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Immunotherapy in calves experimentally infected with *Cryptosporidium parvm*

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크립토스포리디움에 실험적으로 감염된 송아지의 면역요법

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초 록 : 7일령의 송아지 4마리에 실험적으로 크립토스포리디움을 감염시키고 면역혈청, 면역초유 그리고 단크론항체(C6)을 투여하여 이들의 면역요법제로서의 효과를 측정하였다. 크립토스포리디움 감염후 4일째부터 3일간 하루 2회(200~500ml)씩 3종의 면역요법제를 송아지 1마리씩 각각 경구로 투여하였으며, 나머지 한마리의 대조송아지에는 인산완충액을 경구로 투여하였다. 크립토스포리디움에 감염된 송아지들은 설사를 나타냈는데 대조송아지의 경우 감염후 3일째부터 9일간 설사증상을 나타냈다. 면역혈청, 면역초유 그리고 C6로 치료한 송아지들은 치료후 각각 3일, 2일, 5일째부터 정상에 가까운 분변을 배출하기 시작하였다. 면역혈청과 면역초유로 치료한 송아지들의 경우, 분변으로 배출되는 오시스트의 수가 대조송아지에 비해 현저하게 줄어들었다. 이러한 결과들은 실험에 제공된 면역요법제중 면역초유나 면역혈청이 크립토스포리디움에 감염된 송아지의 설사증상과 오시스트의 배출을 억제하는 효과가 있음을 나타내는 것이다.

Key words : *Crytosporidium parvum* , calf, immunotherapy, Korea.

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Introduction

Cryptosporidium species is a coccidian parasite of veterinary importance, with a worldwide distribution and a wide range of hosts including man and many other mammalian species, birds, reptiles and fish[1-4]. Neonatal calves infected with the parasite can act as a reservoir of infection for humans.

Management of patients is complicated by the absence of chemotherapeutic agent that are predictably effective in clearing *Cryptosporidium parvum* infection. To determine the efficacy of many chemotherapeutic agents including coccidiostats, anti-protozoal compounds have been tested against *Cryptosporidium* in the course of treatment of infections *in vitro*[5], in experimental animals[6~10], and calves[11]. The development of an effective treatment has been limited by the lack of *in vitro* cultivation systems, which are necessary for the study of their biochemical characteristics and by the lack of a reliable, small-animal model of clinical disease for screening of the efficacy of drug compound[12]. Thus, the efficacy of currently proposed chemotherapeutic agents is limited or doubtful in controlling *C parvum* infection[4,13].

Immunotherapy for persistent infection cuased by *C parvum* was attempted in athymic nude mice or in severe combined immune deficiency mice models. The results demonstrated that discharged oocysts and intestinal infectivity scores were reduced for immunodeficient mice treated with monoclonal antibody (mAb) 17.41[14,15]. Neonatal mice treated orally with mixture of immune colostrum and/or monoclonal antibodies also showed significantly reduced parasite loads compared with control mice, even if infection were not completely interrupted[16]. The present study was undertaken to determine the immunotherapeutic efficacy of immune bovine serum, immune bovine colostrum, and mAb C6 in calves experimentally infected with *C parvum*.

Materials and Methods

C parvum : The *C parvum* isolate used in the present experiment was originally obtained from a BALB/c mouse in Korea[17] and was maintained by passage in 7-day-old calves. Feces containing *C parvum* oocysts were collected from experimentally infected calves and suspended in 2.5% potsssium dichromate. The purification of oocysts was done as previously described[18], using the combination of ether extraction and discontinuous sucrose gradients.

Immune bovine serum : Two female Korean native cows were immunized intramuscularly four times at biweekly intervals with approximately 1×10^7 oocysts. The first immunization was done with Freund's complete adjuvant (FCA, Difco), and the second with Freund's incomplete adjuvant (FIA, Difco) 2 weeks later. The third and fourth immunization at week 4 and 6 were done without adjuvant[19]. Immune sera were obtained from these cattle at two weeks after the last immunization.

Immune bovine colostrum : A Holstein cow at eight months gestation was immunized as is described above[19]. Immune colostrum samples were obtained from the first milking after parturition. Colostral whey was separated from milk solids including cellular constituents and fat.

MAb C6 : The mAb C6 used in this study were derived as described previously[19]. Ascites tumors for hybridoma was produced by intraperitoneal injection of 2×10^6 hybridoma cells per pristane-primed mouse. Ascites fluids were collected and frozen at -70℃.

Experimental infection of calves : Four female Holstein calves 7-day-old were inoculated per os with 1×10^7 *C parvum* oocysts (VRI-CN91). After infection they were kept individually in separate stalls.

Immunotherapeutic treatment : Calf 1 was treated with bovine immune serum (diluted 1 : 10, IFA titer >128), calf 2 with bovine immune colostrum (diluted 1 : 20, IFA titer > 256), and calf 3 with mAb C6 (diluted 1 : 50, IFA titer > 256); calf 4 was given phosphate-buffered saline (PBS) as control. Treatment was initiated at day 4 post-infection (PI) and lasted 3 days. Each calf was administered per os with 200~500ml of the respective immunotherapeutics twice a day.

Observation of clinical signs and detection of oocysts : Feces were recorded from each calf daily until 20 days after the initial infection. The severity of diarrhea was scored : 0,

normal consistency; +, loose and formless; ++, moderately fluidal; +++, diarrheal fluidal but contain a large quantity of vegetable matter; ++++, watery diarrhea. Feces were collected everyday for oocyst detection. Numbers of oocysts (oocysts per gram of feces, OPG) were determined by direct counts in a hemocystometer.

Results

All calves infected with *C parvum* started excreting diarrheal feces at day 3 PI (Table 1). The severity of the diarrhea increased at day 4 PI when the immunotherapy was begun. Diarrhea in the control calf administered with PBS persisted 9 days. The other 3 calves recovered from the diarrhea after the respective immunotherapy: calf 1, 3 days; calf 2, 2 days; calf 3, 5 days.

All the infected calves commenced to discharge oocysts in their feces at day 4 PI (Fig 1). In the control calf, oocyst discharge peaked at day 5 PI and remained until day 7 PI; it then decreased until day 11 PI and increased again. Oocyst discharge in other calves which received immunotherapeutics was somewhat different from the control calf. Calf 1 showed a peak oocyst discharge one day after the immunotherapeutic treatment, and then oocyst shedding decreased sharply. Four days after the treatment oocyst discharge declined sharply in calf 2. Calf 3 showed a peak oocyst discharge 3 days after the treatment, and thereafter the discharge declined sharply.

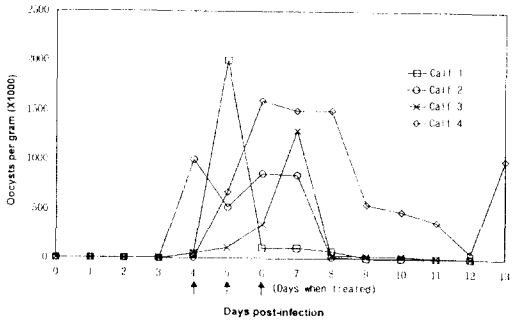

Fig 1. Patterns of oocyst excretion of the calves infected with *Cryptosporidium parvum* oocysts(1×10^7 each) and treated with the respective immunotherapeutic agents at day 4 post-infection for 3 consecutive days.
* Calf 1 was treated with bovine immune serum, calf 2 with bovine immune colostrum, calf 3 with monoclonal antibody C 6, and calf 4 with phosphate-buffered saline.

Discussion

In immunocompromised patients, cryptosporidiosis causes severe, prolonged diarrhea and is considered one of the

Table 1. Diarrheal score in Holstein calves experimentally infected with *Cryptosporidium parvum* and treated with immunotherapeutic agents

Calves	Days post-inoculation													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1	0	0	0	++	++++	+++	+++	++	+	+	+	0	0	0
2	0	0	0	++	+++	++++	++	+	+	0	0	0	0	0
3	0	0	0	++	+++	+++	+++	++	++	++	++	+	0	0
4	0	0	0	+++	+++	+++	++++	++++	+++	+++	++	++	++	+

All calves 7-day-old were inoculated with 1×10^7 *C parvum* oocysts at day 0 and treated with respective immunotherapeutic agents at day 4 post-inoculation for 3 consecutive days: calf 1 with immune bovine serum, calf 2 with immune bovine colostrum, calf 3 with monoclonal antibody C6, and calf 4 with phosphate-buffered saline.

Diarrheal score : 0, normal consistency; +, loose and formless; ++, moderately fluidal; +++, diarrheal fluidal but contain a large quantity of vegetable matter; ++++, watery diarrhea.

most important enteric opportunistic infections in AIDS. So far, on the basis of therapeutic outcomes in *C parvum*-infected AIDS patients, the efficiency of currently porposed anticryptosporidial therapies is limited or doubtful[7,13]. Recently, serotherapy including the various forms of antibody treatment reflected in medicine. Therapeutic mAbs are being investigated in a wide spectrum of clinical setting with very diverse objective. These objective may be classified into four categories: immunosuppression, infectious disease, cancer and intoxication[20]. Investigators have shown that some antibodies protect suckling mice from initial infection[16,21] and reduce the severity of persistent cryptosporidial infection in immunodeficient mice[14,22]. Persistence of the disease in nude mice and acquired immunodeficiency syndrome patients suggests that the immunity is T lymphocyte (probably the T-helper-inducer subset) dependent[2].

C parvum, either alone or with other agents, have been associated with clinical illness expressed primary as diarrhea. Experimental *Cryptosporidium* infection has so far been reported in calves[11,23]. In a resent work[24] it has been shown that 4 Holstein calves infected with *C parvum* began to show mucoid-watery diarrhea at day 3 to 5 PI, and the sign lasted 5 to 7 days. The calves commenced to discharge oocysts in feces at day 3 to 6 PI and remained until day 8 to 10 PI. In this study, the clinical sign was detected in the control calf 3 days postinfection and persisted 9 days. The calf commenced to discharge oocysts in feces at day 4 and lasted 9 days. The results in the present report support the findings of the recent work.

All the infected calves showed diarrhea at day 3 PI, oocysts were detected in fecal samples 4 days postinfection. Treatment with immunotherapeutic agents began 4 days postinfection. Oral treatment with colostral whey and immune serum significantly reduced fecal oocyst excretion and the severity of diarrhea. Fayer and Unger[12] reported that calves appear to be quite susceptible to infection even after they receive colostrum containing antiparasite antibodies. The hyperimmune bovine colostrum with high antisporozoite antibody titers reduced the severity of disease in experimentally infected mice[25]. These results are consistent with those described in the present study and indicate that oral passive immunotherapy with immune bovine colostrum may be a useful treatment approach, although reports of colostrum-mediated protection from cryptosporidiosis among other mammals are contradictory and inconclusive[2,6].

Summary

To determine the efficacy of immunotherapeutic agents, four female Holstein calves 7-day-old were inoculated per os with 1×10^7 *C parvum* oocysts (VRI-CN91). Each calf received twice daily oral dosage of 200~500ml of the immune bovine serum, immune bovine colostrum, mAb C6, and phosphate-buffered saline, respectively. Treatment was initiated 4 days postinfection and laster 3 days. The clinical sign of the calf treated with phosphate-buffered saline lasted 9 days after the initial treatment. The calves treated with those immunotherapeutic agents, however, showed decreased severity of diarrhea at day 3, 2, 5 after the initial administration, respectively. The calves treated with immunotherapeutic agents showed reduced parasite loads compared to control calf. These results suggest that oral passive immunotherapy with immune bovine colostrum and immune bovine serum may be a useful treatment approach.

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