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Lectin-carbohydrate interaction in the immune system

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Abstract

The immune system consists of various types of cells and molecules that specifically interact with each other to initiate the host defense mechanism. Recent studies have shown that carbohydrates and lectins (carbohydrate-binding proteins) play an essential role in mediating such interactions. Both lectins and carbohydrates are widely distributed in the mammalian tissues as well as in microorganisms. Carbohydrates, due to their chemical nature, can potentially form structures that are more variable than proteins and nucleic acids. Lectins can exist in either soluble or cell-associated form, and although overall structures vary, invariably possess carbohydrate-recognition domains (CRD) with various specificities. The interaction between lectins and carbohydrates have been shown to be involved in such activities as opsonization of microorganisms, phagocytosis, cell adhesion and migration, cell activation and differentiation, and apoptosis. The number of lectins identified in the immune system is increasing at a rapid pace. The development in this area has opened a new aspect in studying the immune system, and at the same time, provided new therapeutic routes for the treatment and prevention of disease.

Abbreviations. CL-43, collectin-43; CRD, carbohydrate-recognition domain; CRP, C-reactive protein; FGF, fibroblast growth factor; Fuc, fucose; Glc, glucose; GlcNAc,N-acetylglucosamine; Gal, galactose; GalNAc, N-acetylgalactosamine; Man, mannose; IL, interleukin; Lewis^x, Galβ1-4(Fucα1-3)GlcNAc; Lewis^b, Fucα1-2Galβ1-3(Fucα1-4)GlcNAc; ManNAc, N-acetylmannosamine; NK, natural killer; MBP, mannose-binding protein; MIP-1, macrophage inflammatory protein-1; NeuNAc, N-acetyl-neuraminate; PF-4, platlet factor 4; SAP, serum amyloid P component; SP-A, surfactant protein-A; SP-D, surfactant protein-D; TGF-β, tumor growth factor-β

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1. Overview

Carbohydrates are widely distributed in animal tissues where they have been traditionally viewed as structural components and the source of nutrients. Research in the last 10–15 years however, has shown that carbohydrates have important biological functions, being involved in diverse biological processes such as cell adhesion, cell activation, inflammation, tumor cell metastasis, and apoptosis. The driving forces behind this progress are the studies on biochemistry and biology of carbohydrate–protein interaction which have demonstrated the important role of carbohydrates as recognition molecules involved in many biological processes (Sharon and Lis, 1989; Varki, 1993). The ligands of carbohydrates are called lectins which are defined as carbohydrate-binding proteins other than antibodies or enzymes (Barondes, 1988). A new discipline, glycobiology, has been established and devoted to the studies of biological functions of carbohydrates and lectins. Rapid progress is being made in this field, which is pushing carbohydrates to the forefront of biological science, a position equal to proteins and nucleic acids as important molecules in all living things.

This review is intended to summarize and discuss the recent developments in this field (lectin–carbohydrate interaction) with emphasis on the immune system and how the new information generated has been used in treating diseases.

2. Structural features of carbohydrates

The structure of carbohydrates is complex, more so than that of proteins or nucleic acids. This complexity originates from the following facts: (1) sugar residues can be bound together at three or four different positions; (2) two sugars can be linked together in two isomeric forms, the α- and β-linkages; (3) the carbohydrate chains can have branches, a characteristic separating carbohydrates from other biological macromolecules.

Carbohydrates also can exist in various forms, i.e. as pure carbohydrates, glycoproteins or glycolipids. A term "glycoconjugate" has been used to describe in general carbohydrates conjugated to other molecules like proteins or lipids. Different types of cells may express different carbohydrate structures. In addition, the size of a sugar residue is larger than an amino acid. Thus, a few oligosaccharide chains attached to a glycoprotein can cover a considerable surface area. This implies that the cell surface oligosaccharides are among the first to encounter nearby molecules. All these point to the fact that carbohydrates can serve as functional domains with potentially limitless variability.

Carbohydrates in glycoproteins have been studied most extensively. They can be divided into two major groups: N-glycans which are linked to proteins through the amide group of asparagine and O-glycans which are linked to proteins through hydroxyl groups of serine or threonine. O-glycans are also referred as mucin-type glycans because they were first found in mucin. Both N- and O-glycans can be further divided into subgroups. In N-glycans, for example, there are high mannose, hybrid and complex types. However, it should be noted that tremendous variations exist in the structures of

glycans (Fukuda, 1994). A special type of glycan which is composed of a disaccharide repeat is called glycosaminoglycan. One of the two sugars in the repeating unit is always an amino sugar (N-acetylglucosamine (GlcNAc) or N-acetylgalactosamine (GalNAc)). Glycosaminoglycans include heparin, hyaluronic acid and several other members, and are usually attached to proteins as proteoglycans.

3. Lectins and their classifications

Lectins exist in almost all living organisms. They were first identified before the turn of this century as plant proteins capable of agglutinating red blood cells. Most lectins exist in an oligomeric form. As a result they can bind to their ligands on several different cells and so agglutinate them. Mammalian lectins can be divided into C-, S-, P- and I-type lectins based on the structure of their carbohydrate-recognition domain (CRD) (Table 1) (Drickamer, 1994; Powell and Varki, 1995). The CRD mediates the binding of lectins to carbohydrates and is highly conserved for each lectin type.

Table 1
Mammalian lectin families and their members

Lectin family	Number of members	Examples of members	Cell types or location
C-type	> 20	Collectin	Plasma
		Mannose receptor	Macrophages
		NKR-P1	NK cells
		CD69	Lymphocytes
		Selectin	Endothelial cells and leukocytes
S-type	> 4	Galectin 1	Muscle, neuron, kidney and others
		Galectin 3	Macrophages, basophils, mast cells, and others
P-type	2	46 kDa and 300 kDa mannose-6-phosphate receptors	Mainly intracellular
I-type	> 5	CD22	B cells
		CD33	Myeloid cells
		Sialoadhesin	Macrophage
Heparin-binding proteins	> 20	FGF	Plasma and/or cellular matrix
		IL-8	
		MIP-1	
		Fibronectin	
		Vitronectin	
Hyaluronan-binding proteins	> 5	CD44	Leukocytes and others
Pentraxin	> 5	CRP	Plasma
		SAP	Plasma

The C-type lectins are probably the most diverse group with respect to protein structure, location and sugar-binding specificities. They include members such as collectins in plasma and selectins on endothelial cells and leukocytes. They can be divided into five subgroups based on protein structure features (Drickamer, 1994). The activities of the C-type lectins require the presence of calcium.

The S-type lectins consist of β-galactoside binding lectins, now called galectins (Barondes et al., 1994). So far, at least four galectins (galectin-1, -2, -3 and -4) have been identified, and galectin-1 and -3 are the best studied. They depend on reducing agents (thiols) for full activity. Galectins lack the typical secretion peptide signals and transmembrane domains. However, they are secreted and expressed on the cell surface (Sato and Hughes, 1994). The association with the cell surface is not well understood, but likely achieved by binding to cell surface galactose residues. The externalization of galectins is carried out through the so-called non-classical secretory pathway that is also used by fibroblast growth factor (FGF) and a few other proteins (Sato and Hughes, 1994). Galectin-1, -2 and -4 have two lectin domains, whereas galectin-3 has only one (Fig. 1(a)). Galectin-1 and -2 are homodimers and galectin-4 is composed of two lectin domains linked together with a short peptide chain.

The I-type lectins are a newly established group and include the lectins binding to

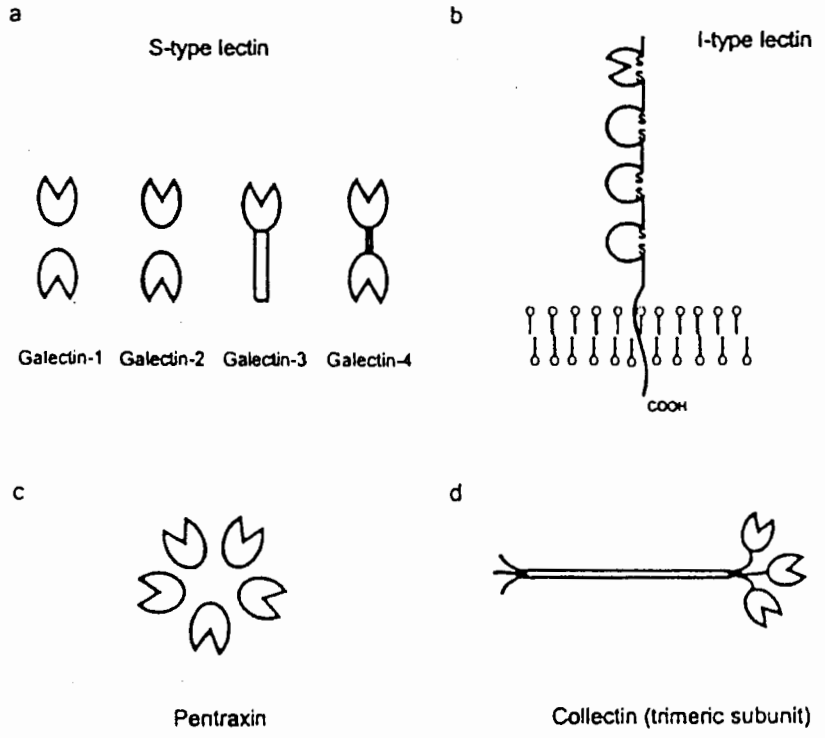

Fig. 1. Schematic of the overall structures of S-type lectin, I-type lectin, pentraxin and collectin (a group of C-type lectins). The oval depicts the globular domain and the CRD is illustrated by a triangular opening on the oval.

sialic acids (Powell and Varki, 1995). Structurally, I-type lectin belongs to the IgG superfamily and the CRD is located on the distal amino-terminal Ig domain (Nath et al., 1995) (Fig. 1(b)). This group now has at least five members, including CD22 and sialoadhesin (Powell and Varki, 1995). The P-type lectin group has only two members, 46 kDa and 300 kDa mannose-6-phosphate binding proteins, which share a common sequence motif. They are found in the Golgi apparatus as well as the plasma membrane in the case of the 300 kDa form, being responsible for transferring newly synthesized glycoproteins to lysosomes and internalization of extracellular glycoproteins.

In addition to the four groups described above, mammalian lectins also include the heparin-binding proteins, hyaluronan-binding proteins, pentraxins, and several others (Table 1) (Drickamer, 1994; Powell and Varki, 1995). The heparin-binding proteins may be the largest group of lectins with at least 20 members including cytokines, growth factors, cellular matrix proteins, and cell surface membrane proteins. The structures of these proteins vary widely. However, a domain characteristically containing a cluster of basic amino acids is always responsible for the binding to heparin by interacting with the negative charges on the latter (sulfate group and uronic acid) (Cardin and Weintraub, 1989; Margalit et al., 1993; Faham et al., 1996).

The pentraxin and hyaluronan-binding protein groups each have about five members. Pentraxins include acute-phase proteins like the classical C-reactive protein (CRP). The pentraxin molecules are homopentamers arranged in a cyclic pentameric symmetry (Fig. 1(c)).

4. Lectins and their carbohydrate ligands in the immune system

4.1. Soluble lectins

4.1.1. Collectins

A group of soluble C-type lectins collectively termed collectins are found in plasma and other body fluids (Holmskov et al., 1994; Malhotra et al., 1994a; Reid and Turner, 1994). They serve as an important part of the innate immune system. Five members, conglutinin, mannose-binding protein (MBP), pulmonary surfactant proteins (SP-A and SP-D) and collectin-43 (CL-43) have been identified (Table 2). MBP is the best studied. Conglutinin and CL-43 so far have only been found in bovids.

Collectins are oligomers of trimeric subunits (Fig. 1(d)). The size of the subunit polypeptides varies among the members and ranges from 28 to 47 kDa (Table 2). The C-terminal portion contains the CRD, and the N-terminal portion consists of the collagen-like domain which allows three molecules to twist into triplets. The trimeric subunits are linked together through disulfide bonding or non-covalently into oligomers. The fully assembled collectins range in size from 600 to 1000 kDa. The structure of collectins resembles that of C1q; both have a N-terminal collagenous domain and a globular C-terminal domain. The difference is that the C-terminal portion of C1q has an immunoglobulin binding domain, instead of a CRD. Competition studies with monosaccharides indicate that the sugar binding specificities vary subtly among the members (Table 2). It should be noted that although collectins can bind to monomeric sugars,

Table 2
Collectins and their biochemical and biological properties

Characteristics	MBP	SP-A	SP-D	Conglutinin	CL-43
Molecular weight of the subunit polypeptide (kDa)	28	28–36	42–44	42–44	42–44
Number of trimeric subunit in mature structure	3–6	6	4	4	1
Sugar preference	GlcNAc Man	ManNAc Man	Maltose Fuc	GlcNAc Man	Man ManNAc
Complement activation	+	–	–	–	–
Binding to C1q receptor	+	+	–	+	+
Binding to influenza virus	+	+	+	+	not done

their natural high-affinity ligands are likely those sugars linked together as part of oligosaccharide chains, e.g. those on bacterial surfaces. Such ligands for collectins in general have not been well defined, although MBP has been shown to preferentially bind to the N-linked bi-antennary complex type oligosaccharides containing two terminal GlcNAc and high mannose type oligosaccharides (Childs et al., 1990). Oligosaccharides with various kinds of sugars are widely distributed in mammalian systems. It is therefore the subtle recognition of foreign natural carbohydrate ligands that makes collectins a part of the host defense systems, i.e. attacking foreign invaders but not self.

The terminal domains of the collectins serve distinct functions; the C-terminal lectin domain mediates the binding to various microorganisms and the N-terminal collagen-like domain interacts with cells and complement components thereby exerting the biological effect. MBP, after binding to bacteria, can mediate the killing of bacteria by activating complement. It interacts with and activates C1r and C1s without the involvement of C1q, essentially substituting for C1q (Lu et al., 1990). Other studies have suggested that a MBP-associated serine protease (MASP) is responsible for complement activation (Matsushita and Fujita, 1992). MBP constitutes a new mechanism of complement activation (lectin pathway), independent of the classical and alternative pathways (Holmskov et al., 1994). Other collectins are so far not known to activate complement (Table 2).

Collectins bind to cells through C1q receptors, which are present on many cell types, including most leukocytes, platelets, endothelial cells, and fibroblasts (Malhotra et al., 1990). The binding to bacteria by MBP facilitates their phagocytosis by macrophages through interaction with the macrophage surface C1q receptor (Fig. 2) (Tenner et al., 1995). That is, the collectins, like antibodies, also act as opsonins. In the case of viruses, binding by collectins to virion surface oligosaccharides prevents viruses from entering target cells. Conglutinin, MBP, SP-A and SP-D have been shown to inactivate influenza virus in this manner (Anders et al., 1990; Malhotra et al., 1994b; Malhotra and Sim, 1995).

The reaction of collectins to infections or injury is immediate, unlike antibodies whose reaction takes at least one to three days. They are especially important to children and young animals when maternal immunity has declined and whose immune system is not fully capable of mounting an efficient humoral response. A deficiency in MBP

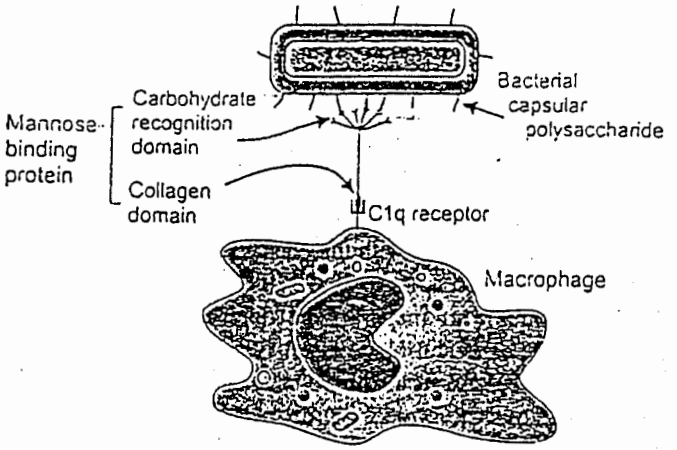

Fig. 2. MBP mediates the phagocytosis of bacteria by macrophages. The CRD of MBP interacts with bacterial surface carbohydrates and the collagenous domain interacts with the C1q receptor on macrophages.

renders children highly susceptible to infections, and has been claimed to account for a significant number of immunodeficiency cases in children (Soothill and Harvey, 1976; Candy et al., 1980; Sumiya et al., 1991).

Bovine conglutinin, primarily produced in the liver (Lu et al., 1993), has been used as a genetic marker of innate immunity (Weigel et al., 1991; Detilleux et al., 1995). Its level in plasma decreases under disease conditions, e.g. mastitis and *Cowdria ruminantium* infection (Rose et al., 1978; du Plessis, 1985; Akiyama et al., 1992). Conglutinin has also been found in tissues, where it is primarily associated with macrophages, dendritic cells and glial cells (Holmskov et al., 1992). This suggests that conglutinin may also be associated with macrophage function.

4.1.2. Pentraxins

Pentraxins are a group of plasma proteins that include CRP, serum amyloid P component (SAP), and possibly a few other members (Steel and Whitehead, 1994; Gewurz et al., 1995). They are acute-phase proteins since synthesis of pentraxins increases upon stimulation by the products of inflammation and tissue injury such as IL-1 and IL-6. Pentraxins have been found in all vertebrates and in some invertebrates (Ying et al., 1992; Gewurz et al., 1995). They have multiple biological functions including activation of complement and stimulation of phagocytic leukocytes.

The pentraxin molecule is composed of non-covalently associated protein subunits arranged in cyclic pentameric symmetry (Fig. 1(c)). The size of the subunit ranges from 25 to 30 kDa. The three-dimensional structure of SAP has been solved and been shown to resemble Concanavalin A (Emsley et al., 1994). Pentraxins bind to multiple ligands in a calcium-dependent manner including polysaccharides (Tennent and Pepys, 1994). SAP has been shown to bind to galactose polymers and glycosaminoglycans (Hind et al., 1984; Tennent and Pepys, 1994). The ability to bind to multiple ligands corresponds to the multiple binding sites on the pentraxin molecules (Emsley et al., 1994).

Both CRP and SAP can activate the classical complement pathway by interacting with the collagenous domain of C1q (Jiang et al., 1992; Ying et al., 1993). They are also known to interact with neutrophils, monocytes/macrophages, and NK cells and augment their activities (Galve-de Rochemonteix et al., 1993; Mortensen, 1993; Landsmann et al., 1994). CRP has been shown to have an antitumor effect (Bama et al., 1993).

SAP plays an important role in the systemic amyloidoses and likely also in Alzheimer's disease (Gewurz et al., 1995; Tennent et al., 1995). SAP is a normal cellular matrix component associated with the elastic fibers and the glomerular basement membrane (Dyck et al., 1980; Breathnach et al., 1981). The coating of these components by SAP may protect them from inappropriate degradation. SAP is also a universal component of amyloid deposits and binds to all types of amyloid fibrils. It is required for the formation of amyloid fibrils from β2 microglobulin (Ono and Uchino, 1994). The binding by SAP inhibits the proteolytic degradation of amyloid fibrils in systemic amyloidoses and Alzheimer's disease, thus contributing to the persistence of amyloid deposits (Hind et al., 1984; Tennent et al., 1995).

4.1.3. Cytokines

Cytokines include a large number of soluble factors that are important regulators or effectors of the immune system. Many of them possess the binding activity to carbohydrates, notably to heparin. They include IL-8, MIP-1, PF-4, TGF-β and various growth factors.

Heparin is a polymer of sulfated disaccharide repeats (hexouronic acid and glucosamine) (Casu, 1989), which normally exists as glycosaminoglycan on the cell surface or on the cellular matrix. The interaction with heparin has been best studied with acidic (FGF-1) and basic (FGF-2) fibroblast growth factors (Burgess and Maciag, 1989; Gospodarowicz, 1991; Ishihara et al., 1994). The negative charges from heparin (sulfate groups and uronic acids) and the positive charges from the basic amino acids of the growth factors are the main factors in mediating the binding (Cardin and Weintraub, 1989; Margalit et al., 1993; Faham et al., 1996). The sequences of the binding domains on cytokines vary among different members, but always contain a cluster of basic amino acids. Due to variations in the extent and position of sulfation, different regions of a single heparin molecule may exhibit different protein binding specificities (San Antonio et al., 1993; Tyrrell et al., 1993).

The effect of heparin binding includes stabilization of the cytokine molecules, adsorption of them onto the cell surface or cellular matrix, and facilitation of their binding to the high-affinity receptors on the cell surface (Burgess and Maciag, 1989; Gospodarowicz, 1991; Witt and Lander, 1994). It has been suggested that chemokines like IL-8 first attach to the glycosaminoglycans on the endothelium and then are presented to neutrophils, initiating their transmigration (Webb et al., 1993; Witt and Lander, 1994).

4.1.4. Monosaccharide-specific antibodies

Polysaccharides are antigenic, especially when conjugated to a protein carrier. Polysaccharide epitopes can be conformational like protein ones. However, some of them seem not to be highly conformationally restricted. That is, the reaction of some

polysaccharide-specific antibodies can be inhibited by simple monosaccharides as demonstrated with monoclonal antibodies. Such monosaccharide-specific antibodies represent a unique group of carbohydrate-binding proteins and are at least in part responsible for the cross-reactivity of the polysaccharide antigens from bacteria of different serotypes (Szu et al., 1981; Ota et al., 1987; Takada et al., 1988) or even different microorganisms (Nnalue et al., 1994). Whether they can be classified as lectins is still debatable. Here we tentatively use the term monosaccharide-specific antibody to describe them.

Although monosaccharide-specific antibodies can be induced by immunization or infections, it seems more important that they can be naturally present in human and animals. Their presence is believed to be the result of humoral response to normal environmental antigens, e.g. normal bacterial flora in the intestine (Galili et al., 1988). One of the best examples is the anti-gal antibody, which is the most abundant natural antibody in human, accounting for ~1% of the circulating antibodies (Galili et al., 1984). These can be either IgG, IgM or IgA. They show a binding preference to galactose in the α-linkage, especially the Galα1-3Gal structure. Their binding to such antigens, however, can be inhibited by simple galactose (Galili et al., 1984, Galili et al., 1985; Wieslander et al., 1990). These antibodies are receiving increasing attention because of their role in pig-to-human xenotransplantation (Sandrin and McKenzie, 1994). The Galα1-3Gal structure is widely present in animals including pigs, but absent in humans (Galili et al., 1988). It is these anti-gal antibodies in humans that are responsible for the acute rejection of the transplanted pig tissues (Sandrin and McKenzie, 1994). The anti-gal antibodies are also responsible for the complement-dependent inactivation of animal C-type retroviruses by human serum (Takeuchi et al., 1996), providing an important mechanism for the lack of cross infection from animal to human. Such natural anti-gal antibodies have also been reported in several animal species including cattle and rabbit, although the fine specificity of these antibodies have not been defined (Hadge et al., 1987; Sugii and Hirota, 1990).

In addition to the anti-gal antibodies, antibodies specific for other monosaccharides have also been detected in normal individuals (Lalezari and Jiang, 1984; Summerfield and Taylor, 1986). In chicken, the major mannose-binding protein in serum and egg york has been identified as the chicken immunoglobulin molecule now termed IgY (Wang et al., 1985, Wang et al., 1986; Warr et al., 1995). Likewise, a GlcNAc-binding protein in chicken serum and egg york has also been identified as IgY (Hoppe et al., 1991). Recently, chickens have been reported to also have antibodies specific for GlcNAc, fucose, melibiose and lactose in their serum (Sugii and Hirota, 1993). The procedures used for the isolation of these carbohydrate-binding chicken antibodies are essentially the same as those used for the isolation of mammalian MBP and other collectins (Wang et al., 1986; Hoppe et al., 1991; Sugii and Hirota, 1993). However, the presence of collectin-like molecules in chicken serum or egg york has so far not been reported.

The presence of natural monosaccharide-specific antibodies appears to be universal. Evidence suggests that they play an important part of innate immunity (Takeuchi et al., 1996). In chickens, they may play an even more important role considering the probable lack of collectin-like molecules. A carbohydrate-binding antibody will essentially have

the same effect as a collectin, i.e. opsonizing microorganisms and activating complement and phagocytic cells. Thus, increasing the level of certain monosaccharide-specific antibodies by immunization with conjugated polysaccharides or monosaccharides may result in the enhancement of innate immunity.

4.2. Cell surface lectins

4.2.1. Phagocytosis

The primary function of macrophages and NK cells is to kill and/or phagocytose foreign organisms and malignant cells. Several lectins found on macrophages and NK cells participate in these processes. They include the mannose receptor and the mouse macrophage galactose/N-acetylgalactosamine-specific C-type lectin (MMGL) on macrophages, and NKR-P1 on NK cells. The macrophage mannose receptor is a 170 kDa C-type lectin present on macrophage surfaces as a membrane glycoprotein (Wileman et al., 1986). It possesses multiple CRDs (Taylor et al., 1992). The interaction of this receptor with mannose residues on microbial surfaces facilitates their phagocytosis (Sung et al., 1983; Blackwell et al., 1985). As discussed above, the opsonization of microorganisms by MBP is another way to enhance their phagocytosis by macrophages.

MMGL is a Gal/GalNAc-specific C-type lectin that is involved in the recognition of tumor cells by macrophages (Yamamoto et al., 1994; Sakamaki et al., 1995). A human analog of MMGL has recently been identified (Suzuki et al., 1996). The MMGL preferentially binds to highly branched N-glycans with terminal galactose residues and clusters of truncated O-glycans, which are characteristic of tumor cells (Springer, 1989). Expression of MMGL therefore allows macrophages to be tumoricidal. It is conceivable that MMGL may also contribute to the phagocytosis of microorganisms by macrophages.

NKR-P1 is a C-type lectin originally identified on rat NK cells that binds to GalNAc/GlcNAc (Bezouska et al., 1994a). It is a dimeric type II transmembrane protein. Its human (NKG2) and mouse (Ly-49) analogs have also been identified. These lectins are involved in cytotoxic killing of virus-infected and malignant cells (Chambers et al., 1993). Recently, several high-affinity oligosaccharide ligands for NKR-P1 have been identified (Bezouska et al., 1994b). These include blood group antigens and glycosaminoglycans. These oligosaccharide ligands are directly involved in the NK cell-mediated lysis of tumor cells.

4.2.2. Inflammation

A key component of inflammation is the emigration of leukocytes through vascular endothelium. This process is divided into three steps, rolling, attachment and migration (Carlos and Harlan, 1994). Selectins, the C-type lectins found on cell surfaces, play a key role in the rolling step in which leukocytes roll along the surface of vascular endothelium before establishing firm adhesion through their surface integrin molecules (Lowe, 1994; Varki, 1994). There are three types of selectins, L-selectin (CD62L) on leukocytes, E-selectins (CD62E) on endothelial cells, and P-selectin (CD62P) on both platelets and endothelial cells. All of them are membrane glycoproteins. They all specifically bind to a carbohydrate structure called sialyl Lewis^x (CD15s) although they

may also bind to other carbohydrates such as heparin (Varki, 1994). Recently, soluble E-selectin was found to be angiogenic (Kock et al., 1995). Angiogenesis is a normal part of the inflammation process. E-selectins can be shed from endothelial cells (Newman et al., 1993). This finding therefore has provided a direct link between leukocyte adhesion and angiogenesis.

Integrins are responsible for the firm adhesion of leukocytes to vascular endothelial cells (Carlos and Harlan, 1994). One key integrin involved in this step is MAC-1 (CD11b/CD18 or CR3). The major ligand of MAC-1 is ICAM-1, but MAC-1 is also known to bind to carbohydrates such as zymosan and bacterial lipopolysaccharide (Ross et al., 1985; Wright and Jong, 1986; Thornton et al., 1996). Recently, MAC-1 has been shown to bind to heparin, and such binding is involved in the firm adhesion of neutrophils to endothelial cells (Coombe et al., 1994; Diamond et al., 1995).

4.2.3. Cell activation and differentiation

Expression of lectins is often associated with cell activation and differentiation. This implies that these lectins are necessary for the function of activated or differentiated cells. Several lectins are expressed in this manner. They include the selectins, CD69, galectin-3 and the macrophage mannose receptor.

Selectins play an important role in leukocyte trafficking. At inflammatory sites, endothelial cells are exposed to numerous cytokines or chemokines. These agents stimulate endothelial cells to express or increase the expression of E-selectin, P-selectin and L-selectin which normally is only expressed on leukocytes (McEver et al., 1995). The expression or enhanced expression of these selectins on endothelial cells increases the adhesiveness of the endothelial cells and is directly responsible for recruitment of leukocytes to inflammatory sites.

CD69 is a GlcNAc/GalNAc-specific C-type lectin found on lymphocytes and other hematopoietically-derived cells (Testi et al., 1994; Bezouska et al., 1995). It is a dimeric membrane glycoprotein capable of signal transduction (Gerosa et al., 1991). Its expression in lymphocytes increases upon cell activation (Cosulich et al., 1987). CD69 is important in the maturation and proliferation of lymphocytes. It is expressed at the very early stage of lymphocyte differentiation in the thymus (Vanneaske et al., 1995).

Galectin-3 on macrophages, previously called Mac-2, is a differentiation and activation marker (Leenen et al., 1986; Nangia-Makker et al., 1993; Sato and Hughes, 1994). It is either not present or is present at a low level on unstimulated monocytes, but is abundantly expressed on thioglycolate-induced peritoneal macrophages and phorbol ester-treated monocytic cell lines such as HL-60. Galectin is probably important for the adhesion of macrophages to the cellular matrix since it is known to bind to laminin which contains galactose-rich oligosaccharide chains. As mentioned earlier, galectins can also be secreted. The soluble galectin-3 can cause superoxide release (Liu et al., 1995). Thus, galectin-3 may also act in an autocrine fashion. Like galectin-3, the mannose receptor is found on macrophages but not on monocytes (Ezekowitz et al., 1984).

Galectin-3 has also been identified on mast cells, eosinophils and neutrophils (Frigeri and Liu, 1992; Truong et al., 1993a, Truong et al., 1993b). It binds to IgE and so has previously been called the IgE-binding protein (εBP) (Frigeri and Liu, 1992). The

soluble galectin-3 can potentially form aggregates, thus achieving multivalence (Hsu et al., 1992). It has been postulated that galectin-3 in aggregated form may cross-link the receptor-bound IgE molecules on mast cells and eosinophils, causing cell activation and degranulation (Liu, 1993). On the other hand, the cell surface-associated galectin-3 can also bind to IgE, leading to cell activation as shown in the case of neutrophils (Truong et al., 1993b).

It is interesting to note here that the low-affinity IgE receptor (CD23), a transmembrane protein, is also a lectin (C-type). It is unique among the immunoglobulin receptors in that it is a lectin. Its sugar binding specificity has not been well characterized. However, it is the lectin domain that binds to IgE molecules. Besides binding to IgE, CD23 interacts with CD21(CR2) on B cells and MAC-1 (CD11b/CD18) and p150 (CD11c/CD18) on monocytes. These observations suggest that CD23 may be more important as a regulator of B cell and monocyte functions (Aubry et al., 1992; Lecoanet-Henchoz et al., 1995).

Members of the I-type lectin family, such as CD22 on B cells, CD33 on myeloid cells, and sialoadhesin on macrophages, have also been suggested to be involved in cell differentiation and signaling (Powell and Varki, 1995).

4.2.4. Apoptosis

Apoptosis, also referred to as programmed or physiological cell death, is common in the immune system. Most of the hemopoietic cells have a very limited life span with neutrophils surviving for only one day or less. They all die by apoptosis. A mannose/fucose-specific lectin has been suggested to be involved in the phagocytosis of apoptotic neutrophils by fibroblasts (Hall et al., 1994).

Soluble recombinant galectin-1 induces the apoptosis of activated T cells (Telford et al., 1992). This activity seems well correlated with its inhibitory effect on autoimmune disease by limiting the number of antigen-reactive T cells. Recently, galactin-1 expressed on endothelial cells has been shown to induce apoptosis of activated T cells (Perillo et al., 1995). CD45, a membrane glycoprotein expressed on activated T cells, is involved in the interaction with galectin-1; the oligosaccharides on this protein are rich in polylactosamine sequences (Galβ1-4GlcNAcβ1-3) that are preferentially recognized by galectin-1. Galectin-1 is expressed on the stromal cells of the thymus and lymph node. This finding therefore further indicates the direct role of galectin-1 in T cell development and maturation as well as modulation of the T cell immune response.

5. Carbohydrate-based therapeutics

The recognition of lectin–carbohydrate interaction has provided a new route for the development of carbohydrate-based therapeutics (Karlsson, 1991; Beuth et al., 1995). The lectin–carbohydrate interaction is involved in many pathological processes such as tumor cell metastasis (Muramatsu, 1993; Lotan et al., 1994), host–microbial interaction (Lentz, 1990; Ofek and Doyle, 1994; Karlsson, 1995), and inflammation (Lowe, 1994; Varki, 1994). By blocking such glycobiological interactions in these processes, carbohydrates can potentially be of therapeutic use. For example, synthetic sialylated Lewis^x-

containing oligosaccharides have been designed to disrupt selectin-sialylated Lewis^x interaction during leukocyte migration. Some of them have been found to be effective in blocking the binding of leukocytes to the surface of endothelial cells, and also in preventing inflammation under *in vivo* conditions (Mulligan et al., 1993; Burke et al., 1994; Han et al., 1995). Some other carbohydrates, e.g. heparin (Nelson et al., 1993), have also been found to be strong antiinflammatory agents. Heparin is known to interact with selectins (Imai et al., 1991; Varki, 1994). In veterinary medicine, a polysulfated glycosaminoglycan has been used to treat equine synovitis (Yovich et al., 1987; White et al., 1994).

Carbohydrates can also be used to engage specifically the cell-associated lectins thereby stimulating cellular functions. Complex carbohydrates such as yeast β-glucan, inulin and lentinan have long been reported to have immunostimulating and/or adjuvant effects (Chihara, 1992; Williams et al., 1992; Cooper et al., 1993). These effects probably result from their ability to interact with lectins on the immune cells such as macrophages thereby stimulating their functions. Yeast β-glucan has been reported to interact with MAC-1 (CD11b/CD18; CR3) of macrophages (Thornton et al., 1996). It has been noted that insoluble or particulate carbohydrates are better adjuvants than the soluble ones (Cooper et al., 1993). This may be due to enhancement of phagocytosis of the antigens (Falo et al., 1995).

6. Conclusion

The information reviewed here clearly shows that the interactions between carbohydrates and lectins are an integral part of host defense. This has thus opened up a new component of the immune system with fundamental and practical implications. Many known immunological processes have been found to have a basis in such glycobiological interactions. Future studies will certainly reveal more roles played by carbohydrates and lectins and provide more clues for designing therapeutics based on their interactions.

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