Brazilian Journal of Medical and Biological Research (2001) 34: 1033-1036
ISSN 0100-879X Short Communication

Production and characterization of a monoclonal antibody against an *Ascaris suum* allergenic component

R.R. Pires, T.M. Oshiro, D.M. Itami, I. Fernandes and M.F. Macedo-Soares

Laboratório de Immunopatologia, Instituto Butantan, São Paulo, SP, Brasil

Abstract

Ascaris suum allergenic components (PIII) separated by gel filtration chromatography of an adult worm extract were used to immunize BALB/c mice. Popliteal lymph node cells taken from the immunized animals were fused with SP2/O myeloma cells using polyethylene glycol (MW 1450) as fusogen. The hybridomas were cultured in HAT-containing medium and cloned at limiting dilutions. Supernatants from the growing hybrids were screened by ELISA using plates coated with PIII or the *A. suum* crude extract. The monoclonal antibody obtained, named MAC-3 (mouse anti-*A. suum* allergenic component), is an IgG1 kappa mouse immunoglobulin that specifically recognizes a 29,000 molecular weight protein (called allergenic protein) with an affinity constant of 1.7×10^9 M^{-1}. The *A. suum* components recognized by MAC-3 induce specific IgE antibody production in immunized BALB/c mice. Ascitic fluid induced in Swiss mice by injecting *ip* the hybridoma cells and incomplete Freund's adjuvant was purified by affinity chromatography using a protein A-Sepharose column. The purified monoclonal antibody was then coupled to activated Sepharose beads in order to isolate the *A. suum* allergenic component from the whole extract by affinity chromatography.

Correspondence
M.F. Macedo-Soares
Laboratório de Imunopatologia
Instituto Butantan
Av. Vital Brasil, 1500
05503-900 São Paulo, SP
Brasil
E-mail: fmacedo@usp.br

Presented at the XVI Annual Meeting of the Federação de Sociedades de Biologia Experimental, Caxambu, MG, Brazil, August 22-25, 2001.

R.R. Pires, T.M. Oshiro and D.M. Itami are recipients of FAPESP fellowships.

Received April 12, 2001
Accepted June 12, 2001

Key words
- *Ascaris suum*
- Allergen
- IgE
- Monoclonal antibodies

Helminth infections are associated with production of substantial amounts of both parasite-specific and nonspecific IgE, occurrence of immediate hypersensitivity reactions and proliferation of hypersensitivity-associated cells, such as mast cells and eosinophils. The ascaroid nematodes are particularly active in inducing atopic reactions (1,2). Regarding *Ascaris suum*, extracts in various stages of purification have been widely used for allergen characterization in different animal species (3-5). Several allergens have been isolated from worm extracts, body fluid or culture supernatant. A potent allergen was identified in the body fluid of adult *A. suum* and characterized as a 14,000 molecular weight protein that induces an IgE antibody response in a rat model (6). It was also demonstrated that *A. suum* potentiates the IgE response in different species, although attempts made using the mouse model were unsuccessful (7). Working with *A. suum*, we have previously shown that whole extract obtained from adult worms suppresses the production of antibodies to an unrelated antigen in the mouse system (8). Moreover, we

have demonstrated that the suppressive components present in the *A. suum* extract could be isolated from a 29,000 molecular weight component which induces high levels of IgE antibody (9). In order to further characterize this allergenic component, the aim of the present study was to produce a specific monoclonal antibody (mAb) which provides an important tool for a better identification of the allergen.

BALB/c mice weighing 18-22 g (provided by the animal house of Butantan Institute) were injected into the footpad with an emulsion of complete Freund's adjuvant containing 40 µg of *A. suum* allergenic component purified as previously described (8). Two weeks later, the animals received 40 µg of the antigen in incomplete Freund's adjuvant by the same route and the popliteal lymph nodes were excised 3 days later for fusion with a nonsecreting SP2/O cell line using 50% polyethylene glycol (MW 1450). The hybridomas were cultured in HAT (hypoxanthine-aminopterin-thymidine)-containing medium and cloned under limiting dilution conditions. Supernatants from growing hybrids were screened by ELISA using plates coated with *A. suum* allergenic components purified by gel filtration (PIII) and visualized with 0.1 µg of peroxidase-labeled rat mAb against different mouse Ig isotypes (kindly provided by Dr. H. Bazin, Experimental Immunology Unit, University of Louvain, Brussels, Belgium).

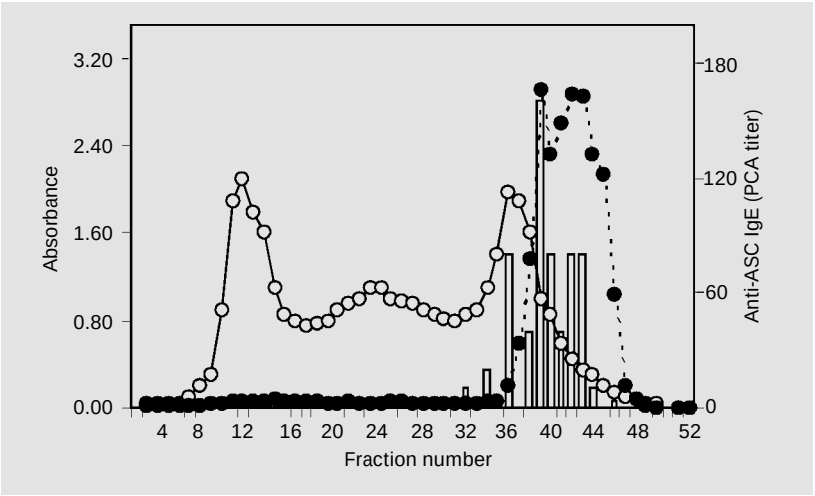

Figure 1. Specificity of mouse anti-*Ascaris suum* component (MAC-3) monoclonal antibodies. The open circles represent the gel filtration profile of *A. suum* crude (ASC) extract (peak I, fractions 11 to 16; peak II, fractions 26 to 31; peak III, fractions 37 to 50). Thirty milligrams of ASC was applied to a Sephacryl S-300 column and fractions of 2.0 ml were collected with PBS, pH 7.2. Absorbance was determined at 280 nm. Each fraction was injected with alumn adjuvant into BALB/c mice in order to evaluate the IgE antibody production. The bars represent the passive cutaneous anaphylaxis (PCA) titer of mouse sera three weeks after immunization. The specificity of MAC-3 was determined by ELISA using polystyrene plates absorbed with each fraction and incubated with MAC-3 plus a peroxidase-labeled rat monoclonal antibody against mouse IgG1. The filled circles represent the absorbance obtained at 492 nm.

The cloned hybrid cells produced an IgG1 kappa mAb named MAC-3 - mouse anti-*A. suum* allergenic component. The specificity of MAC-3 mAb was determined by ELISA using plates coated with each fraction eluted from a Sephacryl S-300 column to which 30 mg of *A. suum* extract was applied. At each step, the plates were incubated for 1 h at 37°C and washed five times with PBS, pH 7.2. They were then saturated with 5% defatted milk in PBS and 1 µg/well of MAC-3 was added followed by peroxidase-labeled rat anti-mouse IgG1 mAb and 100 µl/well of *o*-phenylenediamine diluted in sodium citrate buffer, pH 5.0, at a concentration of 0.4 mg/ml. The mAb was visualized by the addition of 0.012% H_2O_2. Plates were read in a Titerteck Multiskan apparatus at 492 nm. The MAC-3 mAb recognized only fractions eluted in the third peak (fractions 37 to 50), represented by the low molecular weight proteins of the *A. suum* extract (Figure 1).

The allergenic properties of *A. suum* components were determined by their ability to induce IgE measured by passive cutaneous anaphylaxis (PCA) in rats according to the method of Mota and Wong (10). Several dilutions of the serum from mice immunized with $Al(OH)_3$ (7.5 mg) plus 250 µl of each fraction of *A. suum* crude extract eluted on a Sephacryl S-300 column were injected intradermally into the shaved back of three rats. The animals were challenged intravenously with 0.5 mg of *A. suum* crude extract in 0.25% Evans blue solution after a sensitization period of 18-24 h. The PCA titer was

expressed as the reciprocal of the highest dilution that produced a lesion of more than 5 mm in diameter. The results shown in Figure 1 demonstrate that the same fractions recognized by MAC-3 induced specific IgE antibody production.

A large amount of MAC-3 was purified from ascitic fluid of Swiss mice injected with the secreting hybridoma and incomplete Freund's adjuvant by affinity chromatography using a protein A-Sepharose column. Purified MAC-3 was conjugated with activated Sepharose and the adult worm crude extract was applied to the column. The allergenic protein was then eluted using citrate buffer, pH 2.8. Allergenic protein purity was evaluated by SDS-PAGE and by immunoblotting. The protein banding pattern of the whole extract was quite complex, as revealed by SDS-PAGE performed on a 7.5% acrylamide gel at 20-mA constant current and visualized with silver staining (Figure 2). On the other hand, the material eluted from the MAC-3-Sepharose column (allergenic protein) showed a single band corresponding to a 29,000 molecular weight protein. The different components of the whole extract separated by SDS-PAGE were transferred to a nitrocellulose membrane that was then treated with MAC-3, followed by the addition of anti-mouse IgG1 mAb labeled with peroxidase plus H_2O_2 and 4-chloro-1-naphthol. As shown in Figure 2, the mAb MAC-3 recognized only the 29,000 molecular weight protein present in the whole extract.

MAC-3 mAb binds to allergenic protein with an affinity constant of 1.7×10^9 M^{-1}, as determined by ELISA according to Van Heymingen et al. (11).

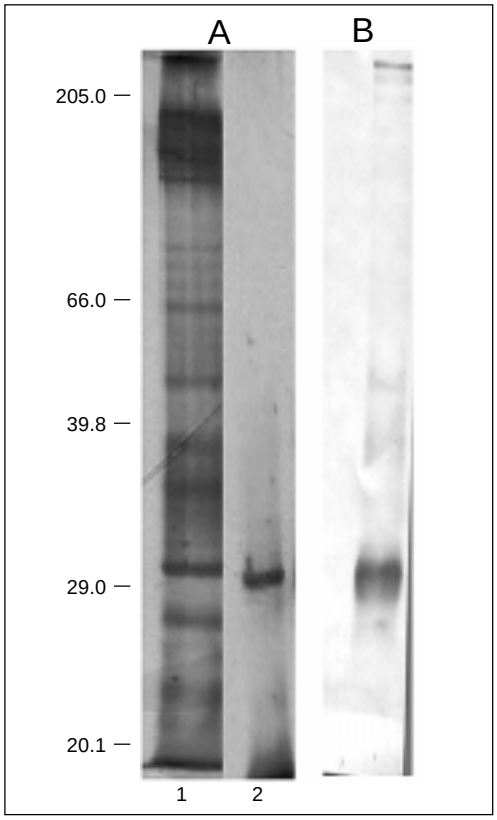

Figure 2. SDS-PAGE patterns (A) of whole extract (lane 1) or the allergenic protein from *Ascaris suum* recognized by MAC-3 monoclonal antibody (lane 2). The molecular weight markers are represented at the left. *B*, Immunoblotting of *A. suum* components using MAC-3 plus a peroxidase-labeled rat monoclonal antibody against mouse IgG1.

We produced a mouse mAb against the *A. suum* allergen that is an IgG1 kappa immunoglobulin with an affinity constant of 1.7×10^9 M^{-1} and which recognizes specifically a 29,000 molecular weight protein from the adult worm extract. This mAb will be used for the identification of allergenic protein in different stages of the *A. suum* life cycle as compared to immunosuppressive components.

References

1. Jarret EE & Miller HRP (1982). Production and activities of IgE in helminth infection. *Progress in Allergy*, 31: 178-233.
2. Barriga OO (1984). Immunomodulation by nematodes: A review. *Veterinary Parasitology*, 14: 299-320.
3. Weizger I, Patterson R & Pruzanski JD (1968). *Ascaris* hypersensitivity in the rhesus monkey. I. A model for the study of immediate type hypersensitivity in the primate. *Journal of Allergy*, 91: 14-22.
4. Bradbury SM, Percy DH & Strejan GH (1974). Immunology of *Ascaris suum* infection. I. Production of reaginic antibodies to worm components in rats. *International Archives of Allergy and Applied Immunology*, 46: 498-511.
5. Stromberg BE (1980). Potentiation of the reaginic (IgE) antibody response to ovalbumin in the guinea pig with a soluble metabolic product from *Ascaris suum*. *Journal of Immunology*, 125: 833-836.
6. McGibbon AM, Christie JF, Kennedy MW & Lee TD (1990). Identification of the ma-

jor ascaris allergen and its purification to homogeneity by high-performance liquid chromatography. *Molecular and Biochemical Parasitology*, 39: 163-171.
7. Crandall CA (1976). *Ascaris suum*: homocytotropic antibody responses in mice. *Experimental Parasitology*, 39: 69-73.
8. Soares MFM, Macedo MS & Mota I (1987). Suppressive effect of an *Ascaris suum* extract on IgE and IgG antibody responses in mice. *Brazilian Journal of Medical and Biological Research*, 20: 203-211.
9. Macedo-Soares MF, Mota I & Macedo MS (1992). Isolation of *Ascaris suum* components which suppress IgE antibody responses. *International Archives of Allergy and Applied Immunology*, 97: 37-43.
10. Mota I & Wong D (1969). Homologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sciences*, 8: 813-820.
11. Van Heymingen V, Brock DJ & Van Heymingen S (1983). A simple method for ranking the affinities of monoclonal antibodies. *Journal of Immunological Methods*, 62: 147-153.