Available online at www.sciencedirect.com
ScienceDirect
Antiviral Research 71 (2006) 237–247
Antiviral Research
www.elsevier.com/locate/antiviral

Mini-review

Inhibition of HIV entry by carbohydrate-binding proteins

J. Balzarini

Rega Institute for Medical Research, K.U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received 10 January 2006; accepted 1 February 2006

Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus Professor at the Katholieke Universiteit Leuven, Belgium.

Abstract

Carbohydrate-binding proteins (CBP) can be isolated from a variety of species, including procaryotes (i.e. cyanobacteria), sea corals, algae, plants, invertebrates and vertebrates. A number of them, in particular those CBP that show specific recognition for mannose (Man) and *N*-acetylglucosamine (GlcNAc) are endowed with a remarkable anti-HIV activity in cell culture. The smallest CBP occur as monomeric peptides with a molecular weight of ~8.5 kDa. Many others are functionally dimers, trimers or tetramers, and their molecular weight can sometimes largely exceed 50 kDa. CBP can contain 2 to up to 12 carbohydrate-binding sites per single molecule, depending on the nature of the lectin and its oligomerization state. CBP qualify as potential anti-HIV microbicide drugs because they not only inhibit infection of cells by cell-free virus (in some cases in the lower nano- or even subnanomolar range) but they can also efficiently prevent virus transmission from virus-infected cells to uninfected T-lymphocytes. Their most likely mechanism of antiviral action is the interruption of virus entry (i.e. fusion) into its target cell. CBP presumably act by direct binding to the glycans that are abundantly present on the HIV-1 gp120 envelope. They may cross-link several glycans during virus/cell interaction and/or freeze the conformation of gp120 consequently preventing further interaction with the coreceptor. Several CBP were shown to have a high genetic barrier since multiple (≥5) glycan deletions in the HIV envelope are necessary to provoke a moderate level of drug resistance. CBP are the prototypes of conceptionally novel chemotherapeutics with a unique mechanism of antiviral action, drug resistance profile and an intrinsic capacity to trigger a specific immune response against HIV strains after glycan deletions on their envelope occur in an attempt to escape CBP drug pressure.

Keywords: Carbohydrate-binding agents (CBA); Carbohydrate-binding proteins (CBP); Lectins; HIV; Virus entry; gp120 envelope

Contents

E-mail address: jan.balzarini@rega.kuleuven.be.

0166-3542/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2006.02.004

1. Introduction

The entry of the human immunodeficiency virus (HIV) into its target cells is mediated by the glycoproteins gp120 and gp41 that are present on the viral envelope. They sequentially bind to the cellular receptor protein CD4 and a co-receptor, mainly CXCR4 and/or CCR5. Both gp120 and gp41 are heavily glycosylated (Leonard et al., 1990; Gallaher et al., 1995; Wyatt et al., 1998; Kwong et al., 1998). It is estimated that gp120 consists of glycans for almost 50% of its molecular weight. The carbohydrates are probably exclusively N-linked. Whereas all glycans share a common pentasaccharide $Man_3GlcNAc_2$ bound to the amide nitrogen of asparagines that are part of a glycosylation motif (i.e. NXT/S), the glycans of HIV-1 gp120 consist for ~33% of the high-mannose type, 4% of the hybrid type and 63% of the complex type, the latter being predominantly glucosylated and/or sialylated (Mizuochi et al., 1988a,b). Obviously, agents that interact with the glycans on the viral envelope may disturb the efficient interaction between gp120 (or gp41) and its (co)receptors through steric hindrance, prevention of necessary conformational changes of the *env* and/or cross-linking of several glycans present on the *env* and/or the target cell. There exist several types of carbohydrate-binding agents (CBA) for which anti-HIV activity has been reported. They are virtually exclusively of protein nature and can be divided in at least seven distinct groups of molecules depending their origin (i.e. procaryotes, sea corals, algae, fungi higher plants, invertebrates, vertebrates (i.e. mammalians)). They may have different carbohydrate (i.e. mannose (Man), *N*-acetylglucosamine (GlcNAc), galactose (Gal) oligomers) specificities. Similar to other entry inhibitors, these agents are not only able to inhibit infection of cells by cell-free virus particles, but they are also able to prevent syncytium formation (virus transmission) between virus-infected and uninfected cells. Keeping in mind that virus transmission *in vivo* occurs both through cell-free virus and virus-infected cells, the capacity to inhibit both modes of infection by a single drug is one of the reasons why carbohydrate-binding proteins (CBP) have recently been suggested as potential candidate microbicide drugs for prevention of HIV infection. Interestingly, carbohydrate-binding agents may also have the potential to block the binding of HIV to disseminating cells (e.g. DC-SIGN-expressing dendritic cells) (Geijtenbeek et al., 2000) preventing both localized infection and HIV dissemination pathways, or to macrophages through their mannose receptor (Nguyen and Hildreth, 2003). This property may make CBA superior over other entry inhibitors as microbicide drugs. In this review, an overview is given of different types and origins of carbohydrate-binding proteins for which anti-HIV activity has been demonstrated and the carbohydrate specificity determined. An excellent extended overview of the structural and biological properties of a number of CBP described in this mini-review has been recently published by Botos and Wlodawer (2005).

2. CBA from procaryotic origin

2.1. Scytovirin (SVN)

SVN is a 9.7 kDa lectin (95 amino acids) isolated from aqueous extracts of the cyanobacterium *Scytonema varium* (Bokesch et al., 2003) (Table 1). It was shown to bind to HIV-1 gp120, gp160 and gp41 but not CD4, and to inhibit laboratory strains and primary HIV-1 isolates at low nanomolar concentrations. The inhibition involves a selective interaction between SVN and high-mannose oligosaccharide-bearing glycoproteins. In particular, interaction with α(1-2),α(1-6)-trisaccharide units was demonstrated (Adams et al., 2004). Recently, recombinant SVN was produced at 5–10 mg/l yield for structural (crystallographic) studies and for further preclinical investigations as a potential topical microbicide drug for HIV prophylaxis (Xiong et al., in press).

2.2. Microcystis viridis lectin (MVL)

MVL is a protein with a 13 kDa (monomer) size isolated from a unicellular freshwater bloom-forming cyanobacterium (*Microcystis viridis* NIES-102 strain), and shown to bind mannan (Yamaguchi et al., 1999) (Table 1). It consists of 113 amino acid residues and is composed of 2 tandemly repeated homologous domains of 54 amino acids separated by a 5 amino acid linker. Interestingly, no sequence homology of MVL to any other lectin or proteins was found. Recently, this carbohydrate-binding protein was revisited and found to bind oligomannosides such as $Man_6GlcNAc_2$ at sub-micromolar affinity (Williams et al., 2005). The Man β(1-4)GlcNAc conformation in the context of a larger oligomannoside proved important for efficient recognition by MVL. Interestingly, it was demonstrated that its minimal target comprises of the Manα(1-6)Manβ(1-4)GlcNAcβ(1-4)GlcNAc tetrasaccharide core of oligomannosides. Evidently, in contrast with Cyanovirin N (see below), it does not bind to the terminal [α(1-2)mannose oligomer] branching area of high-

Table 1
General properties of anti-HIV carbohydrate-binding proteins from non-mammalian origin

Species	Lectin name	Abbreviation	Carbohydrate specificity	Oligomerization state	MW monomer (kDa)
Cyanobacteria					
Nostoc ellipsosporum	Cyanovirin-N	CV-N	α(1-2)Man	Dimer	11
Scytonema varium	Scytovirin	SVN	α(1-2),α(1-6)Man	?	9.7
Microcystis viridis	–	MVL	Manβ(1,4)GlcNAc	Dimer	13
Sea corals					
Gerardia savaglia	–	GSL	D-Man	Dimer	14.8
Algae					
Griffithsia sp.	Griffithsin	GRFT	Man, Glc, GlcNAc	Dimer	13
Fungae					
Longispora albida	Actinohivin	AHA	Man	?	12.5
Annelida					
Chaetopterus variopedatus	–	CVL	β-Gal	?	30
Laxus oneistus	Mermaid	–	Man	Dimer?	16.4
Plant monocotyledoneae					
Orchidaceae					
Listera ovata	Twayblade lectin	LOA	α(1,3)Man	Dimer	12.5
Epipactis helleborine	Broad-leaved helloborine lectin	EHA	Man	Dimer	12.5
Cymbidium hybrid	–	CHA	Man	Dimer	12.5
Amaryllidaceae					
Galanthus nivalis	Snowdrop lectin	GNA	α(1,3)Man	Tetramer	12.5
Hippeastrum hybrid	Amaryllis lectin	HHA	α(1,3); α(1,6)Man	Tetramer	12.5
Narcissus pseudonarcissus	Daffodil lectin	NPA	α(1,6)Man	Dimer/trimer/tetramer	12.5
Alliaceae					
Allium porrum	Leek lectin	APA	Man	Tetramer	13
Allium ursinum	Ramsons lectin	AUA	Man	Dimer	12 or $11.5\alpha + 12.5\beta$
Plant dicotyledoneae					
Moraceae					
Artocarpus integrifolia	Jacalin, jack fruit lectin	Jacalin	Galα(1,6)/Galβ(1,3)GalNAc	Dimer/tetramer	$14.7\alpha + 2\beta$
Fabaceae					
Canavalia enformis	Jack bean lectin	ConA	Man > Glc > GlcNAc	Tetramer	26.5
Pisum sativum	Garden pea lectin	PSA	Man > Glc/GlcNAc	Tetramer	$17\alpha + 6\beta$
Lens culinaris	Lentil lectin	LCA	Man > Glc > GlcNAc	Tetramer	$17.5\alpha + 5.7\beta$
Vicia faba	Broad bean, faba bean lectin	VFA	Man > Glc/GlcNAc	Tetramer	$5.6\alpha + 20.7\beta$
Lathyrus odoratus	Sweet pea lectin	–	Man > Glc > GlcNAc	Tetramer	$6\alpha + 20\beta$
Urticaceae					
Urtica dioica	Stinging nettle lectin	UDA	GlcNAc oligomers	Monomer	8.5
Cecropiaceae					
Myrianthus holstii	Myrianthin	MHA	GlcNAc	?	9.2
Euphorbiaceae					
Hevea braziliensis	Rubber tree lectin, hevein	HBA	GlcNAc	Monomer	~8.5

mannose structures, but rather to tetra- or penta-oligosaccharides located in the internal core of gp120. The MVL monomer has two carbohydrate recognition domains making numerous intermolecular contacts with their carbohydrate ligands (including seven to eight intermolecular hydrogen bonds). Since crystallographic analysis revealed that MVL is a monodisperse homodimer in solution with a symmetrical three-dimensional structure (MW 25.4 kDa), it contains a total of four independent carbohydrate recognition sites (Fig. 1A). It inhibits HIV-1 *env*-mediated cell fusion at an IC_{50} as low as ~30–37 nM as measured in a quantitative vaccinia virus reporter gene assay that reproduces the events that lead to membrane fusion (Bewley et al., 2004). Both X4 and R5 HIV strains are equally inhibited. Additional in-depth antiviral studies are still awaited to fully enable estimation of the antiretroviral potential of MVL.

2.3. Cyanovirin N (CV-N)

Cyanovirin N, an 11-kDa protein originally purified from extracts of the cultured cyanobacterium *Nostoc ellipsosporum*

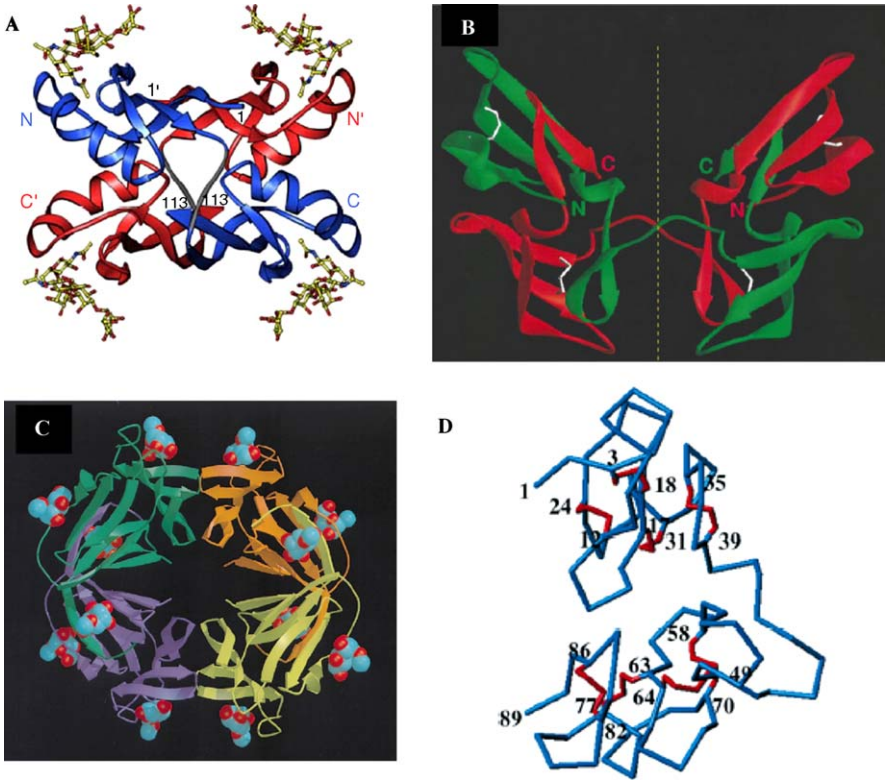

Fig. 1. Panel A: Ribbon diagram of the MVL-$Man_3GlcNAc_2$ complex with the bound carbohydrate depicted as ball-and-sticks (carbon, yellow; nitrogen, blue; oxygen, red) (Williams et al., 2005). Panel B: The domain-swapped CV-N structure. The crystallographic two-fold symmetry is shown as a yellow broken line (Yang et al., 1999). Panel C: GNA tetramer substituted by MeMan at all 12 binding sites. The crystallographically independent monomers are distinguished by color (Hester and Wright, 1996). Panel D: Backbone structure of the UDA-1 isolectin. The eight disulfide bridges are indicated in red (Harata et al., 2001). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)

(Boyd et al., 1997) (Table 1), comprises a unique sequence of 101 amino acids consisting of two sequence repeats (residues 1–50 and 51–101) with a unique unprecedented tertiary structure that reveals two symmetrically-related domains formed by strand exchange between the two repeats (Fig. 1B). The overall fold of CV-N is dependent on numerous contacts between both repeats (Bewley et al., 1998). Indeed, crystal structures revealed the existence of a domain-swapped dimer with two primary carbohydrate-binding sites and two secondary carbohydrate-binding sites on the opposite ends of the dimer (Yang et al., 1999; Botos et al., 2002). It clearly has a binding geometry of high-mannose sugars, in particular α(1-2)-linked mannose rings (Bewley, 2001). Cell culture experiments performed by Bolmstedt et al. (2001) reached similar conclusions. Recently, CV-N was shown to inhibit HIV infections in a vaginal transmission model (i.e. female *Macaca fascicularis* infected with a pathogenic recombinant chimeric SIV/HIV-1 (SHIV 89.6P) strain), suggesting that CV-N is a good candidate for testing in humans as a potential anti-HIV topical microbicide (Tsai et al., 2004). Interestingly, recombinant CV-N could be expressed in commensal *Streptococcus gordonii* and was attached to the bacterial surface or secreted in soluble form in the supernatant of liquid bacterial cultures (Giomarelli et al., 2002). It was shown that the secreted form of CV-N could tightly bind to HIV-1 gp120, whereas CV-N displayed on the bacterial cell wall surface was able to efficiently capture HIV virions. Very recently, Lagenaur et al. (2005) have managed to engineer CV-N expression in the commensal *Lactobacillus jensenii* 1153 at high yield (4–5 μg/ml) and shown that *L. jensenii*-derived CV-N markedly inhibits HIV-1 (BaL and III_B) infection in cell culture. These findings represent an important first step in the development of a system to efficiently deliver and maintain an antivirally active protein in the vaginal mucosa.

3. A CBP from the sea coral *Gerardia savaglia* (GSA)

The carbohydrate-binding protein derived from the sea coral *G. savaglia* was one of the very first lectins shown to be endowed with anti-HIV activity in cell culture (Kljajic et al., 1987). The agglutinin is composed of two polypeptide chains with a MW of 14.8 kDa for each monomer (Table 1). Ca^{2+} was required to preserve full activity. GSA shows specificity for D-mannose, but proved to be mitogenic and agglutinates human red blood cells. GSA was later shown to prevent infection of H9 cells by HIV-1 at 0.2 μM (complete protection). Also, it inhibits syncytia formation in co-cultures of virus-infected H9/HIV-1 and uninfected T-cells. At that time, it was already shown that GSA reacts with glycans of HIV-1 gp120 (Muller et al., 1988). Unfortunately, no further studies on GSA with regard to its anti-HIV potential have been performed.

4. Griffithsin derived from a red alga

Griffithsin (GRFT) is isolated from an aqueous extract of the red alga *Griffithsia* sp. as a 13 kDa protein (Table 1) consisting of 121 amino acids one of which [amino acid 31 (molecular mass 151.05)] did not correspond with any of the 20 known common amino acids. Most likely, it occurs as a dimer in solution (Mori et al., 2005). Both native and recombinant GRFT potently inhibit X4 and R5 HIV replication and HIV transmission at unusual low concentrations (0.04–0.6 nM). It binds to gp120 in a glycosylation-dependent but Ca^{2+}-independent manner, and prevents gp120 binding to 2G12 mAb which interacts with terminal α(1-2)mannose oligomers. It also prevents gp120 binding to 48d mAb which binds to the CD4-induced epitope on gp120. Interestingly, in contrast with the above-described cyanobacterial lectins, soluble gp120 binding to GRFT was inhibited by the monosaccharides glucose, mannose and GlcNAc but not by galactose, xylose, fucose, GalNAc or sialic acid-containing glycoproteins. Also, GRFT is believed to contain four carbohydrate-binding domains, separated by three linker sequences Gly-Gly-Ser-Gly-Gly (Mori et al., 2005). Such an organization could explain its unusual potent activity due to the possibility of the formation of multivalent bonds between GRFT and oligosaccharides on gp120. No homology of GRFT to any other primary amino acid sequence could be found so far. It is clear that GRFT may qualify as a potential candidate microbicide to prevent the sexual transmission of HIV and AIDS.

5. Actinohivin derived from an actinomycete

Actinohivin is derived from the actinomycete *Longisporum albida*, and found to be endowed with anti-HIV activity in cell culture. This compound is a 114 amino acid protein (~12.5 Da), with an IC_{50} of 60–700 nM against HIV. It binds to high mannose-type glycans of HIV gpl20 (Takahashi et al., 2005; Inokoshi et al., 2001; Chiba et al., 2001, 2004). Given its unusual origin and potent anti-HIV activity in cell culture, it is worth further investigating for its antiviral (i.e. microbicidal) activity.

6. Plant lectins

Plant lectins probably are the most diverse group of CBP with a wide variety of carbohydrate-binding properties depending the plant species and even the anatomic site of isolation (i.e. bulbs, leaves, roots, etc.). Strikingly, the vast majority of plant lectins that are active against HIV are endowed with a carbohydrate specificity directed against mannose oligomers. So far, only one plant lectin that shows specificity for GlcNAc found in the rhizomes of the stinging nettle *Urtica dioica* (UDA) has shown pronounced anti-HIV activity. Jacalin, derived from the jack fruit, was also reported to show activity against HIV and recognizes, besides mannose, also other monosaccharides such as galactose. Most HIV-inhibitory plant lectins are derived from the monocot families *Amaryllidaceae*, *Orchidaceae* and *Alliaceae* or the dicot families *Fabaceae*, *Moraceae*, *Urticaceae* and *Cecropiaceae* (Table 1).

6.1. Mannose-specific plant lectins from the Amaryllidaceae family

A variety of lectins were found in several members of the *Amaryllidaceae* family (Van Damme et al., 1988a). The plant lectins derived from *Galanthus nivalis* (Snowdrop) (GNA), *Hippeastrum* hybrid (Amaryllis) (HHA) and *Narcissus pseudonarcissus* (Daffodil) (NPA) are tetramers of 4 × 12.5 kDa, and for GNA, it has been shown that each monomer contains two carbohydrate-binding sites, but a third site is created once tetramerization occurs (Hester and Wright, 1996; Sharon and Lis, 2003) (Fig. 1C). Thus, this additional carbohydrate-binding site is composed of peptide strains of 2 neighbouring monomers in the tetrameric structure resulting in a total of 12 carbohydrate recognition sites. For NPA, also dimers of 2 × 12.5 kDa and trimers of 3 × 12.5 kDa have been reported (Van Damme and Peumans, 1989; Botos and Wlodawer, 2005). The plant lectins selectively inhibit a wide variety of HIV-1 and HIV-2 strains and clinical isolates in various cell assays (Balzarini et al., 1991, 2004a). They also markedly prevent syncytium formation between persistently HIV-infected cells and uninfected T-lymphocytes. Moreover, it has recently been demonstrated that the plant lectins GNA, HHA and CHA and also UDA (see supra) inhibit dendritic cell HIV-1 infection and dendritic cell-directed HIV-1 transfer albeit at differential potency (Turville et al., 2005). Interestingly, it has been shown that short exposure of GNA and HHA to cell-free virus particles or to persistently HIV-infected cells markedly decreased HIV infectivity and increases the protective (microbicidal) activity of these plant lectins (Balzarini et al., 2004a). It was shown that GNA and HHA interrupt the virus entry process by interfering with the virus envelope glycoprotein. In fact, BIAcore studies revealed that HHA strongly associates but poorly dissociates from recombinant HIV-1 gp120 (Rusnati and Balzarini, unpublished). A beneficial property of these plant lectins to be used as potential microbicides is their resistance to denaturation at low pH (≥2) and at high temperatures (≥50 °C for prolonged time periods), and their lack of measurable mitogenic activity and human red blood cell agglutinating activity.

6.2. Mannose-specific plant lectins derived from the Orchidaceae monocot family

Mannose-specific lectins have been isolated from at least three different orchid species (i.e. *Listera ovata* (LOA), *Epipactis helleborine* (EHA) and *Cymbidium* hybrid (CHA)) (Van Damme et al., 1987, 1994). They are dimers with a MW of 12.5 kDa for the monomer. These lectins were found to be highly inhibitory against HIV-1 and HIV-2, but also SIV in cell culture (Balzarini et al., 1992). Recently, Liu et al. (2005) has isolated a monomeric orchid lectin. They belong to the most antivirally active plant lectins reported so far, and consistently inhibit a broad variety of clinical HIV-1 clade isolates (Balzarini et al., 2005a). They are, in this respect, more consistent and more potent HIV inhibitors than those lectins isolated from the *Amaryllidaceae* family.

6.3. Mannose-specific plant lectins derived from the Alliaceae monocot family

The mannose-specific lectin from *Allium porrum* (a 4 × 13 kDa tetramer) (APA) proved markedly more inhibitory to HIV than the lectin derived from *Allium ursinum* (a 2 × 12 kDa or a 11.5 + 12.5 kDa dimer) (AUA) (Balzarini et al., 1992). Interestingly, whereas the closely related lectins from *Allium sativum* (garlic) bulbs were inactive against HIV, those derived from the leaves and roots of the garlic plants show pronounced anti-HIV activity (Van Damme and Balzarini, unpublished). These findings illustrate that subtle differences in the CBA three-dimensional structure and/or preference for unrecognized carbohydrate oligomer structures may play an important role in the eventual efficient recognition of the HIV-1 gp120 envelope.

6.4. Mannose-specific plant lectins derived from the Fabaceae dicot family

The first reports on the antiviral activity of mannose-specific lectins derived from the Fabaceae family came from Lifson and co-workers (1986) who reported the anti-HIV activity of a variety of bean- and pea-derived lectins. It was soon realized that these CBP inhibited entry of HIV into its target cells and that mannose-specificity was an important prerequisite for antiviral activity of these lectins. Unfortunately, these plant lectins proved mitogenic and/or agglutinated human red blood cells, and are most likely too toxic for an eventual antiviral application *in vivo*.

6.5. Jacalin derived from the Moraceae dicot family

Jacalin, the major protein from jack fruit seeds, is a tetrameric lectin of 65 kDa that recognizes Gal and Galβ(1-3) GalNAc which are often present on *O*-linked glycans (Bourne et al., 2002). Recent reports suggest also mannose, glucose, *N*-acetylneuraminic acid and *N*-acetylmuramic acid as additional carbohydrates recognized by jacalin (Bourne et al., 2002). However, it is believed that this rather unusual lectin exerts its anti-HIV activity by interacting with the CD4-expressing cells rather than with gp120 of the virus particle. Moreover, jacalin is highly mitogenic and has a variety of other biological effects (Kabir, 1998) that may preclude its use as a potential antiviral drug *in vivo*.

6.6. The N-acetylglucosamine-specific lectin from the dicot plant U. dioica

The lectin from the stinging nettle (*U. dioica*) rhizomes (UDA) is one of the few plant lectins that are thought to exist as a monomer in solution. It has been isolated for the first time in 1984 by Peumans et al. (1984) and found to be a complex mixture of isolectins (Shibuya et al., 1986; Van Damme et al., 1988b). The molecular weight of UDA is 8.5 kDa (∼89 amino acids). It has two carbohydrate-binding sites with different affinities, and, together with hevein (derived from the rubber tree *Hevea braziliensis*), is one of the smallest plant lectins ever reported (Harata and Muraki, 2000; Saul et al., 2000; Harata et al., 2001) (Fig. 1D). Both UDA and hevein are *N*-acetylglucosamine (GlcNAc) specific. In the crystal structure of UDA in complex with a $(GlcNAc)_3$ trimer, extensive interactions of two UDA molecules and one carbohydrate oligomer is found, and predominantly consists of aromatic stacking of tyrosine, tryptophan and histidine from UDA with the sugar rings of the carbohydrate, as well as a variety of (five) hydrogen bonds, and various van der Waals interactions. UDA prevents HIV entry and transmission upon co-cultivation of persistently HIV-infected cells and uninfected cells in the lower μg/ml range (higher nanomolar range) (Balzarini et al., 1992, 2005b). It strongly binds to HIV-1 gp120 (Rusnati and Balzarini, unpublished) and its molecular interaction with the $(GlcNAc)_2$Man part of a glycan on gp120 could be modelled without steric hindrance with the remaining glycan part and the gp120 peptide (Balzarini et al., 2005b). A striking property of UDA is its consistent suppression of a broad variety of HIV-1 clade isolates in PBMC (Balzarini et al., 2005b). These properties may make UDA a promising candidate for further (pre)clinical development as an anti-HIV microbicide. Surprisingly, whereas crystallographic analysis revealed that UDA has a typical hevein structure, the hevein lectin from the rubber tree is completely devoid of antiviral activity as also observed for the GlcNAc-specific weat germ agglutinin (WGA) (Balzarini and Van Damme, unpublished). This observation may suggest that the lectin from the stinging nettle roots may have additional properties that differ from those of the closely related hevein lectin, and/or that UDA might recognize GlcNAc oligomers or GlcNAc-Man oligomers in a different conformational manner than hevein or WGA.

6.7. Myrianthin derived from the Cecropiaceae dicot family

MHL was purified from the African plant *Myrianthus holstii* by Charan et al. (2000). It belongs to the *Cecropiaceae* family which is closely related to the *Urticaceae* family. It is a small cysteine-rich lectin (MW 9.2 kDa) with presumed GlcNAc affinity. Pure MHL has anti-HIV activity at an EC_{50} of 150 nM, that is at a concentration that is quite comparable with that of UDA. MHL has been shown to bind to HIV gp120 and likely to inhibit the virus infection process after CD4 binding occurred. No structural (crystallographic) data are available. Unfortunately, no further investigations on this lectin have been reported, which makes it impossible to speculate on its potential as anti-HIV (microbicidal) compound.

7. CBP from invertebrate origin

From the sea worm *Chaetopterus variopedatus* a 30 kDa lectin was isolated and designated as CVL (Table 1). The protein was recently defined to have β-galactose specificity (Wang et al., in press). However, it is unclear whether it also recognizes other mono- or oligosaccharides. If not, this is the very first lectin with exclusive β-galactose specificity that has such a pronounced anti-HIV activity in cell culture. The lectin is active against HIV-1 infection in the nanomolar range (4–60 nM) and prevents virus infection at an early stage of the HIV replica-

tion cycle, presumably the entry process. Cell-to-cell fusion of HIV-infected (C8166/HIV-1) and uninfected (H9) cells is also blocked at equally low CVL concentrations (70 nM). Although this novel type of lectin looks an interesting addition to the current armamentarium of predominantly mannose- and GlcNAc-specific lectins, further in-depth studies are required to reveal the molecular basis of its unique position in the anti-HIV CBA families and its potential as an anti-HIV microbicide drug.

Another mannose-specific lectin has been identified from *Laxus oneistus*. The Ca^{++} dependent mannose-specific lectin has a molecular weight of 16.4 kDa with some evidence of dimerisation (Bulgheresi et al., 2006). Interestingly, the lectin proved structurally and functionally similar to the human dendritic cell-specific DC-SIGN receptor molecule. Given this similarity, it would be of particular interest to further investigate the lectin for its potential to inhibit HIV replication and transmission in cell culture.

8. Mammalian mannose-binding lectins

8.1. Mannose-binding lectin (MBL)

MBL is a Ca^{2+}-dependent mannose-specific serum lectin and binds pathogens as the initiating step of the lectin pathway of the innate immune system to opsonize the pathogen (Ji et al., 2005; Klein, 2005). It is found as multimers in serum (reviewed in Hansen and Holunskov, 1998). The MBL subunit molecular weight is ~31 kDa. Its carbohydrate recognizing domain exists of 115 amino acids, and strongly binds to D-mannose, GlcNAc and fucose (Ji et al., 2005; Hansen and Holunskov, 1998). However, its carbohydrate-binding site has a rather low affinity for the carbohydrates, but oligomerization of the MBL molecules show much lower carbohydrate dissociation rates. It has been shown that MBL efficiently binds to HIV in cell culture, although primary HIV strains are less efficiently bound. Interestingly, MBL may also block efficient binding of HIV to DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin) that functions as a mannose-specific lectin in dendritic cell recognition and uptake of pathogens (i.e. HIV) leading to antigen presentation to T-cells. Such interaction of DC-SIGN with gp120 involves trisaccharide residues of high-mannose oligosaccharides of gp120 but not the terminal Manα(1-2)Man residues (Feinberg et al., 2001). However, the eventual role of MBL in the *in vivo* setting is unclear and still a considerable matter of debate in terms of its role in preventing HIV infection and transmission and in the evolution and speed of HIV-induced pathology (Ji et al., 2005; Klein, 2005). A recent study of Heggelund et al. (2005) suggests a modulatory role of MBL on cytokine responses and HIV replication after stimulation with microbial products. These effects of MBL on inflammatory responses and viral replication may be clinically relevant for the HIV infection. Further studies are needed to define the *in vivo* contribution of MBL to clearance and destruction of HIV and the reasons for the rather low neutralization capacity by MBL against primary HIV-1 strains.

8.2. Other carbohydrate-binding lectins from the innate immune system

A variety of carbohydrate-binding proteins, other than MBL or DC-SIGN are part of the innate and/or adaptive immune system. In this respect, mammalian defensins are probably the best studied peptides. They consist of small cationic antimicrobial peptides predominantly found in leukocytes and epithelial cells and were shown to possess anti-HIV activity. One discriminates between α-, β- and the cyclic θ-defensins (Chang and Klotman, 2004). It is, however, not very clear how these agents exert their eventual anti-HIV activity. Although binding to the glycosylated gp120 has been demonstrated, for several of the β- and θ-defensins, their antiviral action may be predominantly at the level of direct virus inactivation or even at a stage in the virus replication cycle that occurs after the entry and reverse transcription process (Chang and Klotman, 2004; Sun et al., 2005; Chang et al., 2005).

9. The 2G12 monoclonal antibody

The mAb 2G12 belongs to one of the very few broad neutralizing anti-HIV antibodies directed against an epitope at gp120 that lies around the C4/V4 region. A wide spectrum of HIV-1 clade isolates except clade E is efficiently neutralized in cell culture in the lower μg/ml range of the mAb. Interestingly, the recognized epitope on gp120 contains high-mannose glycans at several *N*-glycosylation sites (Scanlan et al., 2002; Calarese et al., 2003, 2005). This is very unusual: glycosylated peptide epitopes often contain microheterogenic carbohydrates (broad range of glycoforms) causing dilution of a single antigenic response; the glycans may easily cover potential immunogenic epitopes; and since they are synthesized by the cellular glycosylation machinery, glycans are often well tolerated by the host organism. Though, the 2G12 mAb interacts with very specific, highly conserved glycosylation sites on gp120 (i.e. N-295, N-332 and N-392), and its binding to HIV-1 gp120 is further influenced by the presence of the N-339 and N-386 glycans (Zhu et al., 2000; Scanlan et al., 2002). Moreover, strong evidence is provided that the terminal α(1-2)mannose oligomers on the high-mannose glycans are the predominant interaction sites of 2G12. The 2G12 mAb is unique among the studied antibodies in that it appears like a tightly packed dimer formed by an unprecedented V_H domain swapping (Calarese et al., 2005). This results in the creation of multiple distinct carbohydrate-binding sites: two for the normal antibody site and two novel sites within the new $V_H/V_{H'}$ interface, which may contribute to the multivalent binding of 2G12 to HIV-1 gp120. Whether antibodies such as 2G12 may eventually play a role as an anti-HIV agent (drug) still needs to be demonstrated. However, a recent report of Trkola et al. (2005) demonstrated that a delay of HIV-1 rebound could be obtained after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies such as 2G12, 2FS and 4E10. Therefore, the *in vivo* activity of neutralizing antibodies should be further explored.

10. HIV resistance to carbohydrate-binding proteins

Any antiviral drug that is frequently or persistently administered to HIV will soon or later select for mutant virus strains that may display several degrees of resistance to the particular drug. Understanding the molecular basis of drug resistance is important for every new agent that may enter clinical trials irrespective whether it will be applied for systemic use or as a topical microbicide. Only a few resistance selection studies have been recently performed for the mannose-specific lectins HHA, GNA, Concanavalin A and CV-N, for the GlcNAc-specific UDA and for the mAb 2G12. Interestingly, these agents invariably seem to select for virus strains that contain mutations in HIV gp120, predominantly at *N*-glycosylation sites. Indeed, up to eight to nine glycosylation sites could be annihilated in gp120 upon prolonged exposure of HIV-1 to HHA, GNA or UDA (Balzarini et al., 2004b, 2005a,b). This phenomenon was later also confirmed for CV-N (Witvrouw et al., 2005) and 2G12 (Pashov et al., 2005; Nakowitsch et al., 2005). This drug resistance profile is unique in several aspects: (i) no other anti-HIV drugs that are currently under (pre)clinical investigation, including the different entry inhibitors, select for such a targeted deletion of glycans of HIV gp120; (ii) no cross-resistance of CBP-resistant mutant virus strains to other HIV-1 drugs has been observed; (iii) CBP such as HHA, GNA and in particular UDA and CV-N are endowed with a high genetic barrier because multiple mutations (at least four to five) need to be present in gp120 to exert a (still) moderate drug resistance profile; and (iv) last but not least, it may be speculated that HIV-1 strains that develop glycan deletions in their gp120 envelope upon prolonged CBP exposure, may expose previously hidden immunogenic epitopes to the immune system, making the mutant virus now vulnerable to inactivation by an extensive neutralizing antibody (Nab) response (Balzarini, 2005). Although this concept has yet to be proven for CBP, it may represent an attractive property of CBP to be endowed with a potential dual mechanism of antiviral action: either a direct inhibitory effect at the level of entry (by binding to the gp120 glycans) and an indirect effect by triggering Nab production upon the selection of mutant virus strains. It seems now imperative to prioritarily test this hypothesis in the *in vivo* setting.

11. CBP versus other entry inhibitors as potential microbicide drugs

Beside CBP, a variety of other microbicide candidate drugs exists, and many are currently subject of (pre)clinical evaluation. They range from non-specific (i.e. detergents, buffering agents) and more specific (i.e. anionic polymers) to highly specific anti-HIV microbicides (such as soluble CD4, CXCR4 or CCR5 antagonists, reverse transcriptase (NtRTI and NNRTI) inhibitors, etc.) (for an overview, see Balzarini and Van Damme, 2005).

NNRTIs have the advantage of a high therapeutic window but they are solely inhibitory to HIV-1 strains. The presence of a lipophylic tight-binding NNRTI at the site of virus infection or transmission may result in a virtual direct inactivation of RT in the virus particle. Because of its lipophylic nature, it can easily be incorporated into the drug-exposed target cell membrane, which exerts a local protective barrier if the virus tries to enter the cells. However, systemic absorption of such microbicide drugs may result in systemic side effects or viral drug resistance or both.

Although anionic substances that target adsorption of the virus are efficient in preventing contact between cell-free virus and its target cell, they are generally less efficient in preventing transmission of the cell-associated virus to uninfected cells. Also, antiviral activity of polyanions sometimes markedly varies depending the nature of the HIV strain. Although they may have a rather limited general toxicity (compared with detergents or surfactants), several polyanions have shown to be endowed with an anticoagulant effect.

CBP may have the advantage of tight binding to the HIV envelope and a number of them have broad-spectrum HIV clade inhibitory potential. Moreover, they are colorless, odorless and tasteless, resistant to higher temperatures and low (vaginal) pH. These are favorable properties for a microbicide drug. However, due to their protein nature, CBP may be expensive to produce and to scale-up. A commensal bacterial (i.e. *Lactobacillus*) expression system may be an interesting alternative approach to deliver the microbicide in the vaginal environment. The choice of the nature of the CBP to be used as microbicide drug candidate should be carefully made. Some lectins can indeed be endowed with unfavorable properties such as hemagglutination of human erythrocytes, mitogenic stimulation of human peripheral lymphocyte cells, inflammatory activity, cellular toxicity, interference with protein synthesis, etc. (Van Damme et al., 1998; Sharon and Lis, 2003). CBP may of course also interact with cellular glycoproteins, and it has recently been demonstrated that galectin-1, a dimeric β-galactoside-binding protein, rather promotes infection with R5 and X4 HIV-1 variants by facilitating attachment of HIV-1 to the cell surface. Obviously, galectin-1 can cross-link HIV-1 to its target cells and promotes a firmer adhesion of the virus to the cell surface (Ouellet et al., 2005). All these above mentioned potential unfavorable interactions should be taken into account before making the choice of the type of CBP for clinical investigations.

12. Conclusion

Carbohydrate-binding proteins consist of a wide range of different structures, sizes and carbohydrate oligomeric specificities. They are found in a variety of different species, ranging from procaryotes to corals, algae, fungi, plants, invertebrates and vertebrates (i.e. mammalians). For several of them, details on the crystal structure of the CBP in complex with specific carbohydrate oligomers are available. This should allow to understand the interaction of these proteins with their target carbohydrates and to eventually design novel CBA (including peptidomimetics and small-size CBA) that are further optimized to prevent or inhibit HIV infection and transmission. The potential microbicidal activity of at least one CBP has already been demonstrated in virus-exposed monkeys, and efficient engineered expression of this CBP in commensal bacteria has also been shown. The above described efforts, achievements and insights will eventually payoff to transform these drug leads into affordable, useful and

efficient drugs for antiviral (i.e. HIV) therapy, either as microbicide or to be applied for systemic use.

Acknowledgements

The research of the author has been supported by the European Commission (R. Descartes Prize-2001 HPAW-90001 and EMPRO), the Flemish "Geconcerteerde Onderzoeksacties" (Krediet no. 05/19), the Flemish "Fonds voor Wetenschappelijk Onderzoek" (Krediet no. G-067-04), and the Centers of Excellence of the K.U. Leuven (Krediet no. EF/05/15). The author thanks Prof. Els Van Damme at the Ghent University, Belgium, for critical reading of the manuscript. The dedicated editorial help of Mrs. C. Callebaut is gratefully acknowledged.

References

Adams, E.W., Ratner, D.M., Bokesch, H.R., McMahon, J.B., O'Keefe, B.R., Seeberger, P.H., 2004. Oligosaccharide and glycoprotein microassays as tools in HIV glycobiology: glycan-dependent gp120/protein interactions. Chem. Biol. 11, 875–881.

Balzarini, J., Schols, D., Neyts, J., Van Damme, E., Peumans, W., De Clercq, E., 1991. α-(1-3)- and α-(1-6)-D-mannose-specific plant lectins are markedly inhibitory to human immunodeficiency virus and cytomegalovirus infections in vitro. Antimicrob. Agents Chemother. 35, 410–416.

Balzarini, J., Neyts, J., Schols, D., Hosoya, M., Van Damme, E., Peumans, W., De Clercq, E., 1992. The mannose-specific plant lectins from *Cymbidium* hybrid and *Epipactis helleborine* and the $(N$-acetylglucosamine$)_n$-specific plant lectin from *Urtica dioica* are potent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication in vitro. Antiviral Res. 18, 191–207.

Balzarini, J., Hatse, S., Vermeire, K., Princen, K., Aquaro, S., Perno, C.-F, De Clercq, E., Egberink, H., Vanden Mooter, G., Peumans, W., Van Damme, E., Schols, D., 2004a. Mannose-specific plant lectins from the *Amaryllidaceae* family qualify as efficient microbicides for prevention of human immunodeficiency virus infection. Antimicrob. Agents Chemother. 48, 3858–3870.

Balzarini, J., Van Laethem, K., Hatse, S., Vermeire, K., De Clercq, E., Peumans, W., Van Damme, E., Vandamme, A.-M., Bolmstedt, A., Schols, D., 2004b. Profile of resistance of human immunodeficiency virus to mannose-specific plant lectins. J. Virol. 78, 10617–10626.

Balzarini, J., 2005. Targeting the glycans of gp120: a novel approach aimed at the Achilles heel of HIV. Lancet Infect. Dis. 5, 726–731.

Balzarini, J., Van Damme, L., 2005. Intravaginal and intrarectal microbicides to prevent HIV infection. Can. Med. Assoc. J. 172, 461–464.

Balzarini, J., Van Laethem, K., Hatse, S., Froeyen, M., Van Damme, E., Peumans, W., De Clercq, E., Schols, D., 2005a. Marked depletion of glycosylation sites in HIV-1 gp120 under selection pressure by the mannose-specific plant lectins of *Hippeastrum* hybrid and *Galanthus nivalis*. Mol. Pharmacol. 67, 1556–1565.

Balzarini, J., Van Laethem, K., Hatse, S., Froeyen, M., Peumans, W., Van Damme, E., Schols, D., 2005b. Carbohydrate-binding agents cause deletions of highly conserved glycosylation sites in HIV gp120. A new therapeutic concept to hit the Achilles heel of HIV. J. Biol. Chem. 280, 41005–41014.

Bewley, C.A., 2001. Solution structure of a cyanovirin-N:Man alpha 1-2Man alpha complex: structural basis for high-affinity carbohydrate-mediated binding to gp120. Structure 9, 931–940.

Bewley, C.A., Cai, M., Ray, S., Ghirlando, R., Yamaguchi, M., Muramoto, K., 2004. New carbohydrate specificity and HIV-1 fusion blocking activity of the cyanobacterial protein MVL: NMR, ITC and sedimentation equilibrium studies. J. Mol. Biol. 339, 901–914.

Bewley, C.A., Gustafson, K.R., Boyd, M.R., Covell, D.G., Bax, A., Clore, G.M., Gronenborn, A.M., 1998. Solution structure of cyanovirin-N, a potent HIV-inactivating protein. Nat. Struct. Biol. 5, 571–578.

Bokesch, H.R., O'Keefe, B.R., McKee, T.C., Pannell, L.K., Patterson, G.M.L., Gardella, R.S., Sowder III, R.C., Turpin, J., Watson, K., Buckheit Jr., R.W., Boyd, M.R., 2003. A potent novel anti-HIV protein from the cultured cyanobacterium *Scytonema varium*. Biochemistry 42, 2578–2584.

Bolmstedt, A.J., O'Keefe, B.R., Shenoy, S.R., McMahon, J.B., Boyd, M.R., 2001. Cyanovirin-N defines a new class of antiviral agent targeting N-linked, high-mannose glycans in an oligosaccharide-specific manner. Mol. Pharmacol. 59, 949–954.

Botos, I., O'Keefe, B.R., Shenoy, S.R., Cartner, L.K., Ratner, D.M., Seeberger, P.H., Boyd, M.R., Wlodawer, A., 2002. Structures of the complexes of a potent anti-HIV protein cyanovirin-N and high mannose oligosaccharides. J. Biol. Chem. 277, 34336–34342.

Botos, I., Wlodawer, A., 2005. Proteins that bind high-mannose sugars of the HIV envelope. Prog. Biophys. Mol. Biol. 88, 233–282.

Bourne, Y., Astoul, C.H., Zamboni, V., Peumans, W.J., Menu-Bouaouiche, L., Van Damme, E.J., Barre, A., Rouge, P., 2002. Structural basis for the unusual carbohydrate-binding specificity of jacalin towards galactose and mannose. Biochem. J. 364, 173–180.

Boyd, M.R., Gustafson, K.R., McMahon, J.B., Shoemaker, R.H., O'Keefe, B.R., Mori, T., Gulakowski, R.J., Wu, L., Rivera, M.I., Laurencot, C.M., Currens, M.J., Cardellina II, J.H., Buckheit Jr., R.W., Nara, P.L., Pannell, L.K., Sowder II, R.C., Henderson, L.E., 1997. Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. Antimicrob. Agents Chemother. 41, 1521–1530.

Bulgheresi, S., Schabussova, I., Chen, T., Mullin, N.P., Maizels, R.M., Ott, J.A., 2006. A new C-type lectin similar to the human immunoreceptor DC-SIGN mediates symbiont acquisition by a marine nematode. Appl. Environmental Microbiol. 72, in press.

Calarese, D.A., Scanlan, C.N., Zwick, M.B., Deechongkit, S., Mimura, Y., Kunert, R., Zhu, P., Wormald, M.R., Stanfield, R.L., Roux, K.H., Kelly, J.W., Rudd, P.M., Dwek, R.A., Katinger, H., Burton, D.R., Wilson, I.A., 2003. Antibody domain exchange is an immunological solution to carbohydrate cluster recognition. Science 300, 2065–2071.

Calarese, D.A., Lee, H.-K., Huang, C.-Y., Best, M.D., Astronomo, R.D., Stanfield, R.L., Katinger, H., Burton, D.R., Wong, C.-H., Wilson, I.A., 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.

Chang, T.L., Klotman, M.E., 2004. Defensins: natural anti-HIV peptides. AIDS Res. 6, 161–168.

Chang, T.L., Vargas Jr., J., DelPortillo, A., Klotman, M.E., 2005. Dual role of alpha-defensin-1 in anti-HIV-1 innate immunity. J. Clin. Invest. 11, 765–773.

Charan, R.D., Munro, M.H., O'Keefe, B.R., Sowder II, R.C., McKee, T.C., Currens, M.J., Pannell, L.K., Boyd, M.R., 2000. Isolation and characterization of *Myrianthus holstii* lectin, a potent HIV-1 inhibitory protein from the plant *Myrianthus holstii*. J. Nat. Prod. 63, 1170–1174.

Chiba, H., Inokoshi, J., Okamoto, M., Asanuma, S., Matsuzaki, Ki., Iwama, M., Mizumoto, K., Tanaka, H., Oheda, M., Fujita, K., Nakashima, H., Shinose, M., Takahashi, Y., Omura, S., 2001. Actinohivin, a novel anti-HIV protein from an actinomycete that inhibits syncytium formation: isolation, characterization, and biological activities. Biochem. Biophys. Res. Commun. 282, 595–601.

Chiba, H., Inokoshi, J., Nakashima, H., Omura, S., Tanaka, H., 2004. Actinohivin, a novel anti-human immunodeficiency virus protein from an actinomycete, inhibits viral entry to cells by binding high-mannose type sugar chains of gpl20. Biochem. Biophys. Res. Commun. 316, 203–210.

Feinberg, H., Mitchell, D.A., Drickamer, K., Weis, W.I., 2001. Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. Science 294, 2163–2166.

Gallaher, W.R., Ball, J.M., Garry, R.F., Martin-Amedee, A.M., Montelaro, R.C., 1995. A general model for the surface glycoproteins of HIV and other retroviruses. AIDS Res. Hum. Retroviruses 11, 191–202.

Geijtenbeek, T.B., Kwon, D.S., Torensma, R., van Vliet, S.J., van Duijnhoven, G.C., Middel, J., Cornelissen, I.L., Nottet, H.S., KewalRamani, V.N., Littman, D.R., Figdor, C.G., van Kooyk, Y., 2000. DC-Sign, a den-

dritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 100, 587–597.

Giomarelli, B., Provvedi, R., Meacci, F., Maggi, T., Medaglini, D., Pozzi, G., Mori, T., McMahon, J.B., Gardella, R., Boyd, M.R., 2002. The microbicide cyanovirin-N expressed on the surface of commensal bacterium *Streptococcus gordonii* captures HIV-1. AIDS 16, 1351–1356.

Hansen, S., Holunskov, U., 1998. Structural agents of collectins and receptors for collectins. Immunobiology 199, 165–189.

Harata, K., Muraki, M., 2000. Crystal structures of *Urtica dioica* agglutinin and its complex with tri-*N*-acetylchitotriose. J. Mol. Biol. 297, 673–681.

Harata, K., Schubert, W.D., Muraki, M., 2001. Structure of *Urtica dioica* agglutinin isolectin I: dimer formation mediated by two zinc ions bound at the sugar-binding site. Acta Crystallogr. D Biol. Crystallogr. 57, 1513–1517.

Heggelund, L., Mollnes, T.E., Espevik, T., Müller, F., Kristiansen, K.I., Aukrust, P., Frøland, S.S., 2005. Modulatory effect of mannose-binding lectin on cytokine responses: possible roles in HIV infection. Eur. J. Clin. Invest. 35, 765–770.

Hester, G., Wright, C.S., 1996. The mannose-specific bulb lectin from *Galanthus nivalis* (snowdrop) binds mono- and dimannosides at distinct sites. Structure analysis of refined complexes at 2.3 Å and 3.0 Å resolution. J. Mol. Biol. 262, 516–531.

Inokoshi, J., Chiba, H., Asanuma, S., Takahashi, A., Omura, S., Tanaka, H., 2001. Molecular cloning of actinohivin, a novel anti-HIV protein from an actinomycete, and its expression in Escherichia coli. Biochem. Biophys. Res. Commun. 281, 1261–1265.

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

Kabir, S., 1998. Jacalin: a jackfruit (*Artocarpus heterophyllus*) seed-derived lectin of versatile applications in immunobiological research. J. Immunol. Methods 212, 193–211.

Kljajic, Z., Schroder, H.C., Rottmann, M., Cuperlovic, M., Movsesian, M., Uhlenbruck, G., Gasic, M., Zahn, R.K., Muller, W.E., 1987. A D-mannose-specific lectin from *Gerardia savaglia* that inhibits nucleocytoplasmic transport of mRNA. Eur. J. Biochem. 169, 97–104.

Klein, N.J., 2005. Mannose-binding lectin: do we need it? Mol. Immunol. 42, 919–924.

Kwong, P.D., Wyatt, R., Robinson, J., Sweet, R.W., Sodroski, J., Hendrickson, W.A., 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393, 648–659.

Lagenaur, L.A., Liu, X., Simpson, D.A., Essenmacher, K., Parker, C., Chang, C.-H., Tsai, D., Rao, S., Hamer, D., Parks, T.P., Lee, P.P., Xu, Q., 2005. Development of vaginal Lactobacilli for mucosal delivery of a topical microbicide, Cyanovirin-N (CV-N). Abstracts of the International Meeting of the Institute of Human Virology, Baltimore, USA, 29 August–2 September. Retrovirology 2 (Suppl. 1), S93.

Leonard, C.K., Spellman, M.W., Riddle, L., Harris, R.J., Thomas, J.N., Gregory, T.J., 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.

Lifson, J., Coutre, S., Huang, E., Englemann, E., 1986. Role of envelope glycoprotein carbohydrate in human immunodeficiency virus (HIV) infectivity and virus-induced cell fusion. J. Exp. Med. 164, 2101–2106.

Liu, W., Yang, N., Ding, J., Huang, R.-h., Hu, Z., Wang, D.-C., 2005. Structural mechanism governing the quaternary organization of monocot mannose-binding lectin revealed by the novel monomeric structure of an orchid lectin. J. Biol. Chem. 280, 14865–14876.

Mizuochi, T., Spellman, M.W., Larkin, M., Solomon, J., Basa, L.J., Feizi, T., 1988a. 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., Spellman, M.W., Larkin, M., Solomon, J., Basa, L.J., Feizi, T., 1988b. Structural characterization by chromatographic profiling of the oligosaccharides of human immunodeficiency virus (HIV) recombinant envelope glycoprotein gp120 produced in Chinese hamster ovary cells. Biomed. Chromatogr. 2, 260–270.

Mori, T., O'Keefe, B.R., Sowder 2nd, R.C., Bringans, S., Gardella, R., Berg, S., Cochran, P., Turpin, J.A., Buchheit Jr., R.W., McMahon, J.B., Boyd, M.R., 2005. Isolation and characterization of griffithsin, a novel HIV-inactivating protein, from the red alga *Griffithsia* sp. J. Biol. Chem. 280, 9345–9353 (2004).

Muller, W.E., Renneisen, K., Kreute, M.H., Schroder, H.C., Winkler, I., 1988. The D-mannose-specific lectin from *Gerardia savaglia* blocks binding of human immunodeficiency virus type 1 to H9 cells and human lymphocytes in vitro. J. Acquir. Immune Defic. Syndr. 1, 453–458.

Nakowitsch, S., Quendler, H., Fekete, H., Kunert, R., Katinger, H., Stiegler, G., 2005. HIV-1 mutants escaping neutralization by the human antibodies 2F5, 2G12, and 4E10: in vitro experiments versus clinical studies. AIDS 19, 1957–1966.

Nguyen, D.G., Hildreth, J.E., 2003. Involvement of macrophage mannose receptor in the binding and transmission of HIV by macrophages. Eur. J. Immunol. 33, 483–493.

Ouellet, M., Mercier, S., Pelletier, I., Bounou, S., Roy, J., Hirabayashi, J., Sato, S., Tremblay, M.J., 2005. Galectin-1 acts as a soluble host factor that promotes HIV-1 infectivity through stabilization of virus attachment to host cells. J. Immunol. 174, 4120–4126.

Pashov, A., MacLeod, S., Saha, R., Perry, M., VanCott, T.C., Kieber-Emmons, T., 2005. Concanavalin A binding to HIV envelope protein is less sensitive to mutations in glycosylation sites that monoclonal antibody 2G12. Glycobiology 15, 994–1001.

Peumans, W.J., De Ley, M., Broekaert, W.F., 1984. An unusual lectin from stinging nettle (*Urtica dioica*) rhizomes. FEBS Lett. 177, 99–103.

Saul, F.A., Rovira, P., Boulot, G., Van Damme, E.J.M., Peumans, W.J., Truffa-Bachi, P., Bentley, G.A., 2000. Crystal structure of *Urtica dioica* agglutinin, a superantigen presented by MHC molecules of class I and class II. Struc. Fold. Des. 8, 593–603.

Scanlan, C.N., Pantophlet, R., Wormald, M.R., Ollmann, S.E., Stanfield, R., Wilson, I.A., Katinger, H., Dwek, R.A., Rudd, P.M., Burton, D.R., 2002. The broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2G12 recognizes a cluster of alpha $1 \rightarrow 2$ mannose residues on the outer face of gp120. J. Virol. 76, 7306–7321.

Sharon, N., Lis, H. (Eds.), 2003. Lectins, second ed. Kluwer Academic Publishers, Dordrecht, pp. 1–452.

Shibuya, N., Goldstein, I.J., Shafer, J.A., Peumans, W.J., Broekaert, W.F., 1986. Carbohydrate binding properties of the stinging nettle (*Urtica dioica*) rhizome lectin. Arch. Biochem. Biophys. 249, 215–224.

Sun, L., Finnegan, C.M., Kish-Catalone, T., Blumenthal, R., Garzino-Demo, P., La Terra Maggiore, G.M., Berrone, S., Kleinman, C., Wu, Z., Abdelwahab, S., Lu, W., Garzino-Demo, A., 2005. Human beta-defensins suppress human immunodeficiency virus infection: potential role in mucosal protection. J. Virol. 79, 14318–14329.

Takahashi, A., Inokoshi, J., Chiba, H., Omura, S., Tanaka, H., 2005. Essential regions for antiviral activities of actinohivin, a sugar-binding anti-human immunodeficiency virus protein from an actinomycete. Arch. Biochem. Biophys. 437, 233–240.

Trkola, A., Kuster, H., Rusert, P., Joos, B., Fischer, M., Leemann, C., Manrique, A., Rehr, H.M., Rehr, M., Oxenius, A., Weber, R., Stiegler, G., Vcelar, B., Katinger, H., Aceto, L., Gunthard, H.F., 2005. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat. Med. 11, 615–622.

Tsai, C.C., Emau, P., Jiang, Y., Agy, M.B., Shattock, R.J., Schmidt, A., Morton, W.R., Gustafson, K.R., Boyd, M.R., 2004. Cyanovirin-N inhibits AIDS virus infections in vaginal transmission models. AIDS Res. Hum. Retroviruses 20, 11–18.

Turville, S.G., Vermeire, K., Balzarini, J., Schols, D., 2005. Sugar-binding proteins potently inhibit dendritic cell human immunodeficiency virus type 1 (HIV-1) infection and dendritic-cell-directed HIV-1 transfer. J. Virol. 79, 13519–13527.

Van Damme, E.J.M., Peumans, W.J., 1989. Isolectins in *Narcissus*: complexity, inter- and intraspecies differences and developmental control. Physiol. Plant 79, 1–6.

Van Damme, E.J.M., Allen, A.K., Peumans, W.J., 1987. Leaves of the orchid twayblade (*Listera ovata*) contain a mannose-specific lectin. Plant Physiol. 85, 566–569.

Van Damme, E.J.M., Allen, A.K., Peumans, W.J., 1988a. Related mannose-specific lectins from different species of the family *Amaryllidaceae*. Physiol. Plant 73, 52–57.

Van Damme, E.J.M., Broekaert, W.F., Peumans, W.J., 1988b. The *Urtica dioica* agglutinin is a complex mixture of isolectins. Plant Physiol. 86, 598–601.

Van Damme, E.J.M., Peumans, W.J., Pusztai, A., Barocz, S. (Eds.), 1998. Handbook of Plant Lectins: Properties and Biomedical Applications. John Wiley & Sons, Chichester, NY.

Van Damme, E.J.M., Smeets, K., Torrekens, S., Van Leuven, F., Peumans, W.J., 1994. Characterization and molecular cloning of the mannose-binding lectins from the *Orchidaceae* species *Listera ovata*, *Epipactis helleborine* and *Cymbidium* hybrid. Eur. J. Biochem. 221, 769–777.

Wang, J.H., Kong, J., Li, W., Molchanova, V., Chikalovets, I., Belogortseva, N., Lk'yanov, P., Zheng, Y.T. A beta-galactose-specific lectin isolated form the marine worm *Chaetopterus variopedatus* possesses anti-HIV-1 activity. Comp. Biochem. Physiol. C Toxicol. Pharmacol., in press.

Williams Jr., D.C., Lee, J.Y., Cai, M., Bewley, C.A., Clore, G.M., 2005. Crystal structures of the HIV-1 inhibitory cyanobacterial protein MVL free and bound to $Man_3GlcNAc_2$: structural basis for specificity and high-affinity binding to the core pentasaccharide from N-linked oligomannoside. J. Biol. Chem. 280, 29269–29276.

Witvrouw, M., Fikkert, V., Hantson, A., Pannecouque, C., O'Keefe, B.R., McMahon, J., Stamatatos, L., De Clercq, E., Bolmstedt, A., 2005. Resistance of human immunodeficiency virus type 1 to the high-mannose binding agents cyanovirin N and concanavalin A. J. Virol. 79, 7777–7784.

Wyatt, R., Kwong, P.D., Desjardins, E., Sweet, R.W., Robinson, J., Hendrickson, W.A., Sodroski, J.G., 1998. The antigenic structure of the HIV gp120 envelope glycoprotein. Nature 393, 705–711.

Xiong, C., O'Keefe, B.R., Botos, I., Wlodawer, A., McMahon, J.B. Overexpression and purification of scytovirin, a potent, novel anti-HIV protein from the cultured cyanobacterium *Scytonema varium*. Protein Expr. Purif., in press.

Yamaguchi, M., Ogawa, T., Muramoto, K., Kamio, Y., Jimbo, M., Kamiya, H., 1999. Isolation and characterization of a mannan-binding lectin from the freshwater Cyanobacterium (blue-green algae) *Microcystis viridis*. Biochem. Biophys. Res. Commun. 265, 703–708.

Yang, F., Bewley, C.A., Louis, J.M., Gustafson, K.R., Boyd, M.R., Gronenborn, A.M., Clore, M., Wlodawer, A., 1999. Crystal structure of cyanovirin-N, a potent HIV-inactivating protein, shows unexpected domain swapping. J. Mol. Biol. 288, 403–412.

Zhu, X., Borchers, C., Bienstock, R.J., Tomer, K.B., 2000. Mass spectrometric characterization of the glycosylation pattern of HIV-gp120 expressed in CHO cells. Biochemistry 39, 11194–11204.