Age-dependent resistance to *Cryptosporidium muris* (strain MCR) infection in golden hamsters and mice

Jae Ku RHEE*, Wang Su SO and Hyeon Cheol KIM

Department of Parasitology, Veterinary School, Chonbuk National University, Chonju 561-756, Korea

Abstract: An age-dependent aspect of resistance to *Cryptosporidium muris* (strain MCR) infection was monitored in Syrian golden hamsters, *Mesocricetus auratus*, at 1-, 5- and 10-week of age and in ICR mice, *Mus musculus*, at 3-, 12-, and 15-week of age orally inoculated with a single dose of 2×10^6 oocysts, respectively. The prepatent periods for both animals were similar, independent of age, but the patency was significantly longer in younger hamsters ($P < 0.001$) and a long tendency in younger mice. Hamsters infected at 1-week of age excreted about 10 times higher oocysts than those at 5- and 10-week of age. However, the total oocyst output was similar among mice of different ages. There was a good correlation between the length of the patency and the total oocyst output in hamsters ($R = 0.9646$), but not in mice ($R = 0.4561$). The immunogenicity of the parasite to homologous challenge infections was very strong in hamsters and relatively strong in mice. These results indicate that acquired resistance to *C. muris* infection is age-related and the innate resistance is independent of age of hamsters, and that both innate and acquired resistance, on the contrary, are irrespective of age of mice.

Key words: *Cryptosporidium muris* (strain MCR), golden hamster, mice, transmission experiment, age-dependent resistance, immunogenicity

INTRODUCTION

Cryptosporidium spp. can cause chronic life-threatening diarrhea in neonatal and immunodeficient individuals. In recent years, increasing interest for *Cryptosporidium muris* infection in cattle has led to the finding of reduced body weight gain and milk production (Anderson, 1987; Esteban and Anderson, 1995). Several transmission experiments to develop laboratory animal model of *C. muris* have been done in various laboratory animals (Iseki et al., 1989; Aydin, 1991; Matsui et al., 1994; Rhee et al., 1995; Aydin and Özkul,

1996). Meanwhile, age-dependent aspects of resistance to *C. parvum* and *C. baileyi* infections were investigated in mice, calves, lambs and chickens, respectively (Sherwood et al., 1982; Heine et al., 1984; Harp et al., 1990; Ortega-Mora and Wright, 1994; Sreter et al., 1995). However, no transmission experiment has been performed, so far, to clarify infectivity of *C. muris* to golden hamster except for the report of Pavlasek and Lavicka (1995) who revealed a spontaneous gastric cryptosporidiosis from desert hamster (*Phodopus roborovskii* Satunin, 1903) in the Czech Republic. Although Matsui et al. (1994) described that mice infected with *C. muris* (strain RN 66) showed a uniform degree of susceptibilities with different ages, experimental evidence in details for age-related resistance to *C. muris* has not been known in

• Received 20 November 1998, accepted after revision 1 February 1999.

*Corresponding author (e-mail: jkr@moak.chonbuk.ac.kr)

animal models. The present study was to observe the age-dependent resistance to the coccidium in orally infected hamster and mouse by understanding the discharge pattern of oocysts. Additionally, this is the first report which presents the findings of an experimental infection with *C. muris* (strain MCR) to elucidate basic host-parasite relationship in golden hamsters.

MATERIALS AND METHODS

Ten specific pathogen free (SPF) Syrian golden hamsters at 1-, 5- and 10-week of age and 10 SPF ICR mice at 3-, 12- and 15-week of age were each orally inoculated with a single dose of 2×10^6 oocysts of *C. muris* (strain MCR) isolated from mice, respectively, as previously described (Rhee et al., 1995). Each of another five age-matched both animals served as uninoculated controls. The animals were housed as single in wire-bottomed cages and given a commercial nonmedicated concentrates and water ad libitum. Each cage was placed on a tray containing 5 mm depth of water to keep the excrements wet. Five days after oocyst shedding ceased, all the animals

were each inoculated again with a single dose of 2×10^6 oocysts to determine the establishment of a homologous challenge infection. Following inoculation, examination of fecal samples was subjected to the methods previously described by Rhee et al. (1995). Mean values of the lengths of the patent periods in each age for both hosts were compared using Student's *t*-test. The relationship between the lengths of the patent periods and the total oocyst excretions in each animal was analyzed by linear regression.

RESULTS

The prepatent periods of *C. muris* infection in hamsters ranged from 10 to 13 days (Table 1) and in mice from 4 to 6 days (Table 2), irrespective of their ages at inoculation of oocysts. The standard deviation of the mean prepatent period was low for both animals (Tables 1, 2). A significant difference was found in the length of the patent period, ranging from 29.7 to 63.3 days, in hamsters infected at 10-, 5- and 1-week of age ($P < 0.001$), showing an inverse relationship between the age at inoculation and the length

Table 1. The length of prepatent and patent period, and the total oocyst excretion of golden hamsters inoculated with 2×10^6 *Cryptosporidium muris* (strain MCR) oocysts at different ages

Case number	Ages at inoculation								
	1-week old			5-week old			10-week old		
	PP ^{a)}	P ^{b)}	TOO ^{c)}	PP ^{a)}	P ^{b)}	TOO ^{c)}	PP ^{a)}	P ^{b)}	TOO ^{c)}
1	11	80	176.2	11	49	15.2	11	36	10.4
2	12	80	64.3	13	44	6.5	11	34	1.5
3	11	70	56.9	10	44	6.1	12	33	4.2
4	13	66	85.6	11	43	6.9	10	32	7.7
5	11	65	102.3	11	36	21.3	12	30	6.5
6	11	64	75.3	10	33	10.0	10	29	20.6
7	11	58	161.9	11	29	4.0	10	29	10.5
8	11	57	122.4	10	26	3.9	10	28	13.6
9	11	57	44.0	10	25	3.5	11	27	11.2
10	11	36	44.6	11	16	2.1	12	19	3.7
Mean	11.3	63.3	93.4	10.8	34.5	8.0	10.9	29.7	8.0
± SD	0.67	12.76	46.97	0.92	10.55	6.03	0.86	4.72	3.92

^{a)}prepatent period (days); ^{b)}patent period (days); ^{c)}total oocyst output ($\times 10^5$).

The patent period was significantly longer in 1-week old than 5-week and 10-week old hamsters ($P < 0.001$), while that was not significantly different between the latter two groups ($P > 0.2$).

Table 2. The length of prepatent and patent period, and the total oocyst excretion of mice inoculated with 2×10^6 *Cryptosporidium muris* (strain MCR) oocysts at different age

Case number	Ages at inoculation								
	3-week old			12-week old			15-week old		
	PPa)	Pb)	TOOc)	PPa)	Pb)	TOOc)	PPa)	Pb)	TOOc)
1	5	55	178.0	5	39	68.6	5	45	112.2
2	5	51	114.0	5	35	127.8	5	45	93.9
3	5	51	113.7	5	35	114.3	5	41	127.1
4	5	49	142.5	5	35	57.0	6	38	50.2
5	5	49	83.6	5	35	50.9	5	35	134.3
6	5	49	54.6	5	35	48.7	5	35	65.3
7	5	39	88.1	5	35	48.3	5	35	56.1
8	5	39	65.8	5	35	46.2	5	35	49.4
9	5	39	44.2	5	35	28.1	5	35	48.4
10	4	36	78.2	6	34	118.0	5	35	31.8
Mean	4.9	45.7	96.3	5.1	35.3	70.8	5.1	37.9	76.9
± SD	0.3	6.36	39.20	0.3	1.27	35.55	0.3	4.01	34.98

a)prepatent period (days); b)patent period (days); c)total oocyst output ($\times 10^6$).

The patent period was significantly longer in 3-week old than 12-week and 15-week old mice ($P < 0.001$), while that was not different between the latter two groups ($P > 0.08$).

Fig. 1. Mean daily oocyst shedding in hamsters inoculated with 2×10^6 oocysts of *Cryptosporidium muris* (strain MCR) at 1-, 5- and 10-week of age. (+) and (-) indicate that less than 10^3 oocysts were detected, and oocysts were not detected, respectively.

of the patent period (Table 1). In contrast to hamsters, a slight difference was found in mice at 12-, 15- and 3-week of age (Table 2).

Oocysts were not observed in fecal samples of uninoculated controls. Hamsters infected at 1-week of age excreted a significantly higher number of oocysts (93.4×10^5 , on the average)

Fig. 2. Mean daily oocyst shedding in mice inoculated with 2×10^6 oocysts of *Cryptosporidium muris* (strain MCR) at 3-, 12- and 15-week of age. (+) and (-) are the same as in Fig. 1.

than those infected at 5- (8.0×10^5) and 10-week (8.0×10^5) of age, while mice at 3-, 12- and 15-week of age discharged a similar number of oocysts (from 70.8×10^6 to 96.3×10^6). The standard deviations of the means of the patency and those of the total oocyst shedding were high for both animals (Tables 1, 2). The patterns of oocyst excretion in both hosts are depicted in Figs. 1 and 2. Peaks of

oocyst productions in hamsters at 1-week of age occurred between day 34 and 43 post-inoculation (PI) and in mice at all ages between day 16 and 27 PI, giving rise to one peak. But those in hamsters at 5- and 10-week of age were not prominent. As shown in Tables 1 and 2, the longer its patency, the higher its oocyst excretion was observed in hamsters ($R=0.9646$), however such relationship was not revealed in mice ($R=0.4561$).

Hamsters challenged with oocysts of *C. muris* did not excrete any oocysts during 30 days post-challenge-infection (PCI), while all mice excreted a few oocysts on day 16 and 31 PCI.

DISCUSSION

The acquisition of age-related resistance to infection with *C. parvum* has been well characterized in rodent and ruminant models (Sherwood et al., 1982; Harp et al., 1990). However, the age-related aspects of resistance to *C. muris* were poorly understood in animal models. The present study demonstrates the experimental evidence for the resistance to experimental cryptosporidiosis in hamster.

The prepatent periods of *C. muris* for both animals were similar irrespective of age, suggesting that the innate resistance to the protozoon is independent of age. Low standard deviation suggests slight individual difference in the innate resistance to *C. muris* infection for both animals. This is in line with a study that showed a uniform prepatent periods of 7-10 days in mice orally inoculated with *C. muris* (strain RN 66) at 3 to 29-week of age (Matsui et al., 1994).

In hamster, the older the host, the shorter the patency, suggesting that acquired resistance is age-related in this case, as shown in mice with *C. parvum* and in chicks with *C. baileyi* infections (Sherwood et al., 1982; Sreter et al., 1995). However, such phenomenon was scarcely demonstrated in mice, as shown in kids and lambs infected with *C. muris* (strain MCR) (Rhee et al., 1998).

Interestingly, there was a good correlation between the length of the patent period and the total oocyst excretion in hamsters, but relationship was not shown in mice. High

standard deviations of the mean of the patency and those of the total oocyst shedding indicate considerable individual differences in terms of the effectiveness of the acquired resistance to *C. muris* infection. It is reasonable to think that oocyst production gave rise to one prominent peak in both younger animals due to excretion of oocysts in maximum number, as was found in *Cryptosporidium* spp. infections.

A single oral infection with *C. muris* elicited protective immune response of sufficient magnitude to clear the parasite from the gastric mucosa and to make both animals completely resistant to the oral homologous challenge infections. It implies that the immunogenicity of hamsters against *C. muris* (strain MCR) is very strong even at the age of 1-week.

Based on these experimental results, the acquired resistance to *C. muris* infection in hamsters is age-related in contrast to mice (possibly independent of age). Additionally, we also revealed that *C. muris* (strain MCR) is capable of infecting hamsters although a large number of oocyst shedding was not observed in comparison with mice, and hamsters are, therefore, not considered as a favourable experimental animal. All things considered, the mouse has been proven to be the most suitable host of *C. muris* by a great deal of oocyst shedding in comparison with a small body size and continued infection for a long time.

REFERENCES

- Anderson BC (1987) Abomasal cryptosporidiosis in cattle. *Vet Pathol* **24**: 235-238.
- Aydin Y (1991) Experimental cryptosporidiosis in laboratory animals: Pathological findings and cross-transmission studies. *Fac Vet Med Univ Ankara* **38**: 465-482.
- Aydin Y, Özkul IA (1996) Infectivity of *Cryptosporidium muris* directly isolated from the murine stomach for various laboratory animals. *Vet Parasit* **66**: 257-262.
- Esteban E, Anderson BC (1995) *Cryptosporidium muris*: Prevalence, persistency, and detrimental effect on milk production in a drylot dairy. *J Dairy Sci* **78**: 1068-1072.

- Harp JA, Woodmansee DB, Moon HW (1990) Resistance of calves to *Cryptosporidium parvum*: Effects of age and previous exposure. *Infect Immun* **58**: 2237-2240.
- Heine J, Moon HW, Woodmansee DB (1984) Persistent *Cryptosporidium* infection in congenitally athymic (nude) mice. *Infect Immun* **43**: 856-859.
- Iseki M, Maekawa T, Moriya K, Uni S, Takada S (1989) Infectivity of *Cryptosporidium muris* (strain RN 66) in various laboratory animals. *Parasitol Res* **75**: 218-222.
- Matsui T, Fujino T, Kobayashi F, Morii T, Tsuji M (1994) Oocyst production and immunogenicity of *Cryptosporidium muris* in the experimental mice. *Jpn J Parasitol* **43**: 199-204.
- Ortega-Mora LM, Wright SE (1994) Age-related resistance in ovine cryptosporidiosis: Patterns of infection and humoral immune response. *Infect Immun* **62**: 5003-5009.
- Pavlassek I, Lavicka M (1995) The first finding of a spontaneous gastric cryptosporidiosis infection in hamsters. *Vet Med (Praha)* **40**: 261-263.
- Rhee JK, Kim HC, Eun GS (1998) Infection kinetics and developmental biology of *Cryptosporidium muris* (strain MCR) in Korean native kids and Corriedale lambs. *Korean J Parasitol* **36**: 171-181.
- Rhee JK, Yook SY, Park BK (1995) Oocyst production and immunogenicity of *Cryptosporidium muris* (strain MCR) in mice. *Korean J Parasitol* **33**: 377-382.
- Sherwood D, Angus KW, Snodgrass DR, Tzipori S (1982) Experimental cryptosporidiosis in laboratory mice. *Infect Immun* **38**: 471-475.
- Sreter T, Varga I, Bekesi L (1995) Age-dependent resistance to *Cryptosporidium baileyi* infection in chickens. *J Parasitol* **81**: 827-829.