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Interactions with Lectins and Agglutination Profiles of Clinical, Food, and Environmental Isolates of *Listeria*

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On the basis of preliminary trials with 14 collection strains of *Listeria*, five lectins (*Canavalia ensiformis*, concanavalin A; *Griffonia simplicifolia* lectin I; *Helix pomatia* agglutinin; *Ricinus communis* agglutinin; and *Triticum vulgaris* wheat germ agglutinin) were selected to set up a microtiter agglutination assay. The lectin agglutination profiles of 174 clinical, food, and environmental strains of *Listeria monocytogenes*, *Listeria innocua*, and *Listeria seeligeri* were investigated. Data on the standard determination of the antigenic structure were available for clinical strains; nonclinical isolates were assigned to serogroup 1 or 4 with commercial antisera. The listeria-lectin interaction was related to serological type rather than species; in particular, the strains assigned to serogroup 1 or belonging to serovars 1/2a, 1/2b, 1/2c, 3a, 3b, and 7 were never agglutinated by *G. simplicifolia* lectin I. The five-lectin set proved to be capable of detecting differences between serologically identical isolates of *L. monocytogenes*. Of the 150 isolates of this species, 144 were distributed over 15 different lectin agglutination profiles and 6 autoagglutinated, the overall typeability being 96%. However, the profiles encountered among *L. monocytogenes* isolates were not randomly distributed. With strains assigned to serogroup 1 or belonging to serovars 1/2a, 1/2b, 1/2c, and 3b, the clinical isolates fell into only two of the eight patterns recorded overall; with strains of serogroup 4 and serovar 4b, food and environmental isolates were distributed over eight of the nine patterns found in total, while clinical isolates were distributed over five patterns. In a comparative study of 15 epidemiologically relevant isolates of *L. monocytogenes* from five distinct outbreaks, strains with identical phage types and/or DNA fingerprints displayed identical lectin profiles. The heterogeneity of agglutination profiles may form the basis of a new approach to *L. monocytogenes* typing.

Listeriae are widely distributed in the environment and also occur in the intestinal tracts of healthy animals and humans. Of the currently recognized species of the genus *Listeria*, only *Listeria monocytogenes* is an important human and animal pathogen. The other species are generally believed to be nonpathogenic, although *Listeria ivanovii* has sometimes been found to cause infections in humans and animals (31). In humans, *L. monocytogenes* is increasingly involved in both community- and hospital-acquired infections (12). Listeriosis is a serious invasive disease mainly of immunocompromised hosts, elderly people, neonates, and fetuses, leading to septicemia, abortion, stillbirth, meningitis, and meningoencephalitis.

Epidemiologic studies and surveillance of listeriosis have been hampered for many years by the lack of suitable typing systems. *L. monocytogenes* is divided into four serological groups and is subdivided into 13 serovars according to somatic and flagellar antigen components (30, 31): serogroup 1 (serovars 1/2a and 1/2b), serogroup 2 (serovar 1/2c), serogroup 3 (serovars 3a, 3b, and 3c), and serogroup 4 (serovars 4a, 4ab, 4b, 4c, 4d, 4e, and 7). Nevertheless, serotyping is only of minimal help in epidemiologic studies, since more than 90% of all clinical isolates belong to only three serovars (1/2a, 1/2b, and 4b) (21, 29). Phage typing, the other conventional typing approach, is performed in very few laboratories worldwide; it appears to be reproducible and discriminatory, but it suffers from a lack of standardization and a low typeability rate (2, 22, 27). In recent years, a variety of molecular approaches, such as multilocus enzyme electrophoresis (4, 25), DNA restriction enzyme analysis (3, 5, 10, 11, 24), rRNA typing (34), and random amplified polymorphic DNA typing (20), have proved to be of great value in establishing additional typing schemes for *L. monocytogenes*. Largely on the basis of these techniques, ingestion of foods contaminated with *L. monocytogenes* was demonstrated to be a major route of infection not only in outbreaks but also in sporadic cases of human listeriosis (12, 29). In general, however, these molecular methods are labor-intensive and do not permit the rapid screening of a large number of isolates (29). Thus, subtyping of *L. monocytogenes* is still a problem, warranting a search for alternative methods.

In the study described here, we assessed the possibility of exploiting lectin agglutination to differentiate listerial isolates. Lectins are carbohydrate-specific, sugar-binding proteins that can precipitate polysaccharides or agglutinate cells (14). Most lectins are derived from plants, but lectins or lectin-like substances have also been found in bacteria, invertebrates, and mammalian tissues. The earliest applications of lectins were for blood typing, but since then the availability of a large number of lectins with different carbohydrate specificities has led to their use as recognition molecules in biological and medical research (19, 32). Microbial surfaces are rich in potentially lectin-reactive sites (7); peptidoglycan, teichoic acids, lipopolysaccharides, and the capsular material of bacteria all bind lectins, while fungi, viruses, and protozoa also exhibit numerous surface-binding sites. The unique property of lectins of binding noncovalently to specific sugars favored several attempts to use lectins as reagents for the identifica-

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tion, taxonomy, and epidemiologic monitoring of microbial agents. This matter has recently been reviewed by Slifkin and Doyle (33), who showed in particular that *L. monocytogenes* can interact with lectins. More recently, encouraging data on the use of lectin typing systems have been reported with staphylococci (15, 16), streptococci (17), and gonococci (23). In the present study, we selected a battery of five lectins which proved to interact variably with clinical, foodborne, and environmental isolates of *Listeria*. The heterogeneity of agglutination profiles may form the basis of a new approach to *L. monocytogenes* typing.

MATERIALS AND METHODS

Bacterial strains. A total of 203 *Listeria* strains were studied. Of these, 14 were obtained from official culture collections (see Table 1). Another 174 were recently isolated from clinical specimens, foods, or the environment (see Table 2). In particular, the 67 clinical *L. monocytogenes* strains were all isolated from patients (having clinical symptoms consistent with listeric infection) in different Italian hospitals; multiple isolates from the same patient were avoided. The foodborne isolates of all species were collected in Italy from different kinds of meat (beef, pork, chicken, and turkey; 43 strains), from soft cheeses (mostly mozzarella and Gorgonzola; 23 strains), or from other foods (16 strains) and included some foodborne isolates reported previously (8, 9). The seven environmental isolates were all *L. monocytogenes* strains isolated in Italy from sewage. The remaining 15 strains (all of *L. monocytogenes*) were isolated in the course of five distinct outbreaks of listeriosis. Strains M4 and M5 and strains Cr69 and Cr70 were two baby-baby pairs resulting from two different cases of hospital cross-infection; the former pair of isolates has been described previously (5, 10). Strains M9 and M10 were a patient-food pair involved in a previously described case of foodborne listeriosis, and strain M11 was another food isolate shown to be unrelated in the same studies (5, 11). Strains An67 and An68 were a previously undescribed mother-baby pair. The remaining six strains (kindly placed at our disposal by the Presidio Multizonale di Prevenzione, Unitá Sanitaria Locale 28, Bologna, Italy) included two isolates from patients (Bo71 and Bo72), three isolates from foods (Bo73, Bo74, and Bo75), and one isolate from a refrigerator (Bo76) involved in an outbreak of foodborne listerial gastroenteritis (28).

Strains were identified on the basis of standard criteria (31): morphology, Gram staining, tumbling motility, catalase production, and hemolytic activity on horse blood agar. The CAMP test was performed with *Staphylococcus aureus* ATCC 25923. Additional identification tests included nitrate reduction, urease and H_2S production, the methyl red–Voges-Proskauer reaction, and the ability to ferment glucose, esculin, maltose, mannitol, rhamnose, and xylose with acid production. All strains were maintained in soft agar after the first isolation.

Serotyping, phage typing, and DNA fingerprinting. Standard determination of the antigenic structures of the clinical strains was carried out at the Listeria Referenz Laboratorium, Würzburg, Germany. Nonclinical strains were assigned to serogroup 1 or serogroup 4 with listerial O antisera (Difco Laboratories, Detroit, Mich.). In order to distinguish between these two levels of serotyping, we arbitrarily use the term "serovar" to refer to the results obtained by the former method and the term "serogroup" to refer to the results yielded by the latter method.

Phage typing and DNA fingerprinting information was already available for some previously described outbreak-related isolates (5, 10, 11). For the remaining epidemiologically relevant isolates, DNA for restriction endonuclease analysis was extracted as described elsewhere (5) and was digested with *Eco*RI restriction endonuclease (Boehringer Mannheim GmbH, Mannheim, Germany) under the conditions specified by the manufacturer. Restriction fragments were separated by electrophoresis in 1% agarose gels containing 0.5 μg of ethidium bromide per ml. Restriction fragments of phage lambda DNA were included as molecular weight standards. The gels were photographed under UV light (254 nm) with P/N type 665 film (Polaroid Corporation, Cambridge, Md.).

Lectins. The lectins assayed and their sugar specificities (32) are outlined in Table 1 (Sigma, St. Louis, Mo.). The lectins were dissolved in phosphate-buffered saline (PBS; 0.05 M disodium phosphate, 0.15 M sodium chloride [pH 7.2]), and the mixtures were stored at −20°C. The lectin concentration of the stock solutions was 1 mg/ml, while the working concentration was 50 μg/ml.

Agglutination assays. The 14 collection strains of *Listeria* were used to evaluate the conditions affecting agglutination by lectins. Brain heart infusion broth, brain heart infusion agar, and blood agar base (all from Difco), the last two supplemented with 5% sheep blood, were used as growth media. For agglutination assays, cells harvested after overnight growth at 37°C were suspended in PBS to an optical density of 1.0 ± 0.1 at 540 nm. Agglutination tests were carried out on sterile U-well microtiter plates (Greiner Labortechnik, Frickenhausen, Germany). In each well, 50 μl of cell suspension, either heated or unheated, was added to 50 μl of the working solution of each lectin. As a control, 50 μl of PBS was added to 50 μl of each lectin solution and to 50 μl of each cell suspension. Plates were incubated at 37°C overnight and were then visually inspected. Lectin agglutinations of commercially available listerial O antigens of serogroup 1 and serogroup 4 (Difco) were also assayed.

In the case of heat pretreatment, cell suspensions adjusted for optical density as described above were heated at 80°C for 1 h before being assayed for lectin agglutination.

In the case of further protease treatment, heated cells were harvested, resuspended in PBS containing 50 μg of proteinase K (Sigma) per ml, and incubated at 37°C for 30 min. Enzyme-free suspensions were also prepared as controls. Cells were then washed three times in PBS, resuspended at the usual optical density (1.0 ± 0.1 at 540 nm), and assayed for lectin agglutination as described above.

Carbohydrate inhibition tests. The following sugars were used in agglutination-inhibition studies: Gal, Glc, Man, GlcNAc, and GalNAc. A 10-mg amount of the carbohydrate to be tested was added to 1 ml of the lectin working solution. The sugar-containing lectins were then used in agglutination tests as described above.

Statistical analysis of data. The distribution of lectin agglutination profiles among *L. monocytogenes* strains was studied by the run-test (6).

RESULTS

Selection of conditions affecting the interaction between listeriae and lectins. Preliminary experiments aimed at investigating and selecting the conditions affecting the listeria-lectin interaction were performed with the 14 collection listeriae, another 14 *L. monocytogenes* strains freshly isolated from various sources, and listerial O antigens of types 1 and 4 (Difco). The main results obtained with the 14 collection strains and with the listerial antigens are shown in Table 1.

The differences observed in the interaction between test strains and lectins were mainly dependent upon the growth

TABLE 1. Interactions between lectins and 14 collection strains of *Listeria* (grown on blood agar base plus 5% sheep blood) or listerial O antigens

Strain or antigen	Serotype	Microtiter agglutination by lectin[a]:									
		WGA		GS-I		ConA		RCA-I		HPA	
		u	h	u	h	u	h	u	h	u	h
L. monocytogenes											
NCTC 7973	1/2a	+	+	−	−	−	+	−	−	−	+
NCTC 5105	3a	+	+	−	−	+	+	−	−	−	+
NCTC 5214	4a	−	−	−	−	−	−	−	−	−	−
NCTC 10528	4ab	−	−	−	−	−	+	−	−	−	−
NCTC 4883	4c	−	+	+	+	−	+	−	+	−	−
NCTC 10888	4d	−	−	−	−	−	−	−	−	−	−
NCTC 10890	7	−	+	−	−	−	+	−	W	−	−
ATCC 984	1	+	+	−	−	−	W	−	−	−	W
ATCC 9525	4	−	+	−	−	+	+	−	+	−	+
L. innocua NCTC 11288	6a	−	+	+	+	−	W	−	−	−	+
L. ivanovii											
NCTC 11846	5	−	−	−	−	−	+	−	+	−	−
CIP 7842	5	−	−	−	−	−	+	−	+	−	−
L. seeligeri NCTC 11856	1/2b	+	+	−	−	−	−	−	−	−	−
L. welshimeri NCTC 11857	6a	−	−	+	+	−	−	−	−	−	−
Listerial O antigen	1	+		−		+		−		−	
Listerial O antigen	4	−		+		−		+		+	

[a] The lectins used and their sugar specificities were as follows: *Arachis hypogaea* (PNA), galactose (Gal), and galactosamine ($GalNH_2$); *Canavalia ensiformis* (ConA), mannose (Man) and glucose (Glc); *Glycine max* (SBA), *N*-acetyl-galactosamine (GalNAc), and Gal; *Griffonia simplicifolia* (GS-I), Gal and GalNAc; *Helix pomatia* (HPA), GalNAc, *N*-acetyl-glucosamine (GlcNAc), and Gal; *Ricinus communis* (RCA-I), Gal; *Triticum vulgaris* (WGA), $(GlcNAc)_{1-3}$; and *Vicia villosa* (VVA), GalNAc. No strains were agglutinated by the lectins PNA, SBA, or VVA. Abbreviations: u, tested with unheated strains; h, tested with heated strains; w, weak.

medium and cell heating. A poor interaction with lectins was shown by strains (whether they were heated or not) grown in brain heart infusion broth or agar with 5% sheep blood and by unheated strains grown on blood agar base with 5% sheep blood. Under these conditions, only *Triticum vulgaris* (WGA), *Griffonia simplicifolia* (GS-I), and *Canavalia ensiformis* (concanavalin A [ConA]) interacted with some listeriae. After growth on blood agar base plus 5% sheep blood with heating, *Listeria* strains other than *Listeria welshimeri* (agglutinated by GS-I only) and serovars 4ab (agglutinated by ConA only) and 4a and 4d (refractory to all lectins) of *L. monocytogenes* could interact with two additional lectins (*Helix pomatia* agglutinin [HPA] and *Ricinus communis* agglutinin [RCA-I]). The remaining three lectins (*Arachis hypogaea* [PNA], *Glycine max* [SBA], and *Vicia villosa* [VVA]) failed to agglutinate any of the test strains under any conditions. Listerial O antigen type 1 was agglutinated by WGA and ConA, whereas listerial O antigen type 4 was agglutinated by GS-I, RCA-I, and HPA.

On the basis of these preliminary trials, we decided to use a five-lectin set (GS-I, ConA, WGA, RCA-I, and HPA) to investigate the lectin agglutination profiles of listeriae more extensively. Agglutination assays were carried out with cells grown on blood agar base plus 5% sheep blood and heated.

To check the reproducibility of the assay, the collection strains were subcultured 10 times, and the lectin profiles of three different colonies from the last subculture of each strain were assayed. All of the colonies of each strain showed the same profile as that exhibited by the original culture. No difference in the agglutination profiles was observed when suspensions of heated listeriae were retested after 2 weeks at 4°C. WGA, GS-I, and ConA retained unmodified agglutinating properties when they were kept in solution for 6 months at −20°C, whereas RCA-I and HPA were slightly less stable.

Agglutination tests with 174 listerial isolates. The 174 clinical, food, or environmental isolates of the species *L. monocytogenes*, *Listeria innocua*, and *Listeria seeligeri* were assayed with the five-lectin agglutination set (Table 2).

Of the 56 test strains of *L. monocytogenes* belonging to serovars 1/2a, 1/2b, 1/2c, and 3b or assigned to serogroup 1, 3 strains failed to interact with any lectin and 3 strains autoagglutinated. The remaining 50 strains did not react with GS-I but reacted with one or several of the four other lectins. For the remainder of the report we refer to these strains as "lectin group 1."

Of the 92 test strains of *L. monocytogenes* belonging to serovar 4b or assigned to serogroup 4, 88 interacted with variable patterns with one or several lectins of the set; 2 strains failed to interact with any lectin and 2 autoagglutinated. For the remainder of the report we refer to these strains as "lectin group 4."

Of the two unserotypeable strains of *L. monocytogenes*, one interacted with ConA and RCA-I and one autoagglutinated.

The overall lectin typeability of *L. monocytogenes* strains was 96%.

No autoagglutination was observed with strains of either *L. innocua* or *L. seeligeri*. Strains of these two species either failed to show any interaction with or were variably agglutinated by the lectins. However, strains of *L. seeligeri* were never agglutinated by GS-I.

Agglutination profiles of *L. monocytogenes* isolates. Overall, 15 distinct agglutination profiles were recognized with *L. monocytogenes* isolates (Table 3).

Eight agglutination profiles (none of which showed GS-I agglutination) were observed with lectin group 1 *L. monocyto-*

TABLE 2. Serotyping and source of 174 *Listeria* field isolates and their interaction with lectins

Species and serotype[a]	No. of strains								
	Total	Isolated from			Agglutinated by:					Autoagglutinating
		Clinical material	Foods	Environment	WGA	GS-I	ConA	RCA-I	HPA	
L. monocytogenes	150									6
1/2a	7	7			7	0	7	1	6	
1/2b	8	8			8	0	8	1	7	
1/2c	2	2			2	0	2	0	2	
1	35		30	5	24	0	28	23	16	3
3b	4	4			4	0	4	0	4	
4b	46	46			32	45	43	37	12	1
4	46		44	2	28	37	38	38	9	1
NT	2		2		0	0	1	1	0	1
L. innocua, ND	18									
	18		18		5	5	9	4	5	
L. seeligeri	6									
1	2		2		2	0	2	2	2	
ND	4		4		2	0	0	0	1	

[a] NT, not serotypeable; ND, serotype not determined.

genes isolates. However, while the 32 isolates of food or environmental origin were distributed over all of these eight patterns, the 21 clinical isolates fell into two patterns only: 19 strains were agglutinated by WGA, ConA, and HPA and 2 strains were agglutinated by WGA, ConA, and RCA-I. Of these two patterns, the former was shared by 1 (isolated from sewage) and the latter by 6 (isolated from foods and sewage) of the 32 nonclinical isolates. These differences in the lectin profile distributions of clinical and nonclinical strains of lectin group 1 were statistically significant ($P < 0.01$), mainly because of the greater agglutination frequencies by HPA ($P < 0.01$) and WGA ($P < 0.05$) and the lower agglutination frequency by RCA-I ($P < 0.01$) of clinical versus food or environmental strains.

Nine agglutination profiles were recognized with lectin group 4 *L. monocytogenes* isolates. Of these, two patterns (ConA and RCA-I and that of nonreactive strains) were also encountered with group 1 strains (see above). Food and environmental isolates were distributed over eight patterns; the clinical isolates were distributed over five patterns. Of these five patterns, four were shared by nonclinical strains, whereas the other (GS-I and ConA) was found in clinical isolates only. Differences in the lectin profile distributions of clinical and nonclinical strains of lectin group 4 were not statistically significant ($P > 0.05$).

Lectin typing versus other typing systems in the investigation of epidemiologically relevant strains of *L. monocytogenes*. Both previously characterized and newer sets of epidemiologically relevant isolates were studied (Table 4).

The same lectin profile (WGA, ConA, and HPA) was exhibited by two phage-untypeable, serovar 3b isolates (M4 and M5) from a previously reported case of hospital cross-infection confirmed by DNA fingerprinting (5, 10).

TABLE 3. Agglutination profiles recognized by the five-lectin set with *L. monocytogenes* isolates

Lectin agglutination profile					No. of strains from the indicated lectin group and source[a]			
					Lectin group 1		Lectin group 4	
WGA	GS-I	ConA	RCA-I	HPA	Clinical[b] ($n = 21$)	Food or environmental ($n = 32$)	Clinical[b] ($n = 45$)	Food or environmental ($n = 45$)
+	+	+	+	+			12	9
+	+	+	−	+				1
+	+	+	+	−			20	19
+	+	+	−	−				1
−	+	+	+	−			5	6
−	+	+	−	−			6	
−	+	−	−	−			2	3
+	−	+	+	+		15		
+	−	+	+	−	2	6		
+	−	+	−	+	19	1		
+	−	+	−	−		1		
+	−	−	−	−		1		
−	−	+	+	−		2		4
−	−	+	−	−		3		
−	−	−	−	−		3		2

[a] The test strains are the same as those reported in Table 3 except for the not serotypeable and the autoagglutinating strains, which are not included here.

[b] Differences in the lectin profile distributions of clinical and nonclinical isolates were statistically significant ($P < 0.01$) with strains of lectin group 1 but not ($P > 0.05$) with those of lectin group 4.

TABLE 4. Comparative results of lectin typing and other typing systems determined in five sets of *L. monocytogenes* isolates investigated in the course of distinct outbreaks

Outbreak and isolate	Source	Serotype	Phage type[a]	DNA fingerprinting (reference)[b]	Lectin agglutination profile				
					WGA	GS-I	ConA	RCA-I	HPA
Outbreak 1									
M4	Bronchial aspirate	3b	NT	Facinelli et al. (10), Casolari et al. (5)	+	−	+	−	+
M5	Blood	3b	NT	Facinelli et al. (10), Casolari et al. (5)	+	−	+	−	+
Outbreak 2									
M9	Cerebrospinal fluid	4b	340/108/107/2389/52/47/3552/312/1444	Facinelli et al. (11), Casolari et al. (5)	+	+	+	+	+
M10	Fish	4b	340/108/107/2389/52/47/3552/312/1444	Facinelli et al. (11), Casolari et al. (5)	+	+	+	+	+
M11	Meat	4b	340/108/107/2389/52/47/3552	Facinelli et al. (11), Casolari et al. (5)	−	+	+	−	−
Outbreak 3									
An67	Vagina	4b	ND	Fig. 1a	+	+	+	+	−
An68	Cerebrospinal fluid	4b	ND	Fig. 1a	+	+	+	+	−
Outbreak 4									
Cr69	Blood	4b	ND	Fig. 1a	−	+	+	+	−
Cr70	Cerebrospinal fluid	4b	ND	Fig. 1a	−	+	+	+	−
Outbreak 5									
Bo71	Blood	1/2b	ND	Fig. 1b	+	−	+	+	−
Bo72	Blood	1/2b	ND	Fig. 1b	+	−	+	+	−
Bo73	Fish	1/2b	ND	Fig. 1b	+	−	+	+	−
Bo74	Cheese	1/2b	ND	Fig. 1b	+	−	+	+	−
Bo75	Fruit pie	1/2b	ND	Fig. 1b	+	−	+	+	−
Bo76	Refrigerator	1/2b	ND	Fig. 1b	+	−	+	+	−

[a] NT, not typeable; nd, not determined.
[b] With the isolates from outbreaks 3 to 5, data on DNA fingerprinting are reported in this paper in the indicated figure.

Of three serovar 4b isolates, previously investigated by phage typing and DNA fingerprinting (5, 11), the two shown to be related by these methods (M9 and M10, a patient-food pair) shared the same lectin profile (WGA, GS-I, ConA, RCA-I, and HPA), whereas the third, unrelated isolate (M11, another food isolate) exhibited a different lectin profile (GS-I and ConA).

The same profile (WGA, GS-I, ConA, and RCA-I) was shown by a mother-baby pair of serovar 4b isolates (An67 and An68) with identical *Eco*RI fingerprints (Fig. 1a).

Another lectin profile (GS-I, ConA, and RCA-I) and another *Eco*RI restriction pattern were shared by a baby-baby pair of serovar 4b isolates (Cr69 and Cr70) (Fig. 1a).

Finally, six isolates investigated following a foodborne outbreak of gastroenteritis and shown to belong to the same serovar (1/2b) and to share the same multilocus enzyme pattern (28) also shared the same lectin profile (WGA, ConA, and RCA-I) and the same *Eco*RI fingerprint (Fig. 1b).

Carbohydrate inhibition test. Carbohydrate inhibition studies were performed with four *L. monocytogenes* strains including three clinical isolates (two of serovar 4b and one of serovar 1/2b) and one food isolate (of serogroup 1). The inhibition assay was also performed with listerial O antigens types 1 and 4. WGA was consistently inhibited by GlcNAc, GS-I by Gal and GalNAc, and RCA-I by Gal. ConA was consistently inhibited by Man, was inhibited in only one strain by Glc, and was never inhibited by GlcNAc. HPA was consistently inhibited by GalNAc and GlcNAc, and HPA in only one strain was inhibited by Gal.

Lectin agglutination after protease treatment. Twelve *L. monocytogenes* isolates selected to represent a variety of sero-

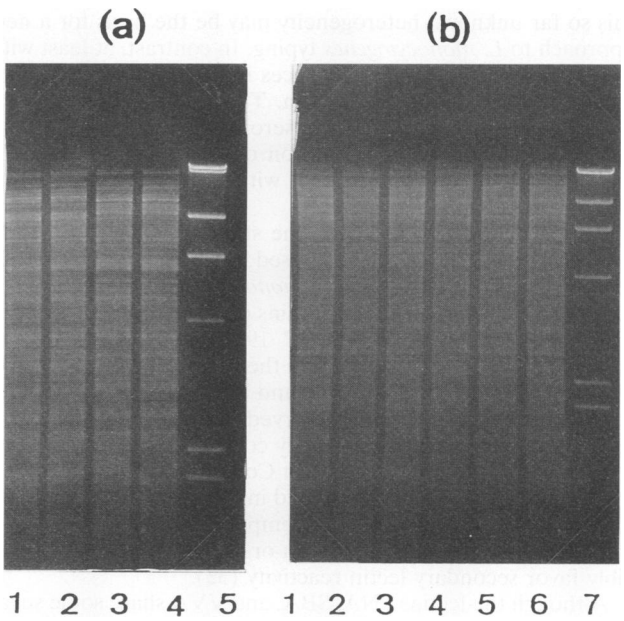

FIG. 1. *Eco*RI-generated DNA digest patterns of 10 epidemiologically relevant isolates of *L. monocytogenes*, including a mother-baby pair and a baby-baby pair (a) and 6 isolates involved in an outbreak of foodborne gastroenteritis (b). (a) Lane 1, An67; lane 2, An68; lane 3, Cr69; lane 4, Cr70; lane 5, *Hin*dIII-digested phage lambda DNA. (b) Lane 1, Bo71; lane 2, Bo72; lane 3, Bo73; lane 4, Bo74; lane 5, Bo75; lane 6, Bo76; lane 7, *Hin*dIII-digested phage lambda DNA.

TABLE 5. Lectin agglutination profiles of 12 *L. monocytogenes* strains after protease treatment

Strain	Serotype	Lectin agglutination profile[a]									
		WGA		GS-I		ConA		RCA-I		HPA	
		u	p	u	p	u	p	u	p	u	p
NCTC 7973	1/2a	+	+	−	−	+	−	−	−	+	−
LM37	1/2b	+	+	−	−	+	−	−	−	+	−
A23	1	+	+	−	−	+	−	+	−	−	−
W34	1	−	−	−	−	+	−	+	−	−	−
NCTC 5105	3a	+	+	−	−	+	−	−	−	+	−
LM6	3b	+	+	−	−	+	−	+	−	−	−
NCTC 5214	4a	−	−	−	−	−	−	−	−	−	−
M9	4b	+	−	+	+	+	−	+	−	+	−
Pd17	4b	+	−	+	+	+	−	+	−	−	−
An67	4b	+	−	+	+	+	−	+	−	−	−
Cr69	4b	−	−	+	+	+	−	+	−	−	−
NCTC 4883	4c	+	−	+	+	+	−	+	−	−	−

[a] u, untreated, i.e., without proteinase K treatment; p, after proteinase K treatment.

logical types, lectin agglutination profiles, and origins were retested for lectin agglutination after treatment with proteinase K (Table 5). Under these conditions, while no previously undetected agglutination was observed, ConA, RCA-I, and HPA failed to agglutinate any strains. WGA agglutination was still observed with the isolates of lectin group 1 but not with those of lectin group 4. With the latter strains, agglutination by GS-I was not affected by proteinase K treatment.

DISCUSSION

The present study shows that listeriae interact with lectins, giving rise to different agglutination patterns, and suggests that this so far unknown heterogeneity may be the basis for a new approach to *L. monocytogenes* typing. In contrast, at least with the lectins that we used, differences in agglutination were not suitable for species identification. The listeria-lectin interaction appears to be related to serological type rather than species, even if a large proportion of the *L. innocua* and *L. seeligeri* isolates did not interact with any of the five lectins used.

Among the lectins tested in the study, GS-I, ConA, HPA, and VVA have previously been used by Slifkin and Doyle (33) in agglutination assays with *L. monocytogenes*. Those investigators tested seven collection strains of *L. monocytogenes*, four of which (ATCC 19111, ATCC 19113, ATCC 19114, and ATCC 19117) were also tested in the present study (as NCTC 7973, NCTC 5105, NCTC 5214, and NCTC 10888, respectively). The lectin interactions observed in the two studies with these four strains are substantially comparable, despite minor differences in the interaction with ConA, which might depend on the longer incubation time used in our experiments (18 h at 37°C instead of 15 min at room temperature). In fact, increasing incubation periods of a lectin-organism mixture may possibly favor secondary lectin reactivity (33).

Although the lectins PNA, SBA, and VVA share some sugar specificities with GS-I, HPA, and RCA-I (Gal and GalNAc), they failed to agglutinate the test strains. In fact, even though several lectins possess the same specificities, it does not follow that each of them will interact with a given microbial structure (33). Not only must an organism possess the proper carbohydrate determinants on its surface but other factors (e.g., lectin molecular weight, hydrophobic group stabilization, hydrogen ion concentration, and ionic strength) may also affect the lectin-sugar interaction (26).

Given the sugar-binding specificities of lectins, inhibition studies may provide valuable information about the specific sugars present at the cell surface of an organism. Our inhibition tests suggest that GlcNAc, Glc, GalNAc, Gal, and Man are present on the surface of *L. monocytogenes*. The presence of GalNAc and Man has not been reported earlier to our knowledge. GlcNAc, Glc, and Gal are known to be major substituents of teichoic acids in *L. monocytogenes*, although they are limited to particular serovars (13). The serovar-related differences in the presence of GlcNAc and Gal as teichoic acid substituents in *L. monocytogenes* might account for differences in the interaction of particular serovars with such lectins as WGA and GS-I (the former having affinity for *N*-acetyl-β-D-glucosaminyl residues and *N*-acetyl-β-D-glucosamine oligomers and the latter having affinity for α-galactosyl end groups [18]). Strains of serovars 1/2a, 1/2b, 1/2c, 3a, and 3b were agglutinated by WGA, irrespective of the heating regimen, whereas strains of serovars 4b and 4c were agglutinated only after heating. This difference might reflect a diverse location of the carbohydrate receptors on the cell surface. In fact, in serovars 1/2a, 1/2b, 1/2c, 3a, and 3b GlcNAc is present as a glycosyl substituent of the ribitol residues, whereas in serovars 4b and 4c GlcNAc is integrated into the chain (13). In this latter case, the heating treatment probably causes WGA-reactive sites to become exposed. With regard to GS-I, the occurrence of agglutination with strains of serovar 4b or assigned to serogroup 4 is consistent with the fact that only in the teichoic acid of serovar 4b strains has Gal been demonstrated to be a glycosyl substituent (13).

Although carried out with a limited number of selected isolates, experiments with proteinase K suggest that certain lectin receptor sites (e.g., those for ConA, RCA-I, and HPA) are usually removed or modified by protease treatment, whereas others (e.g., that for GS-I) are not. The finding that the WGA receptor site is apparently removed or modified in the isolates of lectin group 4 but not in those of lectin group 1 appears to be an additional differential element between the two groups of organisms.

In 1988, after reviewing foodborne listeriosis, the World Health Organization Working Committee concluded that *L. monocytogenes* should be considered an environmental contaminant (1). Therefore, when outbreaks of human listeriosis occur, there is the need to type associated isolates, particularly those yielded by the suspected vehicle of infection. However, it may be critical to establish the relationship between patient and food isolates in investigations of foodborne listeriosis. Lectins may offer a new method for *L. monocytogenes* typing, allowing an easy comparison of strains. In particular, the five-lectin set enabled us to detect differences between *L. monocytogenes* isolates of the same serovar and to type phage-untypeable isolates, the overall typeability of *L. monocytogenes* strains being 96%. Strains having identical phage types and/or DNA fingerprints always displayed identical lectin profiles. Thus, examination of the lectin agglutination profile may be a viable alternative in the initial determination of whether two strains are different. Further improvement of the lectin typing set might result from the search for additional listeria-interacting lectins.

Important differences emerged in the lectin profiles of *L. monocytogenes* strains depending on the source of isolation (clinical material or food or environmental samples). Among lectin group 1 isolates, all clinical strains interacted with three lectins of the set (WGA, ConA, and either RCA-I or HPA); on the contrary, the food or environmental strains showed more

variable patterns, interacting with four, three, two, one, or none of the five lectins of the set. Thus, the agglutination profiles were not randomly distributed in lectin group 1: two profiles only (WGA, ConA, and HPA, which was the most frequently encountered profile, and WGA, ConA, and RCA-I) were recognized among clinical isolates, in contrast to the nine profiles (including the two listed above) recognized among food or environmental isolates. Among lectin group 4 isolates, differences in agglutination profiles between clinical and nonclinical strains were less pronounced. However, even among those strains, the no-agglutination pattern was observed in nonclinical isolates only. Overall, food and environmental strains appear to be more heterogeneous than clinical strains as far as the lectin interaction is concerned, probably because of greater variabilities in the surface receptors of these strains. On the other hand, a lower variability among clinical isolates of *L. monocytogenes* (most being related to only three serovars, serovars 1/2a, 1/2b, and 4b) in comparison with that among food and environmental isolates of the same species is well-established (29). Thus, our findings are in accordance with the hypothesis that some particular strains are more frequent than others among clinical isolates (4, 25), which implies that strains of *L. monocytogenes* do not all have the same potential as agents of human listeriosis.

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