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ORIGINAL ARTICLE

Reduction of enterotoxin induced fluid accumulation in ileal loops of neonatal calves with anti-F5 fimbriae recombinant antibody

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Abstract Neonatal calf colibacillosis caused by enterotoxigenic *Escherichia coli* (ETEC) is an economically significant problem in most parts of the world. The most common ETEC found in calves express the F5 (K99) fimbriae, which are necessary for the attachment of the bacteria to the ganglioside receptors on enterocytes. It is known that prevention of ETEC $F5^+$ adhesion to its ganglioside receptors with specific antibodies protects calves from colibacillosis. Previously we have described the development and characterization of a mouse recombinant antibody fragment (moRAb) that prevents F5 fimbrial protein induced agglutination of horse red blood cells (HRBC), which exhibit the same gangloside receptor for F5 fimbriae. Here we demonstrate that this recombinant antibody fragment inhibits *in vitro* the attachment of ETEC $F5^+$ bacteria to HRBC as well as isolated calf enterocytes, and *in vivo* it decreases fluid accumulation in intestinal loops of calves. Thus, correct oral administration of this anti-F5 moRAb may serve as an immunoprophylactic for cost effective control of colibacillosis in calves.

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Introduction

Enterotoxigenic *Escherichia coli* (ETEC) infection, one of the most common causes of diarrhea in newborn calves, is characterized by excessive fluid loss resulting in dehydration, and mortality in severe cases within 24 to 48 h from the onset of infection (Acres 1985). Subsequent to oro-feacal transmission, ETEC colonizes the posterior half of the small intestine in high numbers after attachment to the enterocytes; secretes toxins whose action on the enterocytes leads to diarrhea. Attachment of bacteria to the enterocytes is mediated by fimbriae or pili, mostly of the F5 (K99) type. The F5 fimbriae bind to the surface of the enterocytes, via a ganglioside [N-glycoloyl-GM3 (NeuGc-GM3)] receptor that is also found on the horse red blood cells (HRBC) (Hernández et al. 1989; Smit et al. 1984; Teneberg et al. 1993). The interaction of F5 fimbriae with HRBC causes a strong agglutination (Gaastra and De Graaf 1982; Tixier 1975), which we used previously to characterize an anti-F5 mouse recombinant antibody fragment (moRAb) (Bhaskaran et al. 2005). This anti-F5 moRAb, a single chain fragment of the variable region (scFv), inhibited the F5 fimbriae-induced hemagglutination indicating that the moRAb would prevent adhesion of ETEC to the enterocytes via F5 fimbriae, a critical early step in the pathogenesis of colibacillosis.

The importance of passive lacteal immunity, and more specifically local immunity within the intestinal lumen, in protection against ETEC mediated neonatal calf diarrhea has long been recognized (McEwan et al. 1970). Calves are protected from pathogens through the passive transfer of specific

immunoglobulins in colostrum ingested soon after birth (Gay 1983; McGuirk and Collins 2004). Protection may be incomplete or absent if there is insufficient oral acquisition of the immunoglobulins through colostrum, resulting in illness and death of calves. Hence, in case of colibacillosis, supplementary oral administration of hyperimmune sera from cattle immunized with F5 fimbrial proteins prevents ETEC infections in calves (Acres 1985), emphasizing the importance of providing adequate levels of specific immunoglobulins either by immunization of dams or by supplementation.

A commercial supplement containing an anti-F5 monoclonal antibody (mAb) used for prophylaxis reduces the mortality in neonatal calves (Morter 1984). However, in vitro mass production of mAbs is cost prohibitive, and justified animal welfare concerns limit the use of in vivo ascites for obtaining concentrated immunoglobulin preparations. Alternatively, antibody engineering allows us to produce the necessary antibodies in various recombinant formats without the use of animals.

Since functional antibody fragments can be expressed in *E. coli* or other expression hosts readily, an advantage for certain applications utilizing low cost production and purification, recombinant antibody technology has gained importance in the development and production of diagnostic and therapeutic molecules (Morter et al. 1984). In this study, anti-F5 moRAb, a single chain fragment of the variable region (scFv), previously shown to inhibit F5 fimbriae induced agglutination of HRBC was tested for its ability to prevent the adhesion of ETEC $F5^+$ bacteria to HRBC and isolated calf enterocytes (Bhaskaran et al. 2005). Furthermore, we tested its ability to decrease fluid accumulation in calf intestinal loops, which would be indicative of inhibition of ETEC infection (Myers et al. 1975; Smith and Halls 1967). Thus, the decrease in fluid accumulation would demonstrate the potential use of anti-F5 moRAb as an orally administered prophylactic for neonatal calf colibacillosis.

Materials and methods

Anti-F5 mouse recombinant antibody

The phagemid clone pSHOB-52 used to prepare soluble anti-F5 mouse recombinant antibody fragment (anti-F5 moRAb) was selected from clones obtained following insertion into the vector pCANTAB 5E of the linked variable heavy-chain (V_H) and variable light-chain (V_L) genes of an anti-F5 mAb, which protects calves against ETEC $F5^+$ infections (Bhaskaran et al. 2005). Briefly, the V_H and V_L genes isolated by RT-PCR from the murine hybridoma cells 2BD4E4 K99 (ATCC # HB-8178, Rockville, MD, USA) were bridged with an oligonucleotide encoding a $(Gly_4Ser)_4$ linker by performing splice overlap extension. The composite single-chain fragment of variable region (scFv) gene was then inserted into the phagemid pCANTAB 5E for phage display and the clone pSHOB-52 in the suppressor *E. coli* strain TG1 was selected after biopanning with F5 fimbrial protein. The pSHOB-52 recombinant phage rescued from TG1 cells with a helper phage was used to infect a non-suppressor HB2151strain of *E. coli* for the expression of soluble recombinant antibody by growing overnight in a 2X YT media containing 100 μg/ml ampicillin and 1 mM IPTG. The soluble anti-F5 moRAb, purified from, either the growth media (moRAbSup) or the bacterial periplasmic extract (moRAbPE) by affinity to an E-Tag, was used for the inhibition studies.

Hemagglutination inhibition

Hemagglutination inhibition was carried out essentially as described previously (Bhaskaran et al. 2005) except that a 100 μl of the bacterial suspension containing 2 x 10^8 CFU/ml of ETEC strain B41 ($O101:K^-:K99^+:F41^+$) (ATCC, Rockville, MD, USA) was used to produce agglutination. The ETEC strain B41 was grown in for 18 h at 37 °C in minimal medium containing 50 μg/ml tetracycline to promote the maximal expression of F5 fimbriae. Minimal medium was made with 0.8 mM $MgSO_4$-$7H_2O$, 9.5 mM citric acid-H_2O, 57.4 mM K_2HPO_4, and 16.75 mM $NaNH_4HPO_4$-$4H_2O$ in 950 ml dH_2O and autoclaved to sterilize. The medium was cooled to 56 °C, and 9.8 mM L-proline, 10 ml 50 % filtered dextrose, 10 ml 100× MEM vitamins (Sigma, Chemical Co., St. Louis, MO, USA) and 20 ml 50× MEM amino acids (Sigma Chemical Co., St. Louis, MO, USA) added aseptically (Jay et al. 2005). *E. coli* strain BL21 (DE3) pLys (Invitrogen, Carlsbad, CA, USA) that do not express F5 fimbriae was used for the negative control. For inhibition, the bacterial suspension in the wells of a 96 round-bottom-well microtiter plate was preincubated for 5 min at room temperature with two fold serial dilutions of either anti-F5 moRAb, anti-F5 mAb or an isotype anti-*Anaplasma marginale* mAb (anti-Am mAb) control, with starting concentration of 1.5×10^{-7} mmol (Waghela et al. 2000). After adding and mixing 100 μl of 0.25 % HRBC suspension to each well, the plates were incubated at 4 °C for 4 h and then checked for hemagglutination.

Inhibition of attachment of ETEC $F5^+$ to HRBC by anti-F5 moRAb

The attachment of the ETEC bacteria to either HRBC or enterocytes was blocked with anti-F5 moRAb and using anti-F5 mAb as a positive control and anti-Am mAb as a negative control. Various dilutions of these reagents in PBS were mixed separately in glass tubes with five microliters of 10^8 CFUs of ETEC strain B41, and incubated at 37 °C with gentle shaking for 30 min. Then, 50 μl of 0.25 % HRBC were added to the tubes, mixed and the suspension further incubated for 30 min. A 5 μl aliquot from each tube was spread on

glass slides, air-dried and then heat fixed at 56 °C for 5 min and in methanol for another 5 min. HRBC spread on triplicate slide was stained with Giemsa (Accustain, Sigma Diagnostics, St. Louis, MO, USA) and examined with a light microscope under oil immersion at 100X magnification. A total of 200 HRBCs on each of the three slides were counted, and the number of HRBCs with attached bacteria used to calculate mean percentage of the total cell count. Another similar set of tubes but with ETEC strain BL21 (DE3) pLys were as used as bacterial strain control.

Inhibition of attachment of ETEC F5$^+$ to enterocytes by anti-F5 moRAb

The inhibition of attachment of ETEC strain B41 to the enterocytes was similarly performed, except that HRBC were replaced with 8.6 x 10^5 enterocytes prepared as previously described (Jay et al. 2004). A total of two hundred enterocytes per each of the three slides were counted and cells with attached bacteria were differentially counted. The number of cells with attached bacteria was expressed as mean percentage of the total cell count.

Calf intestinal loop experiments

Five one-day-old colostrum-deprived healthy Holstein bull calves were fed with 2 liters of pasteurized skim milk at birth and then taken off feed 3 h before surgery. A laparotomy was performed on the calves that were anesthetized with 5 % thiopental sodium after tranquilization with 2 % xylazine. At laparotomy, the exteriorized ileum, kept moist with sterile prewarmed PBS, was ligated into 6–7 segments of approximately 7 cm lengths separated by interspaces of approximately 2 cm. In each animal, the segmented ligated loops received one of the treatments or their respective controls: purified anti-F5 monoclonal recombinant antibody from bacteria periplasmic extracts (moRAbPE) [T1] or anti-F5 moRAbSup from the bacterial growth medium [T2]; heat inactivated *E. coli* B41 [T3]; parental anti-F5 mAb [T4] or an isotype mAb control (anti-pseudorabies mAb, VMRD Pullman, WA) [T5]; plain media [T6] or plain media [T7]. Ten minutes after the initial treatment above, each loop, except the ones in plain media group T7, was inoculated with 2 × 10^8 CFU of live ETEC B41. Loops of group T7 were used as basal control. The intestinal segments were replaced into the abdominal cavity and the surgical incision closed with sutures. Each calf received an intramuscular dose of the analgesic Vetalgina prior to euthanasia 12 h later. At necropsy, the loops were retrieved from the peritoneal cavity and length (L), circumference (C) and weight (W) of each segment measured to calculate the ratio K = [(C/L) * W] for each intestinal segment, as the response to challenge with ETEC B41. The volume of fluid collected from each loop was noted and aliquots were taken to determine bacterial count (cfu/ml). A small piece of intestinal tissue from each loop was taken for histology and immunohistochemistry. For histology, thin 5-μm sections were stained with hematoxylin and eosin and examined for the degree of exfoliation, villus atrophy, fusion and inflammation to score the lesions as previously described (Bellamy and Acres 1983). Intestinal lesions were evaluated microscopically and scored on the severity of the lesions as 0 = no apparent injury, + = Slight inflammatory infiltrate and edema in the submucosa, ++ = Moderate inflammatory infiltrate, submucosal edema and villous atrophy, +++ = Moderate inflammatory infiltrate, submucosal edema, villi atrophy and necrosis. For immunohistochemistry, thin sections were deparaffinized, washed with xylol and rehydrated with successive washes with graded reducing concentrations of ethanol. Endogenous tissue peroxidase activity was removed by treatment with H_2O_2 in methanol. Antigenic activity was recovered by heating the tissue sections on slides in citrate buffer in a microwave oven at high power for 3 min. To observe *E. coli* attachment, tissues were reacted with anti-F5 mouse monoclonal antibody (MCA, IgG1 Santa Cruz) at a concentration of 0.0033 μg/μl after blocking with the buffer provided in Avidin-Biotin Blocking kit (Zymed Histochemical Reagents). After washes, the tissues were treated with a goat anti-mouse IgG peroxidase conjugate and the binding visualized using the H_2O_2 as substrate for deposition of the dye amino-ethyl-carbazol,

Statsitical analysis

Mean K values and bacterial counts were analyzed by one way ANOVA and Tukey's multiple comparison test.

Results

Hemagglutination inhibition assay

Hemagglutination induced with ETEC strain B41 was completely inhibited when the bacteria were preincubated with anti-F5 mAb at a concentration of ≥3.75 × 10^{-8} mmol (Fig. 1). However, nearly two times more molecules of anti-F5 moRAbPE were necessary for complete inhibition of hemagglutination. Inhibition was not observed in wells with bacteria incubated with the isotype control anti-Am mAb. Also, hemagglutination was absent when control bacteria were incubated with the HRBC (not shown).

Effects of anti-F5 mAb and anti-F5 moRAb on binding of ETEC F5$^+$ to HRBC or bovine enterocytes

Microscopic observations of HRBC spreads showed that a large number of ETEC strain B41 bacteria attached to the HRBC when the preincubation mixture did not contain either

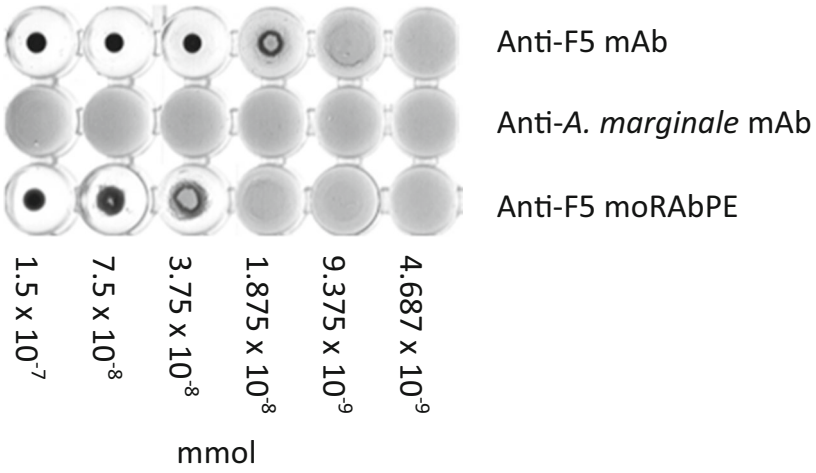

Fig. 1 Inhibition of ETEC strain B41 induced hemagglutination with anti-F5 monoclonal antibody and anti-F5 moRAbPE. Anti-*A. marginale* monoclonal antibody was used as an isotype control. The mAbs and moRAb were serially diluted from the starting concentrations of 1.5×10^{-7} mmol

anti-F5 mAb or anti-F5 moRAb, and hemagglutination was induced in the absence of these reagents (Fig. 2, panel A). However, in the presence of either anti-F5 mAb or anti-F5 moRAbPE (Fig. 2, panels B and C, respectively), the numbers of bacteria attached to HRBC was reduced or absent. Clumping of bacteria was observed in the samples preincubated with anti-F5 mAb, due to agglutination. However, such bacterial agglutination was not seen in samples, which had been preincubated with anti-F5 moRAbPE.

A similar picture was observed when enterocytes were used instead of HRBC. Attachment of ETEC strain B41 to enterocytes observed in the presence of anti-Am mAb (Fig. 2, panel D) was reduced when the bacteria were preincubated with either anti-F5 mAb or anti-F5 moRAb (Fig. 2, panels E and F). Again, in the sample with anti-F5 mAb, reduced binding to enterocytes was accompanied by bacterial agglutination. We also measured the total number of cells with adhering bacteria in the presence of various concentrations of control anti-Am mAb, anti-F5 mAb or anti-F5 moRAb. The percentage of HRBCs with attached ETEC strain B41 inversely correlated with the concentration of either anti-F5 mAb or anti-F5 moRAb (Fig. 3). There was a drop of 32 % in the number of HRBCs with attached bacteria when the moRAb concentration was increased from 2×10^{-7} mmol to 2.5×10^{-6} mmol in the preincubation mixture. The values for HRBC attached with bacteria were nearly 10 fold lower with 5×10^{-7} mmols of anti-F5 mAb as compared to 25 % with 4.0×10^{-8} mmol in the preincubation buffer. Fig. 3 also shows the decrease in numbers of enterocytes with attached bacteria when ETEC strain B41 had been preincubated with increasing concentrations of either the mAb or the moRAb. At the lowest concentrations tested, 2×10^{-8} mmol of mAb and 1×10^{-7} mmol of moRAbPE, nearly all of the enterocytes had one or more of bacteria attached. This number had decreased to approximately 50 % when there was a 10 fold increase in the concentration of either anti-F5 mAb or anti-F5 moRAbPE in the preincubation step. In contrast to the HRBC, there was a high background number of enterocytes with attached *E. coli*, even with the highest concentration of both the moRAb and the mAb. This finding might reflect additional means for adhesion to the natural target cell by *E. coli* B41 (Morris et al. 1980; Morris et al. 1982).

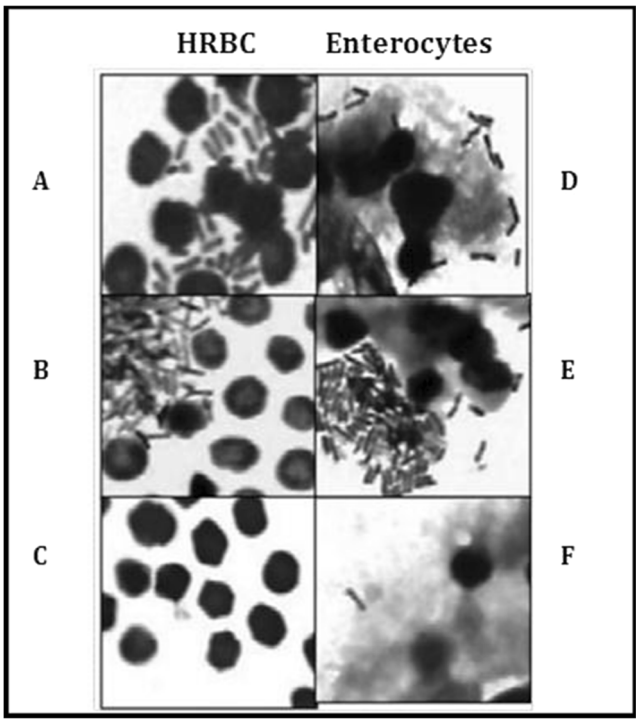

Fig. 2 In vitro inhibition of attachment of ETEC strain B41 to horse red blood cells (panels **a** to **c**) and to bovine enterocytes (**d** to **f**). Panels A and D: isotype anti-Am mAb control; B and E: anti-F5 mAb; C and F: anti-F5 moRAb

Calf intestinal loop experiments

The ratio K was used as an index for fluid accumulation within each loop due to the effects of enterotoxins produced 12 h after challenge with ETEC B41 (Fig. 4). As expected, the

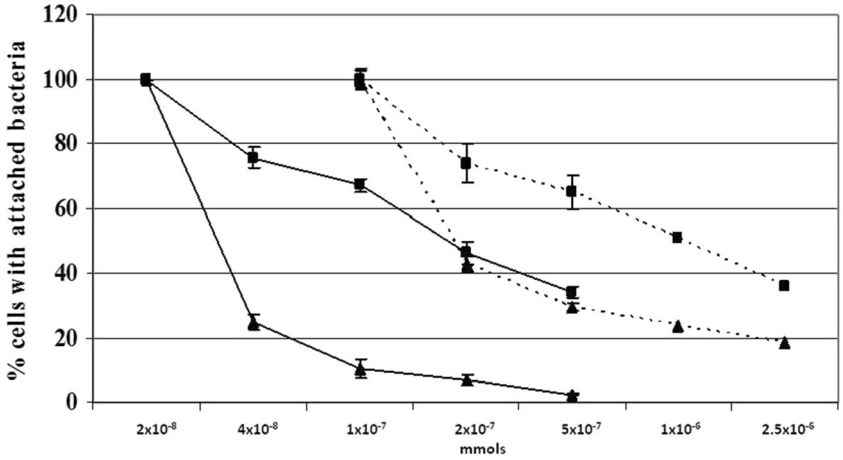

Fig. 3 Adhesion inhibition of ETEC strain B41 binding to host cells. Percent (±SE) of HRBC (▲) and bovine enterocytes (■) with attached bacteria after preincubating the ETEC with various concentrations of either anti-F5 mAb (—) or anti-F5 moRAb (—) calculated by microscopic observation of each treated sample spread on triplicate slide

mean ratio K in groups T1 (anti-F5 moRAbPE), T2 (anti-F5 moRAbSup) and T4 (anti-F5 mAb) was significantly lower as compared to group T6 (media alone) ($P \leq 0.0005$) and group T5 (mAb isotype control) ($p \leq 0.05$ or 0.0005). The response in groups T1, T2 and T4 is comparable to the control group T3 (inactivated *E. coli* B41) and group T7 (basal control) indicating the absence of effects of enterotoxins. Inactivated ETEC B41 expressing F5 is likely to prevent attachment of live ETEC B41 challenge (T3). These groups' ratios were significantly lower than group T6 (media alone) ($P < 0.0005$).

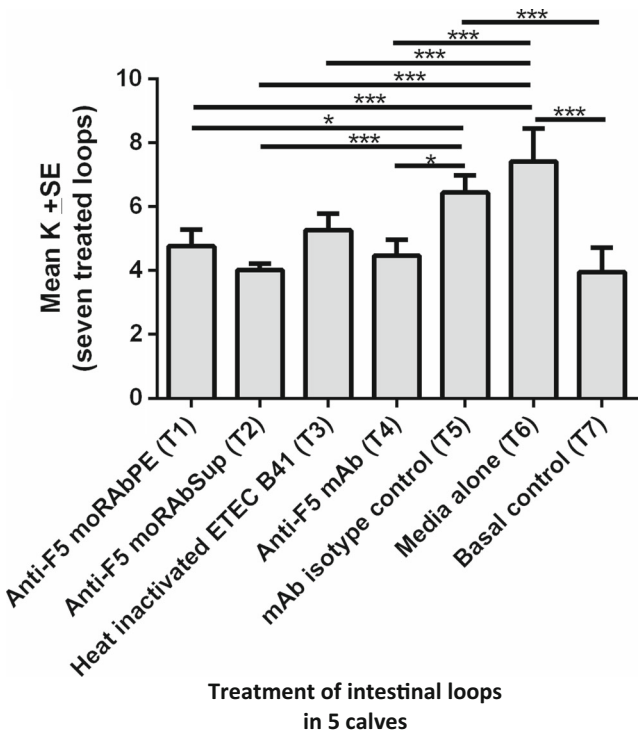

Fig. 4 Accumulation of fluid within treated intetinal loops of five calves following challenge with ETEC B41. The mean ± SE of the ratio K obtained from seven loops of each treatment were used for analysis. Treatments: T1: Anti-F5 moRAbPE = anti-F5 monoclonal recombinant antibody from periplasmic extract; T2: Anti-F5 moRAbSup = anti-F5 moRA from supernatant; T3: Heat inactivated ETEC B41; T4: Anti-F5 mAb = monoclonal antibody; T5: mAb isotype control; T6: Media alone; T7: Basal control = media alone – not challenged. For statistical analysis one way ANOVA and Tukey's multiple comparison test were used (* is $p < 0.05$, *** is $p < 0.0005$)

Bacterial counts, histopathology and immunochemistry

There was no statistical difference in the mean cfu recovered from anti-F5 moRAb (groups T1 and T2) treated versus control challenged loops (group T6), except for the loops in group T4 treated with anti-F5 mAb (Fig. 5). The mean cfu ranged from 4.5×10^8 to 2.9×10^9 in the harvested fluid from moRAb and control groups, whereas cfu for anti-F5 mAb was in the range of 10^3. Thus, although moRAb treated groups had live bacteria, the results in Fig. 4 illustrate that anti-F5 treatment reduces/inhibits the effects following ETEC B41 challenge. However, fluid recovered from loops treated with either moRAbPE (group T1), moRAbSup (group T2) or mAb (group T4) was three times less in volume then that recovered from loops of control group T6. Although, some differences were observed in cellular morphology, the lesion score, based on inflammation, degree of exfoliation, villus atrophy and fusion, did not indicate any differences between the groups. The mean lesion score ranged from minimum of 2.0 in T3 group to maximum of 2.6 in T6 group. For all the other groups except for uninoculated control T7, the score was in-between these minimum and maximum scores. The presence or the absence of ETEC B41 attached to the brush border of the intestinal epithelium was observed by immunochemical staining with anti-F5 mAb (Fig. 6). Morphologically there was less intense staining of the brush borders in the samples that were treated with either anti-F5 moRAb or anti-F5 mAb compared to the controls. However, regardless of this qualitative difference in staining a quantitative assessment was difficult.

Discussion

A key step in the pathogenesis of bovine colibacillois is the F5 fimbriae mediated attachment of ETEC to the enterocytes

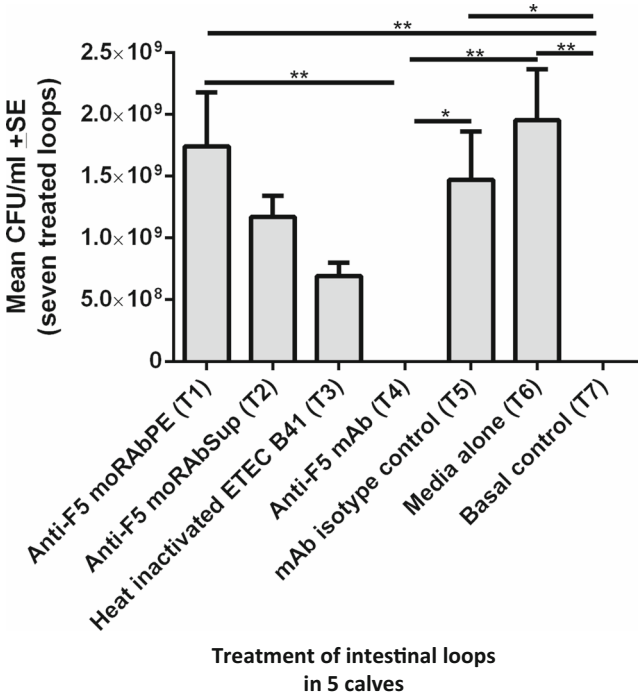

Fig. 5 Colony forming unit per milliliter (CFU/ml) recovered from each of the treated intestinal loops. The mean ± SE of CFU/ml from seven loops of each treatment were used for analysis. Treatments: T1: Anti-F5 moRAbPE = anti-F5 monoclonal recombinant antibody from periplasmic extract; T2: Anti-F5 moRAbSup = anti-F5 moRA from supernatant; T3: Heat inactivated ETEC B41; T4: Anti-F5 mAb = monoclonal antibody; T5: mAb isotype control; T6: Media alone; T7: Basal control = media alone – not challenged. For statistical analysis one way ANOVA and Tukey's multiple comparison test were used (* is $p < 0.05$, ** is $p < 0.005$)

prior to colonization and toxin production. The effects of colibacillosis is reduced or prevented if antibodies specific to the F5 are given orally within the first 24 h of a calf's birth. Anti-F5 mAb used in a commercial preparation for prophylaxis reduces the mortality in neonatal calves probably by interfering with the attachment of the bacteria to the glycoside receptor found on enterocytes of some animal species (Sherman et al. 1983). MAbs against F41 and F4/K88

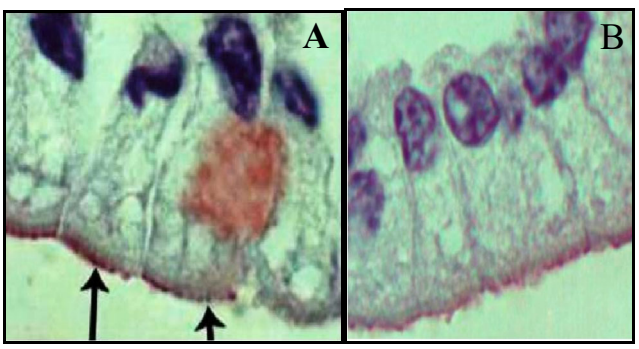

Fig. 6 Immunohistochemisry of calf ileum using specific anti-F5 mAb to detect attached bacteria. Panel A: ETEC B41 attached to enterocyte brush border of group T6 loops (arrows); panel B: enterocyte brush border of loops from group T1

fimbriae of different ETEC strains have been shown to inhibit fimbrial adhesion to isolated calf and porcine epithelial cells/brush borders (Van Zijderveld et al. 1998). Similarly, Fab fragments prepared from mAbs reacting with either ETEC fimbriae F5/K99 or F4/K88 or F41 have been shown to inhibit the binding of *E. coli* strains to their respective receptors (Sun et al. 2000; Isaacson et al. 1978).

ScFv molecules or similar recombinant antibody molecules developed by antibody engineering have proven to be effective in inhibiting the binding of ligands to their respective receptors (Casalvilla et al. 1999; Cirino et al. 1999). One study demonstrated that scFv was more efficient than the parental mAb in inhibiting the growth of *Spiroplasma citri* in liquid medium (Malembic et al. 2002). Another study reported that a scFv had a comparable titer to a mAb in neutralizing the IBD virus infection in vitro (Sapats et al. 2003). It has been suggested that scFv molecules, which neutralize the Rabies virus in vitro or in vivo in mice may have potential as an alternative to human rabies immunoglobulin in post-exposure preventive treatment (Muller et al. 1997; Ray et al. 2001). ScFv recognizing a linear peptide of the hypervariable region 1 of the hepatitis C virus blocked the binding of the peptide to Molt-4 cells (Li and Allain 2005; Zhai et al. 1999), however, the hypervariability of the antigen limits the potential use of the scFv for passive immunization. In converse to this, the binding region of F5 for the enterocytes is conserved indicating that recombinant antibody inhibiting the attachment is a good candidate for use in passive immunization.

In the present study, we have demonstrated that a moRAb developed from hybridoma cells secreting a mAb that reacts with the F5 fimbriae of ETEC (Bhaskaran et al. 2005) will reduce or prevent the attachment of ETEC $F5^+$ bacteria to HRBC as well as isolated bovine enterocytes. Previously, we demonstrated that this moRAb inhibited hemagglutination since the ganglioside receptor for the F5 is common to enterocytes and red blood cells of certain animal species. Expectedly, the hemagglutination inhibiting capacity of intact mAb is better than that of anti-F5 moRAb since higher molar concentrations of moRAb were required to cause the same level of inhibition as its parental mAb. This may be related to the functional affinity of the moRAb being lower than the bivalent mAb, in addition to the fact that the bivalent mAb causes bacterial agglutination, which translates into increased capacity for attachment inhibition.

In calf ligated loop experiments, ratio K used as an index for accumulation of fluids following ETEC B41 challenge indicated strongly that moRAb, prepared either from the periplasmic extract or the growth media of pSHOB52 transformed *E. coli* HB2151, prevented effects of enterotoxin, most likely by inhibiting the attachment of ETEC 41 to and subsequent colonization of intestinal epithelium. Although monomeric scFv may neutralize certain viral infections (Delagrave et al. 1999; Kalinke et al. 1996), dimerized scFv are necessary for effective neutralization for other virus infections (Drew et al. 2001; Lantto et al. 2002). The potential of recombinant

antibodies for prophylaxis is underscored by demonstration that intravenous injections of a human scFv, which recognizes an epitope on a fungal heat shock protein, significantly improves conditions of mice infected with various species of *Candida* (Matthews et al. 2003). Thus, the fact that the anti-F5 moRAb inhibits the adhesion of ETEC $F5^+$ bacteria to bovine enterocytes, albeit less effectively than the parental mAb, suggests that it may be a more economical alternative for preventing colibacillosis of neonatal calves especially if the recombinant antibody is made bivalent. We have previously described the expression of this anti-F5 moRAb in rice (Sunilkumar et al. 2009) wherein, the rice produced moRAb was able to inhibit attachment of B41 to both HRBC as well as bovine enterocytes.

Local passive immunization is a safe procedure for controlling growth of microorganisms and preventing disease in the gastro-intestinal tract (Weiner et al. 1999). It is well known that antibodies present in the colostrum protect young animals from increased morbidity and mortality caused by septicemia, diarrhea and respiratory diseases. Literature on the use of antibodies, including topical application, for prevention as well as therapy of infectious diseases is extensive (Ma and Lehner 1990; Zeitlin et al. 1999; Casadevall and Dadachova 2004). Monoclonal antibodies that neutralize infectivity can be produced rapidly in large quantities for prophylaxis but the procedures are not cost effective. Thus, recombinant antibodies with similar functional properties to the original mAb are an attractive substitute since they can be produced in alternative expression systems in larger quantities at comparatively reduced costs (Ma et al. 1998; Fischer et al. 1999). In this study, we have shown that a recombinant anti-F5 moRAb does prevent attachment of ETEC F5 to enterocytes – a step that is a prerequisite of infection and pathogenesis of the enterotoxigenic bacteria, and prevents fluid accumulation in intestinal loops. Further in vivo experiments with orally administered anti-F5 moRAb in neonatal calves would confirm the utility of this recombinant antibody as supplement in providing an inexpensive means of passive protection of calves against colibacillosis.

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Compliance with ethical standards All animal experiments were conducted after approval of the protocol by the Institutional Committee for the Care and Use of Experimental Animals (CICUAE), College of Veterinary Medicine, National Autonomous University of Mexico.

Conflict of interest The authors declare that they have no conflict of interest.

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