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Protective Immune Response of Bacterially-Derived Recombinant FaeG in Piglets

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FaeG is the key factor in the infection process of K88ad enterotoxigenic *Escherichia coli* (ETEC) fimbrial adhesin. In an attempt to determine the possibility of expressing recombinant FaeG with immunogenicity for a new safe and high-production vaccine in *E. coli*, we constructed the recombinant strain, BL21 (DE3+K88), which harbors an expression vector with a DNA fragment of *faeG*, without a signal peptide. Results of 15% SDS-polyacrylamide slab gel analysis showed that FaeG can be stably over-expressed in BL21 (DE3+K88) as inclusion bodies without FaeE. Immunoglobulin G (IgG) and M (IgM) responses in pregnant pigs, with boost injections of the purified recombinant FaeG, were detected 4 weeks later in the sera and colostrum. An *in vitro* villius-adhesion assay verified that the elicited antibodies in the sera of vaccinated pigs were capable of preventing the adhesion of K88ad ETEC to porcine intestinal receptors. The protective effect on the mortality rates of suckling piglets born to vaccinated mothers was also observed one week after oral challenge with the virulent ETEC strain, C_{83907} (K88ad, CT^+, ST^+). The results of this study proved that the adhesin of proteinaceous bacterial fimbriae or pili could be overexpressed in engineered *E. coli* strains, with protective immune responses to the pathogen.

Keywords: Enterotoxigenic *Escherichia coli* (ETEC), proteinaceous fimbriae, recombinant FaeG, adhesion

Adhesins, which form primarily in polymeric protein fimbrial structures on the surfaces of bacterial cells, are involved in the specific adherence of bacterial cells to host tissues. Practically all gram-negative bacterial species thus far examined have been determined to generate one or more types of fimbriae. A few gram-positive bacteria have also been shown to harbor fimbriae (Wizemann, 1999). Most members of the Enterobacteriaceae are "normal" inhabitants of mammalian intestinal flora. Pathogenic *E. coli* strains, including enterotoxigenic *E. coli* species (ETEC), enteroinvasive *E. coli* species (EIEC) and enteropathogenic *E. coli* species (EPEC), have been determined to express several different types of fimbriae, including type 1 fimbriae (fim), K88 (fae), and K99 (fan) fimbriae. Fimbriae (or pili) are the primary pathogenic factors of these bacteria, and are responsible for its adhesion to enterocyte receptors (Mol and Oudega, 1996). The disruption of bacterial attachment by specific anti-adhesin antibodies appears to be an effective way to incapacitate the potential pathogens.

ETEC expressing various types of fimbrial antigens, including K88 (*fae*), K99 (*fan*), or 987p (*fas*), are causative agents of neonatal diarrhea in piglets. The fimbrial protein, K88, which is found on the surface of ETEC isolates from pigs, along with other fimbriae, mediates the adhesion of bacteria to the small intestinal epithelia of newborn piglets. Susceptibility to K88-fimbriated *E. coli* infection is most profound in the neonatal stage and immediately thereafter, during weaning (Willemsen and de Graaf, 1992). In the case of K88 ETEC, vaccines containing purified K88

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fimbriae, including formalin-inactivated ETEC or engineered bacteria, which harbor the gene cluster expressing K88 fimbriae, have been utilized in the vaccination of pregnant sows, thereby enabling the passive transfer of lacteal immunity from vaccinated dams, which can protect young piglets from ETEC infection (Rutter and Jones, 1973; Furer *et al.*, 1982; Hong *et al.*, 1985; Barman and Sarma, 1999). Vaccines developed thus far, however, even those which produce a satisfactory return on investment, are not exempt from undesirable effects due to the presence of endotoxins, or due to manufacturing difficulties (Greenwood *et al.*, 1988).

Adhesins are generally synthesized as precursors and translocated into the periplasm. Within the periplasm, the majority of subunits have been shown to bind to the periplasmic chaperone encoded by the fimbrial gene cluster. Thus, the adhesins are protected against proteolytic degradation and premature polymerization or aggregation (Mol and Oudega, 1996).

The expression of recombinant proteins in *E. coli* is a common method for the acquisition of a large quantity of the desired proteins, and the bacterial strain BL21 (DE3) is the most extensively utilized host bacterium for protein expression. In an attempt to generate adhesins with proteinaceous fimbriae in *E. coli*, we cloned the DNA fragment of *faeG* encoding for K88ad fimbrial adhesin (without signal peptide), and inserted it into the *E. coli* high-level expression vector, pET-28(a+). It was observed that recombinant FaeG protein could be overexpressed in *E. coli* as inclusion bodies, and the recombinant protein evidences passive immunization activity against K88ad expressing ETEC in piglets. The results of this study should provide information necessary for the development of new alternative vaccines against pathogenic bacteria with proteinaceous fimbriae or pili.

Materials and Methods

Bacterial strains and growth conditions

Enterotoxigenic *E. coli* (ETEC) C_{83907} was a standard ETEC strain expressing K88ad fimbriae, assigned by the China Institute of Veterinary Drug Control, which was used to isolate the plasmid employed as a template for the PCR amplification of *faeG*. Strain C_{83907} was cultured for 24 h in Luria-Bertani (LB) medium at 37°C. The presence of K88ad fimbriae was determined via slide agglutination assays using K88ad fimbriae-specific antibody (Levine, 1987).

E. coli DH5α was utilized in order to replicate the carrying vector, pET-28(a+) (Novagen, USA). *E.coli* BL21 (DE3) (Novagen, USA) was used for transformation with the recombinant bacterial expressing vector, p8801, which harbors *faeG* without a coding region for the signal peptide. The resultant bacterial strain, BL21(DE3+K88), was grown for 18 h in LB broth supplemented with kanamycin (50 μg/ml) at 37°C at 200 rpm.

Construction of p8801

The *faeG* gene was PCR amplified using the plasmid purified from C_{83907} as a template. According to the reported nucleotide sequence of the FaeG mature peptide-coding region (GenBank accession no. M25302), the synthetic oligonucleotides corresponding to its 5' and 3' regions with added restriction enzyme sites were employed as primers. The forward primer was 5'-AAAA**CCATGG**CTGGTGATTTCAATGG-3', and the reverse primer was; 5'-AAAA**GAGCTC**TTATTATTAG TAATAAGTTATTGCTACGTTCAG-3'. The *Nco*I and *Sac*I restriction sites are shown in bold, and the start and stop codons were underlined (Synthesized at the Sangon Biological Company, Shanghai, China). *Pfu* DNA polymerase, dNTPs, and buffers were also purchased from the Sangon Biological Company.

PCR was conducted using a PTC-100^{TM} programmable thermal controller (MJ Research Inc., USA) with the following program: 94°C 5 min, then 30 cycles at 94°C 1 min, 56°C at 1 min, 72°C for 2 min, followed by a final extension at 72°C for 5 min. The 789 bp PCR products and the pET-28(a+) vector were digested with *Nco*I and *Sac*I, purified, and ligated with T_4 DNA ligase (Sambrook *et al.*, 2001). After sequencing, the obtained expression plasmid, p8801, harboring the *faeG* fragment and the control plasmid, pET-28(a+), were utilized to transform *E. coli* strain BL21(DE3)-competent cells, which harbor an integrated copy of the T_7 RNA polymerase gene, under the control of the inducible Lac UV 5 promoter (Studier *et al.*, 1990). The transformed cells were grown at 37°C in LB medium, supplemented with kanamycin (50 μ g/ml) to a cell density of OD_{600} = 0.4-0.6. Recombinant protein expression was induced by 1 mM IPTG, and incubation continued for 4 h at 37°C. The cells were pelleted, resuspended in gel loading buffer, and heated for 10 min at 100°C. The cell extracts were then analyzed via electrophoresis on SDS-15% polyacrylamide slab gels.

Preparation of recombinant FaeG for vaccination

Recombinant FaeG-producing *E. coli* cells were collected from one liter of culture via centrifugation, washed once with washing buffer A [50 mM Tris-HCl (pH 8.0) buffer with 100 mM NaCl and 5 mM EDTA], then resuspended in 10 ml of buffer A. The cells were lysed by three cycles of freezing and thawing, and the DNA was sheared via sonication. The lysates were centrifuged for 10 min at 4000 x g. The over-expressed insoluble and inactive 27.6 kDa FaeG

protein was contained in the pellet. After three washings with 25 ml of buffer A, the insoluble proteins were suspended to 0.5 mg/ml in it, and dialyzed against 50 volumes of 50 mM Tris-HCl (pH 8.0) solution containing urea in a concentration range from 6 M to 1.5 M, and finally dialyzed against 100 volumes of 50 mM Tris-HCl (pH 8.0) solution. Each dialysis step was conducted for 10 h at 4°C. After dialysis, the solution was centrifuged in order to remove insoluble agents. Protein concentrations were determined using a spectrophotometer (DU^R640 nucleic acid and protein analyzer, Beckman Counter Inc., USA) with bovine serum albumin used as a standard, then diluted to 5 mg/ml for animal immunization with PBS (pH 7.4, 150 mM). Also the protein was separated by SDS-PAGE, and the purity of the protein was then analyzed with Tanon Gel Documentation and Analysis System (Tanon Inc., China).

Sow immunization

Six pregnant sows at second lactation were selected. ELISA and slide agglutination assays were used to determine that there were no specific antibodies against K88ad ETEC in the pre-immunization sera. Three of the six pregnant sows were intra-muscularly vaccinated with purified recombinant FaeG (5 mg each) six weeks prior to farrowing. One milliliter of recombinant protein was emulsified in an equal volume of Freund's Complete Adjuvant (Sigma, USA). Booster vaccinations were administered 2 weeks prior to farrowing with one ml of protein (5 mg each) emulsified in an equal volume of Freund's Incomplete Adjuvant (Sigma, USA). Three other pregnant sows were unvaccinated as negative controls.

Antibody response

Sow sera were bled from the jugular vein, one week after booster vaccination. Humoral antibody responses were monitored via ELISA, using purified C_{83907} fimbriae-coated ELISA plates for the detection of specific antibodies. K88ad fimbriae from the C_{83907} bacteria were isolated in accordance with the methods described by Van den Broeck *et al.* (1999). Purity was assessed via electrophoresis on SDS-15% polyacrylamide slab gel.

ELISA was conducted as described by Turnes *et al.* (1999). Alkaline phosphatase-conjugated goat anti-pig IgG (Sigma, USA) (1:5000) was employed as a secondary antibody. The color reaction time was 30 min, and the reactions were visualized with 4-nitrophenyl phosphate (pNPP). Absorbance was determined at a wavelength of 405 nm using a microtiter reader (ELx 800 automated microplate reader; Bio-Tech Instruments, Inc.). The average serum titers were expressed as the log10 of the reciprocal of the highest serum dilution that provided optical density (OD_{405}) reading 3 standard deviations (SD) over the OD_{405} of the pre-immunized sera.

Western blotting analysis was also utilized to verify the production of antibodies against K88ad ETEC in the sera of vaccinated sows as described by Turnes *et al.* (1999). The membranes were probed with vaccinated sows' sera (diluted 1:200) and alkaline phosphatase-labeled anti-pig goat IgG (Sigma, USA) was used as a secondary antibody.

In order to analyze antibodies in the colostrums of vaccinated pigs, ELISA was also conducted. Colostrum whey was obtained via the method described by Barman and Sarma (1999). The sow colostrums were titrated by ELISA, following the same protocol as was used with the serum. In order to detect specific isotype antibodies, alkaline phosphatase-conjugated rabbit anti-sow IgA (BETHYL, USA) and IgM (KPL, USA) were added.

Slide agglutination test

The slide agglutination assay was conducted essentially as described previously (Lopez-Vidal and Svennerholm, 1990). In brief, bacterial cells were harvested from LB broth and adjusted to a concentration of 10^{10} CFU/ml in PBS. Twenty microliter of bacterial suspension were then applied to glass slides. One microliter of vaccinated sow antisera were added, then mixed with a wooden applicator stick. Bacterial agglutination was evaluated via phase-contrast microscopy (Nikon FDX-35, Japan) at a magnification of 400x. Visible agglutination within 2 min was considered a positive reaction.

In vitro villius-adhesion assay

In order to determine whether pig antisera could react with ETEC and inhibit the binding of ETEC to isolated villi, the small intestinal villi of pigs were collected as described by Van den Broeck *et al.* (1999). The *in vitro* villius-adhesion assay was modified as described by Girardeau (1980). Prior to the assay, the villi were washed four times in Krebs-Henseleit buffer without formaldehyde, and finally suspended in PBS supplemented with 1% (w/v) D-mannose (Sangon, China). D-Mannose was added in order to prevent the adhesion of *E. coli* by type 1 pili (F1). Subsequently, 50 μl of immunized pig's antisera and 4×10^8 of C_{83907} organisms were added to an average of 50 villi in 0.5 ml of PBS with 1% D-mannose, then incubated for 1 h at room temperature with gentle shaking. After incubation, the villi were visualized by phase-contrast microscopy (Nikon FDX-35, Japan) at a magnification of 400x. The control experiment was conducted with 50 μl of unvaccinated pig serum and 4×10^8 C_{83907} organisms added to 50 villi in 0.5 ml of PBS with

1% D-mannose.

Protection test

The protection test was conducted as described by Barman and Sarma (1999) with minor modifications Three-day piglets (18 males and 6 females) born to recombinant FaeG-vaccinated sows, and six piglets (5 males and 1 female) from the unvaccinated sows were orally challenged with 1×10^9 CFU of live C_{83907}. The bacterial suspension prepared in 10 ml of sterile PBS (pH 7.4) was administered into the back of the mouth. The gastric acid was neutralized 15 min prior to the administration with 1.4% $NaHCO_3$. The piglets were examined at challenge, then two times daily for 7 days' post-challenge (PC). At each examination, rectal temperature was detected. The excretion of ETEC in feces was demonstrated via the inoculation of fecal samples onto blood agar plates at 37°C for 24 h. Hemolytic *E. coli* colonies were examined for the production of K88 fimbriae via agglutination with K88ad-specific antibodies.

Results

Expression and purification of recombinant FaeG

We attempted to generate the recombinant adhesin of proteinaceous bacterial fimbriae at a high level in engineered *E. coli* strains with protective immune responses to pathogen. We isolated the gene of the K88 adhesin of ETEC encoding for mature FaeG via PCR amplification, and subcloned it into the bacterial expression vector, pET-28(a+). The DNA sequencing of the PCR products indicated that no mutations were introduced by the amplification reaction. The resultant recombinant plasmid (p8801) was utilized to transform *E. coli* BL21(DE3) cells, which resulted in BL21(DE3+K88). IPTG (at 1 mM) was added to the resulting transformants, in order to induce the generation of the recombinant protein. After induction, a major protein of 27.6 kDa (Fig. 1, lane 2), with the expected size of the FaeG of K88ad fimbriae, was observed. As is shown in Fig. 1, the recombinant FaeG accounted for more than 30% of the total cellular proteins. Electrophoretic analysis on 15% SDS polyacrylamide slab gel also showed that the expressed protein was recovered primarily in the form of inclusion bodies in the protein pellets. In order to purify the protein, the pellet was washed three times in order to remove the soluble proteins, and recombinant FaeG protein was obtained with a purity in excess of 90% via the denaturing and refolding of the insoluble FaeG by dialysis against the concentration gradient of the denaturing agent (6 to 0 M Urea) in (50 mM, pH 8.0) Tris-HCl buffer (Fig. 1, lane 1). Electrophoretic analysis on 15% SDS polyacrylamide slab gel revealed that the refolded protein remained undigested under storage conditions of −20°C for at least six months (data not shown).

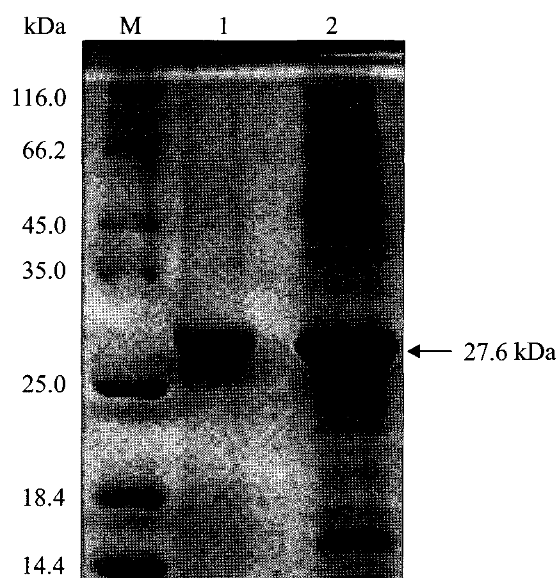

Fig. 1. Analysis of the purified recombinant FaeG with electrophoresis on 15% SDS polyacrylamide slab gel. M: protein molecular weight standards; lane 1: purified recombinant FaeG; lane 2: total protein extract from *E. coli* BL21(DE3) harboring p8801 after IPTG induction.

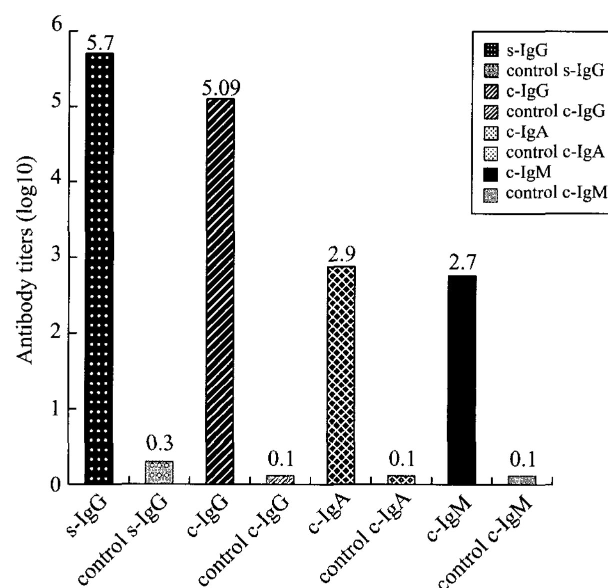

Fig. 2. Antibody titers against K88ad ETEC fimbriae in the sera and colostrums of the immunized sows. s-IgG means anti-K88ad IgG antibodies in immunized sow sera. Control s-IgG means anti-K88ad IgG antibodies in unvaccinated sow sera. c-IgG, c-IgA and c-IgM mean isotype-specific antibodies against K88ad ETEC in the colostrums of the immunized sow sera. Control c-IgG, control c-IgA, and control c-IgM mean isotype-specific antibodies against K88ad ETEC in colostrums of the unvaccinated sow sera.

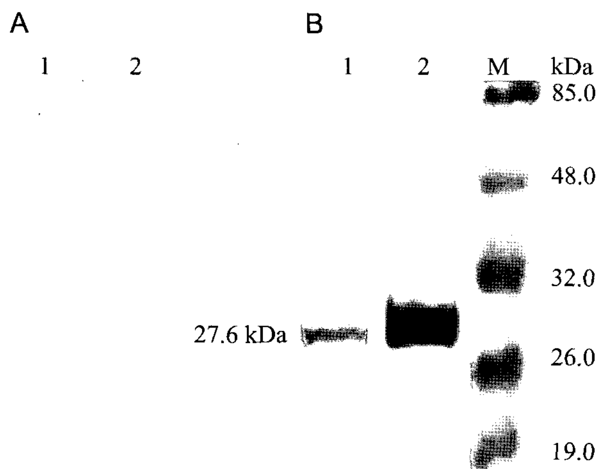

Fig. 3. Western blot analysis for the identification of the FaeG-specific antibody in immunized porcine sera. The proteins were separated via electrophoresis on a 15% SDS polyacrylamide slab gel. Blots were detected using immunized porcine sera as the primary antibody, and alkaline phosphatase-conjugated goat anti-pig IgG was used as the secondary antibody. (A) Sera from unvaccinated sows, Lanes 1: 2 μg total cellular protein from induced *E. coli* BL21(DE3) containing p8801, Lanes 2: 2 μg purified K88ad fimbriae from C83907. (B) Sera from immunized sows. Lane 1: 2 μg total cellular protein from induced *E. coli* BL21(DE3) containing p8801. Lane 2: 2 μg purified K88ad fimbriae from C83907. Lane M: Protein molecular weight standards.

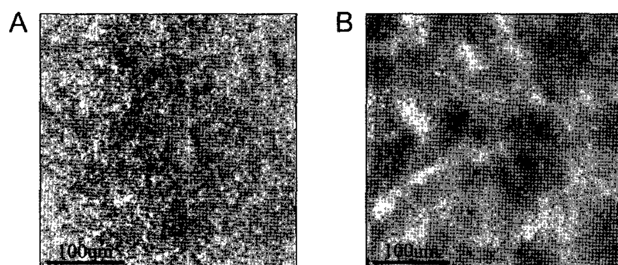

Fig. 4. Results of the slide agglutination assay. (A) No agglutination with the sera from unvaccinated sows to K88 ETEC C83907. (B) K88 ETEC C83907 was agglutinated by antibodies in the sera from immunized sows.

Immune response

ELISA

In order to determine whether the recombinant adhesin proved effective in the induction of immunological responses in animals, three sows inoculated with the purified recombinant FaeG were bled 1 week prior to farrowing. IgG antibodies against K88ad fimbriae in immunized sows were separately detected using ELISA. The average serum titer of the specific IgG immunoglobulin was increased to 1:512,000 dilutions.

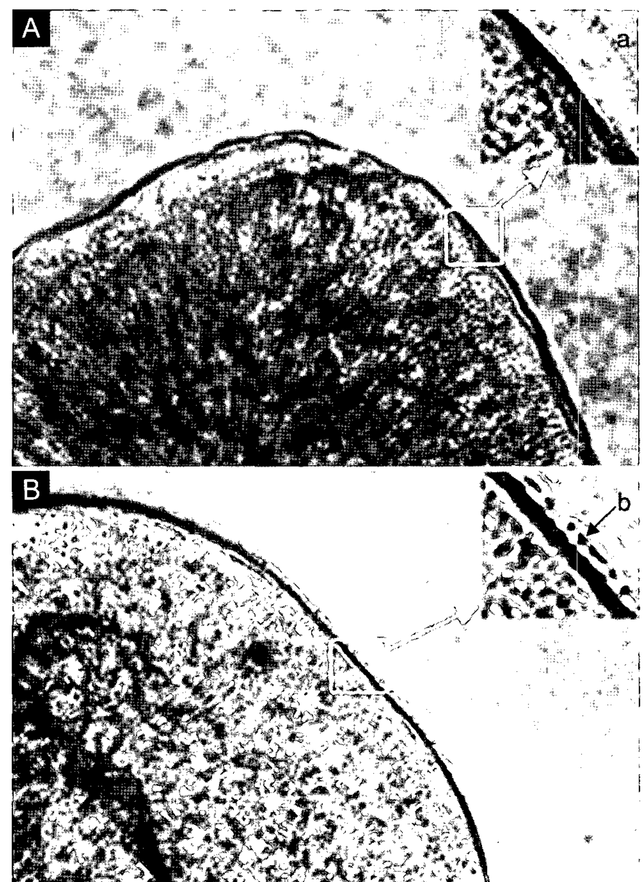

Fig. 5. Small intestinal villous brush borders after the *in vitro* adhesion assay with immunized and non-immunized porcine sera neutralized K88ad ETEC. (A) No adhesion of intestinal villi incubated with recombinant FaeG immunized porcine serum neutralized K88ad ETEC. (B) Significant adhesion of brush borders of intestinal villi incubated with non-immunized porcine serum neutralized K88 ETEC. (a) and (b): enlarged image of brush borders to highlight the adhesion of ETEC to the brush border. Black arrow indicates the adhesion.

By way of contrast, no specific immune responses to the K88ad fimbriae were detected in the sera of unvaccinated sows (Fig. 2, s-IgG).

As the piglets were passively protected by the transfer of lacteal immunity from the vaccinated sows, the colostrums of sows were collected 2 h after farrowing, and specific antibodies against K88ad fimbriae were tested separately. In three whey samples from the immunized sows, isotype-specific antibodies were detected (colostrums IgG 1:124,000, IgA 1:800 and IgM 1:500). By way of contrast, no specific antibodies were detected in the lacteal secretions of the unvaccinated control sows (Fig. 2).

Western blot analysis

In order to further confirm the production of antibodies against K88ad fimbriae in the vaccinated sow sera, Western blot analysis was conducted using the purified recombinant FaeG and K88ad fimbriae as standard

Table 1. Protection of piglets fed with colostrums and milk from vaccinated sow after ETEC challenge

Animal group	No. of animals	Piglets with diarrhea/total (%)	Duration of diarrhea (day)	No. of piglets dead/total (%)
A	24	6/24 (25.0)	2	0/24 (0.0)
B	6	6/6 (100.0)	3-4	6/6 (100.0)

A: piglets received colostrums from vaccinated sow.
B: piglets received colostrums from of non-vaccinated sow.

antigens. Specific recognition was observed between the immunized porcine sera and the two antigens (Fig. 3), thereby indicating the presence of antibodies.

Slide agglutination test

If the antibody induced by recombinant FaeG was clinically effective, it would not only react with purified K88 fimbriae, but would also inactivate K88ad ETEC. In order to determine whether the sow antibodies could interact with the K88ad ETEC, a slide agglutination assay was conducted. It was determined that the sera from immunized sows, used at a dilution of 1:20, could induce the agglutination of C_{83907}. By way of contrast, the sera from unvaccinated sows evidenced no agglutination (Fig. 4).

In vitro villous-adhesion assay

Host cell surfaces harbor a repertoire of proteins, glycoproteins, and glycolipids, to which bacteria are able to adhere and initiate infection. The colonization of K88 ETEC in the intestine is a crucial step in the pathogenesis of porcine neonatal diarrhea. In order to further confirm whether the K88ad specific antibodies could inhibit the ability of the pathogen to adhere to porcine intestinal receptors, an inhibition assay was conducted via the co-incubation of villi with K88ad ETEC and immunized porcine serum. The resultant mixture was then evaluated via phase-contrast microscopy at a magnification of 400x. As is shown in Fig. 5b, the attachment of the pathogen to the villi was observed after incubation with K88ad ETEC and unvaccinated porcine serum, whereas such attachments were not observed with the vaccinated porcine serum (Fig. 5a). This indicates that the specific antibodies induced by recombinant FaeG in porcine serum are capable of inhibiting the adhesion of K88ad ETEC to porcine intestinal villi.

Protection of ETEC challenged piglets

As one of the ultimate objectives of this study was to develop recombinant adhesin with the protection effect on piglets against ETEC infection, the protection effect was evaluated in challenged piglets. In the control group without immunization, all six of the piglets exposed to ETEC developed diarrhea on the second day of challenge, and became profuse and watery on the fourth day. The challenged ETEC bacteria were detected in large numbers in these piglets' faeces on the fourth day post-challenge. These animals became weak and lost body weight. All six of the piglets died prior to the seventh day post-challenge. Unlike the control animals, 18 of 24 piglets born to sows immunized with recombinant FaeG did not develop diarrhea. Only 6 of the 24 piglets evidenced mild symptoms and excreted challenged bacteria up to the fourth day post-challenge. These six piglets recovered prior to seventh day post-challenge, and no body weight losses were observed. The fact that none of the piglets born to sows immunized with the recombinant FaeG died of diarrhea indicates that the recombinant adhesin we developed was clinically effective (Table 1).

Discussion

To date, infection by pathogenic *E. coli* species and the resultant disease remains one of the most serious problems associated with animal and human health. The concept of fimbriae as a colonization factor has resulted in the development of fimbriae-based vaccines for the control of pathogenic bacterial infection. In a previous study, Mooi *et al.* (1983) reported that the adhesin precursors of certain bacterial strains harbor signal sequences, and are likely translocated into the periplasm, which might be stabilized by chaperone molecules. In the case of K88 ETEC, the fimbrial subunit, FaeG, is transported to the periplasmic space, where it associates with the 27 kDa polypeptide chaperone, FaeE. In FaeE-defective K88 ETEC mutants, the FaeG fimbrial subunit was synthesized, but was subsequently degraded by the cell-envelope protease, DegP (Mooi *et al.*, 1982; Mooi *et al.*, 1983). The association of FaeE with FaeG was hypothesized to protect the fimbrial subunits against proteolytic degradation within the periplasm (Bakker *et al.*, 1991).

It has also been shown that the immunization of purified K88 fimbriae might induce protective immune responses in pigs (Van den Broeck, 1999). In order to utilize K88 fimbriae in immunization experiments, it is first necessary to develop a system which can readily generate K88 adhesin with protective immunogenicity

at maximum yield. In our previous study, we determined that recombinant FaeG could be generated in plants with immunogenicity; however, the expression levels are far lower (Huang *et al.*, 2003).

The expression of recombinant proteins in *E. coli* is a common method for the acquisition of a large quantity of the desired protein. Thus, in this study, we have demonstrated the generation of recombinant FaeG in the *E. coli* strain, BL21(DE3), in the form of inclusion bodies with immunological protective effects. Recombinant FaeG accounted for more than 30% of the total cellular proteins. Similarly, in our previous study, we determined that bacterially-derived FanC protein forming inclusion bodies could induce profound immune responses (Huang *et al.*, 2001). The bacterial strain BL21(DE3), which is the most extensively utilized host bacterium for protein expression, was selected. This strain is deficient in both *lon* and *ompT* proteases, and the overexpressed protein normally forms inclusion bodies (Instructions for users provided by Novagen, USA). This may be a drawback with regard to the study of the function and structure of a protein. However, it is actually an advantage with regard to the production of antibodies against the protein, as the inclusion bodies are more stable than the soluble proteins (Ubeidat and Rutherford, 2003). As the recombinant FaeG could be stably maintained in the engineered bacteria, we propose that, in the absence of signal peptide, the synthesized FaeG could not be translocated into the bacterial periplasm, in which the cell-envelope protease is located. Therefore, it accumulates in the cell. In addition, the inclusion body might also protect the recombinant protein against proteinase degradation.

Recombinant FaeG protein was recovered from the inclusion bodies using a 6 M buffered urea solution, and was purified to a purity in excess of 90% via the purification procedure described in this paper. More importantly, the refolded protein remained stable under storage conditions at –20°C for at least six months, thereby suggesting that it is possible to produce the protein in batches, and to store it for long-term use. The fact that pregnant pigs vaccinated with the purified recombinant FaeG developed profound humoral immunological responses suggested that this recombinant adhesin possesses profound immunogenicity. In the receptor inhibition test, we determined that vaccinated porcine sera prevented K88ad ETEC from adhering to receptors on the porcine intestinal villi *in vitro*. Bacterial adhesion to porcine intestinal receptors is an important initial step in ETEC-induced diarrhea. Immunoglobulins with this inhibitory ability in immunized porcine sera can protect piglets against K88ad ETEC infection. This explains why piglets born to sows immunized with recombinant FaeG developed either only mild or no clinical diarrheal symptoms. This passive transfer of the protective effect from immunized pregnant pigs to their piglets is comparable to that of the purified fimbriae and inactivated fimbria-bearing bacterins. Collectively, our findings indicate that recombinant FaeG protein could be produced in *E. coli* without the stabilization provided by chaperone molecules. This product can be employed to replace traditional vaccines that have undesirable effects due to the presence of endotoxins, or as a consequence of manufacturing difficulties. More importantly, considering the fact that in gram-negative bacteria a host of adhesins, including FimA (Type 1 fimbriae), PapG (P fimbriae), FaeG (K88 fimbriae), FanC (K99 fimbriae), and FasA (987P fimbriae), are distributed as proteinaceous fimbriae on bacterial surfaces by a similar chaperone- usher-assisted transport system in bacteria (Mol and Oudega, 1996), this study also provides a new approach for the production of vaccines against infections by gram-negative bacteria with proteinaceous fimbriae or pili.

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