Dose-Dependent Circulating Immunoglobulin A Antibody-Secrating Cell and Serum Antibody Responses in Swedish Volunteers to an Oral Inactivated Enterotoxigenic Escherichia coli Vaccine

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Enterotoxigenic Escherichia coli (ETEC) is the most common cause of diarrhea among children in developing countries and among international travelers to less-developed areas (4). Because of the high morbidity and mortality attributable to ETEC infections, development of vaccines against ETEC is given a high priority. An effective ETEC vaccine should be given orally and ideally should contain an appropriate toxoid in combination with ETEC bacteria expressing the most important colonization factor antigens (CFAs). Significant immunoglobulin A (IgA) antibody-secreting cell (ASC) responses against CTB and the various CFA components were seen in a majority of volunteers after two doses of ETEC vaccine independent of the vaccine lot given. The IgA ASC responses against CTB were significantly higher after the second than after the first immunization, whereas the CFA-specific IgA ASC responses were almost comparable after the first and second doses of ETEC vaccine. Two immunizations with one-third of a full dose of CFA-ETEC bacteria induced lower frequencies of IgA ASC responses against all the different CFAs than two full vaccine doses, i.e., 63 versus 80% for CFA/I, 56 versus 70% for CS1, 31 versus 65% for CS2, and 56 versus 75% for CS4. The proportion of vaccinees responding with rises in the titer of serum IgA antibody against the various CFA antigens was also lower after immunization with the reduced dose of CFA-ETEC bacteria. These findings suggest that measurements of circulating IgA ASCs can be used not only for qualitative but also for quantitative assessments of the immunogenicity of individual fimbrial antigens in various preparations of ETEC vaccine.

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TABLE 1. Comparison of vaccine-specific IgA ASC responses to CFAs and CTB in the peripheral blood of 20 Swedish volunteers after one and two oral immunizations with the ETEC vaccine (lot 003)

| Antigen | After vaccine dose 1 | After vaccine dose 2 | Cumulative frequency (%) |
|---------|---------------------|---------------------|--------------------------|
|         | Frequency (%) | Fold increase<sup>b</sup> | Postimmune ASC levels<sup>c</sup> | Frequency (%) | Fold increase | Postimmune ASC levels | |
| CFA/I   | 89 | 79.4 | 70 | 80 | 28.8 | 35 | 95 |
| CS1     | 84 | 11.2 | 43 | 70 | 8.7 | 49 | 95 |
| CS2     | 74 | 14.8 | 25 | 65 | 10.5 | 28 | 90 |
| CS4     | 78 | 12.0 | 31 | 75 | 10.0 | 43 | 85 |
| CTB     | 68 | 17.0 | 58 | 95 | 209 | 724 | 95 |

<sup>a</sup> Percentage of volunteers with ≥ twofold or higher levels of antigen-specific IgA ASCs per 10<sup>7</sup> MNCs on day 7 after each vaccine dose (lot 003) than before immunization.

<sup>b</sup> Geometric mean increase in numbers of specific IgA ASCs per 10<sup>7</sup> MNCs for the whole group of volunteers.

<sup>c</sup> Median numbers of specific IgA ASCs per 10<sup>7</sup> MNCs for all subjects after each vaccine dose.

Study design. Sixty-seven adult Swedish volunteers (32 women), ages 18 to 46, gave informed consent to participate in the studies, which were approved by the Human Research Ethics Committee at the Medical Faculty, Göteborg University. None of the participants had been traveling to areas where ETEC is endemic for 6 months prior to the study.

In the first study, 20 volunteers received two oral immunizations 2 weeks apart with a full dose of ETEC vaccine lot 003, and another 16 volunteers were given two doses of the same vaccine containing one-third of a full dose of CFA-ETEC bacteria and 1 mg of CTB. Heparinized venous blood (30 ml) for ASC analyses and serum specimens were collected on the day of the first immunization (day 0) and then 7 days after the second vaccine dose. From volunteers receiving the full dose of ETEC vaccine, venous blood samples were also obtained 7 days after the first immunization.

In a second study (performed one year after the first study), the immunogenicity of two doses of a different preparation of ETEC vaccine, lot 005, given 2 weeks apart was investigated in a double-blind, placebo-controlled fashion. Thirty-one subjects were randomly allocated to one of two groups in a 3:2 (vaccine-placebo) ratio. Venous blood samples for ASC analyses were collected 7 days after the first and second immunizations, whereas serum specimens were collected on the day of the first immunization (day 0) and then 7 days after each vaccination.

Detection of circulating ASCs. Mononuclear cells (MNCs) from heparinized venous blood were isolated by standard gradient centrifugation on Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden). The numbers of IgA-secreting cells and antigen-specific IgA ASCs were measured by a micromodification of the ELSA technique (20). Briefly, individual wells of nitrocellulose-bottomed plates were coated with purified CFA/I (20 μg ml<sup>-1</sup>), CS1 (10 μg ml<sup>-1</sup>), CS2 (10 μg ml<sup>-1</sup>), CS4 (10 μg ml<sup>-1</sup>), or GM1 ganglioside (5 μg ml<sup>-1</sup>). The GM1-coated wells were further incubated with purified recombinant CTB (2.5 μg ml<sup>-1</sup>). After blocking with Iscove’s complete medium, the wells were incubated with 5 × 10<sup>4</sup> to 1 × 10<sup>6</sup> peripheral blood cells. Specific ASCs were demonstrated by the addition of affinity-purified goat anti-human IgA antibodies conjugated with horseradish peroxidase (Southern Biotechnology Associates, Birmingham, Ala.) followed by enzyme-chromogen substrate. Spots were enumerated under low magnification (×40), and the number of ASCs was calculated as the mean for four wells. Vaccine-specific IgA ASCs were expressed per 10<sup>7</sup> MNCs to allow comparison with results in other studies (9, 10, 16, 18).

Vaccines that developed a twofold or greater increase in vaccine-specific IgA ASCs between pre- and postvaccination specimens were regarded as responders on the condition that the number of ASCs exceeded 10 per 10<sup>7</sup> MNCs in the postvaccination specimens (10). Since preimmune specimens were not collected in the second study, these volunteers were considered responders if their postvaccination levels of specific IgA ASCs equaled or exceeded by 2 standard deviations the geometric mean of specific IgA ASCs of placebo recipients examined on the same occasion. Thus, postvaccination values of ≥ 10 IgA ASCs per 10<sup>7</sup> MNCs for CFA/I, >15 IgA ASCs per 10<sup>7</sup> MNCs for CS1, CS2, and CS4, and >30 IgA ASCs per 10<sup>7</sup> MNCs for CTB were considered responders.

Serum antibody determinations. Antibodies of the IgA isotype to CTB were measured by the GM1-enzyme-linked immunosorbent assay (ELISA) technique as previously described (1, 8, 20). Antibody responses of the IgA class to CFAs of the vaccine strains in serum were studied by enzyme-linked immunosorbent assay methods (10). In short, individual microtiter wells were coated with 1 μg of purified CFA/I, CS1, CS2, or CS4/ ml, respectively. After blocking was done with 0.1% bovine serum albumin, the wells were incubated with threefold serial dilutions of serum samples at room temperature for 90 min. Bound antibodies were demonstrated by addition of human IgA conjugated with horseradish peroxidase (Jackson ImmunoResearch Laboratories, Westgrove, Pa.) and incubated at room temperature for 90 min followed by addition of orthophenylene-diamine (OPD)-H<sub>2</sub>O<sub>2</sub>. Titors of antibody were assigned as the interpolated dilutions of the samples giving an absorbance value at 450 nm of 0.4 above background when the wells were allowed to react for 20 min with OPD-H<sub>2</sub>O<sub>2</sub>. Pre- and postimmunization specimens from the same subject were always tested side by side. The titer of antibody ascribed to each sample represents the mean of duplicate determinations. A twofold or greater increase in the endpoint titer between pre- and postimmunization specimens was used to signify seroconversion (1, 8).

Statistical analysis. The frequency and magnitude of ASC responses and antibody seroconversion to various immunization schedules and doses of CFA antigens were compared for statistical significance using Fisher’s exact test and Student’s t test, respectively. All statistical tests were interpreted in a two-tailed fashion.

RESULTS

Comparison of responses after one and two doses. immune responses to different vaccine antigens after one and two oral immunizations with ETEC vaccine, lot 003, were determined for 20 volunteers. Prior to immunization, the numbers of CFA- and CTB-specific IgA ASCs were low or negligible, i.e., a mean 1 to 4 ASCs per 10<sup>7</sup> MNCs for each of the various antigens. Significant IgA ASC responses to CFA/I, CS1, CS2, CS4, and CTB were found in 85 to 95% of the vaccinees after either the first or the second dose (Table 1). The frequencies and the magnitudes of the different CFA ASC responses were comparable after the first and the second immunizations, except for the magnitude of the CFA/I response, which was slightly higher after the first dose. There was also a trend of higher median numbers of CFA/I-specific IgA ASCs after the first than after the second vaccine dose, whereas no such difference in the postvaccination levels of IgA ASCs against CS1, CS2, and CS4 was noted (Table 1). The magnitude of the CTB-specific IgA ASC response was significantly (P < 0.001) higher after the second than after the first vaccination (Table 1).

The proportion of volunteers responding to the first dose of vaccine with rises in the titer of specific IgA antibody in serum was further increased by a second dose, i.e., the seroconversion rate was 50% after the first dose versus 65% after the second dose for CFA/I, 0 versus 20% for CS1, 15 versus 40% for CS2, 20 versus 40% for CS4, and 35 versus 95% for CTB. The magnitudes of the different CFA antibody titer increases among responders were similar after the first and second im-
munizations; i.e., the titer increases were, respectively, 2.3-fold and 3.9-fold for CFA/I, not calculable and 3.2-fold for CS1, 4.5-fold and 4.2-fold for CS2, and 3.0-fold and 3.0-fold for CS4. The increase in the titer of IgA antitoxin, on the other hand, was significantly \( P < 0.001 \) higher after the second (50.1-fold) than after the first (1.6-fold) vaccine dose.

**Comparison of responses to various amounts of ETEC bacteria.** Vaccine-specific IgA ASCs and IgA antibody responses in serum were also monitored in 16 volunteers after two oral doses with ETEC vaccine lot 003 containing one-third of a full dose of CFA-ETEC bacteria and 1 mg of rCTB. The immune responses were compared with those found in the above-mentioned group of 20 volunteers given two immunizations with a full dose of ETEC vaccine lot 003. The number of vaccinees responding with circulating IgA ASCs against the different CFAs, i.e., CFA/I, CS1, CS2, and CS4, was lower after immunization with one-third versus a full dose of ETEC bacteria (Fig. 1). The increase in the numbers of IgA ASCs per 10⁷ MNCs was, as a mean, 14.5-fold after the reduced dose versus 28.8-fold after the full dose of ETEC bacteria for CFA/I, 5.8-fold versus 8.7-fold for CS1, 5.9-fold versus 10.5-fold for CS2, and 4.6-fold versus 10.0-fold for CS4. Also, the median postvaccination levels of IgA ASCs were considerably lower in the group of volunteers given the reduced dose of CFA-ETEC bacteria for all tested CFA antigens except CFA/I (Fig. 1); the differences were statistically significant \( P < 0.05 \) for CS1 and CS2.

The proportion of vaccinees responding with rises in the titer of IgA antibody against CFA/I, CS1, CS2, and CS4 in serum was also lower for volunteers receiving the reduced versus the full dose of CFA-ETEC bacteria, whereas the magnitudes of the increases in the titer of antibody were comparable for the two immunization groups (Table 2).

**Comparison of CFA responses in vaccine and placebo recipients.** The immunogenicity of a different preparation of ETEC vaccine, lot 005, was assessed in a placebo-controlled study by measuring circulating IgA ASCs and the IgA antibody responses in serum for 31 volunteers after one and two doses. Based on results from the dose-finding study of ETEC vaccine lot 003, the content of CFA/I was decreased and that of CS2 was increased in lot 005. Most of the vaccinees (63 to 95%) exhibited significant IgA ASC responses to CFA/I, CS1, CS2, and CS4 7 days after either the first or the second immunization with ETEC vaccine, compared with 8% or less for the placebo recipients \( P < 0.05 \) for each vaccine-placebo comparison (Table 3). In analogy with our previous finding, the responses of circulating IgA ASCs to the different CFA antigens were comparable or slightly stronger after the first than after the second immunization (data not shown). The frequencies and the median postvaccination levels of the CFA- and CTB-specific IgA ASC responses did not differ significantly for the two vaccine lots, even though lot 005 contained half as much CFA/I and approximately three times more CS2 than lot 003.

In serum, a twofold or greater increase in the titer of IgA antibody against CFA/I, CS1, CS2, and CS4 was found in, respectively, 84, 21, 53, and 63% of the vaccinees after either the first or second vaccine dose, compared with 8% or less for the placebo recipients (Table 3). The seroconversion rate was always higher after the second than after the first immunization for each of the various CFA antigens, whereas the magnitudes of the increases in the titer of antibody among responders were comparable (data not shown). Both the frequencies and the magnitudes of the serological responses were similar after immunization with ETEC vaccine lots 003 and 005.

**DISCUSSION**

In this study we show for the first time that CFA responses after oral immunization with ETEC vaccine are clearly dose dependent. We also show that the CFA-specific IgA ASC responses in peripheral blood are almost comparable after the first and second immunizations in nonprimed individuals.
These findings contrast with the much higher CFA ASC responses found after one than after two doses of ETEC vaccine for persons who have been primed by previous exposure to ETEC antigens (16, 18). The present study of various preparations of an oral ETEC vaccine confirms and extends the findings from our previous trials of the oral inactivated ETEC vaccine in adult Swedish volunteers (1, 10, 23). Significant IgA ASC responses against CTB and the various CFA components of the vaccine were seen in a majority of volunteers after immunization with two different lots, 003 and 005, of an oral ETEC vaccine containing various amounts of the same CFA-expressing bacterial strains together with recombinant CTB. The mucosal immune responses did not differ significantly for the two vaccine preparations, even though lot 005 contained half the amount of CFA/I and three times more CS2-expressing bacteria than lot 003. However, our small dose-finding study of the ETEC vaccine showed a clear trend toward lower frequencies and magnitudes of circulating IgA ASC responses against all the different CFAs after administration of a vaccine preparation containing one-third of the original dose of CFA-EETC bacteria. Also, the proportion of volunteers responding with rises in the titer of IgA antibody in serum against CFA/I, CS1, CS2, and CS4 was lower after immunization with one-third than with a full dose of ETEC bacteria. The impaired immunogenicity was most pronounced for CS1 and CS2, i.e., the fimbrial antigens of lowest content in the vaccine preparation. Our findings indicate that there is a relation between the mucosal responsiveness and the content of individual CFA and CS components of the ETEC vaccine, but only up to a certain level, above which administration of more fimbrial antigen did not result in any further increase in the immune response. Thus, measurements of circulating IgA ASCs seem to be of value not only for qualitative but also for quantitative assessments of the immunogenicity of individual fimbrial antigens in various preparations of the ETEC vaccine.

An important aspect of our study was to determine when the peak immune responses after ETEC vaccination appear in volunteers who have not been previously exposed to ETEC infection. According to several studies, IgA ASC responses in blood peak after primary immunization with oral live vaccines against cholera, typhoid fever, and Shigella flexneri infection (12, 13, 14). This was also the case when the oral inactivated ETEC vaccine was given to Egyptian and Bangladeshi adults endemically exposed to ETEC infection (16, 18). In all cases the CFA-specific IgA ASC responses were greater (18) or considerably greater (16) after the first than after the second vaccine dose. In contrast to these reports, we found that the frequencies and the magnitudes of circulating IgA ASC responses against the various CFA antigens in adult Swedish volunteers were comparable (or almost comparable for CFA/I) after the first and second immunizations with ETEC vaccine (both lots). In contrast, the CTB-specific IgA ASC responses and the increases in the titer of IgA antitoxin in serum were significantly higher after the second than after the first vaccination. This pattern in antitoxin responses was consistent with those in previous trials of the oral ETEC vaccine and with results from studies with the oral inactivated B subunit-whole cell cholera vaccine in nonprimed individuals (9, 23).

When evaluating the immunogenicity of enteric vaccines, it is important to determine whether responses to the vaccines of persons living in an area where the disease is not endemic will be similar to responses of residents in areas where it is endemic. Hitherto, three randomized, double-blinded, placebo-controlled trials of the oral ETEC vaccine have been per-

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**TABLE 2. IgA antibody responses in serum before and after two oral immunizations with different doses of the whole-cell component of the ETEC vaccine (lot 003)**

| Antigen | One-third dose | | | | Full dose | | |
|---------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|         | Frequency of responders | Preimmune mean titer | Postimmune mean titer | Fold increase | Frequency of responders | Preimmune mean titer | Postimmune mean titer | Fold increase |
| CFA/I   | 7/16 | 45 | 95 | 4.1 | 13/20 | 62 | 166 | 3.9 |
| CS1     | 1/16 | 30 | 38 | 2.9 | 4/20 | 54 | 74 | 3.2 |
| CS2     | 2/16 | 59 | 91 | 6.2 | 8/20 | 81 | 174 | 4.2 |
| CS4     | 5/16 | 29 | 49 | 2.9 | 7/20 | 49 | 93 | 3.0 |

- Responders were defined as having a twofold or greater increase in the titer of IgA antibody between pre- and postvaccination specimens. The number of responders/number of volunteers tested is shown.
- Geometric mean titer of IgA antibody for all subjects before immunization.
- Increase in the geometric mean titer for responding volunteers in relation to the preimmune titer.

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**TABLE 3. CFA-specific responses of IgA ASCs in blood and IgA antibodies in serum of healthy Swedish volunteers after either one or two oral doses of the ETEC vaccine (lot 005) or E. coli K-12 placebo**

| Antigen | No. of responders (postimmune level) | | |
|---------|-------------------------------------|----------------|----------------|
|         | Vaccine | Placebo | Vaccine | Placebo |
| CFA/I   | 14/19(142) | 0/12(0) | 16/19(141) | 0/12(49) |
| CS1     | 15/19(50) | 1/12(5) | 4/19(61) | 1/12(33) |
| CS2     | 12/19(35) | 0/12(0) | 10/19(146) | 0/12(56) |
| CS4     | 18/19(85) | 1/12(0) | 12/19(74) | 0/12(30) |

- Responders were defined as having a postvaccination value of >10 IgA ASCs per 10^7 MNCs for CFA/I and >15 IgA ASCs per 10^7 MNCs for CS1, CS2, and CS4. The number of responders/number of volunteers is shown. The median number of maximal vaccine-specific IgA ASCs per 10^7 MNCs for all subjects after either one or two immunizations is shown within parentheses.
- Number of volunteers exhibiting significantly (i.e., at least twofold) higher titers of IgA antibody in serum after either one or two vaccine doses than before immunization. The geometric mean maximal titer of IgA antibody for the whole group of volunteers after either one or two immunizations is shown within parentheses.
formed with Egyptian adults (18) and children ages 2 to 12 years (19) living in areas where ETEC infections are highly endemic. The present study was designed to assess the immunogenicity of the oral ETEC vaccine, containing the same CFA-expressing bacteria as the vaccine lot used in the Egyptian studies, in persons who had not been primed by previous natural exposure to ETEC antigens. The cumulative proportion of volunteers responding with circulating IgA ASCs to either a first or a second dose of ETEC vaccine was found to be somewhat lower in the Swedish than in the Egyptian studies for all vaccine-related CFA antigens except Cs1. The observed differences in the various studies might be explained by the fact that most Egyptian subjects probably had already experienced infection with ETEC before vaccination, and their immune responses to ETEC vaccine may, in part, have represented anamnestic responses. Whether immunologically naive infants in developing countries (the main target group for ETEC vaccine) will respond to the oral ETEC vaccine in a manner similar to that of Swedish adults previously unexposed to ETEC infection still remains to be investigated.

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REFERENCES

1. Åhrén, C., Wenneřás, J., Holmgren, and A.-M. Svennerholm. 1993. Intestinal antibody response after oral immunization with a prototype cholera B subunit-colonization factor antigen enterotoxigenic Escherichia coli vaccine. Vaccine 11:929–934.
2. Åhrén, C., J. Jertborn, and A.-M. Svennerholm. 1998. Intestinal immune responses to an inactivated oral enterotoxigenic Escherichia coli vaccine and associated immunoglobulin A responses in blood. Infect. Immun. 66:3311–3316.
3. Åhrén, C. M., and A.-M. Svennerholm. 1982. Synergistic protective effect of antibodies against enterotoxigenic Escherichia coli enterotoxin and colonizing factor antigens. Infect. Immun. 38:74–79.
4. Black, R. E. 1993. The epidemiology of diarrheal disease: implications for control by vaccines. Vaccine 11:100–106.
5. Clemens, J. D., D. A. Sack, J. R. Harris, J. Chakraborty, P. K. Neogy, B. Stanton, N. Huda, M. U. Khan, B. A. Kay, M. R. Khan, M. Ansaruzzaman, M. Yunus, M. R. Rao, A.-M. Svennerholm, and J. Holmgren. 1988. Cross-protection by B subunit-whole cell cholera vaccine against diarrhea associated with heat-labile toxin-producing enterotoxigenic Escherichia coli: results of a large-scale field trial. J. Infect. Dis. 158:372–377.
6. Czerkinsky, C., L.-Å. Nilsson, H. Nygren, Ö. Ouchterlony, and A. A. Tarkowski. 1983. Solid-phase enzyme-linked immunosorbent (ELISPOT) assay for enumeration of specific antibody-secreting cells. J. Immunol. Methods 65:109–121.
7. Czerkinsky, C., A.-M. Svennerholm, and J. Holmgren. 1993. Induction and assessment of immunity at enteromucosal surfaces in humans: implications for vaccine development. Clin. Infect. Dis. 16(Suppl. 2):106–116.
8. Jertborn, M., A.-M. Svennerholm, and J. Holmgren. 1986. Saliva, breast milk and serum antibody responses as indirect measures of intestinal immunity after oral cholera vaccination or natural disease. J. Clin. Microbiol. 24:203–209.
9. Jertborn, M., A.-M. Svennerholm, and J. Holmgren. 1994. Immunological memory after immunization with oral cholera B subunit-whole-cell vaccine in Swedish volunteers. Vaccine 12:1079–1082.
10. Jertborn, M., C. Åhrén, J. Holmgren, and A.-M. Svennerholm. 1998. Safety and immunogenicity of an oral inactivated enterotoxigenic Escherichia coli vaccine. Vaccine 16:255–260.
11. Kantele, A., J. M. Kantele, H. Arvilommi, and P. H. Mäkelen. 1991. Active immunity is seen as a reduction in the cell response to oral live vaccine. Vaccine 9:428–431.
12. Kantele, A., M. Hikkinen, Z. Moldoveanu, A. U. Espilahiti, R. D. Alvarez, S. Savarakka, and J. M. Kantele. 1998. Differences in immune responses induced by oral and rectal immunizations with Salmonella typhi Ty21a: evidence for compartmentalization within the common mucosal immune system in humans. Infect. Immun. 66:5630–5635.
13. Kantele, A., T. Pal, and A. Lindberg. 1992. Safety and immunogenicity of the live oral autotaxous Shigella flexneri SFL 124 in volunteers. Vaccine 10:395–404.
14. Losonsky, G. A., C. O. Tacket, S. S. Wasserman, J. B. Kaper, and M. Levine. 1993. Secondary Vibrio cholerae-specific cellular antibody responses following wild-type homologous challenge in people vaccinated with CVD 103-HgR live oral cholera vaccine: changes with time and lack of correlation with protection. Infect. Immun. 61:729–733.
15. Pelto, H., A. Siltosen, H. Kyronooppa, I. Simula, L. Mattila, P. Oksanen, M. J. Kataja, and M. Cadou. 1991. Prevention of travellers’ diarrhoea by oral B-subunit/whole-cell cholera vaccine. Lancet 338:1285–1289.
16. Qadri, F., C. Wenneřás, F. Ahmed, M. Asaduzzaman, D. Saha, M. J. Albert, R. B. Sack, and A.-M. Svennerholm. 2000. Safety and immunogenicity of an oral, inactivated enterotoxigenic Escherichia coli plus cholera toxin B subunit vaccine in Bangladeshi adults and children. Vaccine 18:2704–2712.
17. Quiding-Järbrink, M., I. Nordström, G. Granström, A. Killander, M. Jertborn, E. B. Butler, A. I. Lazarovits, J. Holmgren, and C. Czerkinsky. 1997. Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric and nasal immunisation. A molecular basis for the compartmentalization of effector B-cell responses. J. Clin. Invest. 99:1261–1269.
18. Savarino, S. J., F. M. Brown, E. Hall, S. Bassily, F. Youssell, T. Wierzb, L. Peruski, N. A. El-Masry, M. Safwat, M. Rao, J. M. Jertborn, A.-M. Svennerholm, and J. D. Clemens. 1998. Safety and immunogenicity of an oral, killed enterotoxigenic Escherichia coli-cholera toxin B subunit vaccine in Egyptian adults. J. Infect. Dis. 177:790–799.
19. Savarino, S. J., E. R. Hall, S. Bassily, F. M. Brown, F. Youssell, T. F. Wierzb, L. Peruski, N. A. El-Masry, M. Safwat, M. Rao, H. El Mohamady, R. Abu-Elyazeed, A. Naficy, A.-M. Svennerholm, M. Jertborn, Y. J. Lee, and J. D. Clemens. 1999. Oral, inactivated, whole cell enterotoxigenic Escherichia coli plus cholera toxin B subunit vaccine: results of the initial evaluation in children. J. Infect. Dis. 179:107–114.
20. Svennerholm, A.-M., J. Holmgren, R. Black, M. Levine, and M. M. Merson. 1983. Serologic differentiation between antitoxin responses to infection with Vibrio cholerae and enterotoxin-producing Escherichia coli. J. Infect. Dis. 147:514–522.
21. Svennerholm, A.-M., J. Holmgren, and D. A. Sack. 1989. Development of oral vaccines against enterotoxigenic Escherichia coli diarrhea. Vaccine 7:196–198.
22. Svennerholm, A.-M., C. Åhrén, and J. Jertborn. 1997. Vaccines against enterotoxigenic Escherichia coli infections. I. Oral inactivated vaccines against enterotoxigenic Escherichia coli p. 865–873. In M. M. Levine, G. C. Woodrow, J. B. Kaper, and G. S. Gobon (ed.), New generation vaccines. Marcel Dekker, New York, N.Y.
23. Wenneřás, C., A.-M. Svennerholm, C. Åhrén, and C. Czerkinsky. 1992. Antibody-secreting cells in human peripheral blood after oral immunization with an inactivated enterotoxigenic Escherichia coli vaccine. Infect. Immun. 60:2605–2611.