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REVIEW

Lectins as pattern recognition molecules: The effects of epitope density in innate immunity*

Tarun K Dam and C Fred Brewer[1]

Department of Molecular Pharmacology and Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

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The innate immune response of multicellular organisms is initiated by the binding of soluble and membrane-bound host molecules including lectins to the surface of pathogenic organisms. Until recently, it was believed that the epitopes recognized by host molecules were uniquely associated with the pathogenic organisms. Hence, the term pattern recognition receptors (PRRs) was used to describe their binding specificities. However, with an expanding number of lectin classes including C-type lectins, siglecs, and galectins recognized as PRRs, it is apparent that many of the glycan epitopes recognized on foreign pathogens are present in the host and involved in cellular functions. Hence, the molecular basis for pattern recognition by lectins of carbohydrate epitopes on pathogens is in question. A number of studies indicate that the density and number of glycan epitopes in multivalent carbohydrates and glycoprotein receptors determine the affinity of lectins and their effector functions. This paper reviews lectins that are involved in innate immunity, mechanisms of enhanced affinity and cross-linking of lectins with density-dependent glycan epitopes, density-dependent recognition of glycan receptors by lectins in host systems and lectin–glycan interactions in foreign pathogens. Evidence indicates that lectin pattern recognition in innate immunity is part of a general mechanism of density-dependent glycan recognition. This leads to a new definition of lectin receptor in biological systems, which considers the density and number of glycan epitopes on the surface of cells and not just the affinity of single epitopes.

Keywords: epitope density/glycans/immunosurveillance/innate immunity/lectins

Introduction

The innate immune response of metazoans involves binding of soluble and membrane-bound host molecules to the surface of pathogenic organisms including viruses, bacteria, parasites, and fungi. These interactions can lead to signaling for further immune responses or neutralization of the infectivity of the pathogen (van Kooyk and Rabinovich 2008; Vasta 2009). Host molecules involved in innate immunity include glycan binding receptors (lectins), Toll-like receptors (some of which recognize glycans), nucleotide-binding oligomerization domains (NODs) and NK cell receptors (Medzhitov and Janeway 2000, Vasta 2009). Until recently, innate immune receptors were believed to recognize epitopes uniquely associated with pathogenic organisms (Medzhitov and Janeway 2002). Hence, the term pathogen-associated molecular patterns (PAMPs)[1] was used to describe these epitopes (Medzhitov and Janeway 2002). In turn, pattern recognition receptors (PRRs) are used to describe host molecules that recognize PAMPs (Medzhitov and Janeway 2000). However, with an expanding number of lectin classes including C-type lectins, siglecs, and galectins recognized as PPRs (van Kooyk and Rabinovich 2008; Vasta 2009), it is apparent that many of the glycan epitopes recognized on foreign pathogens are present in the host and involved in functions that include development, immune regulation and homeostasis (Marth and Grewal 2008; Varki et al. 2009). Therefore, the molecular basis for pattern recognition of carbohydrate epitopes on pathogens and hosts by lectins is in question.

Recent studies have shown that the density and number of glycan epitopes in multivalent carbohydrates and glycoprotein receptors are important determinants of the binding and cross-linking activities of lectins and their effector functions. This paper provides a review of lectins that are involved in innate immunity, mechanisms of enhanced affinity and cross-linking activity of lectins with density-dependent glycan epitopes, density-dependent recognition of glycan receptors by lectins in host systems and lectin–glycan interactions in foreign pathogens. The molecular basis for pattern recognition by lectins in innate immunity is discussed in terms of density-dependent glycan recognition.

Lectins in innate immunity

At least three classes of lectins, C-type lectins, siglecs, and galectins, are described as PRRs in the literature (Vasta 2009). C-type lectins are calcium-dependent proteins and comprise a large number of receptors (60–80) that are divided into subfamilies (Zelensky and Gready 2005). Most C-type lectins possess more than one carbohydrate recognition domain (CRD) and hence are multivalent receptors (Drickamer 1993). The majority of C-type lectins are membrane-associated receptors, while a subclass of C-type lectins, the collectins, is soluble molecules. The specificity of C-type lectins can be generally divided into two categories: (1) the mannose (Man)-specific C-type lectins, such as DC-SIGN, that possess specificity for Man- and/or fucose (fuc) terminated glycans, (2) and galactose (Gal)-specific

[1]To whom correspondence should be addressed: Tel: +1-718-430-2227; Fax: +1-718-430-8922; e-mail: fred.brewer@einstein.yu.edu
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C-type lectins, such as macrophage galactose lectin, that recognize Gal- and *N*-acetylgalactosamine (GalNAc) terminated glycans (Weis et al. 1998). The Man-specific C-type lectins are known to bind to multivalent *N*-linked high mannose and Fuc-containing oligosaccharides possessing the Lewis^{a,b,x,y} determinants, while the Gal-specific C-type lectins bind to multivalent glycans possessing GalNAc residues (van Kooyk and Rabinovich 2008). These glycan structures are expressed in mammalian glycoproteins such as the intracellular adhesion molecule ICAM-2, ICAM-3, Mac-1, carcinoembryogenic antigen (CEA), CEA-related cell adhesion molecule (CEACAM1), butyrophilin, CD45, and human milk bile-salt stimulated lipase (BSSL) (van Kooyk and Geijtenbeek 2003). Many of these glycan epitopes are also found in pathogens such as the *N*-linked high mannose oligosaccharides in yeast, viral glycoproteins, *Leishmania pifanoi* lipophosphoglycans, the di- and tri-Man structures of ManLAM in *Mycobacteria tuberculosis*, and Lewis^{x} glycan structures in *Helicobactor pylori* and *Schistosoma mansoni* (van Kooyk and Geijtenbeek 2003). Affinity increases of C-type lectins for polyvalent displays of specific glycans have been posited for their binding to glycan receptors involved in pathogen and self-recognition (Feinberg et al. 2007; van Kooyk and Rabinovich 2008; Garcia-Vallejo and van Kooyk 2009). Interestingly, the specificity of DC-SIGN for high-mannose and Fuc-containing glycans (Lewis^{a,b,x,y}) reflects the apparent ability of this C-type lectin to recognize polyvalent displays of different glycans that are associated with both host and pathogens (Feinberg et al. 2007; Taylor and Drickamer 2007).

Siglecs (sialic acid-binding immunoglobulin-like lectins) are type I membrane proteins with variable numbers of immunoglobulin domains. Siglecs are divided into two types: CD33-related siglecs (Siglecs-3, -5, -6, -7, -8, -9, -10, -11, and -14) and a second group consisting of sialoadhesion (Siglec-1 or CD169), Siglec-2 (CD22) and MAG (Siglec-4) (Crocker et al. 2007). Siglecs from both types show specificity for sialic acid-containing glycans that are found on the nonreducing ends of oligosaccharide chains of *N*- and *O*-linked carbohydrates or on glycosphingolipids. Siglecs are further distinguished by their specificity for sialic acids that possess α(2,3), α(2,6), or α(2,8) linkages to an extended oligosaccharide structure, as well as distinguish the structures of the extended glycans themselves (Crocker et al. 2007). At present, 13 members of the siglec family are known and expressed on a wide variety of host cells as are the sialic acids on glycan receptors recognized by the siglecs. Their functions include cell proliferation, differentiation, induction of apoptosis, negative regulation of B cell signaling, and induction of cytokine secretion (Crocker et al. 2007). Siglecs are also innate immune receptors that bind to sialic acids found on pathogens such as *Neisseria meningitidis*, porcine reproductive and respiratory syndrome virus (PRRSV), *Campylobactor jejuni*, *Trypanosoma cruzi* and group B Streptococcus (Crocker et al. 2007). Thus, siglecs are involved in both pathogen and host recognition functions.

Galectins are a family of lectins with specificity toward β-Gal and LacNAc residues (Varki et al. 2009). To date, 15 galectins have been identified in mammals. Most galectins appear to be divalent proteins with two CRDs, while galectin-3 appears to be pentameric in binding to multivalent glycans (Ahmad et al. 2004). Growing evidence indicates that galectins function as PRRs by binding to glycan epitopes including LacNAc moieties in the *N*-glycans of viruses, lipopolysaccharides, and lipooligosaccharides and capsular oligosaccharides of bacteria, lipophosphoglycans of protozoa, oligomannans of fungi, and LacdiNAc of helminths (Rabinovich and Toscano 2009). Galectins also interact with a variety of host glycoproteins including CD43, CD45, CD7, CD71, CD44, TIM3, CTLA4, MUC1, and MUC16 (Argueso et al. 2009; Rabinovich and Toscano 2009). These interactions lead to different biological functions including apoptosis, cell maturation and cytokine secretion, cell migration, cell growth arrest, and formation of barriers on epithelial surface. Hence, galectins bind to self- and non-self-glycans that include LacNAc epitopes.

In the next section, we will review quantitative studies of the effects of glycan density and numbers on the affinity and cross-linking properties of lectins in order to assess the mechanisms of binding of the lectins involved in innate immunity with glycans found on the host and foreign pathogens.

Quantitative studies of lectin interactions with density-dependent glycans

The binding activity of glycans on a surface (lyposomes, arrays, cells) is represented by density instead of concentration, since the latter is used in solution conditions. Apparent dissociation constants (Kds) can be obtained for the binding of a soluble lectin to glycans on a surface (Oyelaran et al. 2009). The interpretation of Kds obtained from these measurements and the concept of avidity and affinity are discussed at the end of the review.

Several physical studies have demonstrated the dramatic effects of increasing glycan density on the affinity and cross-linking activities of lectins. Rando and coworkers (Orr et al. 1979) were among the first to show threshold effects for the binding and aggregation of liposomes containing varying amounts of synthetic Man-containing glycolipids by the lectin concanavalin A (ConA). Aggregation was shown to be critically sensitive to the mole percent of synthetic glycolipid incorporated, with little aggregation observed at 5% of the synthetic glycolipid and substantial aggregation observed at 7.5% incorporation (Figure 1). Similar threshold effects were reported for varying concentrations of the lectin at a constant mole percent incorporation of the synthetic glycolipid into the liposomes. This group also showed threshold effects for ConA agglutination of modified erythrocytes possessing variable amounts of Man residues synthetically coupled with the outer membrane of the cells (Rando et al. 1979). Hence, these studies demonstrate the importance of glycan density in lectin binding and cross-linking of liposomes containing a synthetic glycolipid.

A recent study provides striking evidence of the importance of glycan density on lectin affinity and selectivity in 2D array experiments (Oyelaran et al. 2009). Bovine serum albumin (BSA) was conjugated with an increasing number of well-defined glycan epitopes and attached to arrays (Figure 2). The arrays were used to evaluate density-dependent binding of three lectins including *Helix pomatia* agglutinin (HPA) and soybean agglutinin (SBA). Table I shows density-dependent binding of HPA and SBA to BSA possessing 4 and 22 α-GalNAc residues, and to BSA attached to 4 and 31 Forssman disaccharide epitopes (GalNAcα1,3GalNAc-). HPA and SBA show little binding to BSA conjugated with four α-GalNAc residues, but both bind well to BSA conjugated with 22 α-GalNAc residues. On the other hand, HPA binds well to BSA conjugated with both 4

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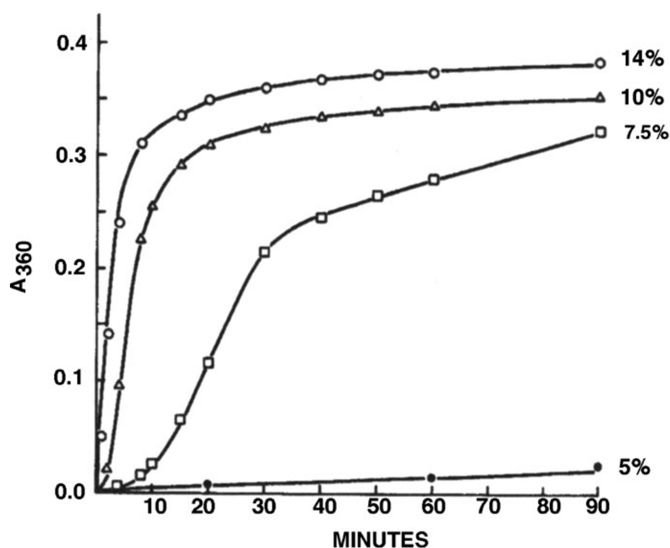

Fig. 1. Aggregation of lecithin liposomes possessing a synthetic mannose glycolipid by ConA (Orr et al. 1979). The mol% of the glycolipid in the liposomes is listed in the figure. The concentration of ConA was 100 mg/mL. (Copyright 1979. American Society of Biochemistry and Molecular Biology).

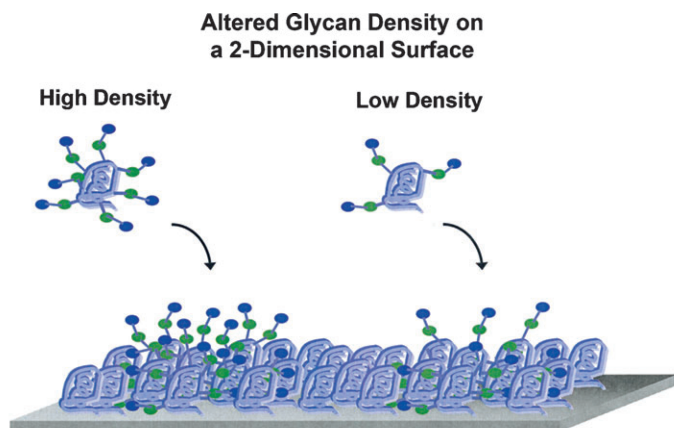

Fig. 2. Density-dependent display of glycans attached to BSA on an array (Oyelaran et al. 2009).

Table I. Glycan density-dependent affinity of *Helix pomatia* agglutinin (HPA) and soybean agglutinin (SBA) for glycan-conjugated BSA[a]

		App Kd (nM)	
Ligand	Number glycan/BSA	HPA	SBA
GalNAc-α-BSA	4	>1270	>4200
GalNAc-α-BSA	22	10	37
Forssman Disacch-BSA[b]	4	6	>4200
Forssman Disacch-BSA[b]	31	5	14

[a]Oyelaran et al. 2009.
[b]Forssman disaccharide is GalNAcα1,3GalNAc-

and 31 Forssman disaccharide epitopes, but SBA does not bind well to BSA possessing four Forssman disaccharides, but does bind to BSA possessing 31 Forssman disaccharides. These results show both density-dependent glycan binding of the lectins as well as selectivity of binding of the lectins to the carbohydrate epitopes attached to BSA. Eight different carbohydrate

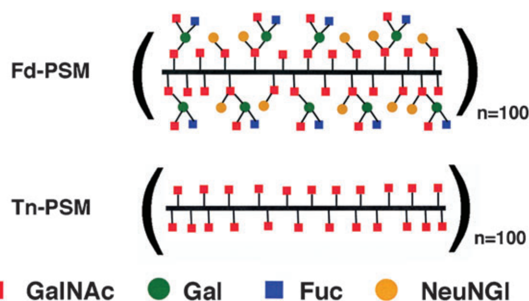

Fig. 3. Schematic representations of porcine submaxillary mucin (PSM) possessing the naturally occurring carbohydrates (Fd-PSM) and only α-GalNAc residues (Tn-PSM). PSM in both samples contain 100 tandem repeats polypeptide domains and a total of ∼2300 *O*-linked glycan chains.

Table II. Relative affinities of Tn-PSM and its fragments for SBA and VML (Dam et al. (2007))

Ligand	Number of GalNAc residues	Relative affinities
	SBA	
GalNAcα1-*O*-Ser	1	1
38/40-mer Tn-PSM	12	120
81-mer Tn-PSM	23	2800
Tn-PSM	2300	850,000
	VML	
GalNAcα1-O-Ser	1	1
38/40-mer Tn-PSM	12	650
81-mer Tn-PSM	23	11,000
Tn-PSM	2300	1,300,000

epitopes were used to show similar density-dependent glycan binding of three lectins to BSA conjugates attached to arrays. Importantly, similar results were observed for the binding of anti-carbohydrate antibodies to density-dependent glycans on the arrays.

While the above studies demonstrate the effects of glycan density on lectin binding, the mechanism of lectin recognition of density-dependent glycan expression is not addressed in these investigations. Recent studies have suggested a mechanism for the enhanced affinity and cross-linking activity of lectins with density-dependent glycan epitopes and provided insight into the magnitude of these effects. Dam et al. (2007) reported thermodynamic data for the binding of soybean agglutinin (SBA) and *Vatairea macrocarpa* lectin (VML), which possess Gal/GalNAc specificity, to chemically and enzymatically modified forms of porcine submaxillary mucin (PSM), which possesses a molecular mass of $\sim 10^6$ daltons and ∼2300 *O*-linked carbohydrate chains. Using isothermal titration microcalorimetry (ITC) and hemagglutination inhibition measurements, both lectins bound to an analog of PSM that possesses only α-GalNAc residues (Tn-PSM) (Figure 3) with affinities that were $\sim 10^6$-fold greater than that of GalNAcα1-*O*-Ser (Table II). A fragment of Tn-PSM with ∼11–12 GalNAc residues showed an affinity ∼120-fold greater than GalNAcα1-*O*-Ser (Table II), while a larger fragment of Tn-PSM with ∼22–24 GalNAc residues possessed an affinity that was ∼3000-fold greater than GalNAcα1-*O*-Ser (Table II). Similar enhancements in the affinity of VML for the two fragments were also observed (Table II). Hence, increasing the number of glycan epitopes in the mucin leads to higher affinities of the lectins.

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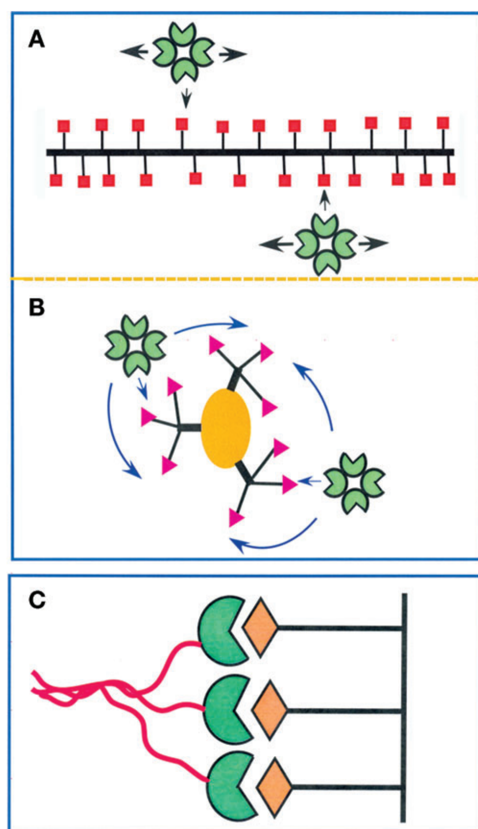

Fig. 4. Schematic representations of lectins binding to (**A**) a linear glycoprotein (mucin) and (**B**) globular glycoprotein (asialofetuin) via the internal diffusion mechanism. In (**C**) an example is given of the 'face to face' lectin-glycan binding mechanism.

These studies also showed that SBA and VML exhibited lower affinity for the natural carbohydrate form of PSM (Fd-PSM) that possesses a nearly equal mixture of single α-GalNAc residues and the blood group A tetrasaccharide (α-GalNAc(1–3)[α-Fuc(1–2)]-β-Gal(1–3)-α-GalNAc-O-Ser/Thr) (Figure 3) (Dam et al. 2007). Importantly, ~40% of the peptide bound α-GalNAc residues were capped with α-*N*-glycoloylneuraminic acid (NeuNGl) residues. The stoichiometries of both lectins binding to Fd-PSM were reduced by a factor of 4 relative to Tn-PSM, consistent with the reduced number of free α-GalNAc epitopes in the former. However, the affinity of SBA for Fd-PSM was reduced by a factor of ~100 relative to Tn-PSM, and the affinity of VML for Fd-PSM was reduced by a factor of ~10 relative to Tn-PSM. Thus, reducing the density of GalNAc epitopes in the mucins significantly reduces the affinity of the lectins.

An internal diffusion mechanism was found to be consistent with the thermodynamic data and dependence of lectin affinity on the length of the mucin peptide chains and density of the glycan epitopes on the mucins (Dam et al. 2007). In this mechanism, a lectin molecule "binds and jumps" from carbohydrate to carbohydrate epitope along the mucin peptide backbone before complete dissociation from the mucin (Figure 4A) (Dam et al. 2007; Dam and Brewer 2008). With a longer life-time of the complex, the affinity of the lectin increases with increasing number of glycan epitopes and spacing of the epitopes (density) in the mucin. Evidence for an internal diffusion mechanism has been previously suggested for the enhanced affinities of ConA and *Dioclea grandiflora* lectin (DGL) binding to bi-, tri- and tetraantennary *N*-linked carbohydrates that possess terminal Man epitopes (Dam et al. 2000, 2002), and for the enhanced affinities of galectins-1, -2, -3, -4, -5 and -7 binding to asialofetuin, a multivalent globular glycoprotein that possesses nine LacNAc moieties (Figure 4B) (Dam et al. 2005). Importantly, the internal diffusion mechanism has been observed in other biological systems (Dam and Brewer 2008). These include proteins that bind to DNA (Blainey et al. 2006), molecular motor proteins that bind and "walk" along filamentous proteins (Dunn and Spudich 2007), and phosphate binding proteins that show threshold binding to proteins that undergo the addition of multiple phosphate groups (Nash et al. 2001).

The cross-linking activities of SBA and VML with the mucins were also enhanced by many orders of magnitude (Dam et al. 2009). Both high affinities and enhanced cross-linking activities of the lectins were associated with the internal diffusion mechanism. This is important since the biological effector functions of lectins such as galectins are often associated with their cross-linking activities (Brewer et al. 2002).

A second mechanism associated with binding of lectins to density-dependent glycan expression is the face-to-face mechanism (Lee and Lee 2000). In this case, the lectin possesses multiple subsites or carbohydrate recognition domains (CRDs) in a common direction that simultaneously bind to a multivalent glycan or glycoprotein with clustered epitopes (Figure 4C). The affinity enhancement in this case can be large. For example, binding of a trivalent carbohydrate with terminal LacNAc residues to the asialoglycoprotein receptor (C-type lectin) results in a ~10^6-fold increase in affinity relative to LacNAc (Lee et al. 1991). The affinity of the receptor for the trivalent carbohydrate is therefore the sum of the free energies of binding of the three subsites of the receptor.

Thus, there are at least two mechanisms that provide density-dependent glycan binding of lectins: (1) binding and internal diffusion of lectins such as galectins among a dense population of glycans (Dam and Brewer 2008), and (2) binding of multiple CRD domains of lectins such as C-type lectins to clustered glycan epitopes (Weis et al. 1998). These insights provide the basis for examining the mechanisms of binding of lectins including C-type lectins, siglecs, and galectins with host glycans and the glycans of foreign pathogens.

Lectin binding to density-dependent glycan expression in host cells

Recent studies involving members of the galectin family have revealed the importance of the carbohydrate epitope density and the number of glycans on galectin binding and effector functions including signaling in biological systems. Lau et al. (2007) showed that the level of cell surface glycoprotein receptors is regulated by the metabolic flux of *N*-glycan biosynthesis. Experiments indicate that the Golgi pathway is sensitive to hexosamine flux for production of tri- and tetra-antennary *N*-glycans on specific glycoprotein receptors, which bind to galectins and form cross-linked complexes (lattices) that oppose glycoprotein endocytosis on the cell surface. Glycoproteins with few *N*-glycans, such as transforming growth factor-β receptor II, cytotoxic T-lymphocyte-associated antigen-4, and

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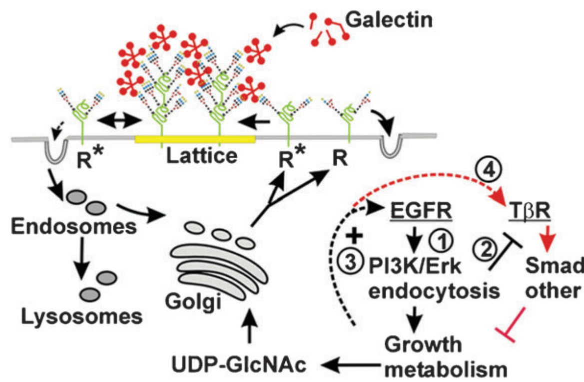

Fig. 5. Model of density-dependant regulation of glycan lattice formation with galectin-3 and the kinetics of endocytosis of surface glycoproteins that are involved in cell growth and differentiation (Lau et al. 2007). (Reprinted with permission from Elsevier).

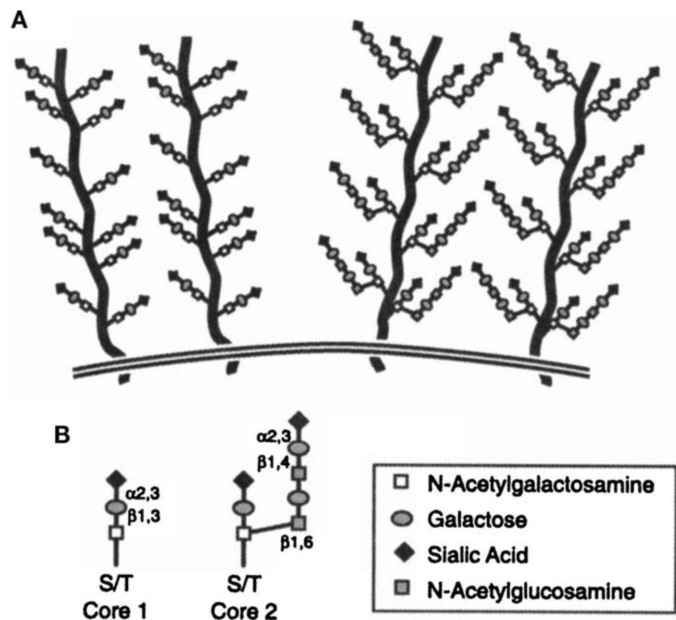

Fig. 6. Schematic representations of CD43 possessing (**A**) core 1 glycans and core 2 *O*-glycans, and (**B**) schematic representations of the structures of core 1 glycans and core 2 *O*-glycans (Hernandez et al. 2006). (Copyright 2006. The American Association of Immunologists, Inc.)

glucose transporter-4 exhibit enhanced cell surface expression with switch-like or rapid responses to increasing hexosamine concentration, whereas glycoproteins with high numbers of *N*-glycans like epidermal growth factor receptor, insulin growth factor receptor, fibroblast growth factor receptor, and platelet derived growth factor receptor exhibit hyperbolic responses (Lau et al. 2007). The authors concluded that enhanced branching of *N*-glycans on specific cytokine receptors and transport proteins regulates their surface retention by lattice formation with galectin-3, which, like LacNAc-specific plant lectins, has been shown to be sensitive to the degree of branching of *N*-linked carbohydrates on the affinity of the lectin (Bhattacharyya et al. 1988; Hirabayashi et al. 2002). Hence, the density of LacNAc epitopes on the *N*-glycans of these cell surface glycoprotein receptors regulates the binding and cross-linking of galectin-3 to the receptors, and hence the cell surface signaling activities of the receptors (Figure 5). These results are consistent with internal diffusion of galectin-3 among the increased number of LacNAc epitopes on the *N*-glycans of the receptors that results in enhanced affinity and cross-linking activity of the lectin.

Demetriou et al. (2001) have provided evidence for galectin-3 regulation of the T cell receptor (TCR) signaling during T-cell response to antigen. They observed that Mgat5-null mice lacking the β1,6 *N*-glycan branch structure on *N*-linked glycans, which is recognized by galectins, reduce galectin-3 association with TCRs on T-cells. As a consequence, TCR signaling and downstream calcium flux in the cells are altered (Demetriou et al. 2001; Chen et al. 2007). The β1,6 *N*-glycan-deficient mice also demonstrate increased susceptibility to autoimmune disease and alterations in T-cell cytokine production, consistent with a role for galectin-3 interactions with TCRs in regulating response to antigen (Demetriou et al. 2001; Lee et al. 2007). These findings suggest that the reduced binding of galectin-3 to TCR molecules is due to a reduction in the density of LacNAc epitopes in the receptor. These results are consistent with internal diffusion of galectin-3 among the *N*-linked glycans of the TCR complex.

Ohtsubo et al. (2005) have demonstrated that the density of LacNAc units on the single *N*-linked complex carbohydrate chain of the glucose transporter 2 (Glut-2) in pancreatic β-cells determines the presence of the transporter and activity on the surface of the cells. These workers showed that the normal *N*-linked carbohydrate of Glut-2 is a complex tetraantennary glycan with four LacNAc epitopes. Interactions of galectin-9 on the surface of the cells with the *N*-linked glycan of Glut-2 were demonstrated to result in stabilization of the transporter and its activity. However, expression of a complex biantennary *N*-linked carbohydrate that possesses two LacNAc units on the receptor leads to attenuation of Glut-2 cell surface half-life and enhanced endocytosis of the receptor with redistribution into endosomes and lysosomes. The impairment of glucose-stimulated insulin secretion leads to metabolic dysfunction, which is characteristic of type 2 diabetes. Expression of a triantennary N-link carbohydrate that possesses three LacNAc units reestablished stable expression of Glut-2 on the surface of the cells along with transport activity of the receptor. Binding of galectins including galectin-9 to *N*-linked carbohydrates possessing the increasing number of LacNAc moieties is known to enhance the affinity and cross-linking activities of lectins (Hirabayashi et al. 2002; Dam and Brewer 2003). Hence, the results are consistent with density-dependent binding and cross-linking of galectin-9 with the multiple LacNAc chains of the *N*-linked carbohydrate of Glut-2 on the surface of pancreatic β-cells.

Galectin-1 binding and cross-linking of CD43, a mucin-like glycoprotein receptor, have been shown to regulate T cell death (Perillo et al. 1998). Interestingly, a different glycoform expressed on CD43 possessing the core 1 glycan, Galβ1,3GalNAc, binds galectin-1 nearly as well as the naturally occurring branched core 2 glycan epitope, Galβ1,4GlcNAc, (Figure 6) even though Galβ1,3GalNAc in solution binds to the lectin 125-fold more weakly than Galβ1,4GlcNAc (Hernandez et al. 2006). Binding of galectin-1 to the core 1 glycoform of CD43 was also shown to regulate T cell death similar to that of CD43 expressing core 2 glycans. Hence, the density and number of Galβ1,3GalNAc epitopes on CD43 compensate for the difference in affinity between the core 1 glycan and the core 2

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glycan when presented as monovalent ligands in solution. These results may relate to the concept of "friction limits" for proteins that diffuse along the backbone of biopolymers in which bond breaking determines the speed of diffusion of the protein ligand along the backbone (Bormuth et al. 2009). In this case, bond breaking between galectin-1 and core 1 glycans is expected to be faster than that with the core 2 glycans, and hence internal diffusion of galectin-1 should be more entropically favorable in the former case. Similar observations have been made for the binding of galectin-1 to GM1 containing the Galβ1,3GalNAc epitope (Siebert et al. 2003) and to core 1 *O*-glycans on the carcinoma mucin CA125 (Seelenmeyer et al. 2003) and on IgA (Sangeetha and Appukuttan 2005).

Binding of host lectins to glycans on foreign pathogens

Evidence exists that the glycans on foreign pathogens including viruses, bacteria, fungi, and parasites are expressed in multi- and polyvalent arrays that bind host lectins and initiate effector functions such as cross-linking and/or stimulation of an adaptive immune response (van Kooyk and Rabinovich 2008; Vasta 2009). In some cases, the glycans expressed on foreign pathogens are similar to those expressed in the host, although at lower levels in the latter such as *N*-linked high mannose oligosaccharides (Garcia-Vallejo and van Kooyk 2009). In other cases, the glycans expressed on the surface of foreign pathogens are not found in the host. The specificity of host lectin–glycan interactions in these cases is discussed in the following section.

Glycans found on foreign pathogens that bind to galectins include multiple *N*-linked glycans found on viruses such as the Nipah virus, a paramyxovirus that causes severe encephalitis. Galectin-1 binds and cross-links the *N*-glycans in the envelope glycoproteins and blocks cell–cell fusion of the Nipah virus (Levroney et al. 2005). Galectin-1 appears to interact with the *N*-linked oligosaccharides of HIV-1 envelope glycoprotein gp120 (Rabinovich and Toscano 2009), which is highly glycosylated with high mannose and complex-type oligosaccharides (Leonard et al. 1990). Different galectins bind to the surface glycans of bacteria including *Klebsiella pneumoniae*, *Helicobactor pylori*, *Streptococcus pneumoniae,* and *Neisseria gonorrhoeae* (Varki et al. 2009; Vasta 2009). For example, galectin-3 recognizes the surface glycans of *Trypanosoma cruzi* (Vasta 2009). The glycan structures of many of these organisms have been shown to be multi- and polyvalent displays of the glycan epitopes (Vasta 2009).

Galectins also bind to the glycans on protozoa. For example, galectin-3 recognizes the surface glycans of *Trypanosoma cruzi* (Vasta 2009). The lipophosphoglycans (LPG) on the surface of *Leishmania* parasites are recognized by both galectin-3 and -9 (Vasta 2009). LPG consists of a phosphoglycan domain linked through a hexasaccharide glycan core to a 1-*O*-alkyl-2-lyso-phoshatidylinositol lipid anchor. The LPG phosphoglycan moieties share a common backbone of PO_{4-}6Gal(β1,4)Manα1 repeating units, where the C3 position of the Gal residue can be either substituted or unsubstituted. In *L. major*, the poly-β-galactose moieties $(Gal\beta1,3)_n$ are recognized by galectin-3 and -9 (Pelletier and Sato 2002; Pelletier et al. 2003). The LPG-associated polylactosamine chains of the protozoan parasite *Trichomonas vaginalis* binds to galectin-1 (Singh et al. 2009). Hence, glycan epitopes on LPG molecules are present in polyvalent displays for galectin binding.

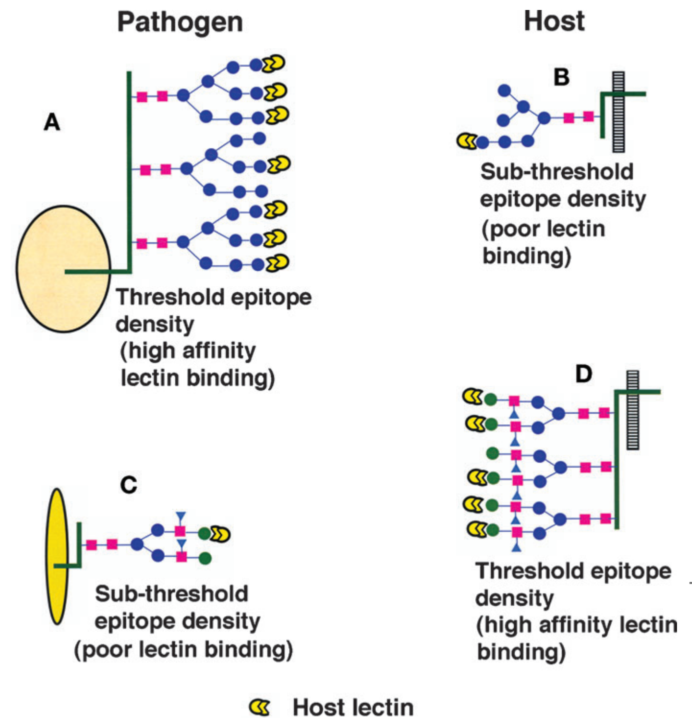

Fig. 7. Schematic examples of density-dependent glycan recognition by host lectins of pathogen (**A**) and (**C**) and host glycans (**B**) and (**D**). Lectins such as DC-SIGN may bind with high affinity to highly dense high-mannose-type glycans on a viral surface glycoprotein (**A**), but only weakly to low density high-mannose-type glycans on the host (**B**). Similarly, the affinity of a lectin such as DC-SIGN for a pathogen with non-clustered (sub-threshold) Lewis^x epitopes may be low (**C**), but would preferentially bind to host glycoproteins with high density Lewis^x epitopes (**D**).

In the case of C-type lectins, DC-SIGN is known to bind to the *N*-linked high mannose chains present on HIV-1 gp120, which are present in higher density than in mammalian glycoproteins (Figure 7) (van Kooyk and Geijtenbeek 2003). Importantly, binding of langerin, a C-type lectin, to the high mannose chains of HIV-1 gp120 on Langerhans cells leads to viral elimination, whereas DC-SIGN on dermal dendritic cells enhances HIV-1 infectivity (van Kooyk and Rabinovich 2008). DC-SIGN also recognizes clustered glycan structures on pathogens such as ebola virus (high mannose oligosaccharides on envelope glycoproteins), *H. pylori* (Lewis^x on LPS), *K. pneumonae* (Man residues on LPS), *M. tuberculosis* (di- and tri-mannose on ManLAM), *L. pifanoi* (high mannose on LPG), and *S. mansoni* (Lewis x epitopes on soluble egg antigens, SEA) (van Kooyk and Geijtenbeek 2003). In all of these examples, the glycan epitopes on the foreign pathogens are expressed in polyvalent displays that aid in binding C-type lectins. Indeed, the high density of *N*-linked high mannose chains on HIV-1 gp120 is also the target of studies of microbicide carbohydrate binding proteins such as actinohivin (Tanaka et al. 2009) and cyanovirin-N (Bewley 2001), which bind to HIV-1 gp120 with nM affinities.

Siglecs are also known to bind to sialic acids expressed on foreign pathogens. Over 20 pathogenic microorganisms are known to extract sialic acids from their hosts and incorporate these moieties into their own glycoconjugates. An example is

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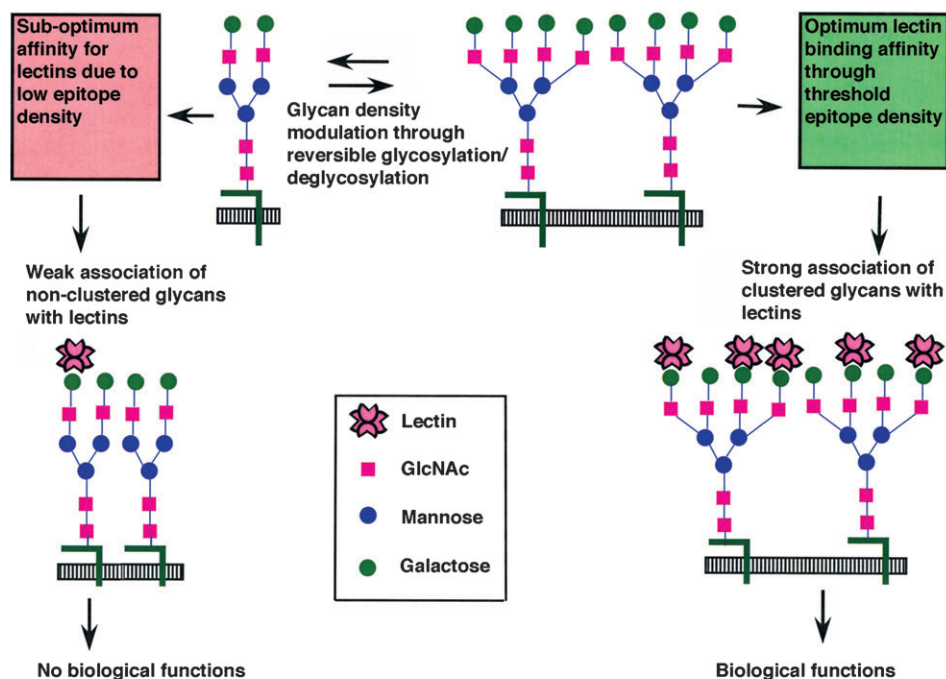

Fig. 8. Model of glycan epitope density modulating biological functions through lectin binding.

the capsular polysaccharide of the human-specific commensal/pathogen group B *Streptococcus* that displays the structure Siaα2–3Galβ1–4GlcNAc, a sequence identical to the termini of human glycoproteins (Carlin et al. 2009). Sialylation of pathogen glycoconjugates appears to be important for the survival in mammalian hosts, presumably acting as molecular mimics of the host cell surface to avoid immune attack. Siglec-1, Siglec-5, and Siglec-7 are known to be involved in host–pathogen interactions of pathogens such as porcine reproductive and respiratory syndrome virus (Crocker et al. 2007). *Neisseria meningitidis*, *Campylobacter jejuni*, *Trypanosoma cruzi,* and Steptococcus group B and pathogen binding to Siglec-1 lead to enhanced endocytosis and uptake of pathogens by macrophages. Thus, the sialic acid moieties on the surface of these organisms are presented in polyvalent arrays that bind to specific Siglecs of the host.

Binding of host lectins to unique glycan epitopes of foreign pathogens

The target of host lectins binding to foreign pathogens is often a glycan that is not expressed in the host. For example, galectin-3 is reported to bind to β1–2 linked mannans of *Candida albicans* and GalNAcβ1–4GlcNAc (LacdiNAc) structures of *Schistosoma mansoni* (Vasta 2009). Both Man and GalNAc β1–4GlcNAc are weak binding epitopes when present as monovalent epitopes to galectins (Shimura et al. 2002). However, the $\sim 10^6$-fold increase in affinity of lectins binding to mucins that possess a high density and large number of a glycan epitope, relative to the monovalent glycan (Dam et al. 2007), suggests that the affinity of even weak binding epitopes can be greatly enhanced when presented in polyvalent arrays. Indeed, the examples of the β1–2 linked mannans of *Candida albicans* (Masuoka 2004) and LacdiNAc structures of *Schistosoma mansoni* (Vasta 2009) binding to galectin-3 are similar to the observation that substitution of the core 1 glycan, Galβ1,3GalNAc, in CD43 results in binding of galectin-1 to this glycoform nearly as well as the naturally occurring branched core 2 glycan epitope, Galβ1,4GlcNAc, even though Galβ1,3GalNAc in solution binds 125-fold more weakly than Galβ1,4GlcNAc (Hernandez et al. 2006). Hence, the density and number of Man epitopes in the β1–2 linked mannans of *Candida albicans* and the LacdiNAc epitopes of *Schistosoma mansoni* appear to compensate for the relatively low affinity of these monovalent epitopes for galectin-1.

Further quantitative measurements of lectins binding to "weak glycan epitopes" in polyvalent arrays are needed to understand the structure–activity relationships in this area.

Conclusions

Evidence shows that binding of host lectins including C-type lectins, siglecs, and galectins to the glycans of foreign pathogens is involved in the innate immune response of metazoans. The glycan epitopes expressed by foreign pathogens are often expressed by the host, which raises the question of the molecular basis of pattern recognition of these epitopes by host lectins. Evidence reviewed in this article suggests that density-dependent glycan binding is a common mechanism of recognition of host lectins to host glycans and the glycans of foreign pathogens (Figure 7 and 8).

Quantitative studies show that lectin binding to glycans is sensitive to glycan density and the number of glycan epitopes (Figure 8). The binding of lectins to high density and the number of glycans can lead to threshold binding effects (Orr et al. 1979) and very large increases in affinity ($\sim 10^6$-fold) (Lee and Lee 2000; Dam and Brewer 2007) and cross-linking interactions (Dam et al. 2009), which can result in a variety of

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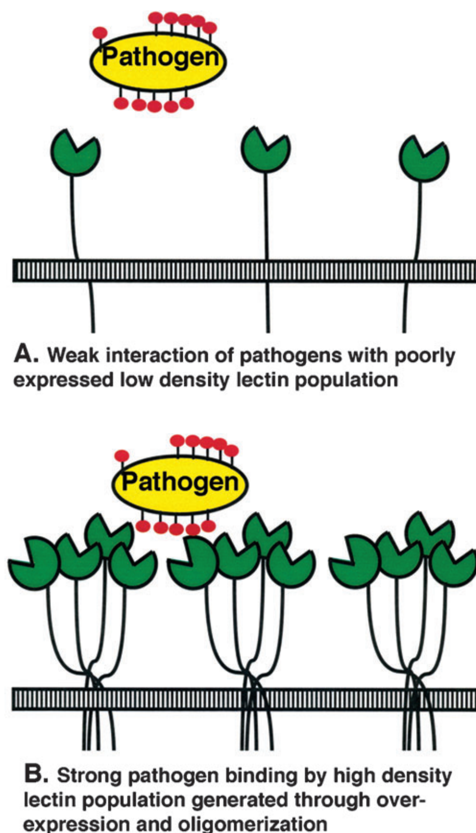

Fig. 9. Model of density-dependent lectin expression on cells and pathogen recognition. In (**A**) low density lectin expression leads to little pathogen binding, and (**B**) high density lectin expression results in pathogen binding and colonization.

"effector" functions in the host (Brewer et al. 2002; Marth and Grewal 2008) as well as pathogenic organism (van Kooyk and Rabinovich 2008; Vasta 2009). Two mechanisms are identified for lectins binding to density dependent glycans: (1) binding and internal diffusion of lectins such as galectins among a dense population of glycans (Figure 4A and B) (Dam and Brewer 2008), and (2) binding of multiple CRD domains of lectins such as soluble C-type lectins to clustered glycan epitopes (Figure 4C) (Weis et al. 1998). The latter mechanism is likely to utilize the first mechanism as well.

Several classes of host lectins that include C-type lectins, siglecs, and galectins are currently defined as PRRs in innate immunity. Evidence indicates that the "pattern" recognized by these host lectins is the high density expression of a glycan epitope on the surface of host cells and foreign pathogens (Figure 7). In many cases, the glycan epitope expressed on the foreign pathogen is a low affinity epitope not found on the host. Host lectins may bind to such "weak epitopes" due to large increases in the affinity of the lectin for polyvalent displays of the glycan by either of the two mechanisms discussed above.

In addition to density-dependent glycan expression, the "threshold" for glycan recognition is also a function of the concentration of the lectin in solution (Rando et al. 1979), such as galectins, and the density of membrane-bound lectins, such as C-type lectins and siglecs (Figure 9) (Vasta 2009). Depending on the concentration or density of host lectins, antagonistic or synergistic activation of pathogen signaling pathways may occur, as well as modulation of immune responses to the pathogen (Vasta 2009). Thus, both density-dependent glycan and lectin expression are important determinants of the innate immune response and host glycan/lectin interactions (Figures 8 and 9).

The above observations suggest that essentially all lectins, whether from animals, plants, or microorganisms, are PRRs, where pattern (often mentioned as glycocode) is defined by the density and total number of glycan epitopes on glycoprotein receptors on the surface of cells. Indeed, the binding of three lectins to eight different carbohydrates conjugated at different densities to BSA in microarray assays support this conclusion (Oyelaran et al. 2009). The same study also demonstrates that binding of anti-carbohydrate antibodies is also sensitive to the density of glycan epitopes (Oyelaran et al. 2009). This suggests that the concept of immunosurveillance by antibodies has as one of its mechanisms "screening" for altered epitope densities in host and foreign cells. Furthermore, a recent review of theoretical and experimental data (Dam and Brewer 2008) suggests that density-dependent ligand–receptor interactions are a general mechanism throughout biology.

Lastly, a new definition of lectin receptor in biological systems emerges that considers the density and number of glycan epitopes on the surface of cells and not just the affinity of single epitopes. Indeed, the concept of single dissociation constants (Kds) for lectins binding to monovalent glycans in solution is replaced by relative Kd's that depend on the density and number of glycans on a surface (Oyelaran et al. 2009). A large range of relative Kd values is also observed as increasing negative cooperativity in the binding of lectins to multivalent glycoproteins as the density of free glycan epitopes decreases with increased binding (Dam and Brewer 2008). Thus, there is no single Kd that describes the affinity of lectins (or antibodies) to multivalent carbohydrates and surface glycans, but instead relative Kd(s) that are dependent on the density and number of glycans on a molecule or surface. This, in turn, impacts the concept of avidity, which describes the affinity of ligands for multivalent epitopes, including lectins for multivalent glycans. The present analysis suggests that the term avidity be replaced by relative affinity for all multivalent binding interactions.

Abbreviations

ConA, concanavalin A; CRD, carbohydrate recognition domain; DGL, *Dioclea grandiflora* lectin; Fd-PSM, fully carbohydrate decorated porcine submaxillary mucin; Fuc, L-fucose; Gal, D-galactose; GalNAc, *N*-acetyl-D-galactosamine; GlcNAc, *N*-acetyl-D-glucosamine; ITC, isothermal titration microcalorimetry; LacNAc; *N*-acetyl-D-lactosamine; NeuNGl, *N*-glycolylneuraminic acid or sialic acid; PAMPs, pathogen-associated molecular patterns; PRRs, pattern recognition receptors; PSM, porcine submaxillary mucin; SBA, soybean agglutinin; Tn-PSM, porcine submaxillary mucin containing GalNAcα1-*O*-Ser/Thr residues.

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