Biomedical Aspects of Glycoconjugate Recognition

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Biomedical aspects of lectins

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Introduction

Studies on lectins are having an increasing impact on medicine, by providing new tools for diagnosis, helping to solve clinical problems, and giving new insights into pathological processes (Lis & Sharon, 1986a, b; Sharon & Lis, 1988). Two early discoveries: that certain lectins are blood-group specific while others are mitogenic, have led to their widespread use for blood typing, for assessing the immunocompetence of patients and for karyotyping. Another early finding, that lectins preferentially agglutinate malignant cells, has led to numerous attempts to use these proteins for cancer diagnosis.

Cell fractionation and bone-marrow transplantation

A more recent development, which has resulted in a clinical application, has been the demonstration that lectins can be used for the identification and fractionation of cells. This may be considered as an extension of the use of lectins for the characterization and separation of glycoproteins, glycopeptides and glycolipids.

In principle, any heterogeneous population of single cells may be sorted into subpopulations with the aid of lectins, provided there are differences in the surface sugars of the cells belonging to the subpopulations. To date, however, lectins are used mainly for the identification and fractionation of immune cells (Sharon, 1983).

Since the binding of lectins to cells can be reversed without damage to the latter by the addition of an appropriate sugar, both the lectin reactive and non-reactive cells are readily recovered, affording high yields of fully viable cells. Lectins therefore offer a distinct advantage over antibodies to cell surface constituents, since as a rule it is impossible to remove the antibodies from the cells to which they are bound.

The first and most popular method for cell fractionation by lectins was developed in our laboratory some 12 years ago (Reisner & Sharon, 1984). It employs selective agglutination with peanut agglutinin (PNA) for separation of murine thymocytes. The fraction agglutinated by PNA consists of immunologically immature cortical cells which represent about 85–90% of the total number of thymocytes. The cells not agglutinated by the lectin (10–15% of thymocytes) correspond to mature, medullary thymocytes. Human thymocytes can similarly be fractionated into immature and mature cells. Separation with PNA provided, for the first time, access to both thymocyte subpopulations and made it possible to examine in vitro the developmental and functional relationship between them (Sharon, 1983).

Another lectin which proved useful for cell fractionation is soybean agglutinin (SBA). It effectively separates SBA mouse splenocytes into B and T cells (Reisner & Sharon, 1984). Sequential agglutination with PNA and SBA of mouse splenocytes yields a fraction that is enriched in haemopoietic stem cells (Reisner et al., 1978). This fraction was also devoid of mature T cells that are responsible for the lethal graft-versus-host reaction which occurs when histo-incompatible (mismatched) bone marrow is used for transplantation. The stem-cell-enriched fraction prepared from one strain of mice was successfully implanted into lethally irradiated mice of another strain, resulting in survival of nearly all the treated animals. In contrast, most irradiated mice that were transplanted with unfractonated splenocytes died. These results suggested that lectin fractionation might also be adapted for the removal of T cells from human bone marrow.

Work carried out by Reisner et al. (1983) has indeed shown that treatment of human marrow with SBA results in agglutination of the bulk of the mature T cells responsible for graft-versus-host disease. The remaining mature T cells are subsequently removed by rosetting with sheep erythrocytes, affording a fraction highly enriched in stem cells. Since 1981, over 100 children born with severe combined immune deficiency (SCID) have been transplanted by lectin-separated marrow from half-identical donors, usually one of the parents. Over 70% of these children have been cured and lead a normal life. The lectin-separation method is being employed also for the treatment of leukemia patients, with encouraging results (O'Reilly et al., 1987). It was used in Moscow in May 1986, in the attempts to rescue the lives of several of the lethally irradiated victims of the Chernobyl accident.

Bacterial lectins and infectious disease

Research on bacterial lectins has provided a better understanding of the development of infection and new ideas about prevention (Sharon, 1987). Work carried out in our laboratory and elsewhere during the last decade has shown that numerous bacterial strains produce surface lectins, commonly in the form of filamentous proteins known as fimbriae or pili. Several types of fimbriae, with respect to their structure and sugar specificity, have been identified. The best characterized of these are Type 1 or common fimbriae of Escherichia coli and related species that are mannose

Abbreviations used: PNA, peanut agglutinin; SBA, soybean agglutinin.
Specific, and Type P fimbriae, also of E. coli, specific for Galα4Gal. Other examples are Type S fimbriae of E. coli, specific for NeuAca2→3Gal, and Type 2 fimbria of oral actinomyces, specific for β-galactosides. Purified fimbriae consist of fimbrillin (or pilin) subunits, the majority of which have a molecular mass in the range of 15–30 kDa. A single fimbria is made up of several hundred subunits, and is a very stable structure. The purified fimbriae bind to cells containing the complementary surface sugars, but frequently they do not act as agglutinins unless they have been polymerized, for example by treatment with glutaraldehyde or anti-fimbrial antibodies.

Detailed characterization of the combining sites of the bacterial surface lectins is important not only for gaining a better insight into the nature of the interaction between bacteria and cell surfaces, but also as a basis for the design of new methods of blocking bacterial adherence and thus for the prevention of infection. We and others have therefore examined the inhibitory effect of a large number of glycosides and oligosaccharides of mannose on the agglutination of yeast and guinea-pig erythrocytes by Type 1 fimbriated E. coli and Klebsiella pneumoniae, as well as on the binding of the bacteria to epithelial cells (Sharon, 1987). In addition, the mannose-specific binding of the bacteria to wild-type and lectin-resistant mutants of BHK cells that have altered surface carbohydrates, has been compared. The major conclusions of these studies are that Type 1 fimbriae: (a) have an extended combining site complementary to the trisaccharide Mana3Manp4GlcNAc; (b) bind preferentially to oligomannose units and hybrid units that are typical components of cell surface glycoproteins, but frequently they do not have a hydrophobic site close to the carbohydrate-binding site.

While all strains of Type 1 fimbriated E. coli examined, as well as K. pneumoniae, exhibited essentially the same pattern of specificity, this was not the case with other enterobacteria. For example, with Salmonella species, aromatic α-mannosides, as well as the trisaccharide Mana3Manp4GlcNAc, were weaker inhibitors than methyl α-mannoside. Therefore, the combining sites of Type 1 fimbriated Salmonella species are probably smaller than that of E. coli or K. pneumoniae, and it is devoid of a hydrophobic region. Different combining sites appear to be expressed by several other mannose-specific bacterial lectins. Although classified under the general term mannose specific (or mannose sensitive), the fimbrial lectins of different genera and species differ in their sugar specificity. Within a given genus, however, all strains tested exhibit the same specificity.

Considerable experimental evidence has accumulated to support the conclusion that bacterial surface lectins, such as those specific for mannose, function in the initiation of infection (Sharon, 1987). For example, methyl α-mannoside caused a significant decrease (by a factor of three compared with the control group) in the extent of bacteriuria in mice infected in their bladders with a mannose-specific strain of E. coli. Methyl α-glucoside, which is not an inhibitor of the mannose-specific adherence, did not affect the incidence of bacteriuria. It was also shown that methyl α-mannoside, but not glucose, caused a large decrease in infection by mannose-specific K. pneumoniae in the bladder of rats.

Further evidence for the role of Type 1 fimbriae in infection is based on comparison of the infectivity of pairs of bacterial variants or phenotypes, only one of which expresses the Type 1 fimbrial lectin. In all cases, the fimbriated phenotype was several times more infective than the non-fimbriated one.

The bacterial surface lectins may function also in lectinophasycytosis, the phagocytosis of bacteria in the absence of opsonins (i.e. antibody and complement) (Ofek & Sharon, 1988). The most thoroughly characterized system of lectinophasycytosis is that of Type 1 fimbriated E. coli and related bacteria which bind to mannose residues on the surface of human, mouse or rat peritoneal macrophages or human polymorphonuclear leucocytes, with resultant stimulation of the phagocytes and internalization and killing of the bacteria.

We have recently isolated, by affinity chromatography on a column of Sepharose-bound Type 1 E. coli fimbriae, three surface glycoproteins (molecular mass 70–80 kDa, 100 kDa and 150 kDa) from human polymorphonuclear leucocytes which serve as receptors for mannose-specific E. coli, and may be involved in the lectin-mediated phagocytosis of the bacteria (Rodriguez-Ortega et al., 1987). Two of these, gp150 and gp100, may be identical with the α- and β-subunits of leucocyte complement receptor and adhesion glycoproteins involved in complement-mediated opsonophagocytosis.

Lectinophagocytosis probably serves as a defence mechanism against microbial infections in the preimmune state and in sites poor in opsonins (e.g. peritoneal cavity of patients undergoing peritoneal dialysis). Evidence for its occurrence in vivo is, however, still limited. We expect that further studies of lectinophasycytosis, as well as of the interaction of bacteria with epithelial cells, will lead to a deeper understanding of the molecular basis of infectious diseases, and perhaps also to new approaches for their elimination.

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