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Passive protection against bovine rotavirus in calves by specific immunoglobulins from chicken egg yolk

Brief Report

M. Kuroki, M. Ohta, Y. Ikemori, R. C. Peralta, H. Yokoyama, and Y. Kodama

Immunology Research Institute in Gifu, Sano, Gifu-City, Japan

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Summary. The efficacy of chicken egg yolk immunoglobulins (yIg) from hens immunized with bovine rotavirus (BRV) serotype G6 (strain Shimane) or serotype G10 (strain KK-3) for protection against homologous BRV in calves was investigated. A significant protection by anti-BRV yIg having 6400 neutralizing antibody titer per dose could be achieved in calves ($P < 0.01$).

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Bovine group A rotavirus (BRV) is an important pathogen in neonatal diarrhea [18]. BRV infection in calves is prevented by oral administration of attenuated live vaccine. However, live vaccine is usually inactivated by neutralizing antibody in colostrum [1, 18]. Passive immunization by feeding bovine immune-colostrum to calves has also been employed [19], but this has some drawbacks: maintaining cows is expensive [5], neutralizing antibody titer (NAT) of colostrum decreases a few days after initial harvest [3, 19]; and the difference of NAT in colostrum between cows and heifers has been reported [6]. Recently, a high antibody response of long duration in poultry against BRV as detected in egg yolk of immunized hens has been documented [12]. The use of egg yolk immunoglobulins (yIg) is promising as it has become possible to mass-produce yIg using improved bioengineering methods [9, 20]. The efficacy of yIg against rotavirus infections in mice has been investigated [2, 7, 12, 21]. Although the murine model is useful for basic characterization of anti-BRV yIg preparations, BRV as well as other non-murine strains of rotavirus do not replicate well in mice, due to natural host range restriction [4, 12]. It is therefore necessary that exact evaluation of anti-BRV yIg be conducted, using susceptible neonatal calves [12]. To our knowledge, there has been no report on the protective effect of specific yIg against any enteropathogenic virus agent, including BRV, using calves as homologous animal host. The present study is aimed at demonstrating

the efficacy of a sustained post-natal oral use of anti-BRV yIg in calves against either of two distinct BRV serotypes (Shimane and KK-3 strains representing BRV serotype G6 and G10, respectively) in calves.

Shimane [15] and KK-3 strains [14] of BRV were kindly supplied by Dr. K. Sato and Dr. Y. Murakami of the National Institute of Animal Health, Tsukuba, Japan, respectively. Virus cultivation with MA-104 cells was done with Eagle's MEM containing 1 μg/ml of trypsin (type III, Sigma, St. Louis, MO). Virus titration was performed with roller tube cultures [10]. The infectivity was expressed by median tissue culture infective doses ($TCID_{50}$) as determined by the appearance of cytopathic effect (CPE) in MA-104 cells. Neutralization test was conducted using the same medium as described previously [10]. The neutralizing antibody titer (NAT) per gram of yIg was expressed as the reciprocal of the highest yIg dilution that showed complete inhibition of CPE of 200 $TCID_{50}$ of homologous BRV.

For production of anti-BRV yIg, Arlacel-adjuvanted (5% mannide monooleate, Maine Biological Laboratories, ME) and formalin-inactivated (0.1%, vol/vol) Shimane or KK-3 virus antigen were each inoculated to one thousand heads of 5-month-old White Leghorn chickens (strain Hyline W36). Each dose of Shimane or KK-3 antigen contained 10^9 $TCID_{50}$ per hen, given twice intramuscularly with one month interval. Two weeks after the second immunization, one thousand eggs laid by the immunized hens were harvested. Purification of yIg from eggs was done with hydroxypropylmethylcellulose phthalate (HPMCP) as described previously [11] except for dilution of yolk with 10 volumes of distilled water. Purified yIg was spray-dried to powder form using a spray-drying machine. Control yIg was prepared with eggs from control sham-immunized hens injected by mixture of virus-free medium and Arlacel following the same immunization schedule of BRV-immunized hens. From 100 liter of yolk supernatant, 350 g each of anti-Shimane, anti-KK-3 or control yIg was prepared. The homologous NATs of anti-Shimane and anti-KK-3 yIg were both 64 000/g, while that of control yIg was 1000/g.

Twenty-four neonatal Holstein calves from a farm that had been free of neonatal diarrhea for the past 3 years were used in this study. The calves were further tested to be negative for group A rotavirus antigen as determined by a commercial latex agglutination test kit (SERODIRECT-ROTA, Eiken Chemical Co. Ltd., Tokyo, Japan). Immediately after birth, each calf was fed with 500 ml of pooled colostrum (40 NAT) obtained from cows in the farm. Within the same day after birth, the calves were transported to the laboratory, housed in individual isolator cages, bled for serum NAT determination and their body weight recorded. The calves were then bottle-fed with a commercial bovine milk formula (Morinaga Milk, Morinaga, Tokyo, Japan) containing <2 NAT for Shimane or KK-3 strain at an amount corresponding to 10% (vol/wt) of body weight per day. Antibiotics were not included in the feed formula and no parenteral antibiotic or any medication was given throughout the experimental period. Before BRV challenge, 24 calves were randomly divided into 6 groups of 4 calves each. Calves were challenged once orally on the second

Table 1. Experimental design of calf treatment

Group	No. of calves	Challenge virus	yIg specificity	Homologous NAT[a]	
				per dose	(per day)
1	4	Shimane	control	100	(300)
2	4		anti-Shimane	3200	(9600)
3	4			6400	(19200)
4	4	KK-3	control	100	(300)
5	4		anti-KK-3	6400	(19200)
6	4			12800	(38400)

[a]Neutralizing antibody titer against challenge virus

day after birth (day 0) with a virulent Shimane BRV (Groups 1 to 3 calves with 1×10^{10} $TCID_{50}$ per calf) or KK-3 BRV (Groups 4 to 6 with 5×10^{9} $TCID_{50}$ per calf) (Table 1). The challenge time was 2 h (11 a.m.) after the first yIg administration on day 0. Daily oral yIg (yIg from eggs of sham-immunized hen for groups 1 and 4, anti-Shimane yIg for groups 2 and 3, and anti-KK-3 yIg for groups 5 and 6) commenced from day 0 until day 9 and was given 3 times a day at 9 a.m., 1 p.m., and 5 p.m. coinciding with feeding time. Antibody administration consisted of reconstituting the yIg powder in 5 ml milk formula and delivering the solution orally via syringe before giving the milk formula ration. Specific anti-BRV yIg were given at two levels of NAT dosages for each BRV strain (Table 1). Within the observation period after challenge, calves which had episode/s of diarrhea or loss of appetite were not milk-fed and given oral electrolyte solution until the cessation of diarrhea or recovery from illness. Clinical response of each calf was recorded and evaluated in terms of daily fecal score, viral excretion in feces and body weight gain. Fecal score was determined every morning by numerical score as follows: 0 = normal feces, 1 = mild diarrhea, 2 = severe watery diarrhea. Excretion of viable virus was examined by the appearance of CPE in MA-104 cells inoculated with 10% (wt/vol) homogenate of feces. Body weight gain of calves was expressed as percentage weight gain at day 10 over initial body weight on day 0.

Data for calves in group 1 to 6 are summarized in Table 2. The NAT against Shimane and KK-3 in sera of calves before challenge varied between 10 to 20. Among Shimane challenged calves, group 3 showed a significant increase in body weight ($P < 0.01$). The reduction of cumulative fecal score and decrease in virus excretion time in group 3 were also statistically significant compared with control group 1 ($P < 0.05$, $P < 0.01$, respectively). In group 2, virus excretion time and loss of body weight were likewise significantly reduced ($P < 0.05$) although the cumulative fecal score was similar to that of the control group. In contrast, calves of group 1 had severe diarrhea, showed sunken eyes indicative of dehydration and registered a negative weight gain.

Table 2. Clinical response of calves to challenge with BRV strain Shimane or KK-3 and protection of calves against challenge by anti-BRV yIg

Group	No. of calves with diarrhea (%)	Cumulative fecal score	Total positive days of virus detection	Body weight gain	
				kg	%
1	4/4[a] (100)	12.8 ± 4.8	7.8 ± 1.3	−3.3 ± 1.6	−7.6 ± 3.6
2	4/4 (100)	12.0 ± 3.6	5.0 ± 1.2[b]	−0.4 ± 1.2[b]	−1.3 ± 3.3[b]
3	0/4 (0)	0.0 ± 0.0[b]	2.3 ± 0.5[c]	+1.3 ± 0.4[c]	+3.5 ± 0.7[c]
4	4/4 (100)	14.5 ± 3.7	7.3 ± 1.0	−4.2 ± 0.7	−11.1 ± 2.1
5	3/4 (75)	6.3 ± 4.9[b]	6.3 ± 1.3	+0.7 ± 0.8[c]	+1.9 ± 2.1[c]
6	2/4 (50)	2.3 ± 4.5[c]	4.3 ± 1.3[c]	+1.1 ± 0.8[c]	+3.3 ± 3.1[c]

[a] No. of calves with diarrhea/total no. of calves
[b] $P < 0.05$, compared to control group 1 or 4 by Welch or Student test
[c] $P < 0.01$, compared to control group 1 or 4 by Welch or Student test

Among KK-3 challenged calves, reduction of cumulative fecal score and decreased virus excretion time were observed in group 6 ($P < 0.01$). Body weight likewise significantly increased in this group compared to control group 4 ($P < 0.01$). Calves in group 5 showed a significantly reduced cumulative fecal score and loss of body weight compared with control group 4 ($P < 0.05$ and $P < 0.01$, respectively). However, the virus excretion time in this group was comparable to that of the control. In contrast, calves of group 4 had severe diarrhea, dehydration and a marked body weight loss.

In the present study, we have demonstrated that a sustained oral treatment with yIg within the immediate post-natal period clinically protected calves against a homologous BRV challenge infection. This has also been documented in mice given heterologous BRV challenge using a BRV homotypic yIg preparation [12]. Chicken egg yolk IgG appears to be a suitable source of specific immunoglobulin comparable to immune-colostrum of cow because there are very few viral agents of chicken origin which can affect calves.

The minimum effective dose of anti-BRV yIg used in this study was lower compared to that of immune-colostrum of cow used in another study [19]. An exact comparison could not be made for the following reasons: (1) possible difference in passage levels of challenge strain, (2) different challenge exposure system, (3) mode of treatment used, (4) the specificity and affinity of neutralizing yIg to BRV which may be high because homologous virus antigen was used for immunization, (5) withdrawal of milk when calves showed clinical signs such as diarrhea, loss of appetite or fever which may have aided in preventing non-specific diarrhea by bacterial action in milk [1, 8] and (6) calves used in this study were fed a limited amount of colostrum. The use of anti-BRV yIg using colostrum-deprived-calves is not advisable due to lack of a pre-existing

local gut immunity which make them highly susceptible to virulent rotavirus infection. We are currently conducting a field test to determine the efficacy of anti-BRV yIg against field BRV infections in a beef herd under field conditions. This field trial is expected to provide data on yIg efficacy against a background of heterotypic BRV infection where different combinations of G (VP7) and P (VP4) types may co-circulate [13, 16, 17].

In conclusion, the data reported herein indicate that oral administration of anti-BRV yIg given as a regular supplement to calves within the immediate post-natal period may be a clinically amenable option for controlling BRV disease particularly in neonatal calves which for various reasons fail to maintain an effective local gut immunity via passive colostral transfer.

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Authors' address: Dr. M. Kuroki, Immunology Research Institute in Gifu, 839-1 Sano, Gifu-City 501-11, Japan.

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