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Serum antibodies to secreted proteins in patients infected with *Escherichia coli* O157 and other VTEC

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SUMMARY

Certain strains of verotoxigenic *Escherichia coli* (VTEC), and in particular those belonging to serogroup O157, cause attaching and effacing (AE) lesions of the host gut mucosa during pathogenesis. The mechanisms involved with bacterial attachment and the destruction of microvilli are determined by a cluster of genes within the LEE region, which also encode five secreted proteins. Sera from patients with antibodies to the lipopolysaccharide (LPS) of *E. coli* O157 and other VTEC were tested for antibodies to these secreted proteins. Twenty-one of 34 (62%) sera with antibodies to the lipopolysaccharide (LPS) of *E. coli* O157 also contained antibodies to one or more of the secreted proteins. Five of 12 sera containing antibodies to the LPS of a range of other VTEC serogroups also contained antibodies to 1 or more of the 5 secreted proteins, as did 16 of 70 (23%) sera from patients with haemolytic uraemic syndrome (HUS), haemorrhagic colitis (HC) or diarrhoea, but without bacteriological evidence of infection with VTEC and which did not contain antibodies to VTEC serogroups O5, O115, O145, O153 or O157. The detection of serum antibodies to secreted proteins may provide additional information for interpreting the results of established lipopolysaccharide-based VTEC serology.

INTRODUCTION

Strains of VTEC cause HUS and HC, with strains belonging to serotype O157:H7 most frequently associated with cases of HUS [1, 2]. However, strains belonging to serotypes O5:H–, O26:H11, O55:H7, O104:H2, O105:H18, O111:H–, O115:H10, O128:H2, O145:H25, O153:H25, O163:H19 and O165:H25 have also been identified [3]. Therefore, the isolation and characterization of VTEC from patients' stools identifies the cause of disease, whereas the detection of VT genes or free VT in faeces only indicates the possible involvement of VTEC [3]. VTEC in patients' stools can be detected using laboratory media; however, since the numbers of faecal VTEC decrease following onset of disease [4], more sensitive methods such as immunomagnetic separation and PCR may be required to detect these organisms [5–7].

Serological tests based on LPS have been developed to provide evidence of infection with *E. coli* O157 [8–11] and the major antibody-antigen cross-reactions with other enteric bacteria have been investigated [12–15]. Serological tests for VTEC other than those belonging to serogroup O157 have also been investigated [16, 17], and patients have been shown to have antibodies to the LPS of *E. coli* belonging to serogroups O5, O115, O145 and O153. Because of the range of LPS antigen-types exhibited by VTEC, an antibody test involving a single common VTEC antigen has been sought.

Certain strains of enteropathogenic *E. coli* (EPEC) have been shown to attach to epithelial cells and cause destruction of the microvilli and the underlying

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cytoskeleton, a process described as 'attaching and effacing' (AE) lesions [18]. The genes encoding the AE mechanism are located on the LEE (locus for enterocyte effacement) region of the *E. coli* chromosome, and this locus has been the focus of much attention [19–24]. The key genes in this locus are *eae*A which encodes the 97 kDa intimin protein [19], and *eae* B which encodes a secreted protein of 37 kDa which is involved in triggering host cell signal transduction and in the phosphorylation of tyrosine [21]. Within the LEE region are located also the *sep*A, B, C and D genes which encode the transport proteins necessary for the membrane translocation of proteins involved in the attaching and effacing process. The LEE locus also encodes proteins of 40, 39 and 25 kDa [22]. The 39 kDa protein has been identified as the enzyme glyceraldehyde-3-phosphate dehydrogenase but the functions of the remaining proteins are unknown though they are all actively secreted by a type III secretory system [23, 24]. LEE genes have been detected in strains of *E. coli* other than EPEC, including *E. coli* O157 and certain other VTEC serogroups [25–29].

In this study, sera from patients with antibodies to the LPS of *E. coli* O157 and other VTEC were screened for antibodies to these 5 proteins.

MATERIALS AND METHODS

Bacteria and bacterial culture

Enteropathogenic *E. coli* strain E20513 (O111:H2) carried the *eae*A gene and *E. coli* strains E45037 (O91:H21), E71341 (0102:H27) and E35990 (O143) did not. Non-*E. coli* control strains comprised *Yersinia enterocolitica* (E58475), *Salmonella munchen* (JT4), *Vibrio cholerae* (E119528), *Citrobacter freundii* (J1351) and *Shigella sonnei* (E119302). Bacteria were identified and serotyped by established methods [30], and stored on Dorset's egg agar slopes at room temperature. Bacteria were grown in 50 ml volumes of L-broth (Oxoid Ltd) with shaking (16 h, 37 °C, 120 r.p.m.).

Sera

One hundred and forty-one sera were tested. Sera from 34 patients were known to contain antibodies to the LPS of *E. coli* O157, as demonstrated by tests established in the LEP [10]. Twelve sera contained antibodies to LPS from *E. coli* O5 (8), O115 (2) O145 (1) and O153 (1) as described previously [16]. Sera from 70 patients with HUS, HC or diarrhoea, but without bacteriological evidence of infection with VTEC and which were known not to contain antibodies to the LPS of *E. coli* O5, O115, O145, O153 or O157, as described previously [17], were also tested. Twenty-five control sera were from healthy blood donors.

Eae A gene probe tests

Strains were tested by colony hybridization with an *eae*A probe which comprised a 1 kb *Kpn*I–*Sal*I fragment.

Preparation of secreted proteins

Bacteria were sedimented by centrifugation (5000 ***g***, 4 °C, 30 min) and supernatants millipore filtered (0·45 μm), and bacterial proteins concentrated by acetone precipitation.

SDS–PAGE and immunoblotting

SDS–PAGE was performed using the method of Laemmli [31] with an Atto mini-gel system (Genetic Research Instrumentation Ltd). Stacking gels were cast with a continuous sample trough as opposed to individual wells. One ml volumes of sterile culture supernatant were acetone precipitated and the resultant protein pellet suspended in 60 μl SDS–PAGE solubilization buffer prior to incubation at 100 °C (5 min) and loading onto gels comprising a 4·5% acrylamide stacking gel and a 12·5% acrylamide separation gel. Following electrophoresis, gels were either stained with Coomassie brilliant blue [32] or used for immunoblotting. For immunoblotting, protein profiles were transferred onto nitrocellulose sheets (0·05 A, 1·5 h) using the method of Towbin and colleagues [33]. Following transfer, immunoblots were cut into strips and each profile reacted with sera (10 μl), from patients with HUS, and known to contain antibodies to *E. coli* O157 or other VTEC. Antibody-antigen complexes were detected using a goat anti-human polyvalent antibody conjugated with alkaline phosphatase (Sigma Chemical Co. Ltd, UK.), and an enzyme substrate buffer comprising nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate [17].

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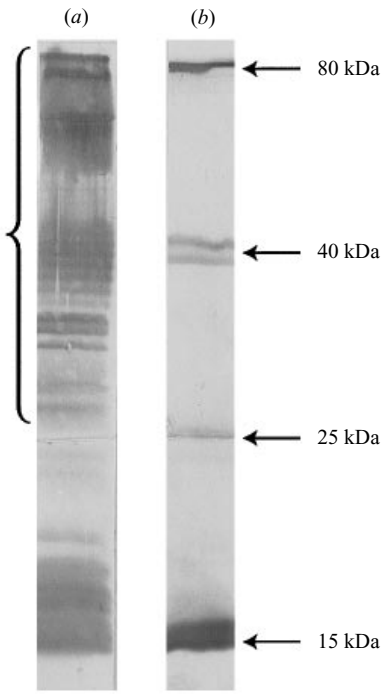

Fig. 1. An example of human antibodies binding to the lipopolysaccharide of *E. coli* O157 (lane A); lane B demonstrates human antibodies binding to secreted proteins of 80, 40, 39, 25 or 15 kDa (arrowed).

RESULTS

Expression of secreted proteins

SDS–PAGE profiles of culture supernatants from *E. coli* strain E20513 were reacted with human sera, known to contain antibodies to the LPS of *E. coli* O157 (Fig. 1, lane 1). Serum antibodies were found to bind to proteins of 15, 25, 39, 40 and 80 kDa (for example see Fig. 1, lane 2). *E. coli* strains E45037, E71342 and E5990, and strains of *Y. enterocolitica*, *S. munchen*, *V. cholerae*, *Citrobacter* spp. and *Shigella sonnei* did not express any of the five proteins made by *E. coli* strain E20513. The kinetics of expression of the five proteins were assessed by examining culture supernatants for these proteins at 2-h intervals from 6–24 h post-inoculation. All five proteins could be detected in culture supernatants throughout the growth cycle of *E. coli* strain E20513.

Serology

Twenty-one of 34 patients' sera known to have antibodies to the LPS of *E. coli* O157 also contained antibodies to 1 or more of the 5 proteins secreted by *E. coli* strain E20513. Twelve sera, shown to contain antibodies to the LPS from *E. coli* O5 (8), O115 (2) O145 (1) and O153 (1) [16], were also reacted with profiles of the secreted proteins. Three of eight sera with antibodies to the LPS of *E. coli* O5, produced antibodies to one or more of the five proteins. These antibodies were not detected in either of the two sera with antibodies to the LPS of *E. coli* O115. Sera from patients with antibodies to the LPS of *E. coli* O145 and O153, both contained antibodies to 1 or more of the 5 proteins.

Sixteen of 70 sera known not to contain antibodies to the LPS of *E. coli* O5, O115, O145, O153 or O157, also contained antibodies to one or more of the five proteins secreted by *E. coli* strain E20513. The 25 control sera did not contain antibodies to these proteins.

DISCUSSION

The five proteins secreted by *E. coli* strain E20513, as detected in the present study, were thought to correlate with the proteins of 110, 40, 39, 37 and 25 kDa as described by Kenny and Finlay [22]. The secreted proteins have been shown to be antigenic and produced during infection, since patients with HUS have been shown to produce serum antibodies to these proteins [24]. The reasons why only 62% of patients made antibodies to these proteins is unclear, and may include an inability of certain patients to mount an antibody response to these particular proteins or the possibility that they are not always expressed during pathogenesis.

Only 3 of 8 sera with antibodies to LPS of *E. coli* O5 contained antibodies to one or more of the five eae proteins. Strains of *E. coli* belonging to serogroup O5 have been shown to carry the *eae*A gene [29, 35]. Although antibodies were not detected in either of the sera with antibodies to the LPS of *E. coli* O115, whether strains of *E. coli* belonging to this serogroup carry the *LEE* genes remains to be determined. Sera from patients with antibodies to the LPS of *E. coli* O145 and O153 both contained antibodies to one or more of the five proteins. Strains of *E. coli* belonging to serogroup O145 have been shown to carry the *eae*A gene [29, 35], although these genes have been shown not to be carried by strains of *E. coli* belonging to serogroup O153 [29, 35].

The 25 control sera did not contain antibodies to any of the secreted proteins, suggesting that the 16 of 70 patients with suspected but unconfirmed VTEC infection were infected with bacteria expressing *LEE* genes.

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Culturing VT-producing strains of *E. coli* from patients provides evidence of infection with VTEC. In the absence of a culturable organism alternative tests must be considered to provide evidence of infection. Serological tests involving the detection of serum antibodies to the LPS of *E. coli* O157 provided evidence of infection in 11% of patients where only a serum sample had been submitted to the LEP [34].

We conclude that infection with *E. coli* O157 and other VTEC may result in the production of serum antibodies to these secreted proteins, indicating that they are expressed during infection. Their significance needs to be established. From our observations it would seem that detection of antibodies to the secreted proteins alone would not be a reliable indicator of infection but may provide additional information helpful in interpreting the results of established lipopolysaccharide-based VTEC serology.

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