T cell responses to cholera infection

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Background

•*Vibrio cholerae* O1 infection causes severe, acute secretory diarrhea. Natural infection gives protection against subsequent disease, and immunity may be generated through an anamnestic B-cell response in the gut-associated lymphoid tissue.

•The vibriocidal antibody is a surrogate marker indicating protection from *V. cholerae;* however, no known threshold level of antibody gives complete protection [1]. Serum anti-cholera 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-regulated pilus subunit A (TcpA) 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 TcpA and a cholera 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 role in B-cell responses.

Methods

Antigenic Stimulation

VCC: *V. cholerae* cytolysin/hemolysin from NICED (Dr. KK Banerjee). While VCC's role in cholera infection is unknown, it can assemble into pore-forming heptameric oligomers and causes 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 *hly* gene encoding the VCC protein is widespread in strains of *V. cholerae,* suggesting a potential role in environmental survival and pathogenesis [8-13]. We used monomeric VCC at a concentration of 2.5 ng/mL.

MP: Cholera membrane preparation from organisms grown in AKI media. Concentration: 10 ug/mL.

TcpA: Concentration: 5 ug/mL.

Vibrio cholerae O1 LPS: Inaba or Ogawa serotype matched to the case. Concentration: 2.5 ug/mL.

Positive controls Purified Protein Derivative and Phytohemagglutinin. Concentrations: 5 ug/mL and 1 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.

FASCIA (Flow-cytometric Assay of Specific Cell-mediated Immune response in Activated whole blood) [14]

•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-CD3, -CD4, -CD8 and -CD45R0 monoclonal antibodies.

•We performed red cell lysis with ammonium chloride, red cell removal, cell washing and suspension in paraformaldehyde for flow cytometric analysis.

• Patient serum was also assayed for VCC, TcpA, and LPS specific IgA and IgG, and for vibriocidal 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].

Results

Table 1 Demographic, serologic and clinical characteristics

Patients	n=16
Sex	Male n=8 Female n=8
Median age	34 years (range 13-50)
Serologic subtype	Ogawa n=11 Inaba n=5
Clinical Characteristics	
IV fluids required (SD)	8.6 Liters (+/-1.6)
Duration of diarrhea	21 hours (+/-3.7)
Duration of hospitalization	31 hours (+/- 3.3)
Healthy Controls (HC)	n=10
Sex	Male n=5 Female n=5
Median age	33 years (range 18-45)

Figure 1 Memory-Effector CD4+/CD45R0+ T cell response to cholera antigens and controls

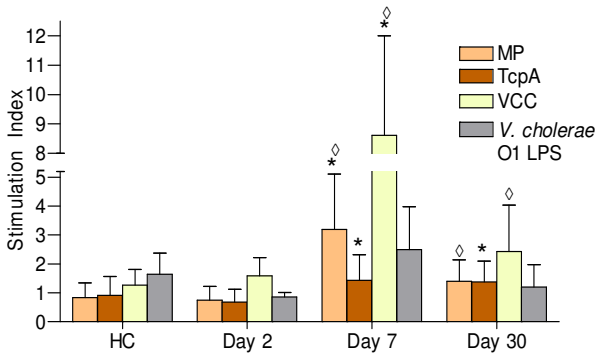

Figure 1: Stimulation Index: Lymphoblast count with antigen stimulation divided by blast count without stimulation (SI = 1 indicates stimulation is equal in samples with and without stimulation). Geometric means with 95% confidence intervals are shown.

* p=<0.05 between day 7 or 30 and day 2, using Wilcoxon matched pairs testing.

◊ p=<0.05 between day 7 or 30 and HC by Mann-Whitney U unpaired testing.

The memory-effector CD4/CD45R0+ population after stimulation is 80% CD45RA-/CCR7-, consistent with an effector phenotype derived from memory T cells.

LPS and TcpA specific antibody responses are similar to our previously reported results [3].

Figure 2: All day 7 and day 30 values are p=<0.05 for comparisons with day 2 except memory-effector cells on day 30 using matched pairs testing.
All day 7 and day 30 values are p=<0.05 for comparisons with HC except anti-VCC IgG levels on day 30 using unmatched pairs testing.

Figure 2 Memory-Effector CD4+/CD45R0+ T cell responses and serum antibody response to VCC

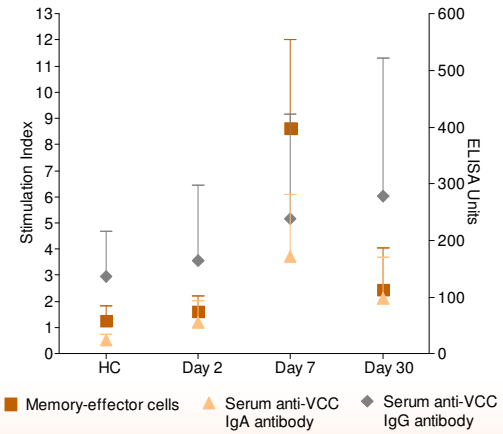

Figure 3 VCC antibody response in lymphocyte supernatant

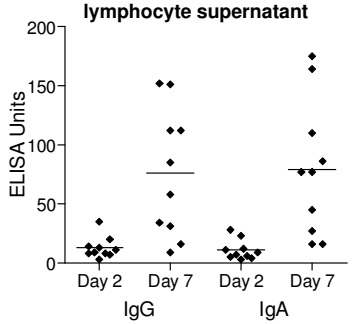

Figure 3: Comparisons between day 2 and day 7 p<0.01 for IgA and p=0.01 for IgG, using matched pairs testing.

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 TcpA increased by day 7 and remained elevated until day 30.

•VCC-specific IgA responses in plasma peaked on day 7 of infection, while VCC-specific IgG responses peaked on day 30. LPS- and TcpA-specific IgA and IgG responses peaked on day 7 and TcpA 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 generate epithelial 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.

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