

Full Length Research Paper

# Effects of FK506 on serum IFN- $\gamma$ and IL-2 levels and liver parasite load in *Toxoplasma gondii*-infected mice

Zheng Shun-li\*, Gong Jiang-jiang, Yang Qing-sheng and Ma Xiao-hong

First Affiliated Hospital of Jinan University, Department of Cardiothoracic Surgery, Guangzhou 510630, China.

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In the present study, we investigated the effects of FK506 (immunosuppressant, Tacrolimus) on the contents of interferon-gamma (IFN- $\gamma$ ) and interleukin-2 (IL-2), and liver parasite load in mice infected with *Toxoplasma gondii* (*T. Gondii*). 120 Kunming mice were intraperitoneally injected with  $10^2$ /mouse *T. Gondii* (RH strain) tachyzoites, and then these mice were randomly divided into FK506 group (group A, 1 mg/kg\*d FK506 was intragastrically administrated) and control group (group B, equal volume of normal saline was intragastrically administrated), and there were 30 male and 30 female mice in each group. 2, 4, 6, 7 and 9-day post infection (PI) was carried out; each of 8 mice were randomly sacrificed to harvest peripheral blood and liver samples, and then serum IFN- $\gamma$  and IL-2 contents and liver parasite loads were detected by ELISA and quantitative fluorescence PCR, respectively. The serum IFN- $\gamma$  and IL-2 contents of another 8 healthy mice served as normal control values. All mice died within 8 and 10 days after infection in group A and B, respectively. On day 2, 4, 6 and 7 post infection, the serum IFN- $\gamma$  content in group A was significantly lower than that in group B ( $p < 0.05$ ). On day 2 post infection, there was no significant difference in serum IL-2 content between groups A and B, but however serum IL-2 content in the group A was lower than that in the group B from day 4 post infection ( $p < 0.05$ ), and subsequently the difference was more and more significant. On day 4 and 7 post infection, liver parasite loads in the group A was significantly higher than that in the group B ( $p < 0.01$ ). FK506 plays a regulatory role in T lymphocyte immunity and decrease host immunity, and then risk of *T. Gondii* infection is also increased.

**Key words:** *Toxoplasma gondii*, FK506, animal model, IFN- $\gamma$ , IL-2.

## INTRODUCTION

*Toxoplasma gondii* (*T. Gondii*), a kind of protozoan parasite, is an important life-threatening opportunistic pathogen especially in recipients with compromised immunity (Singh et al., 1996). The occurrence, development and prognosis of *T. Gondii* infection are closely related to immune status especially cellular immunity in hosts (Nissapatorn, 2009). FK506, a new kind of immunosuppressant, is widely used in liver, heart, lung and other grafts recipients, and thus compromised immunity in recipients is conducive to *T. Gondii* infection. It was previously found that cytokines (IFN- $\gamma$  and IL-2) rather than cytotoxicity-based effector functions were

more critical for protective immunity both during the acute and chronic phases of *T. gondii* infection (Yap and Sher, 1999). Thus, in this study, we compare the content of IFN- $\gamma$  and IL-2, and liver parasite load in mice with acute *T. Gondii* infection which were treated with or without FK506, in order to investigate the effects of FK506 on immunity and cytokines in hosts after *T. gondii* infection.

## MATERIALS AND METHODS

### *T. gondii* tachyzoite purification

*T. gondii* tachyzoites (RH strain) which were preserved in liquid nitrogen were recovered and inoculated in the abdominal cavity of healthy mice. After 3 to 5 passages, the virulence of *T. gondii* tachyzoites was gradually restored. Peritoneal fluid of *T. gondii*-infected mice were harvested and rinsed with phosphate buffer

\*Corresponding author. E-mail: zhengjh0471@163.com. Tel: 0086-20-38688506.

**Table 1.** Serum IFN- $\gamma$  content (pg/ml) in the group A and B ( $x \pm s$ ).

Group	Normal control	Day 2	Day 4	Day 6	Day 7	Day 9
Group A	1.22 $\pm$ 0.12*	2.89 $\pm$ 0.25	4.58 $\pm$ 0.27	8.88 $\pm$ 0.80	7.93 $\pm$ 1.71	**
Group B		5.05 $\pm$ 0.78	10.11 $\pm$ 1.23	43.95 $\pm$ 2.44	30.12 $\pm$ 5.79	10.66 $\pm$ 1.07
p value		<0.05	<0.01	<0.01	<0.01	

\*Compare with the normal control group,  $p < 0.01$ ; \*\*All mice were dead in the group A.

solution, followed by centrifugation and cell counting. *T. gondii* tachyzoites sedimentum was resuspended and diluted for preservation.

#### Preparation and grouping of *T. gondii*-infected mice

There were 64 female and 64 male Kunming mice (body weight from 22 to 26 g) in this study, and 120 mice of them were intraperitoneally injected with  $10^2$ /mouse *T. Gondii* (RH strain) tachyzoites. These *T. gondii*-infected mice were randomly divided into FK506 group (group A, 1 mg/(kg.day). FK506 was intragastrically administered) and control group (group B, equal volume of normal saline was intragastrically administered), and there were 30 male and 30 female mice in each group. 2, 4, 6, 7 and 9-day post infection (PI) was carried out; each 4 male and 4 female mice were randomly sacrificed to harvest peripheral blood and liver samples, and then serum IFN- $\gamma$  and IL-2 content and liver parasite loads were detected. The serum IFN- $\gamma$  and IL-2 content of 8 healthy mice served as normal control.

#### Serum IFN- $\gamma$ and IL-2 detection

Serum IFN- $\gamma$  and IL-2 were detected with a double sandwich ELISA test kit (ShenZhen Jingmei Biotechnology Company, China). Serum samples were diluted in 1:2 before detection, and serum IFN- $\gamma$  and IL-2 detection were performed strictly following the instruction of the ELISA kit. Subsequently, the IFN- $\gamma$  and IL-2 OD values of each sample were measured with a microplate reader at 450 nm, and standard curve was prepared with the OD of standard reagents and the serum IFN- $\gamma$  and IL-2 contents (pg/ml) of each sample were obtained.

#### Liver parasite load detection

During 4 and 7-day post infection (PI), the harvested liver specimens were rinsed with normal saline and preserved at  $-20^\circ\text{C}$ . Liver parasite load in each sample was detected with a quantitative fluorescence PCR kit (Da An Gene Co., Ltd. of Sun Yat-sen University, China). Total RNA was extracted from the ileum, cecum and colon mucus using the TRIzol reagent (Invitrogen). After verification of its integrity, RNA was quantified spectrophotometrically with 1  $\mu\text{g}$  processed for complementary DNA (cDNA) synthesis using SuperScript II reverse transcriptase (Toyobo, Japan). Specific primers for target gene were designed using Primer5 software.

#### Statistical analyses

All statistical analyses were performed with SPSS version 11.0 statistical software. Gene copy of liver parasite load of each sample was expressed by logarithmic transformation before comparison, and all group comparisons were finished with t test. A value of  $p < 0.05$  was considered statistically significant.

## RESULTS

In group A, some mice had peritoneal fluid on day 3 PI, some mice died on day 7 PI, and all mice were dead on day 8 PI. In the group B, some mice had peritoneal fluid on day 4 PI, some mice died on day 9 PI, and all mice were dead on day 10 PI. On day 2, 4, 6 and 7 PI, the serum IFN- $\gamma$  content in the group A was significantly lower than that in the group B ( $p < 0.05$ ) (Table 1). On day 2 PI, there was no significant difference in serum IL-2 content between the group A and B, however serum IL-2 content in the group A was lower than that in the group B from day 4 PI ( $p < 0.05$ ), and subsequently the difference was more and more significant (Table 2). On day 4 and 7 PI, liver parasite loads in the group A was significantly higher than that in the group B ( $p < 0.01$ ) (Table 3).

## DISCUSSION

*T. gondii*, a kind of intracellular parasite, can parasitize in most mammals including humans. Toxoplasmosis's incidence in organ transplantation recipients is closely related to the prevalence of toxoplasmosis in the general population. Toxoplasmosis results mainly from transmission of the parasite with the transplanted organ from a toxoplasma-seropositive donor to a toxoplasma-seronegative recipient. This risk is high in cases of transplantation of organs that are recognized sites of encystation of the parasite, e.g. the heart. Clinical symptoms usually occur within the first 3 months after transplantation, sometimes as early as 2 weeks post transplant, and involve febrile myocarditis, encephalitis or pneumonitis (Derouin and Pelloux, 2008). In clinical practice, it is also reported that long-term administration of immunosuppressants is conducive to *T. gondii* infection in organ transplantation recipients (Mahgoub et al., 2009). However, there have been no studies available to uncover the effects of FK506 on the infection of *T. gondii*. It was previously confirmed that mice was highly sensitive over *T. gondii* infection (Mordue et al., 2001). In this study, a *T. gondii* infection mouse model was established to investigate the serum IFN- $\gamma$  and IL-2 and liver parasite load in mice with and without administration of FK506, and in order to explore the effects of *T. gondii* infection on the immune function and survival time of mice. Biotin - Avidin - double sandwich ELISA method

**Table 2.** Serum IL-2 content (pg/ml) in the group A and B (x±s).

Group	Normal control	Day 2	Day 4	Day 6	Day 7	Day 9
Group A	22.49 ± 2.61	26.01 ± 2.13	30.22 ± 2.31	28.69 ± 2.07	24.93 ± 1.52	**
Group B		30.24 ± 2.18	36.87 ± 2.05	44.15 ± 2.48*	46.04 ± 2.04*	50.18 ± 2.17*
p value		>0.05	<0.05	<0.05	<0.01	

\*Compare with the normal control group, p < 0.01; \*\*All mice were dead in the group A.

**Table 3.** Liver parasites load in the group A and B (x±s).

Group	Day 4	Day 7
Group A	2.99 ± 0.62	7.25 ± 1.11
Group B	1.88 ± 0.32	4.60 ± 0.87
p value	<0.01	<0.01

for cytokine detection have many advantages including high specificity, high sensitivity, simple operation and more objective and accurate results, and thus the serum IFN- $\gamma$  and IL-2 levels were detected by Biotin-Avidin-double sandwich ELISA method in this study.

Cell-mediated immune responses are essential for host control of intracellular infections. Upon initial encounter with the immune system, *T. gondii* rapidly induces production of the type-1 promoting cytokine IL-12 most likely from a subpopulation of dendritic cells (Wang et al., 2005). NK cells, CD<sub>4</sub><sup>+</sup> and CD<sub>8</sub><sup>+</sup> T cells and Th1 lymphocytes are then activated and triggered to synthesize IFN- $\gamma$  and IL-2, the major mediator of host resistance during the acute and chronic phases of *T. gondii* infection (Jongert et al., 2008). Cytokine (IFN- $\gamma$ ) rather than cytotoxicity-based effector functions are more critical for protective immunity both during the acute and chronic phases of *T. gondii* infection (Yap and Sher, 1999). IFN- $\gamma$  is a major cytokine to activate macrophage resistance on *T. gondii*. Mordue et al. (2001) conducted a comparative study with high and low virulent strains of *T. gondii*, and it was found that IFN- $\gamma$  was highly expressed in lethal infection with a low dose of high virulent strains (10<sup>2</sup> RH tachyzoites) and a high dose of low virulent strains (10<sup>5</sup> ME49 tachyzoites) and IFN- $\gamma$  could significantly up-regulate the expression of MHC I and II molecules, and promote the differentiation of T cells and B cells, enhance killing activity of NK cells, and then fully activate mononuclear macrophages and enhance immune function (Treadler et al., 2002). IL-2 is another important cytokine resistant for *T. gondii* infection and IL-2 has many physiologic functions especially in promoting the lethal effects of CTL, NK and LAK cells (Guk et al., 2005). Fang et al. (1999) found that IL-2 could significantly enhance host resistance on acute *T. gondii* infection and prolong the survival time of *T. gondii*-infected mice. At present, it is recognized that IL-2 plays a role of anti-*T. gondii* infection by inducing increased

production of IFN- $\gamma$ , and then activating macrophages and enhancing the killing effects of NK cells (Araujo and Slifer, 2003).

In this study, serum IFN- $\gamma$  content was significantly increased in mice from day 2 PI, but serum IFN- $\gamma$  content of mice in the FK506 group was significantly lower than that in the control group at same time points post infection; serum IL-2 content was significantly increased in mice from day 6 PI, and there was no significant difference in serum IL-2 content between the FK506 and control groups, but serum IL-2 content in the FK506 group was significantly lower than that in the control group from day 4 PI, and subsequently the difference was more and more significant, indicating that FK506 could significantly inhibit the production of IL-2 and IFN- $\gamma$  in mice with *T. gondii* infection, which was coincident with the previous findings (Yi et al., 2010; Liu et al., 2009). *T. gondii* can damage multiple tissues and organs such as brain, eye, heart and liver (Smith et al., 2004). In this study, fluorescent quantitative PCR showed DNA copy of *T. gondii* tachyzoites in liver in the FK506 group was up to 10<sup>7</sup>~10<sup>8</sup> copies/gram of tissue, which was significantly higher than that in the control group, indicating that FK506 inhibited the immune function of mice and diminished the killing roles on *T. gondii*, and thus the growth of *T. gondii* was faster in the FK506 group. On the other hand, earlier death of mice in the FK506 group also confirmed the above presume.

In conclusion, administration of FK506 significantly decreases the production of IFN- $\gamma$  and IL-2 and increases the liver parasite load in mice with *T. gondii* infection. FK506 may decrease the killing roles of host immunity on *T. gondii*, and promote the growth of *T. gondii* and the death of hosts through inhibiting the production of important cytokines such as IFN- $\gamma$  and IL-2.

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