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Chicken egg yolk immunoglobulins as oral prophylactics against bovine rotavirus and coronavirus infections

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ABSTRACT

Chicken egg yolk immunoglobulins (Yig) specific for bovine rotavirus (BRV) serotypes (G6, [P1]), (G10, [P 11]) and bovine coronavirus (BCV) were prepared with egg yolks derived from hens immunized with each viral antigen. Anti-BRV Yig preparations were investigated in suckling mice model, in experimental neonatal calves challenge tests, and in a field condition with an epidemic outbreak of BRV. These results showed significant efficacies of anti-BRV Yig preparations (P<0.01). Anti-BCV Yig preparations were compared with anti-BCV cow colostrums antibody powder. It took about four times more colostral antibody than chicken egg yolk antibody to prevent mortality in neonatal calves (P<0.01). The data of these tests showed that oral administration of Yig had significant efficacies against BRV and BCV infections. These results raise the possibility of wide application of specific Yig against gastro-enteropathogenic diseases caused by other pathogens in animals and human.

INTRODUCTION

Many kinds of innate and acquired defense mechanisms exist that protect the host from potential pathogenic microorganisms like virus and bacteria. The outcome of a particular infection depends on interactions between the virulent capability of the pathogen to evade and damage the host as well as the degree of adaptive immune responses in the host. The adaptive response is quiescent until stimulated by immunizing events, usually infections. Vaccination is the intentional process that can stimulate adaptive resistance in the host by enhancing humoral immune responses. Since a variety of microbial infections occur at the mucosa or penetrate through mucosal surfaces of the body, induction of antibodies in the mucosa is desirable in vaccinations. However, since it is frequently difficult to induce sufficient immunoglobulin levels for protecting the host following current active immunization procedures, passive immunization may be considered as an alternative measure for controlling infectious diseases in animals and humans. Use of chicken egg yolk immunoglobulins (Yig) from hens immunized by specific virulence factors or microorganisms may provide a novel approach to the control of infectious disease; this approach is reviewed in this article.

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NATURALLY OCCURRING PASSIVE IMMUNITY

Empirical observations of the transfer of immunity from mother to offsprings represent perhaps the first observation for passive antibody protection. The factors conferring immunity not produced by the infants or the fetus could be provided by the mother who possessed

antibodies directed against microbes present in her environment [1]. It is now well known that immunoglobulin G (IgG) alone among the five-immunoglobulin classes is actively transported across the placenta in human. This property provides passive immunity to the newborn baby. Evidence indicates that colostrums and mother's milk sustain and even augment the protection of infants against infections in human and in experimental animals prior to the development of gastrointestinal tract immunity. On the other hand, there is essentially no prenatal transfer of immunoglobulin across the placenta in neonatal calves and other ruminants, horses, pigs, and Marsupialia animals. Neonatal calves are therefore born agammaglobulinaemic or severely hypogammaglobulinaemic [2], and they are easy to get septicemia by secondary infection of bacteria after viral infection. They get maternal antibodies by absorption of colostrums from their dams. The absorption of colostral antibodies is limited to the first few hours of life [3]. And the efficiency of absorption of maternal colostral antibody depends on feeding of colostrums and environmental conditions in the farm [4, 5, 6]. In summary, the dam's colostrums is important in neonatal calves for getting maternal antibody, but it is difficult to acquire maternal immunity completely under natural occurring field condition.

BOVINE COLOSTRUM AND YIG

Bovine group A rotavirus is an important virus in neonatal calf diarrhea [7]. As allopathy in the field, antibiotics and electrolyte solution are used for prevention of secondary bacterial infections after viral infection, though the use of antibiotics induces the appearance of drug-resistant bacteria. Although oral administration of attenuated live rotavirus vaccine as soon as possible after birth has successfully reduced morbidity and mortality in neonatal calf [8], live vaccine is usually inactivated by neutralizing antibody titer of colostrums [7, 9, 10]. Therefore, artificial and intentional passive immunization in neonatal calves by oral feeding specific colostrum immunoglobulins derived from hyper-immunized cow has been employed [11, 12]. Bovine colostrum immunoglobulins given orally passively have been shown to prevent infections in the gastrointestinal tract. Colostral IgG and IgA were found to exhibit anti-pathogen activities [13, 14, 15]. There have been reports in some papers concerned with passive protection by bovine immune-colostrum [10, 11, 13, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26]. However, this bovine immune colostrum has some drawbacks in practice. Rapid decrease of neutralizing antibody titer of colostrum in a few days after initial harvest [12, 27], reduction in virus-neutralizing activity by gastric acid and digestive enzymes [15], high cost of feeding cow [28], and the difference of neutralizing antibody titer in colostrum between cows and heifers have been reported [29].

Recently a high specific antibody response of a long duration in poultry as detected in egg yolk of immunized hens has been documented [30]. After immunization of hens, the specific immunoglobulins are transported to the egg yolk and accumulated in it as maternal immunity. Egg yolks from hyper-immunized hens may provide a convenient and economical source of exogenous antibodies for passive immunization because a single chicken can provide up to 30 gram of immunoglobulins per year [31, 32]. Another advantage of yolk immunoglobulins is that collecting eggs from laying hens does not require the bleeding of animals for antiserum production, which is especially suited to current regulations for welfare of immunized host animals [33]. Immunized hens produce IgM and IgG antibodies in serum. Serum IgG is then transported as maternal antibody into egg yolks that are similar to those seen in serum [34, 35]. Both IgM and IgA antibodies are found in egg white, but not in egg yolk. Thus, the yolk is an excellent source of IgG antibody for passive immunity [36]. Hen egg contains as much as 200 mg of immunoglobulins, which is found almost exclusively in the yolk [36]. The chicken egg yolk IgG is described as being similar to mammalian IgG, although recently, evidence has emerged to suggest that this avian immunoglobulin, called either IgG or IgY, is antigenically similar to mammalian IgA [37]. The avian

IgG (200 to 220 kDa) [33, 38] is a monomer with molecular weight slightly higher than that of mammalian IgG (180 kDa) [38]. For purification of egg yolk IgG, egg yolks separated from egg whites must first be delipidated by addition of saline and chloroform [33, 39], propane-2-ol, and acetone [40] or by hydration and sedimentation [34, 41]. After removing lipids, yolk antibodies present in the water-soluble fraction were separated by differential precipitation with ethanol [33, 34], ammonium sulfate precipitations [30, 41], and other procedures. The use of improved bioengineering methods [42, 43, 44, 45, 46, 47] has become to be possible mass-production of Yig.

PASSIVE PROTECTION WITH YIG AGAINST BOVINE ROTAVIRUS AND CORONAVIRUS

Oral administration or feeding of Yig specific for pathogenic agents like virus, bacteria, and protozoa has provided a means for the prevention of infectious diseases of the alimentary tract [30, 31, 32, 33, 39, 41, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 54, 55]. The passage of Yig in neonatal calves [56] and pigs [57] has been researched. The Yig purified with enteric coating polymers (hydroxypropylmethylcellulose phthalate) for oral passage trials was used in neonatal calves. Using enzyme-linked immunosorbent assay, specific antibody activity and pattern of distribution of this Yig preparation in the gastrointestinal tract of neonatal calves were compared with control Yig prepared without hydroxypropylmethylcellulose phthalate. These results showed that the Yig purified with hydroxypropylmethylcellulose phthalate was more resistant against gastric juice in the stomach, thereby, ensuring a transfer of functional antibody activities to the small intestine of neonatal calves after oral administration [56].

(1) BOVINE ROTAVIRUS

Rotaviruses are ubiquitous in animals and human, and infect them worldwide. Group A bovine rotavirus (BRV) is the principal cause of acute diarrhea with dehydration in neonatal calves. Rotavirus serotypes G6 and G10 are major bovine pathogens [58] that have been recovered from diarrheic children and healthy human neonates [59, 60, 61]. This BRV problem has been approached by oral administration of Yig specific for BRV in suckling mice [30], neonatal calves [44], and in field trial with an epidemic outbreak of BRV [45].

Firstly, a standardized murine model for testing anti-BRV Yig preparations against either of two distinct BRV serotypes of strains Shimane [62] and KK-3 [63] representing BRV serotypes (G6, [P1]) and (G10, [P 11]) (Figure 1 and 2) [64] was established [30], because the availability of genetically well-defined strains of mice provides opportunities for elucidating the passive immune mechanisms [65, 66, 67]. Anti-Shimane or anti-KK-3 Yig was prepared from egg yolks derived from hens (strain Hyline, W-36) immunized with Shimane or KK-3 antigen by the use of chloroform extraction and ammonium sulfate precipitations. The protective capacity of Yig was tested against challenge for Shimane or KK-3 strain. There was a significant homotypic and heterotypic protection against diarrhea using 160 anti-Shimane or 160 anti-KK-3 neutralizing antibody titer per dose. The infectious titer of BRV recovered from intestinal tissue or luminal chime decreased with increasing homotypic Yig. A decrease in degree and duration of BRV antigen localization and pathologic change in the villus epithelial lining was observed in mice treated with homotypic Yig at optimum dose for prevention of diarrhea. The neutralizing antibody titer in sera of challenged mice increased with decreasing neutralizing antibody titer in the Yig given before challenge suggesting that protection was dose-dependent. These results indicate that passive protection could be achieved by the use of Yig against BRV-induced diarrhea in this murine model [30].

To evaluate exactly the efficacy of passive immunization requires the use of BRV-seronegative neonatal colostrum-deprived calves [11, 12], because BRV as well as other non-murine strains of rotavirus do not replicate well in mice [30, 68]. Secondly, a

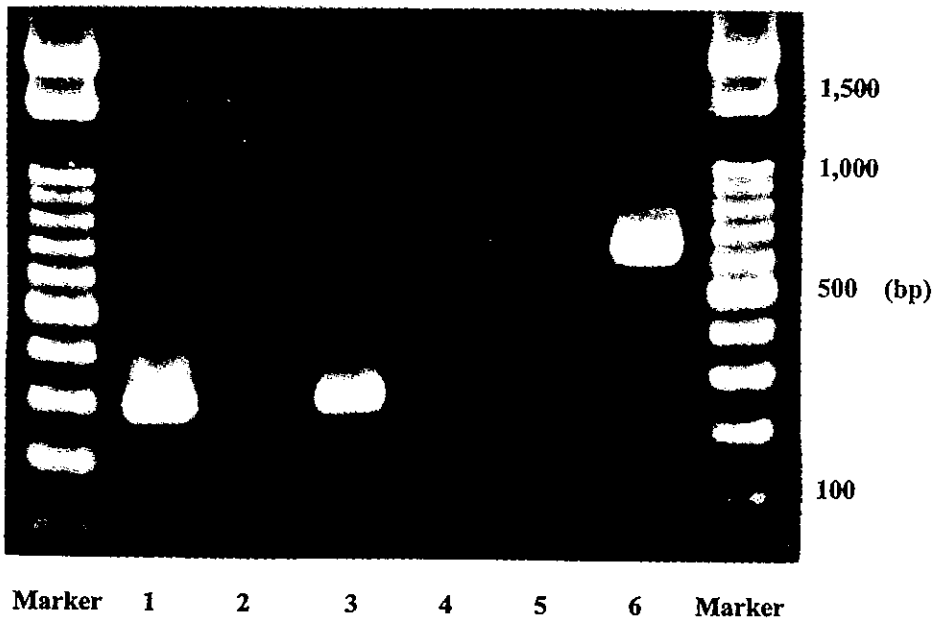

Figure 1. Profiles of PCR products of each BRV amplified with each primer pair. The products were electrophoresed on a 2% agarose gel and stained with ethidium bromide by a method previously described [64]. 100bp DNA ladder markers are shown at both right and left. Lane 1 and 2 contain NCDV with G6 and G10 primers, respectively. Lane 3 and 4 contain Shimane with G6 primer and G10 primers, respectively. Lane 5 and 6 contain KK-3 with G6 and G10 primers, respectively. The PCR results show that NCDV and Shimane have G6 protein, and KK-3 has G10 protein.

colostrum-single-fed neonatal calf model as susceptible homologous animal host for testing anti-Shimane or anti-KK-3 Yig was established [44], because colostrum-deprived calves are difficult to obtain and expensive to maintain in an isolated environment for tests. Anti-BRV Yig was purified from egg yolk derived from hens immunized with Shimane or KK-3 antigen by the use of enteric coating polymers (hydroxypropylmethylcellulose phthalate). Purified anti-Shimane or anti-KK-3 Yig was spray-dried to powder form. Colostrum-fed-neonatal calves (40 serum neutralizing antibody titer) challenged with a virulent Shimane or KK-3 on the second day after birth (day 0) were orally given three times a day from day 0 to day 9. A significant protection by anti-BRV Yig having 6,400 neutralizing antibody titer per dose could be achieved in neonatal calves from the data of clinical sign and body weight gain [44].

Thirdly, the oral efficacy of Yig specific for BRV in protecting neonatal calves was examined in a herd of cattle under field conditions [45]. Anti-Shimane or anti-KK-3 Yig powder was prepared from egg yolks derived from the immunized hens using hydroxypropylmethylcellulose phthalate. The anti-Shimane and anti-KK-3 Yigs were integral components of our bivalent Yig trial product (each with homotypic neutralizing antibody titer of 12,800). A herd of Japanese Black beef calves in Hokkaido Prefecture in Japan was selected for the field trials because the herd had neonatal calf diarrhea and

Figure 2. Profiles of PCR products of each BRV amplified with each primer pair. The products were electrophoresed on a 2% agarose gel and stained with ethidium bromide by a method previously described [64]. 100bp DNA ladder markers are shown at both right and left. Lane 1, 2, and 3 contain NCDV with P 1, P 5, and P 11 primers, respectively. Lane 4, 5 and 6 contain Shimane with P 1, P 5 and P 11 primers, respectively. Lane 7, 8 and 9 contain KK-3 with P 1, P 5 and P 11 primers, respectively. The PCR results show that both NCDV and Shimane have P 1 protein, and KK-3 has P 11 protein.

pneumonia for the three years immediately prior to the field trials. The pathogens of pneumonia in this herd were gram-negative bacteria like *Pasteurella* and *Haemophilus*. The pathogen associated with a serious outbreak of diarrhea in September (12 dead calves) in 1992 was enterotoxigenic *Escherichia coli* K99 (Figure 3). Since October in 1992, the farm workers began to use a commercial vaccine for enterotoxigenic *E.coli* K99 among dams. Since then, there were 18 mortalities associated with diarrhea in February (4 dead calves), August (4 dead calves), and December (10 dead calves) in 1993 (Figure 3). The pathogenic agent inducing this diarrhea in 1993 was confirmed to be BRV. BRV serotyping from feces of the 21 heads of calves during pre-trial survey in April to September in 1993 has been done (Figure 3, open arrow). The number of BRV-positive calves was 14 out of 21 diarrheic calves. BRV isolates from 14 calves were all identified as belonging to serotype G6.

Thereafter, a series of three field trials to evaluate Yig efficacy in neonatal calves were conducted sequentially in the same barn in 1994 (February 21 to March 29, April 2 to May 12, and May 1 to 29) (Figure 3, closed arrow). All of the calves in the Yig-treated groups were orally administered two grams of trial product three times a day for two weeks after birth. All the Yig-treated calves were left in contact with the control (not treated) calves in order to evaluate the efficacy of Yig in preventing natural BRV transmission. Their dams nursed the calves in both groups for the

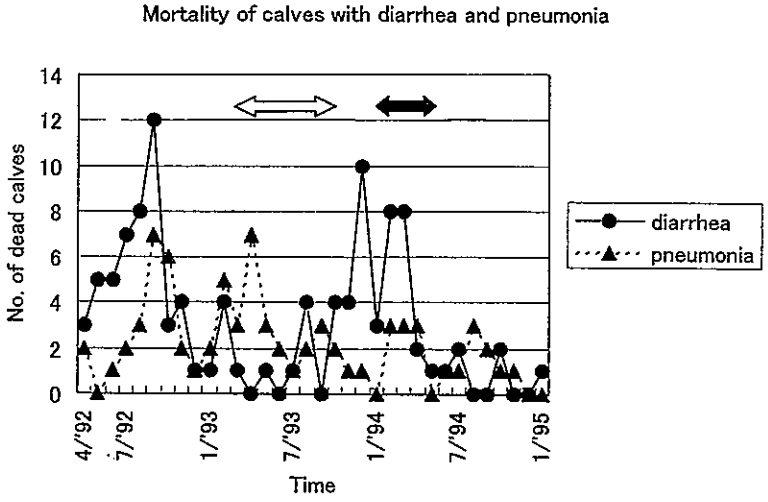

Figure 3. The frequency of mortality associated with diarrhea and pneumonia in the trial herd before, during and after treatment with anti-BRV Yig. The duration of pre-trial field survey for BRV isolation (open arrow) and field trials (closed arrow) for anti-BRV Yig are indicated.

whole duration of the trial including the suckling of the colostrums. Mortalities of calves were found to be associated with diarrhea and pneumonia aside from accidental causes in the farm. Data on body weight gain and BRV excretion in the three field trials are summarized in Table 1. In the first trial, the Yig-treated calves, compared with control calves, showed a significant decrease in the number of calves excreting high titer of BRV (over 10^4 median tissue culture infective dose/gram of feces). The second and third trials did not show significant Yig efficacy (Table 1) due to mildness of symptoms associated with BRV infection, compared to the first trial associated with high relative humidity [69, 70, 71]. The percentage of total rainy days, mean of relative humidity, and mean precipitation during the first trial were significantly higher than the means of the second or third trial. The observation that Yig-treated calves had a marked advantage in overall body weight gain and virus excretion led us to conclude that the efficacy of our bivalent (G6 and G10) trial product derived from specific reactivity between our Yig specific for G6 serotype and the field strain isolated in the farm. Although colostrum-fed calves were used as preferred by the farmers for their livestock in this field trial, the effect of Yig would probably have been more dramatic in a dairy herd in which calves received no colostrum or milk but Yig plus artificial milk replacer. Field trials of Yig in other herds of Japanese Black beef calves in both Miyagi and Hyogo Prefectures also have been done in addition to Hokkaido Prefecture. The summary of results in those three field trials was showed in Table 2. There were positive significant differences for reduction of clinical scores and weight gain in the both herds of Miyagi and Hyogo Prefectures ($P<0.05$).

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 infections particularly in neonatal calves.

(2) BOVINE CORONAVIRUS

Bovine coronavirus (BCV) is an important agent of

Table 1. Summary of results in field trials in a herd in Hokkaido Prefecture

No. of trial	Treatment group (n)	Body weight gain (Mean±S.D.) Kg	Body weight gain (Mean±S.D.) %	No. of calves excreting BRV: Below 10^{2a}	No. of calves excreting BRV: 10^2-10^4	No. of calves excreting BRV: Over 10^4
1st	Yig (10)	3.5±5.2[b]	11.1±17.0	8[c]	0	2[c]
	Control (10)	−0.5±2.5	−1.4±9.3	1	0	9
2nd	Yig (10)	2.9±4.5	9.3±14.6	10	0	0
	Control (10)	3.0±5.6	10.9±20.3	9	0	1
3rd	Yig (10)	2.7±5.4	8.3±18.1	6	4	0
	Control (10)	1.8±3.4	8.0±13.4	5	4	1

[a] Median tissue culture infective doses/gram of feces

[b] $P<0.05$, compared with control group of the first trial

[c] $P<0.01$, compared with control group of the first trial

Table 2. Summary of results in three field trials in Hokkaido, Miyagi, and Hyogo Prefectures

Herd (Pref.)	Species of neonatal calves (n)	Results: Safety	Results: Efficacy: Reduction of clinical scores	Results: Efficacy: Reduction of BRV excretion	Results: Efficacy: Weight gain
A (Hokkaido)	Japanese Black beef (60)	Approval, No accident	*N.S.*[a]	$P<0.01$[b]	$P<0.05$[c]
B (Miyagi)	Japanese Black beef (60)	Approval, No accident	$P<0.05$	N.T.[d]	$P<0.05$
C (Hyogo)	Japanese Black beef (60)	Approval, No accident	$P<0.05$	N.T.	*N.S.*

[a] *N.S.*: No significant difference

[b] $P<0.01$, compared with control group

[c] $P<0.05$, compared with control group

[d] N.T.: Not tested

neonatal calf diarrhea and is associated with acute diarrhea of adult cattle referred to as winter dysentery. This virus is known to cause a more severe disease than those caused by the BRV because it multiplies in both the small intestine and the large intestine whereas the rotavirus infects only the small intestine [72]. BCV vaccines have not been found to be efficacious in protecting against infection [72, 73]. This problem has been approached by oral administration of chicken egg yolk antibody powder (Yap) including Yig. Our group has evaluated the efficacy of Yap and cow colostrums antibody powder (Cap) against BCV-induced diarrhea in neonatal calves, and compared the therapeutic value of Yap and Cap. The protective effect of Yap and Cap prepared from hens and cows vaccinated with BCV-NCDC [74] antigen was evaluated in a challenge model with a virulent BCV strain. All calves in treated groups received either Yap or Cap containing BCV specific immunoglobulins. Control calves received with no antibody powder had severe diarrhea and all died within 6 days after infection. In contrast, all calves fed milk including Yap or Cap with 2,560 or 10,240 neutralizing antibody titer survived and had positive weight gain unlike the other treatment groups. It took about four times more colostral antibody than egg yolk antibody to prevent mortality in neonatal calves. These results indicate that the orally administered Yap and Cap protected against BCV-induced diarrhea in neonatal calves and that the use of Yap provided a higher degree of protection compared to Cap on a titer basis. Two possible explanations may be suggested for the difference in the minimal protective titers between Yap and Cap. Firstly, the avidity of antibodies derived from bovine colostrums is lower than that of antibodies obtained from egg yolk [75]. Secondly, it is suggested that yolk components in the Yap such as proteins and fats may have protected the immunoglobulins fraction from digestive enzymes and allowed safe passage of Yig through the stomach enough to confer protection in the target areas of the small intestine of neonatal calves [50].

WIDE APPLICATION OF YIG

Newly, the efficacies and functions of Yigs specific for human rotavirus [76] and enteric infectious disease pathogens such as bovine and human rotaviruses, bovine coronavirus, *Yersinia ruckeri*, enterotoxigenic *E.coli*, *Salmonella spp.*, *Edwardsiella tarda*, *Staphylococcus*, *Pseudomonas* [77], *Streptococcus mutans* [78], enteropathogenic *E.coli* [79] and *Helicobacter pylori* [80, 81, 82] have been reported. The Yig technology offers great future opportunities for designing prophylactic strategies including improvements of extraction from egg yolks [83], immunization with cholera toxin B and Softigen [84], DNA vaccine [85] and diagnosis applications [86] against infectious gastrointestinal diseases in animals and human.

CONCLUTION

In this review article, these results raise the possibility of wide application of Yig in the treatment of enteropathogenic diseases caused by other pathogens in animals and human; the Yig can be added to feed or formula or applied as a separate therapeutic agent. The Yig may well minimize the dependency on antibiotics inducing appearance of drug-resistant bacteria as the drug of choice against many infectious diseases.

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