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The serological relationship between *Yersinia enterocolitica* O9 and *Escherichia coli* O157 using sera from patients with yersiniosis and haemolytic uraemic syndrome

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SUMMARY

Sera from patients with yersiniosis, shown to contain antibodies to *Yersinia enterocolitica* O9; and sera from patients with haemolytic uraemic syndrome (HUS) caused by *Escherichia coli* O157, were used to investigate serological cross-reactions between *Y. enterocolitica* O9 and *E. coli* O157. Lipopolysaccharide (LPS) was isolated from strains of *Y. enterocolitica* O9 and *E. coli* O157 and reacted with sera by immunoblotting and ELISA. Sera from patients with HUS contained antibodies to the LPS of *E. coli* O157 only; 80% of sera from patients with yersiniosis contained antibodies to the LPS of *Y. enterocolitica* O9 and *E. coli* O157. This one-way cross-reaction was also detected using hyperimmune rabbit antisera.

INTRODUCTION

Yersinia enterocolitica and verocytotoxin-producing *Escherichia coli* have been shown to cause yersiniosis and haemolytic uraemic syndrome (HUS) respectively [1, 2]. Strains of *Y. enterocolitica* isolated from cases of yersiniosis in Europe generally belong to serogroups O3 or O9 [1], and most strains of *E. coli* isolated from cases of HUS belong to serotype O157.H7 [2, 3]. Methods used for the routine isolation of bacteria belonging to the Enterobacteriaceae from clinical specimens, have proved not to be optimal for the growth of *Y. enterocolitica* and consequently this organism can go undetected [4]. Also, since strains of *E. coli* O157 can only be isolated from patients' stools for a limited period following onset of disease [2], the causative organism of HUS may not be isolated. Infection with *Y. enterocolitica* results in serum antibodies to the O-antigen of this organism, and tests using patients' sera have been used to provide evidence of infection. Similarly, we have recently established serological tests for screening patients with HUS for serum antibodies to *E. coli* O157 lipopolysaccharide (LPS) [5–7] when a bacterial agent could not be isolated.

When serology alone is used to provide evidence of a possible cause of yersiniosis or HUS, results of these tests must be considered in the light of antibody–antigen cross-reactions. Chemical analyses of LPS have shown that certain bacteria share

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common sugar sequences, and strains of *Y. enterocolitica*, *E. coli* O157 and *Brucella abortus* have been shown to contain 4-amino-4,6-dideoxy-α-D-mannopyranosyl sugar units [8, 9]. This similarity in LPS composition has been used to explain antigenic cross-reactions between *Y. enterocolitica* O9 and *B. abortus*, and *E. coli* O157 and *B. abortus*, detected with hyperimmune rabbit sera [10–12]. Sera from patients with yersiniosis have been shown to contain antibodies which react with *B. abortus* [4] and, similarly, patients with brucellosis raise antibodies which cross-react with *E. coli* O157 [13]. Studies from this laboratory have shown that patients with HUS, caused by *E. coli* O157, raise antibodies that react with the LPS of *B. abortus* [5, 6].

In the present study we examined these cross-reactions using sera from patients with yersiniosis and from patients with HUS, with LPS purified from *Y. enterocolitica* O9 and *E. coli* O157.

MATERIALS AND METHODS

Bacteria and media

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

Sera

Ten sera, from patients with clinical yersiniosis involving a syndrome of diarrhoea, were referred to the Public Health Laboratory, Leicester for serology. Ten sera from patients with HUS caused by *E. coli* O157.H7 were also used.

Rabbit antisera

Bacteria were suspended in saline and incubated at 100 °C (2·5 h), sedimented (5000 g, 30 min) and suspended in 0·3% (v/v) formol-saline. Rabbits were immunized by injecting (i.v) 0·5 ml, 1·0 ml, 2·0 ml, 2·0 ml and 2·0 ml at 5-day intervals.

LPS

LPS for SDS–PAGE, immunoblotting and ELISA was prepared by the method of Westphal and Jahn [14] as described previously [5, 7]. LPS preparations were examined for contaminating proteins by staining SDS–PAGE LPS profiles using a silver stain for proteins [15]. LPS was also prepared from whole-cells using proteinase K digestion [16] as described previously [5]. The cell mass from 100 μg bacteria was digested with proteinase K (Sigma Chemical Co, St Louis, MO) and used for SDS–PAGE.

SDS–PAGE

SDS–PAGE of LPS was carried out as described [5, 7], using a 4·5% stacking gel and a 12·5% separation gel [17]. Electrophoresis was performed using a

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constant current of 50 mAmp for 3·25 h. Profiles were either stained with silver [15, 18] or used for immunoblotting.

Immunoblotting

LPS profiles were transferred onto nitrocellulose sheets and reacted with antisera (30 μl/lane) as described previously [5, 7, 19]. Antibody–antigen complexes were detected using ^{125}iodinated immunoglobulins 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 c.p.m. and antibody–antigen reactions detected by autoradiography.

ELISA

ELISAs were carried out as described previously [5, 7]. Plates were coated with 0·6 μg of LPS and reacted with sera diluted (×1000) in phosphate buffered saline (PBS) containing 0·5% Tween-20 (PBS-Tween). Antibody–antigen complexes were detected using an alkaline phosphatase conjugated goat-anti-human total Ig antiserum (Sigma Chemical Co, St Louis, MO) diluted to manufacturer's specification in PBS-Tween and used in association with the enzyme substrate, *p*-nitrophenol phosphate (1 mg/ml, Sigma) in diethanolamine buffer [5, 7]. The intensity of resultant colour was determined by measuring the absorbance at 405 nm.

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).

RESULTS

Preparation of LPS

LPS was prepared from *E. coli* O157 and *Y. enterocolitica* O9 using the method of Westphal and Jahn [14]. The SDS–PAGE profile of *E. coli* O157 LPS (10 μg per lane), stained with a silver stain for carbohydrate [18], 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); increasing the amount of LPS used for SDS–PAGE to 100 μg failed to demonstrate high molecular weight LPS.

To ensure that the LPS prepared for the present study was representative of the total cellular O-antigen, LPS purified by the Westphal and Jahn procedure was compared to whole-cell LPs profiles prepared by proteinase K digestion. Silver-stained whole-cell profiles were found to be indistinguishable from profiles of purified LPS (data not shown).

Immunoblotting

Replicate SDS–PAGE profiles of LPS (10 μg per lane) purified from *Y. enterocolitica* O9 and *E. coli* O157 were reacted with ten sera from patients with yersiniosis and ten from patients with HUS. The sera from patients with

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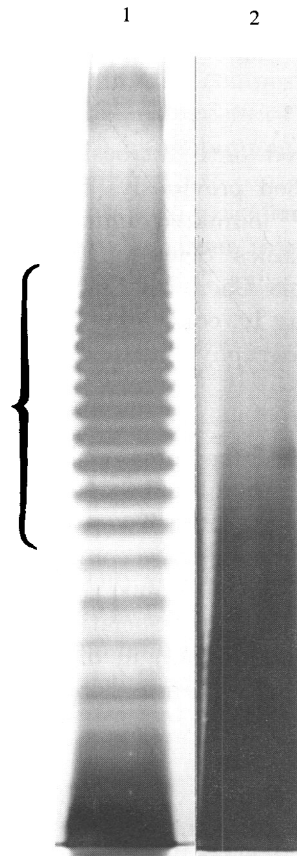

Fig. 1. SDS–PAGE profiles of LPS prepared from *E. coli* O157 E32511 (lane 1) and *Y. enterocolitica* O9 strain E4610 (lane 2) and stained with silver. Strain E32511 (lane 1) produced high-molecular-weight LPS (indicated by brackets), whereas strain E4610 produced only short)chain LPS (lane 2). 10 μg LPS was used per lane.

yersiniosis were found to contain antibodies, of the IgM class, reacting with the LPS of *Y. enterocolitica* O9 (Fig. 2, lane 1); however, eight of these ten sera also contained antibodies reacting with the LPS of *E. coli* O157 (Fig. 2, lane 2). In contrast, the ten sera from patients with HUS did not react with the LPS of *Y. enterocolitica* O9 (Fig. 2, lane 3), but only reacted with the homologous O-antigen (Fig. 2, lane 4).

The above reactions were also carried out using hyperimmune rabbit sera raised to *Y. enterocolitica* O9 and *E. coli* O157. The serum raised to *Y. enterocolitica* O9 reacted with both the homologous O-antigen and *E. coli* O157 LPS (data not shown). The antiserum raised to *E. coli* O157 reacted with O157 LPS only.

The sera from patients with yersiniosis were used to ensure that the LPS prepared by the method of Westphal and Jahn was representative of whole-cell LPS. Profiles of LPS prepared by this method and by proteinase K digestion were reacted with patients' sera. The resultant immunoblot reactions detected with whole-cell LPS were indistinguishable from those obtained using the hot-phenol method (data not shown).

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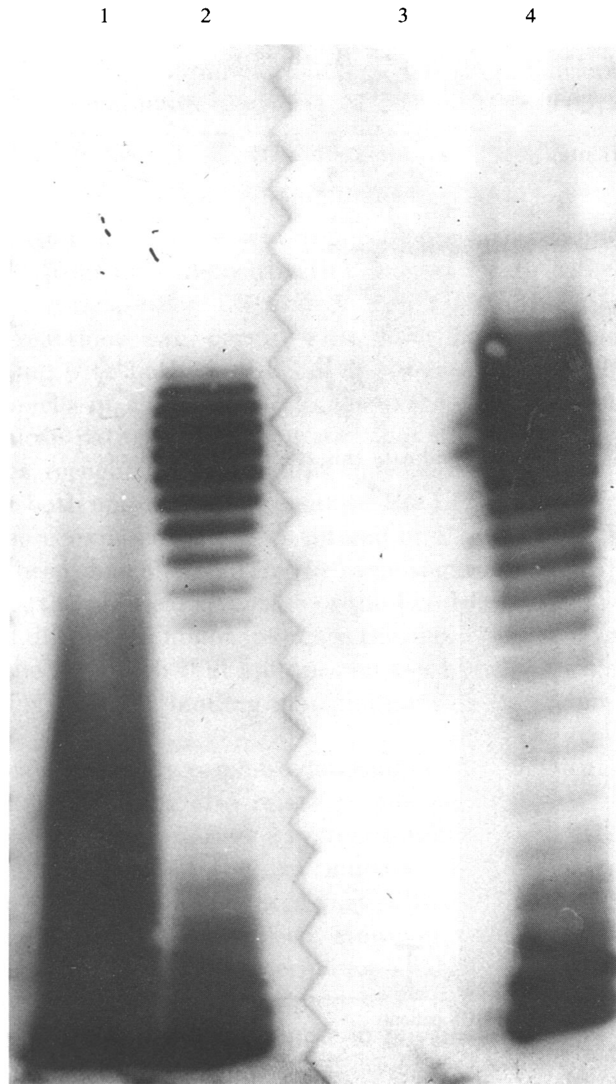

Fig. 2. Replicate profiles of LPS from *Y. enterocolitica* O9 and *E. coli* O157 were reacted with sera from patients with yersiniosis and HUS. Ten sera from patients with yersiniosis reacted with *Y. enterocolitica* O9 LPS (lane 1) and eight of these sera also reacted with the LPS of *E. coli* O157 (lane 2). In contrast, sera from patients with HUS did not react with the LPS of *Y. enterocolitica* O9 (lane 3) but only with the homologous LPS (lane 4). 10 μg of LPS was used per lane, 30 μl of antiserum was used per lane.

Antibody absorptions

Since sera from eight of the ten patients with yersiniosis contained antibodies reacting with the LPS of both *Y. enterocolitica* O9 and *E. coli* O157, sera were absorbed with *Y. enterocolitica* O9 and *E. coli* O157 and reacted with both LPS types by immunoblotting. Absorbing sera with formalin-fixed *Y. enterocolitica* O9 was found to remove antibodies reacting with both *Y. enterocolitica* LPS and *E. coli*

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Table 1. *Serological cross-reactions between* Y. enterocolitica *O9*, E. coli *O157 and* B. abortus

Sera from patients with	Antigen		
	Y. enterocolitica O9	*E. coli* O157	*B. abortus*
Yersiniosis	+*	+*	+[4]
HUS	−*	+*	+[5]
Brucellosis	+[4]	+[13]	+[4]
Rabbit antisera raised to			
Y. enterocolitica O9	+[10]	+*	+[11]
E. coli O157	−*	+*	nd
B. abortus	+[10]	+[18]	+[10]

Superscripts indicate reference; * indicate this study; nd, not done.

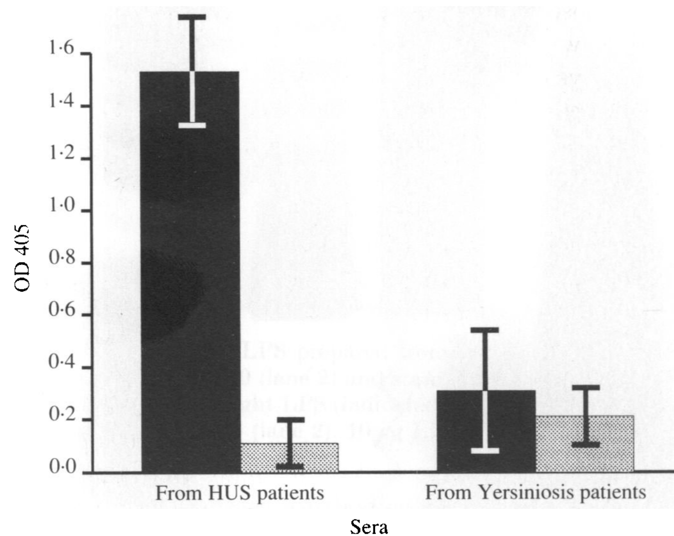

Fig. 3. Histogram showing the reaction of sera, from patients with HUS or yersiniosis, with LPS from E. coli O157 (■) and *Y. enterocolitica* O9 (▨) by ELISA. Sera from patients with HUS reacted predominantly with LPS from *E. coli* O157; in contrast, there was no significant difference in antibody reaction with the LPS of both *Y. enterocolitica* O9 and *E. coli* O157. Bars represent standard deviations from means.

O157 LPS; however, absorbing sera with *E. coli* O157 bacteria removed antibodies to *E. coli* O157 LPS but antibodies reacting with *Y. enterocolitica* LPS remained (data not shown).

ELISA

The qualitative serological results obtained by immunoblotting were quantified using an ELISA. Reacting the ten sera from patients with HUS with *E. coli* O157 LPS gave a mean ELISA value of 1·53 (±0·24) (Fig. 3). These same sera, when reacted with *Y. enterocolitica* O9 LPS, gave a mean value of 0·11 (±0·04) (Fig. 3)

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significantly lower ($P = 0.001$) than the value obtained with O157 LPS. In contrast, when sera from patients with yersiniosis were reacted with the LPS of *Y. enterocolitica* O9, the value obtained, 0·31 (±0·03) (Fig. 3) was not significantly different from the value obtained with O157 LPS, 0·21 (±0·05) (Fig. 3).

DISCUSSION

In the present study we reacted sera from patients with yersiniosis caused by *Y. enterocolitica* and from patients with HUS caused by *E. coli* O157, with LPS purified from *Y. enterocolitica* O9 and *E. coli* O157. Our data showed that antibody cross-reactions can occur, with 80% of sera from patients with yersiniosis reacting with both the LPS of *Y. enterocolitica* and *E. coli* O157. The ability of whole-cells of *E. coli* O157 to remove antibodies recognized as O157 LPS from yersiniosis patients' sera suggested that these sera contained two groups of IgM antibodies, recognizing two distinct epitopes on the LPS of *Y. enterocolitica*. We suggest that both epitopes are present on the LPS of *Y. enterocolitica* O9, but only one of these is exposed on the O-antigen of *E. coli* O157. The LPS of both organisms have been shown to contain the same sugar units [8, 9]; however, as we have shown here, the physical structure of the two LPS types are quite different. These structural differences might influence the accessibility of antibodies to bind to epitopes on the LPS such that epitopes on the LPS of *Y. enterocolitica* O9 are more available for antibody binding than the same epitopes on the LPS of *E. coli* O157.

The differences in antibody response detected between patients with yersiniosis and those with HUS might also relate to the pathogenesis of the respective diseases. Since yersiniosis comprises a systemic infection and HUS appears not to involve bacterial traversal of the gut mucosa, the two bacterial species are presented to the host immune system in very different ways which could result in variation in the class of antibody raised. However, since the same type of cross-reaction was also observed with hyperimmune rabbit sera, where antigens were administered by the same route, it would seem likely that the observed cross-reactions relate directly to the differences in physical structure and not antigen presentation.

From this study and the published literature, antibody–antigen cross-reactions were summarized. Patients with yersiniosis or brucellosis have been shown to raise antibodies which react with the LPS of *E. coli* O157 [this study, 13] in addition to the homologous O-antigen [this study, 4]. Also, patients with HUS caused by *E. coli* O157 raise antibodies which react with the O-antigen of *B. abortus* in addition to *E. coli* O157 [this study, 5]. However, only patients with yersiniosis and brucellosis raise antibodies to *Y. enterocolitica* O9 [this study, 4]. Using hyperimmune rabbit antiserum we were able to show that the antibody cross-reactions described above for human sera also occurred with rabbit sera. Such that antibodies raised to *Y. enterocolitica* O9 or *B. abortus* react with *E. coli* O157 [this study, 12], in addition to the homologous antigen; and antibodies raised to *E. coli* O157 react with *B. abortus* [5] but not *Y. enterocolitica* [this study]. However, antibodies in rabbits immunized with *Y. enterocolitica* and *B. abortus* only cross-reacted with *Y. enterocolitica* O9 and *B. abortus* [10]. Where serology alone

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provides evidence of infection these cross-reactions should be taken into consideration. We conclude from our study that serological testing of patients, with yersiniosis, for antibodies to *Y. enterocolitica* O9 would not be affected by the cross-reactions described. However, tests screening for serum antibodies to the LPS *E. coli* O157 should be interpreted in the light of this study.

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