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Different Secretory Immunoglobulin A Antibody Responses to Cholera Vaccination in Swedish and Pakistani Women

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The capacity of subcutaneous cholera vaccination to induce an antibody response in milk and saliva was studied in lactating Swedish and Pakistani women, since secretory immunoglobulin A (SIgA) antibody responses in these secretions may reflect intestinal immunity. Before immunization, most of the Pakistani women had significant titers of specific SIgA antibodies against *Vibrio cholerae* lipopolysaccharide in milk, whereas only a few of the Swedish women had measurable, low titers. In the Pakistani women a single subcutaneous injection of cholera vaccine gave rise to a significant SIgA titer rise in 70% of the milk and 45% of the saliva samples. The Swedish women, on the other hand, did not respond with a significant antibody response of any immunoglobulin class in milk or saliva, either after a single or after a booster dose 14 days later. In serum, however, the vaccination induced significant titer rises, mainly of IgG antibodies, also in the Swedish women, but these rises were of lower magnitude than those in the Pakistani group. The results suggest a significant difference in the capacity of parenterally administered cholera vaccine to stimulate SIgA antibody formation in naturally primed and nonprimed individuals.

Infection with *Vibrio cholerae* and the action of the disease-producing cholera enterotoxin are restricted to the intestinal epithelium. Therefore, it is probably important that cholera immunization can stimulate an effective mucosal immune response in the intestine.

Methods based on either immunohistological enumeration of antibody-forming cells in lamina propria (6, 9) or measurement of antibodies synthesized by tissue-cultured intestine (10) recently have been elaborated for determination of the local antibody response to cholera antigens in animals. Being invasive, these methods are not suitable for studying mucosal immunity in humans. Local antibody formation in humans has been studied by measuring titer increases in either intestinal aspirates (8) or fecal extracts (12). These methods are far from ideal; aspirates are difficult to collect and represent at best a very limited portion of the intestine, whereas fecal coproantibodies are difficult to extract and subject to extensive proteolytic degradation, invalidating quantitative analysis. Thus, there is a great need for improved methods of studying the intestinal immune response to enteric vaccines in humans.

Recent studies have shown that after antigen stimulation the precursors to the immunoglobulin A (IgA)-producing cells in the Peyer's patches not only migrate to the intestinal lamina propria but also are deposited in various secretory glands (12). This homing mechanism suggests a simpler approach to evaluating intestinal immunity after vaccination by using determination of antibody responses in various secretions such as saliva and milk as a measure of the mucosal immune response in the intestine (4, 13).

In a preliminary study we found that parenteral cholera immunization induced a significant secretory IgA (SIgA) antibody response in milk of women who probably had been exposed naturally to cholera antigens (11). The aim of the present study was to analyze whether such vaccination also gives rise to a mucosal immune response in nonprimed individuals. This was studied by comparing the SIgA antibody responses in milk and saliva from lactating Swedish mothers with no previous exposure to cholera antigen to the responses in naturally primed Pakistani women.

MATERIALS AND METHODS

Ten Swedish and ten Pakistani women were studied during lactation more than 1 month and less than 10 months after parturition. Their verbal consent was obtained before admitting the women to the study. All volunteers were fairly healthy, and to our knowledge none of them had previously been vaccinated against cholera or suffered from this disease. The Swedish

women were immunized subcutaneously (s.c.) with 0.75 ml of bivalent whole-cell cholera vaccine (8×10^9 Inaba and Ogawa vibrios per ml, National Bacteriological Laboratory, Stockholm, Sweden) followed by an s.c. 1-ml booster dose 14 days later. The Pakistani women received a single s.c. dose of 1 ml of cholera vaccine (8×10^9 vibrios per ml, Swiss Serum and Vaccine Institute, Berne, Switzerland). Milk, saliva, and serum samples were taken from all women immediately before and 14 to 16 days after immunization. The specimens were kept frozen at −20°C until analyzed. After thawing, the milk samples were centrifuged at 4,000 × *g* for 15 min to remove cells and lipids. The responses of cholera antibodies of IgG, IgA, and IgM classes and also of antibodies containing secretory component (SC) (essentially SIgA) were tested by means of the enzyme-linked immunosorbent assay method with purified *V. cholerae* lipopolysaccharide as the solid-phase antigen (5, 11).

RESULTS

Before immunization only two of the Swedish women had detectable levels of antibodies against *V. cholerae* lipopolysaccharide in milk, and then in low titer (Fig. 1). In the Pakistani group, on the other hand, significant titers of IgA antibodies against this antigen were registered in 6 out of 10 of the prevaccination milk samples, suggesting earlier natural exposure. In a few of the Pakistani women, very low levels of milk IgG and IgM antibodies were found as well.

Subcutaneous cholera vaccination did not result in significant (greater than twofold) rises of specific IgA, IgG, or IgM antibodies in the milk of any of the Swedish women, nor did the booster immunization induce any milk antibody response in these women (Fig. 1). In the Pakistani women, however, a single s.c. dose of vaccine gave rise to significantly increased IgA antibody titers in 7 out of 10 of the milk samples (Fig. 1). No significant increase was seen in IgG or IgM antibodies. Titration of the Pakistani milk samples with an anti-SC conjugate gave very similar results to those obtained with the anti-IgA conjugate, indicating the SIgA nature of the milk cholera antibodies.

A pattern similar to that in milk was noted for saliva. Thus, none of the Swedish but four out of nine Pakistani women responded to the cholera vaccination with significant titer rises in this secretion. In contrast to the milk response, the cholera antibody titer increase in the saliva of the Pakistani women was not restricted to IgA but also included IgG antibodies.

Significant titer rises of IgG and IgM antibodies in serum were seen both in the Swedish and in the Pakistani women (Fig. 2), but the rises were considerably smaller in the Swedish group. Only one of the Swedish but all the Pakistani women responded with significantly increased IgA antibody titers in serum (Fig. 2).

DISCUSSION

In recent studies, it has been shown that stim-

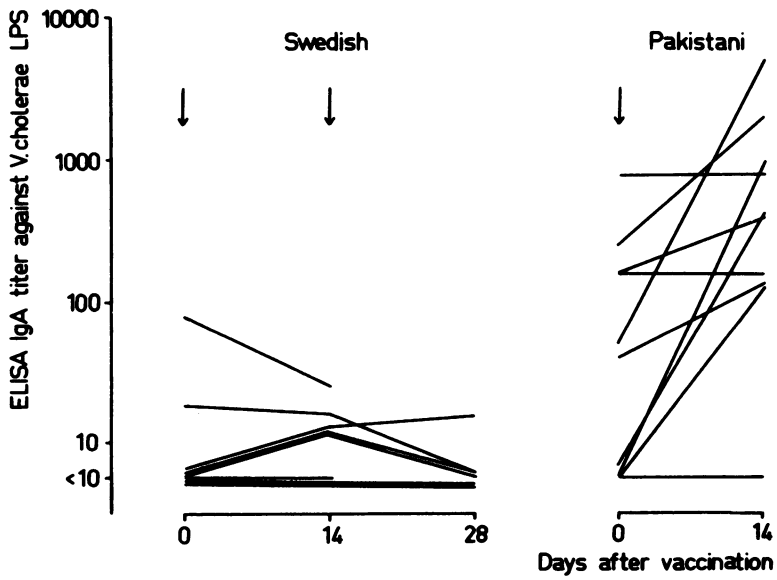

FIG. 1. *Milk IgA antibody response to V. cholerae lipopolysaccharide (LPS) in Swedish and Pakistani (group I) women after s.c. cholera vaccination. Individual titer values are shown. Arrows indicate day of antigen administration.*

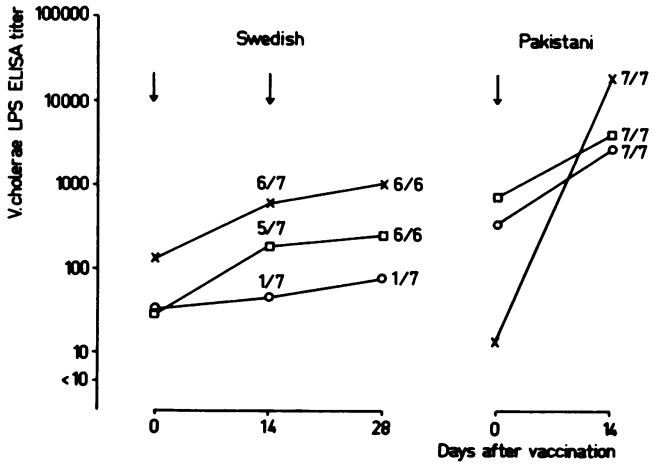

FIG. 2. *Serum antibody response of various classes to V. cholerae lipopolysaccharide (LPS) in Swedish and Pakistani women after s.c. cholera vaccination. Each point represents the arithmetic mean titer from all women in the respective group on the day of immunization. Symbols: ×, IgG; ○, IgA; and □, IgM titers. Frequencies of significant titer increases (>twofold) are indicated. Arrows indicate day of antigen administration.*

ulation with antigen locally in the intestine results in the appearance of specific antibodies as well as antibody-forming cells in milk and other secretions (4). These observations have suggested that determination of antibody responses in various secretions may be used as a measure of intestinal immune responses. It remains to be determined, however, how closely immune responses to various antigens in the gut are reflected in extraintestinal secretions.

In this study it was found that s.c. cholera vaccination frequently gave rise to an SIgA antibody response in milk and saliva of Pakistani but not of Swedish lactating women. Although different batches of cholera vaccine were used in the two groups, it is very unlikely that this could explain the marked difference in response, since the number of killed Inaba and Ogawa vibrios was the same in both vaccines. Only the Pakistani women had significant SIgA cholera antibody levels in milk and saliva before immunization, indicating that they were primed by previous natural exposure to cholera antigens. The results indicate that the vaccination was capable of stimulating a mucosal immune response but only in immunologically primed individuals.

The SIgA nature of the milk and salivary antibodies which increased in response to vaccination indicates that these antibodies were of mucosal or glandular origin. Two different mechanisms have been identified which might explain the appearance of antibodies to enteric antigens in extraintestinal secretions. Weisz-Carrington et al. (12) have shown that in mice antigen-stimulated IgA precursor cells from the intestine home in significant numbers in the mammary glands, where they may develop into specific IgA antibody-secreting cells. This homing or subsequent cell maturation or both are under the influence of lactogenic hormones and may thus be restricted to lactating animals. An alternative explanation for the transfer of intestinal immunity to milk was recently suggested by Halsey et al. (3). They showed that the mammary epithelium has specific affinity for J-chain-containing dimer IgA. Serum IgA antibodies representing "spill-over" from intestinal synthesis (10) may thus be absorbed by the mammary gland epithelium and, under uptake of SC, translocated into the mammary secretion as SIgA antibodies. Either of these mechanisms could explain the results obtained in the vaccinated Pakistani women.

Provided that the milk antibody response correlates with the response in the intestine, which is not yet known, the data suggest that parenteral cholera vaccination in nonendemic areas may be even less effective than in endemic areas (1, 7). The results might also explain the better effect of vaccination in adults than in young children tested in all field trials in endemic areas (7). A single s.c. dose of vaccine is apparently much less efficient as a primer than oral exposure, as indicated by the absence of SIgA formation in the Swedish women even after the booster dose.

This suggests that vaccination against intestinal pathogens in persons living in nonendemic areas should include one or more oral doses of antigen for priming.

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LITERATURE CITED

1. **Finkelstein, R. A.** 1973. Cholera. CRC Crit. Rev. Microbiol. **2**:553–613.
2. **Freter, R.** 1962. Detection of coproantibody and its formation after parenteral and oral immunization of human volunteers. J. Infect. Dis. **111**:37–48.
3. **Halsey, J. F., B. H. Johnson, and J. J. Cebra.** 1980. Transport of immunoglobulins from serum into colostrum. J. Exp. Med. **151**:767–772.
4. **Hanson, L. Å., B. Carlsson, J. R. Cruz, B. Garcia, J. Holmgren, S. R. Kahn, B. S. Lindblad, and A.-M. Svennerholm.** 1979. Immune response in the mammary gland, p. 145–157. *In* P. L. Ogra and D. Dayton (ed.), Immunology of breastmilk. Raven Press, New York.
5. **Holmgren, J., and A.-M. Svennerholm.** 1973. Enzyme-linked immunosorbent assays for cholera serology. Infect. Immun. **7**:753–757.
6. **Lange, S., and J. Holmgren.** 1978. Protective antitoxic cholera immunity in mice: influence of route and number of immunizations and mode of action of protective antibodies. Acta Pathol. Microbiol. Scand. Sect. C **86**: 145–152.
7. **Mosley, W. H.** 1969. Vaccines and somatic antigens. The role of immunity in cholera. A review of epidemiological and serological studies. Tex. Rep. Biol. Med. **27**:227–241.
8. **Northrup, R. S., and S. A. Hossein.** 1970. Immunoglobulins and antibody activity in the intestine and serum in cholera. II. Measurement of antibody activity in jejunal aspirates and sera of cholera patients by radioimmunodiffusion. J. Infect. Dis. **121** (Suppl.):S142–146.
9. **Pierce, N. F., and J. L. Gowans.** 1975. Cellular kinetics of the intestinal immune response to cholera toxoid in rats. J. Exp. Med. **142**:1550–1563.
10. **Svennerholm, A.-M., S. Lange, and J. Holmgren.** 1978. Correlation between intestinal synthesis of specific immunoglobulin A and protection against experimental cholera in mice. Infect. Immun. **21**:1–6.
11. **Svennerholm, A.-M., J. Holmgren, L. Å. Hanson, B. S. Lindblad, F. Quereshi, and R. J. Rahimtoola.** 1977. Boosting of secretory IgA antibody responses in man by parenteral cholera vaccination. Scand. J. Immunol. **6**:1345–1349.
12. **Weisz-Carrington, P., M. E. Roux, M. McWilliams, J. M. Phillips-Quagliata, and M. E. Lamm.** 1978. Hormonal induction of the secretory immune system in the mammary gland. Proc. Natl. Acad. Sci. U. S. A. **75**: 2928–2932.
13. **World Health Organization.** 1979. Intestinal immunity and vaccine development. Bull. WHO **57**:719–734.