# REVIEW

# History of lectins: from hemagglutinins to biological recognition molecules

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*Editor's note:* Dr. Nathan Sharon is among an elite vanguard of scientists who pioneered the study of lectin structure and function. Since his early work on soybean agglutinin in the 1960s, Dr. Sharon has published hundreds of papers covering all aspects of lectin recognition, from the structural basis for lectin–carbohydrate interactions to clinical applications. Along with his longtime collaborator at the Weizmann Institute, Dr. Halina Lis, Dr. Sharon has not only contributed greatly to our understanding of lectins but has been a tireless and highly effective advocate for glycobiology worldwide. It is with great pleasure and appreciation that the editors provide the following historical perspective of these two leaders in the field.

**The occurrence in nature of erythrocyte-agglutinating proteins has been known since the turn of the 19th century. By the 1960s it became apparent that such proteins also agglutinate other types of cells, and that many of them are sugar-specific. These cell-agglutinating and sugar-specific proteins have been named lectins. Although shown to occur widely in plants and to some extent also in invertebrates, very few lectins had been isolated until the early 1970s, and they had attracted little attention. This attitude changed with the demonstration that lectins are extremely useful tools for the investigation of carbohydrates on cell surfaces, in particular of the changes that the latter undergo in malignancy, as well as for the isolation and characterization of glycoproteins. In subsequent years numerous lectins have been isolated from plants as well as from microorganisms and animals, and during the past two decades the structures of hundreds of them have been established. Concurrently, it was shown that lectins function as recognition molecules in cell–molecule and cell–cell interactions in a variety of biological systems. Here we present a brief account of 100-plus years of lectin research and show how these proteins have become the focus of intense interest for biologists and in particular for the glycobiologists among them.**

*Key words:* carbohydrates/functions/glycobiology/microorganisms/plants

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## Introduction

Toward the end of the 19th century, evidence started to accumulate for the presence in nature of proteins possessing the ability to agglutinate erythrocytes. Such proteins were referred to as hemagglutinins, or phytoagglutinins, because they were originally found in extracts of plants. It is generally believed that the earliest description of such a hemagglutinin was by Peter Hermann Stillmark in his doctoral thesis presented in 1888 to the University of Dorpat (now Tartu, Estonia), one of the oldest universities in czarist Russia (reviewed by Franz, 1988). This hemagglutinin, which was also highly toxic, was isolated by Stillmark from seeds of the castor tree (*Ricinus communis*) and was named ricin. Subsequently, H. Hellin, also at Tartu, demonstrated the presence of a toxic hemagglutinin, abrin, in extracts of the jequirity bean (*Abrus precatorius*). Ricin and abrin soon became commercially available, which prompted Paul Ehrlich, at the Royal Institute of Experimental Therapy (Frankfurt), to employ them as model antigens for immunological studies. Although the preparations available to him were very crude by present criteria (we know now that the ricin and the abrin each contained a weakly agglutinating, powerful toxin and a poorly toxic but strong agglutinin, all galactose-specific), he was able to establish with them in the 1890s several of the fundamental principles of immunology. Thus Ehrlich found that mice were rendered immune to a lethal dose of ricin or abrin by repeated small (sublethal), subcutaneous injections of the lectin and that anti-ricin did not protect the animals against the toxic effects of abrin, nor did anti-abrin protect against ricin. This provided clear evidence for the specificity of the immune response. Ehrlich also showed that immunity to the toxins is transferred from a mother to her offspring by blood during pregnancy and by milk after birth. By studying the inhibitory effect of the anti-ricin immune serum on the agglutinating activity of ricin, he demonstrated that there was a quantitative relationship between the amount of antiserum and that of antigen it could neutralize and on this basis performed the first quantitative determination of an antibody *in vitro*. These studies thus demonstrated the specificity of the antibody response, the phenomenon of immunological memory, and the transfer of humoral immunity from a mother to her offspring.

The general public became aware of ricin in 1978, following its use as a weapon in the notorious politically motivated "umbrella murder" of Georgi Markov, Bulgarian opposition writer and broadcaster in exile. Attempts to employ ricin as a potential weapon of war have been carried out by the United States during World War I; during World

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War II a ricin bomb was developed and tested by the British military, but it has never been deployed as a weapon for mass destruction. More recently, ricin has found its way into the arsenals of extremist individuals, groups, and governments.

## Sugar binding and blood type specificity

In 1919, James B. Sumner at Cornell University (Ithaca, New York), well known for being the first to crystallize in 1926 an enzyme, urease (for which he was awarded the Nobel Prize 21 years later), isolated from jack bean (*Canavalia ensiformis*) a crystalline protein that he named concanavalin A and in this way obtained a pure hemagglutinin for the first time. However, nearly two decades passed before Sumner and Howell (1936) reported that concanavalin A agglutinates cells such as erythrocytes and yeasts and also precipitates glycogen from solution. They further showed that hemagglutination by concanavalin A was inhibited by sucrose, demonstrating for the first time the sugar specificity of lectins. With much foresight, they suggested that the hemagglutination induced by concanavalin A might be a consequence of a reaction of the plant protein with carbohydrates on the surface of the red cells.

Already the early results obtained by Stillmark indicated some selectivity in the ricin-induced agglutination of red cells from different animals. This observation was corroborated and further extended by Karl Landsteiner from the University of Vienna, the discoverer of the human A, B, and O blood groups in 1900. Nearly a decade later he reported that the relative hemagglutinating activities of various seed extracts were quite different when tested with red blood cells from different animals (Landsteiner and Raubitschek, 1907). Because of this specificity, Landsteiner concluded that the actions of plant hemagglutinins "resemble antibody reactions in all essentials." He therefore used these proteins to illustrate the specificity concept in the introductory chapter of his classic book *The Specificity of Serological Reactions* (1936).

The 1940s saw the discovery, made independently by William C. Boyd at Boston University and by Karl O. Renkonen at the University of Helsinki, Finland, of the human blood group (or blood type) specificity of the hemagglutinins. They found that crude extracts of the lima bean, *Phaseolus limensis*, and the tufted vetch, *Vicia cracca*, agglutinated blood type A erythrocytes but not blood type B or O cells, whereas an extract of the asparagus pea, *Lotus tetragonolobus*, agglutinated specifically blood type O erythrocytes. Olavi Mäkelä (1957), a doctoral student of Renkonen, examined in 1954–56 extracts from seeds representing 743 plant species and 165 genera, all of the family *Leguminosae*, and detected hemagglutinating activity in more than one-third of them; close to one-tenth of the hemagglutinins exhibited blood type specificity. Although several of the latter were specific either for blood type O or type A, or both type A and B erythrocytes, and one, from *Dolichos biflorus*, reacted much better with $A_1$ erythrocytes than with $A_2$, only the extract from *Griffonia simplicifolia* (previously known as *Bandeiraea simplicifolia*) exhibited almost exclusively type B specificity. Since then, additional hemagglutinins specific for blood types A and O (but not B) have been discovered, as well as several for other blood types, such as N (*Vicia graminea* lectin), T (peanut agglutinin, PNA) and Tn (the lectins of *Vicia villosa* and *Moluccella laevis*).

The blood type–specific hemagglutinins played a crucial role in early investigations on the structural basis of the specificity of the antigens associated with the ABO blood group system. In the 1950s, Walter J. T. Morgan and Winifred M. Watkins at the Lister Institute, London, found that the agglutination of type A red cells by lima bean lectin was best inhibited by α-linked *N*-acetyl-D-galactosamine and that of type O cells by the lectin of *L. tetragonolobus* was best inhibited by α-linked L-fucose. They concluded that α-*N*-acetyl-D-galactosamine and α-L-fucose are the sugar determinants conferring A and H(O) blood group specificity, respectively. Both conclusions have been substantiated by subsequent investigations (for a recent review, see Morgan and Watkins, 2000). The pioneering work of Watkins and Morgan was among the earliest evidence for the presence of sugars on cell surfaces and their potential roles as identity markers, an accepted theme in modern glycobiology. It took a while, however, before the counterreceptors for surface sugars, that is, the endogenous lectins that recognize these sugars, were identified, the first being the mammalian hepatic asialoglycoprotein receptor to be described later.

The ability of plant agglutinins to distinguish between erythrocytes of different blood types led Boyd and Shapleigh (1954) to propose for them the name *lectins*, from the Latin *legere*, to pick out or choose. This term was generalized by us to embrace all sugar-specific agglutinins of nonimmune origin, irrespective of source and blood type specificity (Sharon and Lis, 1972).

## Mitogenic stimulation of lymphocytes and agglutination of cancer cells

Two major discoveries made in the early 1960s were instrumental in bringing lectins into the limelight. The first of these was by Peter C. Nowell (1960) at the University of Pennsylvania, Philadelphia, who found that the lectin of the red kidney bean (*Phaseolus vulgaris*), known as phytohemagglutinin (PHA), is mitogenic, that is, it possesses the ability to stimulate lymphocytes to undergo mitosis. This discovery had a revolutionary impact on immunology in that it shattered the view, held until then, that lymphocytes are dead-end cells incapable of dividing or differentiating further. Within a short time, several other lectins were proven to be mitogenic. Of special significance was the finding that concanavalin A acts as a mitogen because, in contrast to PHA, its activity could be inhibited by low concentrations of monosaccharides, for example, mannose. This finding provided proof that mitogenic stimulation is the result of binding of lectins to sugars on the surface of the lymphocytes and was among the earliest demonstrations for a biological role of cell surface sugars. Mitogenic lectins soon became tools for the study of signal transmission into cells and for the analysis of the biochemical events that occur during lymphocyte stimulation *in vitro*. A most

valuable outcome of such studies was the discovery in the 1970s by Robert C. Gallo and his associates at the National Institutes of Health (Bethesda) of T cell growth factor, now known as interleukin-2, in conditioned medium of normal human lymphocytes stimulated by PHA (Morgan *et al.*, 1976).

The second discovery was made by Joseph C. Aub at the Massachusetts General Hospital in Boston (Aub *et al.*, 1963, 1965). He found that wheat germ agglutinin (WGA) has the ability to preferentially agglutinate malignant cells. This was followed by the reports of Max M. Burger at Princeton University and Leo Sachs and Michael Inbar at the Weizmann Institute (Rehovot) that concanavalin A exhibits the same ability. Together with Sachs and Ben-Ami Sela, we subsequently found that soybean agglutinin (SBA) also possesses the same property. Such investigations provided early evidence that changes in cell surface sugars are associated with the development of cancer and led to the assumption that high susceptibility to agglutination by lectins was a property shared by all malignant cells. Unfortunately, this is now known not to be generally true.

## Lectins galore

Until the early 1970s, the presence of hemagglutinins had been reported in numerous organisms, primarily plants, but only very few had been purified, almost all by conventional techniques. In addition to concanvalin A, they included the plant lectins from soya beans, green peas, *Dolichos biflorus* seeds, wheat germ, and mushroom (*Agaricus campestris*) (reviewed in Sharon and Lis, 1972) and the animal lectins of eel (Springer and Desai, 1971), snail (Hammarström and Kabat, 1969), and horseshoe crab (Marchalonis and Edelman, 1968). The pace of lectin isolation increased dramatically with the introduction of affinity chromatography for lectin purification by Irwin J. Goldstein and Bipin B. L. Agrawal of the University of Michigan, originally for the isolation of concanavalin A on immobilized dextran (Sephadex) (Agrawal and Goldstein, 1967). Numerous lectins have thus become available, for a time still mainly from plants, the number of the latter being now about 500. The interest in these lectins was greatly stimulated by the demonstration that they are invaluable tools for the detection, isolation, and characterization of glycoconjugates, primarily of glycoproteins, for histochemistry of cells and tissues and for the examination of changes that occur on cell surfaces during physiological and pathological processes, from cell differentiation to cancer (Table I).

The occurrence of hemagglutinins in animals was noted quite early, almost all in invertebrates or lower vertebrates, but until the middle of the 1970s, only the three of these mentioned (of eel, snail, and horseshoe crab) were isolated and characterized. The first of the animal lectins shown to be specific for a sugar (L-fucose) was from the eel (Watkins and Morgan, 1952). The isolation in 1974 of the first mammalian lectin, the galactose-specific hepatic asialoglycoprotein receptor, was an outcome of the investigation by Gilbert Ashwell at the NIH together with Anatol G. Morell at the Albert Einstein Medical School (New York) of the mechanisms that control the lifetime of glycoproteins in

**Table I.** Major applications of lectins[a]

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Cell identification and separation

Detection, isolation, and structural studies of glycoproteins

Investigation of carbohydrates on cells and subcellular organelles; histochemistry and cytochemistry

Mapping of neuronal pathways

Mitogenic stimulation of lymphocytes[b]

Purging of bone marrow for transplantation[b]

Selection of lectin-resistant mutants

Studies of glycoprotein biosynthesis

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[a]Lectins from sources other than plants are rarely in use.
[b]In clinical use.

blood circulation (Hudgin *et al.*, 1974; Stockert *et al.*, 1974). At the same time, Vivian Teichberg from our department reported (Teichberg *et al.*, 1975) the isolation from the electric eel of the first member of the family of the β-galactose-specific lectins, designated galectins (Barondes *et al.*, 1994), of which over a dozen members have by now characterized. Since the beginning of the 1980s, the number of purified animal lectins also started to grow quickly, largely thanks to the advent of recombinant techniques.

## From primary to 3D structures

The 1970s also witnessed the intensification of studies of the molecular properties of individual lectins, a prerequisite for a deep understanding of their activities at the molecular level. These studies ranged from the determination of the main physicochemical parameters of lectins to complete amino acid sequencing and elucidation of their 3D structure. Until the advent of recombinant techniques, determination of the primary structure of lectins proceeded rather slowly, and by the end of that decade the complete sequences of only half a dozen lectins, all from plants, were known. In this case, too, concanavalin A led the field, being the first lectin whose primary sequence has been established (Edelman *et al.*, 1972). Concurrently, Edelman's group and independently Karl Hardman with Clinton F. Ainsworth at Argonne National Laboratories (Argonne, Illinois), solved the 3D structure of concanavalin A by high resolution X-ray crystallography, another first for this lectin (Edelman *et al.*, 1972; Hardman and Ainsworth, 1972). This was soon followed by the determination of the structure of WGA as well as of its complexes with its ligands (*N*-acetylneuraminic acid and β4-linked *N*-acetylglucosamine oligomers) by Christine Schubert Wright at the Virginia Commonwealth University (Richmond) even before the complete amino sequence of this lectin had become available (Wright, 1977). The striking difference between the structure of concanavalin A and WGA fully corroborated the suggestion presented by us at the 1973 International Glycoconjugate Symposium in Lille, France, that was based largely on compositional data of these two

proteins, that although "lectins have many biological properties in common, they represent a diversified group of proteins with respect to size, composition and structure" (Sharon *et al.*, 1974).

The availability of the primary structure of numerous lectins allowed the identification of homologies between the sequences of lectins from taxonomically related sources, as originally demonstrated for the legume lectins by one of us (N.S.) in collaboration with Donny Strosberg at the Free University of Brussels (Foriers *et al.*, 1977). By the end of the following decade, homologies were found also for

lectins from other families, such as the galectins and the C-type (Ca$^{2+}$ requiring) lectins (Drickamer, 1988).

During the past few years, the number of lectin primary and 3D structures has increase dramatically, with some 200 of the latter having been elucidated (www.cermav.cnrs.fr/lectines). In addition, many structures of lectin–carbohydrate complexes have been solved. Quite surprisingly, remarkable similarities have been noticed between the tertiary structures of lectins from diverse sources, in spite of the lack of primary sequence similarities (Figure 1). One such common tertiary structure, first observed in the legume lectins, and referred to
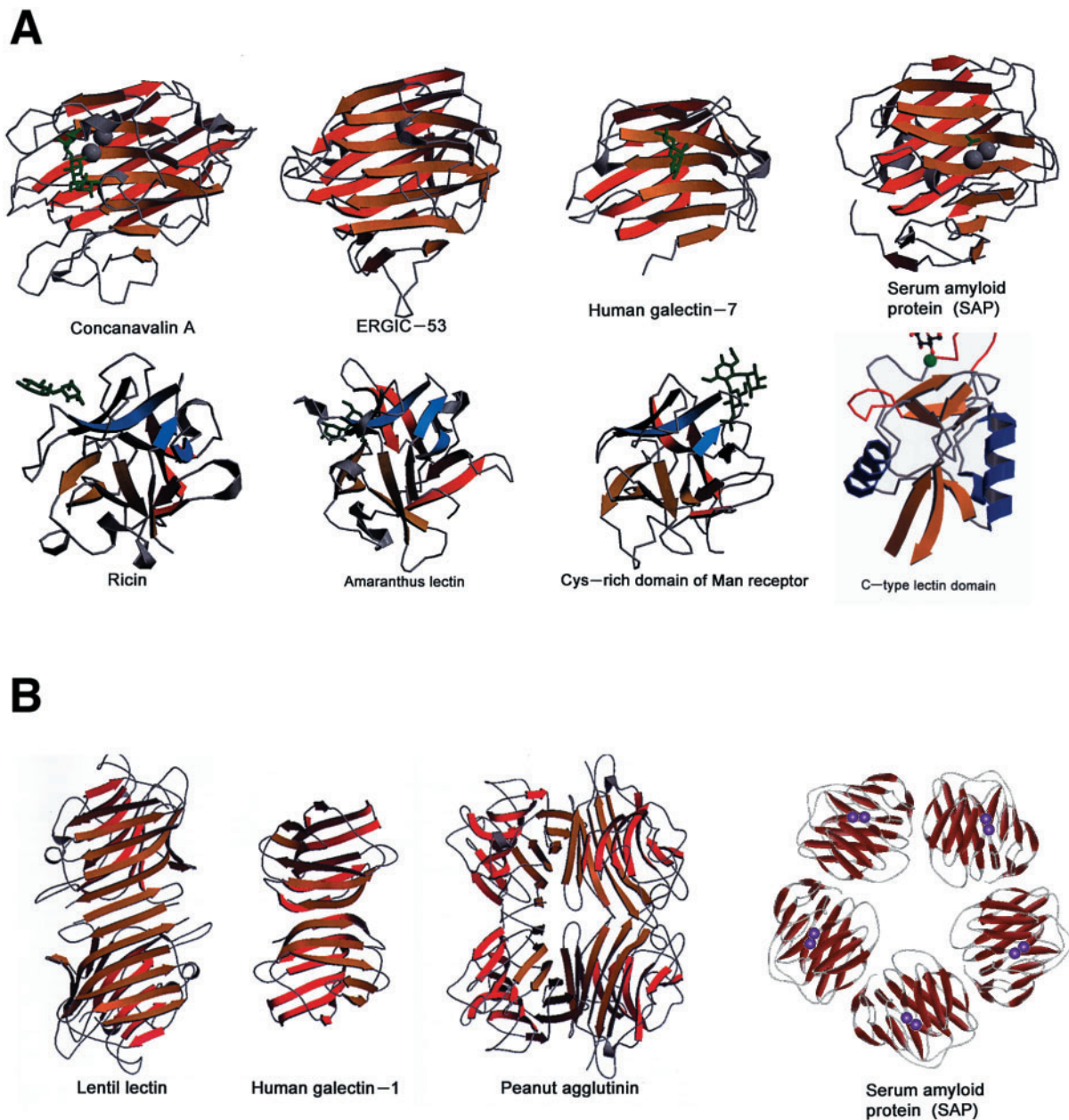


**Fig. 1.** Structures of different lectins represented as ribbon diagrams. (**A**) Upper row shows monomers of lectins from different sources that share the jelly roll or lectin fold. First three lectins (from left) in the lower row all exhibit the β-trefoil fold. (**B**) Variations in quaternary lectins structures. The gray spheres represent metal ions; bound carbohydrate is shown in ball-and-stick representation. All diagrams, except for that of SAP, reprinted with permission from R. Loris (2002) *Biochim. Biophys. Acta*, **1572**, 198–208. The diagram of SAP is from PDB entry sac.

as the lectin fold, consists characteristically of an elaborate jelly roll, derived from antiparallel β-strands, arranged as two β-sheets (Srinivasan *et al.*, 1996). This fold has been found in the legume lectins, the galectins, and in several other animal lectins, such as the pentraxins (Crennel *et al.*, 1994) and ERGIC-53 (Itin *et al.*, 1996; Velloso *et al.*, 2002), as well as in nonlectin molecules, for example, several glycosidases, among them *Vibrio cholerae* sialidase.

Starting in the late 1980s, considerable information has become available, by X-ray crystallography and site-directed mutagenesis, of the chemical groups on the lectin and on the carbohydrates that interact with each other and of the types of bond formed, primarily hydrogen bonds and hydrophobic interactions. It has been concluded that lectins recognize sugars in diverse ways, just like other proteins recognize their ligands (Sharon, 1993).

## Carbohydrate recognition domains

Based on an analysis of the then known amino acid sequences of animal lectins, Kurt Drickamer from Columbia University (New York) proposed in 1988 that the carbohydrate-binding activity of most of them resides in a limited polypeptide segment, designated by him as the carbohydrate-recognition domain (CRD) (Drickamer, 1988). He named the CRD found in the galectins S-CRD and that found in C-type lectins C-type CRD. By now several types of CRD have been discerned, in addition to those just mentioned, each of which shares a pattern of invariant and highly conserved amino acid residues at a characteristic spacing. On this basis it was possible to divide the majority of the animal lectins into structurally related families and superfamilies, the most widely occurring of which is that of the C-type lectins (CTLs). Other families of special interest are the P-type lectins and the siglecs.

The majority of the CTLs are large, asymmetric transmembrane glycoproteins, in which the CRD is attached to a variable number of structurally and functionally different polypeptide domains. In contrast, the galectins are generally small, soluble, nonglycosylated proteins and, unlike the CTLs, do not require $Ca^{2+}$ for their activity. Members of the CTL superfamily are grouped into three families—selectins (the most celebrated one), collectins, and endocytic lectins. The story of the selectins started with attempts to elucidate the molecular basis of lymphocyte homing. These attempts greatly benefited from the availability of an *in vitro* assay for measuring the interaction of lymphocytes with postcapillary high-endothelial venules (HEVs), a known site of lymphocyte exit from the blood stream (Stamper and Woodruff, 1977). Using this assay, which reflects the *in vivo* homing of lymphocytes, Eugene C. Butcher and colleagues at Stanford University obtained a monoclonal antibody (MEL-14) against a murine lymphocyte antigen (Gallatin *et al.*, 1983). The antibody inhibited the binding of the lymphocytes to HEV *in vitro* and their homing *in vivo*, suggesting that the MEL-14 antigen has a direct role in these phenomena. From inhibition experiments of the lymphocyte-HEV binding, Steven D. Rosen and Lloyd M Stoolman at the University of California, San Francisco, have concluded that sugars of the endothelial cell might also be involved in this binding and that the lymphocytes should have a membrane-bound lectin with specificity for fucose and Man-6-P (Stoolman *et al.*, 1984). This lectin was subsequently shown to be identical with the MEL-14 antigen.

In 1987 Bevilacqua and co-workers of Harvard Medical School have developed two monoclonal antibodies that identified a second cell-surface antigen, designated ELAM (endothelial-leukocyte adhesion molecule)-1, expressed on stimulated human endothelial cells but not on unstimulated ones (Bevilacqua *et al.*, 1987). Another vascular cell adhesion molecule was originally isolated from activated platelets independently by Rodger McEver at the Oklahoma Medical Research Foundation, Oklahoma City (McEver and Martin, 1984) and by Bruce and Barbara C. Furie at Tufts University, Boston (Berman *et al.*, 1986; Hsu-Lin *et al.*, 1984), and designated GPM-140 and PADGEM, respectively.

These three cell adhesion molecules, collectively known for a while as LEC-CAMS, were identified as a discrete family of CTLs after the virtually simultaneous publication in 1989 of their primary sequences (Bevilacqua *et al.*, 1989; Johnston *et al.*, 1989; Lasky *et al.*, 1989; Siegelman *et al.*, 1989); these go now under the names L-selectin, E-selectin, and P-selectin, respectively (reviewed in Lasky, 1995). They were all shown to have a similar domain structure, with an extracellular part that consists of an amino terminal CRD, an epidermal growth factor–like domain, and several short repeating units related to complement-binding protein. They bind specifically to the trisaccharide NeuAcα(2-3)Galβ(1-4)[Fucα(1-3)]GlcNAc, known as sialyl-Lewis[x] (siaLe[x] in brief) and its positional isomer, NeuAcα(2-3)Galβ(1-3)[Fucα(1-4)]GlcNAc (siaLe[a]), with both fucose and sialic acid required for binding (Brandley *et al.*, 1990; Stoolman, 1989). The selectins recognize the carbohydrate ligands only when the latter are present on particular glycoproteins, such as cell surface mucins, pointing to the role of the carrier molecule in lectin-carbohydrate interactions; one of the best characterized of such carriers is the P-selectin glycoprotein ligand (Moore *et al.*, 1992).

The paradigm of the endocytic lectins is the mammalian hepatic asialoglycoprotein receptor already mentioned. The collectins, represented by the soluble mannose-binding proteins of mammalian serum and liver, first detected by chance as a contaminant of a preparation of α-mannosidase from human liver (Robinson *et al.*, 1975), subsequently purified and characterized by Toshiaki Kawasaki and Ikuo Yamashina at Kyoto University, Japan (Kawasaki *et al.*, 1978; Kozutsumi *et al.*, 1980), are characterized by an $NH_2$-terminal collagen-like stretch of repeating Gly-X-Y- triplets (where X and Y are any amino acid). The structural unit of the mannose-binding proteins is a trimer of identical subunits with a triple-stranded collagen helix and three CRDs (Weis and Drickamer, 1994). This arrangement of CRDs at a fixed spacing has important biological implications, to be discussed later.

A different kind of CRD has been identified in the siglecs. This family of sialic acid–binding Ig-like lectins, a member of the Ig superfamily, was discovered when the cloning of a macrophage lectin-like adhesion molecule named sialoadhesin (siglec-1) revealed striking structural similarities to a B cell restricted member of the Ig superfamily, CD22 (siglec-2) and to two other members of the Ig superfamily,

CD33 (siglec-3) and the myelin-associated glycoprotein (siglec-4) (Crocker *et al.*, 1994). Members of this family, 11 of which have been identified in humans, are type 1 transmembrane proteins with an extracellular part consisting of a CRD-containing N-terminal V-set Ig-like domain, followed by variable numbers of C2-set Ig-like domains. Except for myelin-associated glycoprotein (siglec-4), exclusively expressed in the nervous system, they are all found on cells of the hematopoietic system. Each siglec has a distinct expression pattern in different cell types, indicating that they perform highly specific functions.

A recent addition to the growing list of mammalian lectins is dectin-1, a β-glucan receptor, identified by Gordon Brown and Siamon Gordon (2001) at Oxford by screening a cDNA library of a macrophage cell line with zymosan. It is a small type II transmembrane receptor containing one CRD, which recognizes β1,3 and/or β1,6-glucans and intact yeasts.

### In protection and symbiosis

The question of the possible physiological role of lectins has intrigued investigators from the start and focused on plant lectins, which for long time were virtually the only ones known (reviewed by Etzler, 1986). It was speculated, for example, that lectins may function as antibodies to protect plants against harmful soil bacteria, control seed germination, or be involved in the transport and storage of sugars, but no evidence for these speculations could be found. However, two proposals put forward in the 1970s still hold. According to first one, lectins protect plants against phytopathogenic microorganisms and insects as well as against predatory animals. The second theory assumes that they are involved in the association between leguminous plants and their symbiotic nitrogen-fixing bacteria.

Probably the earliest publication on the insecticidal action of lectins came in 1976 from the laboratory of Irvin E. Liener at the University of Minnesota, at St. Paul (Minnesota) in which it was reported that feeding bruchid beetles with a diet containing the black bean lectin resulted in the death of the bruchid larvae (Janzen *et al.*, 1976). On this basis the authors concluded that the major role of lectins in legumes is to protect them from attack by insect seed predators. In subsequent studies, several other lectins were shown to be insecticidal, among them WGA, *Galanthus nivalis* lectin and jacalin.

The proposal that lectins may be involved in the protection of plants against pathogenic microorganisms was originally based on the observation made at Rehovot that WGA, PNA, and SBA inhibited the sporulation and growth of fungi such as *Trichoderma viride*, *Penicilium notatum*, and *Aspergillus niger* (see Barkai-Golan *et al.*, 1976). Potato lectin was subsequently shown to act in a similar manner on *Botrytis cinerea*, another fungal phytopathogen. In an extensive study, 11 purified lectins representing the major carbohydrate specificity groups were all found to cause growth disruption during germination of spores of *Neurospora crassa*, *Aspergillus amstelodami*, and *Botryodiplodia theobromae* (Brambl and Gade, 1985). It was also shown that recombinant *Urtica dioica* agglutinin that

has a similar specificity to that of WGA (Broekaert *et al.*, 1989) inhibited the growth of fungal phytopathogens.

The idea that lectins are responsible for the specific association between nitrogen-fixing rhizobia and leguminous plants, which provides the plant with the needed nitrogen, was advanced nearly three decades ago (Bohlool and Schmidt, 1974; Hamblin and Kent, 1973). It was based on the finding that a lectin from a particular legume bound in a carbohydrate-specific manner to the surface polysaccharides or lipopolysaccharides of the corresponding rhizobial species but not to bacteria that are symbionts of other legumes. For instance, SBA agglutinated most strains of *Bradyrhizobium japonicum* that nodulate soybeans but not nonnodulating bradyrhizobial strains. The suggestion has therefore been made that rhizobial attachment to plant roots occurs by interaction between the bacterial surface carbohydrates and lectins present in the roots of the leguminous plants. This became known as the lectin recognition hypothesis, which is still the subject of controversy, because of the lack of unequivocal evidence and of some inconsistencies. Thus for most host–symbiont systems examined, there is no proof for the presence of lectins and their ligands on plant roots and bacteria, respectively, at precisely the right time and location. Moreover, the correlation between the specificity of the host lectin and its ability to recognize the nodulating bacteria of that host is not very strict. Also, several lines of soybeans with no detectable lectin in their seeds or vegetative tissues were nodulated normally by the corresponding rhizobial symbiont.

Application of the techniques of molecular genetics gave results that bolstered the lectin recognition hypothesis but did not fully settle the controversy (reviewed by Kijne, 1996; Hirsch, 1999).

Recently, a variant of the lectin recognition hypothesis has been proposed, that postulates that the host-specific attachment of the rhizobium is achieved through the interaction between species-specific lipo-chitooligosaccharide signal molecules produced by the bacteria, named nodulation factors (Nods), and a new type of a plant root lectin found in different leguminous plants but not in plants of different families (Kalsi and Etzler, 2000).

### Recognition molecules

In a broader sense, the foregoing discussion implies that lectins possess the ability to act as recognition molecules inside cells, on cell surfaces, and in physiological fluids (Figure 2 and Table II). This is in fact the current view of the biological function of lectins, which also evolved during the 1970s (Ashwell and Morell, 1972; Ofek *et al.*, 1978).

Although indications that lectins may function in recognition had appeared in the literature prior to that time, their significance was not appreciated then. A case in point is the demonstration in the 1950s, mainly by of Alfred Gottschalk at the Walter and Elisa Hall Institute (Melbourne, Australia) that the influenza virus hemagglutinin is responsible for the attachment of the virus to the host cells as a prerequisite for infection. However, it was only following the isolation in 1974 of the asialoglycoprotein receptor, a hepatic lectin, and the discovery of its unexpected ability to
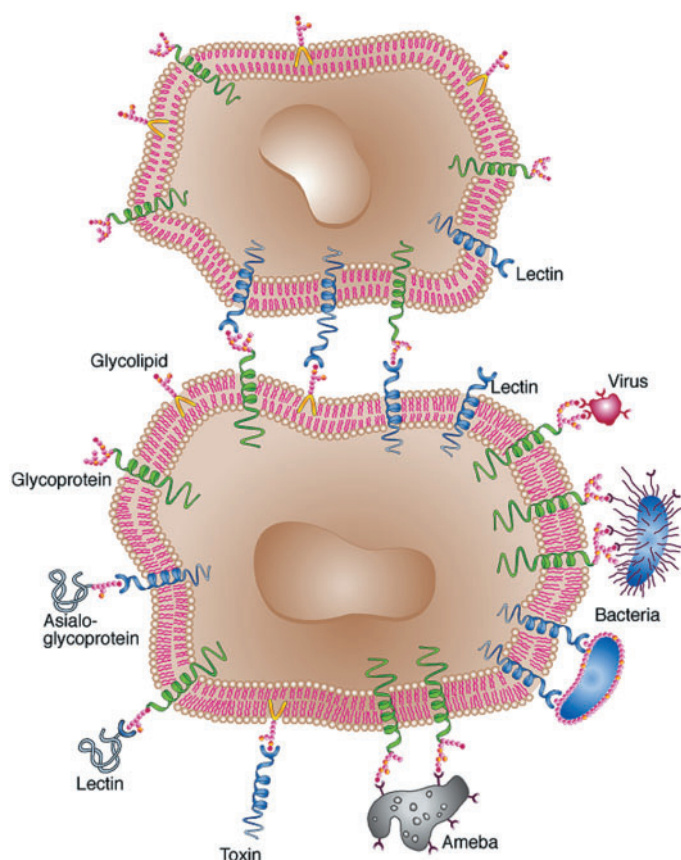
**Fig. 2.** Cell surface lectin–carbohydrate interactions. Lectins serve as means of attachment of different kinds of cell as well as viruses to other cells via the surface carbohydrates of the latter. In some cases, cell-surface lectins bind particular glycoproteins (e.g., asialoglycoproteins), whereas in other cases the carbohydrates of cell surface glycoproteins or glycolipids serve as sites of attachment for biologically active molecules that themselves are lectins (e.g. carbohydrate-specific bacterial and plant toxins, or galectins). Based on an original diagram from BioCarbAB (Lund, Sweden).

**Table II.** Functions of lectins

| Lectin | Role in |
|---|---|
| Microorganisms | |
|   Amoeba | Infection |
|   Bacteria | Infection |
|   Influenza virus | Infection |
| Plants | |
|   Various | Defense |
|   Legumes | Symbiosis with nitrogen-fixing bacteria |
| Animals | |
|   Calnexin, calreticulin, ERGIC-53 | Control of glycoprotein biosynthesis |
|   Collectins | Innate immunity |
|   Dectin-1 | Innate immunity |
|   Galectins | Regulation of cell growth and apoptosis; regulation of the cell cycle; modulation of cell–cell and cell–substratum interactions |
|   Macrophage mannose receptor | Innate immunity; clearance of sulfated glycoprotein hormones |
|   Man-6-P receptors | Targeting of lysosomal enzymes |
|   L-selectin | Lymphocyte homing |
|   E- and P-selectins | Leukocyte trafficking to sites of inflammation |
|   Siglecs | Cell-cell interactions in the immune and neural system |
|   Spermadhesin | Sperm-egg interaction |

recognize and to bind terminal galactose residues on serum glycoproteins (reviewed by Ashwell and Morell, 1974) that the role of lectins in biological recognition started to gain popularity. Support came soon with the identification, by William S. Sly at St. Louis University (Kaplan *et al.*, 1977) of the mannose-6-phosphate receptor crucially involved in intracellular trafficking of lysosomal enzymes. The demonstration that hepatic lectins may also mediate the clearance of bacteria from blood in the absence of opsonins (antibodies and complement), was an early indication of the participation of lectins in non-immune defense, or innate immunity (see later discussion).

Another key finding was made in 1979, when our group, together with others, demonstrated that urinary tract infection in mice by mannose-specific *Escherichia coli* could be prevented by methyl α-D-mannoside (Aronson *et al.*, 1979). It was the first direct evidence for the involvement of bacterial lectins in the initiation of infection, the basis for the present attempts in academia and industry, to apply carbohydrates for antiadhesion therapy of such diseases (reviewed by Mulvey *et al.*, 2001).

Together with Itzhak Ofek, we demonstrated at the same time that the mannose-specific bacterial surface lectins may also mediate attachment of the bacteria to phagocytic cells in the absence of opsonins, leading to engulfment and killing of the bacteria. This process, another example of innate immunity, which we named lectinophagocytosis, may be of importance in the clearance of bacteria from nonimmune patients or from opsonin-poor sites, such as renal medulla or the peritoneal cavity (Ofek and Sharon, 1988). Additional lectins have been implicated in innate immunity. A prominent example is the mannose-specific receptor present on the surface of macrophages; it binds infectious organisms that expose mannose-containing glycans on their surface, leading to their ingestion and killing. Another, recently discovered one, is dectin-1, specific for β1,3 and/or β1,6-glucans, present on fungi.

A similar function, albeit by a different mechanism, is performed by the soluble mannose-binding lectins (MBLs) of mammalian serum and liver (Epstein *et al.*, 1996; Turner, 1996), These proteins bind to oligomannosides of infectious microorganisms, causing activation of complement without participation of antibody, and subsequent lysis of the pathogens, thus acting in innate immunity. The spatial arrangement of the CRDs in the MBLs provides a structural basis for their ability to bind ligands with repetitive, mannose-rich structures, such as

found on fungal and microbial surfaces, but not to the oligomannose units of mammalian glycoproteins (Weis and Drickamer, 1994).

The discovery of the selectins and the demonstration that they play a crucial role in the control of lymphocyte homing and of leukocyte trafficking to sites of inflammation was a landmark in lectin research. Indeed, the selectins provide the best paradigm for the role of sugar–lectin interactions in biological recognition. They mediate the binding of leukocytes to endothelial cells and thereby initiate a rolling phase, in which the lectins interact transiently with glycan ligands, leading eventually to their extravasation. Prevention of adverse inflammatory reactions by inhibition of leukocyte–endothelium interactions, another application of antiadhesion therapy, has become a major aim of the biomedical and pharmacological industry. There are also indications that the selectins may function in the spread of cancer cells from the main tumor to other sites in the body and that by blocking their sugar-binding sites it may be possible to prevent the formation of metastases.

From the late 1980s, evidence started to accumulate that several lectins of different types direct intracellular glycoprotein traffic, by acting as chaperones and sorting receptors in the secretory pathway. Calnexin, a membrane-bound lectin of the endoplasmic reticulum (ER), functions in parallel with calreticulin, its soluble homolog, as part of a quality control system that ensures proper folding of glycoproteins destined to the cell surface. The mannose-specific intracellular lectin, ERGIC-53, first identified as a resident of the ER–Golgi intermediate compartment protein (Schweizer *et al.*, 1988) carries a specific subset of nascent glycoproteins between the two compartments. Two distinct mannose-6-phospate receptors, the only members of the P-type lectin family, mediate the targeting of newly synthesized hydrolases from the rough ER to the lysosomes (Hoflack and Kornfeld, 1985). Both receptors bind their ligands, oligosaccharides bearing terminal Man-6-P residues, most efficiently at pH 6–7, allowing them to interact with hydrolases decorated with such oligosaccharides in the trans-Golgi network, and to release them in the more acidic environment of the lysosomes.

The galectins are believed to act as modulators of cell–substratum interactions and to be essential for the normal differentiation and growth of all multicellular animals. They are capable of inducing cell proliferation, cell arrest, or apoptosis (physiological cell death) and have been implicated in organ morphogenesis, tumor cell metastasis, leukocyte trafficking, immune response, and inflammation, as well as recognition of extracellular matrix.

## Epilogue

As we have shown in this article, during 120+ years, lectins have come a long way since their first detection in plants as hemagglutinins to their present status as ubiquitous recognition molecules with myriad exciting functions and applications.

## Abbreviations

CRD, carbohydrate recognition domain; CTL, C-type lectin; ER, endoplasmic reticulum; HEV, high-endothelial venule; MBL, mannose-binding lectin; PHA, phytohemagglutinin; PNA, peanut agglutinin; SBA, soybean agglutinin; WGA, wheat germ agglutinin.

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