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The Second Datta Lecture

Bacterial lectins, cell-cell recognition and infectious disease

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Numerous bacterial strains produce surface lectins, commonly in the form of fimbriae that are filamentous assemblies of protein subunits. Among the best characterized of these are the type 1 (mannose specific) fimbrial lectins of *Escherichia coli* that consist almost exclusively of one class of subunit with a molecular mass of 17 kDa. They possess an extended combining site corresponding to a trisaccharide and preferentially bind carbohydrate units of oligomannose or hybrid type. Type 1 fimbriae also possess a hydrophobic region close to the carbohydrate-binding site, since aromatic α-mannosides inhibit strongly (up to 1000-times more than methyl α-mannoside) the agglutination of yeasts by the bacteria and the adherence of the latter to pig ileal epithelial cells. The combining sites of type 1 fimbriae of the salmonellae and of other enteric bacteria are different from those of *E. coli* in that they are smaller and do not possess a hydrophobic region. The various bacterial surface lectins appear to function primarily in the initiation of infection by mediating bacterial adherence to epithelial cells, e.g. in the urinary and gastrointestinal tracts. The mannose specific lectins also act as recognition molecules in lectinophagocytosis (i.e. phagocytosis of the bacteria in the absence of opsonins) by mouse, rat and human peritoneal macrophages, and human polymorphonuclear leukocytes. Affinity chromatography of membrane lysates from human polymorphonuclear leukocytes on immobilized type 1 fimbrial lectin, using methyl α-mannoside as eluent, showed that glycoproteins with apparent molecular masses of 70–80, 100 and 150 kDa act as receptors for the bacteria. Inhibition experiments with monoclonal antibodies suggest that the glycoprotein bands of 100 and 150 kDa may be identical with the α and β subunits of leukocyte complement receptors and adhesion glycoproteins involved in complement-mediated opsonophagocytosis. The systems described serve as a fine illustration for the biological role of lectin-carbohydrate interactions. Further studies of these systems will lead to a deeper understanding of the molecular basis of infectious diseases, and perhaps also to new approaches for their prevention.

1. INTRODUCTION

The occurrence in plant seeds of proteins that agglutinate erythrocytes was first noted one hundred years ago [1]. That these hemagglutinins, known for a long time as phytohemagglutinins or phytoagglutinins, are inhibited by simple sugars, i.e. they are sugar specific, was discovered by Sumner and Howell in 1936. During the next decade, Boyd and Renkonen independently found that certain plant agglutinins are blood group specific (e.g. lima bean agglutinin reacts specifically with human blood type A erythrocytes). Boyd [2] in 1954 proposed "that these blood antigen specific plant agglutinins be called *lectins,* from the Latin *legere,* to pick out or choose, intending thus to call attention to their specificity without begging the question as to their nature". The term lectin was subsequently broadened to include all sugar specific and cell agglutinating proteins of nonim-

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mune origin, whether from plants, animals or microorganisms [3,4].

Although scattered reports on the ability of bacteria to agglutinate erythrocytes appeared in the literature during the first half of the century, systematic research on the bacterial hemagglutinins started only in the 1950's, with the work of Duguid in England and of Brinton in the USA [5–7]. Duguid and his co-workers showed that hemagglutinating activity is a property expressed by many bacterial species, most commonly by those belonging to the family of Enterobacteriaceae. They further demonstrated the existence of two major classes of bacterial hemagglutinin: (i) those that are inhibited by low concentrations of mannose, methyl α-mannoside and mannan, which were designated as mannose sensitive (MS in brief); and (ii) those that are not inhibited by the above carbohydrates and which have been designated as mannose resistant (or MR). We now know, however, that many of the hemagglutinins of mannose resistant strains exhibit distinct sugar specificities (table 1). Moreover, bacterial cultures often express three or four hemagglutinins, each with different carbohydrate specificity.

The pioneering work of Duguid and of Brinton also demonstrated that the hemagglutinins described are nearly always associated with the presence on the surface of the bacteria of multiple submicroscopic filamentous appendages which have been designated as fimbriae (Latin for fringes) or pili (Latin for hairs). Several types of fimbriae, with respect to their structure and sugar specificity, have been identified ([7] and table 1). The best characterized of these are type 1 or common fimbriae of *E. coli* that are mannose specific, and type P fimbriae, also of *E. coli*, specific for Galα4Gal. Other examples are type S fimbriae of *E. coli*, specific for NeuAcα2→3Gal, and type 2 fimbriae 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 15–22 kDa. Each filament is made up of several hundred

Table 1

Sugar specificity of bacterial surface agglutinins[a]

Saccharide	Bacteria	Fimbriae
Mannose	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella* spp., *Serratia marcescens, Shigella flexneri*	type 1
Galactose	*Escherichia coli*	
L-Fucose	*Vibrio cholerae*	
N-Acetylglucosamine	*Escherichia coli*[b]	type G
N-Acetylgalactosamine	*Escherichia coli*	
Galα4Galβ-	*Escherichia coli*	type P
Galβ4Glc	*Actinomyces naeslundii, Actinomyces viscosus*	type 2
Galβ4GlcNAc	*Staphylococcus saprophyticus*	
GlcNAcβ3Gal	*Streptococcus pneumoniae*	
NeuAcα2→3Galβ3GalNAc	*Escherichia coli*[c], *Streptococcus mitis, S. sanguis*[d], *Mycoplasma gallisepticum, Mycoplasma pneumoniae*	type S
NeuGcα2→3Galβ4Glc	*Escherichia coli*	K99

[a] For additional references and further details, see [7]
[b] [8]
[c] [9]
[d] [10]

subunits. Most fimbriae are very stable structures, being resistant to detergents and chaotropic agents. The purified fimbriae bind to cells containing the complementary surface sugars, but in most cases they do not act as agglutinins unless they have been polymerized, for example by treatment with glutaraldehyde or anti-fimbrial antibodies. Strictly speaking, they do not conform to the definition of lectins [4], but because they are sugar specific and of nonimmune origin, it is convenient to refer to them as such. Other designations used in the literature for these substances are 'adhesins' or 'agglutinins'.

The study of the bacterial surface lectins is a fast developing area and a most exciting one. It is motivated largely by the realization of the great

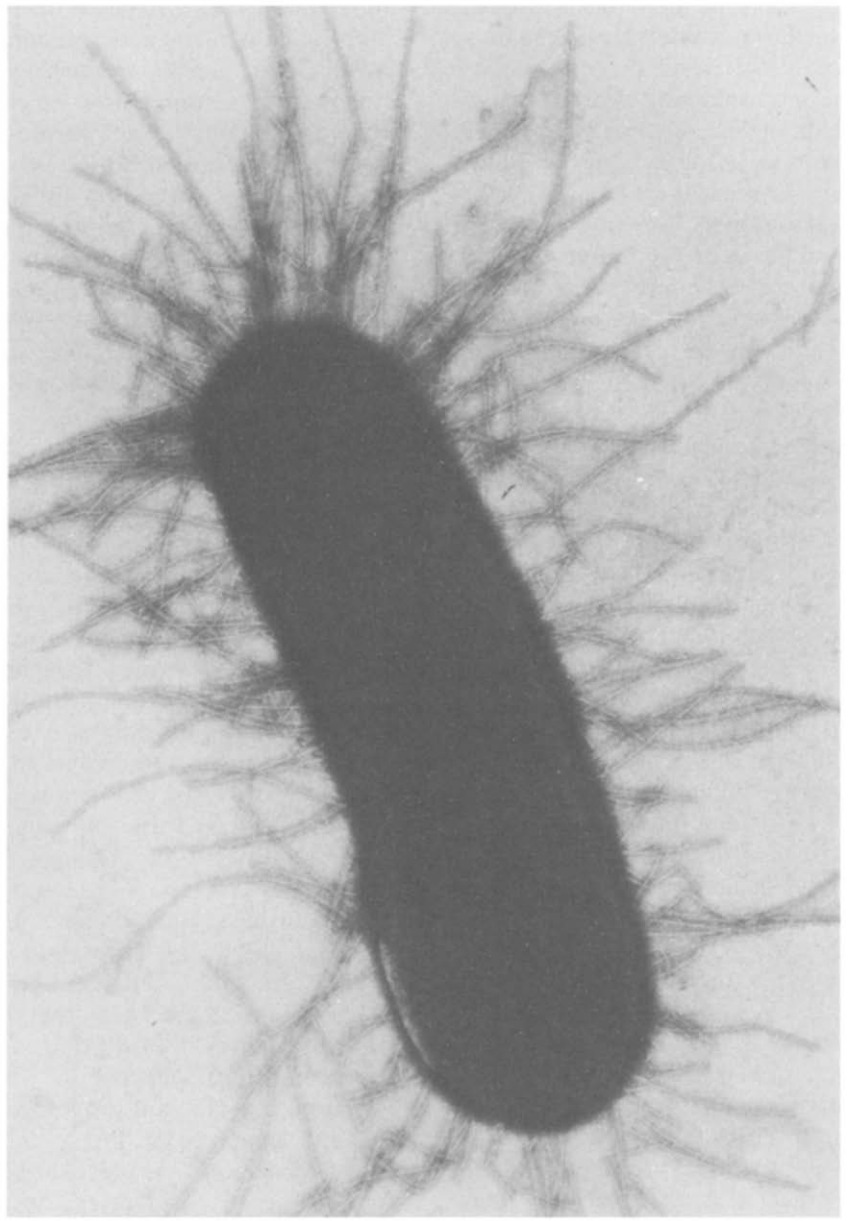

Fig.1. Electron micrograph of type 1 fimbriated *E. coli*.

importance of bacterial adhesion in infection, and by the demonstration that some of these lectins may act as virulence factors. Adherence of the bacteria to host cells protects them from being swept away by the normal cleansing mechanisms operating on mucosal surfaces, such as urinary flow, thereby increasing their ability to colonize the epithelia, multiply and invade the host. This has been most convincingly demonstrated in animal experiments for the mannose and Galα4Gal specific lectins of the Enterobacteriaceae, and may also be the case with the sialic acid specific lectins of several of the mycoplasma. The galactose specific surface lectins of the actinomyces enable the organisms to bind to certain streptococci and, thus, may function in the mixed colonization of teeth and other oral surfaces.

As well as functioning in the initiation of infection, to the disadvantage of the host, certain of the bacterial surface lectins may play a beneficial role, by mediating the adherence of the bacteria to the host phagocytes. In this way they may provide the host with a means of eliminating the bacteria in the absence of opsonins, a mechanism named by us *'lectinophagocytosis'* (Ofek, I. and Sharon, N., in preparation). These varied systems provide an excellent illustration of how lectins can mediate intercellular recognition. More importantly, there is good reason to believe that studies of the bacterial surface lectins and the identification of their receptors will lead to a clearer understanding of the molecular basis of infection and to the development of novel approaches for its prevention.

In this lecture I shall focus on the properties and possible functions of the mannose specific surface lectins, in which I have been interested since the middle 1970's. Further information on these and other bacterial lectins, including soluble ones, may be found in a recently published book [11].

2. TYPE 1 FIMBRIAE

Almost all strains of *E. coli*, as well as many species of other enteric bacteria, express (or have the genetic potential to express) type 1 fimbriae [12,13]. These fimbriae are uniformly distributed on the bacterial cells (fig.1). Their length varies from 0.2 to 1 μm, their width is 7 nm, and their number is commonly between 100 and 400 per cell. They can be isolated and purified by mechanical detachment from the cell surface, followed by differential centrifugation or by salt precipitation. Because of their hydrophobicity, they have a strong tendency to aggregate, which may account for the reports that the isolated fimbriae agglutinate yeasts or guinea pig erythrocytes in a mannose specific manner. The fimbrillin subunit of type 1 *E. coli* is composed of 158 amino acid residues. Evidence, mainly based on genetic experiments, strongly suggests the presence in type 1 fimbriae of a very small amount of an additional subunit, not easily detected by SDS-polyacrylamide gel electrophoresis, which may carry the carbohydrate-binding site of the fimbriae [14,15]. This subunit is probably located on the distal tip of the fimbriae, in a position suitable for mediating the adherence of the bacteria to cells [16].

The fimbrillin subunits of type 1 fimbriae of different *E. coli* strains have the same molecular size and similar amino acid composition. Those of other enterobacteria, e.g. *Klebsiella pneumoniae* and *Salmonella typhimurium,* are different in both size and composition [13].

3. MOLECULAR GENETICS

The early work of Brinton [6] has shown that the synthesis and surface expression of type 1 fimbriae are coded by genes located in the bacterial chromosome. It has also been known for a long time that bacteria genotypically capable of producing the fimbriae may spontaneously shift back and forth from a fimbriated phase to a non-fimbriated one, a phenomenon known as phase variation [5]. In other words, the cells are either fimbriated or bald. This phase variation occurs at a relatively high frequency - approximately one/thousand bacteria per generation - which is several orders of magnitude higher than the rate of mutation. Phase variation should be taken into consideration in studies with fimbriated bacteria. Cultures rich in type 1 fimbriae are best obtained by growth in static conditions in broth, but not on agar, while the presence of glucose in the broth causes overgrowth of the non-fimbriated phenotype over the fimbriated type [5]. Phase variation also occurs in vivo. This was first postulated by us to account for the finding that bacteria recovered from the urine of patients with urinary tract infection are frequently non-fimbriated, although they are

genetically capable of producing the fimbriae [17]. Direct evidence was subsequently presented by several groups. For example, fimbriated *E. coli* cells become non-fimbriated during growth in chambers implanted in the peritoneal cavities of mice [18].

Recent genetic analysis has demonstrated that several genes - designated *fim*A, *fim*B, etc. (or *pil*A, *pil*B) and closely located on the bacterial chromosome - are required for the expression of type 1 fimbriae [13–15]. They code for proteins that are structural or functional subunits of the fimbriae, or those that control their synthesis and assembly. Thus, *fim*A codes for a protein of 19 kDa which is the precursor of fimbrillin, *fim*E for a protein of 31 kDa which is apparently the carbohydrate-binding subunit, and *fim*F for a protein that controls fimbrial length. Formation of the fimbriae and expression of the carbohydrate-binding activity can be genetically separated: mutations outside the fimbrillin gene abolish the ability of the bacteria to bind to cells, but not the expression of the fimbriae. It has further been found that phase variation is genetically controlled at the transcriptional level [19]. The phenomenon occurs due to the periodic inversion of a specific 300-bp DNA segment containing the promoter for the *fim*A gene. The phase switch is controlled by the products of two regulatory genes, *fim*B and *fim*E, located upstream of *fim*A [20]. The *fim*B and *fim*E proteins direct the phase switch into the 'on' and 'off' positions, respectively. These proteins are highly basic, implying that they control the phase switch through interaction at the DNA level.

4. CARBOHYDRATE SPECIFICITY

Detailed characterization of the combining site 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 for the design of more effective inhibitors of adhesion to mucosal surfaces and thus for the prevention of infection. We therefore examined the inhibitory effect of a large number of glycosides and oligosaccharides of mannose on the agglutination of yeasts by *E. coli* (fig.2), *K. pneumoniae, Salmonella* spp. and, in the case of *E. coli*, also by type 1 fimbriae isolated from this organism [21,22].

Among the compounds tested, the best inhibitors of type 1 fimbriae of *E. coli* are the branched oligosaccharides Manα6[Manα3]Manα6[Manα3]-ManαOMe and Manα6[Manα3]Manα6[Manα2-Manα3]ManαOMe, and the trisaccharide Manα3-Manβ4GlcNAc (table 2). Our findings were recently confirmed by Neeser et al. [23], who used guinea pig erythrocytes instead of yeasts as indicator cells. We have also found that the disaccharide Manα3Man, as well as the tetrasaccharide Manα2Manα3Manβ4GlcNAc and the pentasaccharide Manα2Manα2Manα3Manβ4GlcNAc, are poor inhibitors of yeast agglutination by *E. coli*. This had led us to postulate that the combining site of the type 1 fimbrial lectin corresponds to the size of a trisaccharide (fig.3) and that it is in the form of a depression or pocket on the surface of the lectin. Extended carbohydrate-binding sites have been described for enzymes (e.g. lysozyme), several lectins and antibodies. In the case of the *E. coli* lectin, there are probably three adjacent subsites, each of which accommodates a monosaccharide residue.

The presence of a hydrophobic binding region adjacent to the binding site of type 1 fimbriae is suggested by the finding that aromatic α-mannosides are powerful inhibitors of the agglutination of yeasts by *E. coli* and of the adherence of the bacteria to guinea pig ileal epithelial cells [24] (table 3). In both systems, the best inhibitors were 4-methylumbelliferyl α-mannoside and *p*-nitro-*o*-chlorophenyl α-mannoside (500–1000-times more inhibitory than methyl α-mannoside). 4-Methylumbelliferyl α-mannoside was also more effective than methyl α-mannoside in removing adherent *E. coli* from ileal epithelial cells.

The strict specificity of the *E. coli* lectin for α-mannosides is manifested by the findings that: (i) 4-methylumbelliferone is inactive even at a concentration which is well over 100-times higher than the concentration of 4-methylumbelliferyl α-mannoside required for 50% inhibition of the *E. coli* lectin; and (ii) both methylumbelliferyl α-glucoside and *p*-nitrophenyl β-mannoside are not inhibitory. The above results further suggest that 4-methylumbelliferyl α-mannoside and *p*-nitro-*o*-chlorophenyl α-mannoside can provide a basis for the design of therapeutic agents that may prevent

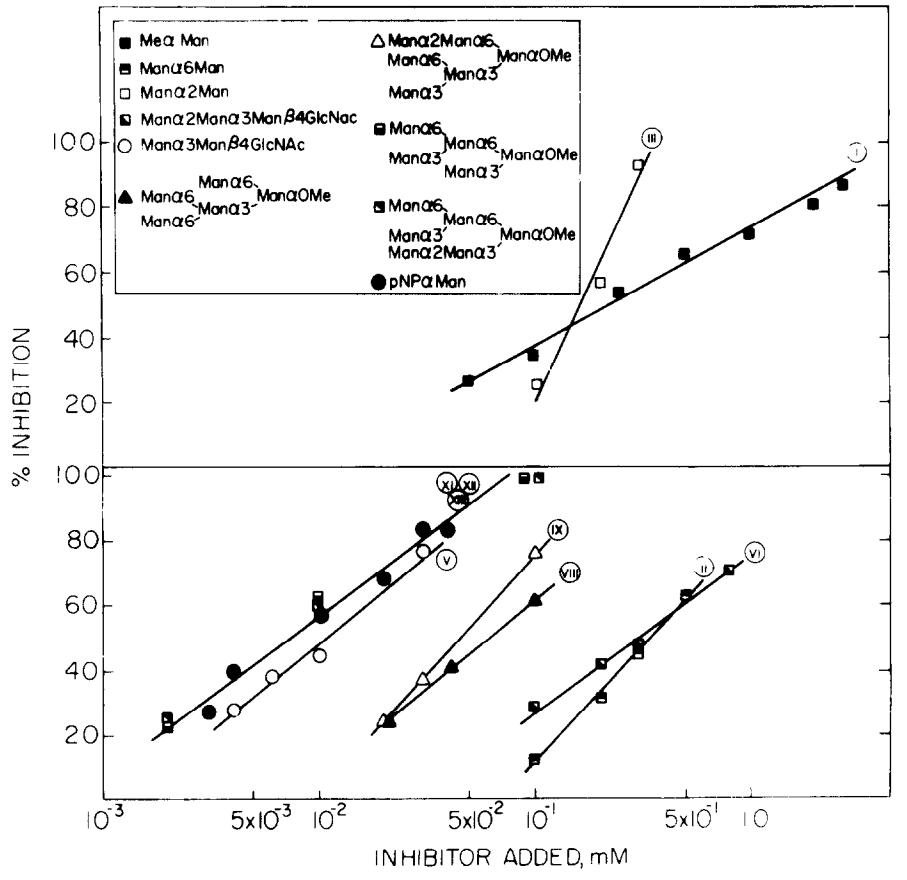

Fig.2. Inhibition by mannose glycosides and oligosaccharides of yeast aggregation by *E. coli* 346 (025). (Data from [21].)

adherence in vivo and infection by *E. coli* strains that express the mannose specific lectins.

The postulated combining site of type 1 fimbriae is different from that of concanavalin A, a plant lectin with a closely related sugar specificity. The agglutinating activity of concanavalin A is inhibited by both mannose and glucose, whereas that of *E. coli* is not inhibited by glucose; Manα2Man is a considerably better inhibitor of concanavalin A than is methyl α-mannoside, in contrast to what is found with the *E. coli* lectin.

Although all strains of *E. coli* examined, as well as *K. pneumoniae,* exhibited essentially the same pattern of specificity, this is not the case with other enterobacteria [22]. For example, with several *Salmonella* species examined, aromatic α-mannosides, as well as the trisaccharide Manα3Manβ4-GlcNAc, were weaker inhibitors than methyl α-mannoside. The combining site of different *Salmonella* species is probably smaller than that of *E. coli* or *K. pneumoniae,* and is devoid of a hydrophobic region. Different combining sites appear to be expressed by several other mannose specific bacterial lectins. Therefore, 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. This pattern probably reflects the conservation of genes which code for the sugar-combining sites in various genera of enterobacteria.

In general, it appears that mannose specific bacteria preferentially bind structures found in short oligomannose chains (and in hybrid units, see below) of *N*-linked glycoproteins. Such structures are common constituents of many eukaryotic cell surfaces [25], which accounts for the fact that

Table 2

Oligomannosides are potent inhibitors of *E. coli* type 1 fimbrial lectin

Compound	Relative inhibition of agglutination[a]	
	Yeasts[b]	Guinea pig erythrocytes[c]
ManαMe	1	1
Manβ4GlcNAc Manα3	21	25–30
Manα6 Manα6 Manα3 ManαOMe Manα3	30	
Manα6 Manα6 Manα3 Manβ4GlcNAcβ4GlcNAc-Asn Manα3		40
Manα6 Manα6 Manα3 ManαOMe Manα2Manα3	30	
Manα6 Manα6 Manα3 Manβ4GlcNAcβ4GlcNAc-Asn Manα2Manα3		7.5

[a] Data are expressed as efficiency of inhibition of agglutination relative to methyl α-mannoside (ManαMe)
[b] [21]
[c] [23]

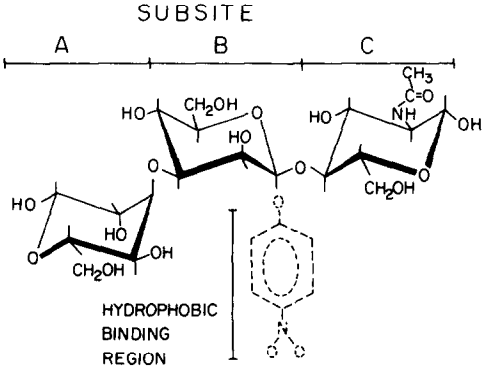

Fig.3. Scheme of combining site of *E. coli* mannose specific surface lectin.

Table 3

Inhibition by aromatic α-mannosides of the interaction of *E. coli* with yeasts and intestinal epithelial cells[a]

Aglycon	Relative inhibitory activity	
	Agglutination of yeasts *E. coli* 025	Adherence to epithelial cells *E. coli* 0128
Methyl	1	1
Phenyl	40	n.t.
p-Nitrophenyl	69	70
p-Bromophenyl	72	n.t.
p-Ethylphenyl	77	150
p-Methoxyphenyl	140	70
p-Ethoxyphenyl	154	240
p-Nitro-*o*-chlorophenyl	717	470
p-Methylumbelliferyl	600	1015

[a] Data from [24]

mannose specific bacteria bind to a wide variety of cells. Animal membrane glycolipids are unlikely to serve as receptors for mannose specific bacteria, since they are devoid of mannose residues.

Further support for these conclusions comes from the measurements of the binding of mannose specific *E. coli* to mammalian cells that differ in the level of oligomannose units on their surfaces. Animal cells treated with swainsonine, an inhibitor of the processing of asparagine-linked oligosaccharide units of glycoproteins, express increased levels of oligomannose or hybrid type oligosaccharides on their surfaces, and decreased levels of complex oligosaccharides. Such cells bound increased numbers (1.5–2-fold) of mannose specific *E. coli*, but binding to the cells of mannose resistant *E. coli* was unaffected [26].

We have shown that mutants of baby hamster kidney (BHK) cells, with increased levels of *N*-linked oligomannose or hybrid units in their glycoproteins, bind considerably larger numbers of mannose specific *E. coli* [27] (fig.4). These cells were also more sensitive to agglutination by the bacteria than the parental wild-type cells. The best example is a mutant ($RicR^{14}$) that lacks the enzyme *N*-acetylglucosaminyltransferase I, which catalyses the first step in the conversion of oligomannose units into complex ones. This mutant bound 4-times more *E. coli* 346, and was agglutinated at a rate more than 10-times faster than the BHK parental cells. Preference for binding to hybrid units was indicated by experiments with mutants which express high levels of such units on their surface.

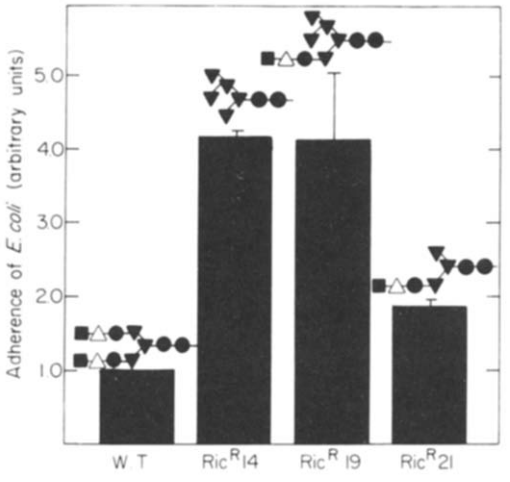

Fig.4. Mannose specific binding of *E. coli* 346 to wild-type and ricin resistant (Ric) baby hamster kidney (BHK) cells. The symbols above each bar denote the predominant *N*-linked oligosaccharide expressed on the surface of the BHK cells: (▼) Mannose; (●) *N*-acetylglucosamine; (△) galactose; (■) sialic acid. (Data from [26].)

5. BIOLOGICAL ROLES

5.1. *Initiation of infection*

There is now considerable evidence to support the idea that the bacterial surface lectins function in the initiation of infection [7]. In one approach the ability of inhibitors of adherence – either sugars or antibodies – to prevent infection was examined.

In the first study of its type, a mannose specific *E. coli* was injected into the urinary bladder of mice in the absence or presence of methyl α-mannoside [28]. The sugar caused a significant decrease (by a factor of 3) in the extent of bacteriuria, compared to the control group (fig.5). Methyl α-glucoside, which is not an inhibitor of the mannose specific adherence, did not affect the incidence of bacteriuria. Inspection under the microscope of stained mice bladders, injected with *E. coli* in methyl α-mannoside, revealed a considerably lower number of adherent bacteria than control mice injected with bacteria in saline. Exposure of methyl α-mannoside (20%) of the *E. coli* strains used in this study did not decrease their viability. When *Proteus mirabilis* was injected into the mice instead of *E. coli*, no effect on the rate of infection was observed with either of the sugars tested, in accordance with the inability of these sugars to inhibit the adherence of *P. mirabilis* to epithelial cells. Subsequent studies showed that methyl α-mannoside caused a large decrease in infection by mannose specific *K. pneumoniae* in the bladder of rats [29] and that mannose decreased markedly the ability of a virulent and non-fimbriated strain of *Shigella flexneri* to cause experimental keratoconjunctivitis in guinea pigs [30]. Glucose had no effect on the infectivity of either of these bacteria (fig.5).

Blocking of the type 1 fimbriae by polyclonal antibodies (in rats) or by monoclonal antibodies (in mice) by active or passive immunization of the animals against the purified fimbriae completely

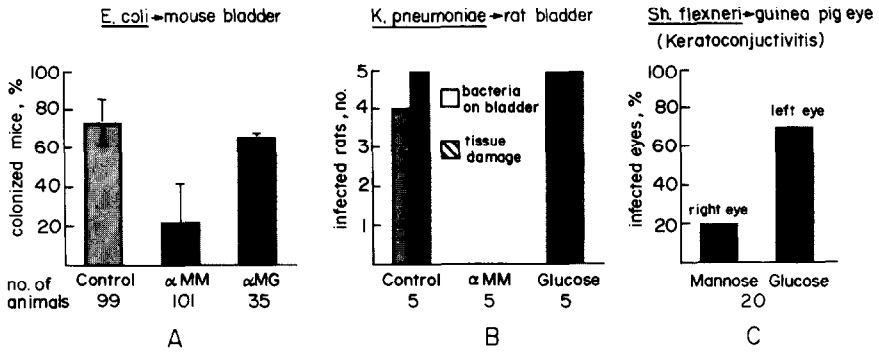

Fig.5. Sugar inhibitors of mannose specific bacterial surface lectins prevent infection. Sugar concentrations and source of data: (A) 10% [28]; (B) 5% [29]; (C) 2% [30]. αMM, methyl α-mannoside; αMG, methyl α-glucoside.

protected them from ascending urinary tract infection by the parent *E. coli* strain [31,32]. A similar result was obtained with monoclonal antibodies against mannose, the sugar determinant in the complementary host receptor, which prevented the attachment of mannose sensitive *E. coli* to various eukaryotic cells. On the other hand, monoclonal antibodies against *N*-acetylgalactosamine lacked protective activity [32].

In another approach, the infectivity of pairs of bacterial variants or phenotypes, only one of which expresses the type 1 fimbrial lectin, were examined. This approach was used in studies of gastrointestinal infection induced by *S. typhimurium* in mice [33], as well as of urinary tract infection induced by *K. pneumoniae* in rats [29] or by *E. coli* in mice [34]. In all cases, the infectivity of the fimbriated phenotype was several times higher than that of the non-fimbriated one.

These and other studies clearly demonstrate that mannose specific lectins are a key factor in the ability of various mannose specific bacteria to cause experimental infection by mediating the attachment of the bacteria to the surfaces of the target tissues. It is also apparent that it is possible to prevent infection by suitable inhibitors of adherence.

In addition to the above direct role ascribed to type 1 fimbriae in the infectious process, they may also function indirectly. They mediate the binding of *E. coli* to *Entamoeba histolytica* and the uptake of the bacteria by the parasites resulting in enhancement of amoebic virulence [35]. They may also be responsible for the association between salmonella and schistosoma species, which may be the reason for the prolonged salmonellosis in schistosoma-infected patients [36].

6. LECTINOPHAGOCYTOSIS

6.1. *Lectinophagocytosis in vitro*

Recognition and ingestion of invading pathogens by phagocytic cells is of major importance in host defense. This process, known as phagocytosis, is a complex one and consists of several key steps. First, bacteria or other particulate foreign material must be bound to the phagocytic surface. This adherence step is generally mediated by specific receptors on the phagocyte plasma membrane that interact with complementary molecular components that coat the surface of the pathogen. Typically, these coating substances are derived from the immune system of the host and are called opsonins, and the resulting process is known as opsonophagocytosis.

Lectinophagocytosis, in contrast, results from the interaction of lectins on either the phagocytes or the bacteria, with complementary carbohydrates on the apposing cells ([37]; Ofek and Sharon, in preparation). The most thoroughly investigated system of lectinophagocytosis is that in which the mannose specific bacteria are the target cells (fig.6). These bacteria bind avidly through their surface lectins to various types of phagocytic cells, such as mouse and rat peritoneal macrophages and human polymorphonuclear leukocytes, in the absence of opsonins [38–40]. Recently, we demonstrated similar binding of the bacteria to human peritoneal macrophages isolated from

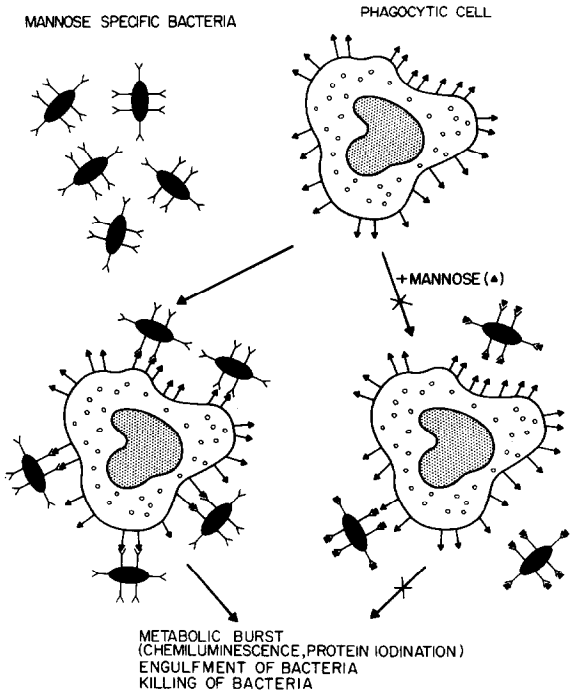

Fig.6. Schematic representation of lectinophagocytosis mediated by the mannose specific surface lectins of *E. coli*.

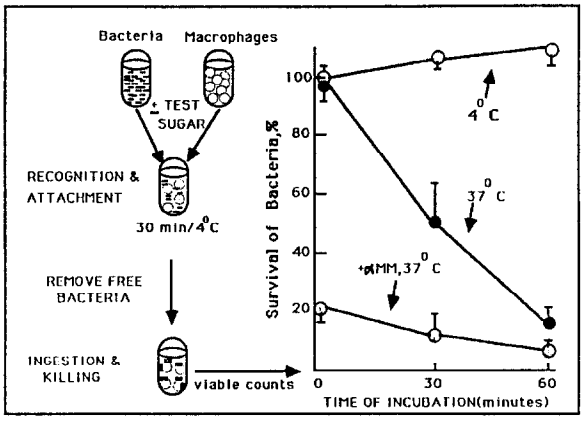

Fig.7. Mannose specific recognition and killing of type 1 fimbriated *E. coli* by mouse peritoneal macrophages. Concentration of methyl α-mannoside (αMM), 250 mM.(Pocino, M., Sharon, N. and Ofek, I., unpublished.)

uremic patients undergoing peritoneal dialysis (Boner, G., Rodriguez-Ortega, M. and Sharon, N., in preparation). Binding elicits a burst of metabolic activity in the phagocytes, including the induction of chemiluminescence and of protein iodination. The degree of metabolic stimulation of phagocytic cells by type 1 fimbriated bacteria may be as high as that observed with bacteria coated with antibody [41]. Binding is frequently followed by ingestion and killing of the bacteria (fig.7), a sequence of steps characteristic for the phagocytosis of opsonized bacteria. All these events are significantly inhibited if the bacteria and macrophages are mixed in the presence of mannose or methyl α-mannoside, sugars which minimally affect immune phagocytosis. Sugars such as galactose or L-fucose, that do not inhibit the lectins of mannose specific bacteria, have no effect on lectinophagocytosis of the bacteria. Also, bacteria from which the fimbriae were removed by ultraviolet irradiation were no longer phagocytised [40].

Attachment of bacteria to macrophages via their mannose specific lectins does not always lead to their ingestion. Whether the attached bacteria are ingested may depend on other surface properties, such as hydrophobicity and electric charge [42].

Several lines of evidence, in addition to specificity studies of the type 1 fimbriae mentioned earlier, suggest that the receptors for these fimbriae on the phagocytic cells are mannose-containing glycoproteins: (i) the same specificity pattern of inhibition is observed irrespective of the target cell used (e.g. yeasts, erythrocytes, BHK cells or phagocytic cells); (ii) a very good correlation was found between the mannan-binding activity of the bacteria and the extent of their attachment to mouse peritoneal macrophages; (iii) the finding that pretreatment of type 1 fimbriated bacteria with yeast mannan inhibited their attachment to mouse and human phagocytes, whereas pretreatment of the phagocytes did not have such an effect, shows that the receptor for the bacterial lectin is on the surface of the phagocytes; (iv) since the only class of mannose-containing compounds in animal membranes are glycoproteins, the receptors for mannose specific bacteria must belong to this class.

We recently isolated the receptors [43] by affinity chromatography on a column of immobilized type 1 fimbriae of a Nonidet P-40 lysate of polymorphonuclear leukocytes surface-labeled with ^{125}I (fig.8). The material eluted from the col-

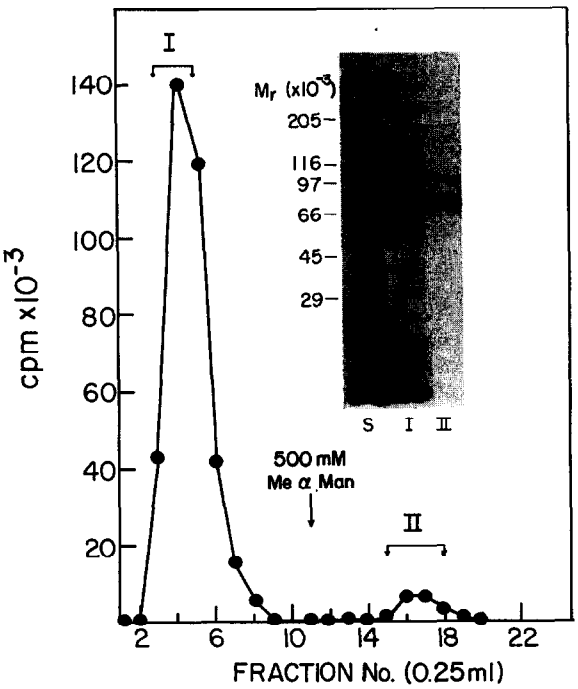

Fig.8. Affinity chromatography on immobilized fimbriae of total cell lysates of ^{125}I-surface labeled polymorphonuclear leukocytes: (I) unbound fraction; (II) fraction eluted with 500 mM methyl α-mannoside. (Inset) Autoradiogram of SDS-polyacrylamide gel electrophoretogram of starting material (S) and column fractions. (Data from [43].)

umn with 500 mM methyl α-mannoside (fraction II) comprised 1–5% of the total trichloroacetic acid-precipitable radioactivity loaded on the column. Analysis of this fraction, by SDS-polyacrylamide gel electrophoresis and autoradiography, revealed a major band of molecular mass between 70 and 80 kDa (gp70–80) and a minor one of 100 kDa (gp100). Upon longer exposure of the gel, a weak band of 150 kDa (gp150) appeared. The latter was the major band observed upon ^{125}I-concanavalin A overlay of blots of fraction II isolated from an unlabeled lysate. Binding of concanavalin A was specific, as demonstrated by its complete inhibition with 500 mM methyl α-mannoside. Additional experiments with isolated membranes have shown that gp150, gp100 and gp70–80 are enriched in the membrane fraction. The strong signal that gp150 gives with concanavalin A indicates that this glycoprotein may be highly glycosylated, or present in many copies on the surface of polymorphonuclear leukocytes.

Our results suggest that gp150, gp100 and gp70–80 are receptors for type 1 fimbriae on human polymorphonuclear leukocytes. This is supported by the finding that fraction II (4.4 μg/ml) obtained from a total cell lysate inhibited (by 70%) yeast aggregation by *E. coli*. No inhibition of yeast aggregation was observed with the whole cell lysate at a concentration of up to 80 μg protein/ml. Preliminary studies with monoclonal antibodies, performed by Morella Rodriguez-Ortega in our laboratory, suggest that gp150 and gp100 may be identical with the α and β subunits of leukocyte complement receptors and adhesion glycoproteins involved in complement-mediated opsonophagocytosis [44].

6.2. *Lectinophagocytosis in vivo*

Although the occurrence of lectinophagocytosis mediated by bacterial surface lectins has been established unequivocally in vitro, little is known about its occurrence in vivo. Most of the evidence available is indirect. One line of evidence comes from studies with Tamm-Horsfall glycoprotein, the most abundant protein in normal human urine. This glycoprotein contains *N*-linked oligomannose units and it is not surprising that it binds to type 1 fimbriated *E. coli* as well as to the isolated fimbriae and that this binding is inhibited by methyl α-mannoside [45,46]. Bacteria coated with the glycoprotein were markedly less susceptible to mannose specific lectinophagocytosis than uncoated ones. Serum (opsonin) mediated phagocytis, on the other hand, was not affected by the glycoprotein. It was suggested that these observations may partially explain the virulence of *E. coli* in the bladder, where serum activity is low and Tamm-Horsfall glycoprotein is abundant.

Further indirect evidence that mannose specific lectinophagocytosis may take place in vivo was obtained in experimental infection with mixed phenotypes, one of which expressed the mannose specific lectin (fim^+) and the other did not (fim^-). Orak [47] or intravesical [48] infection of mice with a mixture of fim^+ and fim^- phenotypes of *E. coli* showed predominance of the fim^+ phenotype in the gut or bladder, and of fim^- in the kidney or peritoneal cavity, respectively. Intravesical infection of mice with a similar mixture of fim^+ and

fim$^-$ phenotypes of *K. pneumoniae* revealed that the predominant phenotype in the bladder 7 days after infection was fim$^+$ while that in the kidney was fim$^-$ [49]. These results were interpreted as being due to selective survival of the fim$^+$ phenotype on mucosal surfaces since it is capable of binding to epithelial cells, and selective survival of the fim$^-$ phenotype in deep tissues (e.g. kidney or peritoneal cavity) where the fim$^+$ phenotype is eliminated by macrophages and/or polymorphonuclear leukocytes.

7. CONCLUDING REMARKS

In this lecture, I have drawn heavily on information obtained from studies of the mannose specific type 1 fimbriae to illustrate the fundamental role that lectin-carbohydrate interactions play in cell-cell interactions, as well as in microbial pathogenicity. On the one hand, these lectins increase the virulence of the bacteria by enhancing bacterial adherence to epithelial cells and, on the other, they decrease virulence by enhancing phagocytosis. Much of what has been learned in this context about type 1 fimbriae is true for other bacterial surface lectins as well, although certain differences are also apparent. Thus, the Galα4Gal specific (type P) fimbriae consist of a variety of protein subunits, only one of which carries the carbohydrate-binding site, and their expression is controlled by multiple genes [50–52]. These fimbriae bind to glycolipids of the globo series [53,54], and in this respect differ from type 1 fimbriae that bind to mannose-containing glycoproteins. However, experimental infection by both types of bacteria can be blocked by inhibitory sugars as well as by anti-fimbrial antibodies. Although type P fimbriated *E. coli* bind poorly to human polymorphonuclear leukocytes, since these cells are deficient in the appropriate receptors, binding is markedly increased if the cells are first coated with globotetraosylceramide (GalNAcβ3Galα4Galβ4-GlcβCer) and binding of the bacteria to the coated cells results in metabolic activation [55]. Thus, type P fimbriated *E. coli* are susceptible to lectinophagocytosis, as are the type 1 fimbriated bacteria. Lectinophagocytosis has recently been shown to be mediated also by type 2 fimbriae specific for β-galactosides that are expressed by certain actinomyces species [56].

It is to be expected that in the near future much more will be learned about the bacterial lectins described in this article and many others will probably be isolated and characterized. A detailed knowledge of the combining sites of these lectins and their receptors should lead to the design of potent inhibitors of adherence, which will hopefully be suitable for testing in humans. We shall also gain a better understanding of lectinophagocytosis, and of its role in natural infection. The day may come when inhibitors of lectin-mediated adherence will be used clinically to prevent colonization before the bacteria have had the chance to overwhelm the host.

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