研究科分　麻布大学雑誌　第13・14巻・2006年

初乳抗体による腸管内での病原性大腸菌の定着阻止および毒素の中和

Inhibition of colonization and neutralization of verotoxin of enterohemorrhagic Escherichia coli 157:H7 by bovine colostral antibodies in beagle dogs

山本静雄[1]，栗林尚志[1]，福山正文[2]，古畑勝則[2]

麻布大学大学院 環境保健学研究科　[1]免疫学分野，[2]微生物学分野

Shizuo Yamamoto [1], Takashi Kuribayashi [1], Masafumi Fukuyama [2], Katsunori Furuhata [2]

Laboratories of [1] Immunology and [2] Microbiology, Graduate School of Azabu University

Abstract. The aims of this study were to evaluate the imhibition of colonization and the neutralization of verotoxin (VT) of enterohemorrhagic *Escherichia coli* O157 colostral antibodies in beagle dogs. Cows were immunized with bacterial cells (O157) purified VT2 14 times at 7-day intervals. Colostral antibodies were obtained from immunized cows after delivery. The titer of bovine colostral antibody to VT was high. But, the titer of antibody to flagellum was very low. IgA antibody titer to VT was higher than IgG or IgM antibody titer. Beagle dogs inoculated with O157 producing VT2 were administered colostral antibody, serum antibody or saline. Furthremore, colostral antibody and colostrum whey obtained from non-immunized cow were administrated. The amount of VT2 in feces was reduced immediately by administration of colostral antibody than plasma antibody, saline or colostrum whey. The residual time in small intestine of bovine colostral antibody and plasma antibody obtained from rabitts immunized VT2 was compared. The residual time of bovine colostral antibody was longer than rabbit serum antibody. This result suggested that bovine colostral antibody resisted to protease in digestive organ.

1. Objective

The aim of this study was to examine whether colostral antibody obtained from immunized cows is able to resist digestive proteases and neutralize Verotoxin (VT) in the digestive system in beagle dogs.

2. Methods

Microorganisms and measurement of VT2

The cultured Enterohemorrhagic *Escherichia coli* (*E. coli)* O157:H7 (O157) was suspended at $1x10^9$ CFU/ml in sterilized physiological saline, avirulent cells used for inoculation. VT2 in culture medium and feces was measured using commercial kit utilizing reversed passive latex agglutination test.

Immunization of cows with O175 cells and verotoxin

2 daily cows aged 6 years were immunized with O157 cells and purified VT2 solution. Colostrum were collected after delivery. After defat and decasein, colostral whey containing antibody was collected and used. Immunoglobulin classes of colostral antibody were isolated by affinity chromatography. Neutralizing antibody titers was measured using vero cells.

Antisera to VT2

Rabbits were immunized with VT2 for 14 times and collected blood.

Experimental infection

9 Beagle dogs were administered fradiomycin sulfate at 50 mg/kg for 3 days prior to inoculation of O157 in order to disturb native enterobacterial flora. Beagle dogs were inoculated with 5 ml of 1×10^9 CFU/ml orally using feeding tube. From the following day, fosfomycin sodium at 50 mg/kg was administered. Feces samples were collected daily after administration of EHEC. 100 ml of colostral antibody, plasma antibody, normal colostral whey or saline were administered orally following confirmation of increased VT2 in feces. Levels of VT2 in feces were measured with a commercial kit utilizing reverse passive latex agglutination test.

Resistance of colostral and serum antibodies against protease in small intestine

Colostral antibody or serum antibody were administered orally to beagle dogs under fasting. Dogs were sacrificed under anesthesia by pentobarbital at 1.5, 2 and 3 hours administration. Small intestine was extirpated after sacrificed. Antibody activity was measured by ELISA.

3. Results and Discussion

Colostral antibody titers showed 1:64 against VT2. Titers for each immunoglobulin class are shown in Table 1.

Table 1. Neutralizing antibody titers of Ig classes isolated from bovine colostral antibody.

Ig class	Titer
IgM antibody	1:8
IgG antibody	1:32
S-IgA antibody	1:128
Plasma antibody	1:8

Protein concentrations of Ig classes were adjusted to 2 mg/ml for neutralization tests.

Changes in fecal VT2 levels after administration of bovine colostral antibody, plasma antibody or saline in beagle dogs inoculation with O157 are shown in Figures 1. Changes in fecal VT2 levels after administration of colostral antibody or colostrum whey in beagle dogs inoculation with *E. coli* O157:H7 are shown in Figures 2. The titers were showed geometric mean.

The ressistance of bovine colostral antibody and rabbit serum antibodies were shown in Figure 3.

Antibiotic treatment in patients infected EHEC is considered to be a risk factor for development of HUS (1, 2, 3, 4, 6). To date, there have been no definitive treatments to prevent HUS. Increases in extracellular VT due to release by bacteria killed by antibiotic treatment is suspected to contribute to development of HUS (3, 5). Suppression of VT toxicity prior to systemic absorption

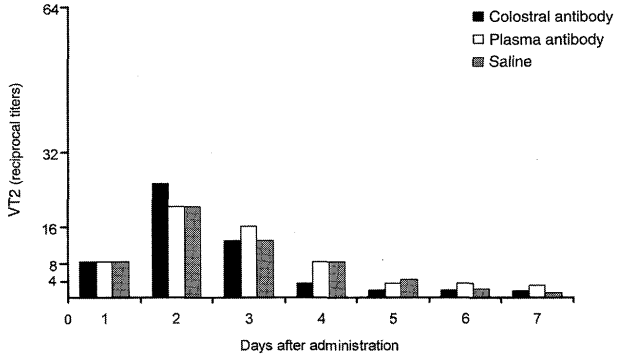

Figure 1. Changes in fecal VT2 following administration of colostral antibody or serum antibody or saline in beagle dogs inoculated with Escherichia coli O157. Each data was presented geometric mean of three dogs.

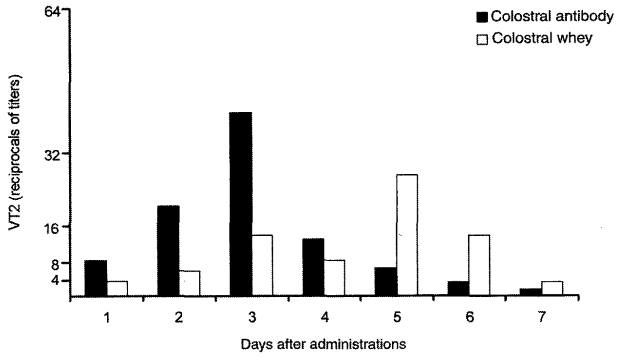

Figure 2. Changes in fecal VT2 following administration of colostral antibody or colostral whey that not contain antibody to VT2 in beagle dogs inoculated with Escherichia coli O157. Each data was presented geometric mean of three dogs.

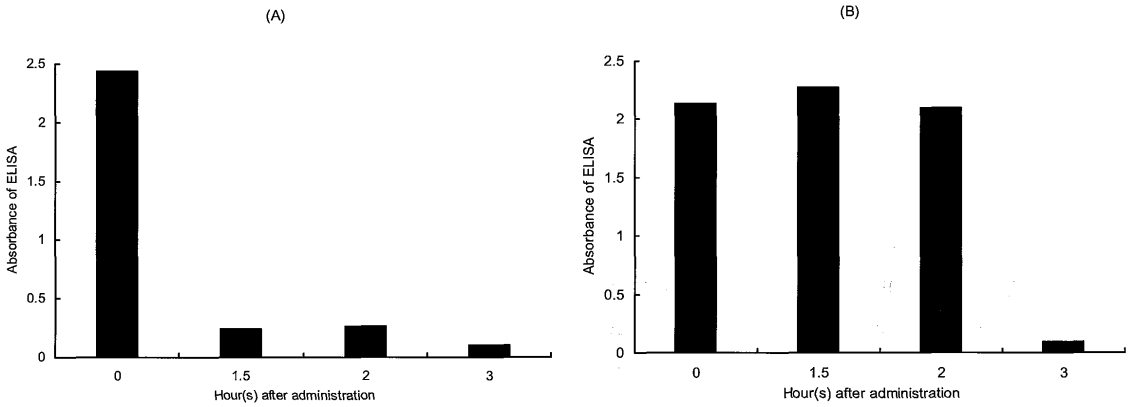

Figure 3. Activity of bovine colostral antibody and rabbit antibody obtained from small intestine of beagle dogs. Antibody activity was measured by ELISA. (A): rabbit serum antibody, (B): bovine colostral antibody.

from the digestive tract is thus considered to be important.

Antibiotic treatment in patients infected EHEC is considered to be a risk factor for development of HUS (1, 2). To date, there have been no definitive treatments to prevent HUS.

Colostral antibody could resistance to protease in small intestine. Enterobacterium flora recovered after administration of colostral antibody caused by VT2 reduced. Then, O157 was impossible to grow in digestive organ and the VT2 amount changed low level.

4. Conclusion

1) The colostral antibody could resist to proteases in gastrointestinal tract.
2) The amount of VT2 in feces was reduced immediately after administration of colostral antibody.
3) These results suggest that colostral antibody to VT2 is useful in treating EHEC infection, and may allow clinicians to administer antibiotics without risk of HUS occurrence.

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