Local Production of Anti–*Vibrio cholerae* Mucosal Antibody in Reproductive Tract Tissues after Cholera

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To investigate whether intestinal presentation of an antigen by *Vibrio cholerae*, a noninvasive organism, could induce an anatomically distant mucosal immune response in reproductive tract tissues, the endocervical immune responses of women in Bangladesh were evaluated after cholera. Endocervical secretions were analyzed for secretory IgA (sIgA) antibody against the B subunit of cholera toxin (CtxB) in 9 women with cholera and 8 women with diarrhea caused by neither *V. cholerae* nor heat labile enterotoxin–producing *Escherichia coli*. Women infected with *V. cholerae* developed significant sIgA anti-CtxB responses in endocervical samples (*P* ≤ .02). Antibody subtype analysis of endocervical IgA was consistent with local mucosal production (*P* ≤ .001). Women with cholera did not develop sIgA anti-CtxB responses in serum. The ability to generate specific mucosal immune responses in reproductive tract tissues after intestinal presentation of antigen could facilitate development of vaccines effective against reproductive tract pathogens.

The ability to induce specific mucosal immune responses in reproductive tract tissues may facilitate development of vaccines protective against reproductive tract pathogens. Parenteral immunization rarely results in mucosal immune responses, and direct immunization of reproductive tract or rectal tissues does not have wide acceptability and results in variable immune responses [1–3]. Immunization of oral or nasal mucosal surfaces may result in specific mucosal immune responses in reproductive tract tissues through activation of the common mucosal immune system [2–4].

In this system, lymphocytes activated at one mucosal surface migrate to another mucosal surface. Immune responses in reproductive tract tissues after oral immunization with purified antigens and/or killed organisms have varied [3–6]. Oral administration of live, attenuated vaccine vectors expressing antigens of reproductive tract pathogens may result in more prominent immune responses in reproductive tract tissues [2].

Human infection with *Vibrio cholerae*, a noninvasive organism, can lead to cholera, resulting in a large volume of secretory diarrhea [7]. To further evaluate whether presentation of antigen by a living organism at the intestinal surface could induce an anatomically distant mucosal immune response in reproductive tract tissues, we evaluated mucosal immune responses to the B subunit of cholera toxin (CtxB) in endocervical secretions of women in Bangladesh after cholera.
Patients and Methods

Study population and study design. The Clinical Research and Service Centre (DHAKA Hospital) of the ICDDR, B: Centre for Health and Population Research, Dhaka, Bangladesh, provides care to >100,000 individuals with diarrhea each year, including 10,000–20,000 patients with cholera [7]. Fifteen women with cholera caused by *V. cholerae* O1 El Tor Ogawa and 15 control women presenting to the Centre with diarrhea not caused by *V. cholerae* or heat labile toxin-producing enterotoxigenic *Escherichia coli* were enrolled in this study. Other than having diarrhea, study participants were healthy, nonpregnant women 18–40 years of age who denied pelvic symptoms. Serum samples were obtained at enrollment and at follow-up visits on days 9 and 23. A pelvic examination was performed on day 9 or 23, depending on which date was less likely to be associated with expected menses.

Stool microbiology. Stool samples were analyzed for the presence of *V. cholerae* by dark-field microscopy and standard microbiological culture; serogrouping and serotyping were done, using specific antisera [7]. Stool samples of control women were analyzed by gene probe for the presence of heat labile toxin–producing enterotoxigenic *E. coli* [8].

Sampling of reproductive tract secretions. Women were excluded from analysis if they were pregnant, if examination by microscopy of vaginal fluid disclosed erythrocytes, yeast, trichomonas, or clue cells indicative of bacterial vaginosis, or if endocervical samples disclosed evidence of *Neisseria gonorrhoeae* by culture or evidence of chlamydial (IDEIA Chlamydia Kit; Dako Diagnostics). For immunologic analysis, endocervical secretions were collected by using 2 antibody-absorbent wicks (2 × 25 mm; Polyfiltronics Group) placed in the cervical os for 1 min while a speculum was in place [2, 9]. Endocervical secretions were recovered and processed, as described elsewhere [2, 9].

Measurement of vibriocidal and anti-CtxB IgG and IgA antibody responses in serum. Using a *V. cholerae* O1 El Tor Ogawa isolate from a study participant as the test strain, we measured serum vibriocidal antibody titers in a standard microassay [10]. Anti-CtxB IgG and IgA antibody responses in serum samples were measured in duplicate samples of 1:200 dilutions of serum samples in PBS–0.05% Tween 20 (PBS-T; Zymed Laboratories) added to wells previously coated with 100 ng of type III ganglioside (Sigma) and 100 ng of CtxB (List Biological). Peroxidase activity was determined by use of a 1:10,000 dilution of mouse monoclonal anti–human IgG biotin and a 1:4000 dilution of streptavidin–horseradish peroxidase conjugate. Anti-CtxB IgG responses were measured similarly in 1:200 dilutions in PBS-T of serum collected on days 0, 9, and 23.

IgA subtype analysis. IgA1 and IgA2 subtype concentrations were measured in serial dilutions of serum and endocervical secretion samples in PBS-T by use of plates previously coated with monoclonal mouse anti–human IgA1 antibody (clone A1-18, Sigma) or monoclonal mouse anti–human IgA2 antibody (clone A9604D2, Southern Biotechnology). Peroxidase activity was determined by use of a 1:2000 dilution of goat anti–human IgG antibody in PBS-T, followed by a 1:2000 dilution of rabbit anti–goat IgG–horseradish peroxidase conjugate (Southern Biotechnology). Samples were compared with standard curves generated by use of purified human IgA1 (catalog no. 400109, Calbiochem) or purified human IgA2 (catalog no. 400110, Calbiochem).

Results

Study enrollment. Three study participants were lost to follow-up. After pelvic examination, 9 study participants with cholera and 8 control subjects met all inclusion/exclusion criteria. For analysis, pelvic samples were obtained from 6 women with cholera and 3 control subjects on day 9 and from 3 women with cholera and 5 control subjects on day 23. Participants who were excluded from the study because of pelvic pathology or evidence of pelvic infection received treatment and follow-up appropriate to their conditions.

Serum responses. Vibriocidal antibody responses in patients with cholera were significantly increased over those of control group patients on day 9 (*P* < .001) and day 23 (*P* < .001) (figure 1). The most prominent serum vibriocidal responses were measured in individuals with cholera on day 9 (*P* < .05, comparing day 9 and day 23 responses). Serum IgG and IgA anti-CtxB responses in patients with cholera were significantly elevated over those of control group patients on day 9 (IgG, *P* < .001; IgA, *P* < .01) and day 23 (IgG, *P* < .001; IgA, *P* < .02). The most prominent serum anti-CtxB responses were also seen on day 9. Of note, even in day 0 samples, there was a small but statistically significant increase of serum IgA anti-CtxB responses in women with cholera, compared with control women (*P* < .01), most likely reflecting an early anamnestic response after infection. Serum sIgA anti-CtxB responses were not detected in samples from women with cholera or from control women on days 0, 9, or 23.
Figure 1. Vibriocidal and anti-cholera toxin B subunit (anti-CtxB) IgG and anti-CtxB IgA responses in serum samples obtained on days 0, 9, and 23. Columns represent the geometric mean titer (GMT) of the reciprocal end dilution (vibriocidal responses) or the geometric mean mOD/min measurement (anti-CtxB responses). Error bars represent the SEM. P values are shown for comparisons of responses in control group patients and patients with cholera.

Analysis of endocervical secretions. Anti-CtxB sIgA responses in endocervical secretions of women with cholera were elevated over those of control group women in samples collected on day 9 (P ≤ .02; figure 2). Even when the sample with the most prominent anti-CtxB sIgA response (262.9 mOD/min) was excluded from analysis, the anti-CtxB sIgA endocervical response in women with cholera was still significantly elevated over that measured in control women (P ≤ .05). There was no significant difference between anti-CtxB sIgA responses in endocervical secretions collected on day 23 from women with cholera and from control women.

IgA subtype analysis. The percentage of IgA2 in total IgA in cervical samples (28.3%) was significantly increased, compared with that in serum samples (5.5%; P ≤ .001).

Discussion

We detected a prominent specific anti–V. cholerae CtxB sIgA response in endocervical secretions of women after cholera. Although assays were different, kinetic measurements of anti-CtxB sIgA responses in endocervical secretions were ~10-fold higher than kinetic measurements of anti-CtxB total IgA responses in serum, demonstrating the magnitude of the immune response. This endocervical response could theoretically represent uterine washout of menses or a transudate of serum IgA; however, we were unable to measure a corresponding anti-CtxB sIgA response in serum, and IgA subtype analysis suggested local, endocervical production of anti-V. cholerae antibody. In the endocervix, IgA2 accounts for ~30%–60% of total IgA, while in serum, IgA2 usually accounts for <5%–7% of total IgA [11]. In our study, 28.3% of the IgA present in endocervical samples was IgA2, while 5.5% of total IgA in serum collected on the same day was IgA2. In addition, the antibody used to detect the anti-CtxB sIgA response in endocervical secretions was a monoclonal antibody directed against human secretory component, a glycoprotein synthesized principally by mucosal epithelial cells.

Figure 2. Secretory IgA (sIgA) anti-cholera toxin B subunit (anti-CtxB) responses in endocervical secretions obtained on day 9. Lines represent the geometric mean mOD/min measurement. The P value is shown for comparison of responses in control individuals and individuals with cholera. Note that if the sample with the most prominent anti-CtxB sIgA endocervical response (262.9 mOD/min) is excluded from analysis, the difference of the mean responses between groups retains statistical significance (P ≤ .05).
The endocervical response could also theoretically be secondary to direct antigenic stimulation of reproductive tract tissues contaminated by *V. cholerae* organisms in diarrheal stools. However, none of the study participants were unconscious or in extremis from cholera, lessening the likelihood of large-volume intravaginal contamination with diarrheal stools. In addition, previous researchers have detected specific antibody-secreting cell responses in uterine tissues of mice and rats after oral immunization with CtxB or cholera holotoxin [5, 6], and specific anti-CtxB and anti-*V. cholerae* lipopolysaccharide responses have been detected in saliva and breast milk samples of human volunteers after intestinal presentation of *V. cholerae* or CtxB [12, 13]. These findings suggest that the endocervical response detected in individuals with cholera in this study may be due to activation of the common mucosal immune system after intestinal presentation of antigen [1].

Other researchers have attempted to use the common mucosal immune system to generate specific mucosal immune responses in reproductive tract tissues after oral immunization with various antigens [1–6, 9, 14]. In many of these studies, sIgA responses were not specifically measured, and immune responses were variable. In general, oral immunization with purified antigens or killed organisms has resulted in poor immune responses in reproductive tract tissues [3, 5, 6]. Intranasal, rectal, and combination oral-local reproductive tract immunization strategies have more reliably induced specific immune responses in reproductive tract tissues, although many of these strategies would have poor acceptance by vaccine recipients [1–6, 9, 14]. Whether ongoing production of antigen by living organisms in the intestine more reliably induces mucosal immune responses in reproductive tract tissues than oral administration of nonviable antigens was not previously clear [2].

Wild-type *V. cholerae* also express cholera toxin, a potent immunoadjuvant, and the ability of cholera toxin to affect the magnitude of immune responses at anatomically distant mucosal surfaces is unclear at present. Notably, derivatives of cholera toxin have been described that retain immunoadjuvanticity and lack enterotoxicity, and such immunoadjuvants can be coexpressed by oral, attenuated *V. cholerae* vaccine vectors to safely augment immune responses [10].

In our study, serum and mucosal anti-*V. cholerae* responses peaked on day 9, and a small but detectable difference in anti-*V. cholerae* CtxB serum IgA response between control group participants and individuals with cholera was evident even at the time of acute diarrhea. These findings strongly suggest that women enrolled in our study were previously exposed to *V. cholerae* and that immune responses detected in this study were anamnestic in nature [7, 12, 13, 15]. Whether repetitive intestinal presentation of antigen is required to induce and maintain immunologic responses in reproductive tract tissues is unclear.

In summary, the results of our study suggest that intestinal presentation of antigen by living organisms may induce specific, locally produced, mucosal humoral immune responses in reproductive tract tissues. Whether such presentation of antigen may also induce nonhumoral immune responses in reproductive tract tissues is unclear [14]. If additional evaluations confirm the results of this study, immunization strategies based on these findings may assist in the development of more effective vaccines against reproductive tract infections.

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**References**

