IMMUNE RESPONSES OF DIFFERENT MOUSE STRAINS AFTER CHALLENGE WITH EQUIVALENT LETHAL DOSES OF *TOXOPLASMA GONDII*

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Summary:

Most immunological studies that utilize different strains of inbred mice following *T. gondii* infection fail to compensate for differences in host susceptibility to the size of the parasite inoculum. To address this concern, susceptible C57BL/6 and resistant CBA/J mice were orally infected with either an equivalent 50 % lethal dose (LD₅₀) of brain cysts of the 76K strain of *T. gondii* (15 cysts in C57BL/6, 400 cysts in CBA/J) or the same dose of parasites in each mouse strain. C57BL/6 mice receiving 400 cysts (LD₅₀ of CBA/J mice) died post infection, whereas CBA/J mice that received 15 cysts (LD₅₀ of C57BL/6 mice) survived. Parasite loads in the brains and serum *Toxoplasma*-specific IgG1 titers of LD₅₀-infected C57BL/6 mice were significantly higher than those in LD₅₀- or 15 cysts-infected CBA/J mice, whereas splenocyte proliferation to *Toxoplasma* antigen and the percentage of CD8 α^+ T cells were reduced in LD₅₀-infected C57BL/6 mice. In contrast, serum IgG2a and IgM titers, the percentage of $\gamma\delta$ T cells and IFN- γ expression of spleen of LD₅₀-infected CBA/J mice were higher than those of either 15 cysts-infected CBA/J mice or LD₅₀-infected C57BL/6 mice. These observations demonstrate that the immune response between LD₅₀-infected C57BL/6 and CBA/J mice was more prominent when compared to C57BL/6 or CBA/J mice receiving the same parasite inoculum. These observations would suggest that caution must be exercised in the planning and interpretation of data when the size of the parasite inoculum has not been adjusted for mouse strain.

KEY WORDS : *Toxoplasma gondii*, toxoplasmosis, lethal equivalence, susceptibility, immune response, mouse.

Résumé : RÉPONSE IMMUNITAIRE DE DIFFÉRENTES LIGNÉES DE SOURIS SOUMISES À DES DOSES LÉTALES ÉQUIVALENTES DE *TOXOPLASMA GONDII*

La plupart des études utilisant différentes lignées consanguines de souris infectées par *T. gondii* ne réussissent pas à compenser les différences de sensibilité des hôtes envers la quantité de parasites inoculée. Face à ce problème, des souris C57BL/6 sensibles et CBA/J résistantes ont été infectées par voie orale soit par des équivalents de 50 % de la dose létale (DL₅₀) de kystes d'origine cérébrale de la souche 76K de *T. gondii* (15 kystes pour C57BL/6, 400 kystes pour CBA/J), soit par une même quantité de parasites chez chacune des lignées de souris. Les souris C57BL/6 qui reçoivent 400 kystes (DL₅₀ des souris CBA/J) en meurent, tandis que les souris CBA/J qui reçoivent 15 kystes (DL₅₀ des souris C57BL/6) survivent. La charge parasitaire cérébrale et les taux sériques d'IgG1 spécifiques de *Toxoplasma* des souris C57BL/6 infectées par la DL₅₀ sont significativement plus élevés que ceux des souris CBA/J infectées par la DL₅₀ ou par 15 kystes, alors que la prolifération de splénocytes et le pourcentage de cellules CD8 α^+ T sont plus bas chez les souris C57BL/6 infectées par la DL₅₀. Par contre, les taux sériques d'IgG2a et d'IgM, le pourcentage de cellules $\gamma\delta$ T et la réaction splénique en IFN- γ des souris CBA/J infectées par la DL₅₀ sont plus élevés que ceux des souris CBA/J infectées par 15 kystes ou des C57BL/6 infectées par la DL₅₀. Ces observations démontrent que la réponse immunitaire des souris C57BL/6 et CBA/J infectées par une DL₅₀ est plus prononcée que celle des souris C57BL/6 ou CBA/J recevant le même inoculum de parasites. Ces observations pourraient suggérer que la prudence doit être de mise dans la présentation et l'interprétation des données quand la quantité de parasites inoculée n'a pas été adaptée à la lignée de souris utilisée.

MOTS CLÉS : *Toxoplasma gondii*, toxoplasmosse, dose létale, sensibilité, réponse immune, souris.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular parasite with a global distribution and an extremely broad host range, including birds, mammals and

humans. Only one species of this parasite has been identified, but there is marked variation in susceptibility, according to its host. Even within the mouse, different inbred strains have markedly different susceptibility to *T. gondii* infection. These susceptibility differences may be due to differences in the virulence of the parasite itself or differences in the genetic make-up of the mice in terms of their immune response (Johnson *et al.*, 2002). The mortality of mouse strains varies widely depending on the parasite strain, size of inoculum, infection route, and gender of the mouse. Earlier studies considered mortality, cytokine production, and parasite burden after challenge infection of susceptible and

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resistant strains of mice with the same or similar doses of *T. gondii* parasites (McLeod *et al.*, 1989; Suzuki *et al.*, 1995; Deckert-Schluter *et al.*, 1995; Luo *et al.*, 1997; Schluter *et al.*, 1999). There have been no reports in which resistant and susceptible mice have been challenged with a physiologically normalized number of organisms. In order to alleviate the parasite dose dependent bias on the immune response in different genetic background, susceptible C57BL/7 mice and resistant CBA/J mice were infected by the oral route with a 50 % lethal dose (LD₅₀) as well as the same dose of parasites in each mouse strain for the particular strain. Following infection, we compared the parasite burden as well as the humoral and cell-mediated immune responses of these two strains of mice.

MATERIALS AND METHODS

MICE AND PARASITE STRAINS

Female inbred C57BL/6 and CBA/J mice were obtained from the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea. All mice used were 8-10 weeks old and documented to be specific-pathogen-free animals. They were maintained under approved conditions in our college's animal research facilities. Two strains of *T. gondii* were used. The RH strain was used to prepare *Toxoplasma* lysate antigen (TLA), and the 76K strain was used to infect mice orally.

PARASITE CHALLENGE OF MICE

To determine the LD₅₀ per oral dose of the 76K strain of *T. gondii*, C57BL/6 mice were infected with four, 15, or 40 brain cysts per mouse, while CBA/J mice were infected with 100, 400, or 1,600 brain cysts. There were 20 mice in each group to study mortality, and checked four weeks daily. After determination of LD₅₀ of each mouse, mice were infected with LD₅₀ of parasites into C57BL/6 or CBA/J mice to compare the immune responses between two strains of mice. To compare the immune responses at the same doses of parasites, mice were also infected with LD₅₀ of C57BL/6 mice into CBA/J mice as well as LD₅₀ of CBA/J mice into C57BL/6 mice. Five mice were used in each value at all time point for evaluation of immune responses and parasite burdens at day 0, 3, 7, 14 and 28 post-infection (PI), respectively. Age- and sex-matched control mice of the same strains were treated with a similar dose of brain homogenate from uninfected mice.

TITRATION OF SERUM ANTIBODIES BY ELISA

We did preliminary experiments to set-up the adequate dilution in ELISA system, and then we determined the

dilution factors of serum as 1:100. Each well of a 96-well plate was coated with TLA (10 µg/ml), and incubated overnight at 4°C. TLA was prepared according to the protocol outlined by Lee *et al.* (1999a). After blocking, serum samples were diluted 1:100 in 0.1 % BSA/PBS containing 0.05 % Tween 20 and added 100 µl per well. After two hours, HRP-conjugated goat anti-mouse IgG1, IgG2a, or IgM (Southern Biotechnology Associates Inc., USA) was applied. After washing, freshly prepared O-phenylenediamine dihydrochloride was added, and the reaction was stopped by the addition of 4N H₂SO₄. Optical density was read at 492 nm using an automatic ELISA reader (Spectra, Molecular Devices, USA).

SPLENOCYTE PREPARATION

AND PHENOTYPIC ANALYSIS BY FLOW CYTOMETRY

Spleens from mice were homogenized, and erythrocytes were lysed by Tris-NH₄Cl (pH 7.2). The splenocytes were washed in RPMI 1640 (Sigma, USA) containing 10 % heat-inactivated FBS, and antibiotics. Splenocyte containing 1×10^6 cells were incubated with either 50 µl of FITC-conjugated anti-mouse CD4, CD8α, and γδ monoclonal antibodies (1:100 dilution in 0.1 % BSA/PBS; PharMingen, USA) or isotype-specific control (PharMingen, USA) for 60 minutes at 4°C. Cells were washed three times in 0.1 % BSA/PBS by centrifugation, fixed with 1 % paraformaldehyde and then analyzed by flow cytometry (FACScan, Becton Dickinson, USA). The data were analyzed using the Cell-Quest program (Becton Dickinson).

SPLENOCYTE *IN VITRO* PROLIFERATION ASSAY

Splenocytes were dispensed in 96-well culture plates at a concentration of 2×10^5 cells per well in RPMI 1640 containing 10 % FBS. Cells were cultured for 72 hours with or without TLA (15 µl /ml) at 37°C, 5 % CO₂. After incubation, the cells were pulsed with 0.5 µCi [³H]-thymidine (Amersham, Belgium) for 12 hr and harvested onto glass-fiber filters by an automatic cell harvester. [³H]-thymidine incorporation was assessed by a liquid scintillation counter, and the results were expressed as counts per minute (CPM). Data reported represent the mean ± standard deviation (SD) of triplicate cultures.

CYTOKINE mRNA EXPRESSION IN TISSUES ASSESSED BY RT-PCR

Cytokine mRNA expression in brains and spleens of mice was assessed according to the method of Deckert-Schluter *et al.* (1995). Each tissue sample was processed for the isolation of total RNA using an RNAgent kit (Promega, USA). A preparation of cDNA was produced using a starting mixture containing 5 µg of total

RNA, 8 µl of 5 × RT buffer, 4 µl of dNTPs, 25 µM oligo-dT20 and 1 µl of AMV reverse transcriptase. The PCR was performed with 2-13 µl of cDNA reaction mixture containing 10 × polymerase buffer, 250 µM dNTPs, 0.4 µM of 3'- and 5'-primer, 2.5 U *Taq* polymerase at 94°C for one minute, 57°C for one minute and 72°C for one minute with Thermal cycler (TaKaRa PCR MP, Japan). The sequences of cytokine-specific primer pairs were as follows: hydroxyphosphoribosyltransferase (HPRT), 3'-GAGGGTAGGCTGGCCTATGGCT-5' and 5'-TGGATACAGGCCAGACTTTGTTG-3'; IFN-γ, 3'-CTCATGGAATGCATCCTTTTCG-5' and 5'-ACGCTACACACTGCATCTTGG-3'; IL-10, 3'-TGTCTAGGTCCTGGAGTCCAGCAGACTCAA-5' and 5'-CCAGTTTACC-TGGTAGAAGTGATG-3'. After 35 cycling, the PCR products were separated by electrophoresis on 2 % agarose gels. Quantification of mRNA was performed with an imaging densitometer (Gel-Doc, BioRad, USA).

QUANTIFICATION OF TISSUE PARASITE BURDEN BY SOUTHERN BLOT

Southern blots for the quantification of tissue parasite burden were done according to the procedure described by Burg *et al.* (1989) and Khan *et al.* (1998). DNA was prepared from the brains and spleens by homogenization of 100 mg of tissue in 2 ml 0.1 M NaCl, 0.2 M sucrose, 0.01 M EDTA and 0.3 M Tris (pH 8.0). The solution was then incubated for two hours at 65°C after the addition of 10 % SDS and proteinase K (100 µg/ml). Potassium acetate (8 M) was then added followed by precipitation of the solution at 4°C for one hour and centrifugation at 5,000 × g for 10 minutes. The aqueous phase was extracted with phenol-chloroform and ethanol precipitated. Oligonucleotide primers used to initiate DNA amplification were complementary to segments of the B1 gene of *T. gondii* (5'-GGAAGTGCATCCGTTTCATGAG and 5'-TCTTTAAAGCGTTTCGTGGTC). DNA was amplified by PCR in a solution containing 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 50 mM KCl, 200 µM of each deoxynucleotide, and 0.4 µM of primers. A positive control (DNA directly extracted from tachyzoites of the RH strain) and a negative control (distilled water) were tested for each reaction. We applied the same volume of DNA already adjusted same optical density, so the applied volume of each lane was same in all experiments. The reaction was carried through 35 cycles, each consisting of 60 s at 94°C, 90 s at 55°C and 60 s at 72°C. After migration on a 2 % agarose gel, the PCR product was passively transferred onto a nylon membrane. The membrane was fixed for 15 minutes at 120°C. Blots were prehybridized at 42°C for three hours. For hybridization, a non-isotopic 5'-GGCGACCAATCTGCGAATA-CCACC probe was used. For developing, it was labelled at the end with digoxigenin

(Boehringer Mannheim, Germany). After rinsing, the membrane was hybridized with an anti-digoxigenin antibody labeled with alkaline phosphatase. After further rinsing, the membrane was developed with a chemiluminescent substrate CSPD (Disodium 3-(4-methoxyspiro[fl,2-dioxetane-3,2'-(5'-chloro)tricyclo [3.3.1.1^{3,7}]decan)4-yl) phenyl phosphate; DIG Luminescent Detection Kit, Roche Molecular Biochemicals, Germany), and detected by exposure of X-ray film. Quantification of DNA was performed using an imaging densitometer.

STATISTICAL ANALYSIS

Statistical evaluations of differences in parasitic loads, antibody titers, proliferation assay, phenotype profiles and mRNA levels were determined by Mann-Whitney U test of nonparametric-independent method and Student's *t* test. Differences between the various groups were considered significant when *p* values were < 0.05.

RESULTS

ESTABLISHMENT OF THE LD₅₀ FOR C57BL/6 (SUSCEPTIBLE) AND CBA/J (RESISTANT) MICE

To determine the LD₅₀ of *T. gondii* in C57BL/6 and CBA/J mice, groups of female C57BL/6 and CBA/J mice were challenged separately with three different doses of brain cysts. Four weeks after infection, the survival rate of C57BL/6 mice receiving four cysts was 85.0 ± 7.1 %, 47.5 ± 10.6 % in the group receiving 15 cysts per mouse, and 0.0 ± 0.0 % for those receiving 60 cysts. The survival rates of CBA/J mice after oral administration of 100, 400, and 1,600 cysts per mouse were 100 ± 0.0 %, 55.0 ± 14.1 %, and 5.0 ± 7.1 %, respectively. Therefore, we determined that the LD₅₀ of the 76 K strain of *T. gondii* was 15 cysts per susceptible C57BL/6 mouse and 400 cysts per resistant CBA/J mouse. In comparison with mortality at the same dose of parasites in each mouse strain, C57BL/6 mice receiving 400 cysts (LD₅₀ of CBA/J mice) were all dead. In contrast, all CBA/J mice receiving 15 cysts (LD₅₀ of C57BL/6 mice) survived.

PARASITE LOADS IN THE BRAIN AFTER INFECTION WERE HIGHER IN C57BL/6 MICE THAN IN CBA/J MICE

As shown in Figures 1A, 1B and 1C, the parasite load in the spleen of both mouse strains increased after infection to reach a maximum at day 7 PI, and decreased thereafter. At day 7 PI, LD₅₀-infected C57BL/6 mice had higher numbers of parasites in the spleen in comparison to LD₅₀- or 15 cysts-infected CBA/J mice (*p* = 0.0025 in LD₅₀-infected C57BL/6 *vs*

Fig. 1. – Levels of parasite DNA in tissues of C57BL/6 and CBA/J mice orally infected with the 76K strain of *T. gondii*. A, *T. gondii* DNA levels of positive control by Southern blot; B, Southern blot of *T. gondii* DNA levels of the spleens and brains from LD₅₀-infected C57BL/6 and CBA/J mice. C, the kinetics of *T. gondii* DNA levels of the spleens and brains from C57BL/6 and CBA/J mice orally infected with the 76K strain of *T. gondii*. The data are expressed as mean $\pm$ standard deviation (SD) of one of two separate experiments ($n = 5$ for each value at all time point). The experiment was repeated two times with essentially similar results.

15 cysts-infected CBA/J, $p = 0.999$ in LD₅₀-infected C57BL/6 *vs* LD₅₀-infected CBA/J). The parasite burdens in the spleen of CBA/J mice receiving 15 cysts were not significant compared to LD₅₀-infected CBA/J mice, except day 7 PI. The parasite burden in the brain of both mouse strains also increased after seven days after infection, and was subsequently maintained at a high level. The parasite burden in the brain was, however, markedly higher in LD₅₀-infected C57BL/6 mice than in LD₅₀- or 15 cysts-infected CBA/J mice at day 14 and 28 PI ($0.0005 < p < 0.007$). The parasite burdens in the brain of CBA/J mice receiving 15 cysts were lower than those of LD₅₀-infected CBA/J mice at day 14 and 28 PI ($p = 0.0544$ at day 14 PI, $p = 0.0155$ at day 28 PI).

DIFFERENTIAL PRODUCTION OF IgG ISOTYPE TITERS BETWEEN RESISTANT AND SUSCEPTIBLE MICE

The IgG1 titer of *Toxoplasma*-infected C57BL/6 and CBA/J mice were similar to that of the uninfected control until day 7 PI. Thereafter, the IgG1 titers of

LD₅₀-infected C57BL/6 mice increased abruptly compared to CBA/J mice at the same time point. At days 14 and 28 PI, IgG1 titers of *T. gondii*-infected C57BL/6 mice were more than two times higher than those of the CBA/J mice (Fig. 2A). IgG2a titers of LD₅₀-infected C57BL/6 mice were consistent with the uninfected control until day 7 PI and increased thereafter. In contrast, the IgG2a titers of LD₅₀- or 15 cysts-infected CBA/J mice significantly increased after infection, and their titers were significantly higher than those of LD₅₀-infected C57BL/6 mice through experiment period. The IgG2a titers of CBA/J mice receiving 15 cysts were lower in LD₅₀-infected CBA/J mice at day 14 or 28 PI ($p = 0.0009$ at day 14 PI, $p = 0.581$ at day 28 PI) (Fig. 2B). The IgM titers of uninfected CBA/J mice were higher than those of uninfected C57BL/6 mice ($p = 0.0005$). The IgM titers of LD₅₀-infected CBA/J mice increased significantly after day 3 PI and peaked at day 7-14 PI, whereas LD₅₀-infected C57BL/6 mice significantly increased after day 7 PI. The IgM titers of CBA/J mice receiving 15 cysts were lower titers to LD₅₀-

Fig. 2. – Time course of the IgG1 (A), IgG2a (B), and IgM (C) antibody titers of sera from C57BL/6 and CBA/J mice orally infected with the 76K strain of *T. gondii*. The data are expressed as mean $\pm$ SD of one of two separate experiments ($n = 5$ for each value at all time point). The experiment was repeated two times with essentially similar results.

Fig. 3. – Proliferative responses of splenocytes from C57BL/6 and CBA/J mice orally infected with the 76K strain of *T. gondii*. Splenic cells were cultured in the presence of *Toxoplasma* lysate antigen for 72 h. The proliferation was assayed by [³H]-thymidine incorporation. Data are presented as the mean $\pm$ SD of one of two separate experiments ($n = 5$ for each value at all time point). The experiment was repeated two times with essentially similar results.

infected CBA/J mice, but not significant differences except day 7 PI ($p = 0.0072$ at day 7 PI) (Fig. 2C).

DIFFERENT PATTERNS OF *IN VITRO* PROLIFERATION OF SPLENOCYTES FROM CBA/J AND C57BL/6 MICE

Thymidine incorporation by TLA-treated splenocytes from both mouse strains was significantly increased at

day 0 and 3 PI as compared to the medium-treated control group. After day 7 PI, DNA synthesis was markedly depressed in TLA-treated splenocytes obtained from C57BL/6 mice, whereas DNA synthesis of CBA/J mice was not effected. Thymidine incorporation of CBA/J mice receiving 15 cysts was similar to that of LD₅₀-infected CBA/J mice through experiment ($p = 0.7531$) (Fig. 3).

CD8⁺ T CELLS ARE DECREASED IN C57BL/6 MICE, BUT NOT IN CBA/J MICE, AFTER *T. GONDII* INFECTION

The percentage of CD4⁺ T cells from LD₅₀-infected C57BL/6 mice did not change significantly during the experiment as compared to uninfected C57BL/6 mice. The percentage of CD4⁺ T cells in LD₅₀-infected CBA/J mice was slightly increased at day 14 PI compared to day 0 ($p = 0.422$), and their pattern of CD4⁺ T cells was similar to CBA/J mice receiving 15 cysts (data were not shown). The percentage of CD8a⁺ T cells from LD₅₀-infected C57BL/6 mice was significantly decreased at day 14 PI compared to day 0 ($p = 0.0002$), whereas the percentage of CD8a⁺ T cells in LD₅₀- or 15 cysts-infected CBA/J mice was increased at day 14 PI ($0.001 < p < 0.0016$). The percentage of $\gamma\delta$ T cells increased abruptly in both strains of mouse after day 3 PI as compared to uninfected controls ($p < 0.0001$). At day 7 or 14 PI, the percentage rise of $\gamma\delta$ T cells in both strains of mouse was approximately three times higher than in uninfected mice, but the percentage of $\gamma\delta$ T cells in LD₅₀-infected CBA/J mice was higher than in *Toxoplasma*-infected CBA/J mice receiving 15 cysts or LD₅₀-infected C57BL/6 mice at day 7 or 14 PI ($0.0068 < p < 0.1026$) (Fig. 4).

Fig. 4. – The kinetics of phenotypic changes of splenocytes from C57BL/6 and CBA/J mice orally infected with the 76K strain of *T. gondii*. Splenocytes were stained with FITC-conjugated anti-mouse CD8α, $\gamma\delta$ TCR mAb, and then analyzed by FACScan. Data shown are the mean $\pm$ SD of one of two separate experiments ($n = 5$ for each value at all time point). The experiment was repeated two times with essentially similar results.

DIFFERENCES IN IFN- γ PRODUCTION BETWEEN RESISTANT AND SUSCEPTIBLE MICE

The splenic and cerebral HPRT mRNA expression was almost equal during the experimental periods (data was not shown). In Figures 5A and 5B, IFN- γ or IL-10 mRNA expression in the spleen and brain of uninfected C57BL/6 mice was set as 1 at each time point of the experiment. The IFN- γ mRNA expression of spleen increased markedly in both strains after infection, and peaked at day 7 PI. IFN- γ expression in LD₅₀-infected CBA/J mice was significantly higher than in LD₅₀-infected C57BL/6 mice before infection, and at days 3 and 7 PI, although the mean increase ratio of IFN- γ after infection was higher in LD₅₀-infected C57BL/6 mice at day 7 PI (1.0 vs 21.0 in LD₅₀-infected C57BL/6 mice, 7.5 vs 28.0 in LD₅₀-infected CBA/J mice). IFN- γ mRNA level in the spleen of CBA/J mice receiving 15 cysts was lower to that of LD₅₀-infected CBA/J mice. IL-10 mRNA expression in spleen in both strains was similar to that in the uninfected control until day 3 PI. Thereafter, mRNA levels of this cytokine increased significantly in LD₅₀-infected C57BL/6 mice, whereas only a slight increase was observed in LD₅₀- or 15 cysts-infected CBA/J mice. IL-10 mRNA level in the spleen of CBA/J mice receiving 15 cysts

Fig. 5. – Cytokine mRNA levels in spleen (A) and brain (B) from C57BL/6 and CBA/J mice orally infected with the 76K strain of *T. gondii*. Data are expressed as relative increase in mRNA levels over the spleen or brain of uninfected C57BL/6 mice at each time point. IFN- γ or IL-10 mRNA expression in the brain and spleen of uninfected C57BL/6 mice was set as 1 at each time point of the experiment. Data shown are the mean $\pm$ SD of one of two separate experiments ($n = 5$ for each value at all time point). The experiment was repeated two times with essentially similar results.

were similar to that of LD₅₀-infected CBA/J mice ($p = 0.9629$).

The constitutive IFN- γ mRNA level in the brains of uninfected CBA/J mice was greater than C57BL/6 mice. IFN- γ mRNA expression in the brains of both strains was not significantly different as compared to uninfected controls until day 3 PI, thereafter a marked increase was observed. At days 14 and 28 PI, IFN- γ expression in the brain was higher in LD₅₀-infected C57BL/6 mice than in LD₅₀-infected CBA/J mice ($0.0316 < p < 0.0019$). IFN- γ mRNA level in the brain of CBA/J mice receiving 15 cysts were similar to that of LD₅₀-infected CBA/J mice ($p = 0.8355$). IL-10 mRNA expression in the brain in both strains was similar to that of uninfected control mice until day 7 and increased significantly in LD₅₀-infected C57BL/6 mice, but not in LD₅₀- or 15 cysts-infected CBA/J mice thereafter. IL-10 mRNA level in the brain of CBA/J mice receiving 1 cysts were similar to that of LD₅₀-infected CBA/J mice ($p = 0.7053$).

DISCUSSION

This study demonstrates that there is considerable difference in the mortality and immune response between resistant and susceptible mice following oral infection with *T. gondii* and these differences were variable according to dose of parasites. When mice were infected with an equivalent LD₅₀ of the 76K strain of *T. gondii*, that is 400 and 15 cysts separately, differences in the immune response were more apparent between resistant and susceptible strains of mice than those mice infected with same dose. This would suggest that an experimental mouse model using an equivalent lethal dose of parasites to compare the immune response is preferable to studies in which mice are infected with the same or similar dose of parasite.

LD₅₀ is widely used to compare the differences among groups in toxicology studies. We utilized the LD₅₀ in order to compare the fundamental differences between two genetically distinct mouse strains. Parasite loads may be related to local immunity or cytopathology. In this study, parasite loads of C57BL/6 and CBA/J mice after *T. gondii* infection were maximal at day 7 PI in the spleens and at day 14-28 PI in the brains. Compared with LD₅₀-infected C57BL/6 mice, both LD₅₀- or 15 cysts-infected CBA/J mice had fewer parasites in the spleen and brain. The patterns of parasite load in the brain and spleen of susceptible and resistant mice infected with *T. gondii* were similar to previously reported by Luo *et al.* (1997). However, there was no significant difference of parasite burden between LD₅₀- and 15 cysts-infected CBA/J mice. *Toxo-*

plasma-specific IgG2a antibody titers of CBA/J mice were significantly greater than those of C57BL/6 mice. Compared to 15 cysts-infected CBA/J mice, IgG2a titers of LD₅₀-infected CBA/J mice were increased. In contrast to IgG2a, IgG1 antibody titers at days 14 and 28 PI were higher in C57BL/6 mice than in CBA/J mice suggesting an enhanced Th1-type immune response in the susceptible strain (Nguyen *et al.*, 1998). *Toxo-plasma*-specific IgM antibody titers of CBA/J mice was higher and increased earlier than those of C57BL/6 mice. These results further support the importance of the IgM response in host protection against parasite infection (McLeod *et al.*, 1989).

T cells respond to foreign antigens by proliferation and the synthesis of cytokines, protective immunity against *T. gondii* is dependent on a strong, cell-mediated, T cell-dependent immune response (Khan *et al.*, 1994; Denkers, 1999; Lee *et al.*, 1999a). In our study, *T. gondii* infection resulted in a temporary decrease in the percentage of CD8a⁺ spleen cells in susceptible C57BL/6 mice, but not in resistant CBA/J mice. Since CD8⁺ T cells are essential in the generation of protective immunity against toxoplasmosis (Khan *et al.*, 1994), a decreased CD8a⁺ T cells in *Toxoplasma*-infected C57BL/6 mice may allow for an increased number of *Toxoplasma* cysts compared with resistant mice (Brown & McLeod, 1990; Schluter *et al.*, 1999). In contrast, the percentage of $\gamma\delta$ T cells was markedly increased after infection in both strains of mice. In this study, we showed a more prominent change in the $\gamma\delta$ T cell proportion in resistant CBA/J mice, especially in LD₅₀-infected CBA/J mice, than in LD₅₀-infected C57BL/6 or 15 cysts-infected CBA/J mice. This finding may be related to the role that $\gamma\delta$ T cells play in the early host response to infection with *T. gondii* (Kasper *et al.*, 1996; Lee *et al.*, 1999b). Suppression of lymphocyte DNA synthesis has been demonstrated in some animal models of *T. gondii* infection and in some acutely infected individuals. A variety of factors, including IL-10, IFN- γ , nitric oxide and parasite factors, may in part be responsible for this down-regulatory event (Candolfi *et al.*, 1994 & 1995; Khan *et al.*, 1995; Haque *et al.*, 1995). In our study, splenocyte proliferation in response to TLA was markedly depressed in *Toxoplasma*-infected C57BL/6 mice from day 7 PI, but not in *Toxoplasma*-infected CBA/J mice. The profile of T cell cytokine synthesis plays an important role in regulating the immune response and determining relative resistance or susceptibility to *T. gondii*. In our study, IFN- γ mRNA expression was significantly increased in the spleen of both mouse strains immediately after *T. gondii* infection. These findings suggest that *T. gondii* infection in mice is predominantly a Th1-polarized response (Denkers, 1999). It is interesting that IFN- γ mRNA expression in spleen were higher in LD₅₀-infected CBA/J mice than in LD₅₀-infected C57BL/6 or

15 cysts-infected CBA/J mice, whereas the increase ratio of IFN- γ mRNA expression after infection was higher in C57BL/6 mice. This would suggest that IFN- γ plays a major role in murine resistance to *T. gondii* infection (Scharton-Kersten *et al.*, 1996) and that overproduction of type 1 cytokines, in particular IFN- γ , may lead to immune hyperreactivity in susceptible hosts (Denkers, 1999; Mordue *et al.*, 2001; Mennechet *et al.*, 2002). Therefore, susceptible C57BL/6 mice may induce more immunopathology than resistant CBA/J mice. Also, the increase of IL-10 mRNA expression was particularly pronounced in the spleens of *Toxoplasma*-infected C57BL/6 mice as compared to *Toxoplasma*-infected CBA/J mice. This increase indicated that IL-10 plays a major role in protecting the host against an excessive Th1 cytokine response (Suzuki *et al.*, 2000), and the progression of toxoplasmic encephalitis in susceptible mice might be the result of elevated levels of IL-10 in the infected brains of susceptible, but not resistant, mice (Hunter *et al.*, 1994; Khan *et al.*, 1995). Together these observations suggest that LD₅₀-infection of C57BL/6 and CBA/J mice may provide a more definitive measure of immune outcome than mice receiving the same dose. This potential difference should be taken into account when studies are planned to evaluate immune parameters between two or more different strains of inbred mice.

ACKNOWLEDGEMENTS

This work was supported by Grant No. 1999-1-20200-002-2 from the Basic Research Program of the Korea Science & Engineering Foundation and National Institutes of Health AI19613 and 30000.

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Reçu le 29 juin 2001

Accepté le 2 octobre 2003