The Korean Journal of Parasitology
Vol. 33, No. 3, 195-200, September 1995

Inhibition of Con A-induced lymphocyte proliferation by peritoneal exudate of *Toxoplasma gondii*-infected mice

Ho-Woo NAM* and Won-Young CHOI

Department of Parasitology, Catholic University Medical College, Seoul 137-701, Korea

Abstract: The presence of biological response modifiers (BRM)-like effect was confirmed in peritoneal exudate (PE) of *Toxoplasma gondii*-infected ICR mice which inhibited Concanavalin A (Con A)-induced peritoneal lymphocyte (PL) proliferation. During 5 days of PL incubation with 10 μg/ml Con A with or without PE, ^{3}H-thymidine uptake was measured for the last 24 hrs. Compared to uninduced control, PL proliferated by 7.3-fold with Con A induction. When PE of infected mice was added, PL proliferation was inhibited by 74.0 ± 11.9% whereas inhibition by PE of normal mice was 16.4 ± 8.3%. Inhibitory effect of PE increased exponentially from 3 days up to 4-5 days of survival after the infection. Inhibitory activity of PE was decreased concentration-dependently. Also the inhibition was diminished when the PE was treated with heat of 95°C for 10 min or precipitated with 10% trichloroacetic acid (TCA). In SDS-PAGE of PE, many minor bands appeared newly. Heat-labile protein molecule in PE exerted inhibitory activity to Con A-induced lymphocyte proliferation.

Key words: *Toxoplasma gondii*, peritoneal exudate, inhibition, Con A, lymphocyte proliferation

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that has emerged as an important opportunistic pathogen in both humans and animals. Only a minority of those infected develop clinical diseases (Kim *et al.*, 1993). Life-threatening toxoplasmosis can occur in individuals with diminished cellular immunity resulting from immunosuppressive agents administered for cancer chemotherapy or in association with organ transplantation (Lowenberg *et al.*, 1983). Similarly, individuals suffering from AIDS frequently experience serious toxoplasmosis involving central nervous system (Luft & Remington, 1988; Ho-Yen, 1992).

The cell-mediated immunity to *T. gondii* represents the major component of host immunity to this organism. *T. gondii* infection itself apppears to change host immunity as determined by several immunologic assays. Lymphocyte proliferation to the T-cell mitogen, Con A, has been found depressed markedly in mice acutely and chronically infected with *T. gondii* (Strickland *et al.*, 1975; Chan *et al.*, 1986; Luft *et al.*, 1987; Yano *et al.*, 1987). All these reports have described the modulation of T cell or T cell subpopulations via antigen-specific priming in acute and chronic infections with *T. gondii*. In this study, to clarify the link between peritoneal exudate (PE) of *T. gondii*-infected mice and peritoneal cells

• Received July 21 1995, accepted after revision Aug. 11 1995.

• This study was supported partly by the Basic Medical Research Grant from the Ministry of Education, Republic of Korea Government (1995).

* Corresponding author

without specific priming, the inhibitory effect of PE was observed in a model of Con A-induced peritoneal lymphocyte (PL) proliferation.

MATERIALS AND METHODS

Parasites

Tachyzoites of RH strain of *T. gondii* were passaged in the peritoneum of ICR mice that had been injected with 2 - 5 × 10^6 organisms 4 days earlier.

Peritoneal exudate preparation

Three ml of saline was injected into the peritoneal cavity and then the exudate was collected. The exudates were centrifuged twice at 13,000 rpm for 5 min to remove cells, debris and *T. gondii*. The exudates were stored at –20 °C until used. Aliquots were electrophoresed under SDS in 7.5% to 15% gradient polyacrylamide gel.

Peritoneal lymphocytes

Normal ICR mice were bred and maintained under conventional conditions. Male mice of 6-8 week old were used for the experiments. Each group consisted of 6 to 8 mice. After injecting 3 ml of Eagle's MEM (Gibco BRL Co., USA) into the peritoneal cavity, peritoneal cells were collected with 3 ml syringes. Peritoneal cells were centrifuged at 6,500 rpm for 5 min and resuspended in EMEM supplemented with 10% fetal bovine serum (FBS, Gemini Co., USA). Cells were cultured for 2 hrs in 35 mm petri dishes (NUNC, Denmark) under 95% air/5% CO_2 incubator to settle down anchorage-dependent cells. Floating cells were harvested and used as peritoneal lymphocytes.

Assay of Con A-induced lymphocyte proliferation

Peritoneal lymphocytes were cultured in 96-well culture plate (Corning Co., USA) for 5 days with 10 μg/ml Con A (Sigma Co., USA) with or without peritoneal exudates by the final concentration 10% (v/v). On the fourth day of the culture, each well was added with 1 μCi of ^{3}H-thymidine (NEN Co., USA) and then incubated for 24 hrs. Cells were harvested onto a glass fiber filter paper by an automatic cell harvester (Dynatech Co., USA). The radioactivity was counted by a liquid scintillation counter (Kontron Co., USA).

RESULTS

Con A-induced PL proliferation was inhibited by adding PE from mice infected with *T. gondii* into the culture medium. As represented in Fig. 1, ^{3}H-thymidine uptake of Con A-induced PL proliferation was increased up to 7.3-fold compared to the control culture of PL without Con A. When both the PE and Con A were treated, the ^{3}H-thymidine uptake was increased as little as 1.6-fold to control. PL culture with PE only did not proliferate no more than 0.8-fold. In the presence of Con A, PE inhibited PL proliferation by 74.0 ± 11.9%.

As shown in Fig. 2, the inhibitory action of PE was resulted from PE of infected mice. When PE of normal uninfected mice was added to PL culture with Con A, 16.4 ± 8.3% of the radioactivity was reduced comparing cultures of PL with Con A. It was significantly lower than that with PE of infected mice ($P < 0.01$). Inhibitory effect of PE from infected mice was increased exponentially from day 1 (16.9 ± 7.9%), 2 (23.4 ± 8.3%), 3 (41.4 ± 7.1%) to 4

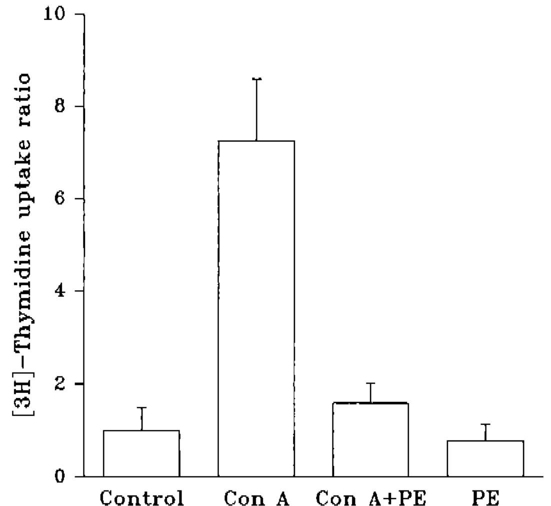

Fig. 1. Inhibition of Con A-induced peritoneal lymphocyte (PL) proliferation by peritoneal exudate of mice infected with *T. gondii*. **Control,** PL only; **Con A,** PL induced with Con A; **Con A + PE,** PL induced with Con A in the presence of peritoneal exudate; and **PE,** PL without Con A but with peritoneal exudate.

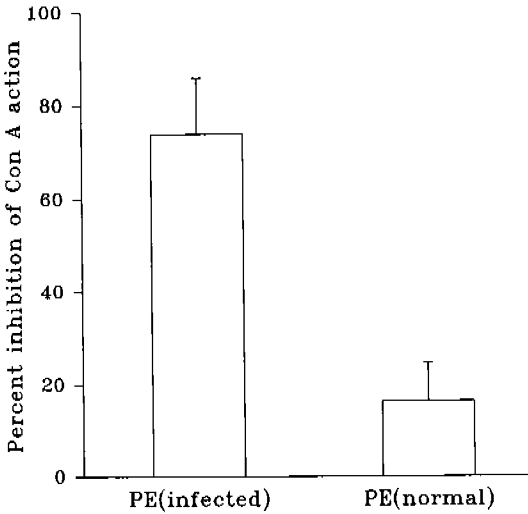

Fig. 2. Percent inhibition of Con A-induced PL proliferation by PE from infected and non-infected mice. Percent inhibition was calculated by the formula: (1- cpm of PL with Con A and PE/cpm of PL with Con A) × 100. **PE (infected),** PE from infected mice; and **PE (normal),** PE from non-infected mice.

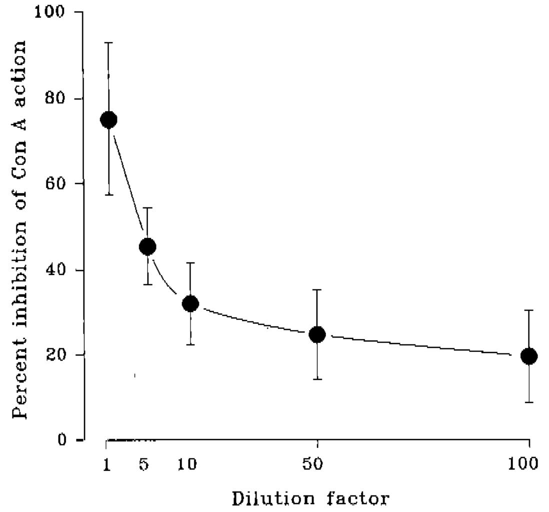

Fig. 4. Effect of PE dilution on inhibition of Con A-induced PL proliferation. PE added to the culture well was 10% (v/v) of media primarily, the dilution factors were further multiplied by 10 actually.

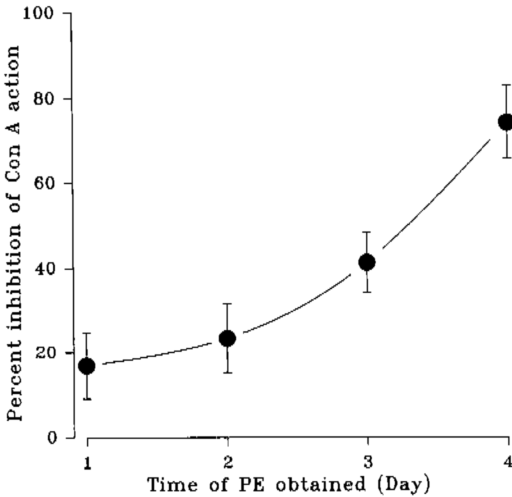

Fig. 3. Changing pattern of inhibitory effects on Con A-induced PL proliferation in PE which were collected consecutively after peritoneal infection with *T. gondii.*

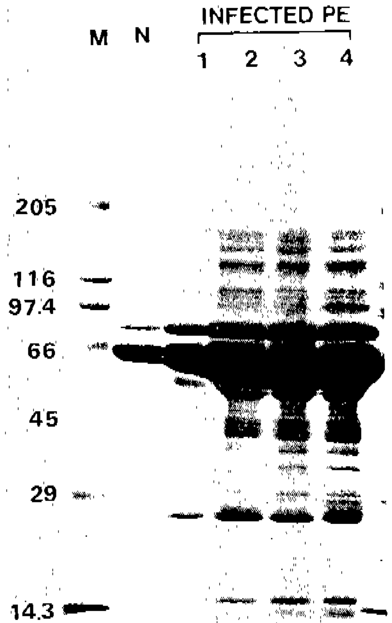

Fig. 5. SDS-PAGE pattern of PE on the time course of postinfection. **M,** molecular weight markers; **N,** PE of uninfected normal mouse, and **1-4,** days after infection.

(74.0 ± 11.9%) up to 4 to 5 days of survival time of mice after the infection (Fig. 3). PE obtained on the first day after infection did not inhibit just as PE of normal mice did.

To characterize the components which

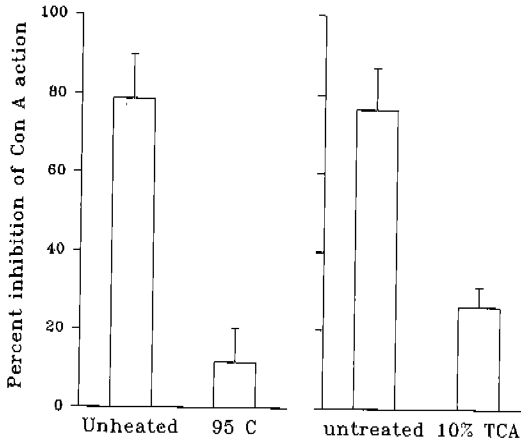

Fig. 6. Effects of heat (95°C for 10 min) and 10% TCA precipitation on inhibition of Con A-induced PL proliferation.

expressed inhibitory action of Con A-induced PL proliferation, the effects of PE dilution by logarithmic factors were observed primarily (Fig 4). The inhibition was diminished by dilution of PE such as dilution factor 1 (74.0 ± 11.9%), 5 (45.4 ± 9.0%), 10 (32.0 ± 9.6%), 50 (24.7 ± 10.5%) and 100 (19.4 ± 10.8%). Same volume of aliquots of PE obtained daily were electrophoresed. Many minor bands appeared newly and increased in quantities according to the time compared to PE of normal mice. In this regard, exact protein concentration of PE was meaningless because serum albumin was a dominant protein in PE as revealed by SDS-PAGE (Fig. 5) which might disturb the quantification of actual concentration of newly appeared minor bands. The inhibitory effect was diminished by heat treatment and TCA precipitation of PE (Fig. 6). The inhibition by PE was lowered down to background level by heating at 95°C for 10 min (11.5 ± 8.5%) and 10% TCA precipitation treatment (26.2 ± 5.1%).

DISCUSSION

Although the responsible factor is yet to be identified, cell-free PE of mice infected with *T. gondii* expressed evidently the inhibitory activity to Con A-induced lymphocyte proliferation in this study. The inhibitory activity was dose-dependent, heat-labile, and increased chronologically after the infection. It was implicated that the factor might be a heat-labile protein molecule.

Immunosuppression, evoked by the parasitic infection, may allow a parasite to survive within the host by not permitting a protective response to develop. The dynamic mechanisms of immunosuppression in murine toxoplasmosis are elicited by different lymphocyte subpopulations involved at different times during the infection. Strickland *et al.* (1975) assessed the blastogenic responses of spleen cells to various mitogens in acute and chronic toxoplasmosis in which mitogen-induced proliferation was reduced. Thereafter, many reports have described similar reduction of lymphoblastogenesis in human and experimental mouse models of toxoplasmosis (Chan *et al.*, 1986; Luft *et al.*, 1987; Yano *et al.*, 1987). Almost all these reported that *T. gondii*-specific T cells (T cells from *T. gondii*-infected humans and animals) failed to proliferate in response to mitogens or antigenic stimulation. This study used non-primed normal lymphocytes instead of spleen cells from infected animals. It was confirmed that proliferation of non-primed, normal lymphocytes by Con A could be suppressed by allotrophic PE from the infected mice with *T. gondii*.

It is well known that macrophages play a central role in the regulation of specific and nonspecific immunity to pathogens. Processing and presentation of the antigen and production of soluble mediators by macrophages are essential steps for the development of T cell-mediated immune responses (Unanue and Allen, 1987; Sypek *et al.*, 1993). On the other hand, macrophages have also been reported to act as natural suppressor cells that down-regulate lymphocyte-dependent immune events. Lymphocyte proliferation *in vitro* after mitogenic stimulation can be suppressed by addition of excess macrophages to the culture system. Evidence has been presented that implicates H_2O_2, prostaglandins, arginase, and other macrophage products (Kung *et al.*, 1977; Allison, 1978; Metzger *et al.*, 1980), and recently nitric oxide (NO) and TNF-α (Albina *et al.*, 1991; Isobe & Nakashima, 1992; Alleva *et al.*, 1994) are concerned in mediating the suppressive effects. Peritoneal injection of *T.*

gondii recruits many macrophages into the peritoneal cavity. We speculate that recruited macrophages themselves may secrete above listed suppressive factors into the PE. Of these, a certain heat-labile protein can be a candidate material which explains the suppressive activity of the PE.

Cytokines related with *T. gondii* infection include interferon, IL-2, TNF-α and IL-12 (Gazzinelli *et al.*, 1992). An important T cell cytokine is IFN-γ which activates macrophages to kill *T. gondii* (Murray *et al.*, 1987; Suzuki *et al.*, 1988; Mellors *et al.*, 1989). IL-2 has been demonstrated to induce a number of cellular immune functions. Sharma *et al.* (1985) showed that administration of recombinant IL-2 resulted in a significant decrease in the mortality in lethally infected mice. Activated macrophages are known to produce TNF-α, a cytokine that demonstrates both antitumor and antimalarial activities. Sibley *et al.* (1991) reported that TNF-α was able to regulate enhanced antimicrobial activity by triggering IFN-γ primed macro-phages to kill or inhibit intracellular *T. gondii*. Alteration of these cytokine responses may result in an increased immunosuppression of infected individuals, which facilitates *T. gondii* to evade from the host protection. Such cytokines as IL-4, IL-6, and IL-10 modulate cell-mediated immune responses directly or indirectly (Kishimoto, 1992). Especially, IL-10 inhibits Con A-induced T cell proliferation or IL-2 production in addition to down-regulation of a number of macrophage function (Ding *et al.*, 1993; Mosmann, 1994). It is suggested that the biased response to suppression overwhelms that of activation of lymphocytes to proliferate in susceptible hosts.

ACKNOWLEDGEMENT

We thank Mr. Nam-Seok Lee for his skillful assistance.

REFERENCES

Albina JE, Abate JA, Henry WL (1991) Nitric oxide production is required for murine resident peritoneal macrophages to suppress mitogen-stimulated T cell proliferation. *J Immunol* **147:** 144-148.

Alleva DG, Burger CJ, Elgert KD (1994) Tumor-induced regulation of suppressor macrophage nitric oxide and TNF-α production. *J Immunol* **153:** 1674-1686.

Allison AC (1978) Mechanisms by which activated macrophages inhibit lymphocyte responses. *Immunol Res* **40:** 3-27.

Chan J, Siegel JP, Luft bJ (1986) Demonstration of T-cell dysfunction during acute *Toxoplasma* infection. *Cell Immunol* **98:** 422-433.

Ding L, Linsley PS, Huang LY, Germain RN, Shevach EM (1993) IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. *J Immunol* **151:** 1224-1234.

Gazzinelli RT, Oswald IP, James SL, Sher A (1992) IL-10 inhibits parasite killing and nitrogen oxide production by IFN-γ activated macrophages. *J Immunol* **148:** 1792-1796.

Ho-Yen DO (1992) Immunocompromised patients. *In* Human toxoplasmosis. p184-203. eds Ho-Yen DO & Joss AWL. Oxford University Press New York USA.

Isobe K, Nakashima I (1992) Feedback suppression of staphylococcal enterotoxin-stimulated T-lymphocyte proliferation by macrophages through inductive nitric oxide synthesis. *Infect Immun* **60:** 4832-4837.

Kim SE, Kim YR, Shin WS, *et al.* (1993) A case of toxoplasmosis infected in the laboratory. *Korean J Infect Dis* **25:** 63-69.

Kishimoto T (1992) Interleukins: molecular biology and immunology. Karger AG Basel Switzerland.

Kung JT, Brooks SB, Jakway JP, *et al.* (1977) Suppression of *in vitro* cytotoxic4 response by macrophages due to induced arginase. *J Exp Med* **146:** 665-672.

Lowenberg B, van Gijn J, Prins E, Polderman AM (1983) Fatal cerebral toxoplasmosis in a bone marrow transplant recipient with leukemia. *Transplantation* **35:** 30-34.

Luft BJ, Pedrotti PW, Engleman EG, Remington JS (1987) Induction of antigen-specific suppressor T-cells during acute infection with *Toxoplasma gondii*. *J Infect Dis* **155:** 1033-1037.

Luft BJ, Remington JS (1988) AIDS commentary. Toxoplasmic encephalitis. *J Infect Dis* **157:** 1-6.

Mellors JW, Debs RJ, Ryan JL (1989) Incorporation of recombinant gamma interferon into liposomes enhances its ability to induce peritoneal macrophage anti-*Toxoplasma*

activity. *Infect Immun* **57:** 132-137.

Metzger Z, Hoffeld JT, Oppenheim JJ (1980) Macrophage-mediated suppression. I. Evidence for participation of both hydrogen peroxide and prostaglandins in suppression of murine lymphocyte proliferation. *J Immunol* **124:** 983-988.

Mosmann TR (1994) Interleukin-10. *In* The Cytokine Handbook. Thomson A ed. p223-237. Academic Press New York USA.

Murray HW, Scavuzzo D, Jacobs JL, *et al.* (1987) *In vitro* and *in vivo* activation of human mononuclear phagocytes by interferon-gamma. Studies with normal and AIDS monocytes. *J Immunol* **138:** 2457-2462.

Sharma SD, Hofflin JM, Remington JS (1985) *In vivo* recombinant interleukin 2 administration enhances survival against a lethal challenge with *Toxoplasma gondii*. *J Immunol* **135:** 4160-4163.

Sibley LD, Adams LA, Fukutome Y, Krahenbuhl JL (1991) Tumor necrosis factor-alpha triggers antitoxoplasmal activity of IFN-γ primed macrophages. *J Immunol* **147:** 2340-2345.

Strickland G, Ahmed A, Sell K (1975) Blastogenic response of *Toxoplasma*-infected mouse spleen cells to T- and B-cell mitogens. *Clin Exp Immunol* **22:** 167-176.

Suzuki Y, Orellana MA, Schreiber RD, Remington JS (1988) Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science* **240:** 516-518.

Sypek JP, Jacobson S, Vorys A, Wyler DJ (1993) Comparison of gamma interferon, tumor necrosis factor, and direct cell contact in activation of antimycobacterial defense in murine macrophages. *Infect Immun* **61:** 3901-3906.

Unanue ER, Allen PM (1987) The basis for the immunoregulatory role of macrophages and other cells. *Science* **236:** 551-557.

Yano A, Norose K, Yamashita K, *et al.* (1987) Immune response to *Toxoplasma gondii* — analysis of suppressor T cells in a patient with symptomatic acute toxoplasmosis. *J Parasitol* **73:** 954-961.

=초록=

톡소포자충 감염 마우스 복수액에 의한 Con A 유도 림프구 증식 억제효과

남호우, 최원영

가톨릭대학교 의과대학 기생충학교실

톡소포자충에 감염된 마우스의 복수액이 Con A로 유도한 정상 마우스 복수 림프구의 아세포화를 억제하는 효과를 관찰하였다. 마우스 감염은 톡소포자충의 RH주를 사용하였다. Con A로 유도한 아세포화 정도는 정상 마우스의 복수 림프구를 얻어 96-well 배양기에 분주하고 Con A를 10 μg/ml로 5일간 처리하면서, 4일째에 ^{3}H-thymidine을 well 당 1 μCi씩 첨가하여 DNA 표지량으로 측정하였다. 아세포화 억제효과는 Con A와 감염 복수액을 동시 처리한 군의 아세포화를 Con A만 처리한 군에서의 아세포화에 대한 비율로 나타내었다. Con A에 의한 아세포화는 7.3배였다. 정상 마우스의 복수액에 의한 아세포화 억제효과는 16.4 ± 8.3%이나, 감염 4일째 마우스의 복수액은 아세포화를 74.0 ± 11.9% 억제하였다. 생존일 4-5일의 감염 마우스에서 복수액을 일자별로 채취하여 처리하면 감염 1일에 16.9 ± 7.9%, 2일에 23.4 ± 8.3%, 3일에 41.4 ± 7.1% 및 4일에 74.0 ± 11.9%로 높은 억제효과가 발현되었다. 감염후 4일에 얻은 복수액을 희석하여 처리하였을 때 아세포화 억제 효과는 농도에 따라 변화하였다. 감염복수액을 95°C에서 10분간 열처리하면 억제 효과가 소멸되었으며, 10% TCA로 침전시킨 후 상층액으로 처리하여도 억제효과가 소멸하였다. 따라서 톡소포자충 감염 마우스 복수액에 복수 림프구의 아세포화를 억제하는 물질이 존재하며, 그 생체활성물질은 열에 약한 단백질이라고 판단하였다.

〔기생충학잡지 33(3): 195-200, 1995년 9월〕