Lectins as Cell Recognition Molecules

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Lectins on cell surfaces mediate cell-cell interactions by combining with complementary carbohydrates on apposing cells. They play a key role in the control of various normal and pathological processes in living organisms.

RECOGNITION IS A CENTRAL EVENT IN A VARIETY OF biological phenomena and the first step in numerous processes based on cell-cell interactions, such as fertilization, embryogenesis, cell migration, organ formation, immune defense, and microbial infection. Improper functioning of cell recognition may cause disease. Thus, defects of leukocyte and platelet adhesion result in recurring bacterial infections and mucosal bleeding, respectively. Furthermore, aberrant cell recognition is thought to underlie the uncontrolled cell growth and motility that characterize neoplastic transformation and metastasis. An understanding of the molecular basis of the cell-surface code, therefore, has implications for intervention in many areas of biology and medicine.

There is little doubt that the high selectivity necessary for the processes mentioned, as well as in related ones, is generally provided by a specific, stereochemical fit between complementary molecules, one a carrier of biological information and the other capable of decoding such information. Cell recognition is thus another aspect of the fundamental concept of lock-and-key complementarity (*1*), which was originally formulated by Emil Fischer in 1897 to account for the specific interactions between enzymes and substrates (that is, molecules in solution). This hypothesis was extended by Paul Ehrlich (in 1900) and Frank Lillie (in 1914) to describe the interactions of cells with soluble molecules and with other cells, respectively. Thus, by the 1920s, the lock-and-key hypothesis had become one of the central theoretical assumptions of cellular biology. The nature of the molecules involved in cellular recognition is still largely a mystery. During the last two decades much attention has been focused on the possibility that such recognition is mediated by carbohydrates and lectins, a class of proteins of nonimmune origin that bind carbohydrates specifically and noncovalently (*2–4*).

In the 1970s it became well established that almost all cells carry carbohydrates on their surfaces in the form of glycoproteins, glycolipids, and polysaccharides (*5*). Concurrently it was realized that carbohydrates have an enormous potential for encoding biological information (*5–7*). In peptides and oligonucleotides, the information content is based only on the number of monomeric units and their sequence, whereas in carbohydrates, information is also encoded in the position and anomeric configuration (α or β) of the glycosidic units and in the occurrence of branch points. Therefore, two molecules of a single monosaccharide (for example, glucose) can join to form 11 different disaccharides, but two molecules of a single amino acid or a single nucleotide can only form one dipeptide or one dinucleotide, respectively. More impressively, four different monosaccharides can form 35,560 distinct tetrasaccharides, whereas four different amino acids or nucleotides can form only 24 tetrameric structures (*8*). Further structural diversification may occur by covalent attachment of sulfate, phosphate, and acetyl groups to the sugars. Thus, in theory, an enormous number of compounds can be derived from a relatively limited number of monosaccharides, leading to the hypothesis that "the specificity of many natural polymers is written in terms of sugar residues and not of amino acids or nucleotides" (*6*, p. 26). The increasingly refined analytical methods used in recent studies have revealed a great diversity of carbohydrate structures associated with soluble and surface-bound glycoconjugates. There are strong indications that this diversity is biologically significant, since in many cases carbohydrates modify the activities of proteins to which they are attached and also serve as markers of cell differentiation, development, and pathological states (*9*).

Although the existence of lectins has been known for more than 100 years (*10*), the idea that they may act as recognition molecules is of recent origin (*11*). It was inspired by the realization that surface carbohydrates may function in cell recognition and was stimulated by advances in lectin research (*2–4*). In particular, it was demonstrated that these proteins are not confined to plants, as originally believed, but are ubiquitous in nature, being frequently found on cell surfaces and intracellular particles. Several membrane-bound lectins were shown to participate in the selective uptake of glycoproteins into cells or in the intracellular trafficking of glycoproteins (*12, 13*). In addition, characteristic changes in lectin expression that coincide with distinct physiological or pathological changes in the life of cells or tissues were observed. The specificity of lectins proved to be much more exquisite than originally assumed, since they not only distinguish between different monosaccharides, but also specifically bind to oligosaccharides, detecting subtle differences in complex carbohydrate structures. Finally, lectin-carbohydrate interactions satisfy additional requirements expected of a cellular recognition system, such as speed and reversibility.

Typically, the lectin and the complementary carbohydrate are located on the surfaces of apposing cells, which may be of the same type or of different types (Fig. 1). Cells may also interact via bridges formed by soluble glycoproteins that bind to the cell surface lectins. Alternatively, the lectins may combine with carbohydrates of insoluble components of the extracellular matrix that promote cell-substrate adhesion. In addition, soluble lectins may act as bridges by binding to carbohydrates on apposing cells.

Various experimental approaches are being used to demonstrate the participation of lectins in cell recognition (*14*). Many are indirect

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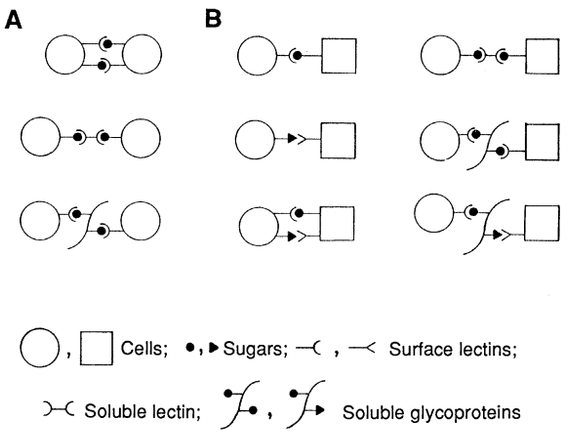

Fig. 1. Different modes of cell-molecule and cell-cell interactions mediated by lectins (**A**) between cells of the same kind (homotypic) and (**B**) between cells of different kinds (heterotypic). [Reprinted from (*4*) with permission, © 1989 Chapman and Hall]

and based on the assumption that whenever such recognition is carbohydrate-dependent, a lectin must be involved. The carbohydrate dependence of a cell-cell interaction can be demonstrated by methods such as inhibition by sugars or glycoconjugates, enhancement or inhibition by enzymatic or chemical modification of cell-surface carbohydrates, or the use of mutant cells—for example, lectin-resistant mutants with altered composition or structure of surface carbohydrates. However, such evidence does not by itself exclude the participation of other types of carbohydrate-binding protein—for instance, cell surface glycosyltransferases—in cell recognition (*15*). To obtain direct evidence, it is essential to isolate the putative lectin and its ligand and establish that they indeed take part in the recognition phenomenon. This should include a demonstration of their surface location and of the ability of the purified lectin and ligand, as well as of antibodies to the lectin and to the ligand, to specifically block the interaction between cells. In addition, changes in the amount of the lectin should be closely linked to physiological events for which cell-cell recognition is required. Besides carbohydrate-binding sites, lectins may contain sites specific for noncarbohydrate ligands, and these sites, too, may be critical for the recognition functions of the lectin (*16*).

In this article we discuss several systems in which there is considerable evidence for the roles of lectins: in viruses, bacteria, and protozoa in the infectious process; in slime molds in cell differentiation; in plants in host-bacteria symbiosis; in animals in uptake and killing of cells, in cell differentiation, organ formation, lymphocyte migration, and metastasis. The implications of the knowledge obtained—for example, for the prevention of infection and the targeting of drugs—are briefly discussed. A listing of lectins thought to be involved in cell-cell recognition is given in Table 1.

Viruses

The oldest, and perhaps best characterized, lectin-carbohydrate recognition system is that governing the interaction of influenza viruses with their target cells (*17*). The ability of the virus to agglutinate erythrocytes has been known since 1941. It took more than a decade before it was shown that the human virus binds to erythrocytes and other cells by recognizing *N*-acetylneuraminic acid, one of the sialic acids, present on the cell surface and that this binding is a prerequisite for initiation of infection. Subsequently, the viral hemagglutinin (lectin) responsible for this binding was purified, crystallized, and studied in detail, culminating in the elucidation of its interaction with *N*-actylneuraminic acid–containing oligosaccharides at the atomic level. The subunit of the lectin is composed of two polypeptides, HA_1 and HA_2 (with molecular masses of 36 and 26 kD, respectively), covalently linked by a disulfide bond, and it associates noncovalently to form trimers that are located on the surface of the viral membrane. The carbohydrate-binding site forms a pocket located in a domain of the lectin protruding from the membrane and is composed of amino acids that are largely conserved in the numerous strains of the virus. Other conserved residues are found behind the pocket and seem to stabilize the architecture of the site without interacting with the carbohydrate.

More than 100 strains of influenza virus, mostly of the A and B types, were examined for their ability to bind to enzymatically modified erythrocytes carrying terminal *N*-acetylneuraminic acid attached to galactose either by an $\alpha 2 \rightarrow 3$ or $\alpha 2 \rightarrow 6$ linkage. Differences in their specificity with respect to this linkage were correlated with the species origin of the virus. Thus, human isolates preferentially agglutinated resialylated erythrocytes containing the NeuAcα2→6Gal sequence, whereas the avian and equine isolates

Table 1. Lectins involved in cell recognition.

Source	Molecular size of subunit (kD)	Sugar specificity	Function
Influenza virus	62*	NeuAcα2→6Gal NeuAcα2→3Gal	Initiation of infection
Escherichia coli†			
Type 1	28	Oligomannose	Initiation of infection; lectinophagocytosis
Type P	35	Galα1→4Gal	Initiation of infection
Type S	12	NeuAcα2→3Gal	Initiation of infection
Entamoeba histolytica	260‡	Galβ1→4GlcNAc	Initiation of infection
Dictyostelium discoideum	28	Gal/GalNAc	Control of differentiation
White clover (*Trifolium repens*)	50	2-Deoxyglucose	Binding of nitrogen-fixing rhizobia
Human, rat, and rabbit macrophages	175	Mannose	Lectinophagocytosis
Mouse peritoneal lymphocytes	80–90	Man-6-P	Homing of lymphocytes
Chicken thymus	15	β-Galactosides	Control of thymocyte maturation
Elicited mouse peritoneal macrophages	45–60	Gal/GalNAc	Killing of tumor cells
Rat cerebellum	31.5	Mannose	Myelin compaction
Melanoma and other cancer cells	34	β-Galactosides	Metastasis

*Composed of S-S–linked polypeptide chains of 36 and 26 kD. †The *E. coli* lectins listed are in the form of fimbriae, which consist of linear assemblies of subunits of different types; only the molecular sizes of the carbohydrate-binding subunits are given. ‡Composed of S-S–linked polypeptide chains of 35 and 170 kD.

exhibited preference for NeuAcα2→3Gal. Strains of influenza C virus [as well as coronaviruses (*18*)] do not bind at all to *N*-acetylneuraminic acid but only recognize another sialic acid, 9-*O*-acetyl-*N*-acetylneuraminic acid; the 9-*O*-acetyl group is critical for mediating cellular attachment.

Comparison of the primary sequences of hemagglutinins of the human virus with those of mutants showing decreased affinity for NeuAcα2→6Gal and markedly increased affinity for NeuAcα2 →3Gal revealed that they differ in a single amino acid substitution, leucine at position 226 in the parental strains being replaced by glutamine in the mutants. Similar studies with avian isolates and their variants showing the reverse change in specificity (from α2 → 3-linked to α2 → 6-linked *N*-acetylneuraminic acid) again revealed a single amino acid substitution at position 226—from glutamine to leucine. This illustrates that a single amino acid substitution can alter the sugar specificity of a lectin. Although residue 226 is located in the carbohydrate-binding site of the influenza virus hemagglutinin, it is not in direct contact with the bound sugar, as shown by crystallographic studies (*19*) of wild-type influenza virus hemagglutinin complexed with NeuAcα2 →6Galβ4Glc and of a mutant hemagglutinin complexed with NeuAcα2→3Galβ4Glc (Fig. 2). The suggestion has therefore been made that the change in specificity is due to conformational differences between the mutant and wild-type proteins.

The role of the hemagglutinin in initiating infection by influenza virus has been convincingly demonstrated. The binding of the lectin to sialic acid–containing carbohydrates on the surface of the target cells leads to the attachment of the virus to the cells. This results in fusion of the viral and cellular membranes, allowing release of the viral genome into the cytoplasm and subsequent replication. Removal of sialic acid from the cell membranes by sialidase abolishes binding and prevents infection, whereas enzymatic reattachment of sialic acid or insertion of sialic acid–containing oligosaccharides (for example, in the form of glycolipids) into the membranes of sialidase-treated cells restores the ability of the cells to bind the virus and to be infected by it. The detailed knowledge of the sialic acid–hemagglutinin interaction provides a possible basis for the design of antiviral drugs that would block viral attachment to cells. An inhibitor targeted to the conserved amino acids of the combining site or to the part of the cellular receptor essential for interaction with the hemagglutinin might be effective against influenza viruses of all subtypes. It would be independent of the antigenic changes that accompany the recurrent epidemics for which these viruses are renowned.

Bacteria

Many bacterial species have the ability to produce surface lectins. In enterobacteria (for example, *Escherichia coli* and *Salmonellae* spp.) and in several other species, the lectins are commonly in the form of submicroscopic hairlike appendages known as fimbriae (pili) that protrude from the surface of the cells (*20–22*). Fimbriae are usually 5 to 7 nm in diameter and 100 to 200 nm in length. The best characterized are type 1 (mannose-specific) fimbriae of *E. coli*, which preferentially bind oligomannose and hybrid oligosaccharides of animal cell surface glycoproteins, and P fimbriae, also of *E. coli*, which interact specifically with glycolipids containing Galα1→ 4Gal. Other examples are S fimbriae of *E. coli*, specific for NeuAcα2 →3Gal, and type 2 fimbriae of oral actinomyces, specific for Galβ1 →3GalNAc, disaccharides found in both animal glycoproteins and glycolipids. Purified fimbriae each consist of several hundred fimbrillin (or pilin) subunits of different sizes, most of which have a molecular mass in the range of 14 to 22 kD. The carbohydrate-binding sites of P, S, and type 1 fimbriae reside in minor subunit constituents with molecular sizes of 28 to 35 kD, located at the tips of the fimbriae and at large intervals along their length (*23*).

Bacterial surface lectins play a key role in the initiation of infection by mediating bacterial adherence to epithelial cells of the host, for example, in the urinary and gastrointestinal tracts (*21, 22*). This has been well documented for type 1 fimbriated *E. coli* and *Klebsiella pneumoniae* and for P fimbriated *E. coli*. The fimbriated strains of these organisms are more infective than their isogenic nonfimbriated

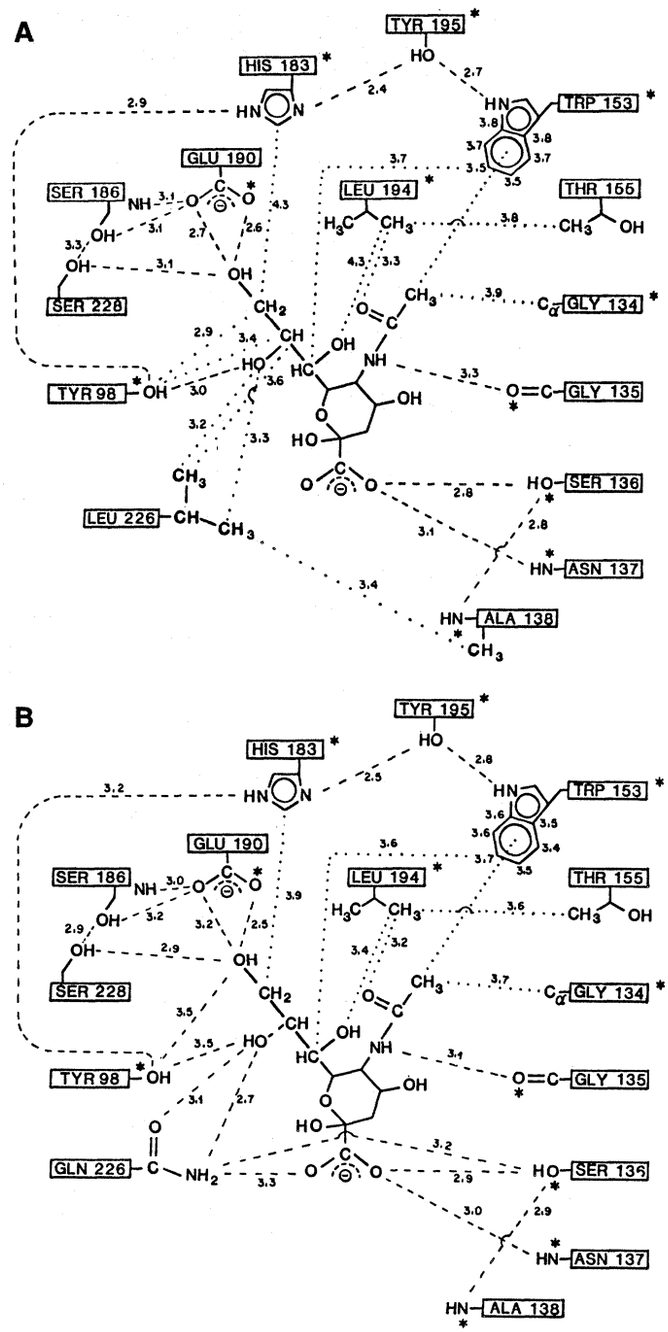

Fig. 2. Potential interactions of *N*-acetylneuraminic acid with wild-type and mutant influenza virus hemagglutinin. Potential hydrogen bonds (dashed lines) and van der Waals contacts (dotted lines) (**A**) in wild-type hemagglutinin with α2→6 sialyllactose and (**B**) in mutant hemagglutinin with α2 → 3 sialyllactose. An asterisk next to a box indicates that the residue is conserved in all known influenza virus hemagglutinin sequences. [Reprinted from (*19*) with permission, © 1988 Macmillan Magazines Ltd.]

counterparts. Furthermore, sugars that inhibit binding of the bacteria to epithelial cells in vitro, as well as antibodies to the lectins or to the lectin receptors, significantly decrease the rate of urinary tract infection in experimental animals (Table 2). Although the bacterial surface lectins may facilitate infection of humans as well, there have been no reports on the application to humans of the findings obtained with animals.

The galactose-specific lectins produced by oral actinomyces, such as *Actinomyces naeslundi* and *A. viscosus*, facilitate initial colonization of epithelial surfaces of the mouth and teeth by mediating the attachment of the bacteria to galactose residues either on the surface of the epithelial cells, or on the surface of other bacteria (for example, *Streptococcus sanguis*), which are adsorbed to the enamel of the teeth (*24*).

Lectin-carrying bacteria may also bind readily to sugars on phagocytic cells, for example, human polymorphonuclear leukocytes or human and mouse peritoneal macrophages (*25*). As demonstrated originally with type 1 fimbriated *E. coli*, and more recently also with oral actinomyces, binding results in metabolic activation of the phagocytes, ingestion of the bacteria, and eventual bacterial death. This sequence of events is characteristic for the well-studied mechanism of phagocytosis mediated by opsonins, that is, antibody and complement. The lectin-mediated, nonopsonic phagocytosis, designated as lectinophagocytosis (*25*), may be of clinical relevance in immunodeficient or unimmunized hosts and in tissues, such as the renal medulla and peritoneum during peritoneal dialysis, where opsonic activity is poor.

Protozoa

Among the numerous protozoa that infect humans and animals, the occurrence of lectins has been best documented in the pathogenic amoeba *Entamoeba histolytica*, which causes dysentery in humans by disruption and invasion of the colonic mucosa (*26*). Various saccharides inhibit amoebic adherence to enterocytes and other mammalian target cells in vitro, which suggests that amoebic adherence is mediated by lectin-carbohydrate interactions. Two distinct lectins, one specific for $\beta 1 \rightarrow 4$-linked oligomers of *N*-acetylglucosamine (*27*) and the other for galactose and *N*-acetylgalactosamine (*28*), have been isolated from *E. histolytica*. The latter lectin (260 kD) is composed of two types of subunit (170 and 35 kD) linked by disulfide bonds. The purified lectins partially inhibit binding of amoebic trophozoites to cultured human intestinal epithelial and Chinese hamster ovary (CHO) cells. In addition, antibodies directed to the heavy subunit of the galactose and *N*-acetylgalactosamine–specific lectin inhibit binding of the amoeba to CHO cells, suggesting that this subunit is primarily responsible for mediating adherence.

The extent of binding of *E. histolytica* to wild-type CHO cells and three lectin-resistant CHO mutants with altered glycosylation patterns is directly correlated with the presence of terminal galactose residues on the cell surface; very poor binding is observed with mutants deficient in galactose (*29*). Moreover, galactose derivatives, in particular *N*-acetyllactosamine, efficiently block binding to CHO cells, whereas no inhibition is seen with *N*-acetylglucosamine or *N,N'*-diacetylchitobiose (GlcNAcβ1→4GlcNAc). These results appear to implicate the galactose-specific rather than the *N*-acetylglucosamine–specific lectin in the recognition of mammalian cells by *E. histolytica*.

Facilitating the adherence of amoebae to intestinal epithelial cells of the host is only one of the ways in which the lectins may enhance the pathogenicity of the parasite (*30*). Once invasion has taken place and the amoebae have spread through the host, the lectins may mediate binding of the parasite to other cells and tissues, in particular to hepatocytes, initiating the killing of these cells. In addition, the lectins enable the amoebae to bind bacteria carrying the appropriate sugars. The bound bacteria are subsequently ingested and serve as a source of nutrition for the parasite, increasing its virulence.

Table 2. Inhibitors of sugar-specific adherence prevent infection (*22*). Abbreviations: UT, urinary tract; and GIT, gastrointestinal tract.

Organism	Animal, site of infection	Inhibitor
Type 1 fimbriated		
Escherichia coli	Mice, UT	MeαMan
	Mice, GIT	Mannose
	Mice, UT	Antibody to mannose
Klebsiella pneumoniae	Rats, UT	MeαMan
Shigella flexneri	Guinea pigs, eye	Mannose
P fimbriated		
Escherichia coli	Mice, UT	Globotetraose
	Monkeys, UT	Galα4GalβOMe

Slime Molds

Aggregation of slime molds, a key event in the differentiation of these organisms from their single-cell, vegetative form to an aggregated form, was for more than a decade considered the most convincing example of the involvement of lectins in cell-cell recognition. This notion was based primarily on studies with *Dictyostelium discoideum* and its developmentally regulated galactose-specific lectin, discoidin I. Discoidin I is a homotetrameric protein with a subunit molecular mass of 28 kD (*31*). Neither the lectin, nor its mRNA, is present at significant levels during vegetative growth, but both become prominent as the mold passes from the vegetative to the aggregating stage, whereupon the cells adhere to each other. The lectin is present on the surface of aggregating cells, and in its isolated form it agglutinates aggregating slime mold cells but not vegetative cells. However, discoidin I, although intimately involved in slime mold aggregation, does not participate in this process by virtue of its ability to bind carbohydrates (*32*). The lectin apparently acts by a sugar-independent mechanism in which it binds to the cells through a short segment of the molecule, the tripeptide Arg-Gly-Asp. The same tripeptide is present in many other adhesion molecules, such as fibronectin, where it also constitutes an important cell-binding recognition site (*33*).

It is now believed that the carbohydrate-binding site of discoidin I is required for packaging the lectin, in the presence of exogenous bacterial polysaccharide, into subcellular particles for eventual secretion from the cell. On exocytosis the particles disintegrate, allowing the lectin to play its part in cell-cell aggregation (*32*).

Plants

Plant lectins were the first proteins of this class to be studied. Because of their broad distribution and ease of isolation, more lectins have been characterized from plants than from any other source (*34*). However, little is known concerning their function. The only hypothesis currently attracting attention is that they serve as mediators of the symbiosis between nitrogen-fixing microorganisms, primarily rhizobia, and leguminous plants, a process of immense importance in both the nitrogen cycle of terrestrial life and in agriculture.

The association between legumes and nitrogen-fixing bacteria is highly specific. For example, rhizobia that infect and nodulate soybeans cannot nodulate garden peas or white clover, and vice versa. The idea that lectins are responsible for this association was initially based on the finding that a lectin from a particular legume—for example, soybean agglutinin—binds in a sugar-specific manner to the corresponding rhizobial species and not to bacteria that are symbionts of other legumes (*35*). A similar specificity pattern was observed with lectins from soybean, pea, red kidney bean, and jack bean seeds and lipopolysaccharides from the respective symbionts (*36*). Although the lectins used were isolated from seeds and there was no proof at the time that roots contain lectins with similar specificities, it was suggested that rhizobial attachment to plant roots occurs by direct interaction between bacterial surface carbohydrates and lectins present in the roots.

Rhizobia contain several *nod* genes essential for host nodulation (*37*). Transfer of certain of these genes from one *Rhizobium* strain to another allowed the recipient strain to infect the particular legume host of the donor *Rhizobium* strain. Thus, in addition to having basic nodulation functions, the *nod* genes are major determinants of host specificity. Although it is not clear how *nod* gene expression leads to host infection, it is possible that some of the genes may affect production of bacterial cell surface carbohydrates. The carbohydrates, in turn, will be recognized by plant root lectins as signals to induce the events leading to nodulation. In fact, transfer of *R. trifolii nod* genes into *R. leguminosarum* induced the hybrid recombinant to synthesize exopolysaccharides characteristic of the donor strain (*38*).

There is convincing evidence in favor of the lectin recognition hypothesis in the symbiosis between white clover, *Trifolium repens*, and *R. trifolii* (*39*). A lectin (trifoliin A) specific for 2-deoxyglucose was isolated from extracts of clover seeds and seedling roots. It bound to infective, but not to uninfective, strains of Rhizobia. Antibodies to the lectin bound mostly to the root hair region of clover roots but did not bind to roots of other, closely related legumes, for example, alfalfa. The lectin was released from the roots by the sugar for which it is specific, suggesting that it associates with the root surface via its carbohydrate-binding site. Trifoliin may thus act as a bridge between similar carbohydrates on both the root hair tips and *R. trifolii*. A polysaccharide that could serve as such a receptor is present on the surface of infective strains of *R. trifolii* but absent (or inaccessible) on noninfective strains.

Other findings, however, cast doubt on the general validity of the notion that lectins serve as recognition molecules in host-symbiont interactions in leguminous plants (*36*). For instance, *R. leguminosarum*, a symbiont of peas, binds to the root hair tips not only of pea but also of other leguminous plants, such as *Canavalia ensiformis* and *Medicago sativa*. The latter, however, are not infected by this bacterium. In addition, heterologous rhizobia attached to pea root hair tips nearly as well as did *R. leguminosarum*. Finally, sugars specific for pea lectin do not inhibit the attachment of *R. leguminosarum* to root hairs. Thus the lectin recognition hypothesis continues to be the subject of controversy.

Higher Animals

Animals produce a variety of lectins, both membrane-bound and soluble, many of which have been implicated in cell recognition phenomena.

Binding, uptake, and killing of cells. The first clue to the possible role of animal lectins came with the discovery of the membrane-bound, galactose and *N*-acetylgalactosamine–specific lectin from rabbit hepatocytes (known as the hepatic-binding protein) and the demonstration that it may be involved in the clearance of glycoproteins from the circulatory system (*12*). The lectin is an oligomeric protein consisting of two types of subunit, with molecular sizes of 40 and 48 kD. Each is inserted into the membrane by means of a hydrophobic region, and the carbohydrate-binding domain is located extracellularly. A similar galactose-specific, membrane-bound lectin was recently isolated from rat peritoneal macrophages (*40*). It mediates binding to the cells not only of galactose-terminated glycoproteins but also of desialylated erythrocytes and other blood cells; after binding, the blood cells are phagocytosed and lysed.

Early experiments in humans and other animals have shown that injected desialylated erythrocytes are cleared rapidly into the liver and spleen, where they are taken up by Kupffer cells (a class of macrophages) and splenic macrophages, respectively (*41*). Because erythrocytes lose sialic acid on aging, and because Kupffer cells also have a galactose-specific lectin (*42*), it was suggested that these lectins are responsible for the physiological clearance of old erythrocytes from the circulatory system. However, the loss of sialic acid is not due to simple desialylation, but results from the elimination of whole carbohydrate chains from the erythrocyte surface (*43*). This is in agreement with the finding that the galactose-specific lectin from peanut, which does not bind to untreated human erythrocytes but interacts avidly with the desialylated cells, does not bind old erythrocytes (*44*). Therefore, the role of lectins in the removal of erythrocytes from circulation remains an open question.

A surface lectin specific for galactose and *N*-acetylgalactosamine appears to be responsible for the ability of activated macrophages to distinguish tumor cells from normal ones and, moreover, to kill the tumor targets. The putative lectin, a glycoprotein with a subunit molecular size of 45 to 60 kD, was purified from activated mouse macrophages and was found to inhibit binding of tumor cells to the macrophages (*45*). Staining with antibodies to the purified lectin revealed that the lectin is present on the surface of activated macrophages but is absent from resident macrophages.

Membrane lectins with specificities other than for galactose have been isolated from different animal cells (*12, 13, 46*). These include the chicken hepatic lectin, which is specific for *N*-acetylglucosamine, two lectins specific for mannose 6-phosphate found in many vertebrate cell types, the lectin from rat liver Kupffer cells specific for L-fucose, and the macrophage lectin specific for mannose and *N*-acetylglucosamine (*46*). A high degree of homology has been found in the carbohydrate-binding domains of many of the membrane-bound lectins.

A novel role ascribed to the mannose and *N*-acetylglucosamine–specific macrophage lectin is as mediator of binding and phagocytosis (that is, lectinophagocytosis) of microorganisms that carry on their surface complementary sugars. Examples are the phagocytosis of *Aspergillus fumigatus* (*47*) and *Klebsiella pneumoniae* (*48*) by mouse and guinea pig alveolar macrophages, respectively, of *Pseudomonas aeruginosa* by monocyte-derived human macrophages (*49*), and of *Leishmania donovani* promastigotes by mouse peritoneal macrophages (*50*). Macrophage membrane lectins with other specificities may also function in lectinophagocytosis. Thus, the galactose and *N*-acetylgalactosamine–specific lectin of mouse peritoneal macrophages appears to be involved in the recognition and phagocytosis of trypomastigotes of the parasite *Trypanosoma cruzi*, the etiological agent of Chagas' disease (*51*).

Differentiation and organ formation. Another role ascribed to animal lectins is in the control of differentiation and organ formation. It is perhaps the main function of the ubiquitous β-galactoside–specific lectins (*52*). In contrast to the membrane-bound animal lectins discussed above, the β-galactoside–specific lectins can be solubilized without the aid of detergents and are therefore often referred to as "soluble." In addition, they are of similar molecular size (usually 12 to 16 and 34 kD) and exhibit considerable sequence homologies

(*46*). There is, however, no homology with the known membrane-bound lectins. Although classified as β-galactoside–specific, the individual lectins examined (for example, of bovine heart muscle and human and rat lung) differ markedly in their preference for different galactose-containing oligosaccharides, indicating that perhaps no two of them are identical in their fine carbohydrate specificities (Table 3) (*53, 54*).

The appearance and cellular distribution of the β-galactoside–specific lectins is developmentally regulated and is temporally coordinated with the expression of complementary carbohydrate structures in many developing organs and tissues (*52*). A striking illustration is the appearance of two lectins in neurons of the dorsal root ganglion soon after formation of the ganglia, both of which are restricted to a distinct functional subset of the neurons (*55*). The same cells also express a series of developmentally regulated carbohydrate structures for which the lectins are specific. The carbohydrates differ from those found in other subsets of dorsal root ganglion neurons.

There are also changes in the cellular location of the β-galactoside–specific lectins during development. For example, chicken lactose lectin I, which is concentrated intracellularly in developing muscle, is externalized upon maturation. Once outside the cells, the lectin may mediate cell-matrix and cell-cell interactions by binding to proteoglycans, constituents of the cell surface, and the extracellular matrix. A soluble lectin, isolated from rat cerebellum, was shown to participate in myelin formation in cultured rat oligodendrocytes (*56*). Monovalent fragments (Fab) of the antibody to the lectin disrupted the myelin structure by causing separation of adjacent lamellae. In addition, the antibody fragments caused almost complete detachment of the oligodendrocytes from the culture substratum, indicating that the lectin is also involved in cell adhesion. Although these results were obtained with cultured cells, the lectin may function in vivo, because it is present in oligodendrocytes and in white matter of the brain.

A β-galactose–specific lectin is found in the epithelium of the thymus and is postulated to be responsible for holding immature thymocytes in the thymic cortex by binding to galactose residues on the surface of these cells (*57, 58*). On maturation of the thymocytes, the galactose residues become masked by attachment of sialic acid, and the cells lose their ability to bind the lectin. They are thus free to migrate to the thymic medulla, where the mature thymocytes reside, or directly to enter the circulatory system.

Migration of lymphocytes. During their normal life span, lymphocytes migrate from the bloodstream into the lymphoid organs, such as lymph nodes and Peyer's patches. An adhesive interaction between lymphocytes and the endothelium of postcapillary venules is the first step in this migratory or "homing" process. Insight into the molecular basis of the adhesive interaction was obtained by experiments in vitro, which demonstrated that binding of both mouse and human lymphocytes to frozen sections of syngeneic lymph nodes was inhibited by L-fucose and mannose 6-phosphate, as well as by fucoidin, a polymer of L-fucose, and a phosphomannosyl-rich polysaccharide from yeasts (*59*). Fucoidin also blocked the migration of lymphocytes into lymph nodes in vivo. Thus, the recognition between lymphocytes and the cells of the lymphoid organs is based on lectin-sugar interactions. The lectin has recently been purified from mouse spleen by affinity chromatography on a monoclonal antibody (Mel-14) against mouse lymphocytes. It is a glycoprotein with a molecular mass of 90 kD and contains a domain with a high degree of homology with other membrane-bound animal lectins such as the various hepatic lectins from chicken, rat, and man (*60*). The identity of the natural ligand for the lymphocyte lectin, however, is not known.

Metastasis. The formation of secondary tumors by circulating cancer cells (blood-borne metastasis) correlates with an increased tendency of the cells to form emboli by aggregating with other tumor cells or host cells. Several lines of evidence strongly suggest that lectins on human and murine metastatic tumor cell surfaces may be involved in the formation of the emboli (*61–63*). In addition, the lectins may facilitate adhesion of the aggregates to endothelial cells of capillaries. They may function by binding complementary glycoconjugates on the surface of other tumor cells to mediate homotypic aggregation, or on the surface of host cells to mediate heterotypic aggregation or attachment to endothelial cells or extracellular matrix.

Table 3. Carbohydrate specificity of three rat lung lectins. The inhibitory activity of galactose was arbitrarily set as 1. Data are from (*53*).

Carbohydrate	Relative inhibitory activity		
	RL-14.5	RL-18	RL-29
Galactose	1	1	1
Galβ1→4Glc	130	60	100
Galβ1→4GlcNAc	650	66	700
Galβ1→3GlcNAc	155	60	270
Galβ1→3GalNAc	5	60	7
Galα1→3GalαOMe	5	18	6
GalNAcβ1→3GalαOMe	4	60	40
GalNAcα1→3Galβ1→4Glc L-Fucα1→2	40	120	2500

β-Galactoside–specific surface lectins (with molecular masses of 14.5 and 34 kD) are present on different murine and human tumor cells, including melanoma (for example, B16), fibrosarcoma, and carcinoma. The amino acid sequence of the 14.5-kD lectin is homologous to that of other β-galactoside–specific lectins of 14 kD—for example, from human lung and placenta. Lectin expression, as measured by the ability of the cells to undergo aggregation in the presence of asialofetuin (Fig. 3) or by the extent of binding of a monoclonal antibody to the lectin, correlates well with the metastatic potential of the tumor cells. Significantly, highly metastatic melanoma and fibrosarcoma cells that had been treated with the antibody to the lectin before their injection into mice showed decreased metastatic potential (*61, 62*). The 14.5-kD lectin is also found on normal embryonal fibroblasts, whereas oncogene-transfected cell clones derived from these cells, as well as established tumor cells, express both the 14.5- and the 34-kD lectins (*64*). The

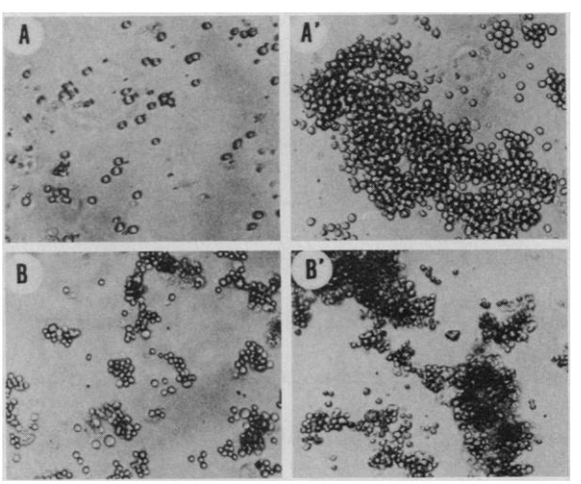

Fig. 3. Agglutination of tumor cells by asialofetuin. (**A** and **A'**) Melanoma cells and (**B** and **B'**) fibrosarcoma cells without (A and B) and with (A' and B') asialofetuin (25 μg/ml). [Reprinted from (*61*) with permission, © 1987 Kluwer Academic Publishers]

levels of mRNA coding for the 14.5-kD lectin are the same in normal cells and in their transformed variants, whereas the mRNA encoding the 34-kD lectin is much more abundant in the transformed cells.

Tumor metastasis and invasion may be mediated not only by tumor cell lectins but also by carbohydrates on the surfaces of these cells. Thus, a direct correlation between sialylation and metastatic capacity has been observed in several tumor cell lines. A case in point are the two related cell lines: Eb, a line of low metastatic potential, and ESb, a spontaneous variant of Eb with high capacity for liver metastasis (*65*). In vitro, ESb cells bind to isolated hepatocytes, whereas Eb cells do not bind unless first treated with sialidase. Enzymatic removal of β-galactose residues from ESb cells or from sialidase-treated Eb cells greatly decreased their ability to bind hepatocytes. The hepatocytes were found to bind ESb cells through galactose and *N*-acetylgalactosamine–specific, lectin-like proteins with molecular sizes of 52, 56, and 110 kD. On the basis of size, these proteins differ from the galactose and *N*-acetylgalactosamine–specific, hepatic-binding protein.

Studies with lectin-resistant glycosylation mutants of a highly metastatic tumor cell line have revealed that the loss of metastatic potential observed with some of the mutants was related to specific glycosylation defects, such as deficiency of sialic acid and galactose or the absence of β1 → 6-linked branches of N-linked complex oligosaccharides. Revertants that regained the wild-type glycosylation profile were again highly metastatic (*66*). In addition, treatment of B16-F10 murine melanoma cells with swainsonine, an inhibitor of protein glycosylation, resulted in a dramatic inhibition of pulmonary metastasis but did not affect tumorigenicity after subcutaneous implantation (*67*). Although lectins recognizing the altered carbohydrates have not yet been identified, most of the host cells that are targets for the metastatic tumors are known to contain lectins.

The distribution patterns of lectins and carbohydrates on normal and malignant cells are being studied, in the hope of finding clear-cut differences that may be useful for diagnostic purposes. An intense effort is also under way to exploit cell surface lectins as targets for the controlled and selective delivery of drugs to malignant cells (*63*).

Concluding Remarks

When the subject of lectins was first reviewed in *Science* nearly 20 years ago (*68*), hardly anything was known about their role in nature, and the idea that they may act as recognition molecules was not mentioned at all. During the intervening years, much evidence has accumulated to support the assumption that lectins play a key role in cell recognition, and thus act as determinants of the social behavior of cells. Some of the evidence has been summarized in this article. Other studies along the same lines have not been dealt with, mainly because there is little, if any, information on the putative lectins. These include investigations on the interaction between fungi and mycoparasites (*69*), invasion of erythrocytes by malaria parasites (*70*), fertilization in algae (*71*), higher plants (*72*), and animals (*73*), as well as control of differentiation of cells of the hematopoietic system (*74*).

From the examples discussed, where lectins have been purified, characterized, and proven to be involved in cellular interactions, at least in vitro, the impression may be gained that cell recognition is mediated by a single type of lectin combining with its complementary carbohydrate. It is, however, difficult to imagine that the high specificity requirements for cell interactions in living organisms are satisfied by such a simple model. In reality, recognition may require for each system a multiplicity of lectins with distinct specificities, as well as other classes of molecule, such as integrins (*33*), cell adhesion molecules (CAMs) (*75*), and antibodies. The availability of new powerful techniques, especially genetic engineering, should make it possible to obtain cells that express lectins or carbohydrates they do not normally produce. This could lead to more precise assessment of the role of lectins in cell recognition, and perhaps also allow the modification of the social behavior of cells, both in vitro and in vivo. The knowledge gained may provide a basis for improved diagnosis and treatment of many diseases.

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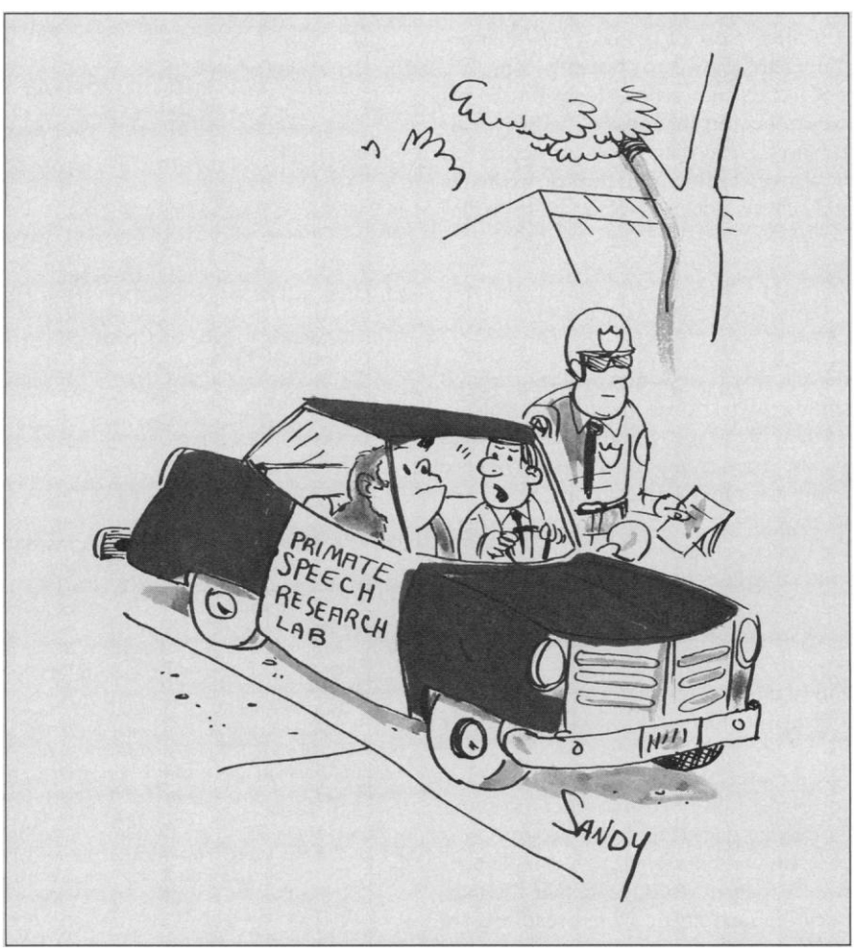

" Let ME do the talking."