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IMMUNOLOGIC CONTROL OF DIARRHEAL DISEASE DUE TO ENTEROTOXIGENIC ESCHERICHIA COLI: REACTOGENICITY, IMMUNOGENICITY, AND EFFICACY STUDIES OF PURIFIED ESCHERICHIA COLI (H10407 AND B7A) PILI VACCINES

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PREFACE

Acute diarrheal infections comprise one of the paramount health problems of military importance. Travelers' Diarrhea is a major cause of morbidity when U.S. military personnel travel to less-developed areas of the world where levels of sanitation and personal hygiene practices are primitive. Travelers' Diarrhea occurs with greatest frequency within the first few days and weeks of arrival, thereby impairing efficiency of military personnel at a time when they may be most needed.

Enterotoxigenic Escherichia coli (ETEC) have been shown to be the most common etiologic agent of Travelers' Diarrhea, accounting for approximately one-half of cases. Control of ETEC infections would be a major step toward diminishing the toll of Travelers' Diarrhea.

Two years ago, in studies supported by the U.S. Army Medical and Research Development Command, we demonstrated that clinical diarrheal infection due to ETEC stimulated homologous immunity to re-challenge. We hypothesized that the mechanism of immunity was operative at the intestinal mucosal surface and involved prevention of adherence of ETEC to proximal, small intestinal mucosa. We suggested a role for vaccines consisting of purified adhesion pili.

Vaccination of pregnant gilts with purified K88, K99, and 987-type purified pili vaccines has been shown to provide striking clinical protection against challenge with ETEC bearing the homologous antigen for piglets suckled on the immunized dams.

Purified pili vaccines could theoretically significantly reduce the incidence of ETEC Travelers' Diarrhea. First, the relevant adhesion factors that allow ETEC to interact with proximal intestinal mucosal cells must be identified; they must then be purified and tested for safety, immunogenicity and efficacy as vaccines in man. During the past year our research has focused on the above-mentioned tasks.

ABSTRACT

Laboratory Studies:

Enterotoxigenic Escherichia coli (ETEC) must possess accessory virulence properties, in addition to heat-labile (LT) or heat-stable (ST) enterotoxins, to be virulent for man or animals. Amongst these additional virulence properties are adhesion or colonization factors that enable ETEC to adhere to mucosa of the small intestine, thereby escaping the potent peristalsis defense mechanism. Colonization factor antigens I and II (CFA/I, CFA/II), which cause mannose-resistant hemagglutination (MRHA) of human type A and B and bovine erythrocytes, respectively, and type 1 somatic pili, which cause mannose-sensitive hemagglutination (MSHA) of guinea pig erythrocytes, all allow E. coli to attach to epithelial cells.

We tested ETEC and enteropathogenic E. coli (EPEC) strains utilized in volunteer challenge studies for CFA/I, CFA/II and type 1 somatic pili after growth on CFA agar and in resting broth. The first group of strains tested included 7 ETEC, and 3 EPEC strains (8 of these 10 caused diarrhea when fed to volunteers) and 15 normal flora control E. coli strains. Of the 6 ETEC and 2 EPEC strains that caused diarrhea in volunteers, only 1 had CFA/I (E. coli H10407) and 1 had CFA/II (B_2C). The majority of the virulent and normal flora strains possessed type 1 somatic pili. One normal flora strain had CFA/II. Thus, there remain six virulent strains (4 ETEC, 2 EPEC) which unequivocally cause diarrhea in man yet which do not cause MRHA and lack CFA/I or II. Five of these 6 possess type 1 somatic pili, which may serve as the organelles of adhesion to epithelial cells. In at least one strain (E. coli 214-4) that produces only ST, neither CFA/I, CFA/II, nor type 1 somatic pili were found. Other surface structures not presently identified must serve as adherence factors in this strain.

In a second laboratory study we examined 41 ETEC strains from cases of Travelers' Diarrhea in Africa and 12 EPEC strains from cases of infant diarrhea in

Hungary. Results are summarized below:

Toxin Type of Strains	No. Strains Tested	Strains with: CFA/I	CFA/II	Type 1 Somatic Pili
LT+/ST+	11	2(18%)	5(45%)	1(9%)
LT+/ST-	14	0	2(14%)	11(79%)
LT-/ST+	16	2(13%)	0	4(25%)
EPEC	12	0	0	10(83%)

These data suggest that CFA/I and CFA/II are frequently encountered in LT+/ST+ strains, rarely in LT- alone or ST-alone strains, and never in EPEC strains. In contrast, LT-alone strains and EPEC typically have type 1 somatic pili. The adhesion factors possessed by the majority of ST-alone strains are unidentified.

In order to examine the stability of CFA plasmids in ETEC strains we serially passed on CFA agar 7 ETEC strains having CFA/I or II. The strains were sub-cultured 7 times. Only 1 of the 7 strains lost its CFA(I) plasmid after the 7 subcultures, establishing that the CFA plasmids are generally quite stable.

Clinical Studies:

We tested a parenteral purified type 1 somatic pilus vaccine from *E. coli* H10407 prepared by Dr. C.C. Brinton, Jr. of the University of Pittsburgh. Twenty-one volunteers received primary IM inoculations of 45, 90, 900 or 1800 mcg of purified pili. No adverse reactions were encountered after a single dose, and all vaccinees had four-fold or greater rises in anti-pili antibody. The geometric mean titer increased as dosage of vaccine increased. Fifteen persons received an 1800 mcg.booster inoculation 28 days following the primary dose. Five of these persons experienced local adverse reactions consisting of tenderness, heat, erythema and induration. Pili vaccines did not disrupt intestinal transit (measured with Carmine red dye), D-Xylose absorption or alter the prevalence of normal colonic flora *E. coli* that possess type 1 somatic pili of the H10407 antigenic variety.

Six volunteers who received two doses of pili vaccine and 7 control volunteers were challenged with $5x10^8$ virulent ETEC H10407. Only 2 of 6 vaccinees developed diarrheal illness versus 7 of 7 unimmunized control volunteers ($p=0.04$). While severe abdominal cramps, malaise and vomiting accompanied the diarrheal syndrome in the controls, these symptoms and signs were not seen in the 2 ill vaccinees. These preliminary results represent the first reactogenicity/immunogenicity/efficacy studies of E. coli pili vaccines in man. The encouraging, positive results suggest that by stimulating immune mechanisms involving interference with attachment of ETEC to intestinal epithelial cells, we are on the correct path toward eventual control of ETEC disease via immunologic methods.

A. HEMAGGLUTINATION, COLONIZATION FACTOR PILI AND TYPE 1 SOMATIC PILI IN ENTEROTOXIGENIC AND ENTEROPATHOGENIC ESCHERICHIA COLI THAT CAUSE DIARRHEA IN VOLUNTEER STUDIES

Introduction:

Enterotoxigenic *Escherichia coli* (ETEC) must possess accessory virulence properties, in addition to heat-labile or heat-stable enterotoxin, to be virulent for man or animals. The best-recognized accessory virulence properties are colonization factors that enable ETEC to adhere to mucosa of the small intestine thereby escaping the potent peristalsis defense mechanism.[1]

Some animal strains possess plasmid-mediated, pili-like, surface organelles of attachment, such as K88[2] and K99[3] antigens, that are associated with mannose-resistant haemagglutination (MRHA)[4,5]. Analogs, colonization factor antigens I[6] and II[7] (CFA/I, CFA/II), have been described in human strains that cause MRHA of human type A and B[8,9] (CFA/I) or bovine (CFA/II) erythrocytes.[7,8] Many animal ETEC strains which lack MRHA and K88 or K99 are, nevertheless, virulent.[1,10] Porcine *E. coli* 987 is an example;[11-13] it attaches to intestinal epithelium by another class of pili not associated with haemagglutination.[11-14] A third class of pili, type 1 somatic ("common") pili,[15,16] also manifest adhesive properties for mucosal cells[14,15,17-19] and cause mannose-sensitive haemagglutination (MSHA) of guinea pig erythrocytes;[20-23] their role in pathogenesis of ETEC diarrhea is under investigation.[14,23,24]

The concept of immunologic control of ETEC diarrhea via multivalent, purified pili vaccines is highly attractive. Indeed, vaccination of pregnant gilts with purified K88[25], K99[26] and 987-type[27] purified pili vaccines has been shown to provide striking clinical protection against challenge with *E. coli* bearing the homologous antigen for piglets suckled on the immunized dams. A trivalent K88, K99, 987-type pili vaccine is expected to reduce piglet colibacillosis by 50-80%.

Purified pili vaccines could theoretically significantly reduce the incidence of ETEC Travelers' Diarrhea but the relevant colonization factor antigens in human ETEC strains must first be identified. Evans et al reported that 25 of 29 of their ETEC strains from Travelers' Diarrhea (86%) exhibited MRHA of human type A erythrocytes and possessed CFA/I.[6] In contrast, while Orskov and Orskov[8] found MRHA and CFA/I commonly in 078 serogroup isolates from human diarrhea, they occurred in none of 49 ETEC pathogens from five other serogroups. Similarly, Gross et al[28] examined 89 ETEC strains from patients with diarrhea and found that only nine strains (10%) exhibited MRHA and only six strains (7%) were CFA/I-positive; four of these were serogroup 078. Since the MRHA-type pili are plasmid-mediated, one possible explanation for the differences noted in these reports is that the strains tested by Orskov and Orskov and Gross et al could have lost their plasmids at the time of testing. Alternatively, other colonization factors may exist in human ETEC strains that are not associated with MRHA, and are perhaps analogous to 987-type pili of porcine strains.

In an attempt to help resolve this confusion we examined the various ETEC and enteropathogenic E. coli (EPEC) strains that have been used in volunteer challenge studies at the University of Maryland (whose virulence, or lack thereof, is thus indisputable) for the presence of CFA/I, CFA/II and type 1 somatic pili.

Materials and Methods:

Bacterial Strains:

Strains B7A, B2C and H10407 were supplied by Samuel Formal, Washington, D.C.; strains 214-4, E2528-C1, TD225-C4 and H10407P were provided by Joy Wells, Atlanta, Ga.; strain H10407P was also provided by Dolores Evans, Houston, Texas; Bernard Rowe of Colindale, England sent strains E2348/69, E851/71 and E74/68. After receipt from donors, the strains were stored until utilized for clinical

or bacteriologic studies by inoculating multiple clones into skimmed milk which was aliquoted into 12-24 individual glass vials and frozen to -70°C.

The origin, serotype, enterotoxin type and ability to cause diarrhea in healthy volunteers of each strain are listed in Table 1. Strains were tested for LT by Y-1 adrenal cell assay and for ST by the infant mouse assay.

Fifteen control E. coli strains representing normal flora were randomly selected from stool cultures of 15 healthy young adults that were plated on Eosin-Methylene-Blue Agar. These strains were shown to be negative for LT or ST.

Cultivation of Strains:

A vial containing each strain was defrosted and 0.01 ml. of milk suspension was plated onto 5% sheep's blood in trypticase soy agar. Each E. coli strain was cultivated by two separate methods and then tested independently; multiple E. coli clones were picked and subcultured either onto CFA agar[9] or into Mueller-Hinton broth (15 ml.).[22] CFA agar plates were incubated aerobically for 24 hours at 37°C prior to testing. Mueller-Hinton broth cultures (15 ml.) were incubated aerobically for 48 hours at 37°C, sub-cultured into Mueller-Hinton broth for 48 additional hours and tested.[22] The tubes of broth were centrifuged, decanted and the pellets resuspended with saline to a concentration of 10^{10} organisms/ml.

Haemagglutination:

CFA/I and CFA/II were identified by MRHA of human type A or bovine erythrocytes, respectively, while MSHA of guinea pig cells was used to demonstrate type 1 somatic pili. Human type A, guinea pig and bovine erythrocytes were obtained fresh, washed twice in 0.85% saline and divided to prepare a 3% suspension in saline or mannose (1.0%). Haemagglutinations were carried out on glass slides at 24°C with human and guinea pig cells and at 4°C with bovine cells. Several bacterial colonies were harvested with a sterile wooden applicator stick and mixed on the slide with 0.025 ml. of erythrocyte suspension by the method of Evans et al. Agglutination

was graded from 0 to 4+ depending on rapidity and strength of reaction.

Agglutination with Specific Antisera:

Anti-CFA/I antibody was prepared by the method of Evans et al[7,19] wherein E. coli H10407 organisms grown on CFA agar were used to repeatedly inoculate two 2.5 kg. albino rabbits intravenously. The animals were exsanguinated after 21 days when the serum agglutinated H10407 to a titer of 1:512. The sera were repeatedly absorbed with E. coli H10407P (which lacks CFA/I) until the sera strongly agglutinated H10407 but failed to agglutinate H10407P.

Results:

The seven ETEC and three EPEC strains that were fed to volunteers as part of a long-term program involving studies of pathogenesis, immunity and vaccine development are described in Table 1. All ETEC strains except H10407P, the laboratory mutant of H10407, caused diarrhea in doses of 10^6-10^{10} organisms (Table 1). When grown on solid CFA agar, one ETEC strain, H10407, caused MRHA of human erythrocytes (signifying CFA/I) and one strain, B2C, caused MRHA of bovine but not human cells (indicative of CFA/II) (Table 2). H10407 was strongly agglutinated by anti-CFA/I; no other strains were positive.

When broth-grown organisms were tested, different results were encountered (Table 3). All strains except 214-4, B2C and E74/68 exhibited strong MSHA of guinea pig and human erythrocytes which is characteristic of type 1 somatic piliation. Neither H10407 nor B2C manifested MRHA after cultivation in broth.

Four ETEC strains (B7A, TD225-C4, E2528-C1 and 214-4) and two EPEC strains (E851/71 and E2348/69) that caused diarrheal illness in volunteers did not exhibit MRHA of human or bovine erythrocytes after growth on CFA agar or in broth and were not agglutinated by anti-CFA/I or anti-CFA/II antibody.

Results of testing the normal flora control strains are seen in Tables 4 and 5. One strain exhibited MRHA of bovine but not human erythrocytes after growth on CFA agar and in broth. Type 1 somatic pili were frequently found in broth-grown strains as evidenced by MSHA of guinea pig and human cells.

Discussion

If a small number of antigenic types of colonization factors could be identifie that were common to human ETEC pathogens, the feasibility for immunoprophylaxis of human ETEC disease, particularly Travelers' Diarrhea, would be great. Evans et al identified a plasmid-mediated,[29] pilus-like organelle,[6] CFA/I, in human pathogen H10407 which caused MRHA of human type A erythrocytes,[8,9] and thus is analogous to the K88 and K99 adhesion pili of animal ETEC strains.[2-5] A second antigen, CFA/II, was described which causes MRHA of bovine but not human erythrocytes.[7] Independent observers were unable to demonstrate MRHA of human erythrocytes of CFA/I in series of ETEC isolates from human diarrhea with anywhere near the high frequency reported by Evans et al.[6] In order to rule out the role of plasmid loss as an explanation for these discrepancies, we undertook characterization of the ETEC and EPEC strains that had been fed to volunteers, i.e. strains whose virulence for man is unequivocal.[30-34] The strains, which were stored in milk at -70°C, were passed only two or three times in preparation for haemagglutination and agglutination testing, which is equivalent to the number of passages involved in preparation of inocula for human challenge.

MRHA of human erythrocytes and CFA/I were found in only one strain, H10407 (Table 2). One other strain that caused diarrhea, B2C, manifested MRHA of bovine but not human cells, indicative of CFA/II.

There remain four ETEC strains and two EPEC which do not cause MRHA of human, bovine or guinea pig erythrocytes, and do not possess CFA/I or CFA/II, yet are clearly virulent for man. The ETEC strains include all enterotoxigenic phenotypes: LT+/ST+, LT+/ST-, and LT-/ST+. Our interpretation of these findings is that there exist other classes of adhesion pili which are not associated with MRHA or other surface structures that serve as colonization factors such as poly-

saccharides or slime layers. Of the eight ETEC and EPEC strains that resulted in diarrhea when fed to volunteers, six caused MSHA of guinea pig erythrocytes after static growth in broth, indicative of type 1 somatic piliation. These results confirm the findings of Brinton et al who first demonstrated the high frequency of somatic type 1 pili in ETEC strains from man. It is also feasible, as suggested by Brinton, that type 1 somatic pili serve as colonization factors in some ETEC and EPEC strains that lack the MRHA class of pili and that this antigen may have an important role as a component in a pili vaccine to prevent human diarrhea due to ETEC.

B. HEMAGGLUTINATION, COLONIZATION FACTOR PILI, AND TYPE 1 SOMATIC PILI IN ENTEROTOXIGENIC AND ENTEROPATHOGENIC E. COLI FROM AFRICA AND EUROPE.

After our challenge *E. coli* strains had been tested for HA and piliation and certain patterns were apparent, we became eager to examine a larger number of ETEC and EPEC from diverse geographic areas. A collaboration was undertaken with Dr. R. Bradley Sack of Baltimore City Hospital and Dr. Joo of Budapest, Hungary. Dr. Sack provided 23 ETEC isolates from proven cases of Travelers' Diarrhea in Morocco and 18 strains from cases in Kenya. Dr. Joo provided 12 strains of EPEC from cases of infant diarrhea.

These strains were examined for CFA/I, CFA/II and Type 1 somatic pili as described above. The occurrence of these factors in the strains is shown in Table 6 (Morocco), Table 7 (Kenya) and Table 8 (Hungary). The results are summarized in Table 9. There was a relationship between type of toxins produced by the strains and their piliation. Of 11 ETEC strains that produced both LT and ST, 2 had CFA/I (18%) and 5 had CFA/II. Thus, 67% of LT+/ST+ strains elaborate one of the known CFA antigens. In contrast, CFA/I and II were distinctly rare in LT+/ST- and LT-/ST+ strains (Table 9). Type 1 somatic pili, on the other hand, occurred in 79% of LT+/ST- strains and in 83% of EPEC strains.

These data suggest that a multivalent pilus vaccine containing CFA/I, CFA/II and one or two common antigenic varieties of type 1 somatic pili could theoretically provide broad immunity.

In an effort to assess the lability of the CFA/I and CFA/II phenotypes, at least one of which (CFA/I) is plasmid-mediated, we serial passaged 7 of Dr. Sack's strains that showed CFA/I or II upon initial examination (Table 10). These 7 strains were sub-cultured seven times on CFA agar and re-examined (Table 10). Despite multiple passage, only one isolate changed and lost its CFA/I. These data demonstrate that the CFA/I and CFA/II properties are quite stable and in true pathogens would not expect to be easily lost in the course of routine culture and subculture.

C. PURIFIED TYPE 1 SOMATIC PILUS VACCINE FROM ENTEROTOXIGENIC ESCHERICHIA COLI H10407

Introduction

When travelers from the industrialized countries work, study or vacation in less-developed areas of the world, as many as 30-70% develop acute diarrheal disease during the first weeks of residence.[35-40] Enterotoxigenic *Escherichia coli* (ETEC) are the most frequent etiologic agent of Travelers' Diarrhea, accounting for 30-60% of cases.[35,37,40] Thus, a highly-effective vaccine to prevent ETEC diarrhea could diminish the attack rate of Travelers' Diarrhea by as much as 60%.

It has been shown in recent years that various types of surface pili serve as virulence factors for ETEC pathogens of man and animals. Purified pilus vaccines have been shown to be safe and highly effective in preventing severe ETEC diarrhea in piglets. Piglets suckled on dams immunized with purified pili vaccines were significantly protected against diarrhea upon challenge with the homologous ETEC, in comparison with piglets suckled on control dams.

ETEC strain H10407 produces two recognized types of non-conjugation pili. One pilus causes agglutination of guinea pig erythrocytes but this phenomenon is

inhibited by D-mannose; these are referred to as type 1 somatic pili. The other pili promote agglutination of human type A or B erythrocytes, even in the presence of D-mannose; these are referred to as non-mannose sensitive (NMS) pili of H10407 and are probably identical to the colonization factor antigen I of H10407 described by Evans et al. Studies by Brinton et al and Levine et al have shown that the great majority of ETEC strains isolated from persons with diarrhea, irrespective of the type of enterotoxin produced, elaborate type 1 somatic pili. Approximately 40% of the type 1 somatic pili found on human ETEC enteropathogens, according to Brinton et al, are identical to or closely resemble the antigenic variety found on E. coli H10407. This observation suggests that this antigen could serve as an important component in a multiple pilus antigen vaccine against human ETEC diarrhea.

In this study we examined the reactogenicity, immunogenicity and efficacy in adult volunteers of a purified E. coli H10407 type 1 somatic pilus vaccine.

Vaccine:

Pili were purified in the laboratory of Dr. C.C. Brinton, Jr. by the method previously described. Briefly, E. coli H10407 was cloned and colonies were selected that expressed type 1 somatic pili. Piliated phase colonies were inoculated into glucose/yeast extract/tryptone (GYET) still broth. After overnight incubation at 39°C, the still broth culture was used to inoculate trays containing GYET agar. Following overnight incubation at 39°C, the confluent bacterial growth evident on the agar was harvested with 0.01 u phosphate-buffered saline (PBS) (0.85%), pH 7.2. The bacterial suspension was blended at 13,000 rpm for 5 minutes in a Sorvall Omnimixer to shear pili from the cells. Cells were then removed by centrifugation leaving pili in the supernatant. Type 1 pili were crystallized by addition of $MgCl_2$ to 0.1 Molar. Crystals were removed by centrifugation and retention of the pellet. The pellet was redissolved with the PBS and the cycle of crystallization, centrifugation and redissolution was repeated four times after

which the pili suspension was sterilized by filtration. Purity of the pili preparation was documented by Brinton and co-workers using darkfield microscopy, electron microscopy, ultraviolet spectroscopy, polyacrylamide gel electrophoresis and agglutination with antibody to H10407 type 1 and NMS pili. Vaccine was prepared to a concentration of 1800 mcg. of purified pili per ml.

Reactogenicity:

Twenty-one volunteers received primary immunization with purified type 1 pili vaccine given IM in doses of 45, 90, 900 or 1800 mcg. Neither erythema, induration, heat, tenderness, fever, malaise nor other adverse reactions were encountered (Table 11). Fifteen volunteers received a booster IM inoculation of 1800 mcg. of pili vaccine; five individuals developed local reactions including induration, heat or erythema (Table 11). Local reaction after the booster occurred in persons who had received primary inoculations with 45 (2), 900 (2) and 1800 (1) mcg doses of vaccine. Onset of the local reactions was approximately 24 hrs. The reactions were described as mild to moderate by the volunteers. No nausea, vomiting or diarrhea was encountered.

Immunogenicity:

The definitive serologic results from this type 1 pili vaccine study will be forthcoming at some future date from Dr. Brinton's laboratory. In the meantime, Charles Young in the CVD rapidly developed an ELISA technique for measurement of type 1 somatic pili antibody in serum; this development allowed us to get preliminary impressions of the antigenicity of the vaccine.

Antibody to H10407 type 1 somatic pili was measured by an enzyme-linked immunosorbent assay (ELISA). Polystyrene, 96 well, microtiter plates were coated with 0.1 ml. of purified pili (100 mcg/ml); each antigen well had a corresponding blank well with washing buffer (phosphate-buffered saline, 0.5% Tween 20, 1% fetal calf serum). Two-fold dilutions (1:100-1:12,800) of unknown sera and positive control sera (0.025 ml) were added to antigen-containing and blank wells. After

incubation for 60 min. at 37°C, the wells were rinsed thrice with washing buffer and reacted for 60 min. at 37°C with goat anti-human IgG conjugated with alkaline phosphatase. The plates were again washed three times and enzyme substrate was added (p-nitrophenylphosphate). The reaction was stopped after 30 minutes by the addition of 3M NaOH. Optical density (O.D.) was read with an ELISA colorimeter. The optical density of the blank well was subtracted from that of the antigen-containing well to derive the net O.D. The highest serum dilution giving a net O.D. $\geq$ 0.15 was considered the titer for that serum.

Anti-Type 1 Pili Antibody:

Following immunization with a single IM inoculation of purified pili vaccine, all volunteers demonstrated four-fold or greater rises in pili antibody by ELISA (Table 12). Geometric mean titer (GMT) rose with increasing vaccine dose (Table 13). Some volunteers who received a high initial dose (900 or 1800 mcg) followed by a booster had titer as high as 1:56,000 (Table 12).

Vaccine Efficacy:

One month after administration of the booster dose (1800 mcg.) of parenteral pili vaccine, six vaccinees agreed to participate in a challenge study along with seven matched control volunteers. Following ingestion of 5×10^8 virulent H10407 organisms, all seven controls developed diarrheal illness (Table 14). Three controls purged copious rice-water stools resulting in total diarrheal stool volumes of 3.8, 7.5 and 9.9 liters and two required intravenous fluids to maintain hydration. In contrast, only 2 of 6 vaccinees developed diarrheal illness ($p=.04$, Fisher's Exact Test). While 6 of 7 ill controls experienced malaise and vomiting, none of the vaccinees, ill or well, had these complaints (Table 14). The diarrheal illness manifested by the two ill vaccinees was similar in incubation, total volume, number of loose stools and duration to that seen in the controls.

Despite clinical protection, all vaccinees, as well as controls, excreted virulent H10407. Within 48 hrs. post-challenge, all challenged volunteers, vaccinees

and controls, were shedding E. coli H10407 as the predominant aerobic coliform. Of 1200 E. coli colonies picked from stool culture plates during the first five days post-challenge, 1183 (98.6%) were strongly agglutinated by H10407 antiserum. Six hundred and two of these clones were tested for the presence of H10407 type 1 somatic and NMS pili by agglutination with specific antiserum; all 602 had both types of pili.

Gastrointestinal Function:

Intestinal Transit Time:

Intestinal transit time was measured by the Carmine red dye method[41] prior to immunization and 28 days thereafter in 16 vaccinees. The mean transit time was similar before (29 hrs.) and 28 days after immunization (20 hrs.). Six vaccinees (whose mean transit time was 29 hrs. pre-immunization and 28 hrs. one month later) also had transit times measured on day +56 (one month after booster immunization) and five days after challenge with virulent ETEC. Mean transit time in these six persons was 51 hrs. on day +56 and 24 hrs. on the fifth day post-challenge.

D-xylose Absorption and Excretion Tests:

To establish the mean and range of normal values for the one hour blood xylose absorption test[42-44] of a normal adult population tested by our laboratory, specimens from 30 healthy adults were run. These 30 individuals comprised 21 pili vaccinees prior to immunization, seven unimmunized control volunteers prior to challenge, and two other healthy adults. The mean blood D-xylose level one hour post-ingestion of the monosaccharide was 14.2 mg/dl $\pm$ 5.2 (mean $\pm$ S.D.).

In sixteen recipients of pili vaccine who had D-xylose absorption tests performed prior to and one month post-immunization, the levels before (mean 12.6 mg/dl $\pm$ 4.5) and one month after vaccine (mean 14.7 mg/dl $\pm$ 4.1) were similar ($p>0.1$, paired Student's t test).

Six vaccinees received an 1800 mcg. booster dose of vaccine and participated in a vaccine efficacy challenge one month thereafter. These persons provided an opportunity to analyse D-xylose absorption test values from four points in time: pre-immunization (Day 0), pre-booster (Day +28), pre-challenge (approximately Day +57) and post-challenge (Day +64). There were no significant differences in this group among mean one hour blood xylose on day 0 (14.8 mg/dl $\pm$ 2.7), day +28, 14.4 mg/dl $\pm$ 4.0) or day +57 (15.7 mg/dl $\pm$ 2.8). However, the one hour D-xylose levels of the group post-challenge (mean 9.3 mg/dl $\pm$ 4.2) were significantly lower ($p<0.01$, Student's paired t test) compared with pre-vaccine and pre-challenge levels. The two vaccinees with diarrhea had the most prominent falls in blood xylose between pre- and post-challenge specimens; 16.7 mg/dl fell to 2.1 and 13.6 dropped to 2.8 mg/dl.

The one-hour blood xylose levels also fell significantly ($p<0.05$) in seven control volunteers between pre-challenge (16.9 mg/dl $\pm$ 7.1) and post-challenge (9.3 mg/dl $\pm$ 4.2) examinations.

The one hour D-xylose absorption test was sufficiently sensitive to detect small bowel dysfunction associated with overt diarrheal illness in nine persons (seven controls, two vaccinees) as well as a defect associated with subclinical infection due to ETEC in the four vaccinees who remained clinically well after challenge. In contrast, no small bowel dysfunction could be detected by xylose absorption tests following primary or booster immunization.

We also collected urine for five hours and performed D-xylose excretion tests in the six vaccinees and seven controls who participated in the challenge study. Pre-challenge all 13 volunteers had normal values ($\geq$1.2 gm/5 hr. urine volume). Post challenge six of seven ill controls, both ill vaccinees and one of four well vaccinees had abnormal test results.

Stool specimens from 15 vaccinees were cultured pre- and 28 days post-challenge. Stool was plated on EMB agar and 15 E. coli clones per specimen were sub-cultured twice into Mueller-Hinton broth and incubated resting for 48 hrs. at

37°C. The cultures were spun, supernatant discarded and the bacterial pellet tested for agglutination with antiserum to H10407 type 1 somatic pili antibody. Results are shown in Table 15. Vaccine did not alter the prevalence of normal colonic flora E. coli that possess type 1 somatic pili of the H10407 antigenic variety.

These data provide the first tangible results in humans that control of ETEC diarrhea is a feasible goal by use of pili vaccines. Further steps along the way will involve testing of other pili antigens to devise a multivalent, "broad-spectrum" vaccine and evaluation of orally-administered vaccine.

Summary

Purified type 1 somatic pili vaccine from E. coli H10407 was given parenterally to 21 healthy adults. The vaccine was found to be non-reactogenic after one dose, gave some local reactions upon booster inoculation and was highly immunogenic. When challenged with virulent E. coli H10407, the attack rate in vaccinees was significantly lower (2/6) than in controls (7/7) (p=0.04).

REFERENCES

1. Bertschinger, H.U., Moon, H.W., Whipp, S.C. Association of Escherichia coli with the Small Intestinal Epithelium. I. Comparison of Enteropathogen and Nonenteropathogenic Porcine Strains in Pigs. Infect. Immun. 5:595-605, 1972.

2. Jones, G.W., Rutter, J.M. Role of the K88 Antigen in the Pathogenesis Neonatal Diarrhea Caused by Escherichia coli in Piglets. Infect. Immun. 6:918-927, 1972.

3. Moon, H.W., Nagy, B., Isaacson, R.E., et al. Occurrence of K99 antigen on Escherichia coli Isolated from Pigs, and Colonization of Pig Ileum by K99+ Enterotoxigenic E. coli from Calves and Pigs. Infect. Immun. 15:614-620, 1977.

4. Jones, G.W., Rutter, J.M. The Association of K88 Antigen with Haemagglutinating Activity in Porcine Strains of Escherichia coli. J. Gen. Microbiol. 84:135-144, 1974.

5. Burrows, M.R., Sellwood, R., Gibbons, R.A. Haemagglutinating and Adhesive Properties Associated with the K99 Antigen of Bovine Strains of Escherichia coli. J. Gen. Microbiol. 96:269-275, 1976.

6. Evans, D.G., Evans, D.J., Jr., Tjoa, W.S., et al. Detection and Characterization of Colonization Factor of Enterotoxigenic Escherichia coli Isolated from Adults with Diarrhea. Infect. Immun. 19:727-736, 1978.

7. Evans, D.G., Evans, D.J., Jr. New Surface-Associated Heat-Labile Colonization Factor Antigen (CFA/II) Produced by Enterotoxigenic Escherichia coli of Serogroups 06 and 08. Infect. Immun. 21:638-647, 1978.

8. Orskov, I., Orskov, F. Special O:K:H Serotypes Among Enterotoxigenic E. coli Strains from Diarrhea in Adults and Children. Occurrence of the CF (Colonization Factor) Antigen and of Hemagglutinating Abilities. Med. Microbiol. Immunol. 163:99-110, 1977.

9. Evans, D.G., Evans, D.J., Jr., Tjoa, W. Hemmagglutination of Human Group A Erythrocytes by Enterotoxigenic Escherichia coli Isolated from Adults with Diarrhea: Correlation with Colonization Factor. Infect. Immun. 18:330-337, 1977.

10. Hohmann, A., Wilson, M.R. Adherence of Enteropathogenic Escherichia coli to Intestinal Epithelium In Vivo. Infect. Immun. 12:866-880, 1975.

11. Isaacson, R.E., Nagy, B., Moon, H.W. Colonization of Porcine Small Intestine by Escherichia coli: Colonization and Adhesion Factors of Pig Enteropathogens that Lack K88. J. Infect. Dis. 135:531-539, 1977.

12. Nagy, B., Moon, H.W., Isaacson, R.E. Colonization of Porcine Intestine by Enterotoxigenic Escherichia coli: Selection of Piliated Forms In Vivo, Adhesion of Piliated Forms to Epithelial Cells In Vitro, and Incidence of a Pilus Antigen among Porcine Enteropathogenic E. coli. Infect. Immun. 16:344-352, 1977.

13. Moon, H.W., Nagy, B., Isaacson, R.E. Intestinal Colonization and Adhesion by Enterotoxigenic Escherichia coli: Ultrastructural Observations on Adherence to Ileal Epithelium of the Pig. J. Infect. Dis. 136 Supp. S124-129, 1977.

14. Isaacson, R.E., Fusco, P.C., Brinton, C.C., et al. In Vitro Adhesion of Escnerichia coli to Porcine Small Intestinal Epithelial Cells: Pili as Adhesive Factors. Infect. Immun. 21:392-397, 1978.

15. Brinton, C.C. The Structure, Function, Synthesis and Genetic Control of Bacterial Pili and a Molecular Model for DNA and RNA Transport in Gram Negative Bacteria. Trans. N.Y. Acad. Sci. 27:1003- , 1965.

16. Swaney, L.M., Ying-Ping, L., Chuen-Mo, T., et al. Isolation and Characterization of Escherichia coli Phase Variants and Mutants Deficient in Type 1 Pilus Production. J. Bacteriol. 130:495-505, 1977.

17. Duguid, J.P., Gillies, R.R. Fimbriae and Adhesive Properties in Dysentery Bacilli. J. Path. Bact. 74:397-411, 1957.

18. Ofek, I., Mirelman, D., Sharon, N. Adherence of Escherichia coli to Human Mucosal Cells Mediated by Mannose Receptors. Nature 265:623-625, 1977.

19. Salit, I.E., Gotschlich, E.C. Type 1 Escherichia coli Pili: Characterization of Binding to Monkey Kidney Cells. J. Exp. Med. 146:1182-1194, 1977.

20. Salit, I.E., Gotschlich, E.C. Hemagglutination by Purified Type 1 Escherichia coli Pili. J. Exp. Med. 146:1169, 1977.

21. Duguid, J.P. Functional Anatomy of Escherichia coli with Special Reference to Enteropathogenic E. coli. Rev. Latino. Microbiol. 7:Suppls. 13-14, 1-6, 1964.

22. Old, D.C. Inhibition of the Interaction between Fimbrial Hemagglutinins and Erythrocytes by D-Mannose and Other Carbohydrates. J. Gen. Microbiol. 71:149-157, 1972.

23. Brinton, C.C., Jr. The Piliation Phase Syndrome and the Uses of Purified Pili in Disease Control. In Proceedings of the XIIIth Joint U.S.-Japan Conference on Cholera, Atlanta, Ga., September, 1977, p. 33-70. DHEW publication No. (NIH) 78-1590. National Institutes of Health, Bethesda,Md.

24. Levine, M.M., Daya, V. Haemagglutination, Pili and Diarrhoeagenic Potential of Escherichia coli Strains in Man. 18th Interscience Conference on Antimicrobial Agents and Chemotherapy; Abstract. Atlanta, Ga., 1978.

25. Rutter, J.M., Jones, G.W. Protection against Enteric Disease Caused by Escherichia coli - a Model for Vaccination with a Virulence Determinant. Nature 242:531-532, 1973.

26. Isaacson, R.E., Morgan, R.L., Moon, H.W., et al. Immunization against Enterotoxigenic Escherichia coli Infection by Vaccination and Purified Pili. In Proceedings of the XIIIth Joint U.S.-Japan Conference on Cholera, Atlanta, Ga., September, 1977, p. 285-293. DHEW publication No. (NIH) 78-1590. National Institutes of Health, Bethesda, Md.

27. Nagy, B., Moon, H.W., Isaacson, R.E., et al. Immunization of Suckling Pigs Against Enteric Enterotoxigenic Escherichia coli Infection by Vaccinating Dams with Purified Pili. Infect. Immun. 21:269-274, 1978.

28. Gross, R.J., Cravioto, A., Scotland, S.M., et al. The Occurrence of Colonization Factor (CF) in Enterotoxigenic Escherichia coli FEMS Microbiol. Letters 3:231-233, 1978.

29. Evans, D.G., Silver, R.P., Evans, D.J., Jr., et al. Plasmid-Controlled Colonization Factor Associated with Virulence in Escherichia coli Enterotoxigenic for Humans. Infect. Immun. 12:656-667, 1975.

30. Levine, M.M., Caplan, E.S., Waterman, D., et al. Diarrhea caused by Escherichia coli that Produce Only Heat-Stable Enterotoxin. Infect. Immun. 17:78-82, 1977.

31. Levine, M.M., Nalin, D.R., Hoover, D.L., et al. Immunity to Enterotoxigenic Escherichia coli. Presented at 17th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York City, October 14, 1977.

32. Levine, M.M., Bergquist, E.J., Nalin, D.R., et al. Escherichia coli Strains that Cause Diarrhea but do not Produce Heat-Labile or Heat-Stable Enterotoxins and are Non-Invasive. Lancet I:1119-1122, 1978.

33. DuPont, H.L., Formal, S.B., Hornick, R.B., et al. Pathogenesis of Escherichia coli Diarrhea. N. Eng. J. Med. 285:1-9, 1971.

34. Levine, M.M., Nalin, D.R., Hoover, D.L., et al. Immunity to Enterotoxigenic Escherichia coli. Infect. Immun. 23:729-736, 1979.

35. Gorbach, S.L., B.H., Kean, D.G., Evans, et al. Travelers' Diarrhea and Toxigenic Escherichia coli. N. Engl. J. Med. 292:933-936, 1975.

36. Kean, B.H. The Diarrhea of Travelers to Mexico: Summary of Five-year Study. Ann. Intern. Med. 59-605-614, 1963.

37. Merson, M.H., G.K., Morris, D.A., Sack, et al. Travelers' Diarrhea in Mexico: a Prospective Study of Physicians and Family Members Attending a Congres. N. Engl. J. Med. 294:1299-1305, 1976.

38. Rowe, B. Escherichia coli 0148 and Diarrhoea in Adults. Br. Med. J. 3:741,1974.

39. Sack, D.A., D.C., Kaminsky, R.B., Sack, et al. Prophylactic Doxycycline for Travelers' Diarrhea. Results of a Prospective Double Blind Study of Peace Corps Volunteers in Kenya. N. Engl. J. Med. 298:758-763, 1978.

40. Shore, E.G., A.G., Dean, K.J., Holik, et al. Enterotoxin-Producing Escherichia coli and Diarrheal Disease in Adult Travelers: a Prospective Study. J. Infect. Dis. 129:577-582, 1974.

41. Dimson, S.B. Carmine as an Index of Transit Time in Children with Simple Constipation. Arch. Dis. Child. 45:232-235, 1970.

42. Buts, J.P., Morin, C.L., Roy, C.C., et al. One Hour Blood Xylose Test: A Reliable Index of Small Bowel Function. J. Pediat. 90:729-733, 1978.

43. Christie, D.L. Use of the One Hour Blood Xylose Test as an Indicator of Small Bowel Mucosal Disease. J. Pediat. 92:725-728, 1978.

44. Morin, C.L., Buts, J.P., Weber, A., et al. One-Hour Blood Xylose Test in Diagnosis of Cow's Milk Protein Intolerance. Lancet I:1102-1104, 1979.

Table 1

ORIGIN, SEROTYPE, ENTEROTOXIN TYPE AND ABILITY TO CAUSE DIARRHEA IN MAN OF VARIOUS *E. COLI* STRAINS

Strain	Serotype	Origin	Enterotoxin Type	Caused Diarr in Volunteer
H10407	O78:H11	Diarrhea case, Bangladesh	LT^+/ST^+	+
B7A	O148:H28	Diarrhea case, Vietnam	LT^+/ST^+	+
TD 225 C4	O75:H9	Diarrhea case, Mexico	$LT^+/ST-$	+
E 2528-C1	O25:NM	Cruiseship diarrhea outbleak, Caribbean	$LT^+/ST-$	+
214-4	Non-typable	Diarrhea case Mexico	$LT-/ST^+$	+
B_2C	O6:H16	Diarrhea case, Vietnam	LT^+/ST^+	+
H10407-P	O78:H11	Mutant of H 10407	$LT^+/ST-$	-
E851/71	O142:K86:H6	Infant Diarrhea	EPEC	+
E2348/69	O1127:K63:H6	Infant Diarrhea	EPEC	+
E74/68	O128:K67:H2	Infant Diarrhea	EPEC	-

Table 2

BIOLOGICAL PROPERTIES OF VARIOUS ESCHERICHIA COLI STRAINS
GROWN ON SOLID CASAMINO/YEAST (CFA) AGAR

STRAIN	CAUSED DIARRHOEA IN VOLUNTEERS	HAEMAGGLUTINATION						AGGLUTINATION BY CFA/1 ANTISERA
		HUMAN RBC*		GUINEA PIG RBC		BOVINE RBC		
		WITHOUT MANNOSE	WITH MANNOSE	WITHOUT MANNOSE	WITH MANNOSE	WITHOUT MANNOSE	WITH MANNOSE	
H10407	+	4+	4+	0	0	0	0	4+
B7A	+	0	0	0	0	0	0	0
TD225-C4	+	0	0	0	0	0	0	0
E2528-C1	+	0	0	0	0	0	0	0
214-4	+	0	0	0	0	0	0	0
B_2C	+	0	0	0	0	4+	4+	0
H10407P	0	0	0	0	0	0	0	0
E851/71	+	0	0	0	0	0	0	0
E2348/69	+	0	0	0	0	0	0	0
E74/68	0	0	0	0	0	0	0	0

*Red blood cells.

Table 3

BIOLOGICAL PROPERTIES OF VARIOUS ESCHERICHIA COLI STRAINS GROWN IN MEULLER-HINTON BROTH

STRAIN	CAUSED DIARRHOEA IN VOLUNTEERS	HAEMAGGLUTINATION						AGGULTINATION BY CFA/1 ANTISERA
		HUMAN RBC*		GUINEA PIG RBC		BOVINE RBC		
		WITHOUT MANNOSE	WITH MANNOSE	WITHOUT MANNOSE	WITH MANNOSE	WITHOUT MANNOSE	WITH MANNOSE	
H1C407	+	2+	0	4+	0	0	0	3+
B7A	+	4+	0	4+	0	0	0	0
TD225-C4	+	4+	0	4+	0	0	0	0
E2528-C1	+	4+	0	4+	0	0	0	0
214-4	+	0	0	0	0	0	0	0
B_2C	+	0	0	0	0	0	0	0
H10407P	0	4+	0	4+	0	0	0	0
E851/71	+	4+	0	4+	0	0	0	0
E2348/69	+	4+	0	4+	0	0	0	0
E74/68	0	0	0	0	0	0	0	0

* Red blood cells

Table 4

BIOLOGICAL PROPERTIES OF NON-ENTEROTOXIGENIC ESCHERICHIA COLI NORMAL FLORA CONTROL STRAINS GROWN ON SOLID CASAMINO/YEAST (CFA) AGAR

STRAIN	HAEMAGGLUTINATION						AGGLUTINATION BY CFA/1 ANTISERA
	HUMAN RBC*		GUINEA PIG RBC		BOVINE RBC		
	WITHOUT MANNOSE	WITH MANNOSE	WITHOUT MANNOSE	WITH MANNOSE	WITHOUT MANNOSE	WITH MANNOSE	
EC 2010-1-1-A	0	0	0	0	0	0	0
EC 2010-3-1-B	0	0	0	0	4+	4+	0
EC 2010-4-1-A	0	0	0	0	0	0	0
EC 2010-5-1-A	0	0	0	0	0	0	0
EC 2010-6-1-A	0	0	0	0	0	0	0
EC 2010-7-1-A	0	0	0	0	0	0	0
EC 2010-8-1-A	0	0	0	0	0	0	0
IN 4017-1-1-A	0	0	0	0	0	0	0
IN 4017-2-1-G	0	0	0	0	0	0	0
IN 4017-3-1-A	0	0	0	0	0	0	0
IN 4017-4-2-A	0	0	0	0	0	0	0
IN 4017-6-1-A	0	0	0	0	0	0	0
IN 4017-7-1-A	0	0	0	0	0	0	0
IN 4017-8-1-A	0	0	0	0	0	0	0
IN 4017-11-1-A	0	0	0	0	0	0	0

* Red blood cells.

Table 5

BIOLOGICAL PROPERTIES OF NON-ENTEROTOXIGENIC ESCHERICHIA COLI NORMAL FLORA CONTROL STRAINS GROWN IN MUELLER-HINTON BROTH

STRAIN	HAEMAGGLUTINATION						AGGLUTINATION BY CFA/1 ANTISERA
	HUMAN RBC*		GUINEA PIG RBC		BOVINE RBC		
	WITHOUT MANNOSE	WITH MANNOSE	WITHOUT MANNOSE	WITH MANNOSE	WITHOUT MANNOSE	WITH MANNOSE	
EC 2010-1-1-A	0	0	0	0	0	0	0
EC 2010-3-1-B	4+	0	4+	0	4+	4+	0
EC 2010-4-1-A	3+	0	4+	0	0	0	0
EC 2010-5-1-A	0	0	0	0	0	0	0
EC 2010-6-1-A	4+	0	4+	0	0	0	0
EC 2010-7-1-A	3+	0	3+	0	0	0	0
EC 2010-8-1-A	4+	0	4+	0	0	0	0
IN 4017-1-1-A	4+	0	3+	0	0	0	0
IN 4017-2-1-G	4+	0	4+	0	0	0	0
IN 4017-3-1-A	0	0	0	0	0	0	0
IN 4017-4-2-A	4+	4+	0	0	0	0	0
IN 4017-6-1-A	4+	0	4+	0	0	0	0
IN 4017-7-1-A	4+	0	4+	0	0	0	0
IN 4017-8-1-A	4+	0	4+	0	0	0	0
IN 4017-11-1-A	4+	0	4+	0	0	0	0

* Red blood cells.

Table 6

OCCURRENCE OF COLONIZATION FACTOR PILI I AND II AND TYPE 1 SOMATIC PILI IN ENTEROTOXIGENIC ESCHERICHIA COLI ISOLATES FROM CASES OF TRAVELERS' DIARRHEA IN MOROCCO

LT^+/ST^+ Strains	serotype	CFA/I HA *	CFA/I AB +	CFA/II HA	CFA/II AB	Type 1 Somatic Pili *
M408 C1	06:K :H16	-	-	+	+	-
M111 C5	0:25:K :H	-	-	-	-	-
M411 C1	0:25:K :H	-	-	-	-	-
M424 C1	06:K15:H16	-	-	+	+	-
M524 C1	06:K15:H16	-	-	+	+	-
M633 C1	020:H-	+	+	-	-	-
M452 C1	020:H-	+	+	-	-	-
M145 C2	0128:Hsp.ag.	-	-	+	+	+
M447 C4	08:K83:H-	-	-	+	+	-
$LT^+/ST-$ Strains						
M403 C3	03,073:H	-	-	-	-	+
M117 C1	0?:K48:H19	-	-	-	-	+
M324 C3	082:K :H12	-	-	-	-	-
$LT-/ST^+$ Strains						
M406 C1	0x2:K?:H-	-	-	-	-	-
M407 C4	027:K :H20	-	-	-	-	-
M109 C2	0 sp.ag.:H12	+	+	-	-	-
M409 C1	0x2:K?:H-	-	-	-	-	-
M415 C1	0x2:K?:H-	-	-	-	-	-
M421 C1	0 sp.ag.:K83:H-	-	-	-	-	+
M326 C3	0x2:K?:H-	-	-	-	-	-
M526 C6B	0x2:K?:H-	-	-	-	-	-
M626 C4	0x2:K?:H-	-	-	-	-	-
M443 C1	0x2:K?:H-	-	-	-	-	-
M321 C6E	0?:K13:H-	-	-	-	-	+

* By hemagglutination pattern

\+ By agglutination with specific antibody

Table 7

OCCURRENCE OF COLONIZATION FACTOR PILI I AND II AND TYPE 1 SOMATIC PILI IN ENTEROTOXIGENIC ESCHERICHIA COLI ISOLATES FROM CASES OF TRAVELERS' DIARRHEA IN KENYA

LT^+/ST^+ Strains	Serotype	CFA/I HA*	CFA/I AB+	CFA/II HA	CFA/II AB	Type 1 Somatic Pili*
A201 C3	O159:H4	-	-	-	-	-
A350 C1	O159:H4	-	-	-	-	-
$LT^+/ST-$ Strains						
A104 C3	O159:H34	-	-	-	-	+
A330 C1	O159:H34	-	-	-	-	+
A348 C3	O18,a,b:H1	-	-	-	-	+
A349 C4	O159:H34	-	-	-	-	+
A375 C4	O159:H4	-	-	-	-	+
A346 C1	O6:H-	-	-	+	+	-
A336 C4b	O159:H4	-	-	-	-	+
A334 C1	O159:H4	-	-	-	-	+
A225 C2	O159:H34	-	-	-	-	+
A226 C2	O159:H34	-	-	-	-	+
A233 C2	O6:H16,40	-	-	+	+	-
$LT-/ST^+$ Strains						
A213 C5	O11:H11	-	-	-	-	-
A338 C5	O27:H7	-	-	-	-	+
A220 C2	O148:H53	-	-	-	-	-
A220 C3	O8,O30:	-	-	-	-	+
A237 C1	O128:H12	+	+	-	-	-

* By hemagglutination pattern

\+ By agglutination with specific antibody

Table 8

OCCURRENCE OF COLONIZATION FACTOR PILI I AND II AND TYPE 1 SOMATIC PILI IN ENTEROPATHOGENIC E. COLI ISOLATES FROM SPORADIC CASES OF INFANTILE DIARRHEA AND NURSERY OUTBREAKS IN HUNGARY*

Strain	Serotype	CFA/I HA+	CFA/I AB**	CFA/II HA++	CFA/II AB**	Type 1 Somatic Pili †
Lt 660	020	-	-	-	-	+
27566/1	0142	-	-	-	-	+
27574	020	-	-	-	-	+
27570	0142	-	-	-	-	+
37789	026:K60(B6)	-	-	-	-	+
22143	0111:K58 (B4)	-	-	-	-	+
46323	0126:K71 (B16)	-	-	-	-	+
80845	0111:K58 (B4)	-	-	-	-	+
15749	055:K59 (B5)	-	-	-	-	+
74971	0119:K69 (B14)	-	-	-	-	-
M 56899	0114:K90	-	-	-	-	-
Sz 011	0119:K69 (B14)	-	-	-	-	+

* Strains provided by Dr. Joo, State Serum and Vaccine Institute, Budapest

** Agglutination with specific antibody

\+ Mannose-resistant hemagglutination (MRHA) of human type A erythrocytes.

++ MRHA of bovine but not human erythrocytes

† Mannose-sensitive hemagglutination of guinea pig erythrocytes

Table 9

OCCURRENCE OF CFA/I, CFA/II OR TYPE 1 SOMATIC PILI IN VARIOUS TYPES OF ENTEROTOXIGENIC OR ENTEROPATHOGENIC E. COLI

Toxin Type of Strains	Number Tested	Percent of Strains with: CFA/I	CFA/II	Type 1 Somatic Pili
LT^+/ST^+	11 *	18	45	9
$LT^+/ST-$	14 *	0	14	79
$LT-/ST^+$	16 *	13	0	25
EPEC	12 +	0	0	83

* Strains provided by R.B. Sack

\+ Strains provided by Dr. I. Joo

Table 10

RE-EXAMINATION OF ENTEROTOXIGENIC *E. COLI* STRAINS KNOWN TO HAVE COLONIZATION FACTOR PILI AFTER SEVEN SERIAL SUB-CULTURES

Strain	Colonization Factors Found: Initial	After 7 Subcultures
M408 C1	CFA/II	CFA/II
M424 C1	CFA/II	CFA/II
M524 C1	CFA/II	CFA/II
M633 C1	CFA/I	-
M452 C1	CFA/I	CFA/I
M145 C2	CFA/II	CFA/II
M447 C4	CFA/II	CFA/II

CLINICAL RESPONSE OF VOLUNTEERS TO PARENTERAL IMMUNIZATION WITH PURIFIED H10407 TYPE 1 SOMATIC PILI VACCINE

Dose	Following Inital Vaccine Dose			Following 1800 mcg. Booster Dose		
	Fever	Malaise	Local Reactions	Fever	Malaise	Local Reactions
45 mcgs.	0/3*	0/3	0/3	0/3	0/3	2/3
90 mcgs.	0/4	0/4	0/4	0/3	0/3	0/3
900 mcgs.	0/4	0/4	0/4	0/3	0/3	2/3
1800 mcgs.	0/10	0/10	0/10	0/6	0/6	1/6

*No. with reactions / No. immunized

Table 11

Table 12 ANTIBODY TO H10407 TYPE 1 SOMATIC PILI MEASURED BY ELISA BEFORE AND AFTER PARENTERAL IMMUNIZATION WITH ONE OR TWO DOSES OF PURIFIED PILI VACCINE

Vaccinee	Reciprocal Titer Day 0	Initial Vaccine Dose (mcgs)	Reciprocal Titer Day +28	Booster Vaccine Dose (mcgs)	Reciprocal Titer Day +56
EC4001-1A	<100	45	800	1800	800
EC4001-2A	<100	"	800	"	400
EC4001-3A	<100	"	6400	"	800
EC4001-5B	<100	90	200	-	NS
EC4001-6B	<100	"	200	1800	800
EC4001-7B	<100	"	200	"	800
EC4001-8B	<100	"	800	"	3200
EC4001-9C	<100	900	800	-	-
EC4001-10C	<100	"	12,800	1800	51,200
EC4001-11C	<100	"	12,800	"	6400
EC4001-12C	200	"	12,800	"	51,200
EC4001-13D	<100	1800	6400	"	NA*
EC4001-14D	<100	"	51,200	"	51,200
EC4001-15D	<100	"	3200	"	3200
EC4001-16D	<100	"	6400	"	3200
EC4001-17D	<100	"	25,600	"	51,200
EC4001-18D	<100	"	6400	"	3200
EC4001-19E	<100	"	800	-	NS
EC4001-20E	<100	"	800	-	NS
EC4001-21E	<100	"	200	-	NS
EC4001-23E	<100	"	3200	-	NS

*Specimen not available
**Day +56 also equals the date just prior to virulent challenge
†Volunteers who participated in challenge study of vaccine efficacy.

Table 13

RELATIONSHIP BETWEEN DOSE OF PARENTERAL *E. COLI* PURIFIED PILI VACCINE AND ANTIBODY TITER

Single Dose

	N	Pre-Immunization		N	Day +28
Low Dose Vaccinees (45 or 90 mcg.)	7	50*		7	594
High Dose Vaccinees (900 or 1800 mcg.)	14	55		14	4307

Two Doses

					Day +56
Low Priming Dose (45 or 90 mcg.)	7	50	1800 mcg. Booster Dose on Day +28	6	898
High Priming Dose (900 or 1800 mcg.)	9	55	1800 mcg. Booster Dose on Day +28	9	13,958

* Reciprocal Geometric Mean Titer

RESPONSE OF VACCINEES IMMUNIZED WITH
TWO PARENTERAL DOSES OF PURIFIED *E. COLI* TYPE 1 PILI VACCINE AND CONTROLS
FOLLOWING INGESTION OF $5X10^8$ VIRULENT ENTEROTOXIGENIC *E. COLI* (STRAIN H10407)

Group	Mean Incubation (hrs.)	Diarrhea	Mean Total Diarrheal Stool Volume per Ill Volunteer	Mean Total No. Loose Stools per Ill Volunteer	Vomiting	Abdominal Cramps	Malaise	Positive Stool Cultures
Vaccinees	24.5	2/6*	3.89+	16	0/6	0/6	0/6	6/6
		p=0.04						
Controls	31	7/7	3.96	18	6/7	6/7	7/7	7/7

Table 14

*No. positive/No. challenged

+Liters

Table 15

PREVALENCE OF NORMAL COLONIC *E. COLI* FLORA
THAT POSSESS SOMATIC PILI OF H10407
ANTIGENIC VARIETY

Volunteer	Day 0	Day +28
4001-1A	0*	100
4001-2A	0	100
4001-3A	0	80
4001-6B	193	87
4001-7B	67	100
4001-8B	93	100
4001-10C	100	100
4001-11C	100	100
4001-12C	100	100
4001-13D	40	73
4001-14D	100	0
4001-15D	73	0
4001-16D	40	60
4001-17D	100	100
4001-18D	87	100

*% of 15 colonies tested that were agglutinated 3+ or 4+ by antibody to type 1 somatic pili of *E. coli* H10407.

PUBLICATIONS 1979 CONTRACT YEAR

Published:

1. Levine, M.M., Nalin, D.R., Hoover, D.L., Bergquist, E.J., Hornick, R.B., Young, C.R.: Immunity to Enterotoxigenic Escherichia coli, Infect. Immun. 23:729-736, 1979.

2. Nalin, D.R., Levine, M.M., Young, C.R., Bergquist, E.J., MacLaughlin, J.: Concentrating Escherichia coli Culture Supernates Increases Detection of Heat-Stable Enterotoxin in the Infant Mouse Assay. J. Clin. Microbiol. 8:700-703, 1978.

3. Greenberg, H.B., Levine, M.M., Merson, M.H., Sack, R.B., Sack, D.A., Valdesuso, J.R., Nalin, D.R., Hoover, D., Chanock, R.M., Kapikian, A.Z. Solid Phase Microtiter Radioimmunoassay Blocking Test for Detection of Antibodies to Escherichia coli Heat-Labile Enterotoxin. J. Clin. Microbiol. 9:60-64, 1979.

In Press:

1. Levine, M.M., Rennels, M.B., Cisneros, L., Hughes, T.P., Nalin, D.R., Young, C.R. Lack of Person-to-Person Transmission of Enterotoxigenic Escherichia coli Dispute Intimate Contact. Am. J. Epidemiol. in press.

2. Huang, T.L., Levine, M.M., Daoud, G.S., Nalin, D.R., Nichols, B.L. "Fecal Steroids in Diarrhea. III. Experimentally - Induced Travelers' Diarrhea". Am. J. Clin. Nutrition, in press.