after the discovery of HIV as the cause of AIDS more than 25 drugs directed against four different viral targets (i.e. reverse transcriptase, protease, integrase, envelope gp41) and one cellular target (i.e. CCR5 co-receptor) are available for treatment. However, the search for an efficient vaccine is still ongoing. One of the main problems is the presence of a continuously evolving dense carbohydrate shield, consisting of *N*-linked glycans that surrounds the virion and protects it against efficient recognition and persistent neutralization by the immune system. However, several lectins from the innate immune system specifically bind to these glycans in an attempt to process the virus antigens to provoke an immune response. Across a wide variety of different species in nature lectins can be found that can interact with the glycosylated envelope of HIV-1 and can block the infection of susceptible cells by the virus. In this review, we will give an overview of the lectins from non-mammalian origin that are endowed with antiviral properties and discuss the complex interactions between lectins of the innate immune system and HIV-1. Also, attention will be given to different carbohydrate-related modalities that can be exploited for antiviral chemotherapy. Med Res Rev, 32, No. 2, 349–387, 2012

Key words: HIV-1; carbohydrate-binding agent (CBA); *N*-linked glycan; lectin; innate immune system

1. INTRODUCTION

Lectins are carbohydrate-binding proteins that do not modify the carbohydrates to which they bind. These proteins can be found across a wide variety of different species in nature, including prokaryotes, sea corals, algae, fungi, higher plants, invertebrates, and vertebrates.[1–3] As also synthetic agents with carbohydrate-binding capacity have been identified, the term carbohydrate-binding agent (CBA) was introduced to include both the peptidic lectins and the non-peptidic low-molecular-weight agents. Binding of the CBAs to *N*-linked glycans of glycoproteins predominantly occurs via mannose, glucose, fucose, *N*-acetylglucosamine, galactose, *N*-acetylgalactosamine, and/or sialic acid residues. The internal linkages between the individual monosaccharides of an *N*-linked glycan play an important

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role in the specificity of binding of a CBA. The human immune system takes advantage of the presence of the dense glycan shield that surrounds certain pathogens. Various C-type lectins, such as DC-SIGN on dendritic cells (DC), the mannose receptor MMR on macrophages, the soluble mannose-binding lectin (MBL) found in plasma and several more, bind the oligosaccharides on the surface of viruses and bacteria in order to remove them and in the case of antigen-presenting cells, present the foreign epitopes to $CD4^+$ T cells to provoke an efficient immune response against the pathogen. However, as will be discussed further in this review, some pathogens such as HIV-1 try to circumvent such eradication and use these C-type lectins of the innate immune system to gain easily access to the lymph nodes populated with $CD4^+$ T lymphocytes.

Recently CBAs were put in the spotlight as new potentially promising antiviral agents, since the discovery that infection of heavily glycosylated viruses, such as human immunodeficiency virus (HIV-1) (for review see[2,3]) and hepatitis C virus (HCV)[4,5] could efficiently be blocked by certain CBAs. Moreover, CBAs not only prevent the virus from entry into its target host cell, but long-term drug pressure results in the selection of virus strains in which several *N*-linked glycans that constitute the protective glycan shield around the viral particles were deleted. As this shield masks important immunogenic epitopes of the viral envelope and often protects the virus against recognition and eradication by neutralizing antibodies of the immune system, CBAs might have the potential to assist the immune system in containing the viral infection. This dual mode of action might also work against other glycan-containing enveloped viruses, as in the case of flaviviruses such as Dengue virus (DENV),[6] coronaviruses like the severe acute respiratory syndrome coronavirus (SARS-CoV)[7] and other *Nidovirales*.[8] Thus, for CBAs to become efficient antiviral compounds, they need to be able to inhibit this specific interaction between viruses and lectins of the immune system that are used by the virus for transmission and establishment of the infection.

In this review, an overview will be given of the CBAs that are able to bind and neutralize HIV. They predominantly include lectins from prokaryotic and plant origin, (semi)synthetic CBAs, but also lectins of the immune system. The CBA concept for the treatment of glycosylated enveloped viruses will be thoroughly covered, and recent advances in this emerging field will be discussed.

2. ENVELOPED GLYCOSYLATED VIRUSES SUSCEPTIBLE TO CBA INTERACTION

The envelope of HIV-1 consists of the highly glycosylated surface protein gp120, and the transmembrane protein gp41. Gp120 first binds to the cellular receptor CD4, undergoes a conformational change and then binds to the coreceptor CCR5 or CXCR4. This binding triggers other conformational changes, resulting in the insertion of the fusion peptide of gp41 in the cellular membrane and eventual fusion of the virus with the cell. Around 18–33 (average ~24) *N*-glycosylation motifs (N-X-T/S, where X is not proline) can be found in the gp120 sequence, and it was shown by Leonard et al.[9] that all motifs are occupied by a glycan. Moreover, 11 oligosaccharides were determined to be high-mannose type or hybrid type, while 13 were complex type. This is a remarkable ratio in favor of high-mannose type glycans, because viruses derive their glycans through the glycosylation machinery of the host cell. Such a high density of high-mannose type glycans has never been observed on mammalian glycoproteins. Therefore, we might expect neutralization of HIV-1 by the mannose-specific lectins of the innate immune system, such as by the MBL, but the opposite is true. As we will discuss further in this review, host lectins bind HIV presumably through the high-mannose type glycans but HIV uses this event for survival and to escape destruction by the immune system.

A variety of other enveloped viruses also contain highly glycosylated envelope proteins, and thus, may be targeted by CBAs. HCV is an enveloped RNA virus classified within the family of *Flaviviridae*. Infection of HCV often leads to chronic disease such as hepatitis, cirrhosis, and hepatocellular carcinoma.[10] The major site of replication is the liver, but other cell types, such as monocytes and macrophages, were also reported to become infected with HCV.[11] Four cellular receptors have already been identified, all of which are required at the same time for HCV-entry to occur.[12] HCV encodes two envelope glycoproteins, E1 and E2 (reviewed in[13]). E1 contains up to six potential *N*-glycosylation sites, while E2 can contain up to 11 *N*-glycans. However, due to a proline immediately downstream of the consensus sequence, one of the potential *N*-glycosylation sites of E1 is not recognized by the oligosaccharyltransferase.[14] All 11 potential glycosylation motifs were occupied with high-mannose type glycans, except for positions N41 and 48, where a complex type glycan was found.[15] It is thought that the E2 glycoprotein is responsible for initiating virus attachment,[16,17] while the E1 glycoprotein is responsible for fusion of the virus with the cell membrane.[18,19]

DENV is a single positive-stranded RNA virus that also belongs to the *Flaviviridae* family. It is transmitted by the *Aedes aegypti* mosquito, and infection can result in flu-like symptoms that can progress to dengue hemorrhagic fever, causing 25,000 deaths, and about half a million patients being hospitalized a year.[20] Two glycoproteins can be found on the surface of immature virus particles, the premembrane (prM) and the envelope (E) protein (reviewed in[21]). Upon maturation of the virus particle prM is cleaved to M. The envelope E protein forms the shell of the virus, and changes conformation during the immature, mature, and fusion-activated state of the virion (reviewed in[22] and references therein). In contrast with other members of the *Flaviviridae* family, the E glycoprotein of DENV contains only 2 *N*-linked glycosylation sites. Asn153 is conserved among all flaviviruses while Asn67 is unique for DENV.[23] It is postulated that the *N*-linked glycan at position 67 is high-mannose type, while the oligosaccharide at position N153 is pauci-mannose type, a glycan type that can be abundantly found on insect cell glycans,[24] but also in plants.[25,26]

Coronaviruses were given renewed interest after the discovery that the severe acute respiratory syndrome (SARS) was caused by a coronavirus (SARS-CoV). Coronaviruses are enveloped single-stranded positive RNA viruses (reviewed in[27]). Several coronaviruses are important veterinary pathogens, causing respiratory or enteric diseases in livestock and poultry. The envelope consists of the membrane protein M, the spike protein S and the envelope protein E. The E protein is not glycosylated. The M protein is a glycoprotein with 1 *N*-linked glycan at position 4, and is the main structural component of the virion that mediates assembly and budding of viral particles.[28] The spike protein S forms a trimer that belongs to the group of class I viral fusion proteins, that also includes Env of HIV-1, and mediates virus–cell attachment and fusion.[27] The S protein is further cleaved by proteases, like trypsin or factor Xa into the S1 and S2 subunits. The spike protein of SARS-CoV is heavily glycosylated, with 23 putative *N*-glycosylation sites, of which at least 12 have been described to be glycosylated.[29] Only four oligosaccharides were structurally determined, of which two were high-mannose type, and two were complex type. Other coronaviruses include feline infectious peritonitis virus (FIPV), mouse hepatitis virus (MHV), and feline enteric coronavirus (FECV).

The recent outbreaks of influenza H1N1 (also known as swine flu) and H5N1 (avian flu) have raised the need for a better understanding of influenza virus infections and how to block them. Influenza type A viruses can be categorized based on the properties of the two membrane glycoproteins (spikes), hemagglutinin (HA) and neuraminidase (NA).[30] To date, there are 9 different HA and 15 different NA variants known. Between different virus subtypes the amino acid sequence of HA can vary up to 70%. HA plays a major role in influenza

virus entry. After binding of HA to the terminal sialic acids of the epithelial receptor, the virus is taken up by endocytosis. The low pH in the endosomes activates fusion of the viral and endosomal membranes (reviewed in[30]). HA is synthesized as a precursor HA0, and is cleaved to generate HA1 and HA2. Both HA1 and HA2 are glycosylated, but among subtypes the variation in glycosylation is more extensive in HA1 than in HA2, where glycosylation is more conserved.[31] Intensive study of the oligosaccharides on HA of fowl plague virus suggested the presence of seven *N*-linked glycans, five of which are complex type, one proved high-mannose type, and one hybrid type.[32] However, the number and structure of the glycans vary greatly among different influenza A subtypes and even during virus evolution in time.

Human T cell lymphotropic virus type 1 (HTLV-1) was the first human retrovirus to be associated with disease, like adult T cell leukemia (ATL), cutaneous T cell lymphoma and HTLV-associated myelopathy (HAM). The surface unit gp46, noncovalently attached to the membrane unit gp21, builds up the envelope.[33] Gp46 contains five glycosylation motifs that are all utilized,[34] while gp21 only contains one *N*-glycan motif.

Over 100 double-stranded DNA viruses constitute the herpes simplex virus family. Herpes simplex virus (HSV) type 1 and type 2 are members of the alphaherpesvirus subfamily.[35] Five glycoproteins are involved in HSV entry, being gB, gC, gD, Gh, and gL.[36,37] These glycoproteins also play a role in immune evasion and cellular responses. The gC family of type I membrane glycoproteins contain both *N*-linked (at least eight potential *N*-glycan sites) and *O*-linked (mucin-like) glycans.[37] Also human cytomegalovirus (CMV) contains several glycoproteins in its envelope (i.e. p86 and p130/55) for which *N*-linked high-mannose type carbohydrates were determined.[38]

Several other viruses contain glycoproteins in their envelope (i.e. parainfluenza viruses, respiratory syncytial virus, etc.), but are not further considered in this review due to their reported insensitivity to CBAs[39] or to lack of reports in literature on potential interaction with CBAs.

3. OVERVIEW OF CBAS ENDOWED WITH ANTIVIRAL ACTIVITY

A. Cyanobacterial CBAs

Several lectins from prokaryotic origin were shown to be able to bind to HIV-1 in a carbohydrate-dependent manner. The most intensely studied prokaryotic antiviral lectin is Cyanovirin-N (CV-N) derived from the cyanobacterium *Nostoc ellipsosporum*. This 11 kDa small virucidal protein is able to inactivate diverse laboratory HIV-strains and primary isolates, as well as HIV-2 and SIV strains at nanomolar concentrations.[40] Crystal structures revealed that CV-N forms dimers by domain swapping, thereby creating two new carbohydrate-binding sites, besides the two primary binding sites.[41,42] CV-N preferentially binds glycans containing Manα1-2Man, which is only present on the terminal branches of high-mannose type glycans. Besides HIV-1, CV-N was also reported to inhibit infection of HCV,[5] influenza virus,[43] Ebola virus[44,45] and HSV type-1 infection in cell culture.[46] The microbicidal potential of CV-N has already been proven in a macaque model[47,48] and attempts to construct live microbicides, such as engineered *Lactobacillus* strains expressing CV-N are on the way.[49,50] However, care should be taken when considering CV-N as a potential microbicide, as it was shown recently that the lectin induces the expression of a wide panel of chemokines and has stimulatory/mitogenic activity in PBMC cultures, what might result in cells being more susceptible to HIV-infection, and in the induction of HIV-1 replication in latently infected cells.[51]

Three other lectins derived from cyanobacteria have been described to possess anti-HIV-activity. MVL, derived from *Microcystis viridis* is a mannan-binding lectin of 13 kDa. It is composed of two tandemly repeated homologous domains of 54 amino acids.[52] Its minimal target comprises the Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc tetrasaccharide core of oligomannosides.[53] Scytovirin, expressed by *Scytonema varium*, consists of 95 amino acids organized in two domains with carbohydrate-binding sites and with five intrachain disulfide brigdes.[54] It binds gp120 through interaction with α(1-2), α(1-2), α(1-6) tetramannoside units on oligosaccharides.[55] Finally, a new lectin from the filamentous cyanobacterium *Oscillatoria agardhii*, OAA, was described to be endowed with antiviral activity against HIV-1.[56] OAA exclusively binds to high-mannose type *N*-glycans. Alike scytovirin, OAA possesses two carbohydrate-binding sites per molecule, and is able to inhibit HIV-1 infection at concentrations in the nanomolar range.

B. Sea Coral-Derived CBAs

The carbohydrate-binding protein derived from the sea coral *Gerardia savaglia* was one of the first lectins shown to possess anti-HIV activity in cell culture.[57,58] It is a 14.8 kDa protein consisting as a dimer and requiring Ca^{2+} for efficient carbohydrate binding. It showed specificity for D-mannose.

C. Actinomycete-Derived CBAs

From the actinomycete strain K97-0003, also known as *Longispora albida*, actinohivin (AH) is derived. AH has a molecular mass of 12.5 kDa and exhibits internal sequence triplication.[59] Like most CBAs with antiviral properties, AH inhibits viral entry by binding high-mannose type glycans on gp120.[60] Recently, the crystal structure of AH was resolved, which revealed a three-dimensional (3D) structure containing three sugar-binding pockets (Fig. 1).[61] Thus, as is the case for the other CBAs described so far (and for the ones that are going to be described), multivalency seems to be common among many, if not all, naturally derived CBAs.

D. Algae-Derived CBAs

Griffithsin (GRFT), extracted from the red alga *Griffithsia* sp. is one of the most potent anti-HIV CBAs described so far, being able to block HIV-1 infection in the picomolar range.[62] For this reason, GRFT is being studied as a potential candidate microbicide agent.[63,64] Determination of the 3D structure revealed that GRFT consists of a domain-swapped dimer, and each monomer has three almost identical carbohydrate-binding sites (Fig. 1).[65]

E. CBAs Derived from Plants

Since the late 1980s/early 1990s, antiviral research has also focused on the potential of plant lectins as antiviral agents.[39,58,66] Plant lectins can be isolated from over a thousand plant species, but no more than 500 have been well documented (for a review, see[67]). Here, only the plant lectins with antiviral activity will be mentioned in brief.

Most plant lectins with anti-HIV activity have specificity for mannose. The mannose-specific plant lectins CHA (*Cymbidium* hybrid agglutinin), EHA (*Epipactis helleborine* agglutinin), LOA (*Listera ovata* agglutinin), NPA (*Narcissus pseudonarcissus* agglutinin), GNA (*Galanthus nivalis* agglutinin), and HHA (*Hippeastrum* hybrid agglutinin) are potent inhibitors of HIV-1 entry into target cells.[39,66] Also the *N*-acetylglucosamine-specific lectin from the stinging nettle *Urtica dioica* (UDA), with a 8.7 kDa size being among the smallest lectins known so far, is able to prevent HIV infection (Fig. 1).[68] MHL derived from

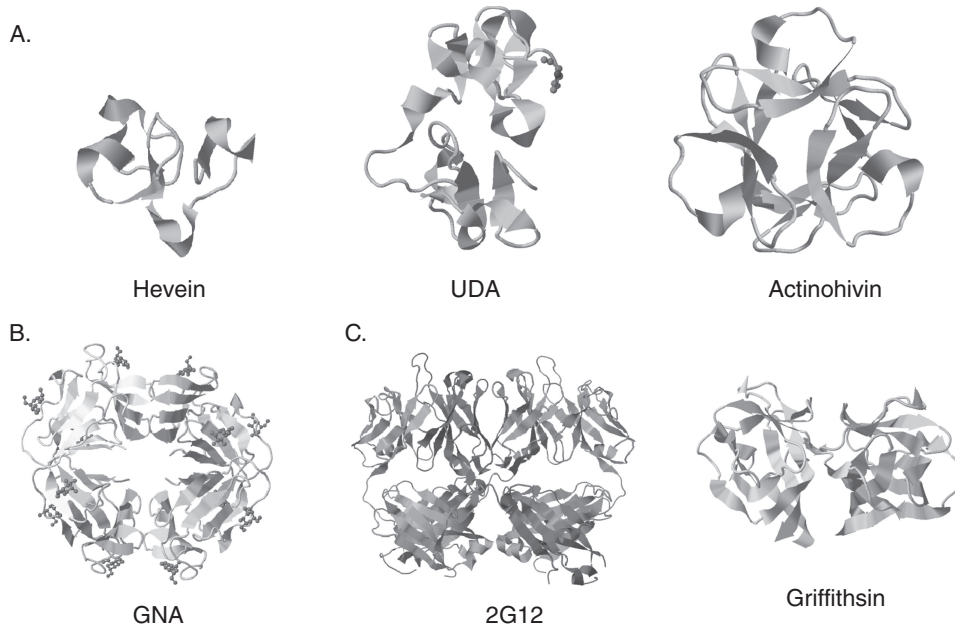


Figure 1. Different manners for CBAs to achieve multivalency. **A**. A single molecule contains several CRDs. Hevein[233] (derived from *Hevea brasiliensis*) is probably the smallest and most simple plant lectin containing one CRD. UDA[234] contains two hevein-like CRDs, while AH[61] has 3 three sugar-binding pockets. **B**. Several monomers combine to form a multimer. GNA[235] is a tetramer, each monomer having two carbohydrate-binding sites. Tertramerization creates four additional CRDs C. Domain swapping. A secondary or tertiary element of a monomeric protein is replaced by the same element of the other peptide, thus forming oligmers. The antibody 2G12[148] and the protein griffithsin[65] achieve multivalency through domain swapping.

Myrianthus holstii is, like UDA, a small cysteine-rich lectin with GlcNAc specificity, being active against HIV at nanomolar concentrations.[69] A variety of other plant-derived lectins, including mannose-specific lectins from *Allium porrum* (APA)[70] and *Allium ursinum* (AUA)[39] and the mannose-specific lectins from the Fabaceae family (peas and beans)[71,72] showed anti-HIV activity in cell culture (Table I). Recently a new plant lectin, derived from the banana *Musa acuminata*, named BanLec, was described to inhibit HIV-1 infection by binding the high-mannose type glycans on gp120.[73] Concanavalin A (ConA), Wheat germ agglutinin (WGA) and Phytohaemagglutinin (PHA) also seem to recognize the carbohydrates on HIV gp120 and show anti-HIV activity, but they are rather cytotoxic (i.e. mitogenic) in cell culture.

F. Invertebrate-Derived CBAs

Three lectins isolated from the Annelida family were also reported to possess anti-HIV activity. The lectin CVL, isolated from the marine worm *Chaetopterus variopedatus* is the only lectin known so far to inhibit HIV-1 by binding β-galactose.[74] A GlcNAc-specific Ca^{2+}-independent lectin was isolated from the sea worm *Serpula vermicularis* (SVL) as a homotetrameric protein with a total molecular mass of 50 kDa.[75] Finally, Mermaid was purified from *Laxus oneistus*. This Ca^{2+}-dependent mannose-specific lectin is structurally homologous to DC-SIGN, and as such, is able to inhibit HIV-1 binding to DC-SIGN.[76]

G. Vertebrate-Derived CBAs

In mammals, protein–carbohydate interactions serve multiple functions in the innate immune system and play a dual role: they are involved in a variety of cell–cell interaction and

Table I. Overview of Carbohydrate-Binding Agents of Non-mammalian Origin, Known to Interact with HIV-1

Species	Lectin name	Abbreviation	Carbohydrate specificity	Reference
Cyanobacteria				
Nostoc ellipsosporum	Cyanovirin-N	CV-N	α(1,2)Man	40
Scytonema varium	Scytovirin	SVN	α(1,2)-α(1,6)Man	55
Microcystis viridis	None	MVL	Manβ(1,4)GlcNAc	53
Oscillatoria agardhii	None	OAA	Man	56
Sea corals				
Gerardia savaglia	None	GSL	D-Man	58
Algae				
Griffithsia spp.	Griffithsin	GRFT	Man, Glc, GlcNAc	62
Fungae				
Longispora albida	Actinohivin	AH	Man	59
Annelida				
Chaetopterus variopedatus	None	CVL	β-Gal	74
Serpula vermicularis	None	SVL	GlcNAc	75
Laxus oneistus	Mermaid	None	Man	76
Plants				
Orchidaceae				
Listera ovata	Twayblade lectin	LOA	α(1,3)Man	66
Epipactis helleborine	Broad-Leaved helleborine lectin	EHA	Man	39
Cymbidium hybrid	None	CA	Man	39
Amaryllidaceae				
Galanthus nivalis	Snowdrop lectin	GNA	α(1,3)Man	66
Hippeastrum hybrid	Amaryllis lectin	HHA	α(1,3)-α(1,6)Man	66
Narcissus pseudonarcissus	Daffodil lectin	NPA	α(1,6)Man	66
Alliaceae				
Allium porrum	Leek lectin	APA	Man	70
Allium ursinum	Ramsons lectin	AUA	Man	39
Moraceae				
Artocarpus integrifolia	Jacalin, jack fruit lectin	Jacalin	Galα(1,6) or Galβ(1,3)GalNAc	232
Fabaceae				
Canavalia ensiformis	Jack bean lectin/ Concanavalin A	ConA	Man > Glc > GlcNAc	71,72
Pisum sativum	Garden pea lectin	PSA	Man > Glc/GlcNAc	71,72
Lens culinaris	Lentil lectin	LCA	Man > Glc > GlcNAc	71,72
Vicia faba	Broad bean, faba bean lectin	VFA	Man > Glc/GlcNAc	71,72
Lathyrus odoratus	Sweet pea lectin	None	Man > Glc > GlcNAc	71,72
Phaseolus vulgaris	Phytohaemagglutinin	PHA		71,72
Urticaceae				
Urtica dioica	Stinging nettle lectin	UDA	GlcNAc oligomers	68
Cecropiaceae				
Myrianthus holstii	Myrianthin	MHA	GlcNAc	69
Poaceae				
Triticum spp.	Wheat germ agglutinin	WGA	GlcNAc	39
Musaceae				
Musa acuminata	Banana lectin	BanLec	Man	73

Table II. Lectins of the Innate Immune System that Interact with HIV-1

Lectin	Found on	Specificity	Reference
Galectins			
Galectin-1	Thymus, endothelial cells, activated T cells, macrophages, activated B cells, trophoblasts, follicular dendritic cells	β-galactose	78,79
Siglecs			
Sialoadhesin	Macrophages, monocytes	Sialic acid	83
C-type lectin			
DC-SIGN	Dendritic cells, macrophages, B cells	Internal trimannose, Manα(1,2)Man, fuc	86,87
L-SIGN	Endothelial cells of liver and lymph nodes, placental villi		106,107
Langerin	Langerhans cells	Man, GlcNAc, fuc	121
MMR	Macrophages, dendritic cells	Man, fuc, GlcNAc, gluc	129
MBL	In serum	Man, GlcNAc, fuc	137–139
SP-D	Lung fluid, mucosal fluid, blood		143
SP-A	Lungs, amniotic fluid, vaginal fluid	Man	144
DCIR	B cells, monocytes, DCs		145
Antibody			
2G12	Blood	Manα(1,2)Man	146,147
Defensins			
α-defensin	Leukocytes, epithelial cells		164
β-defensin	Leukocytes, epithelial cells		165
θ-defensin	Leukocytes, epithelial cells		159,160

functioning as well as in pathogen recognition. Some of them may lead to pathogen neutralization.[77] Three major structural families of vertebrate lectins exist: galectins, siglecs, and C-type lectins.[1] Because an extensive overview of lectins of the immune system is out of the scope of this review, we will mainly focus on lectins that have been reported and/or are potentially able to interact with HIV-1. This includes a subset of C-type lectins but also the carbohydrate-specific antibody 2G12, and the minidefensins (Table II).

1. Galectins

Galectins are a family of soluble lectins that are designated for their galactose specificity. Only galectin-1 (out of the 15 galectins discovered so far) has been reported to interact with HIV-1 to promote viral adsorption.[78,79] In the presence of galectin-1 virus infectivity was increased, and this increase could be blocked with lactose, suggesting an involvement of the carbohydrate-recognition domain (CRD) of galectin-1.

2. Siglecs

The siglecs (sialic acid-binding, Ig-like lectins) constitute a distinct subset of the Ig superfamily.[1] As the name suggests, these glycan-binding proteins are well known for their specificity for sialic acid-containing glycans, of which *N*-acetylneuraminic acid being the most common.[80] Sialoadhesin (CD169, siglec-1) is expressed on activated macrophages in chronic inflammation and in tumors, but also on monocytes from HIV-1 infected individuals.[81] The expression of sialoadhesin is upregulated in $CD14^+$ cells after HIV-1 infection[82] and circulating sialoadhesin-expressing monocytes are capable of binding HIV-1 and exposing the virus to immune target cells.[83]

3. C-type lectins

Most of the vertebrate lectins are members of the C-type or Ca^{2+}-dependent lectin family, also called C-type lectin receptors (CLRs). In fact, any protein containing one or more C-type lectin domains can be classified as a CLR.[84] C-type lectins are either produced as transmembrane proteins or secreted as soluble proteins (reviewed in ref[85]). Soluble lectins include members of the collectin family, such as mannose-binding protein (MBP) or the lung surfactant proteins A (SP-A) and D (SP-D). Based on their molecular structure, two groups of C-type lectins can be distinguished on DCs: type I C-type lectins, with an extracellular *N*-terminus (i.e. MMR and DEC-205), and type II lectins, with an intracellular *N*-terminus (i.e. DC-SIGN, L-SIGN, Langerin). All type II C-type lectins only contain one CRD, while type I lectins contain several CRD or CRD-like domains.[85]

a. DC-SIGN and L-SIGN

DC-SIGN, which stands for DC-specific ICAM-3 grabbing nonintegrin,[86] was the first C-type lectin shown to be able to bind HIV-1, HIV-2 and SIV and enhance *trans* infection of T cells.[87,88] It is a type II integral membrane protein of 404 amino acids, with an ectodomain that contains a short *N*-terminal domain, a repeat region consisting of 7.5 copies of a 23 amino acid sequence, and a *C*-terminal CRD.[86] It forms a tetramer when present in the cellular membrane. Structural studies by Feinberg et al.[89] revealed that the CRD of DC-SIGN interacts with an internal α1-3-linked mannose, but only when present in a high-mannose type glycan. Later on, Appelmelk et al.[90] discovered that DC-SIGN binds also to Lewis blood group antigens that contain fucose residues. Moreover, DC-SIGN binds with much higher affinity to the fucose-containing carbohydrate Le^x than to mannotriose. Thus, DC-SIGN efficiently recognizes at least two classes of glycans: mannose-rich glycans and fucosylated glycans.[91]

DC-SIGN, as the name suggests, can be found on some subsets of immature dendritic cells (IMDCs), but later it was shown that it is also expressed on the surface of $CD4^+$ macrophages in the placenta and the lung,[92] and on a subset of B cells in the peripheral blood and tonsils of healthy, HIV-1 seronegative donors.[93] The lectin is involved in the interaction between DCs and resting T cells by interacting with ICAM-3.[86] IMDCs in peripheral tissues capture antigens and subsequently migrate to the T cell areas of secondary lymphoid organs. On their journey to the lymphoid organs, their expression profile changes, downmodulating the expression of DC-SIGN, and upregulating the expression of chemokine receptors and other cell surface molecules.[87] In the lymph nodes, the mature DCs are able to activate resting T cells.

Instead of being taken up by DCs and degraded in the lysosomal compartments, HIV binds to DC-SIGN and uses DC-SIGN to afford infection of T cells in the lymphoid tissues.[86,87] As mentioned above, at the same time DC-SIGN was discovered, it was also shown that HIV-1 is able to bind DC-SIGN and be transferred to resting T cells in the lymphoid tissues.[86,87] This process was named in *trans* infection of T cells. It was shown by Baribaud et al.[94] that productive infection of PBMCs by HIV could occur with virus amounts that are not sufficient to initiate infection when applied directly to activated T cells as free virus particles. Thus, HIV attached to DCs by DC-SIGN can more efficiently be transmitted to $CD4^+$ T lymphocytes than cell-free virus. Moreover, HIV bound to DC-SIGN can remain infectious for up to 5 days,[87] due to the internalization of intact HIV into a nonlysosomal compartment.[95–97] However, not all virus is saved from degradation, and probably only a minority of the internalized virus is retained and kept intact inside DCs, while the majority of virus particles are degraded, processed and presented to T lymphocytes in the lymph nodes.[97,98]

DCs also express CD4 and CCR5, although the expression levels are too low to support efficient HIV-infection. However, DC-SIGN is able to concentrate HIV on the surface of the DC, thus facilitating the interaction between CD4 and CCR5, and enhancing infection in *cis*.[92]

In fact, it has been shown that DC-SIGN surface levels are upregulated in HIV-infected cells. This is caused by the HIV protein Nef, which acts by inhibiting DC-SIGN endocytosis. This upregulation dramatically increases the interaction of DCs with T lymphocytes and HIV-1 transmission.[99]

Thus, DCs transfer HIV-1 to $CD4^+$ lymphocytes in two distinct phases. In the first phase, the virus is internalized into nonlysosomal compartments and transferred via the infectious synapse to $CD4^+$ T lymphocytes within 24 hr. However, at the same time, the DC can become infected through the CD4-coreceptor complex. This represents the second phase, which is dependent on productive infection of immature DCs by the virus, as it can be inhibited by zidovudine.[97]

Macrophages present in breast milk can be induced to express DC-SIGN and transmit virus to T cells.[100] However, children who were breast fed during the first months of their lives were protected against HIV infection.[101] Recently it was shown that a number of factors present in human breast milk block DC-SIGN-mediated HIV transmission to $CD4^+$ T cells, thus inhibiting mother-to-child transmission. It was first reported that multimeric and protein-associated Lewis X (Le^x) motifs in breast milk could bind to DC-SIGN and prevent the capture of HIV-1 and subsequent transmission to $CD4^+$ T lymphocytes.[102] Later on, also bile salt-stimulated lipase and MUC1, Le^x containing glycoproteins that are abundantly present in breast milk, were also reported to inhibit binding of HIV-1 to DC-SIGN.[103,104] Moreover, IgA and IgG antibodies to the DC-SIGN CRD are also able to bind DC-SIGN and inhibit transmission in *trans* of R5-tropic HIV-1.[105] Thus, although HIV-1 can be transmitted via breast milk, there are factors present in breast milk that suppress the mother-to-child transmission.

L-SIGN (previously named DC-SIGNR), a DC-SIGN homologue, was also described to be able to capture HIV-1, HIV-2, and SIV, and transmit the virus to human PBMCs.[106,107] It exhibits 77% amino acid identity with DC-SIGN. This receptor is expressed on sinusoidal endothelial cells in the liver and on endothelial cells in lymph node sinuses and placental villi. As lymph nodes represent the major site of HIV replication in vivo, the presence of L-SIGN on the surface of endothelial cells in lymph node sinuses represents an obvious mechanism by which virus can be transmitted to $CD4^+$ cells.

DC-SIGN captures not only HIV-1 but also many other viruses containing a glycosylated envelope. CMV expresses the envelope glycoprotein gB, which can bind to DC-SIGN and be transmitted to permissive cells.[108] This is also the case for HCV, where both L-SIGN and DC-SIGN capture and transmit the virus to hepatocytes,[10,109] and for the SARS-CoV.[110] Both lectin receptors can also bind Ebola virus GP and act as cofactors for cellular entry.[111,112] DC-SIGN-bound Ebola virus could also be transmitted to susceptible cells. Infection of Marburg virus is greatly enhanced when DC-SIGN and L-SIGN are expressed.[110] DENV uses DC-SIGN to productively infect DCs.[6,113,114] Finally, also HTLV-1 is able to bind DC-SIGN via its glycoprotein gp46. Moreover, the presence of DC-SIGN significantly enhances viral entry of HTLV-1 into susceptible cells, and like HIV-1, HTLV-1 bound to DC-SIGN can be transmitted to T cells.[115]

However, the presence of a highly glycosylated envelope does not necessarily imply that binding of the virus to DC-SIGN occurs. For example, vesicular stomatitis virus, which contains a highly glycosylated envelope, does not interact with DC-SIGN. These observations indicate that some degree of selectivity exists for recognition of glycoproteins by DC- or L-SIGN.[95]

b. Langerin

Langerhans cells (LCs) are IMDCs located in the skin epidermis and mucosal tissues. They express the unique type II transmembrane receptor Langerin (CD207).[116,117] Like DCs, LCs perform an essential function in the immune response with their ability to take up and

process foreign as well as self antigens and to present the processed antigens to T cells after migration to lymph nodes. As they are present in the stratified squamous epithelia of the genital mucosa, they are probably among the first cells to encounter HIV during sexual transmission. The extracellular region of the receptor exists as a trimer,[118] and the CRD shows specificity for mannose, GlcNAc and fucose. The CRD of Langerin resembles the CRD of other C-type lectins, but with some exceptions: (1) there are several extra secondary structural elements in Langerin, such as an additional 3_{10}-helix and a new two-stranded β-sheet; (2) Langerin possesses only one of the four possible calcium-binding sites described for this fold; and (3) there appears to be a second, calcium-independent sugar-binding site in the CRD.[119]

In 2003, Kawamura et al.[120] reported that immature LCs can be productively infected by R5 HIV. As preincubation of LCs with mannan or EGTA, known inhibitors of C-type lectin binding, did not block infection, they concluded that LC infection by HIV was solely dependent on the presence of CD4 and CCR5 in their cell membrane. However, the function of Langerin in HIV infection and transmission seems to be more complex than initially thought. In contrast to other C-type lectins, like DC-SIGN or mannose receptor (MR), Langerin was shown to prevent HIV-1 transmission by LCs.[121] Indeed, HIV-1 captured by Langerin was internalized into Birbeck granules and degraded. When Langerin was blocked with the antibody 10E2, the protective function against HIV infection disappeared. The story became more complicated when Fahrback et al.[122] discovered that LCs, activated with LPS and TNF-α, could efficiently mediate *trans*-infection of HIV-1. Activation of LCs apparently led to a downregulation of cell surface langerin expression.

The transmission rate of HIV during sexual intercourse is very low; the male-to-female transmission probability through semen is 1 in 200 to 1 in 2000 per exposure.[123] The genital tract is a multilayer barrier against invading pathogens. The first layer consists of mucus, which traps pathogens to prevent further infection.[124] The next layer is the stratified squamous epithelium, where the LCs reside. However, for HIV to reach this epithelium, physical breaches in the epithelial integrity are needed.[125] Pathogens with LPS on their outer membrane or a physical trauma, which is often associated with TNF-α production, can cause lesions in the epithelium. When HIV would cross the epithelium under these circumstances, the LCs would probably be activated, capture HIV-1, and transfer the virus to $CD4^+$ T cells.

c. Macrophage mannose receptor (MMR)

The MMR is a type I transmembrane protein that can be found on macrophages and DCs.[126] It mediates phagocytosis, endocytosis, and pinocytosis of antigens containing mannose, fucose, *N*-acetylglucosamine, and glucose,[127] where they are targeted to lysosomes for proteolytic degradation and presentation on major histocompatibility complex II.[128] The receptor consists of five domains: (1) a cysteine-rich amino terminus, which is homologous to the ricin B chain; (2) a fibronectin type II repeat region; (3) eight calcium-dependent CRDs, which are 30% homologous to each other; (4) a transmembrane region, and (5) a cytoplasmic domain.

Like DC-SIGN, the MMR is able to bind gp120 in a Ca^{2+}-dependent manner and transmit the virus to target cells,[129] but there are some important differences between the two receptors. First of all, MMR oligomerises into a dimer,[130] while DC-SIGN forms tetramers. In fact, HIV gp120 only binds dimeric MMR. Second, the binding of gp120 to MMR cannot be blocked with EGTA, pointing to non-Ca^{2+}-dependent binding to MMR.[130] Indeed, it is postulated that gp120 is able to bind all 8 CRDs of MMR, but only binding to CRD4 and CRD5 is Ca^{2+} dependent. Finally, binding of gp120 to MMR induces phagocytosis, which does not result in viral replication. Thus, in macrophages two independent pathways of HIV

entry may co-exist: pathway 1 is the infectious route by the CD4/coreceptor complex, and pathway 2 is the noninfectious phagocytic route mediated by MMR.[131]

d. Mannose-binding lectin

MBL (also designated as mannan-binding lectin or MBP) is a serum protein of hepatic origin that binds to carbohydrates present on microorganisms and plays a role in their clearance and destruction through complement activation and by inducing opsonization.[132,133] It is part of the collectin family, which is composed of large homo-oligomers that contain collagenous *N*-terminal segments and *C*-terminal C-type CRDs.[134] MBL is organized as bouquets containing two to six building blocks, each of which consists of a trimer of the constituent polypeptide. The CRD of MBL contains two Ca^{2+} ions, and has specificity for D-mannose, *N*-acetylglucosamine and fucose.[134] However, it recognizes mannose in a very different way compared with DC-SIGN or L-SIGN, although their CRDs share 24% homology. In the trimeric conformation, the CRDs of MBL are 45 Å apart. Spectroscopic and model-building studies have shown that terminal mannose residues in vertebrate high-mannose glycans are about 20–30 Å apart. Therefore, the binding sites of MBL are too far apart to bind vertebrate high-mannose type glycans, but are at an optimal distance to bind more widely spaced residues such as those found on the surfaces of bacterial and fungal cells. In this way, MBL cannot mediate complement-mediated lysis or an opsonic reaction to host cells. In contrast, DC-SIGN and L-SIGN may recognize multiple self-oligosaccharides in specific arrangements on the surface of certain glycoproteins,[135] which is important for tolerance toward self-antigens.[136]

As early as in 1989 MBL was described to inhibit HIV infection in vitro.[137] Later on Saifuddin et al.[138] showed that primary isolates of HIV-1 also interact with MBL, and that this interaction occurred through the CRD of MBL, as addition of mannan blocked the binding. These results were contradicted by Ying et al.[139] who demonstrated that MBL binds HIV-1, but could not detect neutralization of HIV at MBL concentrations that were approximately ten times higher than physiologic serum MBL levels. However, MBL could efficiently opsonize primary isolates of HIV for uptake by monocytic cells and can activate the classical complement pathway.[140]

e. Lung surfactant proteins SP-D and SP-A

Another member of the collectin family is pulmonary surfactant D protein,[134] although its organization is different: instead of a bouquet, it forms a cruciform, consisting of four associated trimeric units. SP-D is present in the lung, but also at various mucosal locations[141] and in blood.[142] Alike MBL, SP-D is able to bind gp120 in a Ca^{2+}-dependent manner, albeit at significant lower concentrations than MBL.[143]

Besides SP-D, another pulmonary surfactant, SP-A exists,[134] which has a comparable structure as MBL. SP-A can be found in lungs, amniotic fluid and more importantly in vaginal fluid and the female genitourinary tract. Like MBL and SP-D, SP-A can bind to HIV by targeting the high-mannose glycans on the gp120 envelope in a calcium-dependent manner. However, SP-A also enhances binding of gp120 to DCs and transfer of HIV to T cells.[144]

f. Dendritic cell immunoreceptor (DCIR)

Recently, a new CLR on DCs was described as an attachment factor for HIV-1. DCIR is a prototypic DC-associated CLR, which is downregulated upon maturation of the DC. So far, no ligand of DCIR has been identified, and its in vivo function remains elusive. Various antigen-presenting cells express DCIR, including B cells, monocytes, myeloid DCs, and plasmacytoid DCs. Comparable to DC-SIGN, DCIR is able to capture HIV-1 and promote infection in *trans* and in *cis*.[145]

DCIR is the fourth C-type lectin found on DCs, besides DC-SIGN, Langerin and MMR. It is thought that no unique CLR is fully responsible for HIV-1 attachment to all DC subsets. Therefore, a microbicide that is designed to inhibit the interaction between HIV-1 and DCs should preferentially block all possible CLRs present on DCs.

g. The neutralizing antibody 2G12

2G12 is an antibody that is unique in several ways. First of all, the antibody is able to bind an epitope on HIV gp120 consisting of *N*-linked glycans.[146,147] The discovery of an antibody directed against oligosaccharides was surprising, as glycoproteins are poor immunogens for several reasons. For one thing, carbohydrates exhibit microheterogeneity, which means that one glycoprotein exists in multiple glycoforms. Second, glycans are dynamic structures covering a substantial area of amino acids, being able to cover potential epitopes of a protein. Third, many viruses use the host glycosylation machinery for the glycosylation of their envelope glycoproteins. The host will recognize these structures as "self", not as "foreign". Site-directed mutagenesis studies have implicated that the epitope of 2G12 consists of the Manα1-2Man-linked residues of the *N*-linked oligosaccharides at amino acid positions N295, N332, and N392, while the glycans at positions N386 and N448 play an indirect role in the conservation of the epitope conformation.[147] However, the 3D structure indicates that 2G12 rather binds at amino acid positions N332, N339, and N392 of gp120.[148] The glycan at position N295 may play an indirect role in 2G12 binding by preventing further processing of the glycan at position N332 and by maintaining its oligmannose structure as one that is recognized by 2G12.

2G12 has a very peculiar 3D structure. Two Fab molecules form a dimer through swapping of the V_H domains.[148] This is achieved by twisting the variable regions with respect to the constant region when compared with their standard orientation in a Fab (Fig. 1). This domain swapping results in the creation of multiple binding sites: two corresponding to the normal antibody, and two new sites within the V_H/V_H' interface. Based on the structure of 2G12 a lot of initiatives have been undertaken to design new immunogens that elicit 2G12-like antibodies.[149–155] However, in most of the cases, the elicited 2G12-like antibodies have not been able to neutralize gp120.

h. Defensins

Defensins are a family of cysteine-rich, cationic antimicrobial peptides, expressed by leukocytes and epithelial cells.[156] Three subfamilies can be distinguished: the α-, β-, and θ-defensins. They are all derived from an ancestral gene that existed before reptiles and birds diverged.[157] The α- and β-defensins are most widely distributed among mammalian, and have been studied in detail (see review[158]). The θ-defensins (also called minidefensins) are the smallest cyclic peptides of animal origin known so far. They are 18 residues big, derived from two precursor peptides, each of which contributes nine residues to the mature peptide. Three rhesus monkey θ-defensins were first discovered when it became clear that human bone marrow also expresses the mRNA that is homologous to the precursors of rhesus monkey circular minidefensins. However, a stop codon in the signal sequence silences transcription.[159] Based on the sequence of the human gene, the putative ancestral human circular minidefensin was synthesized and called "retrocyclin". Retrocyclin could protect human $CD4^+$ cells from HIV-1 infection in vitro.[159,160] Based on SPR experiments, it was concluded that retrocyclin binds glycosylated gp120, suggesting it is a lectin.[161] Later on, also the human α-defensins HNP1, HNP2, and HNP3 were shown to bind to glycosylated gp120.[162] However, retrocyclin was shown to bind to gp41 in a non-carbohydrate manner and inhibit the formation of the 6-helix bundle during HIV-1 fusion,[163] making it a fusion inhibitor, and it was shown that α-defensins dramatically downregulate the expression of CD4 on the cell surface. Therefore, α-defensins inhibit the interaction between gp120 and CD4.[164] Finally, β-defensins are also able to inhibit HIV-1 X4 and R5, but its antiviral mode of

action is based on modulation of the CXCR4 coreceptor, and no lectin-like properties are attributed to this defensin.[165]

i. (Semi)synthetic small-size nonpeptidic CBAs

So far, only lectins from natural origin, being proteins, have been discussed. Some of them are potential candidates as anti-HIV microbicides, but the size of the protein, the antigenic properties, their peptidic nature, sensitivity to proteases, and problems associated with large-scale production and purification make them less qualified for systemic use. Synthetic compounds with lectin-like properties would be a gentle solution for these problems. The synthesis of a variety of synthetic CBAs has been well documented in literature, but so far, only a couple of such (semi-)synthetic compounds have been described to be endowed with antiviral (i.e. HIV) activity. Most of them belong to the pradimicin/benanomicin antibiotic family. These antifungal compounds are benzo[a]naphtacenequinones containing a D-alanine and a monosaccharide and disaccharide side chains.[166] Benanomicins A and B were discovered in a fermentation of *Actinomadura* sp., while pradimicins A–E were produced from *Actinomadura hibisca*. Overall, the pradimicins are nearly identical to the benanomicins (Fig. 2), and their antifungal mode of action is probably based on their capacity to bind mannan in a Ca^{2+}-dependent manner. Two molecules of PRM-A interact with one atom of Ca^{2+} creating multiple binding sites that interact with Manα1-2Man.[167] During the late 1980s, both the benanomicins A and B and pradimicin A (PRM-A) were described to possess anti-HIV-activity.[168,169] The antiviral properties of PRM-A have later been studied in more detail.[170] It was shown that PRM-A is able to inhibit HIV-1 X4 and R5 infection at nontoxic concentrations by preventing viral entry. HIV-1 strains selected under increasing pressure of PRM-A contained up to eight different glycan deletions in the gp120 envelope, the majority being high-mannose type glycans, pointing to a highly selective binding of the antibiotic to high-mannose type glycans on the envelope of HIV-1.[170] PRM-A proved poorly soluble. This problem was overcome with the discovery of PRM-S, which has the same cyclic backbone as PRM-A, but with a negatively charged sulfated glucose instead of the terminal xylose moiety found in PRM-A.[171] Besides the higher solubility, PRM-S has a variety of interesting features from a microbicide point of view. For instance, it not only bocks viral entry of HIV-1, HIV-2, and SIV in susceptible target cells, but also prevents DC-SIGN-mediated capture of HIV-1 and SIV and subsequent virus transmission to $CD4^+$ T lymphocytes. The compound is stable at low pH (4 days at pH 4.0) and at high temperature (4 days at 50°C). Also SPR analysis revealed that it still efficiently binds gp120 at low pH.[171] The stability at low pH is of particular importance for a microbicide, as the vaginal pH is acidic (3.5–4.5).

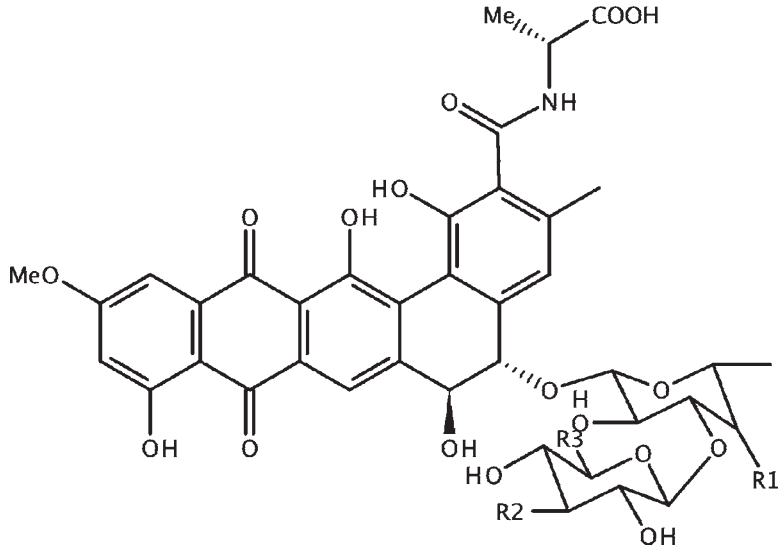
Recently, Alcian Blue (AB), a phtalocyanine derivative, was also described to be able to bind to *N*-linked glycans and as such, inhibit HIV-1 infection.[172] However, it is currently unclear what the sugar specificity is of AB, and whether its antiviral activity is also based on multivalency, as is the case with all CBAs described so far.

The discovery of small-size nonpeptidic compounds that are able to bind the *N*-glycans on the surface of HIV-1 is a new and encouraging step in the further exploration of this type of chemotherapeutics. Further investigations need to reveal whether these compounds are able to block HIV-1 infection in vivo.

4. THE CBA CONCEPT FOR ANTIVIRAL CHEMOTHERAPY

Given the importance of the glycans present on the viral envelope, affecting the carbohydrate structure and/or functioning of the viral envelope can be accomplished by two entirely

A



	R1	R2	R3
Pradimicin A	N(Me)H	OH	H
Pradimicin S	N(Me)H	$SO4^{2-}$	CH_2OH
Benanomicin A	OH	OH	H
Benanomicin B	NH2	OH	H

B

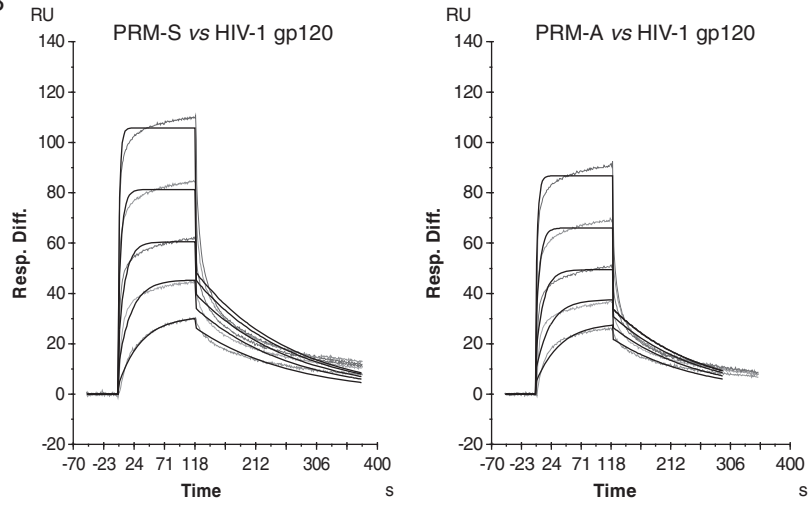


Figure 2. Panel **A**. Structural formula of the low-molecular-weight non-peptidic pradimicin and benanomicin antibiotics. Panel **B**. Interaction between PRM-S and PRM-A with recombinant gp120. Results were obtained from.[171]

different strategies: (1) intracellular interaction of drugs with the glycosylation machinery of the virus-infected cells or (2) extracellular interaction of drugs with lectins from the innate immune system or with the glycans present on the viral envelope.

A. Agents That Interact with the Cellular Glycosylation Pathway

Interfering with the cellular glycosylation pathway might compromise HIV envelope gp120 glycosylation.[173] The α-glucosidase inhibitor *N*-butyldeoxynojirimycin (NB-DNJ) blocks the removal of the glucose residues of the glycan which is a prerequisite for the further trimming of the high-mannose type glycan structures into complex-type glycans. NB-DNJ was demonstrated to be inhibitory to HIV entry.[174] Recently it was shown that encapsulation into lysosomes further significantly increased the antiviral activity of NB-DNJ, up to the nanomolar range.[175] The α(1,2)-mannosidase I inhibitor 1-deoxymannojirimicyn (DMJ) also prevents the conversion of high-mannose type glycans into complex type glycans by blocking the enzymatic removal of the mannose residues after the glycosidases have removed the glucose residues. This results in the appearance of a higher degree of high-mannose type glycans on the HIV-1 gp120 envelope, thus DMJ acts synergistically with CBAs to inhibit HIV-1 infection.[176]

Glycoylation inhibitors would not only affect the glycan synthesis of viral glycoproteins, but also the glycan synthesis of cellular glycoproteins. Therefore, inhibitors of glycosidases have been considered as potential medicines to treat carbohydrate-related diseases such as diabetes, viral infections (i.e. influenza virus, HIV), tumor metastases, and glycosyl sphingolipid (GSL) storage diseases.[177] Thus, iminosugars are being considered for use in cancer therapy, where they can prevent the formation of aberrant *N*-linked glycans and inhibit catabolic glycosidases in order to stop oncogenesis and tumor metastasis.[178] Moreover, NB-DNJ is currently available as Zavesca® for the treatment of type I Gaucher disease, an autosomal-recessive inherited deficiency of the lysosomal enzyme β-glucocerebrosidase resulting in the accumulation of glycosylceramide in macrophages (Gaucher cells).[179] Thus, in vivo application of a glycosidase inhibitor seems to be a feasible goal.

Although not active against HIV-1, but nevertheless interesting to mention, are the anti-influenza drugs oseltamivir (Tamiflu) and zanamivir (Relenza). These drugs are synthetic compounds that bind to, and inhibit the activity of, the virus-encoded sialic acid-binding NA, a glycoprotein that is abundantly present on the surface of influenza virus and whose enzymatic activity is essential for the release of the virus from the infected cells. Inhibition of NA results in an inhibition of the release of progeny (influenza) virions from infected cells.[180] Oseltamivir, and also zanamivir, are now globally used for the treatment of influenza virus infections, and besides some very rare cases of serious side effects, it was reported that the only adverse effect for oseltamivir is nausea and vomiting and for zanamivir diarrhea.[181] Thus, drugs that interfere with the interaction of carbohydrates with their corresponding carbohydrate-binding protein can be useful as medicines.

B. Carbohydrate-Containing Agents That Interact with Lectins of the Innate Immune System

Multivalent carbohydrate-containing agents have also been envisioned in an attempt to prevent pathogen infection/transmission by targeting DC-SIGN. If this task can be afforded in a specific manner, the interaction of DC-SIGN (blocked by the multivalent carbohydrate-containing drug) with the HIV-1 gp120 envelope should be prevented, which may then, in turn, compromise efficient virus transmission. Glycodendrons covered with mannosyl oligosaccharides were shown to be able to inhibit gp120 binding by DC-SIGN in the lower nanomolar range.[155] Also, gold-mannoglyconanoparticles have been synthesized for interaction with DC-SIGN. The nature and the density of the oligosaccharides linked to the gold-nanoparticles influence the eventual antiviral (transmission) activity of the compounds.[182,183] Hyperbranched dendritic polymers functionalized with oligosaccharides proved also able to inhibit virus infection by blockage of the DC-SIGN interaction with the

virus gp120 envelope.[184,185] While this approach has an interesting potential as novel carbohydrate-based antivirals, its inhibitory selectivity to the particular pathogen (i.e. virus) still awaits validation in vivo.

C. Agents That Recognize Glycans on the Viral Envelope

1. Antiviral assays to evaluate the antiviral properties of CBAs

Several assays exist to investigate whether a CBA - peptidic or nonpeptidic - is active against HIV-1 or other enveloped viruses.

In cell free virus assays, HIV is added to peripheral blood mononuclear (primary) cells (PBMCs), monocyte/macrophages or a variety of T cell lines, in the presence of a series of dilutions of the CBA. The percentage of viral inhibition by the compound can be determined based on the production of the virus-induced p24 antigen, viral RNA production, and/or by determining the cytopathogenic effect. Cocultivation assays can also be performed between persistently HIV-1-infected T lymhpocytes and uninfected T cells in order to determine whether the compound is able to inhibit cell-associated viral transfer. DC-SIGN-expressing cells, like the DC-SIGN-expressing B-cell Raji/DC-SIGN or primary DC cultures are often used to study the interaction of HIV-1 gp120 with DC-SIGN, and cocultivation of Raji/DC-SIGN cells, pre-exposed to HIV-1, with noninfected $CD4^+$ T cells mimics the transmission of HIV-1 from DC-SIGN to T cells.

The above-mentioned assays give information on the antiviral properties of the CBAs, but do not provide clues on the antiviral mechanism of the compound. Time-of-addition studies are useful to determine what (time)point in the virus life-cycle is blocked by the inhibitor. Further investigation often includes surface plasmon resonance studies to investigate the direct interaction/affinity between the CBA and the viral envelope glycoprotein such as HIV-1 gp120 or gp41. Last, but not least, phenotypic and genotypic resistance development of the virus against CBAs in HIV-1-infected cell cultures is important to evaluate the role of specific glycan recognition by the CBAs and to reveal particular patterns of glycan configurations/deletions in drug resistance development.

2. Interaction of CBAs with different steps of virus infection and transmission

CBAs could accomplish the extracellular approach. Numerous reports have already shown that CBAs are able to efficiently inhibit the entry of cell-free HIV particles into its target cell[66,170,186,187] but also to prevent giant cell formation that takes place when HIV-infected and -uninfected $CD4^+$ T lymphocyte cells fuse. Moreover, CBAs may also prevent HIV migration through the genital mucosa by blocking the interaction between the glycans on the gp120 envelope of HIV and DC-SIGN,[188–190] the carbohydrate receptor on DCs that are present in the epithelia of the genital tract. Finally, it has been demonstrated that CBAs also prevent the transmission of DC-SIGN-captured virions to $CD4^+$ T lymphocytes.[188] Therefore, CBAs are the only antiviral agents known so far that act at these four important different stages during the HIV-infection/transmission process (Fig. 3).

As CBAs bind the *N*-linked glycans on the gp120 envelope of HIV-1 particles as well as on gp120 present on the surface of HIV-infected cells, these agents do not need to enter the cell to exert their antiviral activity, lowering the risk of cytotoxicity and avoiding interaction of the CBAs with the glycosylation machinery inside the cell.

The binding of CBAs to the envelope of HIV-1 inhibits viral entry at a post-CD4-binding stage.[66] The exact mode of inhibition is still unclear, but Leikina et al.[191] reported that he θ-defensin retrocyclin 2 was able to inhibit influenza virus infection by blocking membrane fusion mediated by the viral HA, through crosslinking and thus by immobilizing

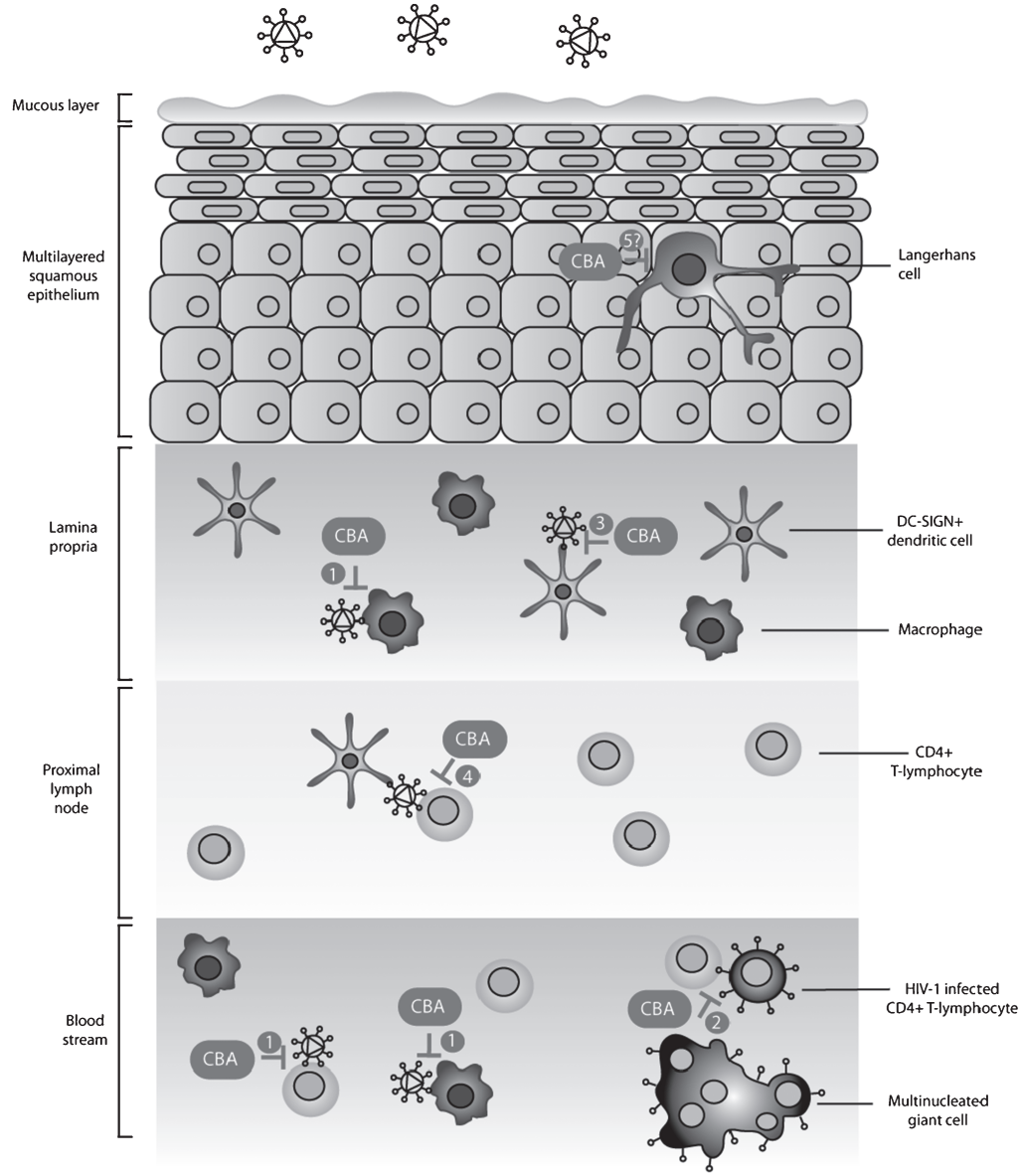


Figure 3. Different modes of antiviral intervention by CBAs. (**1**) CBAs inhibit the infection of macrophages and $CD4^+$ T cells by cell-free HIV-1 particles (**2**) CBAs inhibit syncytia formation between HIV-infected and uninfected $CD4^+$ T cells (**3**) CBAs block the binding of HIV particles to DC-SIGN expressing dendritic cells and (**4**) subsequent transmission of the virus particle to $CD4^+$ T lymphoyctes. (**5**) The interaction between CBAs and Langerin has not been studied yet.

the membrane glycoproteins. Retrocyclin 2 is, like most CBAs, a multivalent lectin. This suggests that CBAs might work in a similar manner as retrocyclin 2. As binding of CD4 to gp120 induces several conformational changes in gp120, necessary to be able to bind to the coreceptor and to insert the fusion peptide in the cell membrane, the multivalent binding of CBAs to gp120 could affect these conformational changes, so that entry cannot efficiently occur anymore. More research is needed to reveal the exact molecular mechanism of antiviral action of the CBAs.

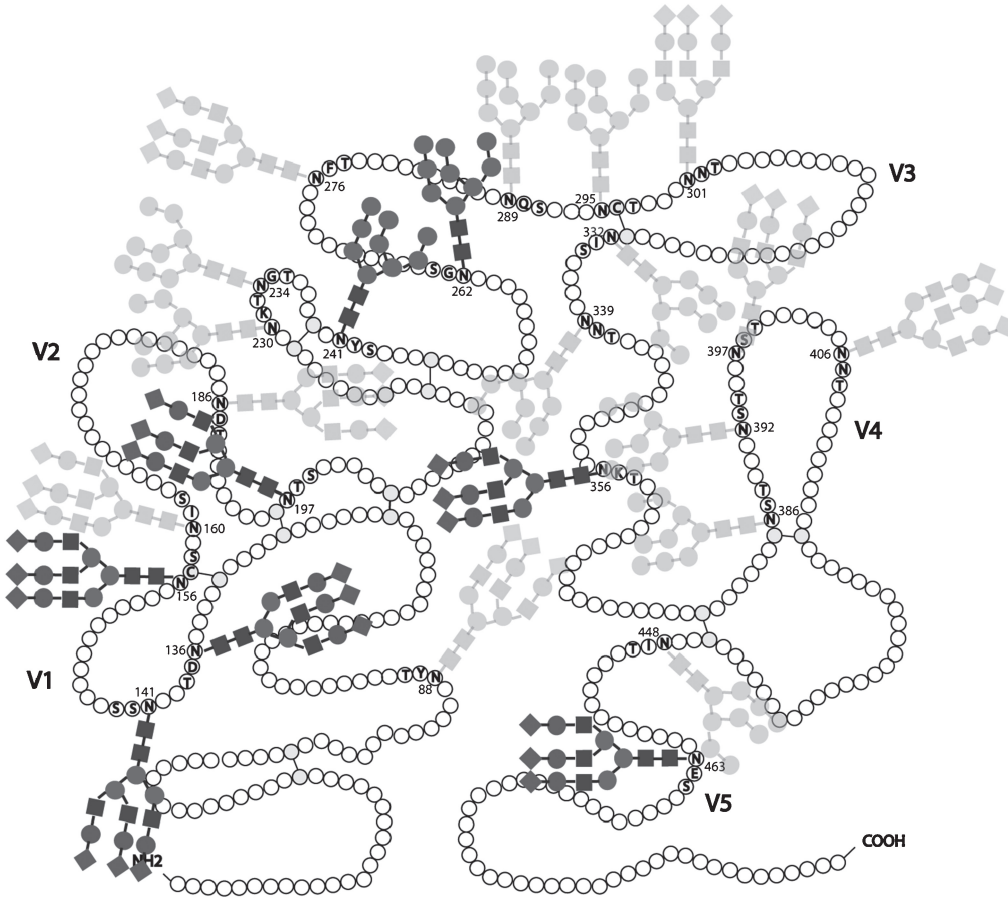


Figure 4. Schematic representation of HIV-1 envelope glycoprotein gp120 (IIIB), showing 24 *N*-linked glycans according to Leonard et al.[9] are complex-type glycans, are high-mannose-type glycans. Glycans deleted under CBA pressure appear as transparent. Only 8 out of the 24 *N*-linked glycans were never deleted, 4 of these being complex-type glycans located on the V1/V2-loop of HIV-1 gp120. Figure adapted from Leonard et al.[9] and Dey et al.[236]

3. CBA resistance profile

To escape the antiviral action of the CBAs, HIV-1 deletes several of the *N*-linked glycans on the viral envelope under increasing CBA pressure (Fig. 4). Several factors affect the dynamics and evolution of CBA resistance development. More than one CBA molecule can bind at several sites on one viral envelope molecule. As high-mannose type glycans are abundantly present on gp120, there are several binding options for the CBAs. It has been estimated that up to 33 PRM-A molecules can bind at the same time to one single HIV-1 gp120 molecule.[170] Although only a few peptidic CBAs (such as HHA, GNA, CV-N,…) can bind to gp120 due to steric hindrance between multiple CBAs, deletion of a few *N*-glycans may create more space for other CBA molecules to bind to the remaining glycans on the mutated gp120 glycoprotein. This implies that deleting one single *N*-linked glycan would not be sufficient for the virus to efficiently escape the antiviral action of the CBAs. In fact, it was demonstrated that multiple oligosaccharide deletions are needed on the envelope of HIV-1 to become phenotypically resistant to a variety of CBAs, including UDA, PRM-A, and PRM-S.[68,170,192–194] The consequences of this high genetic barrier are dual: (1) patients on CBA therapy (or a therapy that includes a CBA) may develop very slow drug resistance against this CBA, and (2) the deleted glycans may create "holes" in the protective glycan

shield, thereby exposing hidden epitopes to the immune system and becoming vulnerable to a (neutralizing) antibody response (Fig. 5). Indeed, as was shown by Reitter et al.[195] as little as two glycan deletions in SIV gp120 can dramatically increase the neutralizing antibody response in mutant HIV-infected monkeys. Hu et al.[196] proved that partly deglycosylated HIV-1 that was made resistant against CV-N, became more sensitive to immunoglobulins directed against the V3 part of the HIV-1 envelope and to sera from HIV-1-infected individuals. In this sense, CBAs may represent a completely new mode of antiviral therapy that actively involves the immune system to attack the virus and virus-infected cells. This dual principle has not been described for any other antiviral compound so far. In addition, CBA-binding to the viral envelope might opsonize gp120, which can result in complement activation and a cellular immune response targeting the gp120-bound CBA. In this regard, the CBAs can act complementary to MBL.

N-linked glycans serve multiple functions, including affording protein stability and resistance to proteolytic degradation, and regulating enzyme activity and signaling, but during glycan synthesis, the most important function of *N*-linked glycans probably is to avoid peptide precipitation and to induce the correct conformation of newly formed proteins in the endoplasmic reticulum (ER).[197] This implies that there would be a limitation to the number of *N*-linked glycans on gp120 HIV-1 can delete in order to escape CBA drug pressure without compromising the correct folding of the envelope protein. Incorrectly folded envelope proteins are expected to have a compromised (co)receptor binding and fusion. In addition, it appears that some oligosaccharides may be indispensable for HIV-1 replication and/or infectivity. In more than 50 HIV-1 strains selected under increasing CBA pressure, 8 *N*-linked

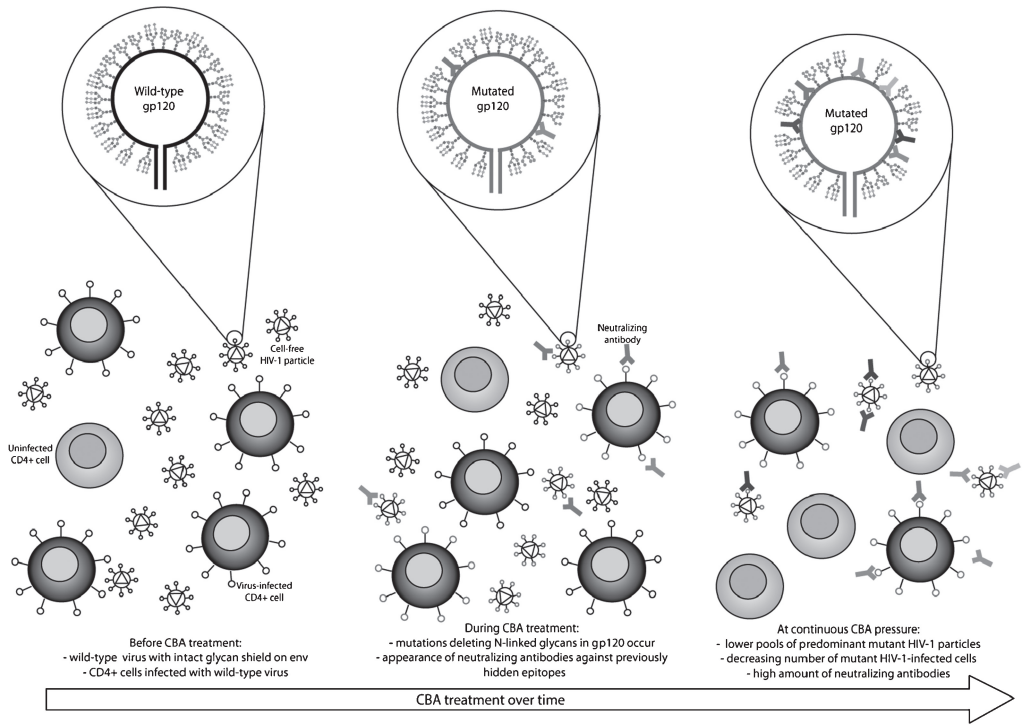


Figure 5. The CBA concept for the treatment of enveloped viruses.

glycans were never found deleted (Fig. 4). Among them, 4 are complex type glycans that are part of the variable V1/V2-loop. It was shown that deletion of these V1/V2-loop glycans on gp120 resulted in severe impairment of the replication capacity and even an increased susceptibility to the inhibitory effect of some CBAs.[198,199] Futhermore, deletion of the high-mannose type glycan at position N260 seems to be detrimental for the infectivity of HIV-1.[200] It would therefore be interesting to develop antibodies against such *N*-linked glycan sites. The neutralizing antibody 2G12 that specifically recognizes *N*-glycan epitopes on HIV-1 gp120 and some recently developed antibodies against a trimannose structure that are able to recognize the HIV-1 and SIV envelope[201] suggests that this would be a feasible goal.

4. Effect of CBAs on other pathogens than HIV

Besides HIV-1, CBAs are also able to prevent viral entry of several other viruses that contain a glycosylated envelope. For instance, the entry of HCV pseudoparticles into Huh7 cells and their capture by DC-SIGN-expressing cells was efficiently blocked by the plant CBAs GNA, HHA, CA, and UDA and the non-peptidic PRM-A.[4] These CBAs are also active against infection of certain viruses of the *Nidovirales* family, like coronaviruses,[4,7,202] arteriviruses, and toroviruses,[8] and against CMV,[66] the feline immunodeficiency virus[203] and DENV[6] infection and cell-to-cell transmission of HTLV-1.[204] CV-N has also been found inhibitory to Ebola virus and influenza virus.[44,205] Conflicting reports, however, exist on the potential of CBAs to inhibit HSV entry. Bertaux et al.[4] did not observe any antiviral activity of HHA, GNA, UDA, or PRM-A against HSV-1, while Tiwari et al.[46] showed that HSV-1 entry could be blocked with CV-N. As CV-N and PRM-A both preferentially bind external α1-2 mannose residues, it is rather surprising that their antiviral spectrum would be different. However, the oligosaccharides present on a virus partly depend on the cell line that has produced the virus. In the case of FIV, it was shown that FIV derived from the fibroblast cell line CRFK was remarkably more sensitive to the inhibitory effects of plant CBAs than FIV derived from thymocyte cell cultures.[203] Also, gp120 produced by insect cells tends to bind CBAs better than when produced by mammalian CHO cells, presumably by the higher density and/or amounts of high-mannose type oligosaccharides on insect glycoproteins.[206] It is therefore important to investigate viruses derived from their naturally infected cell types in order to examine their interaction with CBAs. Thus, the list of enveloped glycosylated viruses that can be inhibited by the CBAs is certainly not limited to HIV and probably other viruses with sensitivity about CBAs will be discovered. In this context, CBAs may also be effective against other pathogens different from viruses. DC-SIGN acts as a universal pathogen receptor, capable of binding microorganisms like *M. tuberculosis*, *Streptococcus pneumoniae*, and *Schistosoma mansoni*.[207–209] Whether the CBAs are also able to block the interaction between these pathogens and DC-SIGN still needs to be investigated, but it is likely that CBAs, by interacting with the glycans on the pathogens, can prevent the binding to DC-SIGN, thereby compromising their DC-SIGN-mediated transmission.

5. Microbicide potential of CBAs

CBAs have favorable properties from a microbicidal point of view. First of all, topical microbicides do not need to be taken up by the cells to be antivirally protective. This lowers the risk of cytotoxicity of the particular agent. However, care should be taken that the product does not damage the cervical epithelium, as was the case with nonoxynol-9, the first microbicide to be tested in a large phase III clinical trial.[210] Second, the plant lectins HHA, GNA, and UDA and the non-peptidic PRM-A and PRM-S do not loose their antiviral activity against HIV-1 after incubation at 50°C or at pH 4–5.[171,211] This is of significant relevance, as the vaginal environment is acidic (pH 3.5–4.5) due to the production of lactic acid by commensal lactobacilli.[212] Third, CBAs are able to prevent the capture of HIV-1 by DC-SIGN-expressing cells, and subsequent transmission to $CD4^+$ T cells.[188,190,212] DCs

expressing DC-SIGN reside in the lamina propria of the vaginal epithelium and are one of the first cells HIV-1 encounters when crossing the epithelium. As discussed before, the capture and transmission of HIV-1 by DC-SIGN represent an important pathway of viral dissemination and host infection, and any effective microbicide should be able to block this mode of transmission. So far, CBAs seem to be the only agents with a demonstrated inhibition of DC-SIGN-mediated virus capture and transmission.

So far, no CBAs have entered in clinical trials for use as a microbicide. Several first generation microbicide candidates, that non-selectively target HIV-1, including nonoxynol-9,[213] cellulose sulfate,[214,215] and PRO2000,[216] were proven either toxic and/or inactive in clinical trials. For obvious reasons, almost all clinical trials currently ongoing are being conducted with antiretroviral compounds that are already approved for clinical use (for more information, see www.avac.org). These compounds are predominantly inhibitors of the HIV-encoded reverse transcriptase (RT), which only acts after the virus has entered a susceptible target cell and starts replication. Recent work indicated that the combination of a nonnucleoside RT-inhibitor with the plant lectin HHA resulted in a synergistic activity against HIV.[217] These observations again support the idea that CBAs might by valuable new entities in a microbicide formulation.

6. Commensal lactobacilli as tool for CBA expression

The vaginal environment has a low pH to create an unfavorable barrier to potential invading pathogens, including viruses such as HIV. The acetic environment is established by lactobacilli that belong to the commensal flora of the vaginal epithelium. Beside producing lactic acid,[218] they also produce H_2O_2 as antimicrobial agent. At their cellular surface, however, lactobacilli contain a variety of surface macromolecules such as exopolysaccharides, glycoproteins, lipoteichoic acid, fimbriae/pili,[219] and lectins. Especially the latter molecules can have a potential effector function to inhibit pathogens. The commensal microorganisms are also thought to enhance the epithelial barrier functions and can modulate immune responses through their surface molecules.

Recently, it was shown that commensal lactobacilli could be engineered to deliver/secrete peptidic CBAs, thus creating a permanent live microbicide in the vaginal environment. The feasibility of this approach is currently under investigation by creating engineered commensal bacteria (i.e. *Lactobacillus jensenii*) that secrete CV-N.[49,50,220] The modified lactobacilli were able to grow intravaginally in mice and to produce CV-N concentrations of at least 10 ng/mL in vaginal washes.[221]

7. Interplay between CBAs and the innate immune system

The interplay between the lectins of our innate immune system and pathogens such as HIV-1 is extremely complicated and care should be taken when administering CBAs as antiviral agents, either for systemic use or as microbicide. Both CBAs and the lectins of the immune system interact with specific glycans on the envelope glycoprotein gp120. Therefore, we must carefully monitor whether the CBAs do not compromise our natural defense system by impeding the binding and/or the interaction of the lectins of the innate immune system to the glycan shield of HIV-1.

Nevertheless, HIV-1 has developed several mechanisms to "abuse" the innate immune system to its own advantage. As described above, HIV-1 hijacks the C-type lectins, DC-SIGN, DCIR, and MMR for direct transport to the lymph nodes, where it can readily infect $CD4^+$ T cells. The possible protective role of Langerin, as reported by de Witte et al.[121] should also be considered. Langerin present on LCs has been proposed to capture HIV followed by its intracellular destruction. It has not been demonstrated yet whether CBAs interfere with the viral binding to langerin.

In addition, HIV-1 has been shown to take advantage of the complement pathway that is activated after MBL binding. MBL binding to gp120 triggers the lectin pathway, leading to the accumulation of C3 fragments on the viral surface that can induce the lytic pathway and/or interact with complement receptors (CR).[222] Host cells protect themselves from lysis by complement by expressing regulators of complement activation on their cellular membrane. As HIV-1 derives its membrane from the host cells, these factors can also be present on the viral membrane and protect HIV-1 from complement lysis. Moreover, opsonization of pathogens with complement factors like C3b, iC3b, and C3d normally induces clearance and elimination of the antigens by binding to CR-expressing cells.[223] However, HIV-1 takes advantage of the opsonization and binding to CRs to infect permissive cells like monocytes, $CD4^+$ T cells, and DCs. Thus, although the innate immune system represents a very efficient first-line defense against invading pathogens, in the case of HIV-1, it has serious shortcomings, failing to efficiently contain the infection. Therefore, external CBAs may play an important role to compensate for these shortcomings.

8. The activity and selectivity of CBAs depend on their glycan specificity and the variability of glycoforms

During the synthesis and processing of the *N*-linked glycans in the ER and the Golgi apparatus, glycosyltransferases and glycosidases add and remove monosaccharides to and from the glycan in order to produce glycoproteins that eventually contain high-mannose type, hybrid type or complex type glycans. Several factors influence the type of *N*-linked glycan that can be found at a certain glycosylation site, including the availability and quantity of different glycosyltransferases or glycosidases in the particular cell, the nucleotide sugar metabolism, and the localization of glycosyltransferases within subcompartments in the Golgi.[224] Glycoproteins with the same protein backbone but different types of *N*-linked glycans are called glycoforms. This implies that the cell-type that produced a particular enveloped virus may influence the activity of CBAs against this virus, as the virus might exhibit a different glycosylation pattern on its envelope glycoprotein. Indeed, FIV produced in CRFK cell culture was highly sensitive to the inhibitory effects of several plant lectins, while FIV derived from thymocyte cell culture was clearly much less sensitive.[203]

Besides the variability in glycoforms, the nature of the lectin also determines the potential to efficiently bind a certain glycoprotein. Although most plant lectins with antiviral activity are claimed or determined to be specific for high-mannose type glycans, this does not imply that they share the exact same carbohydrate specificity. For instance, GNA is specific for α1,3-mannose oligomers, while HHA has been thought to show specificity for both α1,3- and α1,6-linked mannose oligomers. As mentioned above, while PRM-A and CV-N show preference for α1,2-coupled mannoses, only CV-N was proven to be active against HSV-1.[4,46] Thus, although at first glance several lectins share similar carbohydrate-binding specificities, detailed studies of their interaction with carbohydrates often reveals slight but important differences in their sugar affinity. Recently a plant lectin derived from maize was reported to show 64% sequence similarity with GNA.[225] Although both lectins only share 29% of amino acid sequence identity, conserved amino acids are predominantly involved in its 3D conformation and in the formation of the carbohydrate-binding site. Interestingly, while GNA strongly binds high-mannose type glycans, GNA_{maize} almost exclusively binds to complex-type glycans containing β1,2-GlcNAc residues. This difference in carbohydrate specificity results in a 100-fold lower anti-HIV activity of GNA_{maize} compared with GNA.[225]

9. Potential side effects of CBAs

The first lectins were discovered more than 120 years ago when Stillmark observed that preparations from castor bean extracts agglutinated red blood cells[226] (for an overview, see[1]). They were called "agglutinins" due to their ability to agglutinate red blood cells. Nowadays,

the term agglutinin is replaced by lectin, as not all carbohydrate-binding proteins are capable of agglutination.[1] Besides their potential to agglutinate red blood cells, several lectins are known for their cytotoxic, inflammatory, and (anti)proliferative capacities. They can be highly mitogenic and can induce or upregulate a variety of activation markers, chemokines and/or cytokines.[1] Phytohemagglutinin or PHA, a lectin found in beans, is a mitogen that is generally used to stimulate T cell division.[227] When a microbicide containing such a CBA would be applied intravaginally, it could induce inflammation and cause lesions in the epithelium, which may enhance the chances of infection by HIV or other sexually transmitted diseases. Therefore, careful monitoring of potential cytotoxic side effects of such lectins, and CBAs in general, is highly warranted. As mentioned above, CV-N has been studied in great detail for its development as a microbicide,[48,220] but a recent study has shown that CV-N was as efficient as the plant lectins PHA and ConA in expressing and stimulating the activation markers CD25, C29, and HLA-DR on lectin-exposed PBMCs and inducing the expression of a variety of cytokines/chemokines.[51,193] Interestingly, other CBAs such as the mannose-specific plant lectins HHA and GNA, the GlcNAc-specific plant lectin UDA and the small-size non-peptidic pradimicin antibiotics showed hardly any effect on chemokine/cytokine production.[51,171] These observations demonstrate that CBAs may have different biological activities depending on their nature and origin, and thus, should not necessarily be harmful in terms of cell activation, mitogenic activity, and/or cytokine stimulation.

Another point of concern in the development of CBAs as potential microbicides for clinical use is the possibility to elicit antibodies. Although CBAs in the formulation of a microbicide, in particular high-molecular-weight agents such as the peptidic lectins, are not expected to be significantly systematically absorbed, it cannot be excluded that tiny amounts are taken up, reach the blood stream and induce an immune response. Indeed, it has been shown that human serum contains antibodies against dietary lectins, such as the mannose-binding banana lectin BanLec,[228] WGA or the garlic *Allium sativum* agglutinin (ASA).[229,230] These results again indicate that non-peptidic low-molecular-weight CBAs may be preferred over the peptidic lectins, both for systemic as for microbicide use.

So far, no bacterial or plant lectins have made it to clinical practice to treat a disease. Interestingly, oseltamivir and zanamivir, which strongly interact with the envelope NA of influenza, are generally used in the treatment of influenza infections, and except for some very rare cases, no general serious side effect were reported.[181] These drugs are carbohydrate (sialic acid) mimics and inhibit the interaction of the viral NA with α2,3 or α2,6-sialic acid oligomers on the epithelial receptor cells of the host, blocking virus release from the infected cells. Thus, as mentioned before, drugs that block interaction between lectins (i.e. NA) and host carbohydrates can be safe for administration to humans.

Finally, possible cross-linking of the CBA with cellular glycoproteins may be expected to occur and should be prevented as much as possible, as this can affect the function of the glycoprotein, and might even elicit antibodies against self-epitopes. As the vast majority of *N*-linked glycans on mammalian glycoproteins are complex-type glycans,[231] CBAs with a predominant preference for end-standing α1,2-mannose oligomers, which are only present in high-mannose type glycans, should be most likely preferred over other mannose-specific CBAs. In conclusion, caution and careful attention should be given to the choice of CBAs that would be selected for further (pre)clinical development, taken the above-mentioned possibilities of side effects into account.

10. Clinically important issues for CBAs to qualify as potential antiviral medicines

Given the fact that CBAs tightly bind to glycans of enveloped viruses such as HIV, HCV, influenza virus, corona viruses, and some others, they have a number of unique features that discriminate them from other drugs.[70,186] CBAs not only prevent infection of targets cells by

cell-free virus, they also inhibit syncytium formation between infected cells and uninfected ($CD4^+$) cells, block capture of the virus by DC-SIGN and MMR-expressing (dendritic/macrophage) cells and prevent subsequent transmission of the virus to $CD4^+$ lymphocytes.

Moreover, as multiple CBA molecules may bind to one single (HIV gp120) envelope molecule, they are expected to be endowed with a rather high genetic barrier and they proved to display a broad neutralizing capacity against a wide variety of different virus clades. However, one should be cautious to extrapolate cell culture data to the in vivo situation, and therefore animal models should be included in the future studies to enable a realistic estimate of the potential value of this approach. Concern is indeed been given by the fact that CBAs may compete with the lectins of the innate immune systems, and/or may not be selective enough to discriminate between self-glycans and nonself-glycans, resulting in potential side effects. However, thorough investigations on a variety of peptidic prokaryotic and plant lectins, as well as of non-peptidic CBAs have already shown that selectivity and potential side effects such as being mitogenic or inductive for differentiation markers, cytokines, chemokines, and growth factors, highly depend on the nature and source of the CBA. A careful selection of the most promising CBAs for further (in vivo) studies are therefore warranted, not only taking their antiviral and neutralizing properties into account, but also taking their potential side effects such as immunogenicity, mitogenicity, cross-linking of cellular glycoproteins (i.e. red blood cell agglutination), and interaction with the innate immune system, into account.

5. CONCLUSION

Peptidic CBAs and, probably more importantly, synthetic non-peptidic CBAs represent an entirely new class of antivirals targeting the glycan shield on HIV-1, compromising its well functioning to survive the unfavorable environment of the virus. Although more investigations need to be performed to clarify the interaction/competition between the CBAs and natural lectins of the innate immune system, the CBAs are the only antiviral compounds known so far to be able to block HIV-1 infection in four different ways (direct infection, cell-cell syncytia formation, capture by lectins of the innate immune system such as DC-SIGN and MMR, and transmission of captured virus particles to $CD4^+$ T cells). CBA pressure on the virus results in the appearance of mutant virus strains with glycan deletions in their envelope, allowing the immune system to react against previously covered immunogenic epitopes. This concept has already been proven by Reitter et al.[195] in mutant SIV-infected monkeys and by Hu et al.[196] in mutant virus-infected cell cultures pointing to the clinical relevance of this approach. The high genetic barrier of some CBAs and their broad neutralization capacity against a wide variety of HIV strains make them an interesting new class of antivirals, both for systemic and microbicidal application. Although the CBAs are currently not subject of clinical application, they should nevertheless be considered as interesting new drug candidates that deserve further investigation.

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