J. Protozool. Res., 8, 19-27 (1998)

Expression of P30, The Major Surface Antigen of *Toxoplasma gondii* in Baculovirus-insect System and The Evaluation of Immune Response Induced by P30

XIAO-GUANG CHEN[1], XIN MA[1], KUN WU[1], HONG-JUAN PENG[1], SHU-MAN SHEN[1], GUO-ZHANG LIU[1] AND SIU-MING CHAN[2]

[1] *Department of Parasitology, The First Military Medical University, Guang Zhou 510515, China,*

[2] *Department of Zoology, The University of Hong Kong, Pokfulam Road, Hong Kong*

Received 14 May 1998 / Accepted 16 June 1998

Key words : Toxoplasma gondii, major surface antigen, baculovirus, expression system, immune.

ABSTRACT

The major surface antigen (P30) of the *Toxoplasma gondii* was expressed by an insect cell culture system infected with recombinant baculovirus. About 750μg of purified P30 (95% purity) was obtained from a culture of 10^8 insect Sf21 cells. The recombinant P30 was used to immunize mice to induce immune response. Mice injected with the recombinant protein produced specific humoral and cellular immune responses. Immunization with P30 also prolonged the period of survival of mice infected by *Toxoplasma* parasites.

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite. It is widely known to be infective in human, agricultural and domestic animals. It was estimated that as many as one third of the world population show serological evidence of infection (Hughes 1985). Because of the possible long incubation period inside the host, the symptom for toxoplasmosis is usually suppressed and undetected. It is one of the most common opportunistic infectious diseases found in patients who are immunosuppressed. About 6-10% of the AIDS patient developed toxoplasmosis. Of all the encephalitis occurred in HIV positive patients, about 50% is due to the infection caused by *T. gondii*. In chronic infection, infected patients shows symptoms of malaise, fever, sore throat and headache. In pregnant women and animals infected with *T. gondii*, it may cause stillbirth and abortion

(Qian 1993). The surviving infant may suffer from hydrocephalus, intracerebral calcifications, convulsions, blindness and mental retardation. Because of the high infection rate and the serious health threat caused by the *Toxoplasma*, there is an urgency for the production of a diagnostic kit and an effective vaccine for the prevention and curing of the disease. To produce an effective subunit vaccine for the *Toxoplasma*, several surface antigens of the *T. gondii* have been identified. One of the surface antigen, P30, has been isolated from the tachyzoite and identified to have potential use as subunit vaccine (Burl et al. 1988). This protein is highly antigenic and also present in most strains of *Toxoplasma*. It can stimulate both cellular and humoral immune response (Partanen et al. 1984; Khan et al. 1988), inducing the production of IgG, IgM, IgA and IFN-γ. Monoclonal antibody (mAb) against P30 has also been reported to be protective. We have previously expressed P30 in bacterial expression system (Chen et al. 1994), poor reactivity with anti-P30 mAb for this recombinant protein was obtained. Therefore, we attempted to express the protein in an insect baculovirus expression system. The following research reports the cloning of the Toxoplasma (ZS1 strain) P30 gene into a baculovirus expression system. The ability of the purified recombinant P30 to induce immune response was further tested by immunizing the mice with the immunostimulating complex containing the recombinant protein.

MATERIALS AND METHODS

Plasmid and bacterial strain

Plasmid containing the P30 gene (pBV220-P30) was constructed from a pBV220 plasmid vector (Chen et al. 1994). The host for the plasmid is a XL-1 blue bacterial cell (Strategene, USA).

Chemicals and Kits

Baculovirus expression system was from a BaculoGold Transfection kit (Pharmingen, USA). T4 DNA ligase was from GIBCO-BRL (USA) and restriction enzymes were from Sigma (USA). Other common biochemicals were analytical grades produced in China.

Construction of the transfer vector containing P30 gene

The P30 gene was recovered from the pBV220-P30 with EcoR I and BamH 1 digestion and inserted into a baculovirus vector pAcHCL-A previously cut with EcoR I and Bgl II. After confirmation of insertion, the DNA construct was used for the following transfection. The P30 contained transfer vector (pAcHCL-A-30) was co-transfected with baculovirus DNA to insect cell. Recombinant baculovirus was purified by plaque assay as described in the manual of the transfection kit

(Pharmingen, USA). PCR test was further used to confirm recombination (Chen et al. 1996).

Purification of P30 from insect cell

Insect Sf21 cells were infected with recombinant pAcHCL-A-30 and cultured for three days at 27°C. The cells were collected after centrifugation and suspended in a lysis buffer. After incubation in ice for 45 min, the supernatant was passed through a column containing Ni-NTA agarose. The fusion protein composed of poly-His tag and P30 was purified according to the instruction of the kit (Pharmingen, USA). The protein was then concentrated in a spin speed vac and portion of which was used in a SDS-PAGE analysis. A mAb DE53 (provided from Prof. Boothroyd, Stanford University, USA) was used in the Western blot analysis.

Immune response of mice to recombinant P30

Immunostimulating Complex (ISCOM) was prepared based on Lovgren's method (Lovgren et al. 1987). Briefly, Cholesterol (250 mg/ml), Phosphatidyl (250 mg/ml), Quil A (0.1%) and recombinant P30 were mixed in PBS and centrifuged in a 10-50% sucrose gradient at 200,000*g* for 18 hrs. The layer containing the ISCOM was collected and the concentration of protein was determined. Experimental mice were immunized with 0.1 μg, 1.0 μg and 10 μg of P30 in ISCOM by subepidermal injection. The control mice were injected with ISCOM containing only Quil A. For all groups, a booster injection of the same volume of ISCOM containing different amount of P30 or control was performed after 6 weeks.

Immune response of mice

One week after the booster injection, blood samples from experimental mice were taken for measurement of the specific antibodies against P30 by ELISA.

Challenge infection

Tachyzoites of the *Toxoplasma* virulent strain ZS1 were injected intraperitonealy at a concentration of 10^4 into experimental mice. The survival rate of the mice was recorded.

Statistical analysis

A non-parametric U-test was used to analyze the significant differences in experimental and control groups.

RESULTS

Figure 1 shows the construction of the pAcHCL-A-P30. Digestion of plasmid with both EcoR I and BamH I have confirmed that the correct size of the insert. After co-transfection and plaque assay purification, three different recombinant virus was selected. These clones were also shown to contain P30 inserts as detected by PCR.

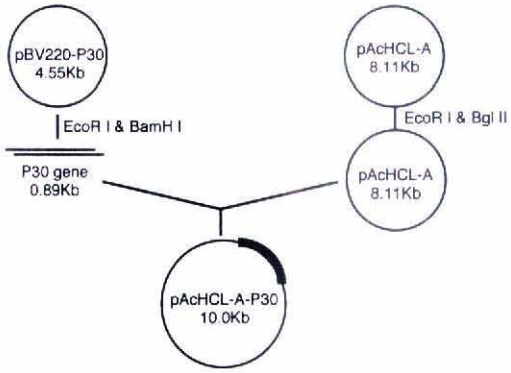

Figure 1 Construction of the recombinant transfer vector pAcHCL-A-P30

Figure 2 shows that P30 gene expressed in Sf21 cell effectively. After further purification of P30 with a Ni-NTA column, P30 of over 95% purity was obtained. The purity can be increase to 98% by passing the purified P30 through the column again. We also optimized the production of P30 using different MOI (3-10) to infect insect cell. It was found that a MOI of 5 produced the highest expression of P30 in 10^8 insect cells. Over 750 μg of highly purified P30 was obtained.

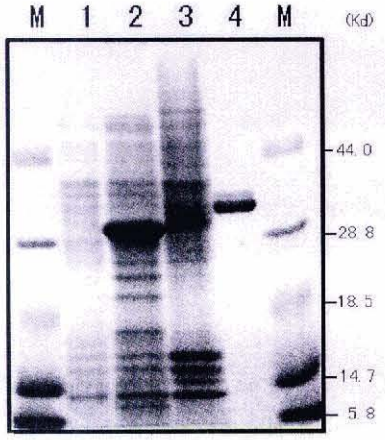

Figure 2 SDS-PAGE analysis on the expression products of P30 in Sf21 cell. Lanes 1: Normal Sf21 cell, 2: Sf21 cell infected by wild type virus AcNPV, 3: Sf21 cell infected by recombinant virus AcNPV-P30, 4: Purified recombinant P30, M: standard molecular weight marker.

The same samples were subjected to Western blot analysis with a mAb DE52. The results indicated that the antibody recognized the P30 protein in both the samples from the transfected insect cells and the purified recombinant fusion P30 (Fig. 3).

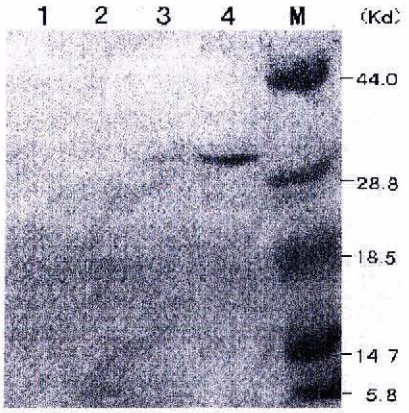

Figure 3 Western blot analysis on the expression products of P30. Lanes 1: Normal Sf21 cell, 2: Sf21 cell infected by wild type virus AcNPV, 3: Sf21 cell infected by recombinant virus AcNPV-P30, 4: Purified recombinant P30, M: standard molecular weight marker.

Immune responses in experimental mice were examined. Antibody titers for P30 of mice from group 1, 2, and 3 were ranged from 1 : 6,400 to 1 : 25,600. The control group showed negative reaction to the antigen. The titers in experimental groups showed dose-dependent response (Table 1). The lymphocyte stimulation test showed that the SI of the experimental groups were significantly higher than that of the control group ($p<0.01$). There also appeared a dose-effect response in different experimental groups (Fig. 4).

Table 1 Antibody response in experimental mice.

Immunized dose	Antibody titer*
0.1μg	6,400, 6,400, 6,400, 12,800, 6,400
1.0μg	12,800, 12,800, 6,400, 25,600, 25,600
10.0μg	25,600, 12,800, 25,600, 25,600, 25,600
10.0μg Quil A	-, -, -, died, -
Positive control**	3,200
Negative control***	-

*Highest dilution with absorbances higher than those of negative controls.
**Pooled sera from chronically infected mice.
***Pooled sera from normal mice.

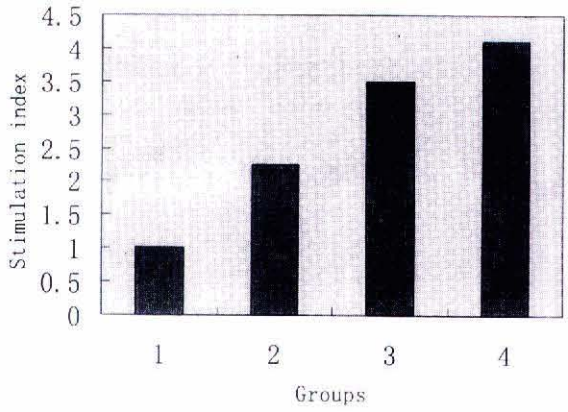

Figure 4 Result of in vitro lymphocyte stimulation test. Columns 1: 10.0 μg of Quil A group, 2: 0.1 μg of recombi-nant P30 group, 3: 1.0 μg of recombinant P30 group, 4: 10.0 μg of recombinant P30 group.

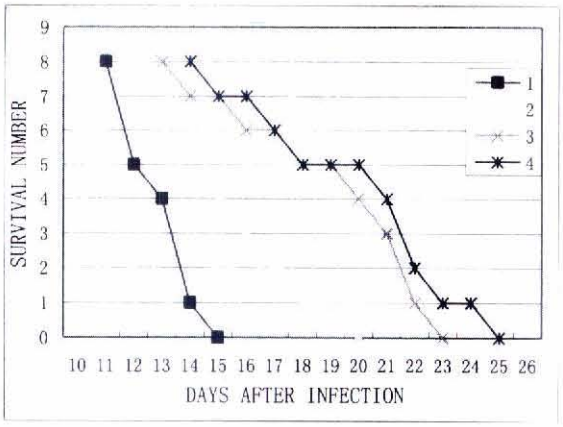

Figure 5 Result of challenge experiment. 1: 10.0 μg of Quil A group, 2: 0.1 μg of recombi-nant P30 group, 3: 1.0 μg of recombinant P30 group, 4: 10.0 μg of recombinant P30 group.

The ZS1 is a virulent *Toxoplasma* strain. Infection of 10^6 tachyzoites via abdominal injection is lethal, the infected mice will die within three days after injection. Using 10^4 tachyzoites to challenge mice, the mortality usually occurs at day 14. As shown in Fig. 5, when P30-immunized mice were challenged with 10^4 tachyzoites, average survival time for these mice was extended. For example, the mice injected with 10μg of P30, the average survival time was prolonged to 20.4 days. Moreover, the difference in survival time between the P30 immunized mice and the control mice is highly significant ($p<0.01$). Although the survival time of P30 injected mice were extended after they were challenged by *Toxoplasma* tachyzoites, the mice did not develop full immune response to toxoplasmosis as P30 immunization could not completely protect the mice against *Toxoplasma*

infection.

DISCUSSION

The major surface antigen (P30) of the *Toxoplasma* has been proved to have good antigenicity and have potential use as subunit vaccine against toxoplasmosis. Most previous research used immunoaffinity approach to purify P30 from the protozoa directly. The quality was not uniform and quantity obtained was limited. The recombinant DNA approach to produce large amount of P30 can provide sufficient material for the diagnosis and production of subunit vaccine for *Toxoplasma*. Although we have previous produced bacterial expressed recombinant P30, the purified P30 had poor reactivity. This could be due to the lack of sufficient protein processing mechanism such as post-translation processing in the bacterial expression system. Our study represents the first report on the expression of a *Toxoplasma* surface antigen in a baculovirus expression system. Our study shows that P30 can be expressed effectively in insect cell. The purified P30 (95%) can also be recognized by mAb against P30. This baculovirus expressed P30 is highly antigenic. This approach can provide sufficient material for the production of a vaccine and a diagnostic kit. The recombinant P30 produced in this study is a fusion protein. The N-terminal of the fusion protein is a small peptide consisting of 6 histidine residues. This peptide can be removed by enzymatic cleavage. Because it would not affect the antigenicity of the P30, it has not been removed in this study.

Previous studies show that there were contrasting results when P30 was used to immunize mice. These results might attribute to the toxicity of the Freund's adjuvant. Our studies using Liposome and Quil A as adjuvant showed improved result to boost immunity of experimental animals (Bulow and Boothroyd 1991; Mowat et al. 1991). ISCOM is a recently recognized adjuvant. Some reports described that IACOM could enhance the immunity of mice when injected with P30 (Lunden et al. 1993; Lunden 1995). In our study, we also showed that P30 in ISCOM also stimulated the immune response of mice to *Toxoplasma* infection. In the challenge study, although survival of the *Toxoplasma* infected mice was prolonged, complete mortality eventually occurred. This suggests that the injection of P30 cannot completely protect the mice from a lethal *Toxoplasma* infection. ZS1 strain is considered as one of the most virulent strains of all the *Toxoplasma* parasites. Direct infection by peritoneal injection is highly stressful to experimental animals. Since only one dose of tachyzoites was injected, further study is needed to optimize the dose and schedule of immunization. The natural infective route of *Toxoplasma* is mostly via mouth by the cyst or oocyst. Therefore, the best challenge way is oral infection with cyst or oocyst. Owing to

the difficulties in collecting and counting of cyst or oocyst, the present study uses tachzoites to get the preliminary data of challenge experiment.

ACKNOWLEDGEMENTS

We thank Dr. J. C. Boothroyd to supply the monoclonal antibody against P30 (DE52). This study was supported by the National Natural Science Foundation of China (No. 39400115) and the Natural Science Foundation of Guang Dong, China (No. 940292).

REFERENCES

Bulow, R. & Boothroyd, J. C. 1991. Protection of mice from fatal *Toxoplasma gondii* infected by immunization with P30 antigen in liposomes. *J. Immunol. 147:* 3496-3500.

Burl, J. L., Perelman, D., Kasper, L. H., Ware, P. L. & Boothroyd, J. C. 1988. Molecular analysis of the gene encoding the major surface antigen of *Toxoplasma gondii. J. Immunol. 141:* 3584.

Chen, X. G., Liu, G. Z., Xu, F., Wang, X. Z. & Jiang, J. B. 1994. Amplification, cloning, sequencing and expression of the gene encoding the major surface antigen of *Toxoplasma gondii* isolated in China. *J. Med. Colleges PLA 9:* 98-102.

Chen, X. G., Liu, G. Z., Tang, Y. M. & Chan, S. M. 1996. Construction of specific PCR kit for *Toxoplasma gondii* DNA and the preliminary assay to rat and human toxoplasmosis. *J. Chinese Parasitic Dis. Prev. 9:* 53-55.

Hughes, H. P. A. 1985. Toxoplasmosis: the need for improved diagnostic techniques and accurate risk assessment. *Curr. Top. Microbiol. Immunol. 120:* 105-111.

Khan, J. A., Eckel, M. E., Pfefferkorn, E. R. & Kasper, L. H. 1988. Production of gamma interferon by cultured human lymphocytes stimulated with a purified membrane (P30) from *Toxoplasma gondii. J. Inf. Dis. 157:* 979.

Lovgren, K., Uggla, A. & Morein, K. 1987. A new approach to the preparation of *Toxoplasma gondii* membrane antigen for use in ELISA. *J. Vet. Med. 34:* 274-282.

Lunden, A., Lovgren, K., Uggla, A. & Araujo, F. G. 1993. Immune responses and resistance to *Toxoplasma gondii* in mice immunized with antigens of the parasite incorporated into immunostimulating complexes. *Inf. Immun. 61:* 2639-2643.

Lunden, A. 1995. Immune responses in sheep after immunization with *Toxoplasma gondii* antigens incorporated into iscoms. *Vet. Parasitol. 56:* 23-

35.

Mowat, A., Donachie, A. M., Reid, G. & Jarrett, O. 1991. Immune-stimulating complexes containing Quil A and protein antigen prime class I MHC-restricted T lymphocytes in vivo and are immunogenic by the oral route. Immunology 72: 317-322.

Partnen, P., Turunen, H. J. & Paasivuo, R. A. 1984. Immunoblot analysis of *Toxoplasma gondii* antigens by human immunoglobulin G, M and A antibodies at different stage of infection. *J. Clin. Microbiol. 20:* 133.

Qian, L. J. 1993. The advance in diagnosis of toxoplasmosis. *Pediatrics foreign Med. 20:* 16-19.