Infectivity of Cryptosporidium andersoni and Cryptosporidium muris to Normal and Immunosuppressive Cynomolgus Monkeys

Koichi MASUNO1,2), Yasuhiro FUKUDA3), Masahito KUBO2,4), Ryo IKARASHI3), Takeshi KURAISHI5), Shosaku HATTORI3), Junpei KIMURA6), Chieko KAI5), Tokuma YANAI2) and Yutaka NAKAI3)*

1)Drug Developmental Research Laboratories, Shionogi & Co., Ltd., 3–1–1, Futaba-Cho, Toyonaka, Osaka 561–0825, Japan
2)Veterinary Medicine, Gifu University, 1–1 Yanagido, Gifu, Gifu 501–1194, Japan
3)Laboratory of Sustainable Environmental Biology, Graduate School of Agricultural Science, Tohoku University, Naruko-Onsen, Osaki, Miyagi 989–6711, Japan
4)Laboratory of Veterinary Pathology, Yamaguchi University, 1677–1 Yoshida, Yamaguchi-City, Yamaguchi 753–8511, Japan
5)The Institute of Medical Science, The University of Tokyo, 4–6–1 Shirogane-dai, Minato-ku, Tokyo 108–8639, Japan
6)Korea Secretary General of Asian Society of Zoo and Wildlife Medicine, College of Veterinary Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151–742, Korea

(Received 9 July 2013/Accepted 24 September 2013/Published online in J-STAGE 16 October 2013)

ABSTRACT. Cryptosporidium andersoni and Cryptosporidium muris infections have been found in the mice and/or cattle. The oocysts of C. andersoni and C. muris have been sporadically detected in human feces, but the infectious capacity and features have been unknown, because of the scarcity of reports involving human infections. To assess the infectivity and the clinical and pathological features of C. andersoni and C. muris in primates, an experimental infectious study was conducted using cynomolgus monkeys. The monkeys were orally inoculated with oocysts of two different C. andersoni Kawatabi types and C. muris RN-66 under normal and immunosuppressive conditions. The feces of the monkeys were monitored for about 40 days after the administration of oocysts using the flotation method, but no shedding oocysts were observed under either both normal or immunosuppressive conditions. Gross and histopathological examinations were performed on the immunosuppressive monkeys, but these revealed no evidence of Cryptosporidium infections, even though the monkeys were subjected to immunosuppressive conditions. It is hypothesized that C. andersoni and C. muris pose little danger of infection in primates even under immunosuppressive conditions.

KEY WORDS: Cryptosporidium, experimental animals, monkey, parasitology, pathology.

doi: 10.1292/jvms.13-0350; J. Vet. Med. Sci. 76(2): 169–172, 2014

Cryptosporidium (Apicomplexa: Cryptosporidiidae) causes emergent diarrhea and/or abdominal pain in both immunocompetent and immunocompromised patients, as well as in agriculturally important livestock species. In normal healthy animals, Cryptosporidium parvum is known to develop as a self-limiting infection localized to the intestinal tract [4]. In immunocompromised animals, infection by this protozoan is potentially life-threatening and may involve extraintestinal organs, such as the trachea, lungs, bile ducts and pancreas [3, 4, 23].

Since the first report of Cryptosporidiosis in humans, it has been recognized that only one species, C. parvum, poses a risk of infection to humans. However, based on analysis of the antigen and housekeeping gene, C. parvum was classified into two genotypes; type I and type II. Type I can infect only humans, and it has been named “C. hominis”. Type II can infect many kinds of animals, including humans, and it has been named “C. parvum”. Most Cryptosporidiosis in humans are caused by these two species of Cryptosporidium.

In one report, 2,414 human feces samples were investigated, and C. parvum was detected in 1,354 samples (56.1%), C. hominis in 1,005 samples (41.7%) and both C. parvum and C. hominis in 21 samples (0.9%) [11]. In another report, 7,758 human feces samples were investigated, and C. parvum was detected in 3,564 samples (45.9%), C. hominis in 3,814 samples (49.2%) and both C. parvum and C. hominis in 40 samples (0.5%) [1]. On the strength of these studies, it was considered that C. parvum and C. hominis cause Cryptosporidiosis in humans in most cases. However, in these and other studies, other species of Cryptosporidium (C. meleagris, C. felis, C. suis, C. muris and C. andersoni) were also detected [1, 11, 16, 20].

Although the major hosts of C. meleagris, C. felis, C. suis, C. muris and C. andersoni are various animals (Table 1), some of those protozoa can cause Cryptosporidiosis in humans and it occasionally proves fatal [14, 22]. However, some earlier investigations may have detected only the elimination of oocysts, which may not suggest evidence of intestinal infection of Cryptosporidium.

A number of drugs, among them nitazoxanide, have been suggested to be effective in both animal models and clinical trials. These drugs improved diarrhea and decreased
In natural infection, *C. andersoni* has been found to have infected the abomasum with fecal excretion of oocysts in cattle, and *C. muris* has been found to have infected the stomach in mice [9, 10, 17]. Although affected cattle exhibit no apparent clinical signs, severe infections of *C. andersoni* can be identified in the abomasum by microscopy [12]. Experimental infectious studies of *C. andersoni* have been reported in SCID mice [6, 8, 21]. Kawatabi type have been reported in SCID mice [6, 8, 21]. Experimental conditions of *C. andersoni* can be identified in the abomasum by microscopy [12].

Table 1. Major host of *Cryptosporidium* spp.

| Species of *Cryptosporidium* | Major host                  |
|------------------------------|-----------------------------|
| *C. parvum*                  | Cattle, sheep, goats, humans |
| *C. hominis*                 | Humans, monkeys             |
| *C. meleagris*               | Turkeys, humans             |
| *C. felis*                   | Feline                      |
| *C. canis*                   | Canine                      |
| *C. suis*                    | Swine                       |
| *C. muris*                   | Rodents, bactrian camels    |
| *C. andersoni*               | Cattle, bactrian camels     |

Same seven monkeys were used in the normal and immunosuppressive experiments.

Table 2. Information of animal in both experimental infections

| Animal No. | Sex  | Origin | Treatment (Cryptosporidium spp.) | Number of dosed oocysts |
|------------|------|--------|----------------------------------|------------------------|
| 1          | Male | Japan  | *C. andersoni* K strain          | 10^6                   |
| 2          | Female | China | *C. andersoni* K strain          | 10^6                   |
| 3          | Female | China | *C. andersoni* A strain          | 10^7                   |
| 4          | Male  | Indonesia | *C. andersoni* A strain     | 10^7                   |
| 5          | Male  | Indonesia | *C. muris* RN66 strain | 10^7                   |
| 6          | Male  | Indonesia | *C. muris* RN66 strain | 10^7                   |
| 7          | Female | Japan  | –                                | –                      |

Protozoa: Two different strains of *C. andersoni* Kawatabi types and one *C. muris* strain (RN-66) were used to induce the experimental infection. In the *C. andersoni* Kawatabi types, K strain and A strain were applied. The former strain was originally established from grazing cattle on the experiment farm belonging to Tohoku university [8, 21], and the latter was newly established from cattle feces on a farm in Aomori Prefecture (Japan). *C. muris* RN-66 was originally isolated from a house rat strain [6]. The strains were successfully subcultured using SCID mice in our laboratory [6, 13, 21]. The oocysts were administered to the monkeys within one month of collection.

Normal animals: Seven cynomolgus monkeys (control; n=1, infected; n=6) were orally inoculated with purified fresh oocysts by means of pelletized meals. The treatments were as follows: 10^6 oocysts of the *C. andersoni* K strain were administered to animal Nos. 1 and 2, 10^7 oocysts of the *C. andersoni* A strain were administered to animal Nos. 3 and 4, 10^7 oocysts of the *C. muris* RN-66 strain were administered to animal Nos. 5 and 6 and no oocysts were administered to animal No. 7 (Table 2). For the administration, water containing the oocysts was poured on two pellets of the meal, air-dried and fed to the monkeys which had been kept under fasting conditions, as soon as possible, and it was confirmed that the monkeys ate the pellets (Fig. 1). The feces of the monkeys were collected into a tube containing 2.5% dichromic acid and were monitored almost every day (from Days 2 to 40 of the study period to observe the shedding of oocysts by the flotation method. The monkeys were not sacrificed and were used again in later experiments.

Immunosuppressive animals: The same seven cynomolgus monkeys were used in the next experiment targeting immunosuppressive animals, and an attempt was made to infect the animals using the same method (Table 2). Before and between this study, monkeys were administered steroids to induce an immunodeficient condition. From 11 days before the study began until Day 10, once every seven days (Days −11, −4, 3 and 10), 10 mg/kg of methyl-prednisolone (Pfizer, New York, NY, U.S.A.) was administered intramuscularly under anesthesia, and 5 mg/kg of methyl-prednisolone was also administered once every seven days (Days 17, 24, 31 and 38) to maintain immunodeficiency. The feces of the monkeys were also collected in a tube containing 2.5% dichromic acid and monitored almost every day (from Days −11 to 40 of the study period, except for Days 1, 2 and 3) to observe the shedding of oocysts by the flotation method.
On Day 44 or 45, all monkeys were euthanized by exsanguination by cutting both the posterior aorta and vena cava under anesthesia, and complete necropsy was done. Organs and tissues, including the liver, pancreas, spleen, kidneys, urinary bladder, adrenal glands, heart, lungs, trachea, thyroid glands, brain, testes, epididymis, prostate, seminal vesicle, esophagus, stomach (fundic and pyloric parts), duodenum, jejunum, ileum, cecum, colon, mesenteric lymph node, sternum (including bone marrow) and femur (including bone marrow) were collected and fixed in 10% neutral buffered formalin. After the fixation, to decalcify the sternum and femur, these tissues were macerated in formalin solution with formic acid. The fixed organs/tissues were routinely processed and embedded in paraffin, and paraffin sections were stained with hematoxylin and eosin and examined microscopically.

RESULTS

Normal animals: No clinical signs or oocysts were detected in any of the animals at any point. Therefore, none of the monkeys were necropsied, and all were used in the immunosuppressive study.

Immunosuppressive animals: These animals were also free of clinical signs and oocysts at all time. No remarkable changes were observed in macroscopic observations.

In histopathological examination, chronic gastritis was observed in monkey Nos. 1, 2, 4, 5, 6 and 7. However, no infection by Cryptosporidium was seen in any of the organs or tissues, including any parts of the stomach. There were decreases in the number and size of the germinal center in the spleen, mesenteric lymph node and mucosal associated lymphoid tissue of the gastrointestinal tract (Fig. 2-1). The lymphoid tissues were compared with normal monkeys in histopathological sections of historical controls (Fig. 2-2). In addition, there were spirillum infections in the gastric pits of the stomach in three animals in the infectious groups.

DISCUSSION

None of the normal or immunosuppressive monkeys exhibited clinical signs or oocysts at any point. It is suspected that they were not infected with C. andersoni or C. muris.

The monkeys were administered Cryptosporidium oocysts orally with the number of oocysts being $10^6$ or $10^7$. These oocysts should have had infectious capacity, because the same method, oral administration, has been used in most other studies, including our previous studies [6, 21], and experimental infection of Cryptosporidium was successfully induced in the other studies. In addition, the number of the oocysts was sufficient for the transmission of infection [19].

In this study, the oocysts were added to the pellet meal and air-dried. Although it is well known that Cryptosporidium oocysts are capable of transmitting an infection under dry conditions [18], this process may have weakened of infectious capacity.

Methyl-prednisolone was administered intramuscularly.
Histopathological examination revealed decreases in the germinal center in the lymphoid tissues. Glucocorticoid suppresses the cells of the germinal center [7], and it is considered that methyl-prednisolone administration caused this atrophic change in the lymphoid tissue in the present study. In addition, there were spirillum infections. This bacterium has occasionally been observed in monkeys fed under conventional conditions, but the incidence seemed to be greater than in other studies. These results may possibly prove the evidence of immunosuppressive conditions in monkeys, and it was suspected that the dose level of methyl-prednisolone was high enough to produce immunocompromised animals. However, no hematological examination or serum cytokine analysis was conducted, and there were no intact control animals that could be compared to the dosed monkeys in the same study. Consequently, it is difficult to conclude that the monkeys were completely immunocompromised.

One report suggested that there are some mouse strains that have been resistant to infection by *C. muris* and *C. andersoni* [15]. A similar mechanism may exist in primates; cynomolgus monkeys may be resistant to gastric *Cryptosporidium* infection, but this hypothesis is considered unlikely.

Our present study suggests that *C. andersoni* Kawatabi types and *C. muris* were not able to infect the targeted animal species, namely monkey, even though natural hosts can be infected under normal condition. Possibly, *C. andersoni* and *C. muris* have no means for establishing infections in human.

In conclusion, *C. andersoni* and *C. muris* pose little danger of infection in primates even under immunosuppressive conditions.

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