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The serological relationship between *Escherichia coli* O157 and *Yersinia enterocolitica* O9 using sera from patients with brucellosis

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SUMMARY

Sera from ten patients with positive brucella serology were used to investigate antibody cross-reactions between the O-antigens of *Escherichia coli* O157 and *Yersinia enterocolitica* O9. SDS–PAGE profiles of lipopolysaccharide (LPS), purified from strains of *E. coli* O157 and *Y. enterocolitica* O9, were reacted with sera by immunoblotting. All ten sera contained antibodies which bound to the LPS of *E. coli* O157, and five of these sera also contained antibodies which bound to the LPS of *Y. enterocolitica* O9. Absorption studies using these five cross-reacting sera indicated the existence of at least three epitopes exposed on the O-antigens of *E. coli* O157 and *Y. enterocolitica* O9. One antigen binding site appeared to be exposed on the LPS of both organisms, while one epitope was exposed on the LPS of *E. coli* O157 only, and another on the LPS of *Y. enterocolitica* O9 only.

INTRODUCTION

Strains of *Escherichia coli* belonging to serotype O157:H7 are a major cause of haemolytic uraemic syndrome (HUS) [1, 2] and haemorrhagic colitis (HC) [3]. Strains of *Yersinia enterocolitica* belonging to serogroup O9 have been shown to cause yersiniosis [4]; and strains of *Brucella abortus* and *B. melitensis* can cause brucellosis [5]. The symptoms of HC and HUS are distinct from those observed in cases of yersiniosis and brucellosis; however, the bacteria that cause these diseases are similar because strains of *E. coli* O157, *Y. enterocolitica* O9, *B. abortus* and *B. melitensis* express O-antigens which contain the same sugar sequences [6–8]. These similarities in lipopolysaccharide (LPS) sugar composition have been used to explain, and predict, serum antibody cross-reactions [9–13]; however, recent studies from this laboratory [14], using sera from patients with HUS and yersiniosis, showed that antibody cross-reactions between strains of *E. coli* and *Y. enterocolitica* are probably more complicated than can be explained by the mutual possession of common sugar sequences. In this instance, sera from patients with HUS were shown to contain antibodies reacting with the LPS of *E. coli* O157 only, whilst 80% of sera from patients with yersiniosis contained antibodies which reacted with the LPS of both *Y. enterocolitica* and *E. coli* O157 [14]. From this study we concluded that patients infected with *E. coli* O157 or *Y. enterocolitica* O9

showed variation in the specificity of the antibodies produced to the O-antigens of these organisms; and also, that either the LPS of *Y. enterocolitica* contained at least two epitopes, of which only one was present on the LPS of *E. coli* O157, or that the observed cross-reactions were due to differences in the LPS structure of these organisms [16].

Since antibody cross-reactions have been reported to occur between strains of *E. coli* O157, *Y. enterocolitica*, *B. abortus* and *B. melitensis*, we used sera from patients with positive brucella serology in an attempt to elucidate the basis of antibody cross-reactions between *E. coli* O157 and *Y. enterocolitica*.

MATERIALS AND METHODS

Bacteria and media

E. coli strain E32511 (O157:H−) and *Y. enterocolitica* strain E4610 (O9) were from the culture collection held by the Division of Enteric Pathogens (DEP). Strains were stored on Dorset's egg agar slopes at room temperature. Bacteria were grown in Hedley–Wright broth (16 h) and used to seed Hartley–Salmonella agar plates prior to incubation (16 h). *Y. enterocolitica* and *E. coli* were incubated at 28 and 37 °C respectively.

Sera

Sera from ten patients, considered antibody-positive to brucella using serology (Table 1), were referred to the DEP. Eight of the patients had clinical brucellosis; *B. abortus* had been isolated from 1 patient and *B. melitensis* had been isolated from a further five patients (Table 1). Two patients did not exhibit symptoms of brucellosis. Secondary serum samples were obtained from 3 of the 10 patients and 2 of these 3 patients (302022, 302085) had been infected with *B. melitensis*.

Agglutination test

A series of dilutions were made with phenol–saline to give titres from 10 to 320. *B. abortus* and *B. melitensis* O-antigens were supplied by the Division of Microbial Reagents and Quality Control (DMRQC), Central Public Health Laboratory, Colindale, London. Aliquots of antigen suspension appropriately diluted were added to each serum dilution, and incubated at 37 °C for 24 h. Known positive control sera were included in each test. After incubation the tests were examined for agglutination, and re-examined after a further 24 h.

Complement fixation test

The antigens employed were *B. abortus* O-antigen supplied by the Central Veterinary Laboratory, Weybridge; and *B. melitensis* O-antigen from DMRQC (as above). Patients' sera were inactivated at 56 °C for 30 min and diluted with veronal diluent in series giving titres of 2–128. One volume of diluted antigen was added to each serum dilution and allowed to react at 4 °C overnight in the presence of complement at minimal haemolytic dose. One volume of sensitized sheep erythrocytes, previously titrated with haemolytic antibody at optimum sensitizing concentration was then added to each serum–antigen mixture and incubated for 30 min at 37 °C. A complement control without serum or antigen, and a serum control without antigen were included in each batch of tests.

Table 1. *Serology and bacteriology of patients*

Patient no.	Age/sex	Clinical brucellosis	Bacteria isolated	Immunoblotting		Agglutination titre			CFT titre	IRMA (U/ml)	
				Ec O157*	Ye O9†	*B. abortus*	*B.melitensis*	*B. abortus*	*B. melitensis*	IgG	IgM
302080	77/F	Yes	*B. abortus*	+	+	1:80	1:80	1:64	1:64	31	3
302084	26/M	Yes	*B. melitensis*	+	+	< 1:20	1:40	1:256	1:256	59	3
302151	26/M	Yes	*B. melitensis*	+	−	< 1:20	< 1:20	1:32	1:32	35	3
302085	6/M	Yes	*B. melitensis*	+	+	< 1:20	1:80	1:64	1:64	57	27
302106	17/F	Yes	*B. melitensis*	+	−	1:40	1:160	1:128	1:64	78	8
302022	49/M	Yes	*B. melitensis*	+	−	1:160	1:80	1:64	> 1:256	12	0
302392	70/M	Yes	None	+	−	1:320	1:320	1:64	< 1:4	33	10
302284	35/F	Yes	None	+	+	1:320	1:320	1:16	1:4	12	2
302164	14/M	No	None	+	−	1:80	1:160	< 1:4	< 1:4	2	8
302465	27/M	No	None	+	+	1:160	1:160	< 1:4	< 1:4	2	5

CFT, Complement fixation test. IRMA, immunoradiometric assay: > 11 U/ml = positive test.
* *Escherichia coli* O157. † *Yersinia enterocolitica.*

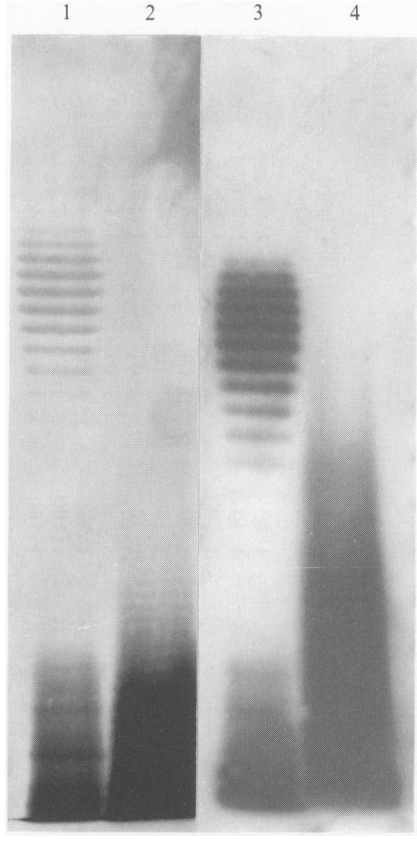

Fig. 1. The SDS–PAGE profile of LPS prepared from *E. coli* O157 contained long-chain LPS giving a typical 'ladder' pattern, the LPS profile of *Y. enterocolitica* O9 contained generally short-chain LPS (lane 2). All ten sera contained antibodies reacting with the LPS of *E. coli* O157 (lane 3) whilst only five sera contained antibodies reacting with the LPS of *Y. enterocolitica* O9 (lane 4). Ten μg of LPS was used per lane, 30 μl of antiserum was used per lane.

Following incubation, the test was left to stand for 2 h before reading. The dilution of serum which produced 50% haemolysis was taken as the end-point for the determination of antibody titre.

Immunoradiometric assay

This test was performed by the method described by Parratt and co-workers [15], and modified by Hewitt and Payne [16]. All sera were heated at 56 °C for 30 min prior to testing. The antigen used was *B. abortus* O-antigen obtained from the Central Veterinary Laboratory (as above). Briefly, 50 μl serum was added to 200 μl of antigen and incubated overnight at 37 °C. Preparations were centrifuged and pellets washed with phosphate-buffered saline containing 2·5 g/l of bovine serum albumin. The washed pellets were resuspended, and 100 μl of iodine-125-labelled anti-human globulin (anti-IgG or anti-IgM) were added. The mixture was incubated at 37 °C for 90 min, and thoroughly washed as described above. The radioactivity present in the washed pellet was measured using a gamma counter. The results were calculated using previously prepared standards.

Lipopolysaccharide

LPS for SDS–PAGE and immunoblotting was prepared from *E. coli* O157 strain E32511 by the method of Westphal and Jann [17] as described previously [2, 3]. LPS was prepared from *Y. enterocolitica* O9 strain E4610 by a modified method based on the hot-phenol extraction procedure of Westphal and Jann [17]. LPS preparations were examined for contaminating proteins by staining SDS–PAGE LPS profiles using a silver stain for proteins [19].

SDS–PAGE

SDS–PAGE of LPS was carried out as described [2], using a 4·5% stacking gel and a 12·5% separation gel. Electrophoresis was performed using a constant current of 50 mA for 3·25 h. Profiles were either stained with silver [20] or used for immunoblotting.

Immunoblotting

LPS profiles were transferred on to nitrocellulose sheets and reacted with human sera (30 μl/lane) as described previously [21]. Antibody–antigen complexes were detected using ^{125}iodinated immunoglobulin raised to human antibodies of classes: IgG (Miles Scientific Div., Miles Laboratories, Inc., Naperville, Ill.) and IgM (Sigma Chemical Co., St Louis, Mo.). Each lane was reacted with approximately 5 μg Ig, containing 10^6 cpm and antibody–antigen reactions detected by autoradiography.

Antibody absorptions

Bacteria grown on HSA were fixed in 3% (v/v) formol–saline, washed in PBS and mixed with sera at a ratio of 50 mg (wet-wt) bacteria with 100 μl serum (16 h, 4 °C). Bacteria were sedimented (5000 ***g***, 15 min, 4 °C) and the supernatants used for immunoblotting.

RESULTS

LPS and immunoblotting

SDS–PAGE profiles of *E. coli* O157 LPS (10 μg/lane), stained with a silver stain for carbohydrate, showed that strain E32511 produced predominantly long-chain LPS giving a typical 'ladder' pattern (Fig. 1, lane 1). In contrast, *Y. enterocolitica* strain E4610 produced LPS without long-chain LPS (Fig. 1, lane 2).

Replicate SDS–PAGE profiles of LPS (10 μg/lane) purified from *E. coli* O157 and *Y. enterocolitica* O9 were reacted with ten sera from patients with positive brucella serology. Five sera were found to contain antibodies, of the IgM class, reacting with the LPS of *E. coli* O157 (for example: Fig. 1, lane 3; Table 1) but not the LPS of *Y. enterocolitica* O9; whilst the remaining five sera contained antibodies which reacted with the LPS of both *E. coli* O157 (for example: Fig. 1, lane 3; Table 1) and *Y. enterocolitica* O9 (for example: Fig. 1, lane 4; Table 1).

Persistence of serum antibodies

Primary and secondary sera were obtained from three patients (302022, 302164, 302085). The initial serum samples from patients 302022 and 302164 contained antibodies to the LPS of both *E. coli* O157 and *Y. enterocolitica* O9, as detected by

Table 2. *Putative epitopes exposed on the O-antigens of* Escherichia coli *O157 and* Y. enterocolitica *O9 as detected using sera from patients with positive brucella serology*

Serum no.	Absorbing antigens	Reaction of antibodies remaining	Antigen(s) recognized by serum following absorption	Antigen(s) recognized by serum
302080	C* and E†	None	E	E (+C?)
	C and Y‡	O157 LPS	E	
302284	C and E	None	E	E (+C?)
	C and Y	O157 LPS	E	
302084	C and E	O9 LPS	Y	Y (+C?)
	C and Y	None	C and Y	
302085	C and E	None	C	C
	C and Y	None	C	
302465	(C?) and E	O9 LPS	Y	Y and E
	(C?) and Y	O157 LPS	E	

* 'C' putative epitope exposed on O-antigen of both *E. coli* O157 and *Y. enterocolitica* O9.
† 'E' putative epitope exposed on O-antigen of *E. coli* O157 only.
‡ 'Y' putative epitope exposed on O-antigen of *Y. enterocolitica* O9 only.

immunoblotting. Sera prepared after 6 and 12 days, respectively, gave identical antibody reactions. The initial serum from patient 302085 reacted with the LPS of *E. coli* O157 only, as detected by immunoblotting; a serum sample taken 54 days later also gave a clear reaction with this O-antigen only.

Antibody absorption studies

The five sera-containing antibodies to the LPS of both *E. coli* O157 and *Y. enterocolitica* O9 were absorbed with whole formalin-fixed *E. coli* O157 and *Y. enterocolitica* O9 prior to reaction with SDS–PAGE LPS profiles by immunoblotting. Absorption of sera 302080 and 302284 with *E. coli* O157 removed antibodies to both *E. coli* O157 and *Y. enterocolitica* O9; however, absorbing these sera with *Y. enterocolitica* O9 failed to remove antibodies which reacted with the LPS of *E. coli* O157. Conversely, absorption of serum 302084 with *Y. enterocolitica* O9 removed antibodies which reacted with the O-antigen of both organisms; whilst absorbing this serum with *E. coli* O157 left antibodies reacting with the LPS of *Y. enterocolitica* O9. In contrast, absorbing serum 302085 with *E. coli* O157 or *Y. enterocolitica* O9 removed antibodies reacting with the LPS of both organisms. Finally, absorbing serum 302465 with *E. coli* O157 failed to remove antibodies reacting with the LPS of *Y. enterocolitica* O9, whilst absorbing this serum with *Y. enterocolitica* O9 left antibodies which reacted with the LPS of *E. coli* O157.

DISCUSSION

Sera from patients with positive brucella serology were reacted with LPS purified from *E. coli* O157 and *Y. enterocolitica* O9, to investigate antibody–antigen cross-reactions between these two LPS types. All ten sera reacted with the LPS of *E. coli* O157, indicating that epitopes on the O-antigens of *B. abortus* and *B. melitensis* were also present on the LPS of *E. coli* O157. Certain biovars of *B. abortus* and *B. melitensis* share common epitopes [8], and at least some of these epitopes appear to be present on the LPS of *E. coli* O157. Five of the sera contained antibodies reacting with the LPS of *Y. enterocolitica* O9, whereas five sera did not, suggesting that infection with *B. abortus* or *B. melitensis* invariably resulted in the production of serum antibodies recognizing distinct epitopes on strains of *E. coli* O157, whilst only certain patients raised antibodies recognizing or capable of binding to epitopes on *Y. enterocolitica* O9. In a recent study [14] using sera from patients infected with *Y. enterocolitica* O9 and *E. coli* O157, we provided evidence to suggest that the LPS of *Y. enterocolitica* O9 probably contains at least two distinct antibody binding sites [14], only one of which was present on the O-antigen of *E. coli* O157. However, results of the absorption studies carried out in the present study suggest that antibody cross-reactions between strains of *E. coli* O157 and *Y. enterocolitica* O9 might involve at least three epitopes.

In an attempt to explain the basis of these antigenic cross-reactions we postulate the following hypothesis. With reference to Table 2, we suggest that one epitope, which we have termed 'E' (*E. coli*), is only exposed on the LPS of *E. coli* O157, and another epitope termed 'Y' (*Y. enterocolitica*) is only exposed on the LPS of *Y. enterocolitica* O9, whilst a third epitope termed 'C' (common) is exposed on the LPS of both organisms. Sera 302080 and 302284 contained antibodies to

epitope E, and possibly C, but not to Y. In contrast, serum 302084 contained antibodies to epitopes C and Y; whilst serum 302085 contained antibodies to epitope C only. Serum 302465 contained antibodies to epitopes Y, E, and possibly C.

The difference in the surface location of epitopes that we have described here might result as a consequence of the physical structure of the respective LPS types. The LPS of *E. coli* O157 migrates during SDS–PAGE to give a typical 'ladder' pattern containing predominantly long-chain LPS which closely resembles the LPS profile obtained with *B. abortus* [2]; however, the LPS of *Y. enterocolitica* O9 migrates in SDS–PAGE gels to give a profile quite distinct from *E. coli* O157. Therefore, although these O-antigens share common sugar sequences, the observed serological reactions might react to the physical configuration of the LPS molecule expressed by *Y. enterocolitica* O9. From this study we conclude that, although serological cross-reactions can occur between strains of *E. coli* O157, *Y. enterocolitica* O9 and certain strains of *Brucella* sp., the nature of these cross-reactions is inconsistent; furthermore, cross-reactions will not always occur as individual patients may raise quite variable antibody responses following brucella infection. Consequently, even though the O-antigens of *E. coli* O157, *Y. enterocolitica* O9, *B. abortus* and *B. melitensis* may share common sugar sequences, these similarities will not necessarily result in identical serological antibody cross-reactions.

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