

Research Article

PAS-1, a protein affinity purified from *Ascaris suum* worms, maintains the ability to modulate the immune response to a bystander antigen

TELMA M OSHIRO,¹ CRISTINA S ENOBE,¹ CLÁUDIA A ARAÚJO,¹
MAHASTI S MACEDO² and MARIA FERNANDA MACEDO-SOARES¹

¹Laboratory of Immunopathology, Butantan Institute and ²Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

Summary Helminth infections and parasite components have potent immunomodulatory effects on a host's immune system. In the present study, we investigated the effect of PAS-1, a protein component of *Ascaris suum* adult worms recognized by a monoclonal antibody (MAIP-1), on humoral and cell-mediated responses to a bystander antigen (ovalbumin [OVA]). MAIP-1 recognized only one of the three polypeptide chains of PAS-1, but neutralized the suppressive effect of the whole worm extract on OVA-specific antibody production. PAS-1 inhibited antibody production against a T-cell-dependent, but not a T-cell-independent, antigen in a dose-dependent way. IgM, IgG₁, IgG_{2b}, and also IgE and anaphylactic IgG₁ levels were downregulated. In addition, PAS-1 inhibited OVA-specific delayed type hypersensitivity reactions in the footpad of mice, showing a potent immunosuppressive activity on both Th1 and Th2 responses that seems to be mediated by the induction of large amounts of IL-10 and IL-4. Indeed, PAS-1-specific spleen cells secreted sevenfold more IL-10 and threefold more IL-4 than OVA-specific cells in response to *in vitro* restimulation with the respective antigens. In conclusion, we showed that PAS-1, a single protein component from *A. suum*, maintains all its immunosuppressive properties.

Key words: antibody response, *Ascaris suum*, cytokine, immunosuppression.

Introduction

Infections by helminth parasites are generally associated with modulatory effects on a host's immune system¹ that either induce eosinophilia and mastocytosis, and increase IgE levels² or suppress the immune response to the parasite or to bystander antigens.^{3–5}

The ability to interfere with the immune response has a considerable biological significance, because infection may suppress protective responses against other pathogens. Coinfection of *Fasciola hepatica* and *Bordetella pertussis* promotes an impairment of the protective Th1 response against *B. pertussis*, exacerbating the bacterial infection.⁶ In humans infected with *Schistosoma mansoni*, *Onchocerca volvulus* or *Ascaris lumbricoides*, bacterial vaccines are less effective in stimulating Th1 responses.^{7–9} In contrast, helminth infections reduce the inflammatory disease caused by *Helicobacter pylori*¹⁰ and *Plasmodium falciparum*.¹¹ Coinfection with *A. lumbricoides* and *P. falciparum* has an important consequence, protecting from the risk of cerebral malaria.¹²

The immunosuppression mediated by helminths can be due to the presence of parasite components that negatively modulate the immune system and interfere in many mechanisms, for instance, blocking antigen processing through the secretion of protease inhibitors, interfering with cytokine production or imitating regulatory cytokine actions.¹³

In relation to *Ascaris suum*, it has been also shown that antibody production and delayed type hypersensitivity (DTH) are markedly suppressed either during the course of acute infection^{14,15} or following injection of crude extracts.^{5,16} *A. suum* suppressive components are distinct from allergenic ones^{17,18} and reduce Th1 and Th2 responses against an unrelated antigen by IL-4 and IL-10.¹⁹ Recently, we identified a 200 kDa protein (PAS-1) from *A. suum* worms using a monoclonal antibody (MAIP-1) produced against high molecular weight suppressive components obtained by gel filtration chromatography.²⁰ PAS-1 has a potent anti-inflammatory activity, probably because of the stimulation of regulatory cytokines (IL-10 and TGF- β) in macrophages.²¹

In this study, we examined the neutralizing capacity of MAIP-1 on the suppressive effect of *A. suum* extract. In addition, the modulatory effects of PAS-1, isolated by affinity chromatography using MAIP-1 coupled to Sepharose 4B, on humoral and cell-mediated immune responses were analysed. Our findings showed that PAS-1, a single protein component of *A. suum*, is responsible for its immunosuppressive activity.

Correspondence: Dr Maria Fernanda Macedo-Soares, Laboratório de Imunopatologia, Instituto Butantan, Avenida Vital Brasil 1500, 05503-900 São Paulo, SP, Brasil. Email: fmacedo@usp.br

Received 8 June 2005; accepted 14 September 2005.

Materials and methods

Animals

BALB/c mice at 4–5 weeks of age, weighing 18–22 g, were supplied by the animal house of Butantan Institute. Food and water were provided ad libitum. All the procedures were approved by the Animal Ethics Committee of Butantan Institute.

Antigens

Ovalbumin (OVA) five times crystallized and keyhole limpet hemocyanin (KLH) were obtained from Calbiochem (La Jolla, CA, USA). DNP-OVA and DNP-hydroxyethylstarch (DNP-HES), thymus-dependent and thymus-independent antigens, respectively, were prepared, as previously described.²² *A. suum* worm extract (ASC) was prepared as described by Strejan and Campbell.²³ The protein component from *A. suum* adult worm extract, PAS-1, was purified by affinity chromatography using the mAb MAIP-1 coupled to Sepharose 4B, as previously described.²⁰

SDS-PAGE and immunoblotting

Samples were heated at 95°C for 5 min in the presence or absence of 5% 2-ME. SDS-PAGE, as described by Laemmli,²⁴ was carried out using 4% stacking and 7.5% resolving gels, and proteins were transferred onto nitrocellulose membranes. For immunodetection, nitrocellulose membranes were blocked with 5% non-fat milk and incubated with mAb MAIP-1. Antigen-antibody complexes were revealed by a second incubation with antimouse kappa chain-horseradish peroxidase conjugate. Colorimetric reaction was carried out using 4-chloro-alpha naphthol.

Immunization protocol

BALB/c mice were immunized with 50 µg of OVA or the same amount of OVA mixed with different doses of ASC or PAS-1 in 7.5 mg of aluminium hydroxide gel as adjuvant. For the neutralization protocol, some groups were treated on days zero, three and six with 1 mg of MAIP-1 or the isotype antibody control (MA-DNP-5) mAb. Other groups were immunized with 10 µg of DNP-OVA or DNP-HES alone or mixed with 500 µg of PAS-1, plus adjuvant. All groups were bled 21 days after immunization and the serum antibody levels were determined by ELISA.

Assessment of DTH reaction

Groups of BALB/c mice were immunized s.c. in the base of the tail with 100 µg of OVA with or without 500 µg of PAS-1 in CFA. A week after, they were challenged with 600 µg of heat aggregated OVA in one hind footpad and saline in the other. The footpad thickness was measured until 72 h after challenge and the net increase expressed as the arithmetic mean ± SD of each group.

Measurement of antibodies by ELISA

Antibody levels were titrated by sandwich ELISA using antigen-coated 96-well plates and peroxidase-conjugated rat antimouse kappa chain mAb (LO-MK-1 PO). For the titration of OVA-specific isotypes, peroxidase-labelled rat mAb against different mouse Ig isotypes (kindly provided by Dr H. Bazin) were used. The reactions were developed with *o*-phenylenediamine and H₂O₂, and the plates were read at 492 nm on an automated ELISA reader. The results are reported as the mean absorbance of samples per group (±SD) at various plasma dilutions.

Measurement of anaphylactic antibodies by passive cutaneous anaphylaxis

The anaphylactic activity of IgG₁ and IgE antibodies was evaluated by passive cutaneous anaphylaxis (PCA) reactions on the back of mice or rats, respectively. Mice were shaved and injected i.d. (50 µL) with three serial dilutions of plasma (inactivated for 1 h at 56°C) in each side of the back.²⁵ After 2 h, they were challenged i.v. with 500 µg of OVA + 0.25% of Evans blue solution. For IgE titration, PCA reactions were carried out in rats using 100 µL of non-inactivated plasma.²⁶ All tests were carried out in triplicate, and the PCA titres were expressed as the reciprocal of the highest dilution that gave a lesion of >5 mm in diameter. The detection threshold of the technique was established at 1:5 dilution. Because variation of PCA titre for the same sample was twofold or less, only differences above this value were considered to be significant.^{27,28}

Cytokine assays

For *in vitro* cytokine measurements, spleen cells were cultured in 24-well tissue culture plates at a final concentration of 2×10^6 cells/well and stimulated with OVA or PAS-1 (100 µg/well) for 72 h. All cytokines were quantified by two-site sandwich ELISA using standards and antibody pairs specific for each cytokine according to the manufacturer's recommendation (R&D Systems, Minneapolis, MN, USA). The detection limits were 15.6 pg/mL for IL-4, IFN-γ and TGF-β, and 31.25 pg/mL for IL-10.

Statistical analysis

Values were expressed as mean ± SD, using groups of four to six animals and three independent experiments. Differences between groups were analysed by Student's *t*-test for unpaired data. For DTH results, a one-way analysis of variance followed by multiple comparisons using the Tukey's method was used. The differences were accepted as significant at $P < 0.05$.

Results

MAIP-1 recognizes one of the three polypeptide chains of PAS-1

We have previously described the production and characterization of a mouse mAb (MAIP-1) that recognizes specifically a 200 kDa component of *A. suum*²⁰ (Fig. 1, lane 3). In addition, we show here by SDS-PAGE and immunoblotting (Fig. 1, lanes 4 and 5) that when PAS-1 is reduced by 2-ME, three important bands of approximately 100, 65 and 45 kDa can be visualized in the gel, and only the 100 kDa band is recognized by MAIP-1.

Neutralization of immunosuppression induced by *A. suum* components using MAIP-1 antibody

Because MAIP-1 recognizes only one polypeptide chain of a single protein of *A. suum*, we asked whether MAIP-1 could neutralize the immunosuppressive effect of the whole extract. Thus, BALB/c mice were immunized with OVA + ASC and treated with MAIP-1 or the isotype control antibody MA-DNP-5. The levels of anti-OVA antibodies detected in plasma from mice treated with MAIP-1 were higher than those present in mice treated with the isotype control and not

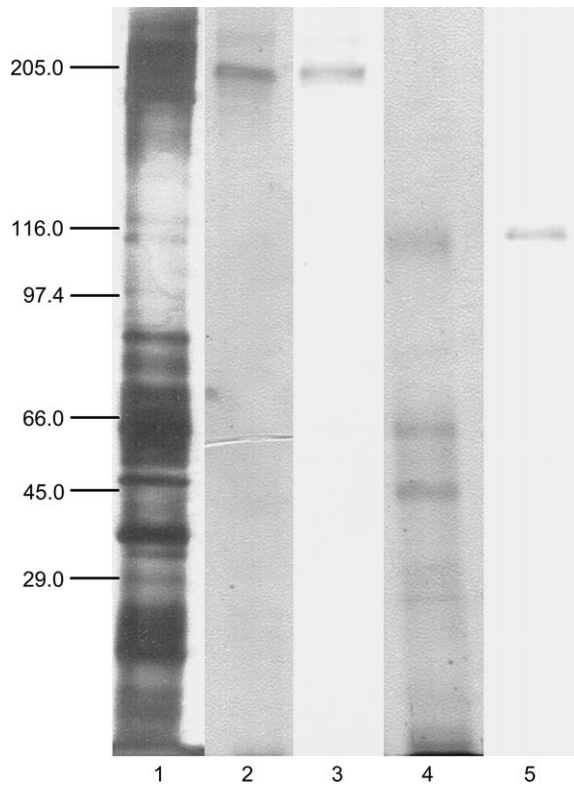


Figure 1 SDS-PAGE profile and immunoblotting analysis of *Ascaris suum* worm extract (ASC) and PAS-1. Silver-stained 7.5% acrylamide gel containing ASC (lane 1), PAS-1 (lane 2) or PAS-1 treated with 2-ME (lane 4). Immunoblotting of PAS-1 (lane 3) and PAS-1 treated with 2-ME (lane 5), probed with monoclonal antibody MAIP-1. Molecular weight markers (kDa) at extreme left.

significantly different from the antibody response of control mice immunized only with OVA (Fig. 2). These results show that the suppression induced by *A. suum* extract was abolished by treatment with MAIP-1.

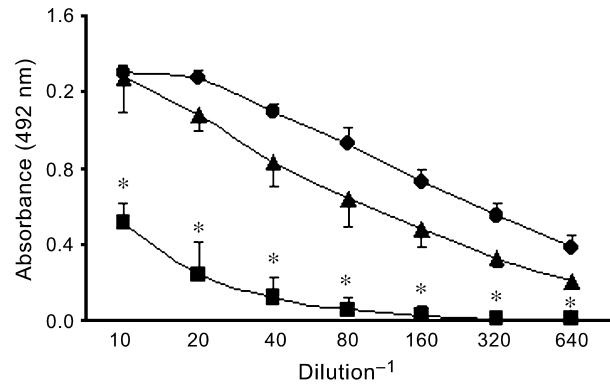


Figure 2 Effect of MAIP-1 on immunosuppression induced by *Ascaris suum* worm extract (ASC). Anti-OVA antibody levels were determined by ELISA in plasma from mice immunized with ●, OVA + adjuvant; ▲, OVA + ASC + adjuvant, treated with MAIP-1 mAb; ■, OVA + ASC + adjuvant, treated with isotype control MA-DNP-5 mAb. The animals were bled 21 days after immunization. The results represent the mean $\pm$ SD absorbance at different plasma dilutions ($n = 6$). * $P < 0.05$ compared with OVA group.

Effect of PAS-1 on humoral and cell-mediated immune responses to a heterologous antigen

We next investigated the effect of the ASC component PAS-1 recognized by MAIP-1 on antigen-specific immune responses. To examine the effect of PAS-1 on T-cell-independent or T-cell-dependent antibody responses, BALB/c mice were immunized with PAS-1 + DNP-HES or PAS-1 + DNP-OVA, respectively. Figure 3 shows the levels of anti-DNP antibodies in both situations. PAS-1 strongly suppressed anti-DNP antibody production induced by a T-cell-dependent carrier (OVA) (Fig. 3B) but had no effect on the T-cell-independent stimulation (HES) (Fig. 3A). Moreover, the suppressive effect of PAS-1 on antibody production induced by OVA was dose dependent (Fig. 4) and affected IgM, IgG₁ and IgG_{2b} antibodies (Fig. 5A,B,D), as well as anaphylactic (IgG₁ and IgE) antibodies measured by PCA (Fig. 5E,F). The levels of IgG₁

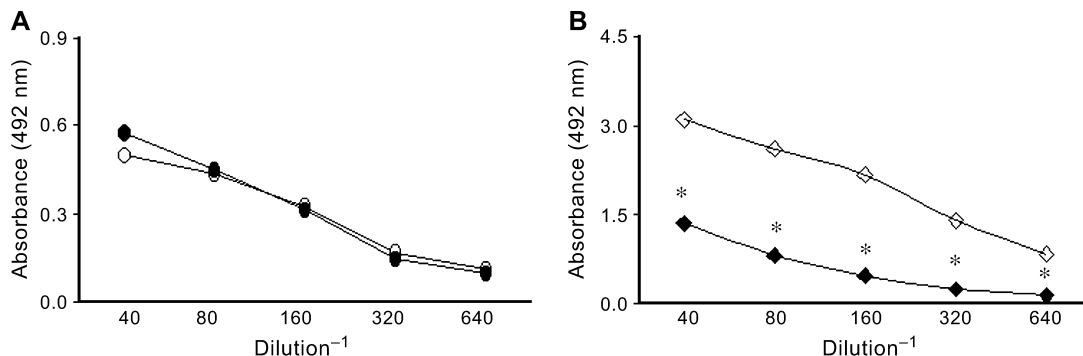


Figure 3 Effect of PAS-1 on thymus-independent (DNP-hydroxyethylstarch [DNP-HES]) and thymus-dependent (DNP-ovalbumin, DNP-OVA) humoral responses. (A) BALB/c mice were immunized with ○, 10 μ g of DNP-HES; or ●, 10 μ g of DNP-HES + 500 μ g of PAS-1; (B) Mice were immunized with ◇, 10 μ g of DNP-OVA; or ◆, 10 μ g of DNP-OVA + 500 μ g of PAS-1. The animals were bled 21 days after immunization, and the levels of anti-DNP antibodies in a pool of five samples were determined by ELISA using a peroxidase-conjugated anti-Ig kappa chain mAb. * $P < 0.05$ compared with DNP-OVA group.

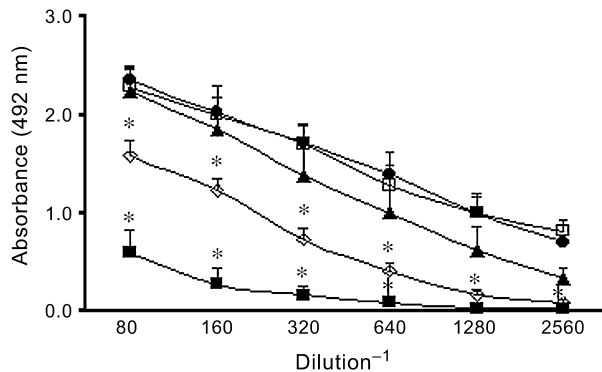


Figure 4 Dose-dependent effect of PAS-1 on antibody production. Mice were injected with ▲, 50 µg of ovalbumin (OVA) + 50 µg; ◇, 200 µg; or ■, 500 µg of PAS-1; in adjuvant. Control groups received ●, 50 µg of OVA; or □, 50 µg of OVA + 500 µg of keyhole limpet hemocyanin, in adjuvant. Plasma obtained 21 days after immunization was titrated by ELISA using a peroxidase-conjugated anti-Ig kappa chain mAb. The results represent the mean $\pm$ SD absorbance at different plasma dilutions ($n = 4-5$). * $P < 0.05$ compared with the OVA group.

antibodies, however, were not decreased with the low dose of PAS-1 (Fig. 5B). IgG_{2a} (Fig. 5C) was not produced in significant amounts in this immunization protocol. We also used KLH, another protein with a molecular weight of 280 kDa (similar to PAS-1), as a control, and this protein had no effect on the antibody response when injected with OVA in a dose equivalent to the highest dose of PAS-1 (Fig. 4).

To study the effect of PAS-1 on the cell-mediated immune response, DTH reaction and cytokine production were measured. As shown in Figure 6, DTH reactions were inhibited in mice immunized with OVA + PAS-1 in comparison with the OVA group during almost all the time course. PAS-1 also influenced the cytokine secretion as shown in Table 1. The production of IL-4 and IFN- γ in cell cultures from OVA + PAS-1-immunized mice stimulated with OVA was suppressed compared with that in cell cultures from OVA-

immunized mice. In contrast, when OVA + PAS-1-primed cells were stimulated with PAS-1, they secreted much more IL-4, IFN- γ , IL-10 and TGF- β than OVA-primed cells stimulated with OVA. The difference was striking in IL-4 levels (2.9-fold) and in IL-10 levels (6.8-fold). *In vitro* stimulation with PAS-1 did not induce any detectable cytokine production in OVA-primed cells (Table 1). Cytokine levels in spleen cell cultures from non-immunized mice stimulated with OVA or PAS-1 were also below the detection limits (data not shown).

Discussion

Fractionation of *A. suum* extract by gel filtration chromatography in a Sephacryl S200 column results in three distinct peaks, namely PI, PII and PIII. The immunosuppressive effect of the extract is restricted to PI, which contains high molecular weight components, and impairs the immune response to bystander antigens and also to PIII. In contrast, PIII is the allergenic fraction, with low molecular weight components that induce IgE and anaphylactic IgG₁ antibodies.^{17,18} The mAb obtained against PI, MAIP-1, allowed the purification of a single component, PAS-1, by affinity chromatography.²⁰

Here we show that PAS-1, a 200 kDa protein with three covalently linked polypeptide chains, mediates the suppressive activity on humoral and cell-mediated immune responses to bystander antigens in a dose-dependent way. MAIP-1, which recognizes an epitope present only in the 100 kDa chain, can neutralize the dampening effect of ASC on antibody production, indicating that either this chain is crucial for this effect or the antibody interferes also with the activity of the other polypeptide chains. The absence of any effect of KLH, a protein with a molecular weight close to that of PAS-1, excludes the possibility that there is an antigenic competition between PAS-1 and OVA at the level of antigen presentation by dendritic cells or macrophages.

PAS-1, however, did not affect the B-cell response to a T-cell-independent antigen. T-cell-independent antigens are nonprotein substances that cannot be processed and presented by MHC molecules. HES is a polysaccharide composed of multiple identical antigenic epitopes that induce cross-linking of membrane Ig present on specific B cells, leading to activation with no requirement for cognate T-cell help.²⁹ Recently, we observed a significant decrease in MHC class II, and CD80 and CD86 costimulatory molecules in APC from PI-injected mice (Silva SR *et al.*, unpubl. data, 2005). These molecules play a crucial role in the activation of T-cell-dependent immune responses.³⁰ Therefore, if PAS-1 also has an inhibitory effect on the expression of MHC class II and costimulatory molecules such as PI, this may explain how PAS-1 impairs antibody production against T-cell-dependent but not T-cell-independent antigens. As a result of this inhibitory effect, the frequency of OVA-specific T cells may be lower in OVA + PAS-1-injected mice than in OVA-injected mice, and consequently, the amounts of cytokines their spleen cells produce when stimulated *in vitro* with OVA will be decreased, as we observed in the experiments reported in this study. Ferreira³¹ has shown by limiting dilution assay that 8 days after immunization, the frequency of OVA-responding cells in OVA + ASC-immunized mice was at least threefold lower than in OVA-immunized mice.

Table 1 Effect of PAS-1 on the cytokine profile of spleen cells from OVA-immunized mice

Cytokine [†] (pg/mL)	Stimulus Immunization	OVA	PAS-1
IL-4	OVA	87.5 $\pm$ 10.2	<15.6
	OVA + PAS-1	66.3 $\pm$ 12.1*	255.1 $\pm$ 22.3*
IFN- γ	OVA	1057.9 $\pm$ 117.2	<31.25
	OVA + PAS-1	554.6 $\pm$ 133.2*	1493.0 $\pm$ 232.0*
IL-10	OVA	283.9 $\pm$ 141.2	<31.25
	OVA + PAS-1	144.6 $\pm$ 65.3	1940.3 $\pm$ 639.5*
TGF- β	OVA	89.2 $\pm$ 4.2	<31.25
	OVA + PAS-1	78.4 $\pm$ 15.9	133.9 $\pm$ 10.2*

* $P < 0.05$ compared with OVA-primed cells stimulated with OVA.

[†]Spleen cells from BALB/c mice immunized with OVA or OVA + PAS-1 were stimulated for 72 h with OVA or PAS-1. Cytokines were measured in culture supernatants by two-site sandwich ELISA and the results represent the mean $\pm$ SD of five mice per group. OVA, ovalbumin.

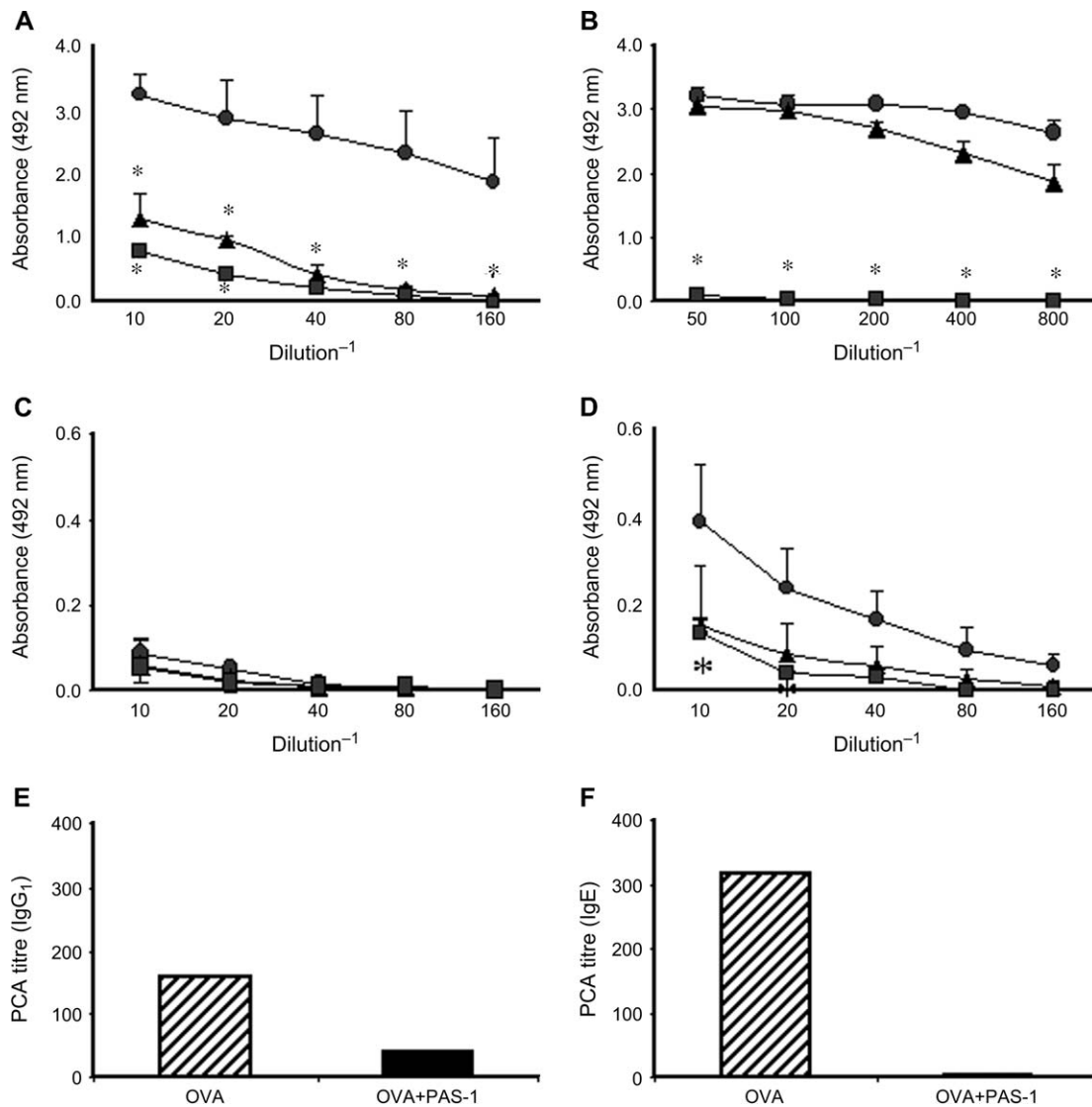


Figure 5 Effect of PAS-1 on the production of different ovalbumin (OVA)-specific isotypes. OVA-specific (A) IgM, (B) IgG₁, (C) IgG_{2a} and (D) IgG_{2b} were titrated by ELISA in plasma of mice immunized with ▲, OVA + 50 µg or ■, 500 µg of PAS-1 in adjuvant and bled after 21 days. The control group was immunized only with ●, OVA. The results represent the mean ± SD absorbance at different plasma dilutions ($n = 4-5$). * $P < 0.05$ compared with the OVA group. (E) Titres of anaphylactic IgG₁ and (F) IgE anti-OVA antibodies were determined by passive cutaneous anaphylaxis for plasma pooled from four to five mice injected with either OVA or OVA + PAS-1.

Besides the T-cell-dependent humoral response, PAS-1 also inhibited DTH reactions elicited by aggregated OVA challenge in the footpad of OVA + PAS-1-immunized mice. The reduction of IFN- γ secretion by OVA-specific cells from these mice correlates well with the suppression of DTH because IFN- γ is known to mediate the effector phase of this type of reaction.³² The ability of the OVA-specific cells to secrete less IL-4 was also accompanied by the suppression of OVA-specific IgG₁ and IgE antibody production, which are regulated by IL-4.³³

In comparison with the cytokine response in OVA-immunized mice, the response to a high dose of PAS-1 in mice immunized with OVA + PAS-1 was biased towards Th2, with much higher levels of IL-4 and IL-10 being produced by PAS-1-specific cells when restimulated *in vitro* with PAS-1. We have

previously shown that neutralization of IL-4 and IL-10 in OVA + ASC-immunized mice leads to a shift towards Th1 with enhancement of IL-2 and IFN- γ production by ASC-specific cells.³⁴ In addition, when a low dose of ASC is used, there is no downregulation of the response against OVA, and the profile of cytokines synthesized in response to ASC changes to a Th1 type, indicating that large amounts of IFN- γ are not suppressive.¹⁶ Our present results also showed that, like in the ASC reports, the suppressive effect of PAS-1 is dose dependent. Therefore, the type of cytokines predominantly induced by different doses of PAS-1 might be crucial in its ability to modulate the response to bystander antigens.

In the experimental protocol reported here, the rise in IL-10 levels in cultures from OVA + PAS-1-primed cells restimulated

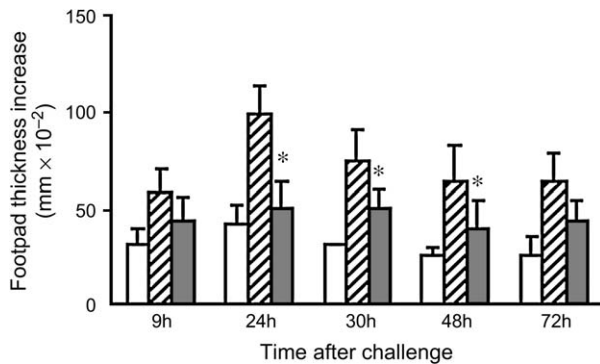


Figure 6 Effect of PAS-1 on delayed type hypersensitivity reactions. Mice were immunized in the base of the tail with ovalbumin (OVA) or OVA + PAS-1, emulsified in CFA. The control group received only CFA. Eight days later, all of the animals were challenged in the footpad with aggregated OVA. The results represent the mean $\pm$ SD for five animals per group. * $P < 0.05$ compared with the OVA group. □, control; ▨, OVA; ■, OVA + PAS-1.

with PAS-1 compared with those from OVA-primed cells restimulated with OVA was remarkable (sevenfold). IL-10, known as a regulatory cytokine, may well be inhibiting the immune response in OVA + PAS-1-immunized mice. The suppressor function of IL-10 has been attributed to its down-regulatory effect on dendritic cell maturation and to the generation of regulatory T cells.^{35,36} Striking production of IL-10 and induction of anergy are distinctive characteristics of regulatory T cells, which can prevent the expansion of Th1 and Th2 cells.^{37,38} Therefore, the source of IL-10 in PAS-1-injected mice might be regulatory T cells. Studies focusing on these cells in our model are already under way. The significant role of IL-10 in the immunosuppressive effect of PAS-1 is further supported by the absence of suppression of Th2-related (IgE and IgG₁) and Th1-related (DTH) parameters in IL-10-deficient mice and of Th1-related parameters (IgG_{2c} and IFN- γ production) in IL-4 + IL-10-deficient mice injected with OVA + PI.¹⁹ In regards to the participation of IL-4, we observed in the experiments reported here that PAS-1-specific cells produced threefold more IL-4 than OVA-specific cells. This cytokine may therefore play a synergistic role with IL-10 in the suppression of the immune response in OVA + PAS-1-injected mice. In addition, the absence of any detectable cytokines in spleen cell cultures from nonimmunized or OVA-immunized mice stimulated with PAS-1 reinforces that its regulatory effect on the response against OVA in the immunization protocol with both antigens is mediated by PAS-1-specific cells.

In conclusion, our results show that the immunosuppressive activity of *A. suum* is mediated by a single protein component. The biological significance of PAS-1 in the expression of a host's immune response is further emphasized by its presence in all stages of the *A. suum* life cycle.³⁹ As well as on the body of adult worms, PAS-1 was detected by immunofluorescence using MAIP-1 mAb on the surface of eggs and in larval stages L1, L2 and L3. The pseudocoelomic body fluid from adult worms also contained PAS-1, as well as the supernatant of 3 day cultures of embryonated eggs, as shown by capture

ELISA. Therefore, early stages of *Ascaris* larvae already release PAS-1 that might modify the immune response.

Acknowledgement

This work was supported by a grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). T. M. O. was in receipt of a doctoral fellowship from FAPESP.

References

- Barriga OO. Immunomodulation by nematodes: a review. *Vet. Parasitol.* 1984; **14**: 299–320.
- Jarret EE, Miller HR. Production and activities of IgE in helminth infection. *Prog. Allergy* 1982; **31**: 178–233.
- Grogan JL, Kremsner PG, Deelder AM, Yazdanbakhsh M. Antigen-specific proliferation and interferon- γ and interleukin-5 production are downregulated during *Schistosoma haematobium* infection. *J. Infect. Dis.* 1998; **177**: 1433–7.
- Soares MFM, Macedo MS, Mota I. Suppressive effect of an *Ascaris suum* extract on IgE and IgG antibody responses in mice. *Braz. J. Med. Biol. Res.* 1987; **20**: 203–11.
- Paterson JCM, Garside P, Kennedy MW, Lawrence CE. Modulation of heterologous immune response by the products of *Ascaris suum*. *Infect. Immun.* 2002; **70**: 6058–67.
- Brady MT, O'Neill SM, Dalton JP, Mills KH. *Fasciola hepatica* suppresses a protective Th1 response against *Bordetella pertussis*. *Infect. Immun.* 1999; **67**: 5372–8.
- Sabin EA, Araujo MI, Carvalho EM, Pearce EJ. Impairment of tetanus toxoid-specific Th1-like immune responses in humans infected with *Schistosoma mansoni*. *J. Infect. Dis.* 1996; **173**: 269–72.
- Cooper PJ, Espinel I, Paredes W, Guderian RH, Nutman TB. Impaired tetanus-specific cellular and humoral responses following tetanus vaccination in human onchocerciasis: a possible role for interleukin-10. *J. Infect. Dis.* 1998; **178**: 1133–8.
- Cooper PJ, Chico M, Sandoval C *et al.* Human infection with *Ascaris lumbricoides* is associated with suppression of the interleukin-2 response to recombinant cholera toxin B subunit following vaccination with the live oral cholera vaccine CVD 103-HgR. *Infect. Immun.* 2001; **69**: 1574–80.
- Fox JG, Beck P, Dangler CA *et al.* Concurrent enteric helminth infection modulates inflammation and gastric immune response and reduces helicobacter-induced gastric atrophy. *Nat. Med.* 2000; **6**: 536–42.
- Nacher M, Singhasivanon P, Silachamroon U *et al.* Helminth infections are associated with protection from malaria-related acute renal failure and jaundice in Thailand. *Am. J. Trop. Med. Hyg.* 2001; **65**: 834–6.
- Nacher M, Gay F, Singhasivanon P *et al.* *Ascaris lumbricoides* infection is associated with protection from cerebral malaria. *Parasite Immunol.* 2000; **22**: 107–13.
- Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat. Rev. Immunol.* 2003; **3**: 733–44.
- Crandall CA, Crandall RB. *Ascaris suum*: immunosuppression in mice during acute infection. *Exp. Parasitol.* 1976; **40**: 363–72.
- Crandall RB, Crandall CA, Jones JF. Analysis of immunosuppression during early acute infection of mice with *Ascaris suum*. *Clin. Exp. Immunol.* 1978; **33**: 30–37.

- 16 Ferreira AP, Faquim-Mauro EL, Abrahamsohn IA, Macedo MS. Immunization with *Ascaris suum* extract impairs T cell functions in mice. *Cell. Immunol.* 1995; **162**: 202–10.
- 17 Soares MF, Mota I, Macedo MS. Isolation of *Ascaris suum* components which suppress IgE antibody responses. *Int. Arch. Allergy Immunol.* 1992; **97**: 37–43.
- 18 Faquim-Mauro EL, Macedo MS. The immunosuppressive activity of *Ascaris suum* is due to high molecular weight components. *Clin. Exp. Immunol.* 1998; **114**: 245–51.
- 19 Souza VMO, Jacysyn JF, Macedo MS. IL-4 and IL-10 are essential for immunosuppression induced by high molecular weight proteins from *Ascaris suum*. *Cytokine* 2004; **28**: 92–100.
- 20 Oshiro TM, Rafael A, Enobe CS, Fernandes I, Macedo-Soares MF. Comparison of different mAb against immunosuppressive proteins of *Ascaris suum*. *Braz. J. Med. Biol. Res.* 2004; **37**: 223–6.
- 21 Oshiro TM, Macedo MS, Macedo-Soares MF. Anti-inflammatory activity of PAS-1, a protein component of *Ascaris suum*. *Inflamm. Res.* 2006; **54**: 17–21.
- 22 Macedo-Soares F, Latinne D, Denis O, Bazin H. In vivo interleukin-4 regulation of antibody responses in euthymic and athymic mice to a T-independent antigen (hydroxyethyl starch). *Int. Arch. Allergy Immunol.* 1996; **110**: 174–81.
- 23 Strejan J, Campbell DH. Hypersensitivity to *Ascaris* antigens. I. Skin-sensitizing activity of serum fraction from guinea-pigs sensitized to crude extracts. *J. Immunol.* 1967; **98**: 893–7.
- 24 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680–85.
- 25 Ovary Z. Passive cutaneous anaphylaxis in the mouse. *J. Immunol.* 1958; **81**: 355–7.
- 26 Mota I, Wong D. Homologous and heterologous passive cutaneous anaphylaxis activity of mouse antisera during the course of immunisation. *Life Sci.* 1969; **8**: 13–20.
- 27 Fox DA, Chiorazzi N, Katz DH. Hapten-specific IgE antibody response in mice. V. Differential resistance of IgE and IgG B lymphocytes to X-irradiation. *J. Immunol.* 1976; **117**: 1622–8.
- 28 Ishizaka K, Adachi T. Generation of specific helper cells and suppressor cells *in vitro* for the IgE and IgG antibody response. *J. Immunol.* 1976; **117**: 40–47.
- 29 Mond JJ, Lees A, Snapper CM. T cell-independent antigens type 2. *Annu. Rev. Immunol.* 1995; **13**: 665–92.
- 30 Bretscher P. The two-signal model of lymphocyte activation twenty-one years later. *Immunol. Today* 1992; **13**: 74–6.
- 31 Ferreira AP. Mecanismos envolvidos na supressão da resposta imune de camundongos por extrato de *Ascaris suum* (PhD thesis). São Paulo, Brazil: Institute of Biomedical Sciences, University of São Paulo, 1995.
- 32 Fong TAT, Mosmann TR. The role of IFN- γ in delayed type hypersensitivity mediated by TH1 clones. *J. Immunol.* 1989; **143**: 2887–93.
- 33 Snapper CM, Finkelman FD, Paul WE. Regulation of IgG1 and IgE production by interleukin 4. *Immunol. Rev.* 1988; **102**: 51–75.
- 34 Macedo MS, Faquim-Mauro E, Ferreira AP, Abrahamsohn IA. Immunomodulation induced by *Ascaris suum* extract in mice: effect of anti-interleukin-4 and anti-interleukin-10 antibodies. *Scand. J. Immunol.* 1998; **47**: 10–18.
- 35 Muller G, Muller A, Tuting T *et al.* Interleukin-10-treated dendritic cells modulate immune responses of naive and sensitized T cells *in vivo*. *J. Invest. Dermatol.* 2002; **119**: 836–41.
- 36 Wakkach A, Fournier N, Brun V, Breittmayer JP, Cottrez F, Groux H. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation *in vivo*. *Immunity* 2003; **18**: 605–17.
- 37 Groux H, Powrie F. Regulatory T cells and inflammatory bowel disease. *Immunol. Today* 1999; **20**: 442–5.
- 38 Cottrez F, Hurst SD, Coffman RL, Groux H. T regulatory cells 1 inhibit a Th2-specific response *in vivo*. *J. Immunol.* 2000; **165**: 4848–53.
- 39 Enobe CS. Imunomodulação induzida por diferentes estágios do desenvolvimento de *Ascaris suum* (MSc thesis). São Paulo, Brazil: Institute of Biomedical Sciences, University of São Paulo, 2001.