CHARACTERISATION AND TYPING OF BACTERIA

Lectin typing of β -haemolytic streptococci of groups A and B

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Summary. Patterns of agglutination of 124 clinical isolates of β -haemolytic streptococci (30 group A and 94 group B isolates) by 21 commercial lectins are reported. Cell suspensions were untreated. Nine (30 %) of the group A isolates, and 23 (24 %) of the group B isolates, were agglutinated by at least one of the lectins. Ten different patterns of agglutination were observed with group A and 15 with group B streptococci. No pattern, except that of non-agglutination by any lectin, was common to group A and group B isolates. In view of the growing interest in the use of lectin typing there is a need for standardisation of assay procedures to enable meaningful comparison of the results of different research groups.

Introduction

Lectins are protein substances that bind specifically to sugar residues¹ and their use as diagnostic reagents in clinical microbiology has been proposed by numerous authors.^{2–10} The selectivity of lectins for microbial surfaces may also be useful in epidemiological studies of bacterial disease, in regard to the genera *Staphylococcus*,^{11,12} *Neisseria*,^{13–15} *Haemophilus*¹⁶ and *Campylobacter*.¹⁷

Group A streptococci (*Streptococcus pyogenes*) have a homogeneous capsular composition in comparison with group B strains (*S. agalactiae*). However, a number of group B serotypes have been identified, and Henrichsen's classification of serotypes on the basis of the presence of capsular polysaccharides Ia, Ib, II, III, IV and V is currently widely used.¹⁸ Although these polysaccharides have different structures, they all consist of repeating units containing galactose, glucose, N-acetylglucosamine and sialic acid. The differences in antigenicity are due to differences in the molar ratios of the monosaccharide residues and in the ways in which the residues are linked.¹⁹

There have been previous studies of lectin agglutination of group A streptococci,²⁰ group B streptococci^{20–24} and group C streptococci.^{25–28} This study reports the patterns of agglutination of 124 clinical isolates of β -haemolytic streptococci (30 group A and 94 group B isolates) by 21 commercial lectins. Cell suspensions were not pre-treated, as the aim was to investigate the binding of lectins to streptococci whose capsular structure had not been modified.

Materials and methods

Lectins

The lectins used (table I) were selected on the basis of their reported specificities, to cover the widest possible range of sugars. All are commercially available (Sigma) except tomentine, a GlcNAc-specific lectin obtained from *Codium tomentosum* (Huds.) Stackh., a green seaweed common in northwest Spain.²⁹ Before use, the lectins were resuspended at 1 mg/ml in phosphate-buffered saline (PBS), pH 7.2–7.4, and stored at –20°C in 500- μ l Eppendorf tubes.

Table I. Panel of lectins

Lectin		Sugar specificity
<i>Anguilla anguilla</i>	(AA)	α -L-Fuc
<i>Arachis hypogaea</i>	(PN)	β -D-Gal(1-3)GalNAc
<i>Canavalia ensiformis</i>	(CC)	α -D-Man, α -D-Glc
<i>Codium fragile</i>	(CF)	D-GalNAc
<i>Codium tomentosum</i>	(CT)	D-GlcNAc
<i>Datura stramonium</i>	(DS)	(D-GlcNAc) ₂
<i>Dolichos biflorus</i>	(DB)	α -D-GalNAc
<i>Glycine max</i>	(SB)	D-GalNAc
<i>Helix pomatia</i>	(HP)	D-GalNAc
<i>Lens culinaris</i>	(LC)	α -D-Man
<i>Limulus polyphemus</i>	(LP)	NeuNAc
<i>Lycopersicon esculentum</i>	(LE)	(D-GlcNAc) ₃
<i>Pisum sativum</i>	(PS)	α -D-Man
<i>Ptilota plumosa</i>	(PP)	α -D-Gal
<i>Solanum tuberosum</i>	(ST)	(D-GlcNAc) ₃
<i>Tetragonolobus purpureas</i>	(TP)	α -L-Fuc
<i>Triticum vulgare</i>	(WGA)	(D-GlcNAc) ₂ , NeuNAc
<i>Ulex europaeus</i>	(UEAI)	α -L-Fuc
<i>Ulex europaeus</i>	(UEAII)	(D-GlcNAc) ₂
<i>Vicia sativa</i>	(VS)	D-Glc, D-Man
<i>Vicia villosa</i>	(VV)	D-GalNAc

Table II. Agglutination of streptococci with lectins

Pattern no.	Agglutinated with lectin*																				Number of isolates		
	AA	PN	CC	CF	CT	DS	DB	SB	HP	LC	LP	LE	PS	PP	ST	TP	WGA	UEAI	UEAII	VS	VV	GAS	GBS
1	-	-	+	+	+	-	+	-	-	-	+	-	-	+	-	-	-	+	-	-	-	1	0
2	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	1	0
3	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	1	0
4	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	1	0
5	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0
6	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	1	0
7	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0
8	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	1	0
9	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	1	0
10	+	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	0	1
11	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	0	1
12	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	0	1
13	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	0	1
14	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1
15	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1
16	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	0	2
17	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	0	2
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	0	1
19	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	0	1
20	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	3
21	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	0	2
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	0	5
23	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19	71

*See table I. GAS, group A streptococci; GBS, group B streptococci.

Bacteria

A total of 124 streptococcal isolates was supplied by the Microbiology Laboratory of the Hospital de Conxo, Santiago de Compostela, from clinical samples from the urogenital tract (76), skin and soft tissues (23), respiratory tract (21) and blood (4). Lancefield groups were determined with the Pastorex® Streptococcal Grouping Kit (Diagnostics Pasteur), in accordance with the manufacturer's instructions. Of the 124 isolates, 30 were group A and 94 group B. After incubation for 24 h at 37°C on blood agar, cells were harvested and washed three times by centrifugation in PBS at 1000 rpm for 10 min. Cell suspensions in PBS were adjusted to obtain an A_{550} of 1.25 in a DMS Varian spectrophotometer.

Agglutination assay

Assays were performed on 12-well glass plates (13 × 9 × 0.6 cm), by adding 15 µl of lectin to 15 µl of bacterial suspension in each well and shaking gently for 30 s. In each series a negative control was run in one well by adding 15 µl of PBS instead of lectin to the bacterial suspension. Agglutination was assessed by microscopic examination. In no case was auto-agglutination observed.

Results

Of the 30 group A isolates, 21 (70%) did not react with any of the lectins; the remaining nine (30%) reacted with at least one lectin. A total of 10 distinct agglutination patterns (including no agglutination) was observed (table II). Nine of the 21 lectins agglutinated at least one group A isolate; *Codium tomentosum* (seven isolates); *Ulex europaeus* I (four);

Table III. Percentages of isolates agglutinated by lectins of the specificities shown

Group A streptococci	%	Group B streptococci	%
D-GlcNAc	78	[D-GlcNAc] ₃	61
D-GalNAc	67	α-D-Man	30
α-L-Fuc	44	[D-GlcNAc] ₂ /NeuNAc	30
α-D-Man/α-D-Glc	33	D-GlcNAc	13
α-D-Gal	33	α-D-Man/α-D-Glc	13
NeuNAc	33	D-GalNAc	13
[D-GlcNAc] ₂	11	[D-GlcNAc] ₂	13
α-D-Man	11	α-L-Fuc	9

C. fragile (four); *Canavalia ensiformis* (three); *Ptilota plumosa* (three); *Limulus polyphemus* (three); *Dolichos biflorus* (two); *Datura stramonium* (one); and *Pisum sativum* (one). The sugars recognised were GlcNAc, GalNAc, Fuc, Man, NeuNAc, Gal and [GlcNAc]₂, and possibly Glc (table III); however, some lectins with specificities for one or more of these sugars did not cause agglutination of any isolates. Of the nine isolates that reacted, 78% were agglutinated by the GlcNAc-specific *C. tomentosum* lectin. However, the response to [GlcNAc]₂-specific lectins was irregular, in that the single isolate agglutinated by the [GlcNAc]₂-specific *D. stramonium* lectin was not agglutinated by the *Triticum vulgare* or *Ulex europaeus* II lectins (both [GlcNAc]₂-specific). None of the isolates was agglutinated by the [GlcNAc]₃-specific *Lycopersicon esculentum* or *Solanum tuberosum* lectins.

Of the 94 group B isolates, 71 (76%) did not react with any of the lectins, whereas the remaining 23 (24%) did so with at least one lectin. A total of 15 distinct agglutination patterns (including no agglutination) was observed (table II). Thirteen of the 21 lectins reacted with at least one isolate. The sugars recognised were GlcNAc, [GlcNAc]₂, [GlcNAc]₃,

Table IV. Lectins that agglutinate group A streptococci

Report	Lectin	Sugar specificity	Number agglutinated/ total isolates
Wagner 1979 ²¹ (pre-treatment)	Wheat-germ agglutinin	D-(GlcNAc) ₄ , NeuNAc	26/26
Kellens <i>et al.</i> 1993 ²⁵ (pre-treatment)	<i>Aegopodium podagraria</i>	D-GalNAc	1/29
	<i>Triticum aestivum</i>	D-(GlcNAc) ₄ , NeuNAc	7/29
	<i>Dolichos biflorus</i>	D-GalNAc	4/29
	<i>Canavalia ensiformis</i>	α -D-Man, α -D-Glc	2/29
	<i>Galanthus nivalis</i>	α (1,3)Man	1/29
This study (no pre-treatment)	<i>Codium tomentosum</i>	D-GlcNAc	7/30
	<i>Ulex europeus</i>	α -L-Fuc	4/30
	<i>Codium fragile</i>	D-GalNAc	4/30
	<i>Canavalia ensiformis</i>	α -D-Man, α -D-Glc	3/30
	<i>Ptilota plumosa</i>	α -D-Gal	3/30
	<i>Limulus polyphemus</i>	NeuNAc	3/30
	<i>Dolichos biflorus</i>	D-GalNAc	2/30
	<i>Datura stramonium</i>	D-(GlcNAc) ₂	1/30
	<i>Pisum sativum</i>	α -D-Man	1/30

Table V. Lectins that agglutinate group B streptococci

Report	Lectin	Sugar specificity	Number agglutinated/ total isolates
Wagner 1979 ²¹ (pre-treatment)	Wheat-germ agglutinin	D-(GlcNAc) ₄ , NeuNAc	53/78
Wagner <i>et al.</i> 1982 ²² (pre-treatment)	<i>Cepaea hortensis</i>	Sialic acids	27/36
Kellens <i>et al.</i> 1993 ²⁵ (pre-treatment)	<i>Triticum aestivum</i>	D-(GlcNAc) ₄ , NeuNAc	4/22
	<i>Iris reticulatum</i>	D-GalNAc	1/22
	<i>Maackia amurensis</i>	Neu5Ac- α (2,3)Gal	11/22
	<i>Arachis hypogaea</i>	β -D-Gal(1,3)D-GalNAc	1/22
	<i>Aegopodium podagraria</i>	D-GalNAc	1/22
	<i>Canavalia ensiformis</i>	α -D-Man, α -D-Glc	1/22
This study (no pre-treatment)	<i>Lycopersicon esculentum</i>	D-(GlcNAc) ₂	7/94
	<i>Solanum tuberosum</i>	D-(GlcNAc) ₂	7/94
	<i>Lens culinaris</i>	α -D-Man	7/94
	<i>Triticum vulgaris</i>	D-(GlcNAc) ₂ , NeuNAc	7/94
	<i>Codium tomentosum</i>	D-GlcNAc	3/94
	<i>Datura stramonium</i>	D-(GlcNAc) ₂	3/94
	<i>Pisum sativum</i>	α -D-Man	2/94
	<i>Codium fragile</i>	D-GalNAc	2/94
	<i>Canavalia ensiformis</i>	α -D-Glc, α -D-Man	2/94
	<i>Anguilla anguilla</i>	L-Fuc	1/94
	<i>Vicia sativa</i>	D-Glc, D-Man	1/94
	<i>Glycine max</i>	D-GalNAc	1/94
	<i>Ulex europeus</i>	α -L-Fuc	1/94

Man, GalNAc and Fuc, and possibly NeuNAc and/or Glc (table III). As with the group A assays, some lectins with specificities for one or more of these sugars did not agglutinate any of the isolates.

Table III lists the percentages of the nine reactive group A and the 23 reactive group B isolates in which each sugar was recognised. Tables IV and V compare the results of this study with those of earlier studies.

Discussion

Kellens *et al.*²⁴ have recently reported the patterns of agglutination of 29 β -haemolytic group A streptococcal isolates by a battery of 25 lectins. In the absence

of pre-treatment none of the isolates was agglutinated; after pre-treatment with trypsin, nine isolates were agglutinated by at least one lectin and five distinct patterns of agglutination were observed. No form of pre-treatment was used in the present study, but despite this, nine of the 30 group A isolates were agglutinated by at least one of the lectins. We obtained 10 distinct patterns of agglutination with group A isolates; a majority pattern of non-agglutination, one pattern involving a single lectin, three involving two, two involving three, two involving four, and one involving seven lectins. These results for group A are compared with those of previous studies (table IV). All the isolates tested, after trypsin treatment, by Wagner²⁰ were agglutinated by wheat germ agglutinin

(WGA). This did not agglutinate any of our isolates. On the other hand, none of the 3560 group A isolates tested by Köhler *et al.*²⁵ again following trypsin treatment, was agglutinated by the D-GalNAc-specific *Helix pomatia* lectin.

To date, the lectins shown to interact with group B streptococci are those of *Cepaea hortensis*,^{21,22} *Streptomyces* 27S5,³⁰ *Lycopersicon esculentum*²³ and *Solanum tuberosum*.²³ Our results revealed 15 distinct patterns of agglutination with group B streptococci; a majority pattern of non-agglutination, as well as five patterns involving a single lectin, five involving two, one involving three, two involving four and one involving five lectins. These results for group B are compared with those of previous studies in table V. Unlike Kellens *et al.*,²⁴ we did not find any patterns (except that of non-agglutination) common to groups A and B.

Some group A and group B strains reacted with a given lectin but not with other lectins of the same nominal specificity and one may ask why lectins with the same nominal specificity sometimes show different reactivities. It is important to be aware that the nominal sugar specificity of a lectin does not necessarily reflect the nature of the carbohydrate to which that lectin binds with highest affinity,³¹ and that differences in the size or molecular conformation, or both, of lectins with the same nominal specificity may affect the ease with which they gain access to sugar residues on the cell surface. Furthermore, a lectin from the same species may have different reported specificities; for example, WGA is described by some authors as [GlcNAc]₄/NeuNAc-specific and by others as [GlcNAc]₂/NeuNAc-specific. In the present study, we have assumed the specificities indicated by the supplier to be correct.

Most previous studies have found that lectins react only with group A and group B streptococci after pre-treatment of cells, most commonly with trypsin. When trypsin or other proteolytic enzymes partially hydrolyse proteoglycans, some carbohydrate residues may become available for binding; equally, other carbohydrate residues may be destroyed or modified. Enzymic pre-treatments may increase the likelihood

that an isolate will be agglutinated by one or more of a given panel of lectins, but at the same time may reduce the total number of distinct agglutination patterns. Furthermore, pre-treatment increases the time required to carry out the assay. Finally, if the aim is to use lectins as tools for epidemiological research, it may not be appropriate to submit bacteria to pre-treatments which alter their surface composition.

Lectins are extremely sensitive to subtle structural differences among bacterial isolates, and lectin typing allows differentiation of a greater number of streptococcal groups than serotyping. The technique, whether used independently or in conjunction with serotyping, is of considerable potential value both for epidemiological studies of streptococci and for routine clinical diagnosis. Lectin agglutination assay is rapid, inexpensive and reproducible, and lectins are stable and easy to store, readily available from major suppliers and active at low concentrations. Furthermore, it may prove possible to extend techniques such as that developed by Slifkin and Gil,²⁸—who identified group C streptococci without antisera by using *Dolichos biflorus* lectin coupled to polystyrene beads—to other lectins.

Despite these advantages, the development of effective diagnostic tests based on lectin typing will remain difficult until some degree of standardisation of procedures is agreed, permitting meaningful comparison of the results obtained by different research groups. Reported specificities of lectins may vary depending on the source consulted and we consider it preferable to use commercially available products and to assume that the specificities cited by the supplier are correct. Lectin agglutination may be affected also by culture conditions, for example, whether the medium is solid or liquid, and is affected by specific treatments, such as trypsin treatment or boiling, before assay. Only when some degree of methodological standardisation has been achieved will the practising clinical microbiologist benefit fully from the extensive research in this field.

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