Bitter-sweet symphony: glycan–lectin interactions in virus biology

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Abstract

Glycans are carbohydrate modifications typically found on proteins or lipids, and can act as ligands for glycan-binding proteins called lectins. Glycans and lectins play crucial roles in the function of cells and organs, and in the immune system of animals and humans. Viral pathogens use glycans and lectins that are encoded by their own or the host genome for their replication and spread. Recent advances in glycobiological research indicate that glycans and lectins mediate key interactions at the virus-host interface, controlling viral spread and/or activation of the immune system. This review reflects on glycan–lectin interactions in the context of viral infection and antiviral immunity. A short introduction illustrates the nature of glycans and lectins, and conveys the basic principles of their interactions. Subsequently, examples are discussed highlighting specific glycan–lectin interactions and how they affect the progress of viral infections, either benefiting the host or the virus. Moreover, glycan and lectin variability and their potential biological consequences are discussed. Finally, the review outlines how recent advances in the glycan–lectin field might be transformed into promising new approaches to antiviral therapy.

Introduction

Many emerging and re-emerging viral diseases in animals and humans pose significant global health problems for which novel antiviral measures are in urgent demand. In general, rational design of new prophylactic and curative antiviral strategies requires a detailed knowledge of the viral infection mechanism and the host's innate and adaptive immune defense. Historically, cell biological and microbiological research was mainly focussed on the nucleic acid and protein level. However, over the last few decades it has become clear that also glycans account to a great extent for the structural and functional diversity displayed by animal and human cells and their pathogens. At this moment, glycobiology is one of the most rapidly expanding disciplines in biology. A rapidly evolving array of powerful, novel techniques for the analysis of glycan structure and function (glycomics) has prompted many scientists to apply these tools to the field of virology, and

this glycovirological approach has yielded a wealth of information on the various glycobiological aspects of viral infection and antiviral immunity. Particularly fascinating in this context are the many distinct glycan-lectin interactions that may occur during viral infection of a host. Virion-associated glycans often serve as ligands for specific host lectins. Conversely, the glycan portions of host glycoconjugates function as receptors for various viruses that employ viral lectins for host cell entry. This review reflects on glycan–lectin interactions in the context of viral infection and antiviral immunity. Following a general introduction on glycan and lectin biology, specific glycan–lectin interactions are highlighted and discussed within the larger framework of viral infection and immunity. Distinction is made between interactions that benefit the host and interactions that benefit the virus. In addition, different factors that contribute to glycan and lectin variation – and that consequently affect glycan–lectin interactions – are explored and various approaches to

modulate specific glycan–lectin interactions in antiviral therapies are briefly discussed.
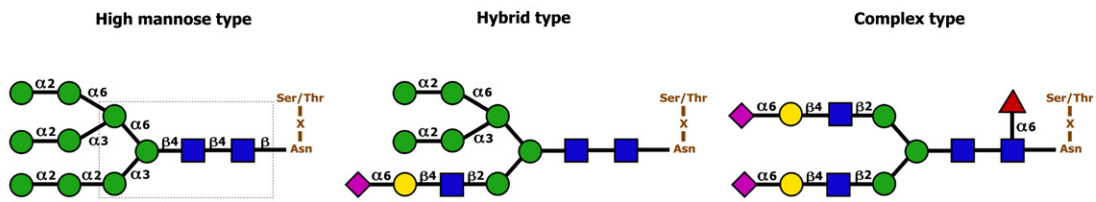
Glycans vs. lectins

Glycans

Like nucleic acids, proteins and lipids, glycans are essential components of the animal cell and organism. The term 'glycan' refers to the carbohydrate portion of glycoproteins and glycolipids typically found at cell surfaces, in extracellular matrices and in cell secretions. Unlike nucleic acid and protein synthesis, the biosynthesis of glycans is not a template-driven process. Instead, glycosylation depends on the concerted action of different glycosyltransferase, glycosidase, and other enzymes. Several important glycan types are exclusively assembled by the enzyme sets present in the endoplasmic reticulum (ER) and the Golgi network: glycan/glycoconjugate synthesis is typically initiated in the ER or early Golgi and gradual processing and diversification – or 'maturation' – occurs as these molecules move further through the different enzyme-equipped compartments of the secretory pathway. The variability inherent to glycan synthesis and maturation forms the basis of the considerable diversity and complexity of the glycan repertoires found on animal glycoconjugates (Varki *et al.*, 2009; Taylor & Drickamer, 2011).

According to the basic glycan 'core' structure, the type of molecule the glycans are linked with and the type of the linkage, glycans and glycoconjugates can be categorized in different classes. Two major classes of protein-linked glycosylation are the *N*- and *O*-linked glycans. *N*-link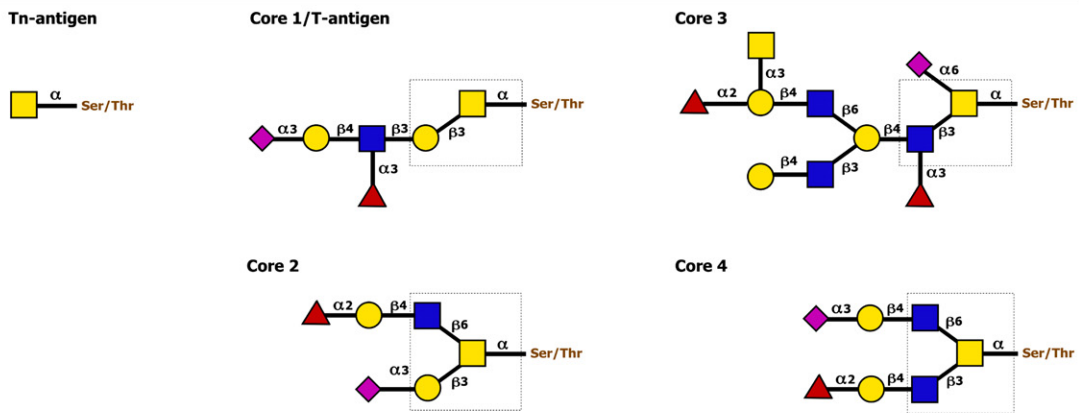ed glycans are covalently linked to the nitrogen atoms of specific amino acid (aa) residues (typically Asparagine) via an *N*-glycosidic bond. *N*-acetylglucosamine–to–Asparagine (GlcNAcβ-Asn) type glycans represent the most common form of *N*-linked protein glycosylation (Weerapana & Imperiali, 2006; Varki *et al.*, 2009; Larkin & Imperiali, 2011; Schwarz & Aebi, 2011; Taylor & Drickamer, 2011). *O*-linked glycans are covalently attached to the oxygen atoms of specific amino acid residues (typically Serine or Threonine) via an *O*-glycosidic bond. A common *O*-glycan type – and potentially the one most studied – is the *N*-acetylgalactosamine–to–Serine/Threonine (GalNAcα-Ser/Thr) type or mucin-type *O*-glycan. Other (nonmucin) *O*-glycan types include α-linked *O*-mannose, α-linked *O*-fucose, β-linked *O*-xylose, β-linked *O*-GlcNAc (*N*-acetylglucosamine), α-/β-linked *O*-galactose, and α-/β-linked *O*-glucose glycans (Van den Steen *et al.*, 1998; Peter-Katalinic, 2005; Varki *et al.*, 2009; Jensen *et al.*, 2010; Gill *et al.*, 2011; Taylor & Drickamer, 2011). Interestingly, various proteins are also modified with glycosaminoglycans (GAGs), linear polysaccharide chains composed of repeated disaccharide subunits consisting of a uronic acid/galactose residue and an amino sugar. Glycosaminoglycan-carrying glycoproteins are generally referred to as proteoglycans (Prydz & Dalen, 2000; Varki *et al.*, 2009; Taylor & Drickamer, 2011). Figure 1 illustrates the general structure and classification of some common types of protein glycosylation. Similarly as for protein-linked glycosylation, different types of lipid-linked glycosylation can be discerned: the glycosphingolipids (Varki *et al.*, 2009; Taylor & Drickamer, 2011; Yu *et al.*, 2011) and the glycophospholipid anchors or glycosylphosphatidylinositol (GPI) anchors (Paulick & Bertozzi, 2008; Varki *et al.*,

Fig. 1. Classification and basic structure of common types of protein-linked glycosylation. (a) GlcNAcβ-Asn type *N*-linked glycans are covalently attached to the amide nitrogen atoms of Asn side chains and are almost exclusively found on Asn residues within the sequence Asn-X-Ser/Thr, in which X can be any amino acid except Pro. The nature of the glycan structures that decorate the common glycan core – the glycan part shown in a dashed box – dictates classification of *N*-linked glycans as high-mannose type, hybrid type or complex type glycans, examples of which are shown in the panel. (b) GalNAcα-Ser/Thr type *O*-linked glycans have a GalNAc residue α-linked to the oxygen atom of the hydroxyl group of Ser or Thr residues. Unlike for GlcNAcβ-Asn type *N*-linked protein glycosylation, there are no clear amino acid motifs that mark these *O*-linked glycosylation sites. A single GalNac residue linked to the Ser/Thr is termed the 'Tn antigen'. Depending on the basic structure of the glycan core, more complex (extended) *O*-linked glycans are categorized into different 'core types'. Cores 1–4 are the most common core structures, but also other core types exist. The Tn antigen and examples of extended core 1, 2, 3, and 4 *O*-glycans are shown in the panel. The distinct glycan cores are shown in dashed boxes. (c) Glycosaminoglycans (GAGs) are linear polysaccharide chains composed of repeated disaccharide subunits of a uronic acid/galactose residue and an amino sugar. Glycosaminoglycans are classified as hyaluronan (HA), heparan sulfate/heparin (HS), chondroitin sulfate (CS), dermatan sulfate (DS), or keratan sulfate (KS), depending on the structure of their basic disaccharide subunits (shown in square brackets) and further modification (e.g. sulfation at different positions) of the glycan chain. With exception of hyaluronan, all major glycosaminoglycan types are sulfated and occur covalently linked to proteins. HS, CS, and DS are found on Ser-linked xylose residues. Although no unambiguous consensus sequence for xylosylation exists, the Ser attachment site is consistently flanked by a Gly residue at its carboxy-terminal side. As depicted in the figure, heparan sulfate and heparin have the same basic structure. Although they share a common biosynthesis, heparin generally undergoes more extensive sulfation and epimerization of uronic acid to iduronic acid. Moreover, heparin is synthesized only in connective tissue mast cells as part of serglycin proteoglycans, whereas heparan sulfate is synthesized in virtually all mammalian cells. KS is found on Asn-linked *N*-glycan core structures (KS I) or Ser/Thr-linked *O*-glycan core 2 structures (KS II). Capping or further modification of the glycosaminoglycan chains – sulfation excepted – is not depicted (adapted from Varki *et al.*, 2009).

(a) GLCNACβ-ASN TYPE N-LINKED GLYCANS

High mannose type

Hybrid type

Complex type

(b) GALNACα-SER/THR TYPE O-LINKED GLYCANS

Tn-antigen

Core 1/T-antigen

Core 3

Core 2

Core 4

(c) GLYCOSAMINOGLYCANS (GAGs)

Hyaluronan (HA)

No covalent link with protein

Heparan Sulphate/Heparin (HS)

Chondroitin Sulphate (CS)

Dermatan Sulphate (DS)

Keratan Sulphate (KS) - Type I

Keratan Sulphate (KS) - Type II
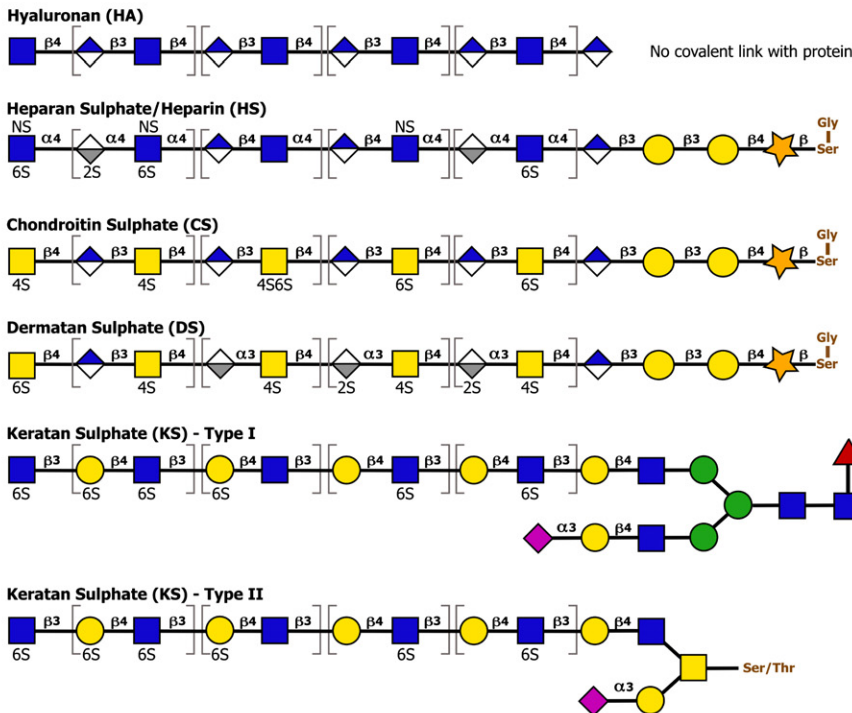
N-Acetylgalactosamine (GalNAc)

N-Acetylglucosamine (GlcNAc)

Mannose (Man)

Galactose (Gal)

Fucose (Fuc)

Xylose (Xyl)

Iduronic Acid (IdoA)

Glucuronic Acid (GlcA)

N-Acetylneuraminic Acid (Neu5Ac)

2009; Taylor & Drickamer, 2011) represent important glycolipid classes. Their basic structure is introduced in Fig. 2.

Despite their different core structures and linkages to carrier molecules, distinct glycan types can still share conserved structural characteristics as they often follow partially overlapping biosynthetic pathways. Although some glycan features may be exclusively found in one specific glycan class, many (sub)terminal glycan modifications can be found in different glycan classes. Common (sub)terminal modifications include poly-N-acetyllactosamine chains, ABH and Lewis histo-blood group antigens (HBGA), and sialic acids in different linkages (Varki et al., 2009; Taylor & Drickamer, 2011). Consequently, the glycan moieties of glycoproteins and glycolipids often have more in common than one would expect based on their core structure.

Apart from the different glycan types introduced here, several other forms of glycosylation exist. However, an in-depth discussion of these is beyond the scope of this review. For more detailed information on glycan structure and biosynthesis, readers may refer to recent reference works on this topic (Varki et al., 2009; Taylor & Drickamer, 2011).

Not only the animal and human hosts, but also their pathogens can benefit from the fine-tuned cellular biosynthetic pathways that govern glycosylation. This is most obvious in the case of obligatory intracellular pathogens such as viruses. Glycans form an important part of the surface of many viruses. As the glycosylation of viral components is facilitated by the cellular glycosylation machinery, viral glycosylation is similar to that of the host. However, important differences have been noted: Viral glycoproteins are often more heavily glycosylated than their cellular counterparts and the composition of individual glycan chains can diverge greatly between virus and host. The biological basis of this variability is further
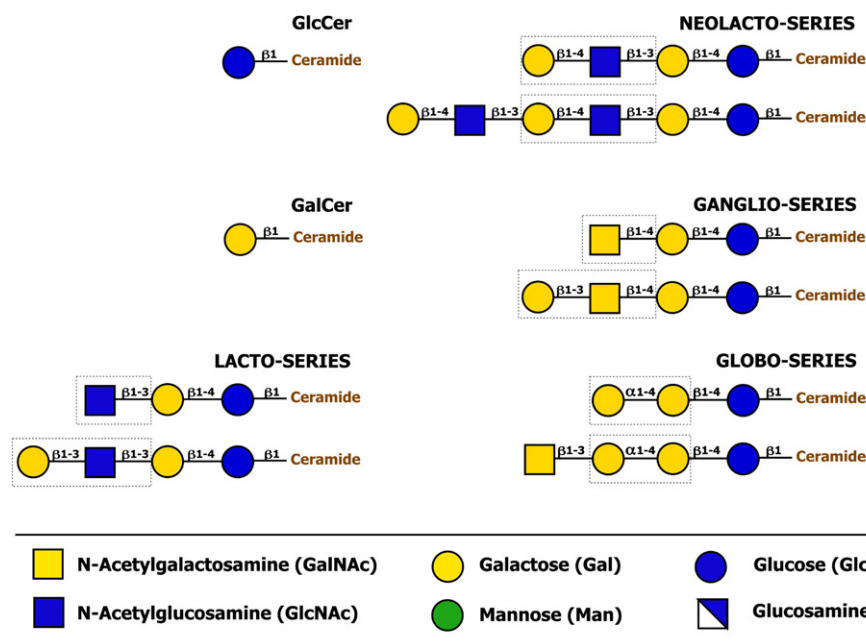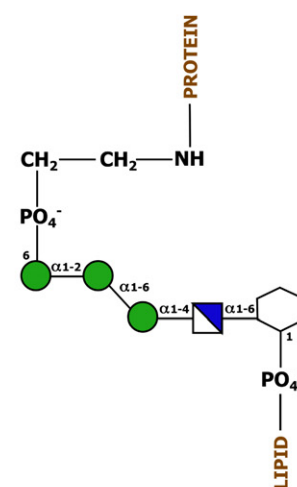

Fig. 2. Classification and basic structure of major types of lipid-linked glycosylation. (a) Glycosphingolipids consist of a hydrophilic glycan moiety linked to a hydrophobic sphingolipid. In higher animals, a ceramide lipid molecule is initially modified with a β-linked glucose or galactose residue, after which further extension and modification of the glycan moiety can occur. Extension to larger glycan chains is common on ceramide-linked glucose residues, whereas further glycan extension on ceramide-linked galactose residues is more rare. Depending on their glycan core structure, glycosphingolipids are classified in 'series'. The figure depicts a number of glycosphingolipid core structures. The key features that characterize each series are shown in dashed boxes. Core structures can be further modified with sialic acids or sulfate groups, which allows subclassification of glycosphingolipids as neutral (lacking charged carbohydrates or ionic groups), sialylated or sulfated. (b) Glycosylphosphatidylinositol (GPI) anchors are found in association with certain membrane proteins and serve as linkers between the protein and the lipid membrane. Glycosylphosphatidylinositol anchors have a common core structure comprising ethanolamine-PO$_4$-6Manα1-2Manα1-6Manα1-4GlcNα1-6myo-inositol-1-PO$_4$-lipid. Differential derivatization of this common core structure through lipid remodeling and modification of the glycan moiety can cause significant glycosylphosphatidylinositol anchor heterogeneity. The protein is linked to the glycosylphosphatidylinositol anchor via an amide linkage between the C-terminal carboxyl group of the protein and the amino group of phosphatidylethanolamine (adapted from Varki et al., 2009).

situated later in this review (see 'Glycan and lectin variation at the virus level').

In line with the similarity between viral and host glycosylation, many of the basic functions covered by glycans in normal animal and human physiology and in viral infection biology – which is intrinsically linked to the biology of the host – are essentially the same. Glycans play important structural roles and are for instance implicated in protein folding and solubility, protease resistance, and masking of highly immunogenic protein stretches ('glycan shielding') (Varki *et al.*, 2009; Taylor & Drickamer, 2011). Alternatively, glycans can also have nonstructural roles and take part in specific recognition events, in which they usually interact with complementary glycan-binding proteins called lectins (Varki *et al.*, 2009; Taylor & Drickamer, 2011).

Lectins

Lectins may simply be defined as carbohydrate-binding proteins, although some definitions are more restrictive and exclude mono-/oligo-saccharide transport proteins, enzymes, glycan-specific antibodies, and even glycosaminoglycan-binding proteins (Elgavish & Shaanan, 1997; Weis, 1997; Loris, 2002; Varki *et al.*, 2009; Gabius *et al.*, 2011). According to the more strict definitions, glycosaminoglycan-binding proteins and lectins are distinguished based on different factors, including their ligand range, the structural basis of their glycan recognition, and their conservation (Varki *et al.*, 2009). In general, glycosaminoglycan-binding proteins interact with negatively charged glycosaminoglycans via clusters of positively charged aa residues and – with exception of hyaluronan-binding proteins, which seem to share an evolutionarily conserved fold – do not appear to be evolutionarily related to each other (Varki *et al.*, 2009). In contrast, most strict sense lectins belong to protein families with defined 'carbohydrate recognition domains' (CRD). The CRDs within a lectin family share structural and functional properties and selectively recognize specific portions of *N*-glycans, *O*-glycans, or glycolipids (sometimes also glycosaminoglycans) (Varki *et al.*, 2009). Although some CRDs can efficiently bind monosaccharides, other CRDs show no apparent affinity for monosaccharides and favor oligosaccharide ligands. The latter CRD type often has a preference for ligands with specific linkages between the monosaccharide subunits, as these linkages determine the 3D structure of the glycan ligand and therefore the portions of the glycan that are available for interaction with the CRD.

Interactions between a single CRD and a single mono-/oligo-saccharide (affinity) are often weak, and strong interactions are usually the result of multivalent binding, i.e. the interaction of multiple CRDs with multiple ligands (avidity). Whereas some lectins contain multiple CRDs that can participate in ligand binding, others contain only a single CRD and rely on clustering of individual lectin molecules for high avidity binding. Clustering of CRDs does not only allow stronger interactions with ligands, but also contributes to the specificity/selectivity of interactions at the multivalent level. The relative spacing of the CRDs allows highly avid, multivalent binding to specific saccharide ligands in a certain density and particular presentation. Ultimately, the avidity of lectins for specific glycoconjugates depends on the structure, multivalency, and density of glycans on these molecules (Elgavish & Shaanan, 1997; Weis, 1997; Loris, 2002; Varki *et al.*, 2009; Gabius *et al.*, 2011).
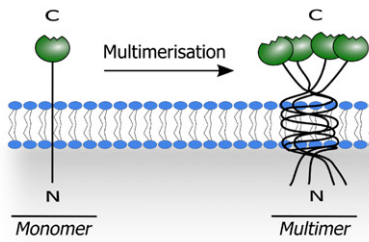
Animal lectins are typically expressed in a cell- and/or tissue-specific manner. They are involved in many different biological processes, including glycoprotein trafficking, cell adhesion and signaling, and their expression is usually tightly regulated (Varki *et al.*, 2009). Particularly striking is the great number of membrane-associated and soluble lectins that are linked with host immunity. Immune system lectins are involved in intercellular communication, positive and/or negative regulation of activation, regulation of inflammation, disposal of damaged and apoptotic cells, etc. Several of these lectins have also been identified as 'pattern recognition receptors' (PRRs), which act as molecular sensors for pathogens and endogenous stress signals and often trigger specific immune reactions/mechanisms in response to their detection (Gordon, 2002; Janeway & Medzhitov, 2002; Cambi & Figdor, 2003; McGreal *et al.*, 2004; Cambi *et al.*, 2005; McGreal *et al.*, 2005; Crocker *et al.*, 2007; Crocker & Redelinghuys, 2008; van Kooyk & Rabinovich, 2008; Garcia-Vallejo & van Kooyk, 2009; Geijtenbeek & Gringhuis, 2009; Sato *et al.*, 2009; Bottazzi *et al.*, 2010; Dam & Brewer, 2010; Kumagai & Akira, 2010; Svajger *et al.*, 2010; Davicino *et al.*, 2011; Osorio & Reis e Sousa, 2011; Sancho & Reis e Sousa, 2012). The capacity of many immune system lectins to couple glycan recognition events with specific signaling and/or effector functions gives them a key regulatory position in the immune system. Figure 3 gives a schematic overview of different types of animal lectins that are considered in this review.

Lectins play a pivotal role in different aspects of the physiology, including the immune defense against (viral) pathogens. However, it has become apparent that several viruses exploit host lectins to promote their spread. In addition, many viruses encode lectins for the recognition of and infectious entry into target cells. The following section explores how glycan–lectin interactions shape the virus-host interplay, mainly focussing on glycan–lectin interactions directly involving infectious virions.

(a) MEMBRANE-ASSOCIATED LECTINS

1. C-type lectins

2. I-type lectins
(Siglecs)

Single CTLD-domain per lectin

Multiple CTLD-domains per lectin

N

N

C

C

N

Multimerisation

Cytoplasm

N

N

C

C

Monomer

Multimer

e.g. DC-SIGN
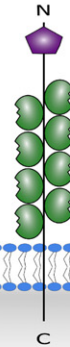
e.g. Mannose receptor
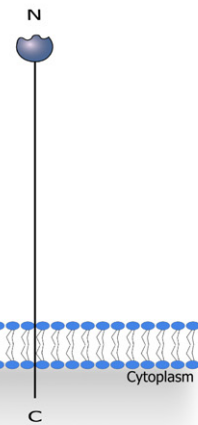
e.g. Siglec-1

(b) SOLUBLE LECTINS

1. C-type lectins
(Collectins)

C

Multimerisation

Multimerisation

N

Monomer

Multimer
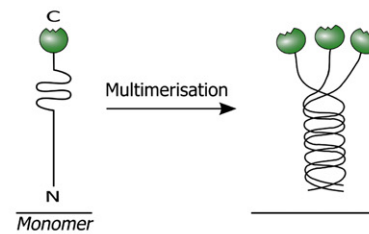
**Collectin Trimer:
e.g. CL-43**
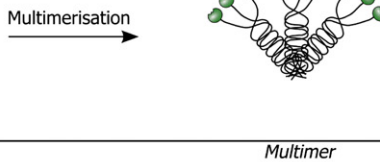
**'Bouquet' organization:
e.g. MBL**
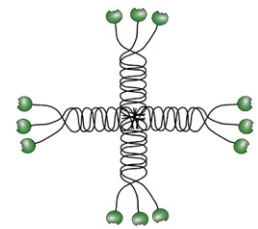
**'Cruciform' organization:
e.g. SP-D**

2. Ficolins
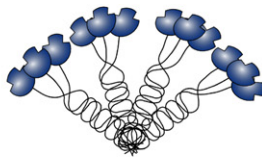
3. Galectins/S-type lectins

4. Pentraxins

e.g. L-Ficolin

e.g. Galectin-1

**e.g. Serum amyloid P
component (SAP)**

C-type lectin domain (CTLD)

I-type lectin domain

Galectin/S-type lectin
domain

R-type lectin domain

Fibrinogen-like domain

Pentraxin domain

Fig. 3. Schematic overview of different types of membrane-associated (a) and soluble (b) animal lectins that are considered in this review. The lectin domains are highlighted and listed in the key. *C-type lectin/C-type lectin domain:* Lectins are classified as C-type lectins based on their Ca^{2+}-dependency and shared primary structure. In the C-type CRD, a Ca^{2+} ion is directly involved in carbohydrate binding by making coordination bonds to both the CRD surface and key hydroxyl groups of the carbohydrate. The C-type lectin family contains both membrane-associated (a.1) and soluble (b.1) lectins. The collectins are soluble C-type lectins characterized by the presence of collagen-like domains. *R-type lectin domain:* This term refers to a CRD that is structurally similar to the CRD in ricin, a toxin found in the plant *Ricinus communis*. *I-type lectin/ I-type lectin domain:* I-type lectins are glycan-binding proteins that belong to the Ig superfamily, but are not antibodies or T-cell receptors. The 'sialic acid-binding Ig-like lectin (siglec)' family of membrane-associated lectins is currently the only well-characterized group of I-type lectins (a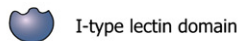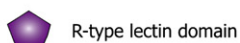.2). *Ficolin:* Ficolins (b.2) are soluble lectins characterized by the presence of collagen-like domains and fibrinogen-like globular domains with a lectin activity. *Galectin/S-type lectin (domain):* Galectins (b.3) are soluble lectins that typically bind β-galactose-containing glycoconjugates and show primary structural homology in their CRDs. Galectins were initially referred to as S-type lectins to reflect their sulfhydryl dependency, the presence of cysteine residues and their solubility; however, at present, not all identified galectins fit this initial description anymore. *Pentraxin/pentraxin domain:* Pentraxins (b.4) are characterized by the presence of pentraxin domains, which contain an eight amino acid long conserved 'pentraxin signature' (HxCxS/TWxS, where x is any amino acid) and display an L-type (Legume-type) lectin fold. SAP is a soluble lectin that requires Ca^{2+} ions for carbohydrate ligand binding (adapted from Fujita, 2002; Varki *et al.*, 2009; Bottazzi *et al.*, 2010).

Glycan–lectin interactions in virus biology

Glycan–lectin interactions that benefit the host

Membrane-associated host lectins can capture viruses for degradation

Lectins cover essential roles in the animal host's immune defense. Importantly, several membrane-associated host (immune system) lectins act as pathogen recognition molecules: they can bind pathogens and activate signaling mechanisms or capture pathogens for subsequent degradation and presentation to cells of the adaptive immune system (e.g. MHCII-restricted presentation of antigens to T cells), resulting in the induction of a pathogen-specific adaptive immune response. Alternatively, pathogens attached to such cell surface lectins may also be directly presented to neighboring immune cells *in trans*, a process that seems especially significant at sites with a high density of immune cells (e.g. the lymph nodes). Hence, binding of a viral pathogen to membrane-associated (immune system) lectins can lead to its clearance and degradation.

A prominent example is the interaction between the human immunodeficiency virus type 1 (HIV-1) and human langerin, a C-type lectin mainly expressed on Langerhans cells (de Witte *et al.*, 2007; van der Vlist & Geijtenbeek, 2010). de Witte *et al.* (2007) reported that HIV-1 interacts with langerin via high-mannose glycans on the gp120 envelope protein and is subsequently internalized into Birbeck granules, leading to virus degradation (de Witte *et al.*, 2007). Also DC-SIGN, a mannose-binding C-type lectin mainly expressed on dendritic cells (DCs), can bind and internalize HIV-1 virions for degradation and promotes MHCII-restricted as well as exogenous MHCI-restricted presentation of HIV-1 antigens

(Moris *et al.*, 2004, 2006). Similarly, various other membrane-associated host lectins can aid as PRRs in the defense against viral pathogens. However, growing evidence illustrates that many of these lectins, including DC-SIGN, are also abused by viruses to gain access to their target cells and facilitate viral spread, as is discussed further below.

Soluble host lectins can block viral infection and target virions for destruction by the immune system

In contrast to the dual role played by different membrane-associated host lectins, soluble host lectins have mainly been associated with protection against viral infection. Several soluble host lectins have been reported to aid in neutralization and clearance of various viral pathogens. Table 1 gives an overview of membrane-associated and soluble host lectins that are linked with the host's defense against different viruses. Current experimental data strongly implicate these host lectins in the defense against the listed viral pathogens and do not attribute explicit proviral effects to the host lectin – unlike for the lectin-virus pairs listed further in Table 2.

The basic principles of soluble lectin-mediated antiviral protection are most easily conveyed using some specific, well-characterized examples. For instance, various studies point out an important role of surfactant protein A (SP-A), surfactant protein D (SP-D), and mannose-binding lectin (MBL) in the defense against influenza A virus (IAV) infection.

SP-A, SP-D, and MBL are all soluble C-type lectins and share a similar basic structure: lectin monomers – consisting of an N-terminal cysteine-rich domain, a collagen-like domain, a coiled coil neck domain, and a C-terminal CRD – assemble into trimers which, under physiologic conditions, further multimerize via their N-termini to form typical cruciform- (SP-D) or bouquet- (SP-A and

Table 1. Overview of host lectins that are linked with antiviral defense

Host lectin	Implicated in defense against …
Membrane-associated lectin	
Langerin	Human immunodeficiency virus[dimt], Measles virus[1]
Soluble lectin	
Collectin-11 (CL-11)	Influenza A virus[2,3]
Collectin-43 (CL-43)	Bovine rotavirus,[4] Influenza A virus[4–6]
Collectin-46 (CL-46)	Influenza A virus[6,7]
Conglutinin	Bovine rotavirus,[4] Influenza A virus[4,6,8–12]
Ficolin A	Influenza A virus[13]
Ficolin-α	Porcine reproductive and respiratory syndrome virus[14]
H-ficolin	Influenza A virus[15]
L-ficolin	Hepatitis C virus,[16,17] Influenza A virus[13]
Galectin-1 (Gal-1)	Hendra virus,[18] Nipah virus[dimt], Parainfluenza virus type 3[18]
Mannose-binding lectin (MBL)	Dengue virus,[19,20] Hepatitis C virus,[21] Human cytomegalovirus,[22] Human immunodeficiency virus,[23–29] Influenza A virus[dimt], Marburg virus,[30] Severe acute respiratory syndrome coronavirus[31,32]
Serum amyloid P component (SAP)	Influenza A virus,[33–36] Influenza B virus,[34] Parainfluenza virus type 3[34]
Surfactant protein A (SP-A)	Herpes simplex virus,[37–39] Human coronavirus 229E,[40] Influenza A virus[dimt], Porcine reproductive and respiratory syndrome virus[41]
Surfactant protein D (SP-D)	Bovine rotavirus,[4] Human coronavirus 229E,[40] Influenza A virus[dimt], Respiratory syncytial virus,[42–44] Sendai virus[45]

Current experimental data implicate these host lectins in the defense against the listed viral pathogens and do not attribute explicit proviral effects to the host lectin.
References in Table 1 are listed in Supporting Information, Data S1.
dimt: discussed in main text.

MBL) like structures (van de Wetering *et al.*, 2004; Veldhuizen *et al.*, 2011). Despite their similar structure, these lectins show distinct glycan ligand specificities (Veldhuizen *et al.*, 2011) and also their interaction with IAV and their effects on infection and spread appear to differ. Studies using distinct IAV isolates indicate that SP-D and MBL bind mannose-rich glycans on the viral surface glycoproteins hemagglutinin and neuraminidase (a viral lectin and a viral glycosidase, involved in IAV entry and release; see discussion on viral lectins) through their CRDs (Malhotra *et al.*, 1994; Reading *et al.*, 1997; Kase *et al.*, 1999; Hartshorn *et al.*, 2000; Hillaire *et al.*, 2011). Although SP-A may interact with some IAV isolates in a similar manner (Malhotra *et al.*, 1994), binding of this molecule to most of the IAV variants tested to date appears not to involve the lectin activity of SP-A; in contrast, virus binding depends on the interaction of the viral

hemagglutinin with a sialylated *N*-glycan on the SP-A CRD (Hartshorn *et al.*, 1994; Benne *et al.*, 1995, 1997; Hartshorn *et al.*, 1997; van Eijk *et al.*, 2003; Mikerov *et al.*, 2008). In other words: SP-D and MBL binding depend on viral glycosylation, whereas SP-A binding mainly depends on the specificity of the hemagglutinin, and this is mirrored in the spectrum of IAV variants these lectins can effectively bind (Hartshorn *et al.*, 1994; Malhotra *et al.*, 1994; Benne *et al.*, 1995, 1997; Hartshorn *et al.*, 1997; Reading *et al.*, 1997; Kase *et al.*, 1999; Hartshorn *et al.*, 2000; van Eijk *et al.*, 2003; Mikerov *et al.*, 2008; Hillaire *et al.*, 2011). Interestingly, the porcine variant of SP-D appears to combine the above-mentioned IAV-binding functionalities: this molecule may not only bind IAV virions through interaction of its CRD with high mannose glycans on the virion surface (similar to other SP-D molecules), but also through interaction of a sialylated glycan on the lateral surface of its CRD with the IAV hemagglutinin (similar to SP-A) and can therefore bind to a broader array of IAV variants (van Eijk *et al.*, 2002, 2003, 2004; Hillaire *et al.*, 2011).

In vitro assays show that SP-A and SP-D can directly neutralize IAV infectivity (Benne *et al.*, 1995; Reading *et al.*, 1997; Hartshorn *et al.*, 2000; van Eijk *et al.*, 2003; Hawgood *et al.*, 2004; Hillaire *et al.*, 2011). Both SP-A and SP-D inhibit the viral hemagglutinating activity that is required for IAV attachment to target cells (Hartshorn *et al.*, 1994; Malhotra *et al.*, 1994; Benne *et al.*, 1995; Hartshorn *et al.*, 1996, 1997, 2000; van Eijk *et al.*, 2002, 2003, 2004; Mikerov *et al.*, 2008; Hillaire *et al.*, 2011) and SP-D was reported to inhibit the viral neuraminidase (Reading *et al.*, 1997; Hillaire *et al.*, 2011). Interestingly, both lectins also induce viral aggregation (Hartshorn *et al.*, 1994, 1996, 1997; van Eijk *et al.*, 2003) and function as potent opsonins. SP-A for instance was identified as an opsonin for IAV phagocytosis by alveolar macrophages (Benne *et al.*, 1997). Moreover, SP-A and SP-D were shown to enhance IAV binding to neutrophils (Hartshorn *et al.*, 1994, 1996, 1997; van Eijk *et al.*, 2003) and SP-D-IAV complexes were found to internalize upon attachment to neutrophils (Hartshorn *et al.*, 1997; van Eijk *et al.*, 2003). Pre-incubation of IAV with SP-A or SP-D also enhances the virus-induced H_2O_2 responses in neutrophils (Hartshorn *et al.*, 1994, 1996, 1997; van Eijk *et al.*, 2003) and pre-incubation of the virus with SP-D can protect neutrophils from IAV-induced deactivation (Hartshorn *et al.*, 1994, 1996, 1997). MBL can counteract IAV by roughly the same mechanisms as SP-D (Hartshorn *et al.*, 1993; Anders *et al.*, 1994; Malhotra *et al.*, 1994; Hartshorn *et al.*, 1996, 1997; Reading *et al.*, 1997; Kase *et al.*, 1999), although its ability to activate the complement cascade expands its capabilities (Anders *et al.*, 1994; Reading *et al.*, 1995; Chang *et al.*, 2010). Ligand binding by MBL (or

Table 2. Overview of host lectins that have been linked with proviral effects

Host lectin	Implicated in infection with/spread or persistence of …
Membrane-associated lectin	
Asialoglycoprotein receptor (ASGPR)	Ebola virus,[1–3] Hepatitis A virus,[4] Hepatitis B virus,[5–10] Marburg virus,[2,11] Sendai virus[12–14]
Blood dendritic cell antigen-2 (BDCA-2)	Hepatitis C virus,[15] Human immunodeficiency virus[16,17]
Dendritic cell immunoreceptor (DCIR)	Hepatitis C virus,[15] Human immunodeficiency virus[18–20]
Dendritic cell-specific intercellular adhesion molecule 3- grabbing nonintegrin (DC-SIGN)	Aura virus,[21] Dengue virus,[22–34] Ebola virus,[1–3,35–41] Feline coronavirus,[42,43] Feline immunodeficiency virus,[44] Hepatitis C virus,[41,45–53] Herpes simplex virus,[54] Human coronavirus NL-63,[55] Human cytomegalovirus,[56–59] Human herpes virus 8/Kaposi's sarcoma-associated herpes virus,[60–62] Human immunodeficiency virus*dimt*, Human T-cell lymphotropic virus,[63–65] Influenza A virus,[66–68] Junin virus,[69] La Crosse virus,[70] Lassa virus,[71] Lymphocytic choriomeningitis virus,[72] Marburg virus,[2,41,73] Measles virus,[74–79] Porcine reproductive and respiratory syndrome virus,[80] Punta Toro virus,[81] Respiratory syncytial virus,[82] Rift Valley fever virus,[70,81] Semliki Forest virus,[83] Severe acute respiratory syndrome coronavirus,[2,41,55,84–87] Severe fever with thrombocytopenia syndrome virus,[70] Simian immunodeficiency virus,[1,40,88–99] Sindbis virus,[21,100] Toscana virus,[81] Uukuniemi virus,[81] West Nile virus[32,33,101]
Liver/lymph node-specific intercellular adhesion molecule 3- grabbing nonintegrin (L-SIGN; DC-SIGN-related protein; DC-SIGNR)	Aura virus,[21] Dengue virus,[28–30,32,33] Ebola virus,[1,2,35,37–41] Hepatitis C virus,[45–52,102,103] Human coronavirus NL-63,[55] Human coronavirus 229E,[104] Human cytomegalovirus,[56] Human immunodeficiency virus,[1,88,99,105–108] Influenza A virus,[66] Junin virus,[69] Marburg virus,[2,41] Respiratory syncytial virus,[82] Semliki Forest virus,[83] Severe acute respiratory syndrome coronavirus,[2,41,55,86,109,110] Severe fever with thrombocytopenia syndrome virus,[70] Simian immunodeficiency virus,[88,90,99] Sindbis virus,[21,100] West Nile virus[32,33]
Liver/lymph node sinusoidal endothelial cell C-type lectin (LSECtin)	Ebola virus,[3,41,111,112] Lassa virus,[71] Lymphocytic choriomeningitis virus,[72] Marburg virus,[41,112] Severe acute respiratory syndrome coronavirus[41]
Macrophage Gal/GalNAc-specific C-type lectin (MGL)	Ebola virus,[113,114] Influenza A virus,[115] Marburg virus[73,113]
Mannose receptor (MR)	Dengue virus,[34] Human immunodeficiency virus,[116–123] Influenza A virus,[115,124] Visna/Maedi virus[125]
Paired immunoglobulin-like type 2 receptor alpha (PILR-α)	Herpes simplex virus,[126–132] Pseudorabies virus[129]
Siglec-1 (Sialoadhesin)	Human immunodeficiency virus,[133–135] Porcine reproductive and respiratory syndrome virus[136–139]
Siglec-4 (Myelin-associated glycoprotein; MAG)	Herpes simplex virus,[140] Varicella-zoster virus[140]
Soluble lectin	
Galectin-1 (Gal-1)	Human immunodeficiency virus*dimt*, Human T-cell lymphotropic virus[141]
Mannose-binding lectin (MBL)	Ebola virus,[36,142–144] Hendra virus,[142,144] Nipah virus,[142,144] West Nile virus[144–146]
Surfactant protein A (SP-A)	Human immunodeficiency virus,[147] Respiratory syncytial virus[148–152]
Surfactant protein D (SP-D)	Human immunodeficiency virus[153,154]

Although capture of a virus by these lectins may have certain antiviral effects or promote the specific immunity against this pathogen, current experimental data suggest that the listed viruses may also employ these lectins to promote viral infection, spread or persistence.
References in Table 2 are listed in Supporting Information, Data S2.
dimt: discussed in main text.

alternatively ficolins) can lead to activation of MBL-associated serine proteases (MASPs) and initiate the complement cascade via the so-called lectin pathway (Blue *et al.*, 2004; Bottazzi *et al.*, 2010). Complement deposition on a virus may interfere directly with crucial steps in the viral infection process (e.g. receptor binding), but can also trigger complement receptor-mediated uptake of the pathogen into immune cells. In addition, for enveloped viruses, complement activation may result in membrane attack complex (MAC) formation on the viral envelope and subsequent virolysis (Blue *et al.*, 2004; Bottazzi *et al.*, 2010).

In line with the available *in vitro* data, recent work with SP-A-, SP-D-, and MBL-knockout mice also confirmed

the antiviral potential of these soluble lectins *in vivo* (LeVine *et al.*, 2001, 2002; Zhang *et al.*, 2002; Li *et al.*, 2002; Hawgood *et al.*, 2004; LeVine *et al.*, 2004; Kingma *et al.*, 2006; Chang *et al.*, 2010). Noteworthy caveats regarding these *in vivo* studies are, however, that direct antiviral effects of these lectins can be hard to uncouple from other – e.g. immune-regulatory – effects and that mice do not represent natural hosts for IAV.

Another interesting example of antiviral activity mediated by soluble host lectins was recently reported for Nipah virus (NiV): the physiologic, homodimeric form of the soluble lectin galectin-1 can inhibit NiV envelope protein-mediated membrane fusion (Levroney *et al.*, 2005; Garner *et al.*, 2010). NiV encodes two viral membrane

glycoproteins – the attachment protein NiV-G and the fusion protein NiV-F – that mediate viral entry and direct the endothelial cell syncytia formation typically associated with NiV infection (Levroney *et al.*, 2005; Garner *et al.*, 2010; Lee & Ataman, 2011). Binding of NiV-G to cell surface receptors induces a conformational change in NiV-F, thereby activating its fusogenic activity (Levroney *et al.*, 2005; Garner *et al.*, 2010; Lee & Ataman, 2011). Galectin-1 associates with glycans on the NiV envelope proteins and interferes with the membrane fusion process in multiple ways (Levroney *et al.*, 2005; Garner *et al.*, 2010). Not only does galectin-1 binding directly inhibit the crucial conformational change in NiV-F associated with membrane fusion, it also reduces the lateral mobility of NiV-F and NiV-G in the lipid membrane and consequently counteracts the physical separation of NiV-F and NiV-G that is essential for this conformation change (Garner *et al.*, 2010). Moreover, galectin-1 binding impedes endocytosis and maturation of the NiV-F precursor NiV-F_0 in infected cells (Garner *et al.*, 2010), further illustrating the capacity of this lectin to block different stages of the viral infection/replication process.

In sum, soluble host lectins can counteract viral infection in various ways: they may directly neutralize virus by destabilizing or aggregating virions, interfere with crucial steps in the viral infection process (e.g. entry), and/or opsonize virus to facilitate uptake and degradation. Upon virus binding, some soluble lectins can trigger complement deposition on the virus, which may inhibit viral infection, enhance viral uptake via complement receptors and subsequent degradation in immune cells, and/or cause virolysis.

Considering the above, it is clear that lectins contribute significantly to the host's antiviral defense. Host lectins are involved in neutralization and clearance of free virus, immune regulation, and – although not extensively discussed in this overview – the detection and clearance of virus-infected cells. Figure 4 illustrates how membrane-associated and soluble host lectins can aid in antiviral defense.

Glycan–lectin interactions that benefit the virus
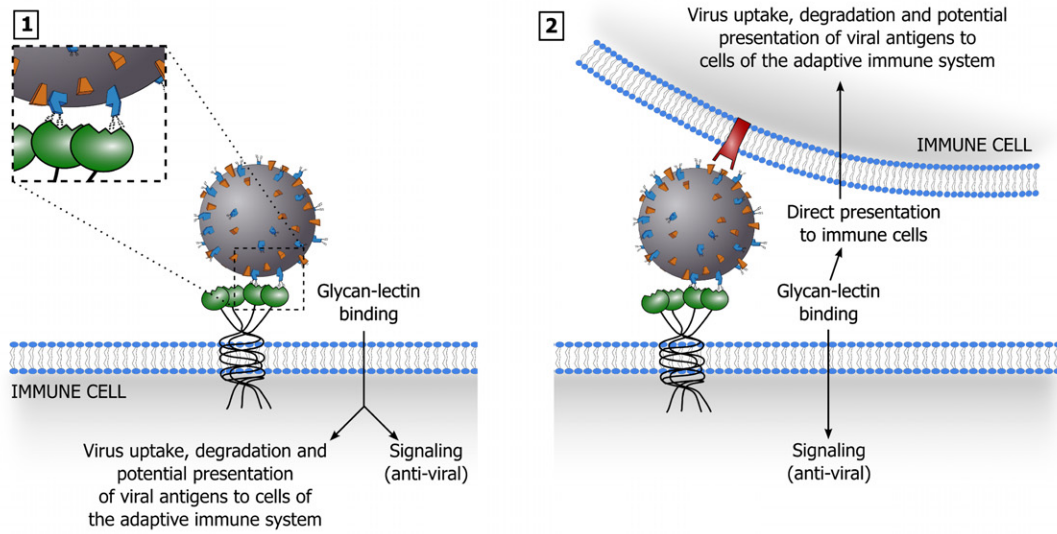
Viruses encode lectins as keys for viral binding and entry into target cells

Like their animal hosts, also viruses can benefit from interactions between glycans and glycan-binding proteins. For instance, many viruses, including HIV-1, herpes simplex virus–1, and Dengue virus, have been shown to interact with glycosaminoglycan molecules present on target cells (Patel *et al.*, 1993; Chen *et al.*, 1997; Krusat & Streckert, 1997; Summerford & Samulski, 1998; Dechecchi *et al.*, 2000, 2001; Vanderheijden *et al.*, 2001; Delputte *et al.*, 2002; Trybala *et al.*, 2002). Viral association with glycosaminoglycans is usually attributed to charge-based attractions between clusters of positively charged aa residues on the virion surface and the negatively charged glycosaminoglycan chains. The interaction with glycosaminoglycans often constitutes the first contact between a virus and its target cell and typically increases infection efficiency. However, it has been documented for several viruses that the ability to interact with glycosaminoglycans can result from adaptation to growth in cell culture (Sa-Carvalho *et al.*, 1997; Klimstra *et al.*, 1998; Hulst *et al.*, 2000; Mandl *et al.*, 2001). Clearly, use of primary virus isolates is of crucial importance when assessing the occurrence and relevance of such interactions *in vivo*.

Aside from potential glycosaminoglycan-binding capacity, several viruses are endowed with a true lectin activity: they carry virally encoded lectins on their surface and use these as keys to gain entry into their target cells (Fig. 5a). Similar to animal lectins, such virally encoded lectins often possess characteristic glycan binding regions ('glycan binding pockets') and recognize specific portions of protein- and lipid-linked glycans. The hemagglutinin protein of IAV is generally regarded as the prototype of a viral lectin. Hemagglutinin is a membrane glycoprotein that forms noncovalently linked

Fig. 4. Schematic overview of how membrane-associated (a) and soluble (b) host lectins are implicated in antiviral defense. (a.1) Binding of virion-associated glycans with membrane-associated host lectins can lead to virus uptake, degradation, and presentation of viral antigens to cells of the adaptive immune system. Binding may trigger specific signaling that promotes an effective antiviral immunity. (a.2) Binding of virion-associated glycans with membrane-associated host lectins may promote direct presentation of the virus to immune cells *in trans*. Binding may trigger specific signaling that promotes an effective antiviral immunity. (b.1) Binding of soluble host lectins to virion-associated glycans may interfere directly with viral infection by destabilizing virions, blocking interaction of the virus with its receptors or interfering with other crucial steps in the infection process (e.g. membrane fusion). Soluble host lectins may also aggregate virions, which often negatively impacts viral infectivity (not depicted). (b.2) Soluble host lectins can act as opsonins: lectin binding to virion-associated glycans may facilitate viral uptake in immune cells via lectin receptors, leading to viral degradation and potential presentation of viral antigens to cells of the adaptive immune system. Lectin binding may also trigger complement deposition on the virus (through the lectin pathway) and facilitate viral uptake via complement receptors. (b.3) Detection of virion-associated glycans by soluble host lectins may trigger complement deposition on the virus (through the lectin pathway), which may directly inhibit viral infection and/or elicit lysis of the (enveloped) virus.

(a) MEMBRANE-ASSOCIATED HOST LECTINS

1

Glycan-lectin binding

IMMUNE CELL

Virus uptake, degradation and potential presentation of viral antigens to cells of the adaptive immune system

Signaling (anti-viral)

2

Virus uptake, degradation and potential presentation of viral antigens to cells of the adaptive immune system

IMMUNE CELL

Direct presentation to immune cells

Glycan-lectin binding

Signaling (anti-viral)

(b) SOLUBLE HOST LECTINS

1 **Direct neutralization**
By destabilizing virions/blocking interaction with virus receptors or other crucial steps in infection (e.g. membrane fusion)/aggregation/...

2 **Enhanced uptake by immune cells**

Virus uptake via cellular receptors for soluble immune system lectin

Degradation and potential presentation of viral antigens to cells of the adaptive immune system

Glycan-lectin binding

MASP

Complement cascade activation

Complement deposition on virus

Virus uptake via complement receptors

Degradation and potential presentation of viral antigens to cells of the adaptive immune system

MASP

Complement cascade activation

Complement deposition on virus

Inhibition of viral infection/virus lysis

3 **Complement-mediated neutralization/virolysis**

Infectious virion	Membrane-associated immune system lectin	S-type lectin	Cellular receptor for soluble immune system lectin
Viral glycoprotein	Soluble immune system lectin (Collectin)	Immune receptor	MASP MBL-associated serine proteases

homotrimers in the (viral) membrane and is responsible for both virus attachment and penetration (Skehel & Wiley, 2000; Harrison, 2008; Gamblin & Skehel, 2010). The mature hemagglutinin protein consists of two disulfide-linked subunits, termed HA1 and HA2 (Skehel & Wiley, 2000; Harrison, 2008; Gamblin & Skehel, 2010). The HA1 subunit forms the globular 'head' region of hemagglutinin that covers the lectin function of this molecule: sialic acid-binding pockets in the membrane distal part of HA1 allow interaction with sialic acid-containing receptors on target cells (Skehel & Wiley, 2000; Harrison, 2008; Gamblin & Skehel, 2010). Variation in the HA1 subunit determines the affinity and specificity (e.g. α2-3- vs. α2-6-linked sialic acids) of this molecule (Skehel & Wiley, 2000; Gamblin & Skehel, 2010). As seen for animal lectins, also hemagglutinin binds with its glycan counterparts with a relatively low affinity and efficient virus attachment and entry depends on the interaction of multiple hemagglutinin molecules with multiple sialic acid-containing receptors (Skehel & Wiley, 2000; Gamblin & Skehel, 2010). The stalk-like HA2 subunit of hemagglutinin mediates the pH-dependent fusion process upon internalization of the IAV virion in the endosomal compartment of the target cell (Skehel & Wiley, 2000; Harrison, 2008; Gamblin & Skehel, 2010).

Similar to IAV, various other enveloped [e.g. influenza B and C viruses (Nakada et al., 1984; Herrler et al., 1985a; Rogers et al., 1986; Vlasak et al., 1987; Herrler et al., 1988; Herrler & Klenk, 1991; Herrler et al., 1991; Rosenthal et al., 1998; Lamb & Krug, 2001; Suzuki & Nei, 2002; Wang et al., 2007b; Wang et al., 2008a), mumps virus (Bowden et al., 2010; Harrison et al., 2010; Chang & Dutch, 2012)] as well as nonenveloped [e.g. murine norovirus (Taube et al., 2009, 2012), feline calicivirus (Stuart & Brown, 2007), and rhesus rotavirus (Dormitzer et al., 2002), among others (Taube et al., 2010)] viruses employ sialic acid-binding viral lectins to infect target cells. Importantly however, viral lectins with a different glycan specificity have also been identified. For instance, many viruses – including human noroviruses (Estes et al., 2006; Le Pendu et al., 2006; Cao et al., 2007; Tan & Jiang,
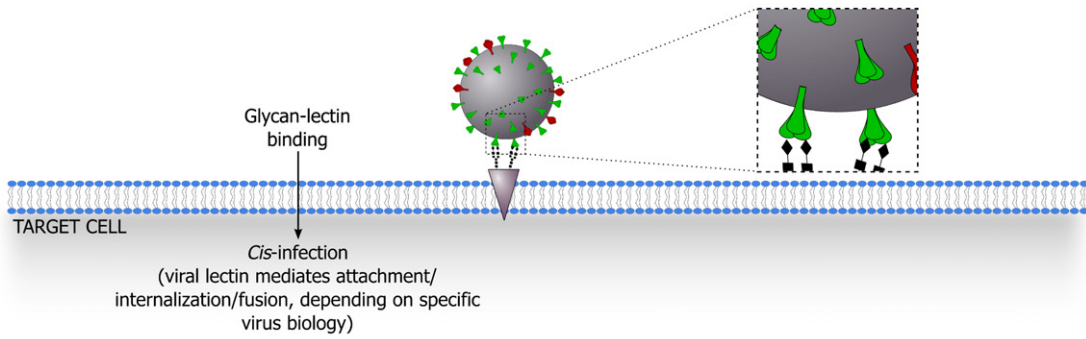
2007; Bu et al., 2008; Choi et al., 2008; Donaldson et al., 2008; Shirato, 2011), rabbit hemorrhagic disease viruses (Ruvoen-Clouet et al., 2000; Rademacher et al., 2008; Guillon et al., 2009; Nystrom et al., 2011), and the rhesus monkey Tulane virus (Farkas et al., 2010) – were found to interact with specific HBGA types.

Although a broad spectrum of viruses has evolved to use viral lectins to secure efficient target cell infection, the use of viral lectins for cellular attachment comes with a price. The glycan receptors for viral lectins are not necessarily target cell-specific and, whereas low affinity/avidity interactions may be reversible, high affinity/avidity binding of viral lectins to nontarget cell-associated glycoconjugates ('decoy receptors') can prevent the virus from efficiently targeting susceptible host cells. In line with this, it was shown for IAV that interaction of the viral lectin hemagglutinin with soluble, sialylated host glycoproteins – e.g. SP-A (cfr. supra) or α2-macroglobulin – can interfere with the viral hemagglutinating activity that is crucial for receptor binding (Rogers et al., 1983; Pritchett & Paulson, 1989; Ryan-Poirier & Kawaoka, 1991; Matrosovich et al., 1992; Ryan-Poirier & Kawaoka, 1993; Hartshorn et al., 1994; Malhotra et al., 1994; Benne et al., 1995; Gimsa et al., 1996; Benne et al., 1997; Hartshorn et al., 1997; Matrosovich et al., 1998; van Eijk et al., 2003; Mikerov et al., 2008; Chen et al., 2010; Cwach et al., 2012).
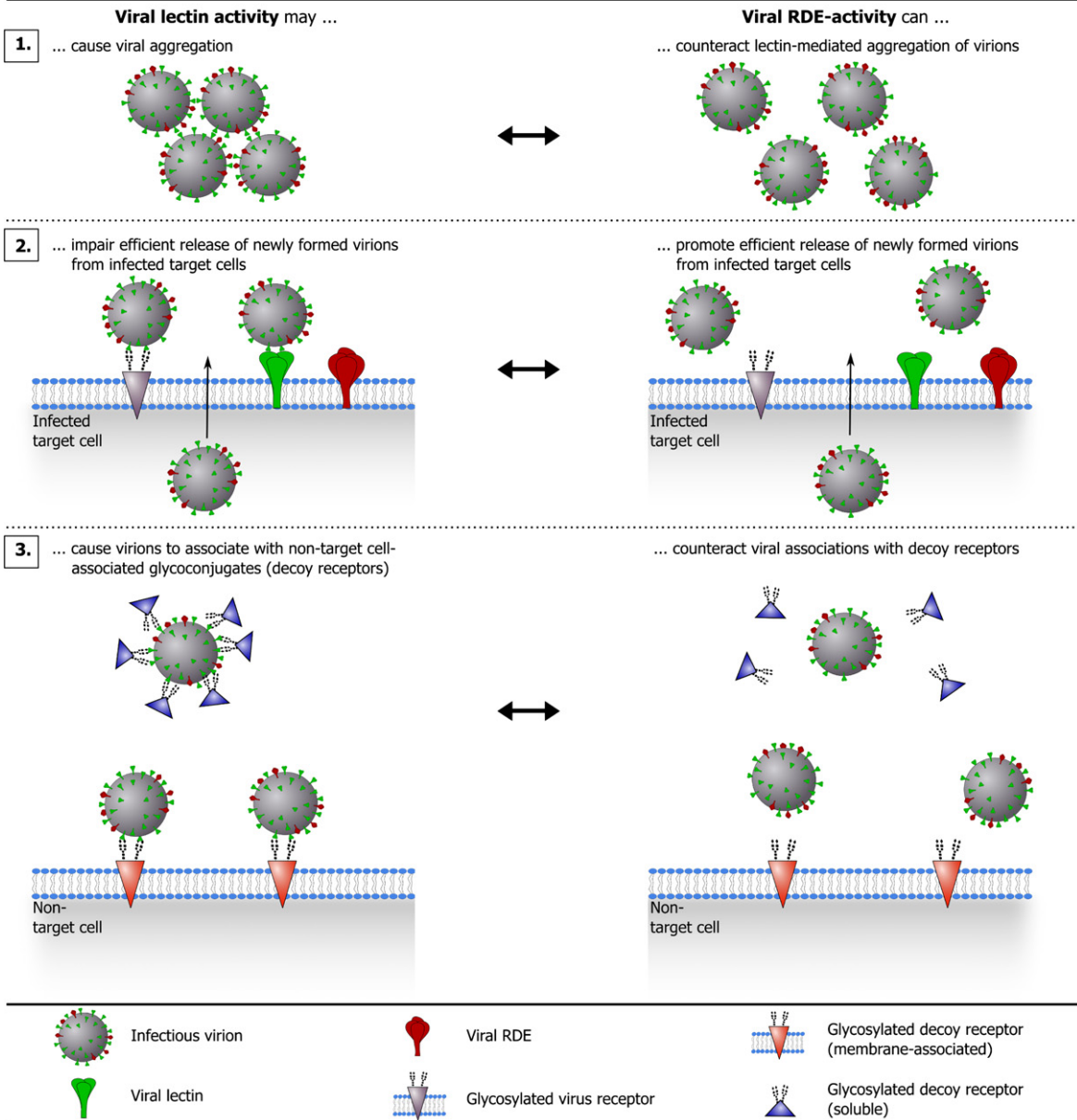
Although the presence of 'high avidity' glycan decoys invariably puts a strain on viral infection efficiency, this burden may be lighter on viruses that are equipped with a receptor destroying enzyme (RDE) that matches the specificity of the viral lectin. Intriguingly, the best-known examples in this context are again influenza viruses. As situated above, both influenza A and B viruses display hemagglutinin proteins on their surface, which bind to sialic acids displayed on the host cell surface and mediate pH-dependent fusion of the viral membrane with the host cell membrane (Skehel & Wiley, 2000; Lamb & Krug, 2001; Wang et al., 2007b; Wang et al., 2008a; Harrison, 2008; Gamblin & Skehel, 2010). An RDE activity was mapped to another viral membrane glycoprotein, designated neuraminidase (Gottschalk, 1957; Colman, 1994;

Fig. 5. (a) illustrates how viral lectins promote target cell infection. (b) shows how many viruses that employ viral lectins also benefit from a matching receptor-destroying enzyme (RDE) activity, which provides a counterweight against (high avidity) lectin activity. (a) Interaction of viral lectins with glycosylated receptors on a target cell promotes viral entry and infection (attachment/internalization/fusion, depending on specific virus biology). (b) Although they clearly benefit the virus, the use of (high avidity) viral lectins comes with a price. For instance, viral lectin activity can cause virions to aggregate (b.1) and can impair efficient release of newly formed virions from (glycosylated) infected cells (b.2). Moreover, binding of viral lectins to nontarget cell-associated glycoconjugates (decoy receptors) can prevent the virus from efficiently targeting susceptible host cells (b.3). Intriguingly, several lectin-carrying viruses are also equipped with an RDE that matches the specificity of the viral lectin and provides a counterweight against lectin-mediated glycan binding. In fact, for viruses equipped with both viral lectins and RDEs, a functional balance between these molecules appears to be an important determinant of the viral (replicative) fitness.

(a) VIRAL LECTINS

Glycan-lectin binding

TARGET CELL

Cis-infection
(viral lectin mediates attachment/
internalization/fusion, depending on specific
virus biology)

(b) VIRAL LECTINS VERSUS VIRAL RDE-ACTIVITY

Viral lectin activity may ...   **Viral RDE-activity** can ...

1. ... cause viral aggregation   ... counteract lectin-mediated aggregation of virions

2. ... impair efficient release of newly formed virions   ... promote efficient release of newly formed virions
from infected target cells   from infected target cells

Infected
target cell   Infected
                                                                     target cell

3. ... cause virions to associate with non-target cell-   ... counteract viral associations with decoy receptors
associated glycoconjugates (decoy receptors)

Non-   Non-
target cell   target cell

Infectious virion   Viral RDE   Glycosylated decoy receptor
                                              (membrane-associated)

Viral lectin   Glycosylated virus receptor   Glycosylated decoy receptor
                                                                 (soluble)

Lamb & Krug, 2001; Gamblin & Skehel, 2010). This enzyme removes sialic acid moieties from glycoproteins and glycolipids by catalyzing the hydrolysis of the α-ketosidic linkage to the subterminal sugar residue and consequently destroys potential receptors for the viral hemagglutinin (Gottschalk, 1957; Colman, 1994; Lamb & Krug, 2001; Gamblin & Skehel, 2010). Viral use of an enzyme that can actually destroy receptors for the virus may seem peculiar at first. Importantly, however, the neuraminidase activity can prevent virions from aggregating via hemagglutinin-sialic acid interactions, promotes efficient release of newly formed virions from (sialic acid-carrying) infected cells, and provides a counterweight to the interaction of hemagglutinin molecules with nontarget cell-associated glycans: neuraminidase-mediated removal of sialic acids from decoy receptors prevents virions from establishing high-avidity interactions with these glycoconjugates and may even provide an escape route for virions after hemagglutinin-mediated binding to nontarget cell-associated glycans (Colman, 1994; Suzuki et al., 1994; Gimsa et al., 1996; Barrere et al., 1997; Gamblin & Skehel, 2010). Conceivably, a functional balance between the hemagglutinin and neuraminidase activities is an important determinant of the (replicative) fitness of IAV variants in vivo. Using IAV as a paradigm, Fig. 5b illustrates how viral RDE activity can balance the high avidity of viral lectins where favorable and consequently improve viral infection efficiency.

In contrast to influenza A and B viruses, other viruses combine both lectin and RDE functions in one protein complex. For example, the viral membranes of mumps virus, Newcastle disease virus, Sendai virus, and human parainfluenza virus 3 and 5 are studded with hemagglutinin-neuraminidase proteins (Bowden et al., 2010; Harrison et al., 2010; Chang & Dutch, 2012). Another example is the influenza C virus, which carries the hemagglutination, RDE and fusion protein functions in one single envelope protein named the hemagglutinin-esterase-fusion protein (Nakada et al., 1984; Herrler et al., 1985a, b; Rogers et al., 1986; Vlasak et al., 1987; Herrler et al., 1988; Schauer et al., 1988; Herrler & Klenk, 1991; Herrler et al., 1991; Rosenthal et al., 1998; Pekosz & Lamb, 1999; Lamb & Krug, 2001; Suzuki & Nei, 2002). Whereas the RDE of influenza A and B viruses is a neuraminidase, which cleaves off entire sialic acid residues, the RDE of influenza C functions as a sialate-O-acetylesterase and cleaves off specific O-acetyl groups (Herrler et al., 1985b; Vlasak et al., 1987; Herrler et al., 1988; Schauer et al., 1988; Pekosz & Lamb, 1999). A similar situation is seen for certain coronaviruses and toroviruses that carry an accessory protein called hemagglutinin-esterase on their surface (de Groot, 2006). As the name implies, functional hemagglutinin-esterase proteins combine a hemagglutinating activity with a sialate-O-acetylesterase activity (de Groot, 2006). Interestingly, for some coronaviruses, the hemagglutinin-esterase protein is not the only envelope protein endowed with a lectin activity. For example, the spike proteins of bovine coronavirus and human coronavirus OC43 have been shown to be potent sialic acid-binding lectins (Schultze et al., 1991; Kunkel & Herrler, 1993). Also the spike protein of the transmissible gastroenteritis coronavirus of pigs has been shown to possess such a lectin activity (Schultze et al., 1996; Krempl et al., 1997, 2000; Schwegmann-Wessels et al., 2011). However, transmissible gastroenteritis coronavirus has no hemagglutinin-esterase protein that serves as RDE to counteract the hemagglutinating activity of the spike protein.

Viruses exploit membrane-associated host lectins to promote infection of target cells and avoid immune recognition

Viruses do not only benefit from virally encoded lectins, but can also use host lectins to their advantage. Paradoxically, many of the host lectins that are exploited by viruses form part of the immune system. Although capture of viral pathogens by these lectins may have certain antiviral effects or promote the specific immunity against this pathogen (cfr. supra), many viruses can also employ such interactions to promote efficient infection and spread or to facilitate persistence (Table 2). Virus binding to membrane-associated lectins can lead to concentration of virions at the cell surface and can facilitate infection of target cells. In many cases, host lectins appear to function as true portals for viral entry: the virus binds to the lectin, which drives subsequent internalization of the virus into specific cellular compartments from which the virus can initiate the next stage of infection. However, it has also been shown that lectins present on non-target cells may facilitate infection of target cells, a process called trans-infection. Many membrane-associated (immune system) lectins also participate in specific signaling pathways and engagement of such lectins by a virus may modulate both viral infection and the immune response in favor of the pathogen.

An immune system lectin that can be used as a paradigm in this context is DC-SIGN. This molecule is mainly expressed on dendritic cells (DCs), but expression on distinct other cell-types – including macrophages, B lymphocytes, platelets, and (immortalized) podocytes – has also been described (Geijtenbeek et al., 2000a, b; Soilleux et al., 2002; Granelli-Piperno et al., 2005; Gurney et al., 2005; Chaipan et al., 2006; Rappocciolo et al., 2006; Mikulak et al., 2010; Svajger et al., 2010). Prototypic DC-SIGN molecules consist of a C-terminal C-type CRD, a

neck region made up of 7 and a half 23 aa residue repeats, a transmembrane domain, and a cytoplasmic domain containing motifs involved in receptor internalization and signaling (Svajger *et al.*, 2010; Tsegaye & Pohlmann, 2010). Lectin monomers typically multimerize via their neck regions to form tetramers, which in turn organize in nanoclusters on the cell membrane (Svajger *et al.*, 2010; Tsegaye & Pohlmann, 2010; Manzo *et al.*, 2012). DC-SIGN functions as a receptor for various ligands. The T cell-expressed molecule ICAM-3 is probably its most prominent endogenous ligand: DC-SIGN binds ICAM-3 on the T cell surface and thereby contributes to the transient, nonantigen-specific interaction of DC with T cells that is required for efficient screening of MHCII-peptide complexes and eventual T cell priming (Geijtenbeek *et al.*, 2000a; Svajger *et al.*, 2010). Moreover, DC-SIGN has been implicated in various other processes, including DC differentiation, migration, and antigen capture (Svajger *et al.*, 2010). Over the last decade, it has become apparent that DC-SIGN interacts with a wide variety of viral pathogens.

A textbook example of a virus that recruits DC-SIGN is HIV-1 (Lekkerkerker *et al.*, 2006; Wu & KewalRamani, 2006; Piguet & Steinman, 2007; Tsegaye & Pohlmann, 2010; da Silva *et al.*, 2011; van der Vlist *et al.*, 2011). DC-SIGN can bind the enveloped HIV-1 particle and mainly recognizes mannose-rich glycans on the viral envelope glycoprotein gp120 (Curtis *et al.*, 1992; Geijtenbeek *et al.*, 2000a, b; Feinberg *et al.*, 2001; Hong *et al.*, 2002; Lin *et al.*, 2003; Su *et al.*, 2004; Hong *et al.*, 2007). The efficiency of HIV-1 capture by DC-SIGN has been linked to receptor density. Experiments in 293 T-Rex cells using an inducible DC-SIGN expression system have shown that high surface expression levels of DC-SIGN correlate with optimal binding of HIV-1 particles, and that lowering the DC-SIGN expression levels can significantly reduce the efficiency of HIV-1 binding (Pohlmann *et al.*, 2001). These data suggest that the high DC-SIGN surface expression levels on certain (immature) DC subsets are compatible with optimal capture of HIV-1 virions, whereas lower DC-SIGN expression levels on B lymphocytes and especially platelets may be mirrored in a less efficient HIV-1 capture (Baribaud *et al.*, 2002; Boukour *et al.*, 2006; Chaipan *et al.*, 2006; Rappocciolo *et al.*, 2006). Nevertheless, studies have shown that also B lymphocytes and platelets can effectively bind HIV-1 virions via DC-SIGN (Boukour *et al.*, 2006; Chaipan *et al.*, 2006; Rappocciolo *et al.*, 2006). Evidently, the interaction between HIV-1 and DC-SIGN is also critically dependent on the viral glycome. Recent research has shown that virion-associated gp120 of peripheral blood mononuclear cell (PBMC)-grown virus predominantly carries oligomannose *N*-glycans (Doores *et al.*, 2010; Bonomelli *et al.*, 2011),

which constitute optimal ligands for DC-SIGN (van Liempt *et al.*, 2006). Nevertheless, virus originating from different host cell types can display a different glycosylation profile, and this may modulate the efficiency of DC-SIGN recruitment (Lin *et al.*, 2003).

Binding of HIV-1 to DC-SIGN can entail both negative and positive effects for the virus. In DC-SIGN-expressing antigen-presenting cells, most of the DC-SIGN-captured virions appear to be internalized into the endolysosomal pathway and rapidly degraded (Moris *et al.*, 2004; Turville *et al.*, 2004). In line with this, DC-SIGN was found to promote MHCII-restricted presentation of HIV-1 antigens (Moris *et al.*, 2006). Intriguingly however, in cells that co-express CD4 and CCR5/CXCR4 (HIV-1 receptor and co-receptors, respectively), DC-SIGN expression also facilitates HIV-1 fusion: DC-SIGN efficiently captures and concentrates viral particles at the cell surface, and binding of this lectin to the HIV-1 envelope protein appears to increase exposure of the CD4 binding site (Lee *et al.*, 2001; Nobile *et al.*, 2005; Burleigh *et al.*, 2006; Hijazi *et al.*, 2011). Although DC-SIGN-mediated enhancement of HIV-1 fusion may promote MHCI-restricted presentation of HIV-1 antigen (proteasome and TAP-dependent pathway) and activation of cytotoxic T lymphocytes (Moris *et al.*, 2004), enhanced HIV fusion inevitably leads to more efficient infection. Indeed, several studies confirm that DC-SIGN facilitates productive (*cis*-) infection in DC-SIGN-expressing cells that also co-express CD4 and CCR5/CXCR4 (Lee *et al.*, 2001; Nobile *et al.*, 2005; Burleigh *et al.*, 2006; Hijazi *et al.*, 2011).

DC-SIGN has also been implicated in HIV-1 *trans*-infection. An initial study showed that DC-SIGN can efficiently capture HIV-1 particles and transfer them to adjacent target T cells, without the need for productive infection of the DC-SIGN-expressing cell (Geijtenbeek *et al.*, 2000b). Subsequent studies on the subject reported DC-SIGN-mediated internalization of infectious HIV-1 virions into low pH nonlysosomal compartments, and advocated that the virus traffics in intracellular compartments towards the zone of T cell contact, where it is released into the infectious synapse (i.e. the contact zone between the virus-loaded cell and the target T cell) (Kwon *et al.*, 2002; McDonald *et al.*, 2003). DC-captured HIV-1 virions as well as T-cell-expressed CD4 and CCR5/CXCR4 were found to concentrate at the DC-T-cell interface, rendering it an ideal micro-environment for efficient infection of target T cells (McDonald *et al.*, 2003). Moreover, it was postulated that DC-SIGN-mediated capture of HIV-1 virions temporarily protects them from degradation and preserves viral infectivity (Geijtenbeek *et al.*, 2000b; Kwon *et al.*, 2002).

The above data supporting DC-SIGN-mediated capture, uptake, intracellular transport and ultimately transfer of intact HIV-1 particles from DCs to target T cells were united in the 'Trojan horse model' of mucosal HIV-1 transmission. This model posits that submucosal DCs capture and internalize HIV-1 virions via DC-SIGN and, by homing to the lymph nodes, provide a means of transport for the virus to a compartment rich in target cells. The virus-loaded DCs then interact with CD4^{+} T cells and the virions are transferred to the target T cell via the infectious synapse, ultimately resulting in efficient target cell infection (Geijtenbeek et al., 2000b; Baribaud et al., 2001; Sewell & Price, 2001). However, recent research is not always in line with this initial model and has challenged several of its key features. Cavrois et al. (2007) reported that HIV-1 trans-infection does not require intracellular virus trafficking, but primarily depends on cell surface-associated virions that reach the infectious synapses via transport on the cell surface (Cavrois et al., 2007). In line with this, Yu et al. (2008) reported that HIV-1 traffics towards the infectious synapse through a specialized, surface-accessible intracellular compartment (Yu et al., 2008). In addition, reports stating that virus capture by DC-SIGN mainly leads to virus internalization into the endolysosomal pathway and subsequent degradation (Moris et al., 2004; Turville et al., 2004; Moris et al., 2006) and that DC-SIGN-mediated trans-infection can only occur within the first hours after virus attachment (Turville et al., 2004) seem to downplay the importance of DC-SIGN-mediated trans-infection for efficient HIV-1 infection and spread. These and other data counter the theory that HIV-1 capture by DCs preserves viral infectivity, and suggest that the presence – and transfer – of infectious virus at later time points may be ascribed to productive DC infection (Turville et al., 2004; Nobile et al., 2005; Burleigh et al., 2006; Wang et al., 2007a). It is also noteworthy that, whereas initial studies identified DC-SIGN as the main factor involved in HIV-1 capture and transmission by DCs (Geijtenbeek et al., 2000b), recent studies also implicate other lectins in this process (Turville et al., 2002; Izquierdo-Useros et al., 2012) or even conclude that DC-SIGN is not involved in DC-mediated HIV-1 trans-infection (Boggiano et al., 2007). Differences in virus strains, cell types, and experimental setup might in part explain these conflicting data. Intriguingly, other recent work indicates that DC-SIGN-expressing B lymphocytes and platelets may effectively capture and transfer infectious HIV-1 via DC-SIGN (Boukour et al., 2006; Chaipan et al., 2006; Rappocciolo et al., 2006), potentially implicating these cells/cell fragments in HIV-1 dissemination in infected patients, although recent work suggests that platelets might negatively regulate viral spread by secretion of CXCL4 (Auerbach et al., 2012; Tsegaye et al., 2013). Clearly, further research is necessary to allow a better understanding of DC-SIGN-mediated HIV-1 trans-infection and its relevance for viral infection and spread in vivo.

Importantly, the role of DC-SIGN in HIV-1 infection appears not to be restricted to purely physical capture of virions for subsequent degradation or cis- or trans-infection. Recruitment of DC-SIGN by HIV-1 also triggers signal transduction that modulates immune responses and infection of DCs and adjacent target cells more indirectly. For example, Hodges et al. (2007) reported that binding of HIV-1 to DC-SIGN compromises DC maturation and primes these cells for trans-infection: Upregulation of CD86 and MHCII is suppressed, whereas synapse formation between DCs and CD4^{+} T cells is promoted (Hodges et al., 2007). Moreover, HIV-1 binding to DC-SIGN was shown to activate Cdc42 and promote formation of membrane extensions that facilitate HIV-1 transfer to CD4^{+} lymphocytes (Nikolic et al., 2011). Other work by Gringhuis et al. (2007, 2010) showed that binding of HIV-1 to DC-SIGN triggers Raf-1 dependent signaling, which modulates toll-like receptor (TLR)-elicited signals to induce synthesis of full-length HIV transcripts as well as production of the immunosuppressive cytokine IL-10 (Gringhuis et al., (2007, 2010). In general, the data discussed above suggest that DC-SIGN recruitment by HIV-1 might affect viral infection and transmission, as well as the host defense against this pathogen in several ways.

Over the last decade, DC-SIGN has become a prototype for lectin-mediated cis- and trans-infection and has been implicated in the infection process of various viruses, including HIV, Dengue virus, Ebola virus, and IAV (see Table 2). Importantly, however, DC-SIGN is not the only host lectin that is (ab)used by viruses to promote target cell infection or avoid immune recognition and clearance. Various other membrane-associated host lectins seem to be exploited by viruses – in ways similar to DC-SIGN – to aid cis-infection, trans-infection and/or viral persistence. Analysis of recent literature suggests that membrane-associated host lectins may constitute weak links in the host's defense against viral pathogens (Table 2).

Soluble host lectins can promote viral infection

Although generally implicated in antiviral defense, soluble host lectins may also support viral infection (Table 2). For example, galectin-1 has been proposed to promote HIV-1 infection (Ouellet et al., 2005; Mercier et al., 2008; St-Pierre et al., 2011; Sato et al., 2012). In vitro experiments pointed out that galectin-1 can enhance HIV-1 infection of different cell types – including human

lymphoid cell lines, PBMC, CD4^{+} T lymphocytes, and monocyte-derived macrophages (Ouellet *et al.*, 2005; Mercier *et al.*, 2008) – and increase HIV-1 infection in an *ex vivo* lymphoid tissue model (Ouellet *et al.*, 2005). Further experiments showed that galectin-1 accelerates virion binding to the target cell surface, probably by crosslinking viral and cellular glycans (Ouellet *et al.*, 2005; Mercier *et al.*, 2008). A more recent study confirmed these findings and showed that galectin-1 binds to clusters of *N*-linked glycans on the viral gp120 envelope protein in a β-galactoside-dependent manner (St-Pierre *et al.*, 2011). Data from the same study identify the HIV-1 receptor CD4 as a ligand for galectin-1 and suggest that galectin-1 can cross-link gp120 and CD4 (St-Pierre *et al.*, 2011). In sum, it appears that the dimeric lectin galectin-1 can enhance HIV-1 infection efficiency by cross-linking viral and host cell glycans and thereby promoting firmer adhesion of the virus to the target cell surface and facilitating virus-receptor interactions (Ouellet *et al.*, 2005; Mercier *et al.*, 2008; St-Pierre *et al.*, 2011; Sato *et al.*, 2012).

Some studies have also attributed proviral effects to the collectins MBL, SP-A, and SP-D. For some viruses, it was reported that – under certain conditions – viral recognition by collectins may enhance *cis*- or *trans*-infection (Hickling *et al.*, 2000; Sano *et al.*, 2003; Gaiha *et al.*, 2008; Brudner *et al.*, 2013; Madsen *et al.*, 2013). It is conceivable that these collectins can bind the virus and subsequently associate with collectin receptors on the surface of target/transmitting cells, thereby concentrating virions at the cell surface and facilitating infection or viral transfer. Nevertheless, involvement of other mechanisms (e.g. collectin-mediated cross-linking of virus- and host cell-displayed glycans, cfr. the galectin-1-HIV-1 example described above) can currently not be excluded. Further research is necessary to elucidate the biology behind the potential proviral effects of collectins and to estimate the occurrence and relevance of these events in an *in vivo* context.

Figure 6 gives a schematic overview of how membrane-associated and soluble host lectins can be implicated in interactions that benefit the virus and facilitate viral infection and spread.

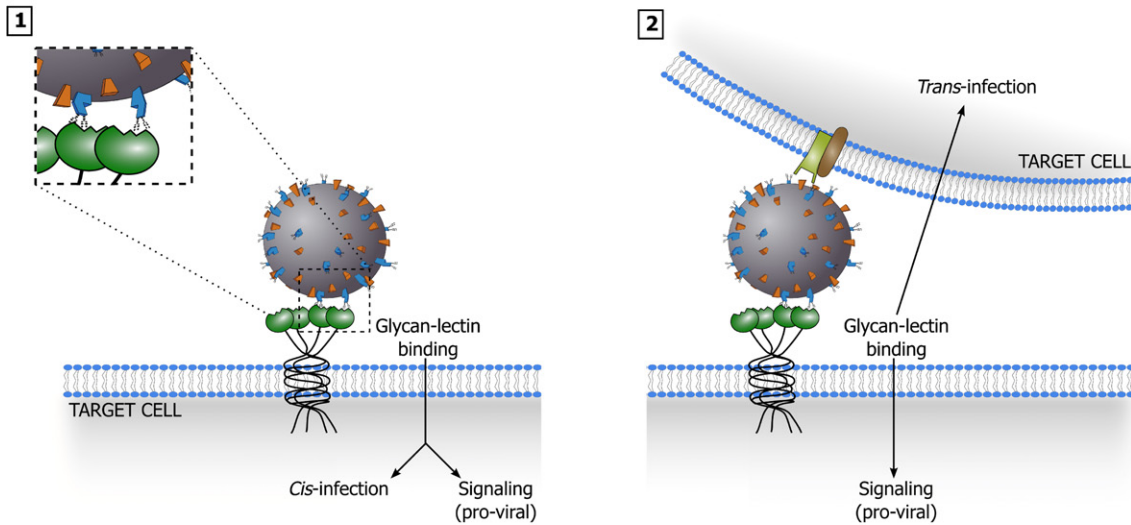## Glycan and lectin: the variable parameters in a biological equation

As glycan–lectin interactions often represent key events in viral infection and/or antiviral immunity, variation in glycan or lectin expression and structure – either at the host or at the virus level – may significantly shift the balance between host and pathogen. A basic insight into the nature and origin of this variability is therefore germane to a proper understanding of glycan–lectin interactions in the context of viral infection biology and immunology.

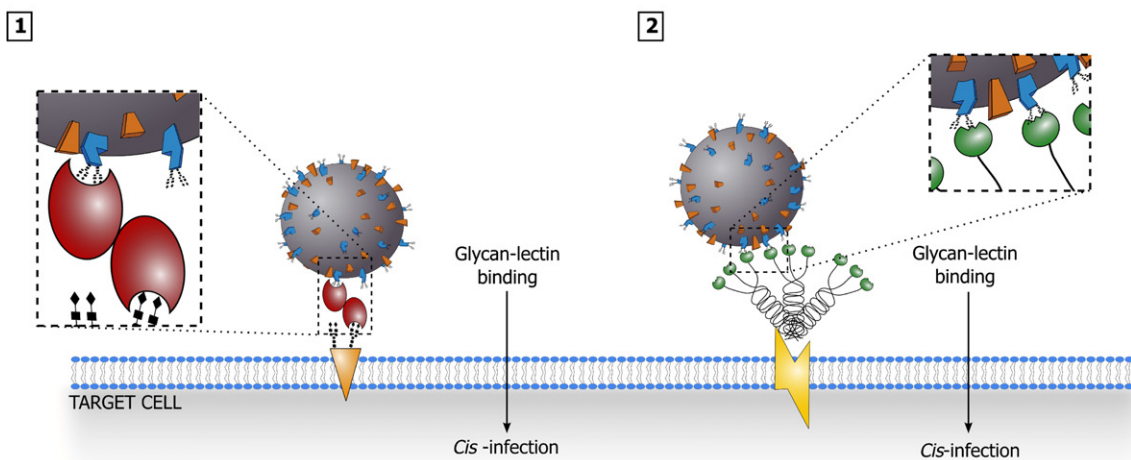## Glycan and lectin variation at the host level

Glycan formation is a very complex and versatile biosynthetic process. In contrast to the primary amino acid sequences of proteins, glycan structures are not directly encoded in the host genome. Instead, they are synthesized in a step-wise manner via the concerted action of various host-encoded glycosyltransferase, glycosidase, and other enzymes. The availability of these glycoenzymes, the availability of precursor molecules and the accessibility of specific glycosylation sites govern (the efficiency of) glycan addition and modification and hence determine glycan variability. The genetic make-up of the host evidently has a major impact on glycosylation, but also other host-related factors can have pronounced effects. Recent research has shown that different cell types within a host can assemble radically different glycomes (Roth, 1996; Haslam *et al.*, 2008) and that factors such as the activation (Comelli *et al.*, 2006; Bax *et al.*, 2007; Haslam *et al.*, 2008) or infection (Lanteri *et al.*, 2003) status of a cell can significantly influence glycosylation processes. Clearly, various biological factors contribute to the high glycan heterogeneity that is seen for many animal glycoconjugates.

Although host glycan variation may influence virtually all viral infections in several ways, its potential impact is probably most evident for viruses that are equipped with viral lectins. A notable example in this context are the noroviruses, a major cause of nonbacterial gastroenteritis in humans. It is well known that the viral capsids of most human noroviruses display an affinity for HBGAs, structurally related but highly polymorphic carbohydrate structures found on proteins and lipids of epithelial cells in the gastrointestinal and respiratory tract, on the surfaces of red blood cells and as free antigens in body fluids such as saliva, blood, and intestinal contents (Bu *et al.*, 2008; Choi *et al.*, 2008; Shirato, 2011). Different noroviruses display distinct HBGA specificities and can be categorized according to the (range of) HBGA structures they preferentially bind (Huang *et al.*, 2005; Shirato, 2011). Human HBGA synthesis is controlled by various enzymes, including the glycosyltransferase enzymes encoded in the *ABO*, *FUT2*, and *FUT3* gene loci. The presence of variant (functional or nonfunctional) alleles at these and other relevant gene loci is a key determinant of HBGA phenotype, as it controls which ABH and Lewis antigens an individual can synthesize (Le Pendu *et al.*, 2006; Shirato, 2011). Although it is still unclear whether they function as primary receptors for noroviruses, current data indicate that HBGAs are important determinants of the noroviral tissue specificity. Moreover, several studies have established a link between HBGA geno-/phenotype and individual susceptibility to (clinical)

(a) MEMBRANE-ASSOCIATED HOST LECTINS

1

Glycan-lectin binding

TARGET CELL

Cis-infection   Signaling (pro-viral)

2

Trans-infection

TARGET CELL

Glycan-lectin binding

Signaling (pro-viral)

(b) SOLUBLE HOST LECTINS

1

Glycan-lectin binding

TARGET CELL

Cis-infection

2

Glycan-lectin binding

Cis-infection

Infectious virion	Membrane-associated immune system lectin	S-type lectin	Glycosylated membrane protein
Viral glycoprotein	Soluble immune system lectin (Collectin)	Virus (co-)receptors	Cellular receptor for soluble immune system lectin

Fig. 6. Schematic overview of how membrane-associated (a) and soluble (b) host lectins can be implicated in interactions that benefit the virus and facilitate viral infection and spread. (a.1) Binding of virion-associated glycans to membrane-associated host lectins can promote (*cis*-) infection of the lectin-expressing cell: host lectins may facilitate viral attachment, internalization, and fusion (depending on specific virus biology). Viral attachment to membrane-associated host lectins may trigger signaling mechanisms that facilitate viral infection, spread, and/or immune evasion. (a.2) Binding of virion-associated glycans to membrane-associated host lectins can promote presentation of the virus to susceptible target cells *in trans*, thereby facilitating target cell infection. Viral attachment to membrane-associated host lectins may trigger signaling mechanisms that facilitate viral infection, spread, and/or immune evasion. (b.1) Multivalent soluble host lectins may facilitate virus attachment and promote viral infection by crosslinking virus- and host cell-displayed glycans. (b.2) Virus recognition by soluble host lectins and subsequent association with target cell-expressed lectin receptors may promote *cis*-infection of target cells. In a similar manner, soluble host lectins may capture and concentrate virions on a cell surface for subsequent presentation to target cells *in trans* (not depicted). Moreover, lectin binding can trigger complement deposition on the virus (through the lectin pathway), which may potentially promote *cis*- or *trans*-infection via cell surface-expressed complement receptors (not depicted).

infection with specific norovirus variants: HBGA phenotypes matching the specificity of the viral lectin correlate with a higher risk of (clinical) infection, whereas non-matching HBGA phenotypes correlate with relative resistance (Le Pendu et al., 2006; Shirato, 2011).

Several studies have revealed significant heterogeneity relating to animal lectins. Lectin expression is governed by various genetic and nongenetic (e.g. hormone balance, immune status) factors. Importantly, gene polymorphisms that affect protein expression and/or functionality have been described for several animal lectins, including MBL and DC-SIGN.

For MBL, mutations in the promoter region of the MBL2 gene were found to affect protein expression levels, probably by influencing binding of transcription factors (Eisen & Minchinton, 2003; Dommett et al., 2006; Heitzeneder et al., 2012). Moreover, specific polymorphisms in MBL2 exon 1, encoding the collagen-like domain of MBL, appear to hinder correct and stable oligomerization of MBL protein chains and impede efficient ligand binding and activation of the lectin complement pathway (Eisen & Minchinton, 2003; Dommett et al., 2006; Heitzeneder et al., 2012). Several studies suggest a correlation between MBL deficiency and susceptibility to HIV infection, but conflicting data have been reported and further research is clearly necessary to corroborate this link (Eisen & Minchinton, 2003; Dommett et al., 2006; Heitzeneder et al., 2012).

Similar findings have been recorded for DC-SIGN. Polymorphisms in the promoter region of the DC-SIGN-encoding CD209 gene can affect protein expression levels and have been linked with altered susceptibility to and/or altered disease progression after infection with several viral pathogens, including HIV-1 and Dengue virus (Martin et al., 2004; Sakuntabhai et al., 2005; Koizumi et al., 2007; Selvaraj et al., 2009; Wang et al., 2011; Boily-Larouche et al., 2012). Moreover, distinct gene polymorphisms in the DC-SIGN-encoding region as well as alternative splicing events give rise to different isoforms of the protein, ranging from variants containing single nucleotide polymorphisms (SNPs) to variants with truncated lectin domains, variable numbers of 23-aa-residue repeats in the neck domain, alternative cytoplasmic domains or a lacking transmembrane region (Mummidi et al., 2001; Liu et al., 2004; Serrano-Gomez et al., 2008; Boily-Larouche et al., 2012). Information on the expression and biological activity of most of these DC-SIGN variants is rather limited. Recently, however, Boily-Larouche et al. (2012) reported that naturally occurring genetic variants of DC-SIGN, carrying specific SNPs in the neck domain-encoding exon 4, have an enhanced capacity to capture and transfer HIV-1 virions to CD4+ T lymphocytes. Moreover, Liu et al. (2004) described differ-

ent neck domain length variants of DC-SIGN – carrying variable numbers of 23-aa-residue repeats in the neck region – and correlated neck domain length heterozygosity with a reduced risk of HIV-1 infection. Recent experimental data provide evidence that naturally occurring DC-SIGN neck domain variants can differ in multimerization competence in the cell membrane and display altered glycan binding capacity (Serrano-Gomez et al., 2008). Moreover, the presence of such neck domain variants appears to modulate multimerization of the prototypic DC-SIGN molecule (Serrano-Gomez et al., 2008). The fact that neck domain variation may influence the presence, stability, and functionality of DC-SIGN multimers on the cell surface can provide a molecular explanation for the link between DC-SIGN polymorphisms and susceptibility to HIV-1 and other pathogens, although further research is needed to substantiate this (Serrano-Gomez et al., 2008).

Glycan and lectin variation at the virus level

Although viruses rely on the host cell machinery for glyco-conjugate synthesis, viral glycosylation profiles can significantly differ from the standard glycosylation profile of their host cell. For instance, it is well known that viral glycoproteins are often more heavily glycosylated than host glycoproteins, and that also the nature of their glycan modifications can significantly differ. A prototypic example in this context is the gp120 glycoprotein of HIV-1. The HIV-1 envelope is studded with trimers of noncovalently associated gp120/gp41 heterodimers (White et al., 2010). Gp120 is one of the most heavily N-glycosylated proteins in nature: it contains more than 20 N-linked glycosylation sites, and N-glycans account for about half of its molecular weight (Zhu et al., 2000; Wei et al., 2003; Pantophlet & Burton, 2006; Scanlan et al., 2007). Intriguingly, whereas mammalian glycoproteins typically carry mainly complex type N-glycans, this is not the case for the viral gp120 glycoprotein. Recent reasearch has shown that virion-associated gp120 of PBMC-grown virus – as opposed to recombinantly expressed monomeric gp120 – predominantly carries oligomannose N-glycans (Doores et al., 2010; Bonomelli et al., 2011). The synthesis of this unusual glycosylation profile appears to be partially directed by the structure of the gp120/gp41 spike itself: the presence of a dense N-glycan cluster in gp120, combined with the steric consequences of gp120/gp41 trimerization, seems to hinder further processing of (normally transient) biosynthetic glycan intermediates by ER and Golgi α-mannosidases, ultimately yielding HIV-1 virions with oligomannose-enriched gp120 glycoproteins (Zhu et al., 2000; Doores et al., 2010; Eggink et al., 2010; Bonomelli et al., 2011). Clearly, although viral glycosylation is

critically dependent on the glycosylation machinery of the host cell, the genetic and structural background of a virus can have a decisive influence in this process.

Importantly, viral infection itself may also have strong repercussions on the glycosylation biology of a host cell. Considering the restricted glycosylation enzyme and precursor availabilities, it is conceivable that overexpression of viral glycoproteins in an infected target cell can result in an increased glycan heterogeneity of both viral and cellular glycoconjugates. Moreover, viruses may also actively modify the host and viral glycome by modulating the expression of host cell glycoenzymes (Hiraiwa et al., 1997; Cebulla et al., 2000; Hiraiwa et al., 2003) or via expression of virally encoded glycoenzymes in infected cells (Jackson et al., 1999; Willer et al., 1999; Nash et al., 2000; Sujino et al., 2000; Vanderplasschen et al., 2000; Markine-Goriaynoff et al., 2003, 2004a, b).

An additional source of glycan variation can be discerned for viruses that can infect multiple cell types, or even different host species. For instance, it is well known that HIV can productively infect multiple cell types, and that HIV glycosylation is cell type-dependent (Liedtke et al., 1994; Willey et al., 1996; Liedtke et al., 1997; Lin et al., 2003). Cell type-dependent glycosylation differences for HIV have been shown to impact viral interaction with and trans-infection via DC-SIGN (Lin et al., 2003), as well as viral sensitivity to antibody neutralization (Willey et al., 1996). Another, particularly fascinating example in this context is Dengue virus (DV). DV is a mosquito-borne flavivirus that can replicate in mosquitos as well as in humans (Navarro-Sanchez et al., 2003; Dejnirattisai et al., 2011). In humans, immature skin DCs are considered the primary target cell for the virus after a mosquito bite, and DC-SIGN is believed to be the main DV receptor on these cells (Navarro-Sanchez et al., 2003; Dejnirattisai et al., 2011). In a recent study, it was shown that insect cell-derived DV can efficiently infect DCs, whereas DC-derived DV is not able to reinfect DCs (Dejnirattisai et al., 2011). Similarly, insect cell-derived DV could efficiently bind and infect a DC-SIGN-expressing cell line, whereas this was not the case for DC-derived DV (Dejnirattisai et al., 2011). Finally, it was found that insect cell-derived DV predominantly contains high-/pauci-mannose-type N-glycans, whereas DC-derived virus contains only complex type N-glycans (Dejnirattisai et al., 2011). Projected against the background of DV infection, these data outline a tentative model of the first stages of DV infection in humans: during DV replication in mosquito cells, newly formed DV virions obtain mannose-rich glycans. Upon viral transfer to a human host, these virions efficiently infect immature skin DCs via DC-SIGN. Importantly, DV replication in skin DCs yields virions with complex type N-glycans,

thus creating a 'glycan mismatch' with DC-SIGN. Due to this mismatch, newly synthesized DC-derived virus will not readily reinfect DCs via DC-SIGN, but preferentially infect other potential host cells via other receptors (Dejnirattisai et al., 2011). Although much more research is needed to verify this tentative model, it elegantly illustrates how cell type-dependent glycan variability may impact a viral infection process.

Another notable factor to be considered in the context of virus-related variability is the rapid evolution of many viral pathogens. This seems especially significant for RNA viruses, as these viruses generally evolve more rapidly than DNA viruses due to factors inherent to their biology and infection strategy (Belshaw et al., 2008; Holmes, 2009; Lauring & Andino, 2010). The higher mutation frequency of many RNA viruses directly implies a higher chance for addition or deletion of putative glycosylation sites. As has been shown for IAV, acquisition or deletion of glycosylation sites may affect crucial steps in the viral infection/replication process (e.g. receptor binding, fusion, release of newly formed virions) (Ohuchi et al., 1997; Wagner et al., 2000; Tsuchiya et al., 2002; Kim & Park, 2012), alter the capacity of the virus to avoid induction of/recognition by virus-specific antibodies (glycan shielding) (Wang et al., 2009; Wei et al., 2010; Wanzeck et al., 2011; Kim & Park, 2012; Job et al., 2013; Sun et al., 2013), and modulate viral interaction with various immune system lectins (Reading et al., 2007; Vigerust et al., 2007; Reading et al., 2009; Tate et al., 2011a, b). Clearly, mutational changes in the viral glycome may affect the virus-host interactome in various ways. Ultimately, the net benefit of a specific glycome change will determine if a glycosylation variant may become dominant in the virus population.

However, not only the viral glycosylation status, but also the affinity and specificity of viral lectins for specific glycoconjugates may change as a result of mutations. For instance, ample data show that amino acid changes at specific sites of the IAV hemagglutinin protein can significantly alter its affinity and/or specificity for particular sialic acid-containing receptors – a factor that is crucial for the virus to infect new host species (Skehel & Wiley, 2000; Wagner et al., 2002; Suzuki, 2005; Gamblin & Skehel, 2010). Finally, functional alterations in the viral RDE due to mutations may also have a strong impact on the interaction of viral lectins with host cell glycans. In the case of IAV, balanced lectin and RDE functions appear to be crucial for efficient viral replication. For IAV variants that are well adapted to a certain host species, the substrate specificity and activity of the neuraminidase generally match the ligand specificity and affinity of the hemagglutinin (Wagner et al., 2002). Disruption of this balance – for instance due to reassortment or transmission to a new host species – often results in a decreased

replicative fitness (Wagner *et al.*, 2002). Interestingly, however, the virus may overcome this hurdle and evolve towards replicative competence by selecting for compensatory mutations in hemagglutinin and/or neuraminidase that restore the functional balance between these molecules (Wagner *et al.*, 2002). Considering these data on IAV, it is conceivable that the balance between viral lectin and RDE is also an important determinant of the replicative fitness of several other viruses.

On a related note, glycan–lectin interactions in virus biology are typically studied using a limited number of (prototypic) virus variants. Although the information obtained in these studies can often be extrapolated to include other virus variants, there are important exceptions. For IAV, for instance, it is well documented that different virus variants can carry hemagglutinin lectins with distinct glycan ligand specificities and therefore associate with distinct spectra of (decoy) receptors. Moreover, IAV variants can display different glycan arrays on their surface, which has been shown to modulate viral infection, glycan shielding, and recognition by various immune system lectins (cfr. supra). The fact that the specific genetic make-up of a virus determines specificity/ affinity of viral lectins, co-directs viral glycosylation, etc., and that this can be mirrored in a distinct virus-host interactome remains an important issue in glycovirology.

In sum, an intricate web of glycan–lectin interactions can modulate viral infection, and host and virus inherent variability in glycans and lectins adds a further layer of complexity to this matter.

Targeting glycan-lectin interactions in antiviral strategies

Considering the pivotal roles of glycan–lectin interactions in many viral infections, interfering with these interactions seems an attractive strategy in the combat against these pathogens. Conversely, strategies that promote recognition of viruses by specific immune system lectins – involved in viral inhibition and clearance – may also prove useful in antiviral therapies. Several possibilities have been and are currently being explored.

Perhaps the most obvious strategy to modulate glycan–lectin binding is the use of molecules that can physically interfere with these interactions. Glycan decoys (e.g. carbohydrate-containing drugs, sugar analogs/glycomimetics) or carbohydrate-binding agents (CBAs) may be used in antiviral therapies to directly block key glycan–lectin interactions at the side of the lectin and at the side of the glycan, respectively. Binding of glycan decoys with a specific lectin can inhibit binding of other ligands by this lectin. For instance, it has been shown that multivalent mannose-containing molecules or mannose-based glycomimetics

can compromise binding of HIV-1 gp120 with DC-SIGN (Wang *et al.*, 2008b; Luallen *et al.*, 2009; Martinez-Avila *et al.*, 2009b; Becer *et al.*, 2010) and inhibit DC-SIGN-mediated *trans*-infection of CD4⁺ T lymphocytes (Martinez-Avila *et al.*, 2009a; Sattin *et al.*, 2010; Berzi *et al.*, 2012). Likewise, sialic acid-containing glycan decoys and sialic acid analogs are being evaluated for their capacity to block the sialic acid binding site of IAV hemagglutinin to inhibit interaction of the virus with sialic acid-containing receptor molecules on the surface of target cells (Landers *et al.*, 2002; Matrosovich & Klenk, 2003; Matsubara *et al.*, 2010; Papp *et al.*, 2010, 2011).

Alternatively, binding of CBAs to glycans displayed on the virion surface can inhibit viral *cis-* or *trans-*infection via host cell lectins. This is elegantly exemplified in several recent studies, showing that mannose- as well as *N*-acetylglucosamine-specific CBAs can effectively prevent DC-SIGN-mediated HIV-1 capture and subsequent transmission to T lymphocytes (Balzarini *et al.*, 2010; Balzarini *et al.*, 2007a; Bertaux *et al.*, 2007; Huskens *et al.*, 2010; Hoorelbeke *et al.*, 2011; Alexandre *et al.*, 2012). Although CBA binding to host cell-associated glycans may inhibit viruses that employ viral lectins, the potential of this strategy for antiviral therapy remains virtually unexplored. It is noteworthy that the antiviral activity of CBAs or glycan decoys that bind to virion surfaces is not necessarily limited to direct inhibition of crucial glycan–lectin interactions, as they can mask greater portions of the virus and interfere with other crucial (including non-glycan-lectin) interactions or steps in the infection process. Moreover, recent research on HIV-1 highlights the antiviral potential of CBAs from yet another angle. Although the heavily glycosylated HIV-1 gp120 protein generally provides multiple ligands for mannose- and GlcNAc-specific CBAs, prolonged CBA pressure selects for HIV-1 variants with multiple *N*-glycosylation site deletions in the gp120 protein that are less sensitive to CBA-mediated neutralization (Balzarini *et al.*, 2004, 2005a, b; Witvrouw *et al.*, 2005; Balzarini *et al.*, 2006; Balzarini, 2007b, c; Balzarini *et al.*, 2007b; Huskens *et al.*, 2007). Interestingly, however, deletion of *N*-glycosylation sites can also increase the immunogenicity of the virus and weaken the glycan shield that protects the virus from recognition by virus-specific antibodies and other (nonlectin) immune receptors (Botarelli *et al.*, 1991; Back *et al.*, 1994; Reitter *et al.*, 1998; Bolmstedt *et al.*, 2001; Kang *et al.*, 2005), and may decrease the efficiency of HIV-1 *trans-*infection via immune system lectins like DC-SIGN (Hong *et al.*, 2007; Liao *et al.*, 2011). In addition, it appears that accumulation of mutations under CBA pressure is often paralleled by a significant reduction of the viral fitness, which is obviously advantageous in the context of an antiviral treatment (Balzarini *et al.*, 2005a; Balzarini, 2007b, c).

For further information on CBAs and their potential in antiviral therapy, readers may refer to recent expert reviews on this topic (Balzarini, 2007c; Francois & Balzarini, 2012).

Although the use of glycan decoys and CBAs may seem the most intuitive strategy to interfere directly with glycan–lectin interaction, other options exist as well. For instance, similar to glycan decoys and CBAs, lectin- or glycoconjugate-specific immunoglobulins may be used to block specific interactions. Evidently, the increasing availability of such 'direct' modulators of glycan–lectin interaction is mirrored in an increasing number of potential antiviral applications.

Apart from direct modulation, also strategies that influence glycan–lectin interactions more indirectly can be employed. In fact, many of the molecules used to examine glycan–lectin interactions *in vitro* suggest themselves as potential therapeutics.

One approach to indirectly govern glycan–lectin interaction is via the use of drugs that alter the host and/or viral glycome. Glycosidases and other enzymes may be used to alter the glycan portions of fully formed and matured glycoconjugates. Alternatively, various drugs may be employed to directly modify glycan synthesis: glycoconjugates produced in the presence of such molecules will obtain aberrant glycosylation, which may promote or annihilate their interaction with specific lectins. Promising results in glycovirological research have highlighted the antiviral potential of such compounds. For instance, ample data indicate that sialidases may be used to counteract infections where sialic acids play important roles as cellular receptors for viral lectins [e.g. IAV binds to sialic acid receptors on the airway epithelium (Skehel & Wiley, 2000; Malakhov *et al.*, 2006; Belser *et al.*, 2007; Harrison, 2008; Chan *et al.*, 2009; Triana-Baltzer *et al.*, 2009a, b, 2010, 2011)] or as viral ligands for host lectins that serve as portals for viral entry [e.g. sialic acids on the porcine reproductive and respiratory syndrome virus bind the macrophage-specific entry mediator sialoadhesin (Delputte & Nauwynck, 2004; Delputte *et al.*, 2007; Van Breedam *et al.*, 2010a, b)]. Sialidase treatment may also enhance recognition of viral glycoconjugates by mannose-specific immune system lectins that can limit viral infection: *in vitro* experiments have shown that enzymatic removal of sialic acids from the HIV-1 virion surface can significantly enhance virus binding and neutralization by MBL (Hart *et al.*, 2002, 2003). In line with this, production of HIV-1 in the presence of the Golgi α1-2-mannosidase I inhibitor 1-deoxymannojirimycin – which blocks the biosynthesis of complex-type, sialylated oligosaccharides – increased susceptibility of the virus to MBL-mediated neutralization (Hart *et al.*, 2003). Interestingly, 1-deoxymannojirimycin treatment of

HIV-1-infected cells was also shown to potentiate the antiviral effects of mannose-specific plant CBAs towards the newly produced HIV-1 virions (Balzarini, 2007a). These and other examples illustrate the potential of these molecules as antiviral drugs.

Considering the various structural and nonstructural roles of glycans, it is clear that glycome modulation can also have effects beyond the alteration of glycan–lectin binding events. For instance, interfering with host cell glycosylation processes using specific inhibitors may inhibit assembly of infectious virions (Leavitt *et al.*, 1977; Katz *et al.*, 1980; Pizer *et al.*, 1980; Herrler & Compans, 1983; Montefiori *et al.*, 1988; Pal *et al.*, 1989; Mehta *et al.*, 1998; Dwek *et al.*, 2002; Wu *et al.*, 2002; Durantel *et al.*, 2007; Lazar *et al.*, 2007; Scanlan *et al.*, 2007; Durantel, 2009; Merry & Astrautsova, 2010). Moreover, glycome modulation may significantly alter the capacity of the virus to evade recognition by virus-specific antibodies and B- and T-cell receptors via glycan shielding (Botarelli *et al.*, 1991; Back *et al.*, 1994; Willey *et al.*, 1996; Reitter *et al.*, 1998; Bolmstedt *et al.*, 2001; Kang *et al.*, 2005; Aguilar *et al.*, 2006; Wang *et al.*, 2009; Francica *et al.*, 2010; Kobayashi & Suzuki, 2012). Clearly, glycome-modifying drugs can counteract viruses in various ways and constitute versatile tools in the control of viral infection.

Also other strategies that can indirectly influence glycan–lectin interactions are certainly worth exploring. For instance, drugs that alter host or viral lectin expression (e.g. cytokines or RNAi) may prove useful in antiviral strategies (Ochiel *et al.*, 2010; Relloso *et al.*, 2002; Ge *et al.*, 2003; Arrighi *et al.*, 2004; Ge *et al.*, 2004; Nair *et al.*, 2005; Yagi *et al.*, 2010; Raza *et al.*, 2011). Furthermore, patients infected with a virus that employs both viral lectins and RDEs may also benefit from treatment with RDE-specific inhibitors, as these can alter the balance between viral lectin and RDE activity which is often crucial for efficient viral replication and spread. Notable examples in this context are the several neuraminidase inhibitors that have been used successfully for the treatment of IAV infections (Kim *et al.*, 1999; Lew *et al.*, 2000; Roberts, 2001; Garman & Laver, 2004; Alymova *et al.*, 2005; von itzstein, 2007; von itzstein & Thomson, 2009; Gamblin & Skehel, 2010; Ikematsu & Kawai, 2011).

In sum, drugs that modulate glycan–lectin interactions – either directly or indirectly – can be powerful instruments in the combat against viral pathogens. However, the antiviral strategies suggested above can also have drawbacks. Intensive use of antiviral therapeutics may elicit rapid selection of drug-resistant virus variants. Another possible drawback relates to the potential off-target effects of these therapies: therapeutics aimed at influencing specific glycan–lectin interactions that play key roles in viral infection processes may also affect general host glycosylation,

lectin expression or glycan–lectin interactions that are crucial for normal functioning of the host and its immune defense. It is also conceivable that such drugs, despite their antiviral effects, may benefit the virus in some ways. For instance, although a glycome-modifying drug may promote viral recognition by specific immune system lectins that aid in viral clearance, it may also promote viral interaction with host lectins that can aid in *cis-* or *trans-*infection. Ultimately, the potential of specific agents as antiviral drugs depends on their net (antiviral) effects *in vivo*. Other potential disadvantages concern the pharmacokinetic properties of specific drugs. For instance, if glycan decoys or CBAs used in antiviral therapy show a broad reactivity and can respectively bind with multiple (nontarget) lectins or glycans in the host, it is possible that much of the antiviral effect is lost. Also, when using peptidic CBAs that are not native to the host, an antibody response might be mounted against these components, leading to neutralization and/or faster clearance of the active compound. In spite of these and other potential pitfalls, it is clear that glycan decoys and CBAs, as well as various indirect approaches to modulate glycan–lectin interaction, show potential for the treatment of diverse viral infections, either or not in combination with other antiviral strategies. A good understanding of relevant glycan–lectin interactions facilitates specific targeting of these binding events and can help to minimize possible off-target effects and to reduce the risk of drug resistance.

Concluding remarks

Glycans and lectins cover crucial roles in virus biology and their interplay often shapes the virus-host interaction. In general, the nature of the glycan, the lectin, and the specific conditions under which their interaction occurs determines the outcome of a specific binding event and directs the virus to a certain fate. Based on current knowledge, it is clear that viral lectins generally facilitate viral infection and spread. On the other hand, although it may seem intuitive that host lectin PRRs and other immune system lectins exclusively act in defense of the host, there is ample evidence that contradicts this. Although many host lectins are involved in the induction of an efficacious immune response against viral pathogens, many viruses can also abuse these lectins to promote infection and spread. Intriguingly, analysis of the many studies regarding the role of host lectins in viral infections suggests that soluble host lectins tend to be associated with antiviral activity, whereas membrane-associated host lectins seem to play a more dubious role and are often implicated in pro- as well as antiviral mechanisms (Tables 1 & 2). It is however noteworthy that the information provided in this manuscript reflects current views on glycan–lectin interactions

in virus biology, and that future research may alter our understanding and interpretation of specific interactions. The fact that (aspects of) viral glycobiology may change during virus-host co-evolution even advocates periodic re-evaluation of specific glycan–lectin interactions.

The biology of glycans and lectins is complex and has long been poorly accessible to virologists and other scientists outside this field. This situation is changing with the emergence of international glycomics consortia (e.g. Consortium for Functional Glycomics), which can provide state-of-the-art techniques and expertise to analyze and interpret virologically/immunologically relevant glycan–lectin interactions. Still, there are specific pitfalls associated with glycovirological research. *In vitro* experiments need to be designed and interpreted considering key issues like glycan and lectin variability, the cell-type dependency of host and virus glycosylation and the influence of the lectin-expressing cell type on the final outcome of a glycan–lectin interaction. In addition, the results of *in vitro* experiments must ultimately be compared with – and re-interpreted in the context of – data obtained in animal models. In fact, our current understanding of specific glycan–lectin interactions in viral infection is mostly based on *in vitro* experiments, underlining the need for experimental validation of these results in the context of the infected host.

In the context of viral infections, many different (lectin-dependent or -independent) interactions and processes take place simultaneously, resulting in a complex network of virus–host factor interaction, signaling, and effector mechanisms, the net effect of which may benefit the host or the virus. Many of the interactions taking place are not yet well defined and probably more are still unknown. Key to combating viral disease is to make these black boxes more transparent. Synergisms between different branches of life sciences are essential to sustain and advance our knowledge in this important field of research.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. References Table 1.
Data S2. References Table 2.