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Short Communication

Preparation of rabbit anti-*Ganoderma sinensis* immunomodulatory protein polyclonal antibody

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***Ganoderma sinensis* immunomodulatory protein (FIP-gsi) was a new protein in fungal immunomodulatory protein (FIP) family. Based on the recombinant FIP-gsi expressed in *Escherichia coli*, the New Zealand white rabbits were immunized with the purity protein to prepare anti-FIP-gsi polyclonal antibody. The efficacy of polyclonal antibody was detected by ELISA and Western blot. The results showed that the anti-FIP-gsi polyclonal antibody with high efficient value and specificity has been successfully preparation, and its efficient value was 1:625,000 detected by indirect ELISA, and a special band had been observed by Western blot method. This study established a method to identify FIP-gsi by immunoblotting, and will lay a foundation for further exploring the immunologic function of FIP-gsi.**

Key words: *Ganoderma sinensis* fugal immunomodulatory proteins (FIP-gsi), polyclonal antibody, ELISA, Western blot.

INTRODUCTION

The first fungal immunomodulatory protein (FIP), LZ -8, was isolated from *Ganoderma lucidum* in 1989 (Kino et al., 1989). Up to now, seven FIPs, LZ-8 (FIP-glu), FIP-gts (Lin et al., 1997), FIP- fve (Ko et al., 1995), FIP-vvo (Hsu et al., 1997), FIP- gja (AY987805), FIP-gmi (Wu et al., 2007) and FIP-gsi (Zhou et al., 2009), were found in *G. lucidum*, *Ganoderma tsugae*, *Flammulina velutipes*, *Volvariella volvacea*, *Ganoderma japoncium*, *Ganoderma microsporum*, and *Ganoderma sinensis*, respectively. FIPs showed to have the same structure features, which had great similarity with heavy chain variable region of immunoglobulin (Tanaka et al., 1989). The first -helix composed by 10 amino acids in the N-terminal was considered to be responsible for the immunomodulatory activity, and Leu-5, Phe- 7, and Leu-9 were regarded as the key amino acids (Lin et al., 1997). FIPs were capable of hemagglutinating mouse, sheep, or human red blood cells. On the function of immunomodulatory activity, on the one hand, FIPs could promote proliferation of mouse spleen cells and human peripheral blood lymphocytes; on the other hand, FIPs could stimulate the expression of cytokines in mouse spleen cells and human peripheral lymphocytes (Kino et al., 1989; Lin et al., 1997; Ko et al., 1995; Hsu et al., 1997). By now, there are not any commercial polyclonal antibodies. Only some publics were reported about polyclonal antibodies against LZ-8 (FIP-glu) (Ding et al., 2006; Bai et al., 2006). The anti-FIP-gsi polyclonal antibody was not reported. In this paper, in order to prepare rabbit anti-FIP-gsi polyclonal antibody, the New Zealand white rabbits were immunized with the purity protein. Further, this research will be expected to provide an immunological method to

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Abbreviation: FIP, Fungal immunomodulatory protein; **TMB,** tetramethyl benzidine; **PBST,** Phosphate buffered saline with tween 20; **HRP,** horseradish peroxidase; **IgG,** immunoglobulin; **SDS-PAGE,** sodiumdodecylsulfate-polyacrylamide gel electrophoresis; **PVDF,** polyvinylidene fluoride; **ELISA,** enzyme-linked immunosorbent assay; **OD,** optical density.

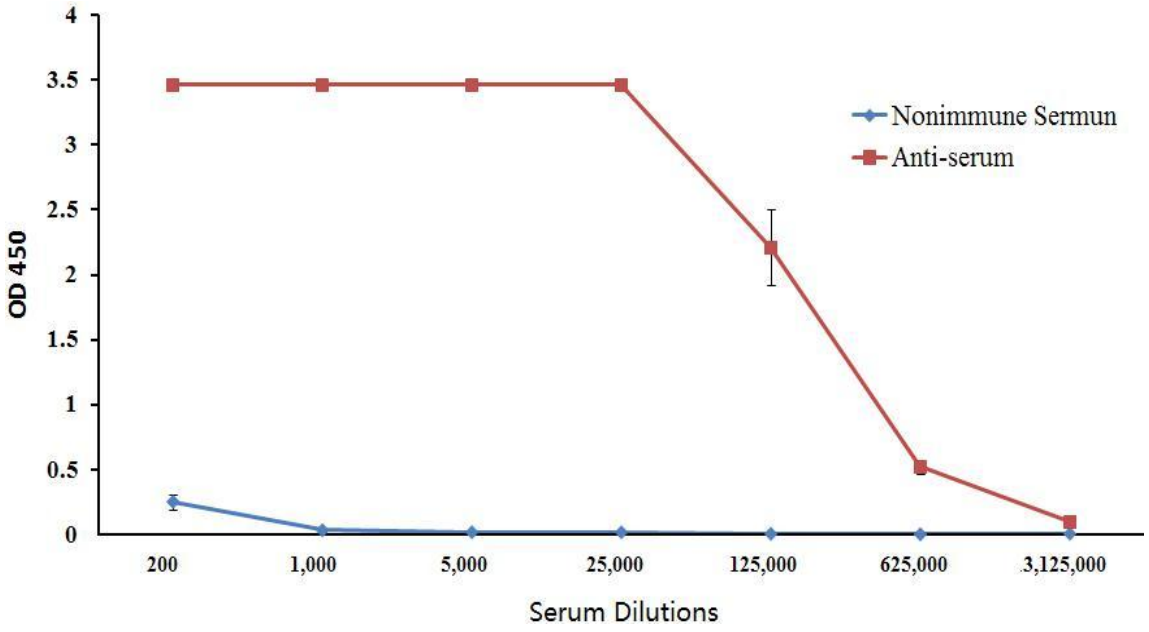

Figure 1. Curve of FIP-gsi polyclonal antibody.

determine FIP-gsi, meanwhile it will also lay a foundation for further study about immunological function of FIP-gsi.

EXPERIMENTAL PROCEDURES

The New Zealand white rabbits were immunized with FIP-gsi purified by our laboratory (Li et al., 2010a). FIP-gsi (600 g/rabbit) dissolved in complete Freund's adjuvant with 1:1 ratio was injected into the rabbits (Day 0). After 21 and 35 days, the rabbits were injected with the protein (400 g/rabbit) dissolved in incomplete Freund's adjuvant with 1:1 ratio. The serum was isolated. The titer of the anti-serum was detected by indirect ELISA. After incubated in 0.2 g of FIP-gsi in 100 l in 96-well ELISA plate at 4°C, the wells were washed with PBST three times, blocked with 5% skim milk at 37°C for 2 h, and washed with PBST three times again. The 100 l anti-serum at different dilutions (from 1:200 to 1:3,125,000) was incubated with wells at 37°C for 1 h. After washing the wells with PBST three times, 100 l horseradish peroxidase (HRP)-conjugated goat anti- rabbit immunoglobulin (Ig G) at 1:10,000 dilution was incubated with the wells at 37°C for 40 min. In order to develop color reaction, 100 l/well tetramethyl benzidine (TMB) solutions was added after washing with PBST three times. The color reaction was finished in 10 min, and was stopped by 50 l/well 2 M H_2SO_4. The absorbance was measured at 450 nm. Ratio of antiserum versus control serum absorbency greater than 2.1 was recognized as positive. The specificity of polyclonal antibody was examined by Western blot. FIP-gsi expressed in *Escherichia coli* was isolated by 15% SDS- PAGE (Li QZ et al., 2010), and the gel was electroblotted onto 0.2 µm microporous polyvinylidene fluoride (PVDF) membrane. After been blocked with 5% skim milk dissolved in TBST for 30 min at room temperature, the membrane was incubated with anti-serum at different dilutions (1:500, 1:2,000, and 1:5,000) at 4°C overnight, and washed with TBST. HRP-conjugated goat anti-rabbit Ig G at 1:10,000 dilution was incubated with the membrane for 40 min. After final washed with TBST, it was reacted by 1 ml ECL for 3 min, exposed for 5 s, developed for 1 min, and fixed.

RESULTS AND DISCUSSION

The anti- serum at different dilutions (200 to 3,125,000-fold) was reacted with 2 g/ml FIP-gsi and examined at OD 450. The result showed that the anti-serum titer was found to be approximately 1:625,000 (Figure 1).

In order to detect the specificity of polyclonal antibody, the analysis was worked on by Western blot. The result showed that a special band was determined at about 14 kD. With the increase in dilution, the specificity was becoming higher. When the polyclonal antibody was diluted 5,000-fold, the specificity was higher (Figure 2).

The family of Ganodermataceae consists of a large group of tree fungi of the class Polyporaceae, specifically the genus *Ganoderma* and other related genera. Among of them, both *G. sinensis* and *G. lucidum* had been written in Pharmacopoeia of China as material raw herbal materials in 2005 (Zhou et al., 2007) . FIP-gsi is one member of FIP family, which has 86.6% homologous rate with FIP-glu (LZ-8) (Zhou et al., 2009). The structures and functions of FIPs have been clearly studied, but little is known about other information, such as the relationship between the gene, structure and function, and other studies of molecular biology. Based on our former research, FIP-gsi had the similar immunological function, and could stimulate the transcriptional expression of cytokines in mouse spleen cells (Li et al., 2010a; Li et al., 2010b). Although FIPs have obvious pharmacologically activities, the natural FIPs yield is low and each kilogram of fungi contain dozens of milligrams of FIP (Kino et al., 1989; Ko et al., 1995; Hsu et al., 1997). Therefore, in order to obtain more sufficient recombinant FIPs which could be used in industrial production, many scientists try

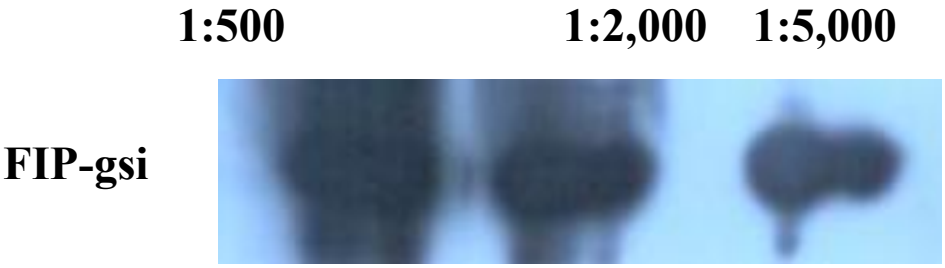

Figure 2. Western blot analysis of polyclonal antibody against FIP-gsi.

to improve the yield of FIPs by genetic engineering methods. Meantime, the detection of FIPs is of great importance. Therefore, establishing a method to detect FIP-gsi and FIPs is necessary. However, there are not any commercial FIPs polyclonal antibodies. So preparing high efficient value and specificity antibody is fully significant. In this study, the obtained rabbit anti-FIP-gsi polyclonal antibody titer was 1:625,000, it was 62.5-fold higher than that of rabbit anti-LZ -8 polyclonal antibody reported by Bai et al. (2006). What is more, the polyclonal antibody would have to dilute 5,000-fold in analysis of Western blot, but the rabbit anti-LZ-8 polyclonal antibody reported by Ding et al. (2006) would only dilute 300-fold. This research will be expected to provide a basis to prepare higher efficient value and specificity of polyclonal antibody.

The immunomodulating activity of FIP played important roles in the prevention and treatment of some diseases without any side effect, so it has good application prospect. With the development of biology and biotechnology, producing medicinal protein by engineering strain will be possible. Thus, it appears to be important to detect recombinant protein, especially in immunology.

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