Toxoplasma gondii infection inhibits the development of lupus-like syndrome in autoimmune (New Zealand Black × New Zealand White) F1 mice

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of autoantibodies and lupus nephritis. In the present study using New Zealand Black (NZB) × New Zealand White (NZW) F1 (NZBW F1) mice, we planned to investigate the effects of Toxoplasma gondii infection on the progress of lupus nephritis. Female NZBW F1 mice at the age of 2 months were perorally infected with T. gondii. The T. gondii infection reduced the number of mice developing proteinuria and immune complex deposits in their kidneys and prolonged their life span. A marked decrease in the levels of IgM and IgG anti-DNA antibodies, especially IgG2a and IgG3 subclasses, was observed in T. gondii-infected NZBW F1 mice at 9 months of age. The level of anti-HSP70 IgG autoantibody in the sera of NZBW F1 mice was significantly higher than that in control mice at 9 weeks after T. gondii infection. Moreover, NZBW F1 mice treated with anti-self heat shock protein 70 (HSP70) monoclonal antibody were substantially protected against the onset of glomerulonephritis. Further, down-regulation of intracellular expression of IFN-γ and IL-10 was shown in spleen cells of T. gondii-infected NZBW F1 mice. This was consistent with the previous data indicating the involvement of Th1-type and Th2-type cytokines in the development of lupus-like nephritis. These results suggest that T. gondii infection is capable of preventing the development of autoimmune renal disorder in NZBW F1 mice.

Introduction

Toxoplasma gondii is among the most prevalent chronic parasitic infections in humans, and toxoplasmosis has emerged as an important opportunistic pathogen in immuno-compromised patients. Cellular immunity is essential for protection against T. gondii infection (1,2). On the other hand, we have demonstrated the production of anti-T. gondii heat shock protein 70 (TgHSP70) antibody cross-reactive to self HSP70, and anti-HSP70 autoantibody was seen to deteriorate the host defense against T. gondii infection (3,4). Moreover, it was earlier demonstrated that VH1-JH1 B-1 cells are responsible for anti-HSP70 autoantibody formation in T. gondii-infected BALB/c (a resistant strain) and C57BL/6 mice (B6 mice, a susceptible strain) (4).

Systemic lupus erythematosus (SLE) is a disease of generalized autoimmunity characterized by B cell hyperactivity, autoantibody production against self-antigens, in particular antibodies to nuclear antigens and DNA, and immune complex deposition in vital organs leading to glomerular nephropathies and proteinuria (5). NZB × NZW F1 (NZBW F1) mice develop a systemic autoimmune disease characterized by lupus nephritis, vasculitis, lymphadenopathy and splenomegaly, closely resembling human SLE (5,6,7). Beginning at the age of 3–4 months, autoantibodies, including anti-DNA antibodies, are developed in these mice and, by 7–8 months, immune complex deposits form in the kidneys. They die of the disease within 1 year (7,8).
The precise etiology of SLE is not yet understood, but genetic, hormonal, environmental and immunoregulatory factors, as well as infectious agents, are thought to contribute to the manifestation of the disease (9–11). Several groups have reported that viral infections might lead to exacerbation of autoimmune diseases (12,13), although the precise mechanisms remain unclear. Lloyd et al. (14) demonstrated that malaria is accompanied by the production of a number of autoantibodies, including some that react with DNA, and DNA-reactive antibodies in malarial infection have the potential to participate in the formation of immune deposits in nephritic malarial kidneys. Conversely, other groups have reported that malaria infection of NZBW F1 mice, one of the best models for SLE animals, retarded the development of their autoimmune disease (15–17).

The aim of this study was to examine the effect of *T. gondii* infection on the development of glomerulonephritis in NZBW F1 mice. We report here that the pathogenic isotypes (IgG2a and IgG3) of the anti-DNA antibodies in the serum of *T. gondii*-infected NZBW F1 mice were significantly reduced and the intracellular expression of Th1-type cytokine (IFN-γ) and Th2-type cytokine (IL-10) in the spleen cells of *T. gondii*-infected NZBW F1 mice was markedly diminished. Additionally, NZBW F1 mice injected with anti-HSP70 autoantibody showed significant retardation of the development of glomerulonephritis. An ameliorating effect of *T. gondii* infection on lupus-like syndrome in NZBW F1 mice was observed.

**Methods**

**Mice and *T. gondii* strain**

Eight-week-old female C57BL/6 (B6, susceptible strain), BALB/c (resistant strain), NZB, NZW and NZBW F1 mice were purchased from SLC (Hamamatsu, Japan). B6, BALB/c, NZB, NZW and NZBW F1 mice were perorally infected with 10 *T. gondii* cysts of Fukaya strain as previously described (3,18).

**Monoclonal antibody (mAb) production, purification and immunization**

Non-cross-reactive anti-TgHSP70 mAb (TgNCR C2, IgG2a) and cross-reactive anti-HSP70 autoantibody (TgCR 20, IgG3) were established as previously described (3). TgNCR C2 and TgCR 20 hybridoma clones were injected in pristane primed BALB/c mice, respectively. TgNCR C2 and TgCR 20 mAbs were purified from mouse ascites on protein G-Sepharose 4 Fast Flow (Amersham Biosciences, Uppsala, Sweden) according to the manufacturer’s instructions. NZBW F1 mice were intraperitoneally immunized with 100 μg TgNCR C2 mAb or 100 μg TgCR 20 mAb twice a month from the age of 2 months. Similarly, NZBW F1 mice were intraperitoneally treated with an isotype-matched control mAb, designated GL 113 (anti-β-galactosidase) (IgG2a. Promega, Madison, WI). At the age of 8 months, protein concentrations in urine, anti-dsDNA antibody formation in the sera of immunized mice and kidney sections were analyzed.

**Proteinuria, histopathology and immunofluorescence**

Protein concentration in urine was evaluated semiquantitatively using albumin reagent strips (Hema-Combistix; Bayer/Sankyo, Tokyo, Japan) on a monthly basis. Mice with proteinuria > 100 mg/dl were scored as positive.

Renal lesions were graded in a blinded manner (19). For light microscopy, kidneys of NZBW F1 mice uninfected and *T. gondii*-infected at 2 months of age were fixed in 4% paraformaldehyde, and 4-μm paraffin sections were stained with periodic acid Schiff (PAS) and H & E at the age of 9 months. Glomerular lesions were graded as follows: 1+ = mild focal mesangial hypercellularity alone; 2+ = moderate mesangial hypercellularity; 3+ = complex endocapillary hypercellularity sometimes with mild sclerosis or necrosis; 4+ = severe endocapillary hypercellularity glomerulonephritis with necrosis or crescent formation. Scores ≥ 1+ were positive.

To detect deposition of immune complexes at glomeruli, frozen sections were incubated for 30 min at room temperature with FITC-labelled goat antibodies to mouse IgM, IgG, or C3 (ICN Pharmaceuticals, Costa Mesa, CA).

**Measurement of antibodies**

B6, BALB/c, NZB, NZW and NZBW F1 mice were perorally infected with 10 *T. gondii* cysts at the age of 2 months as described above. The levels of IgM and IgG anti-ssDNA antibody and anti-dsDNA antibody in the sera of uninfected and *T. gondii*-infected B6, BALB/c, NZB, NZW and NZBW F1 mice at the age of 9 months were determined by ELISA as described previously (20,21). Briefly, wells of ELISA plates (Sumitomo Bakelite Co. Ltd, Tokyo, Japan) were coated for 2 h with 10 μg/ml ssDNA prepared from calf thymus DNA (Sigma Chemical Co., St Louis, MO) or dsDNA [poly(dA-dT)/poly(dA-dT); Sigma]. After washing with PBS and 0.05% Tween 20, plates were blocked with 2% BSA in PBS. 1:200 diluted sera from uninfected and *T. gondii*-infected B6, BALB/c, NZB, NZW and NZBW F1 mice at the same age of 9 months were added for 2 h. After washing with PBS–TWEEN, plates were overlaid with alkaline phosphatase-conjugated anti-mouse IgM antibody (Tago, Camarillo, CA) or anti-mouse IgG antibody (Chemicon International Inc., Temecula, CA) diluted 1:1000 for 2 h at room temperature. Absorbance at OD405 nm was measured. The levels of IgG subclasses with dsDNA in the sera were determined by coating 10 μg/ml dsDNA (Sigma) as described above, using alkaline phosphatase-conjugated rat anti-mouse IgG subclass-specific secondary antibodies (X56, anti-IgG1; R19–15, anti-IgG2a; R12–3, anti-IgG2b; R40–82, anti-IgG3; all from PharMingen, Heidelberg, Germany).

To detect the production of anti-TgHSP70 antibody and anti-HSP70 autoantibody in the sera of *T. gondii*-infected B6, BALB/c, NZB, NZW and NZBW F1 mice, sera were bled via the tail at 2 and 9 weeks post-infection. Sera were collected, and the levels of anti-TgHSP70 antibody and anti-HSP70 autoantibody were tested by ELISA using rTgHSP70 and r mouse (m) HSP70 as described previously (3,4,22). Alkaline phosphatase-conjugated anti-mouse IgM antibody (Tago) and anti-mouse IgG antibody (Chemicon International Inc.) were used as second antibodies.

**Detection of intracellular IL-10, IL-4, IFN-γ and IL-12**

NZBW F1 mice were perorally infected with 10 cysts of *T. gondii* Fukaya strain at the age of 2 months. At 9 months of age, spleen cells and peritoneal exudate cells (PECs) from...
uninfected and *T. gondii*-infected NZBW F1 mice were harvested. Then, the cells were fixed and permeabilized with a Cytotox/Cytoperm kit (PharMingen, San Diego, CA) and stained with 0.1 µg of FITC-conjugated rat anti-mouse IL-10 mAb (JES5-16E3; PharMingen), FITC-conjugated rat anti-mouse IL-4 mAb (BVD-24G2; Immunotech, France), FITC-conjugated rat anti-mouse IFN-γ mAb (XMG1.2; PharMingen), and PE-conjugated rat anti-mouse IL-12 (p40/p70) mAb (C15.6; PharMingen), respectively. Finally, the cells were washed with the permeabilization buffer, and the expressions of IL-10, IL-4, IFN-γ and IL-12 were analyzed by FACScan (Becton Dickinson, Mountain View, CA).

**Anti-dsDNA antibody production by splenic B cells from NZBW F1 mice**

B220+ cells from spleen of NZBW F1 mice at the age of 2 months were obtained by magnetic cell separation (Miltenyi Biotec, Auburn, CA) as previously described (3,4). The purity of these cells was >98%. To investigate the correlation between anti-HSP70 autoantibody and anti-DNA antibody, splenic B cells were stimulated by LPS (15 µg/ml, Sigma Chemical Co., St Louis, MO) and IFN-α (5 U/ml, PharMingen, San Diego, CA) as previously described (23) without or with anti-HSP70 autoantibody (TgCR 18 mAb, IgM) at 1 µg/ml or 10 µg/ml (3). TgCR 18 mAb was purified from mouse ascites as described above. Similarly, splenic B cells were stimulated by LPS and IFN-γ with isotype-matched control mAb, designated J5/D (IgM) (Promega). After 5 days of incubation, the supernatant was collected and the level of anti-dsDNA IgM antibody or anti-dsDNA IgG antibody was assayed using ELISA as described above.

**Statistical analysis**

Differences between mean values were analyzed by unpaired Student’s t-test. P-values < 0.05 were considered significant.

**Results**

**Effects of *T. gondii* infection on survival rate and proteinuria in NZBW F1 mice**

As shown in Fig. 1(A), uninfected NZBW F1 mice began to die at the age of 7 months, and by the age of 10 months, 100% of the mice had died. Conversely, 100% of *T. gondii*-infected NZBW F1 survived beyond 9 months of age, and by 10 months 5% had died. The incidence of proteinuria in NZBW F1 mice gradually increased from the age of 5 months without *T. gondii* infection (Fig. 1B), and then increased sharply from 7 months. Proteinuria was not detected in *T. gondii*-infected NZBW F1 mice at the age of 7 months, but from the age of 7.5 months a low and slightly increasing incidence became evident.

**Retardation of development of glomerulonephritis in *T. gondii*-infected NZBW F1 mice**

At 9 months, there was marked mesangial cell proliferation and an increase in the mesangial matrix in glomeruli, with concomitant glomerular capillary wall thickening, resulting in obliteration of capillary lumina in uninfected NZBW F1 mice (Fig. 2A and B). In some cases, wire loop lesions, sclerosis, crescent formation and adhesion of the glomerular tuft to Bowman’s capsule were noted (grade 4+). However, these changes were less prominent in *T. gondii*-infected NZBW F1 mice (grade 2) (Fig. 2F and G). To verify the development of lupus nephritis in uninfected and *T. gondii*-infected NZBW F1 mice, we examined glomerular deposits of IgM, IgG and C3. IgM (Fig. 2H), IgG (Fig. 2I) and C3 (Fig. 2J) deposits in the glomeruli were suppressed by *T. gondii* infection in NZBW F1 mice at the age of 9 months, as compared with their respective counterparts (Fig. 2C-E) in uninfected NZBW F1 mice. These findings revealed the amelioration of glomerulonephritis in the *T. gondii*-infected NZBW F1 mice at the age of 9 months.

**Effect of *T. gondii* infection on anti-ssDNA antibody and anti-dsDNA antibody formation in NZBW F1 mice**

Although 100% of NZB mice succumbed 3 weeks after *T. gondii* infection and 100% of *T. gondii*-infected NZW mice survived more than 10 months (data not shown), 95% of lupus-prone NZBW F1 mice survived more than 10 months after *T. gondii* infection. High levels of IgM (Fig. 3A) and IgG (Fig. 3B) anti-ssDNA antibodies in the sera of uninfected NZB, NZW and NZBW F1 mice were observed...
at the age of 9 months. At this age, their levels in the sera of *T. gondii*-infected NZW and NZBW F1 mice were significantly lower compared with those of uninfected NZW and NZBW F1 mice. Similarly, the levels of IgM

(Fig. 3C) and IgG (Fig. 3D) anti-dsDNA antibodies in the sera of uninfected NZB, NZW and NZBW F1 mice were high, but their formation in the sera of NZW and NZBW F1 mice after *T. gondii* infection was inhibited.
Reduced production of IgG1, IgG2a and IgG3 anti-dsDNA antibodies in *T. gondii*-infected NZBW F1 mice

To determine whether the IgG subclass pattern of anti-dsDNA antibodies was altered in *T. gondii*-infected NZBW F1 mice, sera from NZBW F1 mice uninfected or infected with *T. gondii* at the age of 2 months were examined at the age of 9 months. Although the level of IgG2b anti-dsDNA antibody was in a similar range in the sera of uninfected and *T. gondii*-infected NZBW F1 mice (Fig. 4C), the levels of IgG1 (Fig. 4A), IgG2a (Fig. 4B) and IgG3 (Fig. 4D) of *T. gondii*-infected NZBW F1 mice were decreased, and especially the formation of IgG2a and IgG3 was significantly reduced.

Anti-HSP70 autoantibody formation in *T. gondii*-infected NZBW F1 mice

The level of anti-TgHSP70 IgM antibody had increased significantly in the sera of B6, BALB/c, NZW and NZBW F1 mice at 9 weeks after *T. gondii* infection (Fig. 5A). Similarly, the level of anti-TgHSP70 IgG antibody had also markedly increased in B6, BALB/c, NZW and NZBW F1 mice at 9 weeks post-infection. The level of anti-TgHSP70 IgG antibody in the sera of *T. gondii*-infected NZBW F1 mice was significantly higher than that in *T. gondii*-infected B6, BALB/c NZW mice (Fig. 5B).

Intracellular cytokine expression in spleen cells and PECs from uninfected and *T. gondii*-infected NZBW F1 mice

In order to determine the influence of *T. gondii* infection in Th1 and Th2 subsets of NZBW F1 mice, NZBW F1 mice were perorally infected with *T. gondii* at the age of 2 months. The expressions of IL-10, IL-4, IFN-γ and IL-12 in spleen cells and PECs from uninfected and *T. gondii*-infected NZBW F1 mice were observed at the age of 9 months (Fig. 6). High levels of IL-10 and IFN-γ expression were detected in the spleen of uninfected NZBW F1 mice, but their expressions in *T. gondii*-infected NZBW F1 mice were significantly decreased. The expression of IL-4 in spleen cells of uninfected and *T. gondii*-infected NZBW F1 mice was low and unchanged, respectively. Low expressions of IL-10 and IL-4 in PECs of uninfected and *T. gondii*-infected NZBW F1 mice were observed. IFN-γ in PECs of uninfected NZBW F1 mice was expressed at a high level, whereas that in *T. gondii*-infected NZBW F1 mice was markedly decreased. In this study, the expression of IL-12 was not detected in PECs from either uninfected or *T. gondii*-infected NZBW F1 mice at the age of 9 months.

Ameliorating effect on lupus-like syndrome in Tg CR 20 mAb immunized NZBW F1 mice

In order to further understand the relationship between high levels of anti-HSP70 autoantibody in *T. gondii*-infected NZBW F1 mice and the suppression of lupus-like syndrome in NZBW F1 mice, NZBW F1 mice were administered with Tg NCR C2 mAb or Tg CR 20 mAb from the age of 2 months. 100% NZBW F1 mice treated with Tg NCR C2 mAb were observed with severe proteinuria (>100 mg/dl) at the age of 8 months. In contrast, the percentage of severe proteinuria in NZBW F1 mice immunized with Tg CR 20 mAb from the age of 2 months was low (Fig. 7A). The production of anti-dsDNA antibody in the sera of Tg CR 20 mAb-injected NZBW F1 mice was significantly decreased when compared with those of Tg NCR C2 mAb-injected NZBW F1 mice (Fig. 7B). Moreover, glomerular deposits of IgG (Fig. 7C) were suppressed in NZBW F1 mice immunized with Tg CR 20 mAb. Glomerular deposits of IgG from NZBW F1 mice immunized with Tg NCR C2 mAb did not show any reduction at the age of 8 months (Fig. 7C). The ameliorating influence on glomerulonephritis was not detected in NZBW F1 mice treated with isotype-matched control mAb (data not shown).

In order to examine the effect of anti-HSP70 autoantibody on anti-DNA antibody formation by B cells from NZBW F1 mice, an *in vitro* culture method of anti-DNA antibody production of NZBW F1 splenic B cells stimulated with LPS and IFN-γ was utilized. The level of anti-dsDNA IgM antibody was not changed in the culture supernatant from B cells stimulated with LPS and IFN-γ plus TgCR 18 mAb, compared with that from B cells stimulated with LPS and IFN-γ plus isotype-matched control mAb (J5/D) (Fig. 8). The levels of anti-dsDNA IgG antibody were low. Thus,
anti-HSP70 autoantibody did not affect anti-dsDNA antibody production directly.

**Discussion**

In the present study, we clearly demonstrated that *T. gondii* infection was sufficient to significantly protect against severe lupus nephritis in NZBW F1 mice. Autoantibodies of the IgG class and particularly IgG anti-DNA antibodies are believed to play a major role in the pathogenesis of lupus nephritis (23,25). It has been reported that the levels of circulating and glomerular deposited IgG3 were associated with glomerulonephritis in the MRL-Fas−/− mouse model of SLE (26), and complement-fixed IgG2a anti-DNA antibodies were demonstrated to be predominant nephritogenic autoantibodies in NZBW F1 mice (27). Moreover, several groups recently revealed that the reduction in anti-DNA antibody, especially in pathogenic IgG2a and IgG3, prevented autoimmune renal injury and increased survival in NZBW F1 mice (28,29). The present data were consistent with the previous data indicating correlation between the down-regulation of IgG2a and IgG3 anti-DNA antibodies and the amelioration of glomerulonephritis in NZBW F1 mice (12,14).

Cytokines have been suggested to play an important role in the immune dysregulation observed in lupus-prone mice and SLE patients (30,31). Peng et al. (32) reported that Th1 and Th2 cells were equally important in SLE, whereas Hasegawa et al. (33) recently demonstrated that IFN-γ but not IL-4 contributed to the development of lupus in NZBW F1 mice. Although IL-12 is a potent regulator of Th1 and Th2 subset development, defective IL-12 production in NZBW F1 mice and SLE patients has been reported (34,35). An amplification loop consisting of B cells and Th cells as a primary event in lupus pathogenesis was proposed (36). In that loop, autoreactive B cells function as antigen-presenting cells and promote T cell activation. Activated T cells provide further help for B cells, and each cycle might further amplify autoreactive B and T cell clones. Although the treatment of NZBW F1 mice with anti-IFN-γ mAb (37) or anti-IL-10 mAb (38) substantially delayed the onset of glomerulonephritis, the basis of cytokine-mediated protection has not yet been elucidated. One possible mechanism of anti-IL-10-mediated protection of NZBW F1 mice against the onset of autoimmunity suggested a protective role for TNF-α in this disease (39,40). Moreover, TNF-α significantly decreased IFN-γ-induced upregulation of MHC class II expression on different mature cells collected from NZBW F1 mice (41). However, there have been some reports that long-term administration of cytokines caused suppression of cell-mediated immunity in NZBW F1 mice (42). Therefore, the long-term toxicity of cytokine treatment remained to be revealed. In the present study, we found that *T. gondii* infection significantly reduced the intracellular expression of Th1 cytokine IFN-γ in spleen cells from NZBW F1 mice at the age of 9 months. At the age of 7 or 8 months, the...
cytokine production pattern in T. gondii-infected NZBW F1 mice was observed to be similar to those at 9 months (data not shown). IFN-γ has been reported to be a cytokine of central importance in murine lupus by promoting the switch of IgM and IgG2a subclass, leading to glomerular injury, and by inducing apoptosis of renal parenchymal cells (43). Thus, T. gondii infection inhibited IFN-γ expression, leading to reduced IgG2a anti-DNA antibody formation and IgG2a deposition in glomeruli, resulting in the amelioration of lupus nephritis in NZBW F1 mice. On the other hand, the intracellular expression of IL-10, a Th2 cytokine, significantly decreased in T. gondii-infected NZBW F1 mice. Ishida et al. (38) reported that the administration of anti-IL-10 antibody delayed the onset of autoimmunity in NZBW F1 mice via inhibition of autoantibody production. Therefore, we postulated that pathogenic T cells were involved in the reduction of anti-DNA antibody formation by T. gondii infection in NZBW F1 mice.

**Fig. 6.** Intracellular expression of IL-10, IL-4, IFN-γ and IL-12 in spleen cells and PECs from uninfected (dotted line) and T. gondii-infected (bold line) NZBW F1 mice was detected by FACS analysis at the age of 9 months. T. gondii-infected mice were perorally infected at the age of 2 months as described. The thin lines depict the isotype control-strain cells. Representative results from three independent experiments are shown.

**Fig. 7.** Effects of TgNCR C2 mAb and TgCR 20 mAb on proteinuria, anti-dsDNA antibody formation and renal immunofluorescence staining in NZBW F1 mice at the age of 8 months. (A) Cumulative incidence of severe (>100 mg/dl) proteinuria in Tg NCR C2 mAb (n = 20) and Tg CR 20 mAb (n = 20) injected NZBW F1 mice at the age of 8 months. ***P < 0.005. (B) The production of anti-dsDNA antibody in the sera of Tg NCR C2 mAb (n = 20)-injected and Tg CR 20 mAb (n = 20)-injected NZBW F1 mice was detected by ELISA as described in Fig. 3. *P < 0.05. (C and D) Sections of kidney from TgNCR C2 mAb (C) and TgCR 20 mAb (D) immunized NZBW F1 mice subjected to immunofluorescence staining with anti-mouse IgG (original magnification, ×400).

**Fig. 8.** Effects of anti-HSP70 autoantibody on anti-DNA antibody formation in vitro. Splenic B cells from NZBW F1 mice at the age of 2 months was purified and stimulated with LPS and IFN-γ plus TgCR 18 mAb as described in Methods. The levels of anti-dsDNA IgM antibody (closed squares) and anti-dsDNA IgG antibody (open squares) were detected by ELISA. Data represent mean ± SD of three to five mice in three separate experiments.
The complexity of cytokine regulation and the imbalance of Th1 and Th2 subsets in SLE animal models and human SLE are well recognized (38,44–48). As previously described, T. gondii infection up-regulated IFN-γ production in wild-type mice (4,49). However, the mechanisms of how T. gondii infection in NZBW F1 mice suppresses both Th1 and Th2 responses are not clear. Several groups previously reported that lactic dehydrogenase virus (LDV)-infected NZBW F1 mice exhibited reduced class II antigen expression on macrophages (50), and that the APC activities of macrophages were suppressed by LDV infection (51). Others and we have previously shown that T. gondii down-regulates MHC class II gene expression and antigen presentation to CD4+ T lymphocytes in extracerebral antigen-presenting cells (49,52–55). Lüder et al. (56) indicated that T. gondii considerably inhibits the IFN-γ-induced MHC class II expression in rat astrocytes and microglial cells at the level of gene transcription, since down-regulation coincided with reduced transcript levels of the most critical transacting factor of class II transcription CIITA. Inappropriate MHC class II expression by endogenous APC of the CNS contributes to the pathogenesis of autoimmune disorders, e.g. multiple sclerosis or experimental allergic encephalomyelitis (57). Hence, impaired APC function may be one of the factors responsible for the suppression of both Th1 and Th2 differentiations in T. gondii-infected NZBW F1 mice.

HSP is a family of ubiquitous and phylogenically highly conserved proteins (58). Autoreactivity to HSP is often associated with autoimmune pathology (59). Anti-HSP70 autoantibody produced by VH1-JH1 B-1 cells was detected in T. gondii-infected BALB/c and B6 mice (3,4). Additionally, Hayakawa et al. (60) previously reported that autoantibody-producing B cells were easily detected in the peritoneal cavity as CD5+ B cells in autoimmune-prone NZBW F1 mice. Our previous study showed that a high level of anti-heat shock cognate protein (HSC) 71 autoantibody was detected in sera from patients with Vogt-Koyanagi–Harada disease (VKH), a systemic disorder that affects various organs and is believed to be an autoimmune disease (61). A high level of anti-HSP70 IgG autoantibody in the sera of NZBW F1 mice was observed after T. gondii infection. In order to clarify the relationship between anti-HSP70 autoantibody and lupus-like syndrome, NZBW F1 mice were treated with anti-HSP70 autoantibody from the age of 2 months twice a month. At the age of 8 months, NZBW F1 mice showed significant retardation of the development of glomerulonephritis. Therefore, anti-HSP70 autoantibody formation in T. gondii-infected NZBW F1 mice was another important factor contributing to retardation of the development of glomerulonephritis. The direct involvement of self-HSP70 molecules in the pathogenesis of SLE disease remains to be further revealed. One possibility is that anti-HSP70 autoantibody may block the high expression of MHC class II gene induced by HSP70. Down-regulation of antigen presentation to CD4+ T lymphocytes may contribute to the amelioration of glomerulonephritis in NZBW F1 mice. Taken together, these results indicated that T. gondii infection inhibited the development of lupus-like syndrome of NZBW F1 mice. Not only reducing the production of IFN-γ and IL-10 but also anti-HSP70 autoantibody formation contributed to retardation of the development of glomerulonephritis in T. gondii-infected NZBW F1 mice. This is the first report regarding the favorable effect of T. gondii infection on glomerulonephritis of NZBW F1 mice.

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Abbreviations

B6 C57BL/6
CR cross-reactive
HSP70 heat shock protein 70
HSC71 heat shock cognate protein 71
LDV lactic dehydrogenase virus
NCR non-cross-reactive
NZB New Zealand Black
NZW New Zealand White
NZBW F1 (New Zealand Black × New Zealand White) F1
PAS periodic acid Schiff
PEC peritoneal exudate cell
TgHSP70 Toxoplasma gondii-HSP70
VKH Vogt-Koyanagi-Harada disease

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