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Memories of a Senior Scientist

A life with lectins

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Introduction

My involvement with lectins is a striking example of the unpredictability of scientific research. I entered the field inadvertently and my path was shaped largely by chance observations and fortuitous personal encounters. It was further driven by my curiosity and the desire to contribute to human wellbeing. The result was an intellectual adventure, exciting and rewarding.

In 1962 I started working on soybean agglutinin (SBA) together with Halina Lis, with whom it has been my good fortune to collaborate to this very day. SBA was the first of several lectins, each unique in its own way, studied extensively in my laboratory. It aroused our curiosity not because of its ability to bind sugars specifically and reversibly or 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 could be used for the detection and isolation of glycoproteins, for the investigation of carbohydrates on cell surfaces, or for cell fractionation, as demonstrated later in my laboratory and elsewhere. Neither did we imagine that they would provide important insights into the molecular basis of protein-carbohydrate interactions, nor that they would be found to function as mediators of cell recognition and as key players in innate immunity [1]. In fact, for a time we were not even aware of the term lectin. This term (from the Latin *legere*, to pick out or choose) was introduced in 1954 by William C. Boyd, from Boston University, for those plant agglutinins, or phytohemagglutinins, that were shown in the 1940s to be blood type specific. Since SBA, like the majority of the phytohemagglutinins, is not blood group specific, we began referring to it as a lectin only in the late 1960s, when it occurred to us that Boyd's narrow definition should be broadened to include all cell agglutinating and sugar specific proteins.

From soy proteins for nutrition to plant glycoproteins

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 US. Depart-

ment of Agriculture that I received in 1961 jointly with Ephraim Katchalski-Katzir, then Head of the Department of Biophysics, of which both Halina and I were junior members. The purpose of the grant was to carry out a fundamental study of these proteins, with the aim of providing information for their better 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 soy 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 most 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 the hemagglutinin, originally isolated from soybeans and characterized in the 1950s by Irvin E. Liener at the University of Minnesota, St. Paul. The first reason for this choice was its possible deleterious effect on the nutritional properties of both raw soybeans and soybean meal, the product obtained after extraction of the oil from the seeds. The other reason was the evidence that SBA contained glucosamine, raising the likelihood that it may be a glycoprotein.

In those days, research on glycoproteins was in its infancy, and nothing was known of their occurrence in plants, but I became intrigued by these compounds because of my interest in carbohydrates. How this interest in carbohydrates came about, and where it led to, besides lectins, is another story [2]. Here I wish only to point out one outcome of this interest, which is my continued teaching the biology of these compounds, primarily at the Feinberg Graduate School of the Weizmann Institute. Originally my course on the subject was named 'Complex Carbohydrates', and it served as the basis of a well-received textbook [3]; during recent years I changed the name of the course to 'Molecular Biology of Glycoproteins and Glycolipids'. It now covers much of glycobiology, a fast growing field that has been referred to as the last unconquered frontier of molecular cell biology, and of which lectins form a substantial part.

Soybean agglutinin, the first known plant glycoprotein

Working on SBA, we soon found that it contains not only glucosamine but also mannose, another typical constituent of glycoproteins. We went on to isolate, from a proteolytic digest of SBA an asparaginyl-oligosaccharide that contained all the *N*-acetylglucosamine and mannose of the lectin [4], and eventually also *N*-acetylglucosaminyl-asparagine [5], the carbohydrate-peptide linking group. We thus proved that the carbohydrate of SBA is covalently attached to the protein by a linkage identical with the one originally found in 1963 by Albert Neuberger in his pioneering studies of ovalbumin. Ours was the first unequivocal demonstration of the presence of glycoproteins in the plant kingdom. Only 15 years later, jointly with Hans (J.F.G.) Vliegenthart from the University of Utrecht, who used high resolution nuclear magnetic resonance, the full structure of the carbohydrate of SBA was established as the branched oligomannoside $Man_9(GlcNAc)_2$, present in animal glycoproteins too. We have thus provided strong evidence for the evolutionary conservation of such protein-linked glycans.

Lectins as markers for cancer cells

Our early work on SBA attracted little attention, and we sometimes felt like wanderers in a desert. Although the studies of lectins were in their seventh decade, and several hundreds of these proteins (almost all from plants) had already been identified, the handful of other researchers active in the field at the time did not fare better.

The situation changed dramatically by the late 1960s, with the accumulation of evidence that cell surface sugars play a key role in cell growth and differentiation, in interactions of cells with their environment, and also in a variety of pathological processes. Much excitement was created by the reports of Max Burger, then at Princeton University, who was 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), that these lectins agglutinated malignantly transformed cells but not their normal parental cells. These 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 that SBA (that we showed to be specific for galactose and *N*-acetylgalactosamine) also possesses the remarkable ability to distinguish between normal and malignant cells [6]. These finding gave a strong boost to the application of SBA and other lectins to the examination of cell surfaces and the changes they undergo during different physiological and pathological conditions.

Early review articles with wide impact

In 1971, while on sabbatical the Department of Biochemistry, University of California at Berkeley, I re-

ceived an invitation to prepare a review on lectins for *Science* magazine. Writing was started by me in the fall of that year in the laboratory of Neuberger at St. Mary's Hospital in London, where I arrived in order to study lysozyme, an enzyme both Neuberger and I were at the time working on. However, I ended up purifying WGA with Tony (A.K.) Allen, separating it into three isolectins and showing that its specificity is similar to that of lysozyme, since it too exhibited a pronounced affinity for oligosaccharides derived from chitin or peptidoglycan [7]. We also demonstrated 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 involved for several years, until his late seventies.

Writing the review for *Science* was completed jointly with Halina Lis upon my return to Rehovot [8]. It summarized the history of lectins since their discovery at the turn of the 19th century, their specificity for monosaccharides and cells, and the properties of the handful of purified lectins, primarily concanavalin A. The changes that occur on cells upon malignant transformation, as revealed by lectins, were discussed, although their significance was not clear, and doubts were raised, amply supported later, as to whether they are a specific characteristic of malignant cells. In spite 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. The review soon gained popularity, in 1982 it became my first Citation Classic - one of seven that I co-authored, all on lectins, and by now has been referred to over 1,500 times. Another major review on lectins was published by us in the following year in the prestigious *Annual Review of Biochemistry*, and a third appeared in the same series in 1986; together they have been cited close to 1,500 times. Throughout the years I have met quite a number of young scientists who told me that these reviews inspired them to do research on lectins. It was probably because we were able to convey to the readers our fascination and enthusiasm for the subject.

The early reviews brought us a large number of invitations to write other ones on lectins; several of these I prepared myself, as the one in *Scientific American* in 1977, but many more were co-authored with Halina, the most recent one for *Chemical Reviews* [9]; we also contributed entries on the subject to half a dozen scientific encyclopedias, among them of immunology, molecular medicine and biological chemistry. In 1989 we published a monograph on lectins [10], a second and greatly enlarged edition of which appeared recently [11]. Some 20 years ago I co-edited a treatise on lectins to which we contributed several chapters [12].

Structure and activity of soybean agglutinin

In the early 1970s, Halina and I embarked on a systematic investigation of the molecular and biological properties of SBA, mainly with Reuben Lotan, a talented and hard working graduate student. Among others we demonstrated that SBA is a tetramer, made up of four nearly identical subunits [13] (all legume lectins consist of two or four subunits) and that the covalently linked carbohydrate was not essential for the biological activity of the lectin. Final proof for the latter conclusion came when carbohydrate-free SBA was obtained in my laboratory in a bacterial expression system, in a fully active form [14].

During the 1960s, several lectins were shown to be mitogenic, i.e. to stimulate lymphocytes to grow and divide. Such lectins became a popular tool in attempts to clarify the mechanism of signal transmission through the cell membrane and of cell activation. Using SBA, Abraham Novogrodsky and Katchalski found in 1973 that the lectin stimulated mouse lymphocytes only after they had been treated with sialidase, which unmasked the subterminal galactose and *N*-acetylgalactosamine residues of the surface glycoproteins and glycolipids to which the lectin must bind in order to initiate the mitogenic signal. With Lotan and others we have subsequently found that SBA was mitogenic only in polymerized form. This was an early demonstration of the effect of lectin valence and/or size on its mitogenic activity, and provided support for the assumption that receptor cross-linking was a prerequisite for cell activation.

For cell separation and bone marrow transplantation

Toward the end of his doctoral research, Lotan, together with Yehuda Marikovsky and David Danon from our Institute, purified peanut agglutinin (PNA) by affinity chromatography and characterized it [15]. The specificity of the lectin has been studied immunochemically by Miercio Pereira in the laboratory of Elvin Kabat at Columbia University, New York [16]. These publications have been widely quoted, primarily because the lectin proved to be useful for numerous applications, especially in immunology.

Just as PNA became available in my laboratory, it was my good fortune that Yair Reisner joined me as a Ph.D. 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, medullary mouse thymocytes were agglutinated by PNA, but that the mature, cortical cells were not. This startling finding served as the basis for the development by Yair of a facile and inexpensive 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, something which was not possible before [17]. The method soon

became popular because it afforded for the first time access to the immature thymocyte subpopulation needed for the investigation of the maturation of T lymphocytes. PNA was also applied as a differentiation marker in other systems, for example in mice, as shown by Yair in a collaborative study with Francois Jacob and Gabriel Gachelin at the Pasteur Institute, Paris, and in humans by others.

Encouraged by the success with PNA, we went on to show, together with Amiram Ravid from our Department, that agglutination by SBA effectively separated mouse splenocytes into T- and B-cells. What proved to be much more significant was Yair's finding, together with Asher Meshorer and Lea Itzicovitch from our Experimental Animal Center, that sequential agglutination of mouse bone marrow or spleen cells by both lectins afforded a cell fraction suitable for transplantation into immunologically unrelated recipients. In the paper describing these results [18], we stated that the same approach 'may prove useful for bone marrow transplantation in humans.' Upon completion of his Ph.D. studies, 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 used for transplantation into children born with severe combined immune deficiency (SCIDs or 'bubble children'). 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 transplants of bone marrow that had been purged with SBA, have been cured and lead a normal life.

Bacterial lectins, cell recognition and anti adhesion therapy of microbial diseases

Although I had been working with bacteria even before getting involved with lectins, I first learned about bacterial adhesion and its possible role in the initiation of infection from Itzhak Ofek, when he joined my laboratory in 1975 as postdoctoral fellow. He set out to examine if carbohydrates on host cells serve as adhesion (or attachment) sites for *Escherichia coli*. Together with David Mirelman, a former graduate student of mine, they found that *E. coli* adhered readily to buccal epithelial cells, and that the adhesion was inhibited specifically by mannose and methyl α-mannoside [19]. Binding was also inhibited by precoating the epithelial cells with concanavalin A, but not by lectins specific for other sugars, Extraction of the bacteria afforded a lectin-like constituent specific for mannose, but no data were obtained by us on its identity with the bacterial fimbriae (or pili), later shown as the mannose-specific bacterial surface lectin. We concluded that mannose, a common constituent of most mammalian cell surfaces, acts as receptor for binding of *E. coli*, thus providing a convincing example, the first of its kind, of the role of lectins in intercellular recognition [20]. Although suggestions that lectins might perform such a function had been made earlier, this concept only became widely accepted at the beginning of the 1990s, with the discovery of the selectins and their role in the control of leukocyte traffic.

The relevance of our findings to bacterial infection was proven in a subsequent 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* was markedly diminished by pre-suspension of the organism in a solution of methyl a-mannoside, but was not affected by glucose, a sugar to which the bacteria do not bind [21]. Our findings were since then confirmed with other infectious organisms and a variety of animals, including calves and monkeys and serve as the basis for the continuing attempts to develop anti-adhesion therapy of microbial diseases.

We have also shown that carbohydrate-lectin interactions play an important role in the recognition between microorganisms and phagocytic cells (such as granulocytes and macrophages) leading to uptake and killing of the organisms. This phenomenon, originally named by us 'lectinophagocytosis' [22], is an early example of innate immunity.

From primary sequences to three dimensional structures

I owe the last turning point in my research on lectins to Jose Luis Iglesias, a young medical student at the University of Montevideo, Uruguay. Having read our publications on lectins, he had become fascinated by these proteins, and found one in the seeds of *Erythrina cristagalli*, an ornamental leguminous tree common in his country. Because of lack of facilities he was however 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, was galactose specific, purified it by affinity chromatography on the immobilized sugar, and defined its specificity pattern [23]. He also showed that ECL is similar in many respects to SBA (except that it is a dimer, while SBA is a tetramer) and that it too is a glycoprotein containing fucose and xylose in addition to the mannose and *N*-acetylglucosamine in SBA. The structure of the carbohydrate of ECL was established in collaboration with the group of Raymond Dwek from Oxford University as the

branched Asn-linked heptasaccharide Manα3(Manα6)-(Xylβ2)Manβ4GlcNAcβ4(Fucα3)GlcNAc. This was one of the first examples of such a plant specific oligosaccharide reported in the literature.

When we had exhausted the supply of *E. cristagalli* flour that Jose had brought with him, I turned my attention to *Erythrina corallodendron*, the coral tree that grows commonly in Israel, the lectin of which (EcorL) was originally isolated in 1980 by Nechama Gilboa-Garber at Bar Ilan University, Ramat Gan. Working on ECorL proved to be highly rewarding. Its primary sequence was established by Rivka Adar by conventional methods, and by her, together with Rafael Arango, a graduate student from Medeillin, Colombia, and Shmuel Rozenblatt from Tel Aviv University, by recombinant techniques [24]. It was homologous to that of other legume lectins, providing further evidence for the proposal, made in 1977 by myself and A. Donny Strosberg from the Free University of Brussels, that despite their distinct sugar specificities, legume lectins are members of one protein family, and that the genes coding for them have a common ancestry [25].

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 [26]. 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; the same was found also for the recently solved structure of ECL [27]. We originally ascribed this difference to the interference by the *N*-linked carbohydrate of ECorL in forming the canonical structure. 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, the quaternary structure of the bacterially expressed ECorL, devoid of carbohydrate, was the same as that of the native one [28].

Examination of the three-dimensional structure of the ECorL-lactose complex, as well as site-directed mutagenesis of the lectin carried out by Rivka Adar together with Hansjörg Streicher, postdoctoral fellow from the University of Konstanz, has led to the identification of the residues of the lectin that are essential for ligand binding; they were found to be identical to those of other legume lectins with different carbohydrate specificities as well [29, 30]. These studies have contributed to a deeper understanding of the molecular basis of carbohydrate-protein interactions [31].

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