

# BACULOVIRUS EXPRESSION OF THE MAJOR SURFACE ANTIGEN OF *TOXOPLASMA GONDII* AND THE IMMUNE RESPONSE OF MICE INJECTED WITH THE RECOMBINANT P30

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**Abstract.** The major surface antigen (P30) of the *Toxoplasma gondii* was expressed by an insect cell culture system infected with recombinant baculovirus. About 750 µg of purified (95% purity) P30 was obtained from a culture of 10<sup>8</sup> 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*. The average survival time of control group is 13.25±1.16 days, but are 16.13±2.1days, 19.50±3.21days, 20.38±3.38days in different immunized groups, respectively.

## 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 *et al*, 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 develop toxoplasmosis. Of all encephalitis occurring 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 (Oian *et al*, 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 *Toxoplasma*, there is an urgency for the production of a diagnostic kit and an effective vaccine for the prevention and curing of the disease. With the aim of producing an effective subunit vaccine for the *Toxoplasma*, several surface antigens of the *Toxoplasma gondii* have been identified. One of the surface antigens, P30, has been isolated from the tachyzoite and identified to have

potential use as subunit vaccine (Burg *et al*, 1988). This protein is also present in most *Toxoplasma*, and is highly antigenic. It can stimulate both cellular and humoral immune responses (Partanen *et al*, 1984; Khan *et al*, 1988), inducing the production of IgG, IgM, IgA and IFN-γ. Monoclonal antibody against P30 has also been reported to be protective. We have previously expressed P30 in a bacterial expression system (Chen *et al*, 1994); poor reactivity with anti-P30 McAb 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 an immune response was further tested by immunizing the mice with the immunostimulating complex containing the recombinant protein.

## MATERIALS AND METHODS

### Plasmid, bacterial and mice

A 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 (Stratagene, USA). The mice used in this experiment were BALB/c.

### Chemical reagents and kits

The baculovirus expression system was from a BaculoGold Transfection kit (Pharmingin, USA). T4 DNA ligase was from GIBCO-BRL (USA) and restriction enzymes were from Sigma (USA). Other

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common biochemical reagents 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 I digestion and inserted into a baculovirus vector pAchCL-A previously cut with EcoR I and Bgl II. After confirmation of insertion, the DNA construct (pAchCL-A-30) was used for the following transfection.

The P30 contained transfer vector (pAchCL-A-30) was co-transfected with baculovirus DNA to insect cells. Recombinant baculovirus was purified by plaque assay as described in the manual of the transfection kit (Pharming, USA). PCR test was further used to confirm the recombination (Chen *et al.*, 1996).

#### Purification of P30 from insect cells

Insect Sf21 cells were infected with recombinant pAchCL-A-P30 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 minutes, 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 (Pharming, USA). The protein was then concentrated in a spin speed vac and portion of which was used in a SDS-PAGE analysis. A monoclonal antibody (DE52, Dr 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 200,000g for 18 hours. 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 subcutaneous 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 amounts 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 mea-

surement of the specific antibodies against P30 by ELISA.

#### Challenge infection

Tachyzoites of the *Toxoplasma* virulent strain ZS1 were injected peritoneously 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 significance differences in experimental and control groups.

## RESULTS

Fig 1 – shows the construction of pAchCL-A-P30. Digestion of plasmid with both EcoR I and Bam H I have confirmed that the correct size of the insert.

After co-transfection and plaque assay purification, three different recombinant viruses were selected. These clones were also shown to contain P30 inserts as detected by nested PCR.

Fig 2 – shows that P30 gene was expressed in Sf21 cells effectively. After further purification of P30 with a Ni-NTA column, P30 of over 95% purity was obtained. The purity can be increased 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 cells. 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.

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

Antibody titers for P30 of mice from groups 1, 2, and 3 were ranged from 1: 6,400 to 1: 25,600. The control group was negative. The titers in experimental groups showed dose-dependent responses (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 to be a dose-effect response in different experimental groups (Fig 4).

The ZS1 is a virulent *Toxoplasma* strain. Infection of  $10^6$  tachyzoites via peritoneal injection is

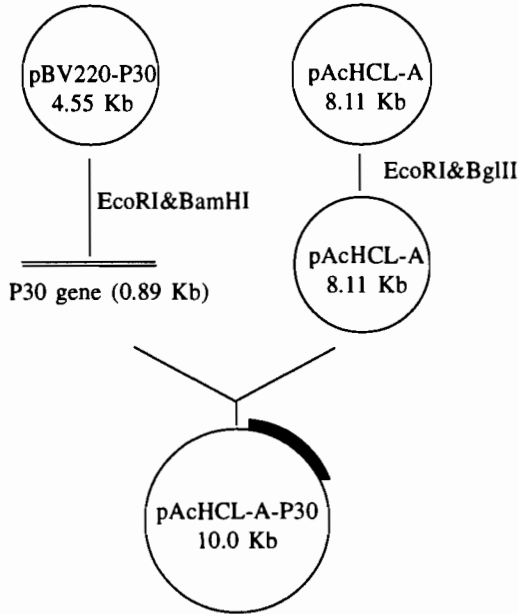


Fig 1—Construction of the recombinant transfer vector pAcHCL-A-P30.

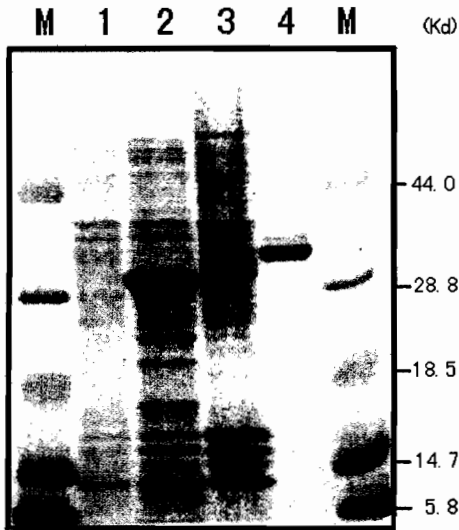


Fig 2—SDS-PAGE analysis on the expression products of P30 in Sf 21 cell.

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 is standard molecular weight marker.

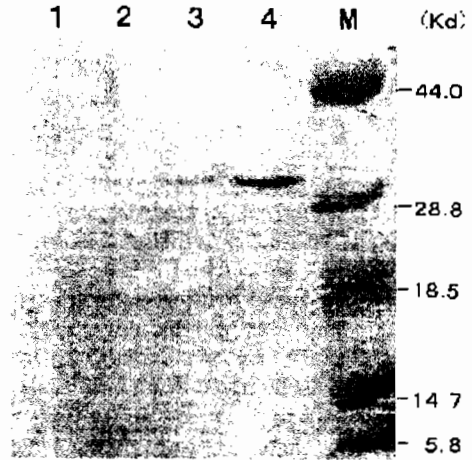


Fig 3—Western blot analysis on the expression products of P30. 1. Normal Sf 21 cell; 2. Sf 21 cell infected by wild type virus AcNPV; 3.Sf 21 cell infected by recombinant virus AcNPV-P30 ; 4.Purified recombinant P30; M is standard molecular weight.

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, the average survival time for these mice was extended. For example, in the mice injected with  $10 \mu\text{g}$  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 times of P30 injected mice were extended after they were challenged by *Toxoplasma* tachyzoites, the mice did not develop full immunity to toxoplasmosis as P30 immunization could not completely protect the mice from *Toxoplasma* infection.

## DISCUSSION

The major surface antigen (P30) of the *Toxoplasma* has been shown to have good antigenicity and have potential use as a subunit vaccine against toxoplasmosis. Most previous research used immunoaffinity approach to purify P30 from the protozoa directly. The quality was not uniform and the quantity obtained was limited. The recombinant DNA approach to produce large amounts of P30 can provide sufficient material for the diagnosis and pro-

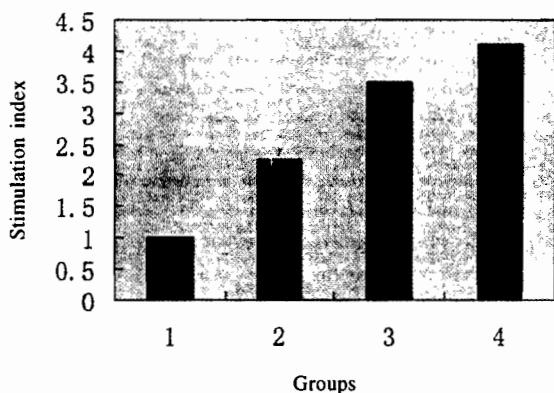


Fig 4—Result of *in vitro* lymphocyte stimulation test.  
 1. 10.0 µg Quil A group ; 2. 0.1 µg recombinant P30 group; 3. 1.0 µg recombinant P30 group; 4. 10.0 µg recombinant P30 group.

duction of a subunit vaccine for *Toxoplasma*. Although we have previously produced 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. The study shows that P30 can be expressed effectively in insect cells. The purified P30 (95%) can also be recognized by monoclonal antibody against P30. This baculovirus expressed P30 is highly antigenic. This approach can provide sufficient material for the production of a subunit vaccine and for 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

Table 1  
 Antibody response in mice

Dose	Number	Titer		
		1:6,400	1:12,800	1:25,600
0.1µg	5	4	1	0
1.0µg	5	1	1	3
10.0µg	5	0	1	4

\*Positive control is 1:3200.

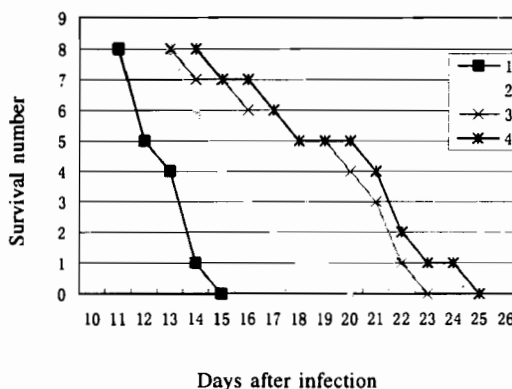


Fig 5—Results of challenge experiment.  
 (a) 10.0 µg Quil A group; (b) 0.1µg recombinant P30 group; (c) 1.0 µg recombinant P30 group; (d) 10.0 µg recombinant P30 group.

results when P30 was used to immunize mice. These results might be attributed to the toxicity of the Freund's adjuvant. Other studies using liposome and Quil A as adjuvants showed improved boosting of immunity in experimental animals (Bulow *et al*, 1991; Mowat *et al*, 1991). ISCOM is a recently recognized adjuvant. Some reports indicated that ISCOM could enhance the immunity of mice when injected with P30 (Lunden *et al*, 1993; 1995). In our study, we also showed that P30 in ISCOM 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*. Direct infection by peritoneal injection is highly stressful in experimental animals. Since only one dose of tachyzoite 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 use tachyzoites to get the preliminary data of challenge experiment.

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