Background

- Vibrio cholerae O1 infection causes severe, acute secretory diarrhea. Natural infection protects against subsequent disease, and immunity may be generated through an anamnestic B-cell response in the gut-associated lymphoid tissue.
- The vibriobial antibody is a surrogate marker indicating protection from V. cholerae; however, no known threshold level of antibody gives complete protection [1]. Serum anti-toxin toxin subunit B (CTB) IgA antibody levels also confer protective immunity, but levels wane rapidly after infection [2].
- Patients with cholera also develop memory B-cell responses to toxin co-regulating subunit A (Tox) and lipopolysaccharide (LPS), detectable for at least one year after infection [3].
- Animal studies indicate that mucosal immune responses to cholera protein antigens are T-cell dependent and mediated by CD4+ T-helper cells. In addition, our group has observed a rapid Th-2 response to Tox and a cholera cell membrane preparation (MP) following cholera infection [4-7].
- B-cell memory responses following cholera waned for the T-cell independent antigen LPS, suggesting that memory B-cell responses may be mediated in a T-cell dependent manner [3].

Objective: We describe both B- and T-cell memory responses after natural V. cholerae O1 induced severe diarrhea in order to investigate the function of T-cell memory in cholera, including a possible B-cell response.

Methods

Antigenic Stimulation

VCC: V. cholerae cytotoxin/colicin from N185 (Dr. KK Banerjee). While VCC's role in cholera infection is unknown, it can assemble into pore-forming, hemolytic, and lipophilic activities leading to immunoglobulin expression and activation in murine B-1a cells. VCC may cause diarrheal disease in infection with non-O1 non-O139 V. cholerae lacking CT. The IgG1 encoding the VCC protein is widespread in strains of V. cholerae, suggesting a potential role in environmental pathogenesis [8-13]. We used moneric VCC at a concentration of 2.5 ng/mL. MP: Cholera cell membrane preparation from organisms grown in AKI media. Concentration: 10 ug/mL. TopA: Concentration: 10 ng/mL. Vibrio cholerae O1 LPS: Inaba or Ogawa serotype matched to the case. Concentration: 2.5 ug/mL. Positive controls Purified Protein Derivative (PPD) and Phthymagemmulin. Concentrations: 5 ug/mL. 10 ug/mL. Samples with media only were also included.

Study Design

Informed consent was obtained from 16 patients with culture-confirmed V. cholerae infection and severe, acute watery diarrhea. Immune responses were compared to those seen in healthy controls from similar socio-economic backgrounds.

FASCA (Flow-cytometric Assay of Specific Cell-mediated Immune response in Activated whole Blood)

- On days 2, 7, and 30 after case presentation, 50uL of peripheral whole blood was diluted eight times with DMEM media, and stimulated with different antigens. After six day in vitro culture at 37°C, supernatant was preserved for cytokine analysis and cells were stained with anti-CD4+, CD8+, CD69 and CD54 monoclonal antibodies.
- We performed red cell lysis with ammonium chloride, red cell removal, washing and suspension in parafomaldehyde for flow cytometric analysis.
- Patient serum was also assayed for VCC, TopA, and LPS specific IgA and IgG, and for vibriobial antibody responses [15].
- Peripheral blood mononuclear cells of 10 different cholera patients were separated by Ficoll technique on day 2 and day 7 after case presentation. After 48 hour in vitro culture in RPMI medium without stimulation, culture supernatant was assayed for anti-VCC antibody [16].

Table 1: Demographic, serologic and clinical characteristics

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Male n=16</th>
<th>Female n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>34 years</td>
<td>(range 13-50)</td>
<td></td>
</tr>
<tr>
<td>Serological subtype</td>
<td>Ogawa n=11</td>
<td>Inaba n=5</td>
<td></td>
</tr>
<tr>
<td>Duration of hospitalization</td>
<td>21 hours</td>
<td>(4-12)</td>
<td></td>
</tr>
<tr>
<td>Duration of diarrhea</td>
<td>31 hours</td>
<td>(9-14)</td>
<td></td>
</tr>
</tbody>
</table>

Results

**Figure 1** Memory-Effecter CD4+ and CD45R0+ T-cell response to cholera antigens and controls

**Figure 2** Memory-Effecter CD4+ and CD45R0+ T-cell response to cholera antigens and controls

**Figure 3** VCC antibody response in lymphocyte supernatant

Summary and Conclusions

- On day 7 after infection, the T-cell memory-effector responses to VCC and MP peaked, and decreased by day 30. Proliferation in response to TopA increased by day 7 and remained elevated until day 30.
- VCC-specific IgA responses in plasma peaked on day 7 after infection, and VCC-specific IgG peaked on day 30. LPS- and TopA-specific IgA and IgG responses peaked on day 7 and TopA responses remained elevated until day 30.
- VCC stimulation generated a significant B-cell antibody response and more lymphoblast proliferation than observed in response to other V. cholerae antigens. The cytolytic activity of VCC may be a compensatory epitherial destruction that allows other cholera antigens to penetrate the mucosa and promote the inflammatory response observed in cholera infection.

- Our results demonstrate that patients with cholera develop a memory-effector T-cell response to cholera antigens by day 7 following infection, in addition to a memory B-cell response. B-cell responses occur during and after T-cell population expansion, suggesting that T cells may play an important role in the activation, development, and maintenance of the B-cell response.

References


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