

Comparison of different monoclonal antibodies against immunosuppressive proteins of *Ascaris suum*

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Abstract

The extract of *Ascaris suum* suppresses the humoral and cellular immune responses to unrelated antigens in the mouse. In order to further characterize the suppressive components of *A. suum*, we produced specific monoclonal antibodies which can provide an important tool for the identification of these proteins. The *A. suum* immunosuppressive fractions isolated by gel filtration from an extract of adult worms were used to immunize BALB/c mice. Popliteal lymph node cells taken from the immunized animals were fused with SP2/O myeloma cells and the cloned hybrid cells obtained were screened to determine the specificity of secreted antibodies. Three monoclonal antibodies named MAIP-1, MAIP-2 and MAIP-3 were selected and were shown to react with different epitopes of high molecular weight proteins from the *A. suum* extract. All antibody molecules have κ -type light chains but differ in heavy chain isotype. MAIP-1 is a mouse IgM, MAIP-2 is an IgA immunoglobulin and MAIP-3 is an IgG1 immunoglobulin and they recognize the antigen with affinity constants of $1.3 \times 10^{10} M^{-1}$, $7.1 \times 10^9 M^{-1}$ and $3.8 \times 10^7 M^{-1}$, respectively. The proteins recognized by these monoclonal antibodies (PAS-1, PAS-2 and PAS-3) were purified from the crude extract by affinity chromatography and injected with ovalbumin in BALB/c mice in order to determine their suppressive activity on heterologous antibody production. It was demonstrated that these three proteins are able to significantly suppress anti-ovalbumin antibody secretion, with PAS-1 being more efficient than the others.

Key words

- *Ascaris suum*
- Immunosuppression
- Immunomodulation
- Monoclonal antibody

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It has been widely reported that nematodes or nematode products can stimulate or inhibit the generation of lymphocyte responses, suggesting that nematodes can affect the host immune response in a number of ways. For example, the nematode *Heligmosomoides polygyrus* contains immunomodulatory factors capable of either stimulating (1,2) or inhibiting (3,4) T and B cell re-

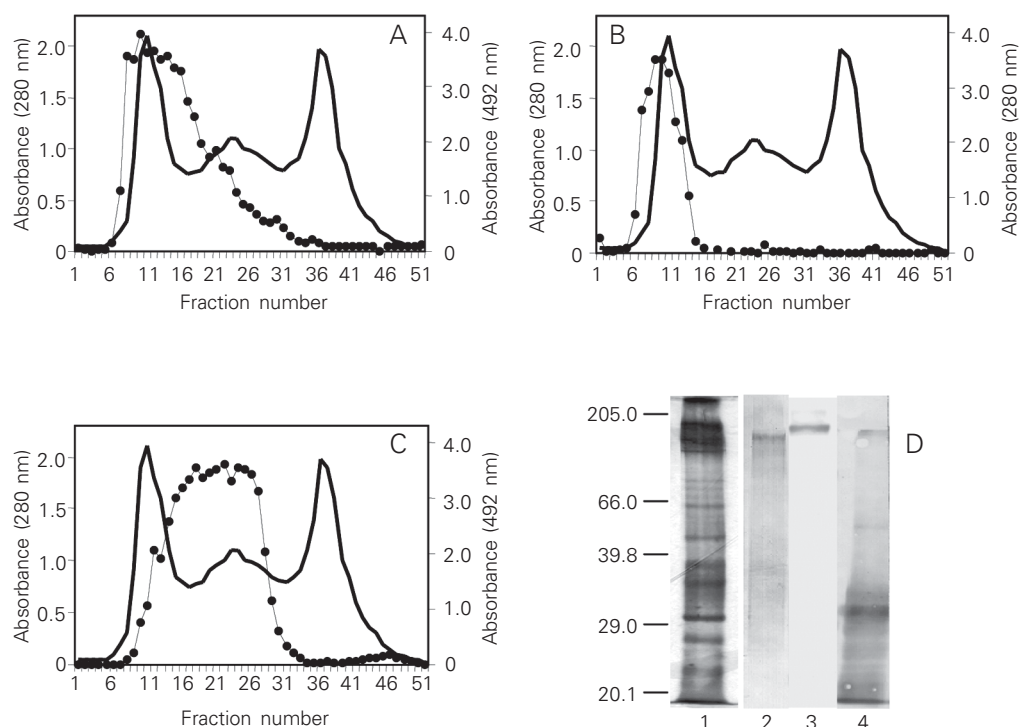
sponses. With respect to *Ascaris suum*, we and others have shown that infection with the nematode or the use of its soluble products or extracts at different stages of purification potentiates or suppresses the immune responses (5-7). Some of the discrepancies and apparent inconsistencies of reports regarding the modulation of immune function induced by *A. suum* extracts are undoubtedly

due to variations in the methods of purification. We have also demonstrated that the immunosuppressive and stimulatory effects are due to distinct components of *A. suum* adult worms (8). While the high molecular mass components strongly suppress the humoral and cellular immune responses (9), the 29-kDa components induce IgE antibody production (10). In the present study we produced specific monoclonal antibodies against *A. suum* immunosuppressive components, which can be important tools for the identification and further characterization of these proteins.

Male BALB/c mice weighing 18-22 g (provided by the animal house of Butantan Institute) were injected in the footpad with an emulsion of complete Freund's adjuvant containing 40 μ g *A. suum* high molecular weight components isolated as previously described (11). Two weeks later, the animals received 40 μ g of the extract in incomplete Freund's adjuvant by the same route and the popliteal lymph nodes were excised 3 days later for fusion with a non-secreting SP2/O

cell line using 50% polyethylene glycol (MW 1450). The hybridomas were cultured in a medium containing hypoxanthine (5×10^{-3} M), aminopterin (2×10^{-5} M) and thymidine (8×10^{-4} M) and cloned under limiting dilution conditions. Supernatants from growing hybrids were screened by ELISA using plates coated with *A. suum* worm extract (12) and developed with 0.1 mg of peroxidase-labeled rat monoclonal antibodies against different mouse Ig isotypes (kindly provided by Dr. H. Bazin, Université Catholique de Louvain, Brussels, Belgium). Three hybridomas producing *A. suum* extract-specific monoclonal antibodies were selected and named MAIP-1, MAIP-2 and MAIP-3 (mouse anti-*A. suum* immunosuppressive protein). The isotypes of these antibodies, determined by ELISA, were respectively IgM, IgA and IgG1, all having κ light chains. In order to analyze the specificity of the monoclonal antibodies, *A. suum* extract was submitted to gel filtration chromatography and the binding of the antibodies to each fraction eluted was evaluated by ELISA. Gel filtra-

Figure 1. Specificity of MAIP-1, MAIP-2 and MAIP-3. The specificity of each monoclonal antibody was determined by ELISA using plates coated with each fraction of *Ascaris suum* extract isolated by gel filtration (Sephacryl S300). The chromatography effluent (solid line) was monitored by absorbance at 280 nm and the binding of MAIP-1 (A), MAIP-2 (B) and MAIP-3 (C) was visualized using a peroxidase-labeled rat anti-mouse κ chain (closed circles). The SDS-PAGE pattern of *Ascaris suum* whole extract is represented in D, lane 1. Immunoblotting of *Ascaris suum* components using MAIP-1 (lane 2), MAIP-2 (lane 3) or MAIP-3 (lane 4) was performed with a peroxidase-labeled anti- κ light chain. The molecular mass markers listed on the left are in kDa.



tion was performed as previously described (8). Briefly, 20 mg *A. suum* extract was applied to a Sephacryl S300 column (2 x 110 cm) equilibrated with PBS, pH 7.2, and 1.5-ml fractions were collected at 20 ml/h. Samples of each fraction were used to coat plate wells for ELISA. The plates were incubated with MAIP-1, MAIP-2 or MAIP-3 (1.0 µg/well) and developed using a peroxidase-labeled rat anti-mouse κ chain monoclonal antibody. The results illustrated in Figure 1 show that *A. suum* adult worm extract is a complex mixture of proteins and that the monoclonal antibodies recognized different fractions of the crude extract.

The specificity of the monoclonal antibodies was also analyzed by SDS-PAGE followed by Western blot. The electrophoretic profile of *A. suum* extract (Figure 1) showed that the crude extract is composed of a mixture of proteins represented by the different bands on the gel. However, MAIP-1 and MAIP-2 recognized only the high molecular mass protein (~200 kDa) while the epitope recognized by MAIP-3 is present in different components of *A. suum*.

A large amount of MAIP-1 (IgM), MAIP-2 (IgA) and MAIP-3 (IgG1) was then purified from defatted ascitic fluid of Swiss mice, injected with the respective hybridoma and incomplete Freund's adjuvant, by affinity chromatography using a Sepharose-4B column coupled with anti-mouse IgM monoclonal antibody, protein A or *A. suum* extract, respectively. Purified MAIP-1, MAIP-2 and MAIP-3 were conjugated with activated Sepharose-4B and *A. suum* extract was applied to the column. The proteins PAS-1, PAS-2 or PAS-3 were then eluted using citrate buffer, pH 2.8. The purified proteins were used to coat plates for ELISA, and the respective monoclonal antibody was added in order to determine the binding affinity by the method of Van Heyningen et al. (13). The affinity constants obtained were $1.3 \times 10^{10} \text{ M}^{-1}$ for MAIP-1, $7.1 \times 10^9 \text{ M}^{-1}$ for MAIP-2, and $3.8 \times 10^7 \text{ M}^{-1}$ for MAIP-3.

To determine the immunomodulatory activity of PAS-1, PAS-2 and PAS-3, BALB/c mice were injected *ip* with 50 µg ovalbumin (OVA) alone or mixed with 500 µg of each protein or bovine serum albumin, in 7.5 mg aluminum hydroxide gel as adjuvant. The animals were bled on day 21 and serum antibody levels were evaluated by ELISA. Briefly, plates were coated with OVA (0.5 µg/well), incubated for 1 h at 37°C, washed five times with PBS, pH 7.2, at each step of the procedure, and then saturated with 5% defatted milk in PBS. Different dilutions of mouse serum were added, followed by peroxidase-labeled rat anti-mouse κ chain monoclonal antibody and 100 µl/well of *o*-phenylenediamine diluted in sodium citrate buffer, pH 5.0. The reaction was visualized by the addition of 0.012% H_2O_2 . Plates were read with a Titertek Multiskan apparatus at 492 nm.

The anti-OVA antibody production, represented in Figure 2, shows a suppression of

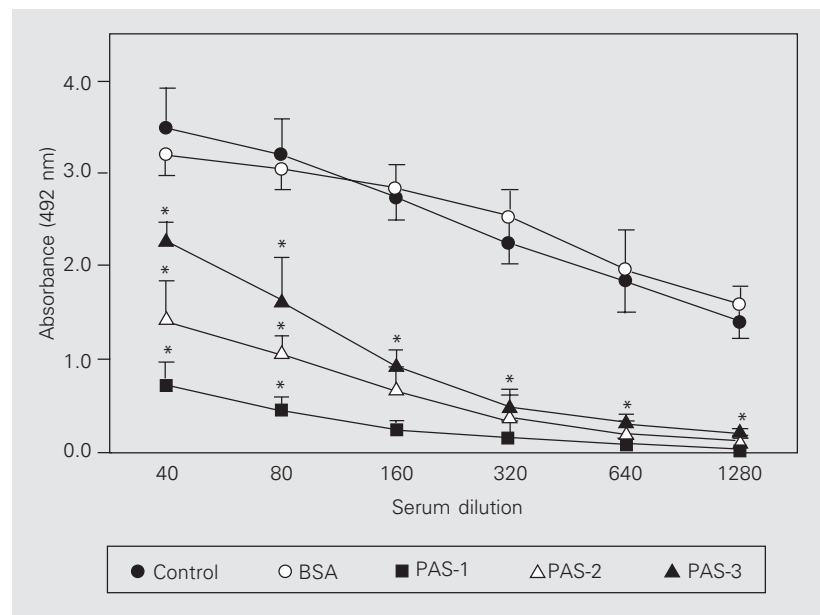


Figure 2. Effect of PAS-1, PAS-2 and PAS-3 on the humoral immune response. Groups of 5 BALB/c mice were immunized with 50 µg ovalbumin (OVA) + 500 µg PAS-1 (filled squares), PAS-2 (open triangles), PAS-3 (filled triangles) or bovine serum albumin (BSA; open circles), mixed with 7.5 mg $\text{Al}(\text{OH})_3$ as adjuvant. In a control group the animals were injected only with 50 µg OVA (filled circles) in the adjuvant. Three weeks later, all mice were bled and anti-OVA antibody levels were determined by ELISA using a peroxidase-labeled rat anti-mouse κ chain monoclonal antibody. * $P < 0.05$ compared to control (Student *t*-test).

the anti-OVA-specific immune response induced by PAS-1, PAS-2 and PAS-3 but not by another unrelated protein such as bovine serum albumin. PAS-1, however, was the most efficient among the suppressive components in reducing the anti-OVA antibody response, especially when compared with PAS-3, probably because the latter is a complex mixture of proteins and some of them might not have the suppressive effect.

The study of the immunomodulation induced by these proteins indicated that PAS-1, PAS-2 and PAS-3 inhibit the heterologous antibody response by a mechanism that is not antigenic competition and/or saturation of antigen-presenting cell receptors, since only the nematode proteins, but not bovine serum albumin at the same concentration, reduced the OVA-specific response.

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