The agglutination of β -haemolytic streptococci by lectins

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Summary. The ability of 25 lectins, isolated from different plants and fungi, to agglutinate 95 clinical isolates of β -haemolytic streptococci was examined. Cell suspensions were untreated, trypsin-treated or boiled at pH 2.0. None of the 95 untreated cell suspensions gave a visible reaction with any of the lectins. When the cells were trypsinised, 42 strains were agglutinated with one or more lectin and after boiling at pH 2, all the strains were agglutinated. After treatment with trypsin, 20 different agglutination patterns were observed, and after boiling, 19 patterns, four of which were similar. A correlation was found between Lancefield group C and some of these patterns. Some lectins reacted specifically with group C streptococci; DBA and WFA, both specific for D-GalNAc, DSA, a GlcNAc-specific lectin, and RPA, which showed a complex specificity, reacted only with group C strains. Furthermore, the lectin of *Maackia amurensis* reacted with 50 % of group B streptococci only. Agglutination assays with lectins were reproducible, easy to perform, relatively inexpensive and, therefore, applicable to studies of cell-wall structure and epidemiology of β -haemolytic streptococci.

Introduction

Lectins are sugar-binding proteins or glycoproteins of non-immune origin that agglutinate or precipitate glycoconjugates.¹ According to their sugar-binding specificity, lectins can be classified into five groups on the basis of their affinity for (i) glucose (Glc) and mannose (Man), (ii) galactose (Gal) and *N*-acetyl-D-galactosamine (D-GalNAc), (iii) *N*-acetyl-glucosamine (GlcNAc), (iv) L-fucose (L-Fuc) or (v) sialic acids.² They can bind to carbohydrate residues of bacterial surface components such as peptidoglycan, teichoic acids, lipopolysaccharides and capsular material.³ In the past, many lectins have been used to study cell-surface carbohydrates of different bacterial species.

Lectins play an important role in studies on the identification of surface structures of streptococci. Lectins from *Helix pomatia*, *Glycine max*, *Dolichos biflorus*, and *Wisteria floribunda* react specifically with group C β -haemolytic streptococci, but not with streptococci of other serogroups.^{4,5} The lectin from *Cepaea hortensis* agglutinated all group B streptococcal strains containing the sialic acid type-specific polysaccharide, whereas other streptococci of group B and other groups, as well as other bacteria—even those containing sialic acid—were not agglutinated.⁶ Group B streptococci were also specifically recognised by the lectins of *Lycopersicon esculentum* and *Solanum tuberosum*, whereas the lectin of *Datura stramonium*, another solanaceous plant, did not agglutinate group

B streptococci.⁷ Furthermore, the use of the *Lotus tetragonolobus* lectin in Western blotting indicated the presence of fucosyl residues in the cell walls of *Enterococcus faecalis*, and lectin probes of wheat germ agglutinin, Concanavalin-A, and soybean agglutinin demonstrated the presence of *N*-acetylneuraminyl, glucosyl, or mannosyl and *N*-acetylgalactosaminyl residues on the surface of this species.⁸

The usefulness of lectin typing for epidemiological purposes for staphylococci, streptococci, neisseria, campylobacters and enterobacteria has been discussed in several studies.^{3, 9–12}

This report describes the agglutination by 25 different lectins from plants and fungi of 95 clinical isolates of β -haemolytic streptococci of different Lancefield groups and assesses the usefulness of lectin agglutination for epidemiological typing.

Materials and methods

Bacterial strains

In the period from Nov. 1992 to Feb. 1993, 95 strains of β -haemolytic streptococci were isolated from clinical samples from the respiratory tract (7), urogenital tract (15), blood (10), skin and soft tissues (61) and central nervous system (2). The strains were stored at -70°C in peptone-glycerol (proteose peptone no. 2 1 % w/v; glycerin 8 % v/v).

Lancefield grouping

Lancefield grouping for groups A, B, C, F and G

Table 1. Lectins used in the agglutination assays

No.	Code	Species name	Common name	Carbohydrate specificity	Reference
1	PNA	<i>Arachis hypogaea</i>	Peanut	β -D-Gal(1,3)D-GalNAc	13
2	AMA	<i>Arum maculatum</i>	Lords and ladies	Complex	UP
3	AMA II	<i>Arum maculatum</i>	Lords and ladies	Complex	UP
4	APA	<i>Aegopodium podagraria</i>	Ground elder	D-GalNAc	14
5	ASA	<i>Allium sativum</i>	Garlic	α (1,3)Man	15
6	AUA	<i>Allium ursinum</i>	Ramsons	α (1,3)Man, α (1,6)Man	15
7	BDA	<i>Bryonia dioica</i>	White bryony	D-GalNAc	16
8	DSA	<i>Datura stramonium</i>	Thorn-apple	D-(GlcNAc) ₂	17
9	GNA	<i>Galanthus nivalis</i>	Snowdrop	α (1,3)Man	15
10	IRA	<i>Iris reticulatum</i>	Iris	D-GalNAc	UP
11	MAA	<i>Maackia amurensis</i>	Maackia	Neu5Ac- α (2,3)Gal	18
12	NPA	<i>Narcissus pseudonarcissus</i>	Daffodil	α (2,6)Man	15
13	RPA	<i>Robinia pseudoacacia</i>	Black locust	Complex	19
14	CCL	<i>Ceratobasidium cornigerum</i>		D-GalNAc	20
15	RSA	<i>Rhizoctonia solani</i>		D-GalNAc	20
16	SMyL	<i>Sclerotinia myabaena</i>		D-GalNAc	21
17	SSL	<i>Sclerotinia sclerotiorum</i>		D-GalNAc	21
18	SNA	<i>Sambucus nigra</i>	Elder	Neu5Ac- α (2,6)GalNAc	22
19	SNA II	<i>Sambucus nigra</i>	Elder	D-GalNAc	22
20	TL	<i>Tulipa</i>	Tulip	D-GalNAc	23
21	WFA	<i>Wisteria floribunda</i>	Wisteria	D-GalNAc	24
22	WGA	<i>Triticum aestivum</i>	Wheat germ	D-(GlcNAc) ₄ , NeuNAc	25
23	Con-A	<i>Canavalia ensiformis</i>	Jack bean	α -D-Man, α -D-Glc	26
24	DBA	<i>Dolichos biflorus</i>	Horse gram	D-GalNAc	27
25	VAA	<i>Viscum album</i>	Mistletoe	β -D-Gal	28

UP, unpublished.

was performed with the Streptex latex agglutination kit (Wellcome Diagnostics) according to the manufacturer's instructions.

Lectins

Lectins from different plants and fungi were extracted, isolated by affinity chromatography and further purified as described previously.¹³⁻²⁸ In table I the lectins are listed, along with the scientific name and the common name of the species from which they were isolated, their carbohydrate specificity and a relevant reference. Lectin solutions of 1 mg/ml w/v in phosphate-buffered saline (PBS; 1.5 mM KH₂PO₄, 10 mM Na₂HPO₄, pH 7.4, 3 mM KCl, 140 mM NaCl) were stored at -20°C until use.

Agglutination assays

Bacterial strains were grown in 50 ml of Todd-Hewitt broth for 18 h at 37°C in air with CO₂ 5% v/v. Cells were collected by centrifugation at 3000 g for 15 min, washed in PBS and resuspended in PBS, adjusting the suspensions to an A₆₂₀ of 5 in a spectrophotometer.

Three ml of each strain suspension were left untreated, 3 ml treated with trypsin and 3 ml boiled in glycine HCl at pH 2. For trypsin treatment, suspensions were incubated with trypsin 0.1 mg/ml w/v for 1 h at 37°C; the cells were washed in PBS to remove the trypsin and resuspended in 3 ml of PBS. For glycine-HCl treatment, the suspensions were centrifuged at 3000 g for 15 min, resuspended in 3 ml of 0.25 M glycine-

HCl buffer, pH 2.0, and boiled for 15 min. The cells were then washed with PBS and resuspended in 3 ml of PBS.

Agglutination assays were performed in small glass tubes in a final volume of 0.1 ml containing 10 μ l of lectin solution and 90 μ l of cell suspension. A negative control was included for each strain by adding 10 μ l of PBS instead of the lectin solution. After the mixtures had been left for 1 h at room temperature, agglutination was assessed visually.

Reproducibility

Reproducibility of lectin agglutination was tested with five strains, one each of Lancefield groups A, B, and G and two of group C. Each strain was subcultured five times and the agglutination assays were performed as described above.

Results

Reproducibility was tested with five strains. Lectin agglutination patterns were not altered on subculture. However, within the same strain there were differences in the agglutination pattern according to the pre-treatment of the bacteria (results not shown).

Without pre-treatment, auto-agglutination of the bacteria prevented visualisation of the lectin agglutination. However, trypsinisation or boiling revealed different agglutination patterns. When the bacteria were treated with trypsin, 42 strains reacted with one or more lectins, giving rise to 20 different agglutination patterns (table II). After boiling the cells in glycine-

Table II. Patterns of lectin agglutination with trypsin-treated cells and their distribution within Lancefield groups

Pattern no.	Agglutination with lectins*																		Number of strains within Lancefield group			
	1	4	7	8	9	10	11	13	14	15	16	17	18	19	21	22	23	24	A (29)	B (22)	C (11)	G (33)
1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	2	0	0	0
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	2	0	0	0
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	3	1	0	0
5	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	0	1	0	0
6	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	0	6	0	0
7	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	0	1	0	0
8	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	0	1	0	0
9	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	0	3	0	0
10	-	+	-	+	-	-	-	+	+	+	-	+	-	+	+	+	-	+	0	0	3	0
11	-	-	-	+	-	-	-	+	+	+	-	+	-	+	+	+	-	+	0	0	5	0
12	-	-	-	+	-	-	-	+	+	+	-	-	-	+	+	+	-	+	0	0	1	0
13	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-	0	0	1	0
14	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	0	0	0	3
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	0	0	0	1
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	0	0	0	2
17	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	0	0	0	1
18	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	0	1	0	1
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	8	1	24
																			29	22	11	33

* Numbers correspond to those in table I.

Table III. Patterns of lectin agglutination with cells boiled in glycine-HCl and their distribution within Lancefield groups

Pattern no.	Agglutination with lectins*																		Number of strains within Lancefield group			
	1	4	7	8	9	10	11	13	14	15	16	17	18	19	21	22	23	24	A (29)	B (22)	C (11)	G (33)
21	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	1	0	0	0
22	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	1	0	0	0
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	27	18	0	20
23	-	+	-	+	-	-	+	-	-	+	-	-	-	+	-	+	-	-	0	1	0	0
24	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	0	1	0	0
25	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	0	1	0	4
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	0	1	0	1
10	-	+	-	+	-	-	-	+	+	+	-	+	-	+	+	+	-	+	0	0	2	0
11	-	-	-	+	-	-	-	+	+	+	-	+	-	+	+	+	-	+	0	0	1	0
26	-	+	-	+	-	-	-	+	+	+	-	+	-	-	+	+	-	+	0	0	1	0
27	-	-	-	+	-	-	-	+	+	+	-	+	-	-	+	+	-	+	0	0	1	0
28	-	-	-	+	-	-	-	+	+	+	-	-	-	-	+	+	-	+	0	0	3	0
29	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	+	0	0	1	0
30	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	0	0	1	0
31	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	0	0	1	3
32	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	0	0	0	1
33	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	0	0	0	1
34	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	0	0	0	2
35	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	0	0	0	1
																			29	22	11	33

* Numbers correspond to those in table I.

HCl, pH 2, all 95 strains agglutinated with at least one lectin, giving 19 different patterns, four of which (patterns 4, 10, 11 and 16) were identical to those obtained with trypsin-treated cells (table II).

The trypsinised cells of the test strains exhibited three agglutination patterns specific for Lancefield group A, five for group B, four for group C, and four for group G streptococci, comprising 17%, 55%,

91% and 15% of strains of each group, respectively (table II). Similarly, lectin agglutination of boiled cells revealed two patterns specific for group A, two for group B, seven for group C and four for group G, comprising 7%, 9%, 91% and 15% of isolates of each group, respectively (table III).

With both methods, seven lectins (AMA, AMA II, ASA, AUA, NPA, TL and VAA) did not react with

Table IV. Agglutination of trypsin-treated cells of different Lancefield groups with different lectins

Lancefield group	Number of strains that agglutinated with each lectin*																		Total
	1	4	7	8	9	10	11	13	14	15	16	17	18	19	21	22	23	24	
A	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	7	2	4	9
B	1	1	0	0	0	1	11	0	0	0	0	0	0	0	0	4	1	0	14
C	0	3	0	9	0	0	0	9	9	10	1	9	0	9	9	10	0	9	10
G	0	3	0	0	2	0	0	0	0	3	0	0	0	0	1	3	3	1	9
Total	1	8	0	9	3	1	11	9	9	13	1	9	0	9	10	24	6	14	42

* Numbers correspond to those in table I.

Table V. Agglutination of boiled cells of different Lancefield groups with different lectins

Lancefield group	Number of strains that agglutinated with each lectin*																		Total
	1	4	7	8	9	10	11	13	14	15	16	17	18	19	21	22	23	24	
A	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	29	0	0	29
B	0	2	0	2	0	0	1	0	0	1	0	0	0	1	0	22	1	0	22
C	0	3	0	8	0	0	0	10	11	11	0	5	0	3	9	10	0	10	11
G	0	8	0	0	1	1	1	0	5	7	0	0	0	0	2	33	1	1	33
Total	0	14	1	10	1	2	2	10	16	19	0	5	1	4	11	94	2	11	95

* Numbers correspond to those in table I.

any of the strains tested. When trypsin-treated cells were used, no lectins were found to be specific either for group A or for group G streptococci. However, the lectin from *M. amurensis* (MAA) reacted with 11 of 22 group B isolates but was non-reactive with other streptococci. In addition, DSA, RPA, CCL, SSL and SNA II were specific for group C streptococci in nine of 11 strains each (table IV). With boiled cells, no lectin was specific for group B, whereas BDA and SNA were specific for one strain of group A each. The lectins RPA and SSL agglutinated 10 and five strains of group C, respectively, in a specific way, while MAA reacted with one strain of group G (table V). Also, 94 of 95 strains were agglutinated with WGA (table V).

Discussion

In this study, agglutination by 25 different lectins of β -haemolytic streptococci, untreated, trypsin-treated or heat-treated, was assessed. Pre-treatment was a prerequisite for agglutination with any lectin and resulted in a reaction of 44% and 100% of the strains, respectively. Recently, Slifkin and Doyle described the applications of lectins in clinical microbiology.⁹ For epidemiological purposes, lectins have to agglutinate the strains reproducibly with a certain discriminating power. Although heat-treatment resulted in an agglutination reaction with all strains tested, the discriminating power was rather low since 65 strains yielded the same pattern (pattern 4) (table III). Furthermore, when divided into Lancefield groups, it became evident that, after trypsinisation, it was possible to differentiate 50% of group B streptococci and 91% of group C streptococci with several lectins. The advantage of lectin agglutination studies is that

strains can be subtyped in more detail than with Lancefield grouping and biochemical tests alone, hence their usefulness in epidemiological studies.

Lectins bind to specific carbohydrate moieties present on the bacterial wall.³ When trypsin or other proteolytic enzymes partially hydrolyse proteoglycans, more carbohydrate residues become available for binding with the lectins. Boiling of the bacteria in 0.25 M glycine-HCl at pH 2 is a more drastic treatment to degrade the bacterial surface structures. As a result, more glycoconjugates dissolve from the cell wall and cannot be detected by agglutination of the bacteria with lectins. It is likely, therefore, that boiled bacteria are less diverse in respect of their agglutination by lectins. This problem could be solved by the use of lectins coupled to latex spheres so the carbohydrates in solutions can be detected.⁷

Lectin agglutination studies are useful for bacterial surface structure studies. When agglutination assays were performed with boiled cells, WGA reacted with all but one strain, whereas DSA, another GlcNAc-binding lectin, bound to only 10 strains. However, WGA is also able to bind to neuraminyl-residues, whereas DSA cannot, indicating that a sialic acid-containing structure is present in almost all streptococcal strains, but is only detectable in most cells after boiling at pH 2. Indeed, only 24 strains reacted with WGA after trypsinisation, whereas 94 strains did so after boiling at pH 2. In an earlier study, WGA, at a concentration of 1 mg/ml, agglutinated all group A and C streptococci and some group B and E strains after trypsinisation. Furthermore, it was demonstrated that the lectin interacted with the teichoic acids and peptidoglycan of streptococci.²⁹

Whereas WGA is not specific for any one of the Lancefield groups, the lectins from tomato and potato,

two other lectins with specificity towards GlcNAc and sialic acid, as well as the sialic acid-specific lectin from *C. hortensis* recognised specifically group B streptococci.⁷ In this study, the lectin from *M. amurensis* (MAA), with specificity for Neu5Ac- α (2,3)Gal³⁰ agglutinated group B streptococci only. The lectin from the elder tree (SNA), which is specific for Neu5Ac- α (2,6)GalNAc,³¹ did not react with any of the group B streptococci. It is evident, therefore, that MAA, with the lectins from tomato, potato and *C. hortensis*, can be helpful in differentiating group B streptococci.³²

The lectins from *H. pomatia* (HPA), *D. biflorus* (DBA), and *W. floribunda* (WFA), all specific for D-GalNAc, agglutinated specifically group C β -haemolytic streptococci.^{8,9} However, in this study WFA and DBA also reacted with a few strains of other Lancefield groups. The lectins from the phytopathogenic fungus *Sclerotinia sclerotiorum*, as well as SNA II, both specific for D-GalNAc, reacted with group C streptococci only. The D-GalNAc-binding lectins, APA, CCL and RSA, agglutinated streptococci of other groups in addition to group C, whereas the D-GalNAc-binding lectins BDA, IRA, and TL did not react with these organisms. On the other hand, only

group C streptococci were agglutinated by the lectin from *D. stramonium*, specific for GlcNAc, as well as by the lectin from *Robinia pseudoacacia*, which has a complex specificity.

The results of the present study indicate that, although some lectins show the same specificity, there is a difference in agglutination activity with different bacteria. Indeed, the carbohydrate-binding activity of most lectins is tested in inhibition studies with simple sugars. However, the carbohydrate most haptenic to the binding site of the lectin has often a more complex structure. Therefore, when lectins are used in structural studies of bacterial walls, it is useful to know their binding specificity in more detail. When the detailed specificity of the lectins is not fully known, the lectin agglutination assay is a cheap and versatile tool for subtyping micro-organisms in the clinical and epidemiological setting. It provides more detailed information than serological techniques or conventional biochemical tests. Moreover, the technique requires neither apparatus nor highly qualified technologists and is less laborious and time-consuming than analyses of proteins or nucleic acids.

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References

- Goldstein IJ, Hughes RC, Monsigny M, Osawa T, Sharon N. What should be called a lectin? *Nature* 1980; **285**: 66.
- Damjanov I. Biology of disease. Lectin cytochemistry and histochemistry. *Lab Invest* 1987; **57**: 5–20.
- Pistole TG. Interaction of bacteria and fungi with lectins and lectin-like substances. *Annu Rev Microbiol* 1981; **35**: 85–112.
- Köhler W, Prokop O, Kühnemund O. Routine identification of group-C streptococci by means of an agglutinin (protectin) from the albumen gland of the edible snail, *Helix pomatia*. *J Med Microbiol* 1973; **6**: 127–130.
- Ottensooser F, Nakamizo Y, Sato M, Miyamoto Y, Takizawa K. Lectins detecting group C streptococci. *Infect Immun* 1974; **9**: 971–973.
- Wagner M. Agglutination of bacteria by a sialic acid-specific lectin of the snail *Cepaea hortensis*. *Acta Histochem* 1982; **71**: 35–39.
- Slifkin M, Cumbie R. Identification of group B streptococcal antigen with lectin-bound polystyrene particles. *J Clin Microbiol* 1987; **25**: 1172–1175.
- Aitchison EJ, Lambert PA, Farrell ID. Antigenic composition of an endocarditis-associated isolate of *Streptococcus faecalis* and identification of its glycoprotein antigens by ligand blotting with lectins. *J Med Microbiol* 1986; **21**: 161–167.
- Slifkin M, Doyle RJ. Lectins and their application to clinical microbiology. *Clin Microbiol Rev* 1990; **3**: 197–218.
- O'Sullivan N, Benjamin J, Skirrow MB. Lectin typing of *Campylobacter* isolates. *J Clin Pathol* 1990; **43**: 957–960.
- Moyes A, Young H. An analysis of lectin agglutination as a means of sub-dividing gonococcal serovars. *J Med Microbiol* 1992; **37**: 51–55.
- Jarløv JO, Hansen J-ES, Rosdahl VT, Espersen F. The typing of *Staphylococcus epidermidis* by a lectin-binding assay. *J Med Microbiol* 1992; **37**: 195–200.
- Lotan R, Skutelsky E, Danon D, Sharon N. The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). *J Biol Chem* 1975; **250**: 8518–8523.
- Peumans WJ, Nsimba-Lubaki M, Peeters B, Broekaert WF. Isolation and partial characterization of a lectin from ground elder (*Aegopodium podagraria*) rhizomes. *Planta* 1985; **164**: 75–82.
- Van Damme EJM, Goldstein IJ, Peumans WJ. A comparative study of related mannose-binding lectins from Amaryllidaceae and Alliaceae species. *Phytochemistry* 1991; **30**: 509–514.
- Peumans WJ, Nsimba-Lubaki M, Carlier AM, Van Driessche E. A lectin from *Bryonia dioica* root stocks. *Planta* 1984; **160**: 222–228.
- Broekaert WF, Peumans WJ, Allen AK. Carbohydrate binding proteins from *Datura stramonium* seeds. In: Bøg-Hansen TC, Breborowicz J (eds) *Lectins*, vol 4. Berlin, New York, Walter de Gruyter Co. 1985; 481–489.
- Kawaguchi T, Matsumoto I, Osawa T. Studies on hemagglutinins from *Maackia amurensis* seeds. *J Biol Chem* 1974; **249**: 2786–2792.
- Wantyghem J, Goulut C, Frénoy J-P, Turpin E, Goussault Y. Purification and characterization of *Robinia pseudoacacia* seed lectins. A re-investigation. *Biochem J* 1986; **237**: 483–489.
- Kellens JTC, Peumans WJ. Occurrence of lectins in different strains of *Rhizoctonia solani*. In: Kocourek J, Freed DJL (eds) *Lectins*, vol. 7. St Louis, Sigma Chemical Co. 1990; 57–62.
- Kellens JTC, Goldstein IJ, Peumans WJ. Lectins in different members of the Sclerotiniaceae. *Mycol Res* 1992; **96**: 495–502.
- Kaku H, Peumans WJ, Goldstein IJ. Isolation and characterization of a second lectin (SNA-II) present in elderberry (*Sambucus nigra* L.) bark. *Arch Biochem Biophys* 1990; **277**: 255–262.
- Cammue BPA, Peeters B, Peumans WJ. A new lectin from tulip (*Tulipa*) bulbs. *Planta* 1986; **169**: 583–588.
- Kurokawa T, Tsuda M, Sugino Y. Purification and characterization of a lectin from *Wisteria floribunda* seeds. *J Biol Chem* 1976; **251**: 5686–5693.
- Nagata Y, Burger MM. Wheat germ agglutinin. Molecular characteristics and specificity for sugar binding. *J Biol Chem* 1974; **249**: 3116–3122.
- Agrawal BBL, Goldstein IJ. Protein-carbohydrate interaction. VI. Isolation of concanavalin A by specific adsorption on cross-linked dextran gels. *Biochim Biophys Acta* 1967; **147**: 262–271.
- Etzler ME, Kabat EA. Purification and characterization of a

- lectin (plant hemagglutinin) with blood group A specificity from *Dolichos biflorus*. *Biochemistry* 1970; **9**: 869–877.
28. Ziska P, Franz H, Kindt A. The lectin from *Viscum album* L. purification by biospecific affinity chromatography. *Experientia* 1978; **34**: 123–124.
29. Wagner M. Interaction of wheat-germ agglutinin with streptococci and streptococcal cell wall polymers. *Immunobiology* 1979; **156**: 57–64.
30. Knibbs RN, Goldstein IJ, Ratcliffe RM, Shibuya N. Characterization of the carbohydrate binding specificity of the leukoagglutinating lectin from *Maackia amurensis*. *J Biol Chem* 1991; **266**: 83–88.
31. Shibuya N, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ. The elderberry (*Sambucus nigra* L.) bark lectin recognizes the Neu5Ac(α 2-6)GalNAc sequence. *J Biol Chem* 1987; **262**: 1596–1601.
32. Lim DV, Morales WJ, Walsh AF, Kazanis D. Reduction of morbidity and mortality rates for neonatal group B streptococcal disease through early diagnosis and chemoprophylaxis. *J Clin Microbiol* 1986; **23**: 489–492.