Vaccines and Other Approaches to the Prevention of Intractable Infant Diarrhea by the Prevention of Intestinal Colonization*

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*The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.
must be approached cautiously, since unwanted effects may occur. As we will see from examples in animals, treatment aimed at one group of pathogens may leave the field open for colonization by others; and induction of active mucosal immunity may only be achieved along with concomitant suppression of systemic immune responses to the same organisms.

PASSIVE ANTIBODY PROTECTION

Piglets and calves can be protected against neonatal *ETEC* infection by suckling dams who have been immunized with appropriate antigens (1). Since transplacental transfer of antibody does not occur in pigs, such protection appears to be transferred via antibody in maternal colostrum or milk (Fig. 1). One common form of neonatal colibacillosis in piglets depends on expression of K88 surface fimbrial antigen and *E. coli* heat stable enterotoxin (ST) as described by Smith and Linggood (1a). This disease can be effectively prevented by parenteral immunization of pregnant gilts in the weeks just before farrowing. In an early study, Rutter and Anderson (2) showed that if gilts received two intramuscular injections of $10^8$ formalin-killed organisms bearing K88 antigens (0149:K91,88ac), mortality in piglets challenged at birth with $10^7$ organisms of the same strain was reduced from 38% to 20%, and there was significantly decreased colonization of the intestinal tract, particularly in proximal portions. High-titer agglutinating antibody against the vaccine strain and other K88 strains that were not homologous for O antigens was found in the IgG fraction of colostrum of the vaccinated sows.

At about the same time, Smith (3) confirmed the nature of the protective effect, since similar protection from diarrhea and inhibition and delay of intestinal colonization could be shown by administering immune serum antibody to piglets in evaporated milk. These studies also confirmed the importance of the anti-K88 antibody in protection, since antiserum protected against challenge with
0141:K85,88ab strains whose only shared antigen with the vaccine strain was K88ab.

Although the study of Rutter and Anderson (2) showed a decrease in mortality in the litters of vaccinated sows that was statistically significant, mortality in the nonvaccinated group was lower than might have been expected, and considerable mortality occurred in the litters of the vaccinated sows. In retrospect, these results must be analyzed in terms of additional knowledge about the virulence mechanisms of the pathogen and the existence of host phenotypes that can modify the susceptibility to infection partly independently of immune mechanisms (4).

The low mortality in the nonvaccinated group is perhaps best explained by the knowledge that some pigs are genetically resistant to infection. Resistance to infection with K88 strains is an autosomal recessive trait at a single locus whose phenotypic expression is a lack of receptors for K88 on intestinal epithelial cells (4). As a result of this mode of inheritance, resistant dams (homozygotes) can bear susceptible piglets (heterozygotes). In an endemic situation, susceptible piglets borne to resistant dams are at the greatest risk of infection, since the resistant dams will never have been colonized with K88 positive strains, and, therefore, they will have lowest spontaneous colostral antibody titers to K88 (5). K88-susceptible dams should have higher preimmunization levels of anti-K88 colostral antibody, and, in fact, detectable low antibody levels to challenge antigen were noted in the milk of nonimmunized sows. Moreover, the ability of a parenteral vaccine to boost mucosal (lacteal) antibody titers will be partly determined by initial antibody levels. Animals with previous antigen exposure (susceptible dams) may be expected to have the greatest memory response to parenteral immunization. Very recently, Sellwood confirmed some of these properties (6). In genetically resistant sows, as compared to K88-susceptible sows, colostrum was less able to inhibit K88 binding to brush border membranes, had lower agglutinin titers for K88 + bacteria, and had less ability to induce opsonic phagocytosis and killing of K88 organisms (a property residing in IgM fractions of colostrum).

Taking the genetic information into account, Rutter et al. (5) vaccinated dams with purified K88 antigen and subsequently challenged piglets at birth with K88 + strains. In this group, mortality was decreased from 69% in litters of unvaccinated sows to 13% in litters born to vaccinated sows. The vaccinated sows had high levels of colostral antibody capable of inhibiting brush border adherence. Antibody level diminished rapidly in milk and was no longer detectable at 7 days post-partum.

The persisting mortality in the vaccinated group may have been caused by the emergence of other enteropathogenic strains whose colonization of the intestinal tract does not depend on K88 antigen. Three E. coli colonization factor antigens have been recognized for pig intestine; these include K99 and P987 in addition to K88. Recently, Soderlind et al. (7) showed that vaccination against a single colonization factor antigen reveals the presence in a herd of bacterial strains that express other colonization factor antigens.
Taken as a whole, these studies are of extreme importance in indicating that antibody in lacteal secretions directed against colonization factor antigens can passively protect otherwise susceptible hosts against infection with enteroadherent *Escherichia coli*. These mechanisms are probably operable in the several commercial vaccines against colibacillosis that are marketed for administration parenterally to pregnant dams or for inclusion in the food, thereby stimulating a mucosal immune response in breast and intestine. The heat-killed vaccines probably do not protect by antiadhesin mechanisms, since K88 antigen is known to be heat labile.

By analogy to these animal studies, infants might be protected against *E. coli* strains pathogenic for humans by a regimen of maternal vaccination using appropriate colonization factor antigens. Although some of these antigens have been identified, and their prevalence among pathogenic *E. coli* in various areas documented, many presumably remain to be discovered (8). Appropriate vaccines would likely need to be multivalent and based on current epidemiologic evidence for the prevalence of pathogenic strains.

Since previous maternal antigen exposure may influence the ability of a parenterally killed vaccine to stimulate a mucosal immunoglobulin rise, knowledge of maternal immune status might dictate the desirability of an oral versus a parenteral vaccine. Mothers in endemic areas may be expected to have been primed by previous exposure to pathogenic strains. Anti-*E. coli* pilus antibodies have been documented in human breast milk (9). On the basis of studies with other antigens, parenteral vaccination should be as effective in inducing a mucosal IgA memory response to boost mucosal immunity as oral immunization in a previously mucosally primed host. Oral immunization might be expected to give the best primary mucosal immune response.

In order to prolong the period of protection beyond the colostral stage, it may be possible to supplement milk or formula with hyperimmune immunoglobulins, as was shown to be effective by Smith (3) using serum antibodies. However, the possible deleterious effect of prolonged passive protection on the development of an active mucosal immune response must be borne in mind. Also, immunoglobulins may not be the only antibacterial agents in the milk, since Otnaess and Svennerholm (10) showed levels of agglutinating activity, which was postulated to reside in a glycoprotein fraction of colostrum. The identity of these substances remains an area for active investigation. An ideal program might include a neonatal period of passive protection followed by a program of active mucosal immunization at a time when the host mucosal immune response is capable of responding.

The abovementioned studies have demonstrated the efficacy of immune colostral IgG or orally administered immune serum (again predominantly IgG) in protecting piglets against subsequent challenge with pathogenic *E. coli*. Similar studies have been performed in calves using K99+ organisms, K99 pili, or 987 pili (11–14). Differences in the predominant immunoglobulin classes among species may be important. Human colostrum and milk contain secretory IgA
as the predominant immunoglobulins, in contrast to the predominance of IgG in the pig colostrum.

Studies demonstrating an *in vivo* capability for IgA have been more limited. Nevertheless, specific secretory IgA has been shown to inhibit attachment of adherent *E. coli* to a variety of epithelial cells *in vitro*. Fubara and Freter (15) have demonstrated that IgA induced by the feeding of heat-killed cholera vibrios to germ-free mice and carefully purified could inhibit the adsorption of vibrios onto the mucosal surface of intestinal loops. This was accomplished by preincubating the organisms with the IgA before challenge, and the IgA did not decrease the total population of vibrios in the loops. The mucosal adherence factors for cholera remain unknown, and the specificity of the IgA preparation was not determined.

Cantey (16) was able to prepare large quantities of purified slgA from the colostrum of rabbits infected with either enteroadherent (RDEC-1) or an enteroinvasive *E. coli* (EIEC). When the colostral IgA was administered just prior to and following challenge doses of the appropriate organisms, colonization was both delayed and diminished, and the occurrence of diarrhea was prevented. This same preparation of IgA was shown by our laboratory (E. C. Boedeker and C. P. Cheney, unpublished results) to be able to inhibit the *in vitro* adherence of RDEC-1 to rabbit intestinal brush borders.

The preceding discussion has provided evidence that active material immunization, with passive protection of progeny, works by inhibiting mucosal colonization by organisms expressing colonization factor antigens. Recent evidence suggests that at least one other mechanism may be operative (17,18). Antibody directed against the product of a bacterial gene or episome (plasmid) may exert a population pressure to eliminate that genetic information from the organism. The genetic information for K88 and other enteroadherence antigens is located on transferable plasmid. Linggood has recently shown that growth of organisms in the presence of antibody directed against their surface components led to the selection of forms that no longer expresses the antigens (nonpiliated forms). Furthermore, the nonpiliated organisms had lost, or were cured of, their plasmids. Thus, antibody provided a selective pressure for loss of plasmid-encoded genetic information. This effect was observed both *in vivo* and *in vitro*. This type of genetic drift in the expression of surface components appears, in the case of enteroadherent *E. coli*, to work to the species' as well as the individual host's advantage by selecting for nonpathogenic strains.

Passive immunization is attractive as a means of preventing enteric disease in the young; however, more work is needed in understanding the precise mechanisms whereby antibody in the lumen prevents or minimizes infection and in choosing the optimal immunizing antigens. Passive immunization has the disadvantage that it must be administered over the entire period of susceptibility of the host to challenge. There is some concern that natural antibody response of the host may be delayed or impaired by a program of passive immunization. Rothberg et al. (18a) have shown that administration of antiserum parenterally
did not delay a systemic response to orally administered antigen. One could postulate that the presence of antibody in the lumen may delay the onset of the host's active mucosal immune response by limiting access of antigens to the immune system. Thus, methods for stimulating an active mucosal response to relevant colonization factor antigens as soon as the host mucosal immune system can respond should be considered.

ACTIVE IMMUNIZATION

It is known from challenge studies in adult human volunteers with *Vibrio cholera* and with *ETEC* that clinical illness results in a prolonged immunity, which renders the host resistant to subsequent infection, at least for a period of months. Thus, in active immunization, the goal is to develop an immunogen that provides protection approaching that seen with the natural disease. In considering appropriate immunogens as vaccines, one must consider both non-infectious bacterial products, which could range from whole killed organisms to purified colonization factor antigens, and attenuated or nonpathogenic organisms expressing the appropriate antigens. The route of antigen administration is also important.

Parenteral immunization has been successfully used to boost preexisting mucosal immune responses in which initial immunity was acquired by exposure to the natural infection. Oral or mucosal immunization appears to be the preferred route for primary mucosal immunization (Fig. 2). There is extensive evidence that the feeding of protein antigens, although inducing a local immune response, leads to a suppression of the systemic immune response to the same antigens. Presumably these effects are caused by the stimulation of antigen-specific suppressor T cells, which act systemically (19). The form and binding capacity of the antigen are both important in determining the magnitude of the mucosal immune response and the degree of suppression of systemic immunity (20). Cholera toxin is highly active in stimulating mucosal immunity, possibly in part because it binds avidly to cell membranes. Particulate antigens seem less likely than soluble antigens to induce systemic unresponsiveness when administered orally. These factors probably determine the rate at which antigen is sampled by the specialized M cells that overly the Peyer's patches of the intestine and whether the antigen reaches the systemic immune system or remains localized in the Peyer's patch.

Porter et al. (21) investigated the ability of heat-killed suspensions of pathogenic *E. coli* strains to stimulate an active mucosal immune response in the intestines of young pigs. These preparations were designed as a prototype vaccine to be included in feed. Antigen was instilled into isolated Thiry-Vella loops in pigs aged 4 to 9 days, and secretions from the loops were measured for 4 to 5 weeks for development of antibody levels. Antibody production of predominantly IgA class could be demonstrated in animals immunized when they were only 10 days old. After antibody levels in secretions had returned to base-line levels,
FIG. 2. Schematic illustration of active mucosal immunization. Bacterial adherence factors (pili) may be presented as purified preparations or on killed or attenuated organisms to the mucosal immune system. These antigens may be processed by specialized epithelial cells (M cells) overlying Peyer's patch and presented to B lymphoblasts. These cells migrate from the intestine and return (home) to the gut-associated lymphoid tissue as mature B cells committed to specific IgA antibody production. Specific IgA secreted in the lamina propria acquires a secretory piece at the basal-lateral border of the intestinal absorptive epithelial cells. It is transported across the epithelium in vesicles and secreted into the lumen.

the loops were rechallenged to determine whether a more rapid (memory) response of immunoglobulin secretion occurred. The response to the second dose of vaccine was similar in time course to the first, with a peak at 9 to 10 days. This suggested that there was no memory response in the intestines of young pigs. A rapid memory response would be required to protect against a disease with a short incubation period and short duration. These experiments suggest that to protect against enteric infection, a single immunization might not be effective but that continuous boosting of the response might be necessary. This might be achieved by continued inclusion of the antigen in feed or formula. Part of the importance of the studies of Porter et al. is that they suggest that active immunization might be achieved in very young animals, at a time when the infant would ordinarily be only passively protected.
These studies did not specifically document that the protective antibody was directed against the colonization factor antigens. Since these antigens, by definition, have a high affinity for the mucosal absorptive epithelial cell surface, absorptive cells may effectively compete for antigen and limit its access to the mucosal immune system. The readily measurable mucosal antibody response may have been related to the particulate nature of the antigen administered (i.e., as whole cell). Keren et al. (22,23) have extensively utilized the Thiry–Vella loop model in adult rabbits to investigate the variables involved in eliciting a mucosal immune response to *Shigella* antigens, particularly the O antigens. We have utilized the same rabbit Thiry–Vella loop to investigate the mucosal immunogenicity of highly purified preparations of colonization factor antigens, specifically CFA/II. We have shown that these molecules are mucosally immunogenic in the adult rabbit and that a durable immune response can be generated. Since the affinity of CFA/II is for human, not for rabbit, intestinal epithelial cell surfaces, the ability of this preparation to induce a relevant mucosal immune memory response in man will only be answered by limited clinical trials for immunogenicity in humans. Such trials are currently under way.

De la Cabada et al. (24) have utilized a rabbit model to demonstrate the protective effect of orally administered CFA/I pili in the rabbit. In earlier studies, Evans et al. (25) had shown that prior administration of CFA/I pili to loops of infant (4-day) rabbits could prevent both mucosal adherence of the organisms and a diarrheal response in the loops. These studies suggested that the infant rabbit might be a useful model for studies of infection with strains bearing human colonization factors. In adult rabbits, however, diarrheal disease and colonization with CFA/I-positive organisms can only be induced if the animals are first subjected to temporary obstruction of the ileum (to prevent clearance of the organisms following challenge) combined with occlusion of the cecum (to prevent it from serving as a reservoir for secreted fluid). When these conditions were met, diarrheal disease could be produced in adult rabbits by challenge with *ETEC* strain H10407. In this model, protection was demonstrated by pretreatment of the animals with orally administered CFA/I pili.

Very recently, we have performed studies of oral immunization with colonization factor antigens using the RDEC-1 model of rabbit diarrhea. In this model, the organism is inoculated into its natural host, and no manipulation is required to produce diarrheal disease in 70 to 80% of animals. Following four 1-mg doses of RDEC-1 pili at 5-day intervals, rabbits were challenged with $10^7$ viable RDEC-1 *E. coli*. At 5 days, colony counts were unchanged in jejunum but were decreased 2 to 3 logs in ileum and 3 logs in cecum. These studies strongly suggest that orally administered pili can protect against subsequent intestinal colonization. They suggest that there is a role for oral vaccination with purified adherence factors. It may be necessary to maintain continuous exposure of the intestine to these antigens to maintain an effective level of immunity. Our studies were performed by challenging the animals during such a period of continued immunization (boosting). Challenge was given long enough
after a dose of pilus vaccine (3 days) to suggest that the mechanism of protection was immunological and not receptor blockade.

Recently, Levine et al. (26) have undertaken clinical trials investigating the reactogenicity, immunogenicity, and efficacy of an *Escherichia coli* type 1 somatic pili parenteral vaccine in man. These are important studies since they represent the first trials of actively induced antipilus immunity in humans in protecting against enteric infection. The type 1 pili chosen for study, the first somatic pili described on *E. coli* by Brinton (27), are also designated common pili because they can be present on the majority of *E. coli* strains, both pathogens and nonpathogens. The genes encoding for type 1 pilus production are chromosomal, but type 1 pilus expression can be controlled by growth conditions. Type 1 pili confer mannose-sensitive adhesive properties for guinea pig red cells and for human buccal epithelial cells, but they do not seem to mediate attachment to human intestinal brush borders (28), and their role in colonization of the gastrointestinal tract is unknown. In particular, it is unknown whether type 1 pili are expressed *in vivo* in the gastrointestinal tract. Nevertheless, it is hoped that immunity to these common *E. coli* surface antigens might confer protection against a wide range of *E. coli* pathogens, including the increasing number of ETEC strains from different areas of the world that do not have presently recognized colonization factor antigens.

For these studies, type 1 pili were prepared from *E. coli* strain H10407, an ETEC strain that also expresses CFA/I under different conditions. Parenteral immunization with two intramuscular doses of type 1 pili was performed. Parenteral immunization was undertaken because of concern that systemic tolerance to these common *E. coli* antigens might develop after oral administration. Because of the likelihood of previous exposure to these antigens, it is reasonable to assume that each immunizing dose was actually a booster dose. Following immunization, all individuals developed serum (IgG) and jejunal (IgA) antibody rises to the type 1 pili. Colonic levels of *E. coli* expressing type 1 pili were not influenced by the immunization. In an initial challenge study, apparent protection was conferred on the vaccinees (2/6 with diarrhea) as compared to nonvaccinated controls (7/7 with diarrhea). Since subsequent studies did not demonstrate equivalent levels of protection, the role of type 1 pilus antigens as vaccines against pathogenic *E. coli* remains a subject for active investigation. Interestingly, none of the controls or vaccinees challenged with H10407 developed antibody rises in serum or intestinal fluid to type 1 pili following challenge with the organisms. This suggests either that pathogenic *E. coli* do not express type 1 pili at the mucosal surface or that most individuals have developed systemic immunologic tolerance to these antigens.

Immunization with living strains of *E. coli* expressing colonization factor antigens is another approach that can be taken. All of the enteroadherent pili described to date are encoded by plasmids; thus, it should be possible to produce strains of *E. coli* that produce only these antigens but not other virulence factors. Separation of CFA production from enterotoxin production has been a problem.
for all of the CFA/I and CFA/II strains since enterotoxin production is often encoded for by the same plasmids as the CFAs. This is in distinction to the separate localization of ENT and K88 plasmids. Recombinant DNA techniques should enable these problems to be solved in the near future. Nevertheless, even if these problems are solved, there remains some concern with feeding live organisms capable of colonizing the gut to infants, since there is at least suggestive evidence that colonization factor antigens themselves may be responsible for diarrhea. This was suggested by the original work of Smith and Linggood (1a) in which animals challenged with K88+, ENT− organisms developed a degree of diarrhea. Morphologic examination of the adherent EPEC strains suggests that close adherence is itself related to mucosal damage. Since pili are lectin analogs and membrane-active molecules, it is possible that they may be responsible for changes in membrane permeability in areas of attachment. Such changes might conceivably induce intestinal secretory events.

Attenuated strains with limited survival capability in the intestine, analogous to the aromatic-dependent (29) and galactose-epimerase-negative mutants of Salmonella (30), may be useful as vaccines. The latter mutants have been shown to be effective in preventing typhoid fever (31). Shigella antigens have been introduced into these strains to produce potential Shigella vaccines (32). Escherichia coli adherence factors could be introduced into these strains or into Shigella strains whose close genetic relatedness to E. coli makes this approach possible. We have recently reported (33) the transfer of RDEC-1 adherence pili into an attenuated Shigella strain together with the transfer of an 85-megadalton plasmid (Fig. 3). This organism did not establish itself in the rabbit gut. Its ability to induce an immune response to pilus antigen is being explored in the Thiry-Vella loop model.

COMPETITIVE COLONIZATION

The concept of using bacterial competition for colonization sites as a means of protecting against colonization by enteropathogens has a long history, including the empiric use of ingested lactobacilli to achieve a "healthy flora." Specific proofs of the efficacy of this therapy are difficult to find. It is frequently overlooked that for most of the types of E. coli diarrheal disease, with the possible exception of the enteroinvasive strains, disease is caused by colonization of areas of the intestinal tract that are normally relatively sterile and in which colonization must be considered abnormal. One must question the wisdom of establishing colonization of the mucosa by any organisms in the proximal gastrointestinal tract, which is primarily utilized for digestion and absorption. Any degree of close mucosal colonization may be the equivalent of injury. Nevertheless, in the cecum, Shedlofsky and Freter (34) have convincingly demonstrated that introduction of a competitive flora in germ-free mice limits the association of cholera vibrios with the mucosa. Moreover, they have shown that this ecologic
effect was synergistic with immune mechanisms in limiting colonization. Thus, if *Vibrio cholera* alone were introduced, they reached levels of $10^9$ to $10^{10}$ in cecum. Vaccination reduced these levels by a factor of 2. Competitive flora alone reduced levels to $10^5$ to $10^6$, but vaccination in this case further reduced levels five- to 60-fold.

Recently, Davidson and Hirsch (35) demonstrated that prior inoculation of intestinal segments of infant mice with K88$^+$, ENT$^-$ strains prevented fluid accumulation when the loops were subsequently challenged with K88$^+$, ENT$^+$ strains. The implication of these studies, which were subsequently duplicated in intact piglets (36), was that the prior colonization with adhesin-positive bacteria prevented subsequent colonization with the fully virulent strains, although these effects were not documented by colony counts. One would not expect that long-term colonization with these strains could be maintained, although they might be useful for establishment of immunity to adherence antigens. Moreover, it is not clear that long-term colonization with such strains would be beneficial. Smith and Linggood’s (1a) initial studies with these organisms revealed a degree of diarrheal disease in the animals colonized with K88$^+$, ENT$^-$ strains. At this point, the role of colonization factor antigens themselves in inducing diarrheal disease remains insufficiently explored to permit one to advocate competitive colonization as an approach to therapy.
RECEPTOR ANALOG THERAPY

As the bacterial (adhesin) and host (receptor) determinants for mucosal colonization are identified and characterized, this knowledge may be utilized to devise analogs of either component that might interfere with bacterial/mucosal interaction in vitro in intestine. Continuous feeding of adhesin or receptor analogs during periods of risk of infection might saturate host or bacterial sites and thereby prevent intestinal colonization (Fig. 4). Of the two methods, receptor analog therapy seems preferable (Fig. 4A). It is unknown whether the host attachment site is a true receptor (in terms of initiating a subsequent response in the host cell) or simply a binding site. Saturation of receptor sites with adhesin analogs (Fig. 4B) might induce unwanted effects or unfavorably influence digestive/absorptive function. The approach has potential, however, in view of the demonstration by Evans et al. (25) that orally administered CFA/I coated the mucosal surface of infant rabbit intestine and prevented subsequent attachment of CFA/I-bearing organisms.

Receptor analog therapy seems more promising. This approach has been used by Stoll et al. (37) with some measurable success in cholera patients to limit the influence of cholera toxin in the epithelium. In this case, the inhibitor was GM₁ ganglioside immobilized on charcoal. Gangliosides immobilized in this way may also influence adhesive mechanisms since Faris et al. (38) have suggested that the mucosal site for both CFA/I and K99 antigens are gangliosides.

A common property of bacteria expressing enteroadherence pili is surface hydrophobicity, as demonstrated for K88 by Smyth et al. (39) and by Wadstrom et al. (40) for K99, CFA/I, and CFA/II. This property can be demonstrated by hydrophobic interaction chromatography using hydrophobically substituted sepharose gels bearing phenyl, octyl, or palmitoyl groups. Organisms with adherence pili are retained on these gels (Fig. 5). We have also shown that RDEC-1 adherence pili confer marked hydrophobicity on these organisms. Wadstrom et al. (41) have utilized the infant rabbit model of ETEC with CFA/I developed by Evans et al. (25) to evaluate the use of an agarose gel substituted with a hydrophilic ligand in prevention of diarrhea. Animals given 0.2 g of the gel 1 hr after challenge with 10⁹ organisms developed diarrhea in only two of 11 cases, whereas controls universally developed diarrhea. These hydrophobic materials are extremely promising, since they utilize a common property of enteroadherence pili and thus should have a broad spectrum of efficacy.

OTHER APPROACHES

Expression of enteroadherence pili is plasmid mediated in all instances described to date. The highly encouraging studies of Linggood and Porter (17) indicating that antibody directed against an as yet unidentified surface component of K88⁺ strains induces the curing of the plasmid responsible for K88 production in vitro suggest that other adverse stimuli in the intraluminal environment may
B. FIG. 4. Receptor (A) and adhesin (B) analog therapy. A schematically diagrams receptor analog therapy. Analogs of the mucosal receptor for bacteria may be immobilized on gel or particulate matrices and administered orally. Such products may effectively compete with the native receptors for bacterial adhesins, bind bacteria, and thereby promote their clearance from the gut lumen. Alternatively (B), analogs of bacterial adhesins might be administered to saturate mucosal receptors, thereby preventing bacterial adherence.

induce similar effects. Control of the intraluminal environment may also influence the phenotypic expression of pili in vivo. Pilus expression tends to be suppressed in media that are complex and enriched. In particular, glucose and
individual amino acids can suppress pilus expression in culture. Dietary manipulation or supplementation might be successful in maintaining an intraluminal environment antithetical to pilus expression. Finally, antibiotics in doses below the minimum inhibitory concentrations can suppress the expression of type 1 pili \textit{in vitro} (42). This may be the mechanism whereby low-dose antibiotic therapy prevents traveler's diarrhea. Because of the well-documented problem of the development of multiple antibiotic resistance in \textit{E. coli}, this approach cannot be embraced without great reservations (43). Nevertheless, other mechanisms for suppression of pilus expression may remain to be discovered, and the concept deserves investigation.

CONCLUSION

Current approaches to the prevention of enteric colonization by pathogenic \textit{Escherichia coli} have been reviewed. At present, the most favorable approaches appear to be the stimulation of maternal lacteal immunity coupled with methods to generate and maintain an active secretory immune response in the intestine of the infant. Purified colonization factors administered orally are likely to be effective stimulants of active immunity. Such materials appear preferable to the live attenuated organisms available at present. Adhesin and receptor analog
therapy, as well as methods to suppress pilus expression \textit{in vivo}, deserve to be explored in devising strategies to protect infants against enteric infections.

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