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A Traditional Oriental Herbal Medicine, Juzen-taiho-to has Suppressive Effect on Non-lethal Rodent Malaria by Means of Stimulation of Host Immunity

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Abstract

The effects of Juzen-taiho-to (TJ-48) on the course of non-lethal *Plasmodium berghei* XAT (Pb XAT) infection in CBA mice were examined. Administration of TJ-48 at a dose of 2 g/kg body weight orally once a day during the observation period suppressed the development of parasitaemia of mice infected with Pb XAT. Mice treated with TJ-48 showed higher levels of specific antibody titers especially IgG2a isotypes of immunoglobulins in the serum compared with the control mice. Production of IFN-γ by spleen cells of mice treated with TJ-48 was also higher than that of the control. These findings suggest that TJ-48 has suppressive effect on the course of parasitaemia caused by stimulation of host immunity.

Key words: *Plasmodium berghei* XAT; traditional oriental herbal medicine; Juzen-taiho-to; IFN-γ; immunoglobulins; antimalarials.

Introduction

Juzen-taiho-to (TJ-48) which originates from Chinese traditional medicine is composed of a mixture of 10 medicinal plants: Angelicae (radix), *Atractylodis lanceae* (rhizoma), Astragali (radix), Cinnamomi (cortex), Cnidii (rhizoma), Ginseng (radix), Hoelen, Paeoniae (radix), Rehmanniae (radix), and Glycyrrhizae (radix). One hundred grams of TJ-48 contains 63.5 g of an extract prepared from a mixture of equal weights of the first nine plants and 3.5 g of an extract from the tenth (Ohnishi *et al.* 1990). It is one of the most commonly used herbal medicines in Japan for treatment of patients with anemia, loss of appetite, extreme exhaustion, and fatigue. TJ-48 has been reported to have immunopotentiating activity (Komatsu *et al.* 1986) and to enhance phagocytosis (Maruyama *et al.* 1988).

All antimalarial drugs exert their effect directly on *Plasmodium* parasites although they have specificity for parasite stages; for example chloroquine is schizontocidal and primaquine is gametocidal. However, there is much evidence from the field observations and experimental results that the effectiveness of chemotherapy is dependent on the host immune status (Targett, 1984). Immunity may work partly as a host defense mechanism whereas it is not apparently efficient enough to affect the infection.

Plasmodium berghei XAT (Pb XAT) is an irradiation-induced attenuated variant of a virulent NK65 strain (Waki *et al.*, 1982). The parasite causes self-limiting infection in mice. The spontaneous recovery from the infection is dependent on $CD4^+$ T cells as mice depleted of the T-cell population are unable to control parasitaemia and the infection becomes lethal (Waki *et al.* 1992). Thus induction of protective immunity in the Pb XAT infection is strongly dependent on the T-cell response of the host.

Hence, we have examined whether Pb XAT

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infection can be interfered by administration of TJ-48 which stimulates immunity in the host.

Materials and Methods

Mice

Female CBA/JNCrj mice were obtained from Charles River Japan Inc. (Kanagawa, Japan). All mice were 6 weeks old at the start of the experiments.

Parasites and infection

An attenuated variant strain of Pb XAT induced from a virulent strain of Pb NK65 (Waki *et al.* 1982) and the original strain were used for the study. A group of 5 mice were inoculated intravenously with an erythrocyte suspension containing 10^4 parasitized red blood cells. Parasitaemia was determined from blood smears stained with Giemsa's stain.

Administration of TJ-48

Juzen-taiho-to (TJ-48) was obtained from the Tumura Company Limited, Tokyo, Japan. Mice were injected with 0.2 ml of TJ-48 suspension in distilled water at a concentration of 400 mg/ml (2 g/kg body weight) directly into the stomach once a day from 2 days before infection for 18 days. Control mice were given distilled water according to the same schedule.

Detection of IFN-γ secreted from spleen cells in vitro

Spleen cells (5×10^6/well) from 3 infected mice per group were cultured at 37°C in an atmosphere of 5% CO_2 in 24-well tissue culture plates containing 1 ml/well RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 20 mM HEPES buffer, 5×10^{-5} M 2-mercaptoethanol, 2.0 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin. After 48 hr, the culture supernatants were removed from each culture and stored at –30°C until assayed. A two-site sandwich ELISA was performed to quantify the IFN-γ in the samples according to the method of Slade and Langhorne (1989), with R4-6A2 for capture and biotinylated XMG 1.2 for detection. Units of IFN-γ were calibrated from a standard curve calculated on the basis of known concentrations of recombinant murine IFN-γ (Genzyme, MA, USA).

Indirect fluorescent antibody test (IFAT)

The anti-plasmodial immunoglobulin isotypes IgM, IgG1, IgG2a, IgG2b, and IgG3 in the serum were titrated by an indirect fluorescent antibody test (IFAT) according to the method of Waki and Suzuki (1974) using acetone fixed parasitized blood smears as antigens and FITC conjugated rat monoclonal antibodies against mouse immunoglobulin isotypes (Zymed laboratories, Inc. San Francisco, U. S. A.) as the second antibodies.

Statistics

The parasitaemias in the groups of mice administered TJ-48 and controls, and production of antibodies and IFN-γ were compared on individual days by using Mann-Whitney U test. Each experiment was repeated 3 times using the same protocol.

Results

Effect of TJ-48 on the course of parasitaemia

Mice administered TJ-48 or distilled water were infected with Pb XAT and the course of parasitaemia was compared. The patent parasitaemia in both groups of mice was nearly the same whereas the course of parasitaemia of mice treated with TJ-48 was significantly lower than that of control mice on day 13 to 17 ($P<0.05$) (Fig. 1). Whereas TJ-48 had no effect on the course of parasitaemia in the infection with virulent Pb NK65 (Fig. 2).

Production of IFN-γ by spleen cells

Production of IFN-γ by spleen cells from mice infected with Pb XAT was examined. Spleen cells obtained 4 days after infection produced IFN-γ most efficiently and then decreased gradually in the production in control mice, whereas those in TJ-48-treated mice showed significantly higher units of IFN-γ on days 4 and 5 ($P<0.05$) (Fig. 3). IFN-γ levels of both drugged and control mice declined to that of uninfected mice by day 7 (data not shown).

Effect of TJ-48 on the development of anti-plasmodial immunoglobulin isotypes in mice infected with Pb XAT

Table 1 shows the serum antibody titers of immu-

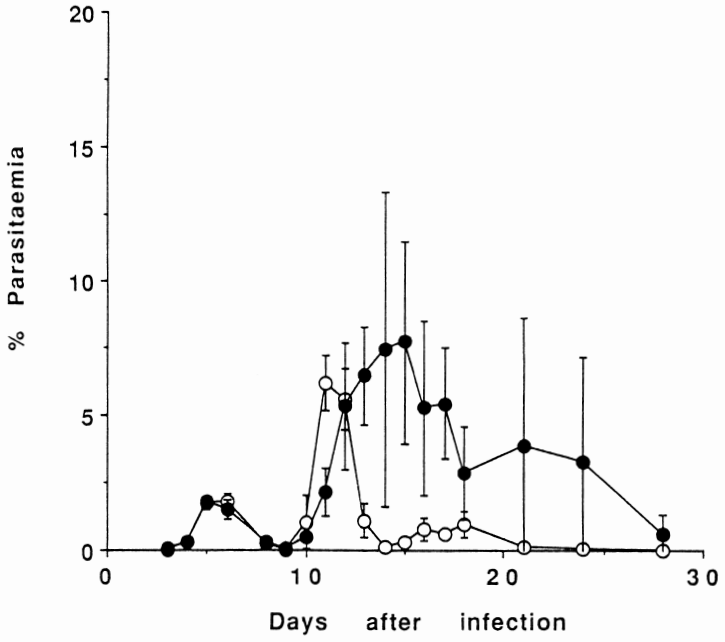

Fig. 1 Effect of TJ-48 on the course of parasitaemia of mice infected with *Plasmodium berghei* XAT. Mice were administered TJ-48 (open circle) or distilled water (closed circle) once a day from 2 days before infection during the observation period.

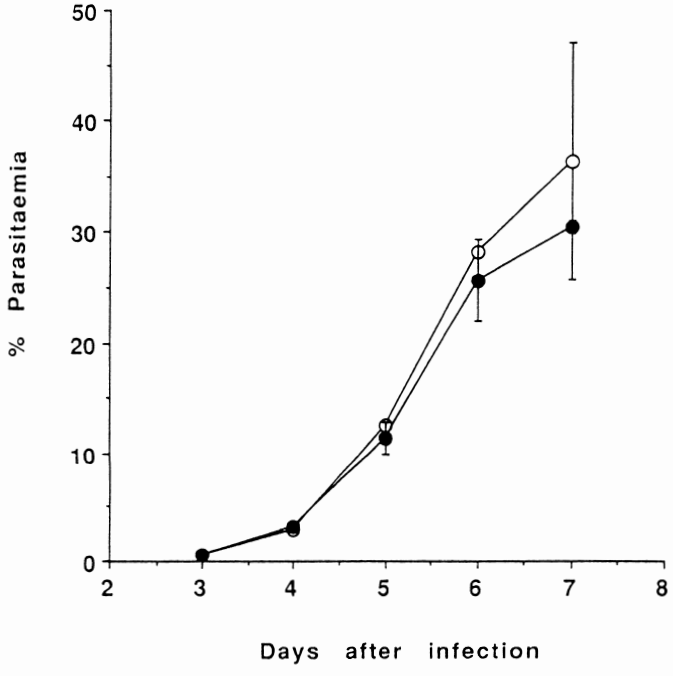

Fig. 2 Effect of TJ-48 on the course of parasitaemia of mice infected with *Plasmodium berghei* NK65. Mice were administered TJ-48 (open circle) or distilled water (closed circle) once a day from 2 days before infection during the observation period.

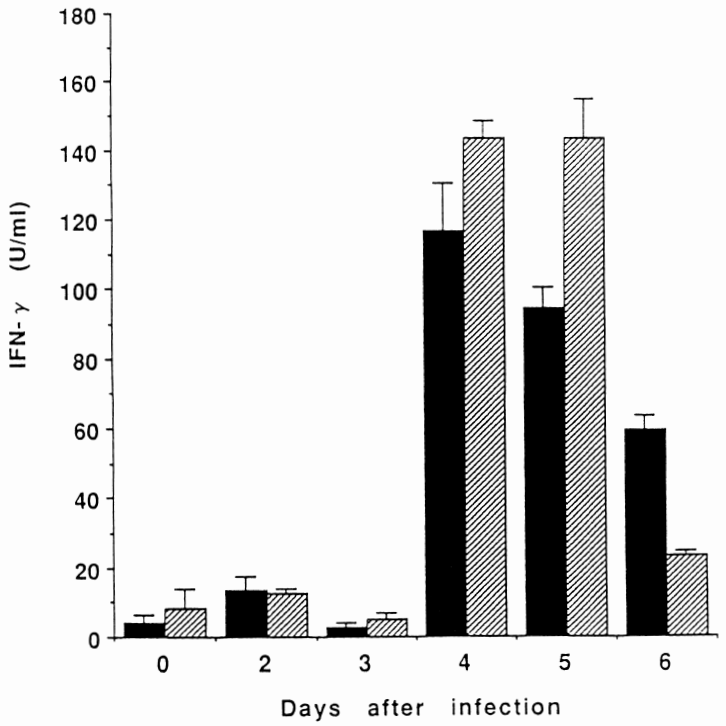

Fig. 3 Production of IFN-γ by spleen cells from CBA mice infected with *Plasmodium berghei* XAT. The data represent the mean±SD for triplicate samples from either untreated mice (closed bars) or those receiving TJ-48 (hatched bars).

Table 1 Effect of TJ-48 on the development of anti-plasmodial immunoglobulin isotypes in CBA mice infected with *Plasmodium berghei* XAT

Ig isotypes	Treatment	1W	2W	3W
Total Ig	*TJ-48	4.8±0	5.6±0.55	5.8±0.45
	Control	3.6±0.55	5.0±0	5.2±0.45
IgM	TJ-48	3.4±0.55	4.0±0	2.6±0.55
	Control	3.2±0.45	3.0±0	1.2±1.1
IgG1	TJ-48	0.0±0	3.6±0.55	4.2±0.45
	Control	0.0±0	3.8±0.45	3.8±0.45
IgG2a	TJ-48	0.0±0	2.2±0.45	3.6±0.55
	Control	0.0±0	2.4±0.55	2.2±0.45
IgG2b	TJ-48	0.0±0	2.8±0.45	3.0±0
	Control	0.0±0	3.0±0	2.6±0
IgG3	TJ-48	0.0±0	3.0±0.71	3.2±0.45
	Control	0.0±0	2.6±0.55	3.0±0.71

*IFA titer: 4^n ± standard deviation

noglobulin isotypes in Pb XAT infected mice treated with TJ-48 or distilled water during the course of infection. The total Ig titer in the sera obtained from TJ-48-treated mice was significantly higher than that in the control mice during the observation period. Among immunoglobulin isotypes, IgM and IgG2a responses were enhanced more markedly by TJ-48 treatment.

Discussion

Mice inoculated with Pb XAT show a low level of parasitaemia with two peaks during which parasites are prevented from multiplying. In the first phase of the infection, neutrophils may have a role in the reduction of parasitaemia (Waki *et al.* 1993). It has become clear that a specific IgG2a isotype of immunoglobulins is responsible for the recovery from the Pb XAT infection (Waki *et al.* 1995). In the present study mice administered TJ-48 showed suppressed parasitaemia in the second phase of the infection. Enhanced production of IFN-γ on day 4 and 5 may induce class switching in B cells to produce protective IgG2a antibodies in the mice administered with TJ-48 which may be responsible for the early outcome of the infection. Satomi *et al.* (1989) demonstrated that pretreatment of mice with TJ-48 had protective effects against *Pseudomonas aeruginosa* which causes an infection often occurring in immunosuppressive patients. This finding is comparable with our result because establishment of *P. aeruginosa* infection is strongly influence by the immune status of the host and the action of TJ-48 may be exerted on the immune system of the host.

Administration of TJ-48 did not affect the course of parasitaemia of mice infected with the lethal Pb NK65. TJ-48 may have no direct effect on the parasites. Furthermore immunity stimulated by TJ-48 might be too weak to affect the course of virulent malaria. TJ-48 has been found to be effective for treatment of tumor-bearing mice when used in combination with anti-cancer drugs (Haranaka *et al.* 1988: Kawamura *et al.* 1989). In cancer therapy, TJ-48 has no direct effect on the target cells but its action may be to regulate the homeostasis of the host's condition. The present findings suggest that some immunopotentiating drugs including TJ-48 can be combined with antimalarials for the treatment of drug-resistant *P. falciparum* malaria.

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References

1) Haranaka, R., Hasegawa, R., Nakagawa, S., Sakurai, A., Satomi, N. and Haranaka, K. (1988): Antitumor activity of combination therapy with traditional Chinese medicine and OK432 or MMC. J. Biol. Response. Modif., 7, 77–90.
2) Kawamura, H., Maruyama, H., Takemoto, N., Komatsu, Y., Aburada, M., Ikehara, S. and Hosoya, E. (1989): Accelerating effect of Japanese Kanpo medicine on recovery of murine haematopoietic stem cells after administration of mitomycin C. Int. J. Immunother., 5, 35–42.
3) Komatsu, Y., Takemoto, N., Maruyama, H., Aburada, M., Hosoya, E., Shinohara, S. and Hamada, H. (1986): Effect of Juzentaihoto on the anti-SRBC response in mice. Jpn. J. Inflammation, 6, 405–408.
4) Maruyama, H., Kawamura, H., Takemoto, N., Komatsu, Y., Aburada, M. and Hosoya, E. (1988): Effect of Kanpo medicines on phagocytosis. Jpn. J. Inflammation, 8, 65–66.
5) Ohnishi, Y., Yasumizu, R., Fan, H., Liu, J., Takao, F., Komatsu, Y., Hosoya, E., Good, R. A., Ikehara, S. (1990): Effects of Juzen-taiho-to (TJ-48), a traditional oriental medicine, on hematopoietic recovery from radiation injury in mice. Exp. Hematol., 18, 18–22.
6) Satomi, N., Sakurai, A., Haranaka, R. and Haranaka, K. (1989): Traditional Chinese medicines and drugs in relation to the host-defense mechanism. In The influence of antibiotics on the host-parasite relationship III, Gillissen, G., Opferkuch, W., Peters, G. and Pulverer, G., ed., Springer-Verlag, Berlin Heidelberg, p.154–163.
7) Slade, S. J. and Langhorne, J. (1989): Production of interferon-gamma during infection of mice with *Plasmodium chabaudi chabaudi*. Immunobiology, 179, 353–365.
8) Targett, G. A. T. (1984): Interactions between chemotherapy and immunity to malaria. In Handbook of Experimental Pharmacology Vol. 68: Antimalarial drugs I, Peters, W. and Richards, W. H. G. ed., Springer-Verlag, Berlin Heidelberg, p.331–348.
9) Waki, S., Kurihara, R., Nemoto, H. and Suzuki, M. (1993): Effect of recombinant human colony-stimulating factor on the course of parasitaemia in non-lethal rodent malaria. Parasitol. Res., 79, 703–705.
10) Waki, S. and Suzuki, M. (1974): Development and decline of antiplasmodial indirect fluorescent antibodies in mice infected with *Plasmodium berghei* (NK65) and treated with drugs. Bull. WHO., 50, 521–526.
11) Waki, S., Tamura, J., Imanaka, M., Ishikawa, S. and

Suzuki, M. (1982): *Plasmodium berghei*: Isolation and maintenance of irradiation attenuated strain in the nude mouse. Exp. Parasitol., 53, 335–340.

12) Waki, S., Uehara, S., Kanbe, K., Nariuchi, H. and Suzuki, M. (1995): Interferon-γ and the induction of protective IgG2a antibodies in non-lethal *Plasmodium berghei* infections of mice. Parasite. Immunol., 17, 503–508.

13) Waki, S., Uehara, S., Kanbe, K., Ono, K., Suzuki, M. and Nariuchi, H. (1992): The role of T cells in pathogenesis and protective immunity to murine malaria. Immunology, 75, 646–651.