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Research Note

Presence of Interferon-Gamma-Mediated Resistance against *Toxoplasma gondii* in T Cell-Deficient Mice

YASUHIRO SUZUKI*, AKIO KOBAYASHI AND HIROSHI OHTOMO

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Toxoplasma gondii is an obligate intracellular parasite among man and animals. Infection in humans usually goes unnoticed or is a benign self-limited illness in immunocompetent individuals. In contrast, congenital infection in the fetus (Remington and Desmonts, 1990) or infection in immunocompromised patients, especially those with acquired immunodeficiency syndrome, may result in debilitating and/or the life-threatening disease (Levy *et al.*, 1985; Levy and Bredensen, 1988; Luft and Remington, 1992). These facts clearly demonstrate that immune responsiveness of the individual is critically important for resistance against this parasite. $CD8^+$ T cells play an important role in the protective immunity (Suzuki and Remington, 1988, 1990; Gazzinelli *et al.*, 1991) but $CD4^+$ T cells are also responsible for immunity to *T. gondii* (Suzuki and Remington, 1988; Araujo, 1991; Gazzinelli *et al.*, 1991; Nagasawa *et al.*, 1991). Interferon-gamma (IFN-γ) has been shown to be the major mediator of resistance against *T. gondii* (Suzuki *et al.*, 1988, 1989; Suzuki and Remington, 1990; Gazzinelli *et al.*, 1991), and $CD8^+$ T cells appear to be a major source of the IFN-γ *in vivo* during the acute infection (Suzuki and Remington, 1990; Gazzinelli *et al.*, 1991). However, this lymphokine could be detected in mice as early as 3 days after infection (Suzuki *et al.*, 1993).

These facts led us to explore whether IFN-γ production by cells other than $CD4^+$ and $CD8^+$ T cells may be involved in resistance in the early stage of the infection. The studies described below reveal that athymic nude and severe combined immunodeficient (SCID) mice treated with monoclonal antibody (mAb) against IFN-γ died of acute toxoplasmosis significantly earlier than control mice. These results indicate the presence of T cell-independent IFN-γ-mediated mechanism(s) of resistance against infection with *T. gondii* in these T cell-deficient mice.

Female BALB/c background nude (nu/nu) and CB-17/Icr scid mice were obtained from Japan Clea Co. (Tokyo, Japan). Female C57BL/6 mice were purchased from Shizuoka Cooperative Association for Laboratory Animals (Hamamatsu, Japan). Mice were 8–10 week old when used. Each experimental group had five to eight mice.

Cysts of the avirulent ME49 strain of *T. gondii* were obtained from chronically infected C57BL/6 mice as previously described (Suzuki *et al.*, 1993). Mice were sacrificed by asphyxiation with CO_2 and their brains removed and trituated in phosphate-buffered saline, pH7.2, with mortar and pestle. An aliquot of the brain suspension was examined for the number of cysts and, after appropriate dilution in phosphate-buffered saline, each mouse received 10 cysts intraperitoneally.

Mice were injected intraperitoneally with anti-IFN-γ mAb (100 μg of H22.1, a hamster mAb, or 1

Department of Parasitology, Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-Ku, Tokyo 105, Japan.
*Present address: Department of Immunology and Infectious Diseases, Research Institute, Palo Alto, Medical Foundation, 860 Bryant Street, Palo Alto, CA 94301, U.S.A.
鈴木康弘　小林昭夫　大友弘士（東京慈恵会医科大学寄生虫学教室）

mg of XMG1.2, a rat mAb) one day before and 6 days after infection. These amounts of the mAb are sufficient to neutralize activity of endogenous IFN-γ produced by immunocompetent mice during *Toxoplasma* infection as described previously (Suzuki *et al.*, 1988, 1991). Control mice were injected with either 100 μg of normal hamster IgG (Cooper Biochemical, Westchester, PA) or 1 mg of normal rat IgG (Cooper Biochemical) in the same manner as for the mAb. The activities of these mAbs have been described previously (Schreiber *et al.*, 1985; Cherwinski *et al.*, 1987).

Levels of significance for comparisons on survival days of mice between experimental groups were determined using Student's *t*-distribution.

Athymic nude mice that were infected and received normal rat or hamster IgG died of toxoplasmosis from day 16 to 20 of infection (Fig. 1). In contrast, all mice that were infected and received either of the mAbs died by day 11 of infection (Fig. 1). ($p<0.0001$). Thus, these athymic nude mice have an IFN-γ-mediated resistance against *T. gondii*.

SCID mice which do not have T as B lymphocytes were infected with *T. gondii*. Those mice that received normal rat IgG died of toxoplasmosis from day 13 to 19 of infection (Fig. 2) whereas those that received mAb against IFN-γ died significantly earlier ($p<0.0001$) (Fig. 2), indicating that SCID mice also have IFN-γ-mediated mechanism(s) of resistance against this parasite.

Studies using immunocompetent mice have previously shown that $CD8^+$ T cells are the major afferent limb of the protective immune response of immunized mice (Suzuki and Remington, 1988, 1990; Gazzinelli *et al.*, 1991) and that their activity is mediated by IFN-γ (Suzuki and Remington, 1990; Gazzinelli *et al.*, 1991). In *in vitro* culture, $CD4^+$ as well as $CD8^+$ T cells can produce IFN-γ in response to *Toxoplasma* antigens (Gazzinelli *et al.*, 1991). In the present study, two strains of immunodeficient mice, athymic nude and SCID, were shown to have IFN-γ-mediated mechanism(s) of resistance against *T. gondii*. This indicates that such mice can produce IFN-γ although they do not have α/ß T cells or both α/ß and γ/δ T cells and B cells, respectively.

The T cell-independent IFN-γ production in these immunodeficient mice appears to occur in the very early stage of infection, possibly within 7 days after infection, since mice died from day 9 of infection when treated with mAb against this lymphokine. However, these IFN-γ production was not sufficient to prevent death of mice infected with an avirulent strain of *T. gondii*. The presence of T cells was required for prevention of death since immunocompetent BALB/c mice survived (data not shown)

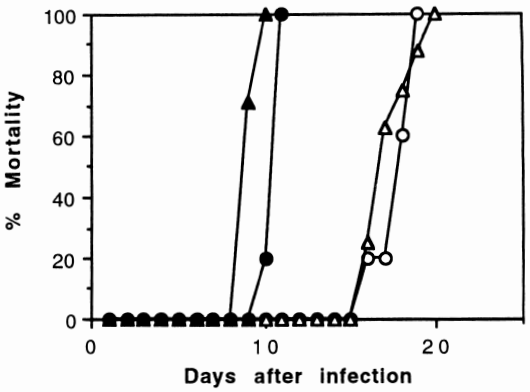

Fig. 1 Effect of mAbs to IFN-γ on resistance of athymic mice against infection with *T. gondii*. Mice were injected ip with either 100 μg of H22.1 (▲) or 1 mg of XMG1.2 (●) one day before and 6 days after infection with 10 cysts of the ME49 strain. Control mice were injected with either 100 μg of normal hamster IgG (Δ) or 1 mg of normal rat IgG (○) in the same manner as mAbs.

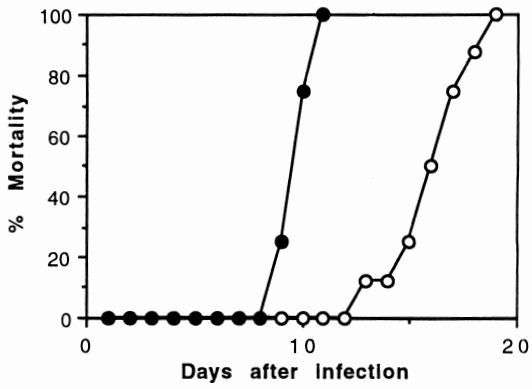

Fig. 2 Effect of a mAb to IFN-γ on resistance of SCID mice against infection with *T. gondii*. Mice were injected ip with either 1 mg of XMG 1.2 (●) or normal rat IgG (○) one day before and 6 days after infection with 10 cysts of the ME49 strain.

but both nude and SCID mice died of acute toxoplasmosis after infection with 10 cysts of the ME49 strain.

The source of IFN-γ in athymic nude and SCID mice during acute toxoplasmosis in the present study is unclear. One possibility for the source is NK cells. NK cell activity has previously been shown to be augmented during the early stage of *Toxoplasma* infection in mice (Hauser *et al.*, 1982; Kamiyama *et al.*, 1982). During infection with *Listeria monocytogenes,* $CD4^-$ $CD8^-$ NK cells were reported to produce IFN-γ (Dunn and North, 1991; Wherry *et al.*, 1991). Recently, Sher *et al.* (1993) reported that NK cells from normal (not infected with *T. gondii*) SCID mice can produce IFN-γ in an *in vitro* culture with *Toxoplasma* antigens.

Another potential source of early production of IFN-γ are γ/δ T cells. However, this appears unlikely in our SCID mouse model since these mice do not have γ/δ T cells (Izzo and North, 1992).

The T cell-independent IFN-γ-mediated mechanism(s) of resistance shown in the present study may be important in immunocompetent mice as well during the early stage of the infection to prevent spread of *T. gondii* before $CD8^+$ and $CD4^+$ T cells begin to produce this cytokine.

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References

1) Araujo, F. G. (1991): Depletion of $L3T4^+$ ($CD4^+$) T lymphocytes prevents development of resistance to *Toxoplasma gondii* in mice. Infect. Immun., 59, 1614–1619.
2) Cherwinski, H. M., Schumacher, J. H., Brown, K. D. and Mosmann, T. R. (1987): Two types of mouse helper T cell clone III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. J. Exp. Med., 166, 1229–1244.
3) Dunn, P. L. and North, R. J. (1991): Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. Infect. Immun., 59, 2892–2900.
4) Gazzinelli, R. T., Hakim, F. T., Hieny, S., Shearer, G. M. and Sher, A. (1991): Synergistic role of $CD4^+$ and $CD8^+$ T lymphocytes in IFN-γ production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. J. Immunol., 146, 286–292.
5) Hauser, Jr., W. E., Sharma, S. D. and Remington, J. S. (1982): Natural killer cells induced by acute and chronic toxoplasma infection. Cell. Immunol., 69, 330–346.
6) Izzo, A. A. and North, R. J. (1992): Evidence for an α/ß T cell-independent mechanism of resistance to Mycobacteria. Bacillus-Calmette-Guerin causes progressive infection in severe combined immunodeficient mice, but not in nude mice or in mice depleted of $CD4^+$ and $CD8^+$ T cells. J. Exp. Med., 176, 581–586.
7) Kamiyama, T. and Hagiwara, T. (1982): Augmented followed by suppressed levels of natural cell-mediated cytotoxicity in mice infected with *Toxoplasma gondii*. Infect. Immun., 36, 628–636.
8) Levy, R. M., Bredensen, D. E. and Rosenblum, M. L. (1985): Neurological manifestation of the acquired immunodeficiency syndrome (AIDS): experience at UCSF and review of the leterature. J. Neuroserg., 62, 475–495.
9) Levy, R. M. and Bredesen, D. E. (1988): Central nervous system disfunction in acquired immunodeficiency syndrome. J. Acquir. Immune Defic. Syndr., 1, 41–64.
10) Luft, B. and Remington, J. S. (1992): AIDS commentary: toxoplasmic encephalitis in AIDS. Clin. Infect. Dis., 15, 211–222.
11) Nagasawa, H., Manabe, T., Maekawa, Y., Oka, M. and Himeno, K. (1991): Role of $L3T4^+$ and $Lyt\text{-}2^+$ T cell subsets in protective immune responses of mice against infection with a low or high virulence strain of *Toxoplasma gondii*. Microbiol. Immunol., 35, 215–222.
12) Remington, J. S. and Desmonts, G. (1990): Toxoplasmosis. In Infectious Diseases of the fetus and newborn infants, Remington, J. S. and Klein, J. O., eds., W. B. Saunders & Co., Philadelphia, 89–195.
13) Schreiber, R. D., Hicks, L. J., Celada, A., Buchmeier, N. A. and Gray, P. W. (1985): Monoclonal antibodies to murine γ-interferon which differentially modulate macrophage activation and antiviral activity. J. Immunol., 134, 1609–1618.
14) Sher, A., Oswald, I. P., Hieny, S. and Gazzinelli, R. T. (1993): *Toxoplasma gondii* induces a T-independent response in natural killer cells that requires both adherent accessory cells and tumor necrosis factor-α. J. Immunol., 150, 3982–3989.
15) Suzuki, Y., Orellana, M. A., Schreiber, R. D. and Remington, J. S. (1988): Interferon-γ: The major mediator of resistance against *Toxoplasma gondii*. Science, 240, 516–518.
16) Suzuki, Y. and Remington, J. S. (1988): Dual regulation of resistance against *Toxoplasma gondii* infection by $Lyt\text{-}2^+$ and $Lyt\text{-}1^+$, $L3T4^+$ T cells in mice. J. Immunol., 140, 3943–3946.
17) Suzuki, Y., Conley, F. K. and Remington, J. S. (1989): Importance of endogenous IFN-γ for prevention of toxoplasmic encephalitis in mice. J. Immunol., 143, 2045–2050.

18) Suzuki, Y. and Remington, J. S. (1990): The effect of anti-IFN-γ antibody on the protective effect of Lyt-2$^+$ immune T cells against toxoplasmosis in mice. J. Immunol., 144, 1954–1956.
19) Suzuki, Y., Joh, K., Orellana, M. A., Conley, F. K. and Remington, J. S. (1991): A gene(s) within the H-2D region determines the development of toxoplasmic encephalitis in mice. Immunology, 74, 732–739.
20) Suzuki, Y., Orellana, M. A., Wong, S. Y., Conley, F. K. and Remington, J. S. (1993): Susceptibility to chronic infection with *Toxoplasma gondii* does not correlate with susceptibility to acute infection in mice. Infect. Immun., 61, 2284–2288.
21) Wherry, J. C., Schreiber, R. D. and Unanue, E. R. (1991): Regulation of gamma interferon production by natural killer cells in SCID mice: Role of tumor necrosis factor and bacterial stimuli. Infect. Immunol., 59, 1709–1715.