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Reflections
Lectins: carbohydrate-specific reagents and biological recognition molecules*

Nathan Sharon, Department of Biological Chemistry, The Weizmann
Institute of Science, Rehovot 76100, ISRAEL

Email: nathan.sharon@weizmann.ac.il

*Dedicated to Ephraim Katchalski-Katzir, mentor and friend, on the occasion of his 90th birthday, May 16, 2006.

Abbreviations used: ECL, *Erythrina cristagalli* lectin; ECorL, *Erythrina corallodendron* lectin; PHA, phytohemagglutinin; PNA, peanut agglutinin; SBA, soybean agglutinin; WGA, wheat germ agglutinin.

INTRODUCTION

The occurrence in nature of proteins with hemagglutinating activity, that in later years were shown to be sugar specific, and eventually named lectins, has been known since the turn of the 19th century, but until about two decades ago they aroused little interest (for a historical survey, see Ref. 1). My own involvement with these proteins began inadvertently and initially on a part time basis in the early 1960s, after my return to the Weizmann Institute from two and a half years of exciting and educational postdoctoral studies in the USA. During the first of these I worked in the laboratory of Fritz Lipmann at the Massachusetts General Hospital, Boston. Lipmann, one of the most influential biochemists of the last century, was then interested in the mechanism of protein biosynthesis. I was assigned to study the amino acid activation reaction, the first step in this process, work that resulted in two publications (2,3). Concurrently, I greatly enriched my knowledge of biochemistry, mainly from my fellow postdocs, and especially from the guest seminars in which ongoing biochemical discoveries and developments were reported. The second postdoc year I also spent at the MGH, but with Roger Jeanloz, a leading carbohydrate chemist, where I got my training in the subject, and also succeeded in isolating an unusual diamino sugar from a Bacillus polysaccharide I had brought with me from Rehovot (4) (see below); the remaining time I worked with Dan Koshland at Brookhaven National Laboratory on the mechanism of action of myosin ATPase (5, 6). Dan was then starting to make his mark on enzymology with his 'induced fit' concept of enzyme action, originally greeted with much skepticism (7).

Back at Rehovot my original aim was to establish the structure of that diamino sugar; I was fortunate to receive for this purpose my first NIH grant, a modest one of some $ 25,000 for three years (this would have been unheard of at the present time, since nothing was known then about the function of the compound). The task took me with a couple of graduate students, over a decade; eventually we were able to prove by degradation and synthesis that the compound in question, which we named bacillosamine, is 2,4-diamino-2,4,6-trideoxy-D-glucose (8, 9). To my delight, the di-*N*-acetyl derivative of bacillosamine has recently been found attached glycosidically to the amide of asparagine or the hydroxyl of serine, in the

carbohydrate-peptide linkage region of several interesting glycoproteins of pathogenic bacteria (10). By a strange twist of fate, these glycoproteins were originally isolated in 2002 by Martin Young and his colleagues at the National Research Laboratories, Ottawa, from *Campylobacter jeujuni* by affinity chromatography on immobilized soybean agglutinin (SBA) (11), the first lectin I got involved with 40 years earlier.

From soy proteins for nutrition to glycoproteins and lectins

My studies of SBA began together with Halina Lis, with whom it has been my good fortune to collaborate to this very day. It aroused our curiosity not because of its ability to bind sugars specifically and reversibly and to agglutinate cells, the hallmarks of proteins of this class, but because of other reasons that I shall presently mention. We did not have the slightest idea that lectins would become extremely useful carbohydrate-specific reagents, that they would be found to function as mediators of cell recognition, or that they would make a major contribution to glycobiology (12). In fact, for a time we were not even aware of the term lectin, which was originally proposed in 1956 by William C. Boyd from Boston University for blood type specific hemagglutinins. Since SBA, like the majority of the hemagglutinins, is not blood group specific, we began referring to it as a lectin only in 1970, when it occurred to us that the original definition should be broadened to include all cell-agglutinating and sugar-specific proteins (13).

Our interest in SBA developed in the course of investigations on soybean proteins carried out within the framework of a generous and long-term grant from the U. S. Department of Agriculture that I received in 1961 jointly with Katchalski-Katzir (14). Katchalski was the founding Head of the Department of Biophysics at the fledgling Weizmann Institute, which was officially inaugurated in 1949. I came to the Department in 1954, after having received my PhD degree from the Hebrew University, Jerusalem; Halina, with a PhD degree from Uppsala University, joined the Department five years later. The purpose of the above grant was to carry out a fundamental study of the soy proteins, with the aim of providing information for their improved utilization for human nutrition. Katchalski and I were persuaded to embark on this

project by Tim (M. L.) Anson and Aaron Altschul, close friends, noted protein chemists and enthusiastic believers in these proteins as the best solution to world hunger. After some time, Katchalski became immersed in his pioneering studies of polyamino acids as protein models and on enzyme immobilization, and turned over the whole project to me, for which I am extremely grateful.

Halina and I set out by trying to obtain pure proteins from soybeans by chromatographic techniques, but this proved to be a difficult task as most of them lack biological activity, are poorly soluble and undergo complex association-dissociation reactions. We therefore chose to focus on SBA, originally isolated and characterized in the 1950s by Irvin E. Liener at the University of Minnesota, St. Paul. The main reason for our choice was the evidence presented by Liener that it contained glucosamine, raising the likelihood that it may be a glycoprotein (15). In those days, research on glycoproteins was in its infancy, but I became intrigued by these compounds because of my interest in carbohydrates, as described elsewhere (16).

Soybean agglutinin, a plant glycoprotein

Working on SBA, Halina and I soon found that it contains not only glucosamine but also mannose. We then isolated from a proteolytic digest of SBA an asparaginyl-oligosaccharide that contained all the *N*-acetylglucosamine and mannose of the lectin (17). Eventually we also isolated from the lectin *N*-acetylglucosaminylasparagine (18), the carbohydrate-peptide linking group, that was identical with the one originally obtained in 1963 by Albert Neuberger, the founding father of modern glycoprotein research, in his pioneering studies of ovalbumin.

As pointed out recently by Liener (19), 'The fact that SBA was shown to be a glycoprotein may not be particularly surprising to the modern-day biochemists, but at the time the finding of a sugar moiety in a plant protein was accepted with reservation. It was thought that glycoproteins were strictly of animal origin and that the finding of a sugar with a plant protein was most likely due to non-covalent contamination'.

In 1981, jointly with Hans (J. F. G.) Vliegenthart from the University of Utrecht the complete structure of the carbohydrate of SBA was established by NMR as the branched

oligomannoside $Man_9(GlcNAc)_2$, found in animal glycoproteins too, demonstrating that protein *N*-glycosylation is a process conserved in plants and animals (20). A unique feature of SBA is that all its molecules carry the same oligosaccharide (21), in contrast to essentially all other glycoproteins, which bear a variety of glycans at each attachment site, i. e. consist of mixtures of distinct glycoforms. SBA serves therefore as an excellent source of this oligosaccharide (for an example, see Ref. 22).

Emerging from obscurity

Our few 1960s publications on SBA attracted little attention, and we sometimes felt like wanderers in a desert. Although the studies of lectins were in their eighth decade, and several hundreds of these proteins (almost all from plants) had already been identified, the handful of other scientists active in the field at the time did not fare better. Irwin J. Goldstein from the University of Michigan at Ann Arbor, still a leading lectin researcher, tells that when he sent a note, in 1963, to *Biochemical and Biophysical Research Communications* describing the purification of concanavalin A by affinity chromatography, it was rejected forthright because 'this represents a modest advance in an obscure area.' The note was eventually published in the *Biochemical Journal* (23), and affinity chromatography soon became the method of choice for lectin isolation.

However, as the 1960s were folding, the attitude towards lectins began to change and a number of leading biochemists and immunologists, among them Gerald Edelman at Rockefeller University, Mel Greaves at London University, Elvin Kabat at Columbia University, Jerker Porath at Uppsala and Jon Singer at UCSD became involved with them. The reasons for this change in attitude were summarized by Kabat, who had become intrigued with lectins primarily because their combining sites seemed similar to those of antibodies, and who in 1977 stated: 'During the past 10 years there has been an extraordinary burst of activity in the study of plant and animal lectins, stimulated largely by the findings that they have specific receptor sites for carbohydrates and react with glycoproteins in solution or on cell membranes…' (24).

In 1970, affinity chromatography of glycoproteins on immobilized lectins was introduced, among others by Goldstein (25). It became a must at one step or another for the isolation of membrane proteins, all of which are glycosylated, a classical case being that of the insulin receptor with the aid of WGA (26). Lectins proved also to be useful for the separation of purified glycoproteins into their glycoforms, i. e., differently glycosylated forms of the same protein. A very recent telling example is of different glycoforms of IgG with different degrees of sialylation, obtained by fractionation on the sialic acid-specific *Sambucus nigra* agglutinin, and shown to differ in their anti-inflammatory activity (27).

Tools for the study of membranes and cells

Interest in lectins intensified with the realization that they are extremely valuable reagents for the investigation of cell surface sugars, for the assessment of the role of the latter in cell growth and differentiation, in interactions of cells with their environment, and also in a variety of pathological processes. In this connection it is instructive to refer to two classical studies with lectins that provided very early evidence for the presence of sugars on cell surfaces and their potential role as cell identity markers, a common theme in modern glycobiology. One came from the laboratory of James Sumner at Cornell University, Ithaca, who in 1919 isolated concanavalin A in crystalline form, but only in 1936, together with Howell, reported that it agglutinates cells such as erythrocytes and yeasts, and that this agglutination is inhibited by sucrose, and thus demonstrated for the first time the sugar specificity of lectins (28). Moreover, with much foresight they suggested that the hemagglutination induced by the lectin might be a consequence of its reaction with carbohydrates on the surface of the red cells. The other study was by Walter Morgan and Winifred Watkins at the Lister Institute, London, who in the early 1950s used blood type-specific hemagglutinins to show that the blood type A immunodeterminant is α-linked *N*-acetylgalactosamine, and that the H(O) determinant is α-L-fucose (reviewed in Ref. 29). This was the first demonstration that cell-surface carbohydrates can serve as carriers of biological information.

Much excitement was created in the following decade by the reports of Joseph C. Aub from the Massachusetts General Hospital (30), of Max Burger, from Princeton University (31), who were both working with wheat germ agglutinin (WGA, specific for *N*-acetylglucosamine and *N*-acetylneuraminic acid) and of Leo Sachs with Michael Inbar from the Department of Genetics of our Institute, who used concanavalin A (specific for mannose and glucose) (32), that these lectins agglutinated malignantly transformed cells but not their normal parental cells. The reports provided compelling evidence that cancer might be associated with a change in cell surface sugars, an idea that only a few years before had been considered completely unfounded. In collaboration with Leo Sachs and Ben-Ami Sela, we found soon thereafter that SBA (specific for galactose and *N*-acetylgalactosamine) also possesses the remarkable ability to distinguish between normal and malignant cells (33). Numerous subsequent studies have demonstrated that high susceptibility to agglutination by lectins is a property shared by many, albeit not all, malignant cells.

Several basic features of membranes were revealed, or their existence confirmed, with the aid of lectins. Thus, using ferritin-conjugated concanavalin A and ricin as an electron-microscopic probe, Garth Nicolson and Jon Singer at University of California, San Diego, found that the lectin derivatives bind specifically to the outer surface of the human and rabbit erythrocyte membrane, and concluded that the oligosaccharides of the plasma membrane of eukaryotic cells are asymmetrically distributed (34). Further support for such distribution was obtained by Vincent Marchesi at Yale University, who used ferritin-labeled PHA and showed that glycophorin, the major sialoglycoprotein of the human erythrocyte membrane, is oriented so that its carbohydrate-carrying segment is exposed to the external medium, while the other segments of the same molecule are embedded in the lipid bilayer or protrude into the cytoplasm (35).

Other ultrastructural studies with lectins provided some of the most convincing evidence for the fluid mosaic membrane model of Singer and Nicolson, according to which the membrane consists of proteins and glycoproteins floating in a lipid bilayer (reviewed in Ref. 36). Prominent among these was the finding of the lectin induced clustering and patching of

the corresponding membrane receptors on lymphocytes and other kinds of cell, as illustrated for example by the treatment with fluorescein-labeled concanavalin A of rat or mouse lymphocytes (37). Reorganization of cell surface carbohydrates was later shown to be required for various activities of lectins on cells, such as mitogenic stimulation and induction of apoptosis.

The toxicity for animals of certain plant lectins has been recognized since the earliest days of lectin research, at the end of the 19th century. However, research on the toxic action of lectins on cells started only many decades later, with special attention being paid to mammalian cell lines (e. g., CHO and BHK) resistant to different lectins, primarily the highly toxic ricin and the less toxic PHA and WGA (reviewed in Ref. 38). Leading the field was one cell phenotype independently isolated in 1974 by Stuart Kornfeld at Washington University, Colin Hughes at the National Institute for Medical Research, Mill Hill, London and Pamela Stanley at Toronto University, Canada. This phenotype lacked GlcNAc-transferase I, the key enzyme in the biosynthesis of complex and hybrid *N*-linked carbohydrate units of glycoproteins. Soon thereafter many other lectin-resistant cell lines with different enzymatic glycosylation defects became available. They proved extremely valuable for the investigation of the biosynthesis of glycoproteins and glycolipids, and of the function of their carbohydrates, especially those expressed on the cell surface. Currently they also serve for the large-scale production of pharmacologically useful glycoproteins, such as erythropoietin.

Review articles

In the fall of 1970 I arrived at the Department of Biochemistry, University of California at Berkeley, for a sabbatical year as Visiting Professor. My host was Clint Ballou, with whom I discussed at length the possibility of using lectins to examine the ideas on the roles of carbohydrates as information and recognition molecules. Such ideas had been entertained by Saul Roseman from Johns Hopkins University (39) and Victor Ginsburg at the NIH (40). Although there existed a few books and several reviews on lectins, none of them dealt with their molecular properties, nor did they indicate their enormous potential as tools for biological

research. Since Dan Koshland from the same department at Berkeley was then member of the editorial board of *Science*, I approached him with the suggestion that I write a review on lectins for that journal. This suggestion was readily accepted by Philip Abelson, then editor of *Science*

Writing was started by me in the fall of that year in the laboratory of Albert Neuberger at St. Mary's Hospital in London, where I arrived for a few months in order to study lysozyme, on which Neuberger and I were at that time working. However, I ended up purifying WGA by ion exchange chromatography from commercial wheat germ together with Tony (A.K.) Allen, separating it into three isolectins and showing that its specificity is similar to that of lysozyme (41), since it too exhibited a pronounced affinity not only for oligosaccharides derived from chitin, as originally demonstrated by Burger and Goldberg (31), but also of peptidoglycan. In addition we also proved that, contrary to suggestions in the literature, WGA was not a glycoprotein. This work further stimulated the interest of Neuberger in lectins, with which he continued to be engaged for several years, until his eighties.

The *Science* review was completed jointly with Halina upon my return to Rehovot early in 1972 (13). It summarized the history of the research on lectins since their discovery, their specificity for monosaccharides and cells, and the properties of concanavalin A and the few other lectins that had been purified at the time. The changes that occur on cell surfaces upon malignant transformation, as revealed by lectins, were discussed, although their significance was not clear and doubts were raised by us, amply supported later, as to whether they are a distinctive characteristic of malignant cells. Regardless of this, we concluded that lectins, both native and modified, provide a new and useful tool for the study of the chemical architecture of cell surfaces. Finally, we dealt in brief with the speculations on the role of lectins in nature, about which nothing was known with certainty. Another review on lectins was published by us in the following year in the *Annual Review of Biochemistry* (42) and a third appeared in the same series 1986 (43). In these reviews we tried to convey to the readers our fascination and enthusiasm for the subject.

In 1989 we prepared a monograph on lectins and in 2003 a second edition of the same (44), both of which have been translated into Japanese. Some 20 years ago I co-edited a treatise

on lectins, to which Halina and I contributed several chapters (45). A related activity of mine was the publication in 1975 of a book entitled '*Complex Carbohydrates'* in which lectins feature high, and where I expressed my firm belief 'that the specificity of many natural polymers is written in terms of sugar residues, not of amino acids or nucleotides' (46). The book was based on notes that I prepared for the graduate students taking my course on the same subject, and remained in use for a long time. I still continue teaching this course, now under the title 'Molecular and Cellular Glycobiology'.

Structural diversity of lectins

The 1970s witnessed the intensification of the study of the molecular properties of individual lectins, a prerequisite for a deep understanding of their activities at the molecular level. In 1972 concanavalin A became the first lectin for which the primary and three dimensional structures have been established, the latter by X-ray crystallography. This was thanks to the efforts of Gerald Edelman's group at the Rockefeller University (47), and of Karl Hardman and Clinton F. Ainsworth at Argonne National Laboratories (48). The fold first observed in this structure, an elaborate arrangement of extended beta strands into two sheets, became known as the jelly roll or lectin fold (48a). The publication of the concanavalin A structure was soon followed by the determination by Christine Schubert Wright at Virginia Commonwealth University of the three dimensional structure of WGA as well as of its complexes with ligands, even before the complete amino acid sequence of this lectin had become available (49). It is worth noting that at present the structures of close to 100 lectins have been solved, almost all also in complexes with ligands (http://www.cermav.cnrs.fr/lectines/).

In my laboratory, we continued to be occupied with SBA, mainly with Reuben Lotan, a talented and hard working graduate student. Among others we demonstrated that lectin is a tetramer, made up of four nearly identical subunits (50) (all legume lectins consist of two or four subunits) and that the carbohydrate of SBA is not essential for its biological activity (51). Final proof for the latter conclusion came when we obtained the carbohydrate-free SBA in a

bacterial expression system in a fully active form (52); still, why SBA, like most lectins, is glycosylated remains an enigma.

Analysis of the amino acid composition of SBA showed that it is similar to that of other legume lectins, the composition of which was known at the time. In particular, it was devoid of sulfur-containing amino acids, in striking contrast to WGA that is rich in such residues. We have therefore proposed that although 'lectins have many biological properties in common, they represent a diversified group of proteins with respect to size, composition and structure', which is indeed the case (53).

The primary sequence of SBA was determined in the early 1980s at The Rockefeller University by conventional methods (reviewed in Ref. 54). Although homologous with the sequences of the other two legume lectins known at the time (from lentil and fava bean), homology with concanavalin A (also a leguminous lectin) could only be obtained by circular permutation of the latter. This means by aligning residue 119 of concanavalin A with the amino terminal residue of the SBA, proceeding to the carboxyl end of concanavalin A (residue 237) and continuing with its amino terminal region along the sequence of SBA. This kind of circular homology, never observed before, was shown by Diana Bowles and her coworkers at York University, UK, to be the result of a unique rearrangement of the peptide chain that occurs in the last step of the biosynthesis of concanavalin A (55).

Crystals of SBA suitable for X-ray diffraction studies were obtained by Boaz Shaanan and colleagues from our Department of Structural Chemistry in 1984 (56), but the high-resolution structure of the lectin, in complex with a ligand, was solved only a decade later by James Sacchettini and coworkers at Albert Einstein College of Medicine, New York (57).

Biological activities and functions of plant lectins

In 1960 Peter Nowell at the University of Pennsylvania discovered that phyto-hemagglutinin, or PHA, the lectin of the red kidney bean, acts as a mitogen for lymphocytes, namely that it has the ability to stimulate these cells to grow and divide (58). This finding shattered the belief, held until then, that lymphocytes are dead-end cells that could neither

divide, nor differentiate further. Within a short time, several other lectins were proven to be mitogenic. Of special significance was the finding, first reported by Werner G. Jaffé at the University of Venezuela, Caracas, that concanavalin A acts as a mitogen (59) since its binding to the lymphocytes could be inhibited and reversed by low concentrations of mannose, in contrast to PHA for which no effective inhibitor was available at the time. It was thus concluded that mitogenic stimulation is the result of binding of lectins to cell surface sugars, providing another early demonstration of a biological function the latter compounds. Not all lectins that bind to cells are, however. mitogenic, indicating that attachment to selected carbohydrates is required for cell stimulation.

In subsequent years, there was an explosive growth in the use of mitogenic lectins in biological research. Until the advent of monoclonal antibodies they served as a popular tool in attempts to clarify the mechanism of signal transmission through the cell membrane and of cell activation. Mitogen stimulated lymphocytes were found, among others, to produce many growth factors, collectively known as lymphokines or cytokines, the first of which was discovered by Robert C. Gallo and coworkers at the National Institutes of Health, Bethesda, as T-cell growth factor in 1976, and later named interleukin-2 (60). When the immune system is malfunctioning, the mitogenic response of lymphocytes is defective. Stimulation by PHA, and to some extent also concanavalin A and pokeweed mitogen, is routinely employed in clinical laboratories as a simple means to assess the immunocompetence of patients suffering from different diseases and to monitor the effects of various immunosuppressive and immunotherapeutic treatments.

Studies with mitogenic lectins have provided information on the cell surface sugars and other factors involved in cell activation. Thus, using SBA, Abraham Novogrodsky and Ephraim Katchalski found that the lectin stimulated mouse lymphocytes only after the cells had been treated with sialidase, which unmasked the subterminal galactose and *N*-acetylgalactosamine residues of the surface glycoproteins and glycolipids to which the lectin bound (61), and we have subsequently demonstrated that peanut agglutinin (PNA, see below) exhibits the same property (62). We then found that SBA was mitogenic only in polymerized

form (63). It was an early demonstration of the requirement of receptor cross-linking for cell activation. The latter findings were published in *European Journal of Immunology* over the objection of one of the referees, in whose opinion papers on lectins had no place in an immunological journal. Luckily, this view was then not generally held; indeed, at the same time Michael Sela invited Halina and me to contribute a chapter on lectins to *The Antigens*, a treatise he was editing (64). A few years later I wrote a review for *Advances in Immunology* on the application of lectins for lymphocyte identification and separation (65). At present lectins form an integral part of immunology, due to their pivotal role in innate immunity (see later).

Since lectins do not stimulate plant cells, it is highly unlikely that they have been selected by evolution for this purpose. Based on findings in my laboratory that SBA (as well as PNA) inhibits the sporulation and growth of fungi such as *Trichoderma viride, Penicilium notatum*, and *Aspergillus niger,* we raised the possibility that lectins may protect plants against pathogenic microorganisms (66). Work in other laboratories has subsequently extended this proposal to include the defense of plants against predatory animals and phytopathogens (reviewed in Ref. 67). According to another suggestion, plant lectins may be responsible for the specific association between leguminous plants and nitrogen-fixing rhizobia that provide the plants with the needed nitrogen (for review, see Ref. 68). However, this suggestion can account for the role of lectins in only one plant family, the Leguminosae.

For cell separation and bone marrow transplantation

Toward the end of his doctoral research, Reuben Lotan, with Yehuda Marikovsky and David Danon from our Institute, purified PNA by affinity chromatography (69). The lectin was also purified at the same time by Toshiaki Osawa from Tokyo University, friend and pioneer lectin researcher (70). The detailed specificity of PNA was then established in collaboration with Miercio Pereira and Elvin Kabat at Columbia University, confirming among others that the lectin has a high affinity for Galβ3GalNAc (known also as T antigen), a characteristic glycan of *O*-glycoproteins (71).

Just as PNA had become available in my laboratory, it was my good fortune that Yair Reisner joined me as a doctoral student. Bright and imaginative, he set his mind to find out whether lectins can serve as markers for lymphocyte subpopulations. He soon found that immature, cortical mouse thymocytes were agglutinated by PNA, but that the mature, medullar cells were not. This served as the basis for the development by him of a facile method (sometimes referred to by us as 'poor man's cell sorter') for separation, by selective agglutination with PNA, of the two thymocyte subpopulations in good yield and with full viability. A manuscript we sent early in 1976 to *Nature* magazine describing the method was promptly rejected by the editor on the grounds that it was not of general interest. Time proved *Nature* wrong, since soon after it was published in *Cellular Immunology* (72), the method became very popular. This was primarily since it afforded access to the immature thymocyte subpopulation needed for the investigation of T lymphocyte maturation, and because it became 'a classic technique for defining the cortical and medullar regions of the thymus' (73). In our study, we have found a marked difference in the level of PNA binding to the thymocyte subpopulations, which is abolished upon treatment of the mature subpopulation with sialidase. We have therefore proposed that sialylation of the PNA receptor may be an important step in the maturation of the thymic cells. Nearly 20 years later evidence was indeed presented by Linda Baum and her coworkers from UCLA and UCSD that regulated expression of a single glycosyltransferase, a Galβ3GalNAc α2,3-sialyltransferase, can account for this glycosylation change (73). This enzyme sialylates Galβ3GalNAc, the preferred ligand of PNA, forming the sequence NeuAcα2,3Galβ3GalNAc and thus masking the PNA binding sites; expression of the enzyme is inversely proportional to that of the PNA receptor. The PNA receptor continues to serve as a differentiation marker for lymphocytes (74). It has also been employed as a similar marker in other systems, for example in embryonal carcinoma cells of mice, as shown by Yair in a joint study with Francois Jacob and Gabriel Gachelin of the Pasteur Institute, Paris (79).

What proved to be highly significant was Yair's demonstration, together with Asher Meshorer and Lea Itzicovitch from our Experimental Animal Center, that sequential

agglutination of mouse bone marrow or spleen cells by SBA and PNA afforded a cell fraction suitable for transplantation across histocompatability barriers. In the paper reporting on these results, we stated that the same approach 'may prove useful for bone marrow transplantation in humans' (76). For his postdoctoral research, Yair joined Robert A. Good, then President of the Sloan Kettering Institute, New York, and Richard O'Reilly, Chief of Bone Marrow Transplantation at that Institute, with the express aim of adapting the lectin separation method to humans. By 1981 he had found that treatment of human bone marrow with SBA alone removed the bulk of the cells responsible for the lethal graft-versus-host disease, and that, after additional processing, such bone marrow, even from haploidentical donors, could be safely transplanted into children born with severe combined immune deficiency (SCIDs or 'bubble children') (77). It is a matter of great pride and satisfaction to Yair, and to me, that over 75% of the hundreds of 'bubble children' who received since then transplants of bone marrow that had been purged with SBA, have been cured and lead a normal life. For several years SBA-purged bone marrow was also used on an experimental basis for treatment of end stage leukemia patients (78).

Legume lectins – a large family of homologous proteins

I owe the last turning point in my research on plant lectins to Jose Luis Iglesias, a young medical student at the University of Montevideo, Uruguay who had become fascinated by these proteins, and found one in the seeds of *Erythrina cristagalli*, an ornamental leguminous tree common in Uruguay. Because of lack of facilities in his country he was unable to isolate the lectin, so he came to my laboratory for a short stay early in 1981, bringing with him six kg of the flour of *E. cristagalli* seeds. In no time he had ascertained that his lectin, which we had designated ECL, is galactose-specific, and purified it by affinity chromatography on the immobilized sugar (79). He also demonstrated that ECL is a glycoprotein containing fucose and xylose in addition to mannose and *N*-acetylglucosamine present in SBA. The structure of the carbohydrate of ECL was established in the laboratory of Raymond Dwek at Oxford University, UK as the branched Asn-linked heptasaccharide Manα3(Manα6)(Xylβ2)-

Manβ4GlcNAcβ4(Fucα3)GlcNAc (80). It was one of the first examples of this plant specific oligosaccharide reported in the literature. The oligosaccharide is allergenic for humans, and presents a major obstacle that needs to be overcome before plants can be employed for the production of pharmacologically useful glycoproteins, such as monoclonal antibodies.

When we exhausted the supply of *E. cristagalli* seed flour that Jose had brought with him from Uruguay, I turned my attention to *Erythrina corallodendron*, the coral tree that grows commonly in Israel, the seed lectin of which (ECorL) was originally isolated in 1980 by Nechama Gilboa-Garber at Bar Ilan University, Ramat Gan (81). Working on ECorL proved to be highly rewarding. Its primary sequence was established by Rivka Adar using conventional methods, and by her, jointly with Rafael Arango, a graduate student from Medeillin, Colombia, and Shmuel Rozenblatt from Tel Aviv University, by recombinant techniques (82). The sequence was homologous to that of other legume lectins, providing further evidence for the proposal, that I made in 1977 with Donny Strosberg, then at the Free University of Brussels, that despite their distinct sugar specificities, legume lectins are members of a single protein family, and that the genes coding for them have a common ancestry (83). According to a recent count, 210 sequences of legume lectins are known, all homologous (84). In the 1980s sequence similarities were found for lectins from unrelated taxonomic families, including lectins from animal sources, starting with the galectins and C-type lectins (85)

Identical tertiary structures, different quaternary structures

In collaboration with Boaz Shaanan, then at the Weizmann Institute, the three-dimensional structure of ECorL in complex with lactose was established by high resolution X-ray crystallography (86). While the tertiary structure observed was superimposable on that of other legume lectins, the quaternary structure was markedly different from the canonical one, such as that of concanavalin A (which is devoid of carbohydrate); the same was found also for the structure of ECL recently solved in collaboration with Ute Krengel and colleagues from Göteborg University, Sweden (87) We originally assumed that the bulky *N*-linked carbohydrate of ECorL interfered with the formation of the canonical structure by the lectin.

This interpretation has been proven to be incorrect, since in a recent joint study with Avadesha Surolia and colleagues from the Indian Institute of Scientific Research, Bangalore (88) as well as by and by K. Ravi Acharya and colleagues at the University of Bath, UK (89), the quaternary structure of the bacterially expressed ECorL, devoid of carbohydrate, was identical with that of the native one. The noncanonical mode of dimerization of the *Erythrina* lectins is therefore most likely due to factors intrinsic to the protein. Indeed, according to Surolia and his coworkers, legume lectins are a family of proteins in which small alterations in essentially the same tertiary structure lead to large variations in quaternary association (90).

An exceptional feature of the three-dimensional structure of the *Erythrina* lectins is that all the seven protein-linked monosaccharide residues are seen with extraordinary clarity, whereas in almost all other such structures of glycoproteins at most 3 or 4 of the protein-proximal monosaccharides have been observed (86-89). The structures also show that in the crystal lattice the glycosylation site and the carbohydrate-binding site are involved in intermolecular contacts through water-mediated interactions.

How lectins combine with carbohydrates

To obtain detailed information on the combining site of ECorL, Rivka Adar together with Hansjörg Streicher, postdoctoral fellow from the University of Konstanz,

carried out extensive site-directed mutagenesis of the lectin. In addition they made a thorough examination of its specificity in collaboration with Jonas Ångstrom and coworkers from University of Göteborg, and with Ray Lemieux from the University of Alberta, Edmonton. Taking also in consideration the three-dimensional structure of the ECorL-ligand complex (87), we have concluded that a constellation of three key amino acid residues, an aspartic acid, an asparagine and an aromatic one, is essential for galactose binding (91-94). The first two residues form hydrogen bonds with the hydroxyls of the ligand, whereas the third interacts with it hydrophobically. Moreover, an identical constellation of amino acid residues is involved also in binding of mannose by other legume lectins, for example by concanavalin A. We suggested therefore that homologous lectins with distinct specificities might bind different

monosaccharides primarily by the same set of invariant residues that are identically positioned in their tertiary structures, although with the ligand in a different orientation (95). Quite surprisingly, the constellation present in the combining sites of legume lectins is also found in certain animal lectins, such as the mannose-specific ERGIC-53 that serves as a carrier of a specific subset of nascent glycoproteins between the ER and Golgi compartments (96). Comparison with the combining sites of lectins from other sources, including of animals, led to an additional conclusion, namely that structurally different lectins with similar specificities may bind the same saccharide by different sets of combining site residues (95). A survey of the literature showed that lectins bind their ligands most commonly by hydrogen bonds (some mediated by water) and hydrophobic interactions, and that in rare cases electrostatic interactions (ion pairing) and coordination with metal ions also play a role. On the whole, most of the side chains of the protein amino acids, as well as main chain groups, can participate in ligand binding. All the above seems to indicate that lectins are products of convergent evolution.

Cell-cell recognition molecules in microbial infection

In the foregoing, I dealt almost exclusively with plant lectins. However, for the last 30 years I have also been interested in microbial lectins, which I wish to describe now. This interest was motivated by the arrival in my laboratory in 1975 of Itzhak Ofek as a postdoctoral fellow. Through him I learned that primarily thanks to the efforts of J. P. Duguid of Ninewells Hospital Medical School in Dundee it had been known that many strains of *E. coli* possess the ability to agglutinate erythrocytes and that this activity is inhibited by mannose and methyl α-mannoside (97), but little attention was paid to these findings. Moreover, the idea that sugar-specific adhesion to host cells might be a prerequisite for bacterial colonization and infection was not considered at all. In retrospect, this is all the more surprising since it had already been established that initiation of infection by influenza virus required its attachment via its hemagglutinin to a sugar (sialic acid) on cells (for an early review, see Ref. 98). Together with David Mirelman, a former graduate student of mine, and Ofek (who later moved to the Sackler Medical School, Tel Aviv University) we found that *E. coli* adheres readily to buccal epithelial

cells and that this adhesion was inhibited specifically by mannose and methyl α-mannoside. The adhesion was also inhibited by pretreatment of the epithelial cells with concanavalin A, but not with other lectins such as SBA. Extraction of the bacteria afforded a lectin-like constituent specific for mannose. We concluded that mannose, a common constituent of most mammalian cell surfaces, acts as receptor for *E. coli* (99). The same conclusion was reached independently and at the same time by Irving Salit and Emil Gotschlich from the Rockefeller University who demonstrated that binding to monkey cells of purified fimbriae obtained from a mannose specific strain of *E. coli* was inhibited by analogues of mannose or by preincubation of the cells with mannose-specific plant lectins (100). These studies provided a clear example, I believe the first of its kind, that lectins function in cell-cell recognition.

With Ofek and Nurit Firon, a graduate student, we have mapped the combining site of the type 1 fimbriae, mainly by the ability of variety of mannose-containing oligosaccharides and α-mannosides to inhibit the agglutination of yeasts by the bacteria or by the isolated fimbriae (101, 102). Hydrophobic mannosides in particular were found to be powerful inhibitors of the agglutination, up to three orders of magnitude more effective than methyl α-mannoside. Based on our results, we proposed that the site is extended, with an adjoining hydrophobic region. A very recent study by Julie Bouckaert and colleagues at the Free University of Brussels of FimH, the carbohydrate-binding subunit of the fimbriae, has confirmed our results and extended them (103). By X-ray crystallography and modeling of FimH-ligand complexes a molecular explanation was obtained for the high affinity of hydrophobic mannosides to the fimbriae, which is based on the interaction of their aglycones with aromatic side chains (referred to as 'hydrophobic gateway') close to the mannose-binding site of the subunit.

That blocking of the bacterial lectins may prevent infection was proven by us in a study carried out in collaboration with Ofek and Mirelman, together with Moshe Aronson from Sackler Medical School, Tel Aviv University. Infection of mouse bladder with a mannose specific *E. coli* strain was markedly diminished by pre-suspension of the organism in a solution of methyl α-mannoside, but was not affected by glucose, a sugar to which the bacteria do not

bind (104). The prophylactic effects of adhesion-inhibitory saccharides have been demonstrated in many other animal models, such as pneumococcal pneumonia in rats (105) and *Helicobacter pylori* gastric infection in monkeys (106). In addition to demonstrating unequivocally that recognition of cell surface carbohydrates is a prerequisite for infection by lectin-carrying bacteria, the above data serve as a definitive proof for the validity of the concept of anti-adhesion therapy of microbial diseases. However, success of such treatment in humans has not yet been achieved (for review, see Ref. 107).

The specificity of the bacterial surface lectins is a key determinant in their animal tropism, as first illustrated by Victor Ginsburg in the case of *E. coli* K99 (108). The fimbrial lectin of this organism is specific for glycolipids containing *N*-glycolylneuraminic acid but not for those containing *N*-acetylneuraminic acid. The former sialic acid is present on intestinal cells of newborn piglets, but is replaced by *N*-acetylneuraminic acid when the animals develop. It is also not normally formed by humans, explaining why *E. coli* K99 can cause lethal diarrhea in piglets, but not in adult pigs or in humans.

In addition to their role in initiation of infection, the mannose-specific bacterial surface lectins may also function in protection against infectious agents. This is the case also for surface lectins of phagocytic cells (such as granulocytes and macrophages). As we have shown some time ago, bacteria and yeasts may bind to these cells in the absence of opsonins, leading to uptake and killing of the organisms. This phenomenon, named by us 'lectinophagocytosis' (109), is an early example of innate immunity, a phenomenon of great importance, in which lectins are now known to be major players (reviewed for example in Refs. 110 and 111).

Concurrent with our studies of the mannose specific *E. coli*, Catharina Svanborg and Hakon Leffler discovered strains of this organism with galabiose (Galα4Gal)-specific fimbriae, that also serve as mediators of attachment of the bacteria to host tissues as a prelude to infection (112). Two surface lectins were discovered soon thereafter in the pathogenic protozoan *Entamoeba histolytica*. One of these, specific for *N*-acetylgalactosamine, was reported by Jonathan Ravdin and Richard Guerrant from the University of Virginia, Charlottesville (113); the other, specific for *N*-acetylglucosamine oligomers, was described at

the same time by David Mirelman and David Kobiler in our Department (114). During the years, compelling evidence has been obtained for the involvement of the former lectin in host cell binding, as well as in a variety of other properties of the ameba, that determine the severity of its infection (for review, see Ref. 115).

Animal lectins in cell recognition

In spite of the evidence presented above, wide acceptance of the concept that lectins function as recognition molecules was slow to come and had to await the isolation and characterization of mammalian lectins. The first of these was the galactose-specific liver lectin, discovered in 1974 by Gilbert Ashwell at NIH with Anatol G. Morell at Albert Einstein College of Medicine, in the course of their investigation of the mechanisms that control the lifetime of glycoproteins in blood circulation (reviewed in Ref. 116). This lectin recognizes and binds asialo-glycoproteins and is responsible for their uptake by the liver and eventual degradation.

In our Department and at about the same time, my former graduate student Vivian Teichberg and Gad Reshef, another graduate student of mine who was killed in the October 1973 war, isolated from the electric eel (*Electrophorus electricus*) and from chicken muscle the first members of the family of the soluble β-galactose-specific lectins (117) designated galectins, several of which are known to be mediators of cellular interactions (for reviews see Refs 118 and 119)). One of these, galectin-3, that is involved in metastatic spread of B16 melanoma cells, was isolated by Reuben Lotan, while still in our Department, together with Avraham Raz from the Department of Membrane Research (120, 121). Another member of this family, galectin-8, has also been isolated at the Weizmann Institute by Yehiel Zick and coworkers from the Department of Molecular Cell Biology (122); by binding to cell surfaces, this lectin modulates cell-matrix interactions and regulates cellular functions in a variety of physiological and pathological conditions.

What fully convinced the skeptics was the discovery at about 1990 of the selectins, a class of C-type mammalian lectins, and the demonstration of their crucial role in the control of

lymphocyte migration (homing) to specific lymphoid organs and to sites of inflammation (for a recent review, see Ref 123). Since then, numerous other mammalian lectins have been discovered, many of which are cell-surface constituents, strategically located to serve as recognition molecules in a variety of systems, both normal and pathological (for reviews, see Refs 110, 111, 122 and 124). Indeed, recognition by lectins in animal tissues is undoubtedly one of the major developments in glycobiology during the last part of the 20th century. But that is another story.

Acknowledgements

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Nathan Sharon

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