SHORT ARTICLE

Cross-reaction between a strain of Vibrio mimicus and V. cholerae O139 Bengal

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Of 200 isolates of Vibrio mimicus screened, one from water (N-57) agglutinated with V. cholerae O139 polyclonal antiserum (absorbed with a rough strain of V. cholerae only) and not with O139 polyclonal diagnostic antiserum (absorbed with the rough strain and V. cholerae O22 and O155). The antigenic relationship between V. cholerae O139 and N-57 is of a,b-a,c type, where a is the common antigenic epitope and b and c are unique epitopes. Strain N-57 was assigned to a new serogroup of V. cholerae O194. It did not possess thectx gene or produce cholera toxin. Antiserum to strain N-57 cross-protected infant mice against cholera on challenge with V. cholerae O139. Structural studies of the surface polysaccharides and studies of the rfb genes will shed more light on the extent of relatedness between V. mimicus N-57 and V. cholerae O139.

Introduction

Since the outbreaks of cholera in 1992-1993, Vibrio cholerae O139 Bengal has become established as a causative agent of endemic cholera in the Indian subcontinent. Genetic studies indicated that V. cholerae O139 is probably a mutant of V. cholerae O1 El Tor, and that it might have arisen by substitution of rfb genes that encode the lipopolysaccharide (LPS) antigen in V. cholerae O1 by genes that encode the LPS antigen and the capsular antigen of V. cholerae O139 [1]. The organism that would potentially donate rfb genes for surface polysaccharide synthesis in V. cholerae O139 has not been identified. V. cholerae O139 also cross-reacts with a number of bacteria, including V. cholerae serogroups O22 and O155 [2], and Aeromonas trota [3]. In addition, as the core antigen is common to all V. cholerae serogroups, V. cholerae O139 also shares this antigen with other V. cholerae serogroups [2]. Therefore, for production of specific diagnostic polyclonal antiserum to V. cholerae O139, the antiserum must be absorbed with a rough strain of V. cholerae and with V. cholerae O22 and O155 [2]. A rapid test based on specific monoclonal antibodies (MAbs) to V. cholerae O139 has been developed that detects the bacterium directly from the samples [4]. The objectives of a search for bacteria that cross-react with V. cholerae O139 are three-fold: to identify the bacterium that would have donated the rfb genes for O139 antigen, to continually ensure the specificity of monoclonal and polyclonal sera to V. cholerae O139 for diagnosis, and to characterise cross-reacting bacteria for general microbiological interest. The continuing search for cross-reacting bacteria identified a water isolate of V. mimicus that agglutinated with V. cholerae O139 antiserum. This isolate was characterised with respect to its antigenic and virulence properties.

Materials and methods

Bacterial strains

One hundred V. mimicus strains isolated from diarrhoeal stools of patients treated at the Dhaka treatment facility of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDBR), during 1968–1993, and 100 strains of V. mimicus isolated from surface and well waters in Dhaka and Matlab, Bangladesh, during 1989–1993, were studied. V. mimicus was identified by standard methods [5].
Production of antiserum

Antisera were produced in adult New Zealand White rabbits against live cells of V. cholerae O139 (strain AI-1852), cross-reacting A. trota (strain 1354) [3] and cross-reacting V. mimicus (strain N-57). The preparation of antigen and the immunisation schedule have been described previously [3]. Antibody to core antigen was removed by absorption of antisera with a rough strain of V. cholerae (strain CA385) [3]. Antisera to V. cholerae O22 and O155 were those prepared previously at the National Institute of Health, Tokyo, Japan [2].

Slide agglutination test

Each of the 200 strains of V. mimicus was tested in a slide agglutination test with two antisera to V. cholerae O139: antiserum absorbed with the rough V. cholerae strain CA385, and diagnostic antiserum absorbed with strain CA385, V. cholerae O22 and V. cholerae O155 [2]. Strains that gave positive results were further tested for agglutination with antiserum to V. cholerae O22 and O155, and A. trota strain 1354, and with two mouse MAb of ICL11 and ICL12 specific for V. cholerae O139, as described previously [6].

Tube agglutination test

The tube agglutination test was performed as described previously. Doubling dilutions of antiserum were tested from a starting dilution of 1 in 20. Agglutination was scored on a 0 to 4+ scale, and the endpoint titre was defined as the highest dilution of antiserum yielding a 2+ agglutination of cells [3].

Agglutinin-absorption test

After incubation of antigen–antibody mixture, the supernate was filter-sterilised. Antisera were absorbed either with a single culture or multiple cultures as required [3].

Serogrouping

The cross-reacting V. mimicus strain was subjected to O serogrouping with antisera to the 155 serogroups of V. cholerae previously recognised [2] and to 38 other serogroups (serogroups O156–O193) recognised subsequently (unpublished data). V. cholerae and V. mimicus have a common serogrouping scheme.

Studies with LPS antigens

LPS was extracted by the hot phenol-water extraction method, separated on SDS-polyacrylamide 13.5% gels and visualised by silver staining. For immunoblotting, the separated LPS was probed with rabbit polyclonal antiserum to the relevant bacteria, and then with alkaline phosphatase-conjugated swine anti-rabbit IgG [3].

Bengal SMART test

The Bengal SMART test, which is a rapid test for V. cholerae O139, is a colorimetric immunoassay that uses the colloidal gold-labelled MAb ICL12 to V. cholerae O139 [4]. The ability of the SMART test to detect cross-reacting V. mimicus was tested according to the manufacturer's instructions.

PCR assay for V. cholerae O139 antigen

The cross-reacting V. mimicus was tested in a PCR assay with primers specific for the O-antigen of V. cholerae O139 as described previously [7].

Enterotoxin production

Culture filtrate was tested in mouse Y1 adrenal tumour cells for cholera toxin-like enterotoxin and in the suckling mouse assay for heat-stable enterotoxin [3]. The criterion for a positive test in the Y1 cell assay was rounding of >50% of the cell monolayer, and that for the suckling mouse assay was a ratio of >0.085 for intestinal body weight to remaining body weight from five mice per test [3].

The cross-reacting V. mimicus strain was also tested in a PCR assay for cholera toxin gene (ctx) with two primers corresponding to the ctx operon of V. cholerae O1 as described previously [8].

Passive protection in infant mouse cholera model

To study the protective efficacy of polyclonal antiserum to the cross-reacting V. mimicus, 0.1 ml of a 1 in 10 diluted antiserum in physiological saline was mixed with a 100 LD50 dose of a clinical isolate of V. cholerae O139 (strain AI-1852) and injected into a group of 4-day-old mice. Mortality was observed for up to 48 h [9].

Results and discussion

Of 200 V. mimicus strains tested, only one strain, isolated from pond water, agglutinated with V. cholerae O139 polyclonal antiserum (absorbed only with V. cholerae CA385) in a slide agglutination test. However, this strain did not agglutinate with the V. cholerae O139 diagnostic antiserum (absorbed with CA385, and V. cholerae O22 and O155). It also agglutinated with antisera to V. cholerae O22 and cross-reacting A. trota (strain 1354), but not with antiserum to V. cholerae O155. The strain was designated N-57.

The results of agglutination and agglutinin-absorption tests are shown in Table 1. Cross-absorption of antiserum to V. cholerae O139 and V. mimicus with heterologous bacteria of V. mimicus and V. cholerae O139 caused reductions in antibody titres to the homologous bacteria. Such reductions in homologous titres occurred when antiserum to V. cholerae O22 and A. trota were
Table 1. Agglutination and agglutinin-absorption tests with antisera to *V. mimicus* (N-57), *V. cholerae* O139 (AI-1852), *V. cholerae* O22, *V. cholerae* O155 and *A. trota* (1354)

<table>
<thead>
<tr>
<th>Antiserum*</th>
<th>Absorbing organism†</th>
<th>Titre of antisera to O antigens from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>V. mimicus</em> (N-57)</td>
<td><em>V. cholerae</em> O139 (AI-1852)</td>
</tr>
<tr>
<td><em>V. mimicus</em> (N-57)</td>
<td>None</td>
<td>2560</td>
</tr>
<tr>
<td><em>V. cholerae</em> O139</td>
<td>640</td>
<td>–</td>
</tr>
<tr>
<td><em>V. cholerae</em> O22</td>
<td>1280</td>
<td>40</td>
</tr>
<tr>
<td><em>A. trota</em></td>
<td>640</td>
<td>40</td>
</tr>
<tr>
<td><em>V. cholerae</em> O139 (AI-1852)</td>
<td>None</td>
<td>320</td>
</tr>
<tr>
<td><em>V. mimicus</em></td>
<td>–</td>
<td>640</td>
</tr>
<tr>
<td><em>V. cholerae</em> O22</td>
<td>None</td>
<td>160</td>
</tr>
<tr>
<td><em>V. mimicus</em></td>
<td>–</td>
<td>640</td>
</tr>
<tr>
<td><em>V. cholerae</em> O155</td>
<td>None</td>
<td>80</td>
</tr>
<tr>
<td><em>V. mimicus</em></td>
<td>–</td>
<td>80</td>
</tr>
<tr>
<td><em>A. trota</em> (1354)</td>
<td>None</td>
<td>320</td>
</tr>
<tr>
<td><em>V. mimicus</em></td>
<td>–</td>
<td>320</td>
</tr>
</tbody>
</table>

*Antibody to core antigen in these sera was absorbed with a rough strain of *V. cholerae* (CA385).
†Absorbed with live and boiled organisms; boiled (100°C for 1 h) cultures were used.
‡No agglutination at a dilution of 1 in >20.

Absorbed with *V. mimicus*. These data suggested that the antigenic relationship between *V. mimicus* N-57 and *V. cholerae* O139, *V. cholerae* O22 and *A. trota* is that of a,b-a,c type where a is the common epitope and b and c are unique epitopes. This is because absorption of an antiserum with a heterologous strain failed to remove antibodies to the homologous strain completely. This type of antigenic relationship has been shown previously between *V. cholerae* O139 and other cross-reacting bacteria such as *V. cholerae* O22 and O155 and *A. trota* [2, 3]. Consistent with the slide agglutination test, *V. mimicus* did not agglutinate with *V. cholerae* O155 antiserum. *V. cholerae* O155 also did not agglutinate with *V. mimicus* antiserum. Different patterns of reactions were observed among *V. cholerae* O139, O22 and O155 and *A. trota* when antisera to these organisms were absorbed with *V. mimicus*.

The SDS-PAGE separation profile of LPS from *V. mimicus* N-57 and *V. cholerae* O139 AI-1852 is shown in Fig. 1. The profile of *V. mimicus* resembled that of smooth bacteria revealing a core antigen (thick band at the bottom of the gel) and an O-antigenic repeating polysaccharide (ladder-like portion in the top part of the gel). However, the LPS pattern of *V. cholerae* O139 resembled that of semi-rough bacteria as reported earlier [1] revealing a modified core structure and no high mol. wt O antigen-specific side chain. Immunoblot studies with antisera to *V. cholerae* O139 and *V. mimicus* recognised homologous and heterologous LPS antigens equally well. In Fig. 2, the immunoblots are shown with antiserum to *V. mimicus* only. Compared with the LPS pattern in Fig. 1, the core antigens in the bottom portion of the gel in Fig. 2 are not stained because the core antibody has been absorbed out. These studies further confirmed the antigenic relationship between *V. cholerae* O139 and *V. mimicus* N-57.

The LD50 of the challenge strain of *V. cholerae* O139, AI-1852, for infant mice was 10^2 cfu in 0.1 ml of inoculum. A 1 in 10 dilution of polyclonal antiserum to isolate N-57 completely protected mice against a challenge dose of 100 LD50 of strain AI-1852. However, all the mice died when a 1 in 10 dilution of pre-immune rabbit serum was used. This suggested
that the shared antigenic epitope(s) between \textit{V. cholerae} O139 and \textit{V. mimicus} N-57 is protective.

Some strains of \textit{V. mimicus} produce a cholera toxin-like enterotoxin and a heat-stable enterotoxin [10]. However, strain N-57 did not produce these toxins, and also gave a negative result for ctx in a PCR assay. This indicated that this strain may not produce watery diarrhoea.

Strain N-57 did not agglutinate with \textit{V. cholerae} O139-specific MAbs (ICL11 and ICL12) and also gave a negative result in the Bengal SMART test, which uses ICL12. It also gave a negative result in the PCR assay specific for \textit{V. cholerae} O139 as it failed to generate an amplicon of 416 bp [7]. These data suggest that such cross-reacting \textit{V. mimicus} strains will not be misdiagnosed as \textit{V. cholerae} O139. The cross-reacting \textit{V. mimicus} strain, N-57, could not be serogrouped with antisera to the 193 \textit{O} serogroups of \textit{V. cholerae}. Therefore, a new serogroup, O194, was assigned to this strain.

Structural analysis of surface polysaccharide of \textit{V. cholerae} O139 suggested that it contains two unique constituents, galactose cyclophosphate and colitose [11]. Some cross-reacting bacteria share either or both of these structures [12, 13]. A previous study found that the cross-reacting between \textit{V. mimicus} strain N-1990 and \textit{V. cholerae} O139 is due to sharing of the galactosyl residue substituted with a cyclic phosphate by both bacteria [14]. The structural basis of cross-reaction as well as the homology of the rfb genes encoding the surface polysaccharides in both \textit{V. cholerae} O139 and strain N-57 will need to be determined to further explore the relatedness between these two bacteria.

Worldwide efforts are being made to contain the threat of 'emerging and re-emerging pathogens'. \textit{V. cholerae} O139 is a newly emerged pathogen, and is considered to be the causative agent of the 'eighth' pandemic of cholera. The potential of cross-reacting bacteria for use as vaccine strains against O139 cholera should also be explored.

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References

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Fig. 2. Immunoblot analysis of LPS from \textit{V. cholerae} O139 strain A1-1852 (A) and \textit{V. mimicus} N-57 (B) separated on an SDS-polyacrylamide 13.5% gel by electrophoresis, blotted on to a nitrocellulose membrane and probed with antiserum to \textit{V. mimicus} N-57.
