The Korean Journal of Parasitology
Vol. 35, No. 4, 245-249, December 1997

Effects of *Cryptosporidium muris* (strain MCR) infection on gastric mucosal mast cells in mice

Jae Ku RHEE*, Chan Gu SURL and Hyeon Cheol KIM

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

Abstract: The responses of gastric mucosal mast cells (GMMCs) to infection with coccidian protozoa, *Cryptosporidium muris*, in mice were examined during primary and challenge infections. Each of three-week-old ICR SPF mice was orally inoculated with a single dose of 2×10^6 oocysts of *C. muris* (strain MCR). After oocyst shedding ceased, the mice were orally challenged with a single dose of 2×10^6 oocysts of the same species. GMMCs reached a peak on days 20-30 postinoculation (PI) in number, and decreased thereafter. An increase on days 20-30 post-challenge-infection (PCI) was also observed. The mice showed, on the whole, normal profiles of oocyst shedding in droppings. The number of the cells of uninfected control mice remained constant. Judging from the above results, it is suggested that mastocytosis correlate with expulsion of *C. muris* in primary infection and the defense mechanism of challenge infection.

Key words: *Cryptosporidium muris* (strain MCR), challenge infection, gastric mucosal mast cell, mouse

INTRODUCTION

Cryptosporidiosis caused by protozoan parasites belong to the genus *Cryptosporidium* is a diarrheal disease that can be life-threatening in immunocompromised hosts. It is obvious that both branches of the immune system are required for complete recovery, since T-lymphocyte dysfunction and/or hypogammaglobulinaemia can lead to the persistence of the infection. An advanced knowledge of host immune responses to *Cryptosporidium* infection is needed to elucidate the immune response mechanism. While mast cells are heterogenous and diverse in functions, they may play an important role as major effector cells in the immune response to infection with helminths (Lee *et al.*, 1986). Since the initial report of Jarrett *et al.* (1968), many investigators have reported a greater number of intestinal mucosal mast cells (IMMCs) to helminth infections (Rhee *et al.*, 1989, 1991 & 1994, Chai *et al.*, 1993). In addition, Woodbury *et al.* (1984) suggested that mucosal mast cells (MMCs) were functionally active during the spontaneous expulsion of primary infections with *Nippostrongylus brasiliensis* and *Trichinella spiralis* in the rat. It is suggested that IMMCs may play an important role in the expulsion of intestinal helminths from the hosts. Therefore, IMMCs are of greater interest to parasitologists.

The better information on mast cells in protozoan infections has been provided by Huntley *et al.* (1985), Rose *et al.* (1980 & 1992), and Harp and Moon (1991). Most

• Accepted 4 October 1997, received after revision 17 November 1997.

• This study was financially supported in part by a Research Grant from Bio-Safety Research Institute, Chonbuk National University in 1997 (CNU-BSRI, No. 97-01).

* Corresponding author

recently, Rhee *et al.* (1997) demonstrated that the increase in appearance of most mast cells was followed by a dramatic decrease of the protozoa in the bursa of Fabricius in chickens experimentally infected with *Cryptosporidium baileyi*. Present study was, therefore, conducted in order to evaluate the effect of *Cryptosporidium muris* (strain MCR) infection on the number of gastric mucosal mast cells (GMMCs) in mice, and thereby, to assess the role of GMMCs in the expulsion of the parasite from the host stomach.

MATERIALS AND METHODS

Oocysts of *C. muris* (strain MCR) used in this study were of the same origin as described earlier (Rhee *et al.*, 1995). Each of three-week-old inbred ICR SPF mice was orally inoculated with a single dose of 2×10^6 oocysts. After oocyst shedding ceased, the experimental mice were challenged with a single dose of 2×10^6 oocysts of the same species on days 50 postinoculation (PI). Keeping the feces wet, fecal examination for oocysts and enumeration of the number of oocysts discharged per day were carried out as described previously (Rhee *et al.*, 1995). Primary-infected mice were sacrificed at an interval of 5 days and challenge-infected mice were 10 days for examination of the mast cells. Uninfected age-matched mice served as the control.

Gastric segments about 0.5 cm in length were removed from the pars glandularis of 5 mice at each stage, fixed in Carnoy's solution (pH 2.0) for 5 to 6 hrs, and submitted to the histology service for routine histologic processing. After being blocked in paraffin, the blocks were sectioned at 5 μm thickness. To observe fluctuations in numbers of GMMCs, the section samples were stained with 1% alcian blue (pH 0.3) for 2 hrs, and counterstained with 0.5% safranin-O (pH 1.0) for 5 min. GMMCs were counted in areas of 0.25 mm^2 (500 × 500 μm) in 5 fields per mouse.

All experiments in the present study were repeated in triplicate.

RESULTS

The primary-infected mice showed slightly shorter patterns of oocyst shedding in droppings than normal profiles shown by Rhee *et al.* (1995). Thus, the mice had a prepatent period of 6 days, with an oocyst plateau production occurring between days 15 and 20 PI with a patent period of 50 days (Fig. 1). Following challenge infection, the mice discharged fewer oocysts on days 16 and 30 post-challenge-infection (PCI) in droppings compared to primary infection alone (data not shown).

GMMCs were observed in mucosa of the pars glandularis of control and infected mice. As shown in Table 1, on days 15-25 PI, the number of GMMCs significantly increased when compared in initial and later stages of primary infection. On day 30, the mast cell number decreased. Significant correlations were observed between the numbers of the mast cells and the oocyst shedding in droppings of mice following primary infection. In the challenged mice, the numbers of the mast cells significantly increased on days 20-30 PCI, too.

In comparison, oocysts did not show up in fecal samples and no change in number of the cells was observed in control group mice during this period.

DISCUSSION

The immunological hallmarks of infection with parasitic helminths, namely eosinophilia, mastocytosis and increased IgE synthesis, all

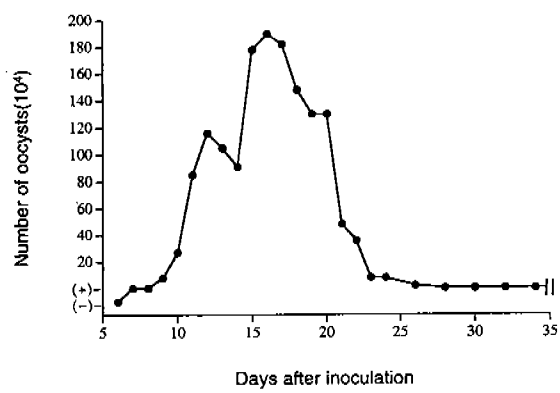

Fig. 1. Pattern of oocyst shedding in mice primarily inoculated with 2×10^6 oocysts of *C. muris* (strain MCR). (+) and (-) indicate that less than 10^3 oocysts were detected and oocysts were not detected, respectively.

Table 1. Fluctuations in numbers of gastric mucosal mast cells in mice infected with *Cryptosporidium muris* (strain MCR)

	Days	Control	Experimental
Postinoculation (PI)	5	5.56 ± 1.06[a)]	7.00 ± 1.78
	10	6.84 ± 1.59	6.75 ± 1.32
	15	5.20 ± 0.69	32.27 ± 4.65
	20	3.97 ± 1.13	36.13 ± 4.64
	25	4.05 ± 0.44	34.40 ± 2.27
	30	4.46 ± 0.29	18.01 ± 0.88
	35	4.16 ± 0.29	7.06 ± 1.23
	40	4.67 ± 0.59	6.17 ± 1.62
	50[b)]	5.44 ± 0.38	7.13 ± 2.02
Post-challenge-infection (PCI)	10	6.72 ± 1.76	6.40 ± 1.49
	20	3.50 ± 0.20	21.69 ± 5.79
	30	6.56 ± 0.82	19.05 ± 3.68
	40	6.85 ± 0.73	6.33 ± 1.67
	50	6.45 ± 0.37	4.18 ± 0.23

[a)]Determinations are expressed as the mean ± SD of 5 fields (0.25 mm^2 per field) per mouse and allocation. [b)]Challenge infection with 2×10^6 oocysts of *C. muris* (strain MCR) on days 50 PI.

appear to be induced by cytokines from the Th2 subset of CD4+ T cell. It is not yet known whether IL-3 and/or IL-4 are always required for the induction of mucosal mastocytosis, or whether IL-9 and/or IL-10 are also important factors in the *in vivo* induction of intestinal mastocytosis (Finkelman *et al.*, 1991).

In hamsters infected with *Entamoeba histolytica*, cell-mediated immunity plays a key role in limiting or inhibiting amoebic proliferation in extraintestinal amoebiasis and macrophages function as effector cells (Ghadirian and Meerovitch, 1983). In actively or adoptively immunized mice challenged with *Eimeria vermiformis*, mastocytosis was not observed, which suggest that the mast cell activity is not instrumental in reducing the numbers of parasites (Rose *et al.*, 1992).

According to Rose *et al.* (1980) in *Eimeria acervulina* or *E. nieschulzi*-infected chickens and rats, there was a small and insignificant initial fall in the numbers of intestinal mast cells in the rats, and a more marked decrease in the chickens. This was followed, towards the end of infection, by a return to (chicken) or an increase above (rat) normal levels. In birds given a large challenge inoculum of oocysts, there was, within hours of dosing, an initial increase in numbers followed by a return to normal by day 2 and this response was similar in both immunized and susceptible birds. This rapid recruitment of cells was not seen in similarly challenged immune rats; in this species the earliest increase was not observed until day 2.

Harp and Moon (1991) reported that mast cell-deficient W/W^v infant mice were similar to normal mice in their susceptibility to and recovery from infection with *C. parvum*, and that W/W^v adult mice were significantly more susceptible to primary infection than were normal adult mice, but both groups recovered at a similar rate. They hypothesized that the mast cells might play a role in this initial resistance of adult mice to *C. parvum*. Because Crowle and Reed (1981) and Erlich *et al.* (1983) attributed increased susceptibility to *N. brasiliensis* and *Giardia muris* in mast cell deficient W/W^v mice, respectively. In *C. baileyi*-infected chickens given 5×10^5 oocysts, the number of mast cells in the bursa of Fabricius rose to a peak at the 3rd and 8th days PI, and then began to decline. It seems likely that a marked increase in the number of mast cells has been associated, at least in part, with the loss of the protozoa (Rhee *et al.*, 1997).

The pathological changes which occur in the small intestine as a result of infection with *E. nieschulzi* in rats are very similar to those induced by infection with helminths (Rose and

Hesketh, 1982). In particular, infections with *E. nieschulzi* in rats (Rose *et al.*, 1980), with *C. muris* in mice (present experiment), with *C. baileyi* in chickens (Rhee *et al.*, 1997), or with helminths (Chai *et al.*, 1993) are characterized by a substantial increase in the number of MMCs. In addition, rat mucosal mast cell protease is released systematically into the blood stream during primary infections of *N. brasiliensis*, *T. spiralis* and *E. nieschulzi* in rats (Woodbury *et al.*, 1984; Huntley *et al.*, 1985). In the present study, the number of GMMCs significantly increased in primary and challenge infections with *C. muris*. It seems that worm expulsion must in some way be causally correlated with MMCs, but it is still not clear exactly what the correlation is.

Based on these experimental results, a similar mechanism of mastocytosis could occur in protozoan infection as in previously reported helminth infection. However, the hypothesis that extracellular worm parasites stimulate Th2 cells that evoke a response characterized classically as a type IV hypersensitivity response and intracellular protozoa Th1 lymphocytes to evoke a classical type I hypersensitivity response (Mossman and Coffman, 1989; Urban *et al.*, 1992) may explain the difference between extracellular and intracellular mechanisms of mastocytosis and the expulsion of parasites. The roles played by these cytokines in promoting or blocking host protective immunity appear to be quite complex, and may differ with species of parasite and host under study. Intensive studies on the function of MMCs during the expulsion process of parasites are needed for better understanding.

ACKNOWLEDGEMENT

We thank Dr. Leslie Lewis, Professor in Biology, City University of New York, for critical reading of the manuscript.

REFERENCES

Chai JY, Kim TH, Kho WG, Chung SW, Hong ST, Lee SH (1993) Mucosal mast cell response to experimental *Metagonimus yokogawai* infection in rats. *Korean J Parasitol* **31**(2): 129-134.

Crowle PK, Reed ND (1981) Rejection of the intestinal parasite *Nippostrongylus brasiliensis* by mast cell-deficient W/W^V anemic mice. *Infect Immun* **33:** 54-58.

Erlich JH, Anders RF, Roberts-Thomson IC, Schrader JW, Mitchell GF (1983) An examination of the differences in serum antibody specificities and hypersensitivity reactions as contributing factors to chronic infection with the intestinal protozoan parasite, *Giardia muris*, in mice. *Aust J Exp Biol Med Sci* **61:** 599-615.

Finkelman FD, Pearce EJ, Urban JF, Sher A (1991) Regulation and biological function of helminth-induced cytokine responses. *Parasit Today* **7**(3): A62-A65.

Ghadirian E, Meerovitch E (1983) Passive transfer of immunity against hepatic amoebiasis in the hamster by cells. *Parasite Immunol* **5**(4): 369-376.

Harp JA, Moon HW (1991) Susceptibility of mast cell-deficient W/W^V mice to *Cryptosporidium parvum*. *Infect Immun* **59:** 718-720.

Huntley JF, Newlands GFJ, Miller HRP, McLauchlan M, Rose ME, Hesketh P (1985) Systemic release of mucosal mast cell protease during infection with the intestinal protozoan parasite, *Eimeria nieschulzi*. Studies in normal and nude rats. *Parasite Immunol* **7:** 489-501.

Jarrett WFH, Jarrett EEE, Miller HRP, Urquhart GM (1968) Quantitative studies on the mechanism of self-cure in *Nippostrongylus brasiliensis* infection. *Proc 3rd Int Congr World Assoc Vet Parasitol* Lyon p191, Elwert Marburg, Lahn.

Lee TDG, Swieter M, Befus AD (1986) Mast cell responses to helminth infection. *Parasit Today* **2**(7): 186-191.

Mosmann TR, Coffman RL (1989) Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol* **7:** 145-173.

Rhee JK, Kim HC, Park BK (1997) Effects of *Cryptosporidium baileyi* infection on the bursa of Fabricius in chickens. *Korean J Parasitol* **35**(3): 181-187.

Rhee JK, Park BK, Jang BG, Yook SY (1994) Effect of immunosuppression on *Ascaris suum* infection in undefinitive hosts III. Investigation in mice. *Korean J Vet Res* **34**(3): 559-567.

Rhee JK, Park BK, Seu YS (1991) Effect of immunoactivity on *Ascaris suum* infection in mice. *Korean J Parasitol* **29**(3): 279-291.

Rhee JK, Yook SY, Park BK(1989) Effect of *Hymenolepis nana* infection on immunological responses of mice to sRBC. *Korean J Parasitol* **27**(1): 23-33.

Rhee JK, Yook SY, Park BK (1995) Oocyst production and immunogenicity of *Cryptosporidium muris* (strain MCR) in mice. *Korean J Parasitol* **33**(4): 377-382.

Rose ME, Hesketh P (1982) Coccidiosis: T-lymphocyte-dependent effects of infection with *Eimeria nieschulzi* in rats. *Vet Immunol Immunopathol* **3**: 499-508.

Rose ME, Millard BJ, Hesketh P (1992) Intestinal changes associated with expression of immunity to challenge with *Eimeria vermiformis*. *Infect Immun* **60**(12): 5283-5290.

Rose ME, Ogilvie BM, Bradley JWA (1980) Intestinal mast cell response in rats and chickens to coccidiosis, with some properties of chicken mast cells. *Int Arch Allergy Appl Immun* **63**: 21-29.

Urban JF, Madden KB, Svetic A, Cheever A, Trotta PP, Gause WC, Katona IM, Finkelman FD (1992) The importance of Th2 cytokines in protective immunity to nematodes. *Immunol Rev* **127**: 205-220.

Woodbury RG, Miller HRP, Huntley JF, Newlands GFJ, Palliser AC, Wakelin D (1984) Mucosal mast cells are functionally active during spontaneous expulsion of intestinal nematode infections in the rat. *Nature* **312**: 450-452.

=초록=

마우스에 있어서 쥐와포자충 감염이 위점막 비만세포에 미치는 영향

이재구 · 설찬구 · 김현철

전북대학교 수의과대학 기생충학교실

마우스에 있어서 쥐와포자충 감염에 대한 면역기전을 해명하기 위하여 이 원충을 최초 및 도전 감염시켜 위점막 비만세포수의 동태를 관찰하였다. ICR계 SPF 3주령 마우스에, 그리고 감염 내과 후 분변 내의 오오시스트 음전 후에 두 번에 걸쳐 각각 2×10^6 의 쥐와포자충 (MCR주) 오오시스트를 경구투여하였다. 최초 감염 후 5일 및 도전 감염 후 10일 간격으로 희생시켜 위를 적출하여 pH 2.0으로 조정된 Carnoy's solution에 5-6시간 고정한 다음 pH 0.3으로 조정된 1% alcian blue에서 2시간, pH 1.0의 0.5% safranin에서 5분간 염색하여 위점막 비만세포수를 계수하였다. 위점막 비만세포수는 최초 감염 후 15일에서 25일까지 최고치에 이른 다음 30일에 감소하여 그 이후에는 정상으로 복귀하였으며, 도전 감염 후 20-30일에도 역시 증가하였다. 최초 감염 후 이 세포의 증가 후에는 분변에 배설되는 오오시스트의 수도 감소하였으며, 도전 감염 후에는 분변에서 오오시스트를 거의 검출할 수 없었다. 이러한 사실로 미루어 보아 이 세포는 최초 감염 원충체 배출, 그리고 도전 감염의 방어기전과 관련이 있다고 생각한다.

〔기생충학잡지 35(4): 245-249, 1997년 12월〕