

Maria Fernanda M. Soares^a

Ivan Mota^a

Mahasti S. Macedo^b

^a Immunology Research and Training Centre, Instituto Butantan, and

^b Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, Brazil

Isolation of *Ascaris suum* Components Which Suppress IgE Antibody Responses

Key Words

Ascaris suum

Helminth infections

IgE

Immune response

Abstract

An extract from adult *Ascaris suum* was fractionated on a Sephadex G-200 column and individual elution fractions assessed for their ability to induce or suppress an IgE antibody response in mice immunized simultaneously with a heterologous antigen (ovalbumin). The results showed that, whilst fractions eluted in the first peak suppressed the anti-ovalbumin IgE antibody production, those eluted in the third peak induced a significant anti-*Ascaris* IgE response. The mixture of the two kinds of fractions resulted in suppression of anti-*Ascaris* IgE antibodies. The apparent molecular weight (MW) of the first and third peaks was 530,000 and 29,000 daltons, respectively. After delipidation, the extract still retained most of its biological activities. SDS-PAGE of this material showed selective loss of high-MW components when compared to the whole extract. No bands were observed in SDS-PAGE of peak I in contrast to peak III, which displayed several bands. Immunoblots of all these samples showed at least 3 bands above MW 150,000, which reacted with anti-peak I antiserum, in the unfractionated extracts and in peak I, but not in peak III. When the extract was fractionated by affinity chromatography using anti-peak I antibodies as ligands, the immunogenic components were present in the effluent, whereas the suppressive components were recovered in the eluate.

Introduction

Helminth infections in man and several animal species have been associated with a large production of IgE antibodies [1] and with the modulation of the immune responsiveness of these hosts [2]. Thus, *Trichinella spiralis*, *Ascaris suum*, *Nippostrongylus brasiliensis*, *Nematospiroides dubius* and diverse filariae can depress or enhance the heterologous humoral or cell-mediated immunities and affect macrophage activity or the response to other invaders [2]. Using the *N. brasiliensis* infection to study the mechanisms

of IgE regulation by helminths, Ishizaka [3] identified two IgE-binding factors which potentiating or suppressive activity on the IgE antibody production depends on two other glycosylation-controlling lymphokines. In relation to *A. suum*, it was shown that this nematode potentiates the IgE response in different species [4-6], although attempts made in the mouse model were unsuccessful [7]. The worm extracts in various stages of purification have been also widely used as immunizing antigen in studies on IgE antibody formation [8-12]. It is interesting to notice, however, that high titres of IgE antibody were only formed

Received:
November 12, 1990
Accepted after revision:
September 12, 1991

Correspondence to: Dra. M.F.M. Soares
Immunology Research and Training Centre
Instituto Butantan, CP 65
05504 São Paulo, SP (Brazil)

© 1992 S. Karger AG, Basel
1018-2438/92/0971-0037
\$ 2.75/0

in immunized animals after inactivation of the isotype-specific regulatory mechanisms [9, 13]. Besides, when the extract was injected with an unrelated antigen like ovalbumin (OA), a marked suppression of cytotropic antibody production to this protein was observed [14, 15]. The effect on IgE but not on IgG1 and IgG2 antibodies was abolished by treatments such as X-irradiation and administration of cyclophosphamide before immunization [16, 17]. Moreover, we have demonstrated in an adoptive secondary IgE antibody response that T and B cell activities were dampened upon concomitant immunization of cell donors with antigen and *Ascaris* extract [18]. We have also reported that the integrity of heat-stable proteins present in the whole extract is essential for its suppressive effect [19]. The present experiments were performed in an attempt to isolate and further characterize the components of *A. suum* responsible for the above observations.

Materials and Methods

Animals

Six- to 7-week-old male A/SN mice were used for immunization. Outbred Wistar rats weighing between 150 and 200 g were used for skin tests.

Antigens

OA, 5 times crystallized, was obtained from Calbiochem (La Jolla, Calif., USA). Adult worm extract from *A. suum* (ASC) was prepared as described earlier [14].

Analytical Methods

Protein and carbohydrate content of whole extract and gel filtration column effluents were determined using the Lowry et al. modification of the Folin assay [20] and the phenol-sulphuric acid assay [21], respectively. The primary standards used in these assays were bovine serum albumin (BSA) and *D*-galactose (Sigma Chemical, St. Louis, Mo., USA). The amount of ASC employed in the experiments was based on the protein content.

Gel Filtration

The parasite extract (40 mg) was fractionated by upward elution at 20 ml/h on a column (80 × 3 cm) of Sephadex G-200 or a column (110 × 2 cm) of Sephacryl S-200 (Pharmacia, Uppsala, Sweden), equilibrated with 0.05 *M* borate-buffered saline (BBS), pH 8.0. Fractions of 1.5 ml were collected after an initial elution volume of 40 ml. Protein elution profile was determined by measuring the absorption at 280 nm and appropriate fractions were pooled and concentrated by ultrafiltration under nitrogen using UM 10 membrane in Diaflo cells (Amicon, Danvers, Mass., USA).

Molecular Weight Determination

The apparent molecular weight (MW) of the ASC components was determined by gel filtration [22] on Sephacryl S-200, using the conditions described above. Their distribution was obtained by reference to a standard curve of K_{av} versus log MW. The MW markers

were obtained from Pharmacia and included thyroglobulin (MW 660,000), ferritin (440,000), catalase (232,000), BSA (67,000), OA (43,000) and chymotrypsinogen (25,700). Data were analysed by linear regression.

Delipidation

The extract was delipidated according to Gidz [23]. Fifty milligrammes of ASC in 5 ml of phosphate-buffered saline were incubated with 0.1 ml of 10% dextran sulphate and 0.5 ml of 1 *M* CaCl₂ for 10 min at room temperature. After centrifugation for 30 min 10,000 rpm the supernatant was separated and exhaustively dialyzed against saline with EDTA (2 g/l). The precipitate was discarded.

Antisera to Extract Components

Rabbits were immunized intramuscularly with complete Freund's adjuvant containing 1 mg of pooled fractions obtained from the Sephadex column. Secondary subcutaneous injections (200 µg) were performed 20 days later and blood collected 10 days after this and 3 subsequent booster injections. Sera from different bleedings were pooled and tested for specificity by double immunodiffusion analysis and titrated by ELISA.

SDS-PAGE and Immunoblots

Samples of the whole extract or gel filtration column fractions were diluted in 0.125 *M* Tris-glycine buffer, pH 8.6, containing 2.5% SDS, 10 mM EDTA, 1 mM TLCK, 1 mM PMSF and 0.05% bromophenol blue. After heating (100 °C, 3 min), 0.3 ml of the mixtures were analysed by SDS-PAGE on 4% stocking and 6–16% resolving gradient gels at 30 mA constant current [24]. Prestained MW markers (Sigma Chemical) were included on each gel. Gels were stained for protein with Coomassie blue. The molecules separated by SDS-PAGE were transferred to nitrocellulose membranes according to Towbin et al. [25]. The membranes were then treated with rabbit anti-peak I serum at 1:50 dilution, followed by addition of antirabbit IgG labelled with peroxidase and H₂O₂ plus 4-chloro-1-naphthol as the enzyme substrate.

Affinity Chromatography

Rabbit anti-peak I IgG antibodies were obtained by 50% ammonium sulphate precipitation of immune serum, followed by gel filtration on a Sephacryl S-200 column [26]. Seventy milligrammes of purified IgG were then coupled to CNBr-Sepharose 4B (Pharmacia) and packed in a column (3.0 × 2.0 cm) equilibrated with 0.1 *M* BBS, pH 8.0. Two milliliters of ASC (1 mg/ml) were filtered through this column. Unbound ASC was collected, pooled and concentrated to 2.0 ml, and recycled 3 times through regenerated columns until depleted of reactive components. Bound components were eluted after each of the 3 cycles with 0.05 *M* diethylamine, pH 11.0, and monitored at 280 nm. Finally, bound and unbound fractions were concentrated to the original volume (2 ml).

Experimental Design

Groups of 8 mice were immunized intraperitoneally with 200 µg of ASC (pre-treated or not) plus 50 µg of OA in 7.5 mg of aluminium hydroxide gel as adjuvant. To test the individual elution fractions from the gel filtration columns, 50 µl of each fraction were mixed with the same amount of OA and adjuvant. Control groups received only 50 µg of OA in adjuvant. In some experiments, the animals were boosted 28 days after immunization with 1/5 of the first dose of antigen.

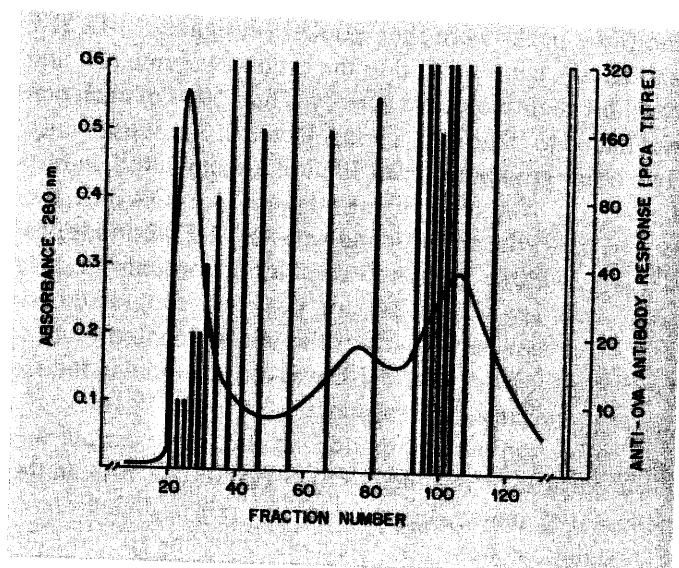


Fig. 1. Sephadex G-200 elution profile of ASC (40 mg). Fractions were analyzed for protein by absorbance at 280 nm (curve). The black bars represent the anti-OA IgE antibody titres (on day 21) of mice immunized with individual fractions plus OA and adjuvant. The white bar represents the PCA titre of a group injected only with OA and adjuvant.

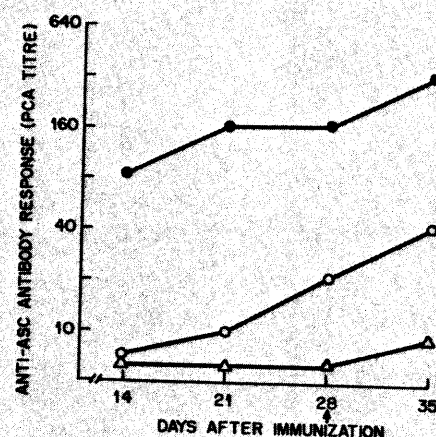


Fig. 3. IgE antibody production in mice immunized with 50 μ l of fractions 94 (●) or 26 (Δ) or a mixture of both (○), in adjuvant. PCA reactions were elicited with ASC. The arrow represents a booster of 10 μ l of the respective fraction.

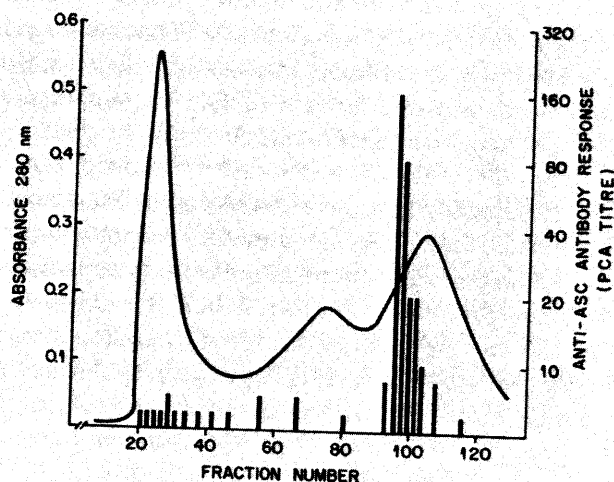


Fig. 2. Anti-ASC IgE antibody response determined in the groups described in figure 1. The same chromatographic profile is also presented.

Measurement of IgE Antibodies

IgE anti-OA and anti-ASC antibodies were measured by passive cutaneous anaphylaxis (PCA) in rats according to Mota and Wong [27]. The animals were challenged intravenously with 0.5 mg of OA or

ASC in 0.25% Evans blue solution, after a sensitization period of 18–24 h. The PCA titre was expressed as the reciprocal of the highest dilution that gave a lesion of more than 5 mm in diameter. Since variation of the PCA titre for the same sample was 2-fold or less, only differences above this value were considered significant [28, 29]. As plasma samples were pooled routinely for each group of mice to provide sufficient material for estimations, the variation in response between individuals was not determined.

Results

ASC was fractionated by gel filtration on Sephadex G-200, as described previously by Ambler et al. [30] and the individual elution fractions were analysed for their ability to induce or suppress an IgE antibody response in mice immunized concomitantly with OA and adjuvant. As depicted in figure 1, only fractions numbered 24–29, corresponding to peak I, suppressed the anti-OA antibody response (up to 32-fold). In contrast, anti-ASC antibodies (fig. 2) were solely induced by fractions confined to peak III (94–101). New groups were then immunized with a mixture of 50 μ l of fractions 94 and 26. As shown in figure 3, the anti-ASC IgE response was very much dampened by the simultaneous injection of fraction 26. Thus, the suppressive activity could be demonstrated in the homolo-

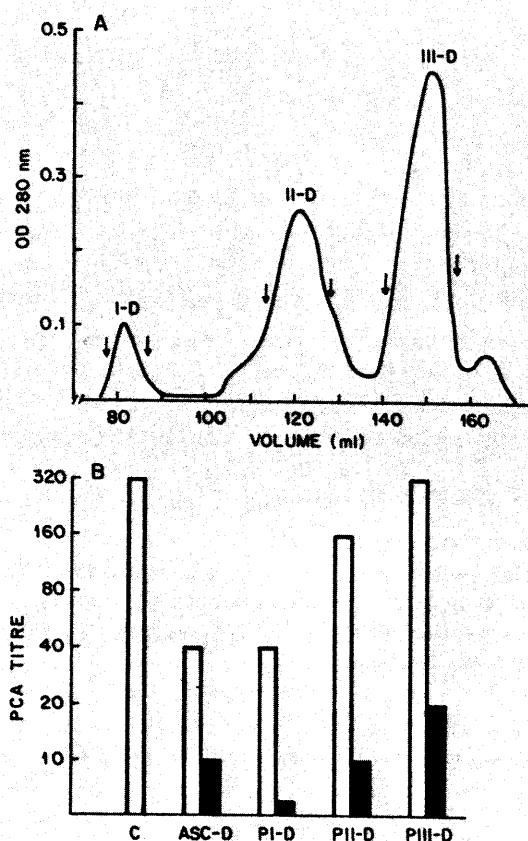


Fig. 4. A Gel filtration of delipidated ASC on a Sephacryl S-200 column. The arrows delimit the fractions pooled in each peak. **B** Anti-OA (□) and anti-ASC (■) IgE antibody titres 21 days after immunization of mice with OA (C) or OA plus delipidated-ASC (ASC-D), peaks I-D, II-D or III-D, in adjuvant.

gous (anti-ASC) as well as in a heterologous (anti-OA) IgE antibody response.

ASC was also fractionated on a Sephacryl S-200 column, where the apparent MW of the peaks was determined as being 530,000, 80,000 and 29,000 daltons, respectively. Analysis of the carbohydrate content of the fractions showed also that most of the sugar residues were eluted in peak I. Furthermore, the biological activity displayed by each fraction resembled that obtained in the Sephadex G-200 column (data not shown).

When lipids and lipoproteins of the extract were precipitated by dextran sulphate, the same elution profile was obtained in the Sephacryl S-200 column, although with a lower yield in the first peak (fig. 4A). Fractions comprising

the three peaks were then pooled (designated I-D, II-D and III-D), concentrated to the original volume and analysed for their biological activity. Thus, 50 µl of each pool was added to 50 µg of OA and injected in 3 groups of mice. Two other groups received 50 µl of a delipidated, unfractionated ASC containing 10 mg/ml plus OA or OA only. Figure 4B shows the anti-OA and anti-ASC IgE antibody production in these groups 21 days after immunization. In spite of its low OD at 280 nm, peak I-D suppressed the anti-OA response as much as the unfractionated sample. On the other hand, peak III-D was the only pool to induce a reasonable anti-ASC response.

The protein banding pattern of the whole extract was quite complex as revealed by SDS-PAGE (fig. 5A). In the delipidated material, however, the polypeptides above 67,000 daltons were not detected. Analysing the pools of fractions eluted in peaks I and III, we noticed that no bands were stained in both peaks I and I-D, whereas peaks III and III-D displayed several bands below 40,000 daltons.

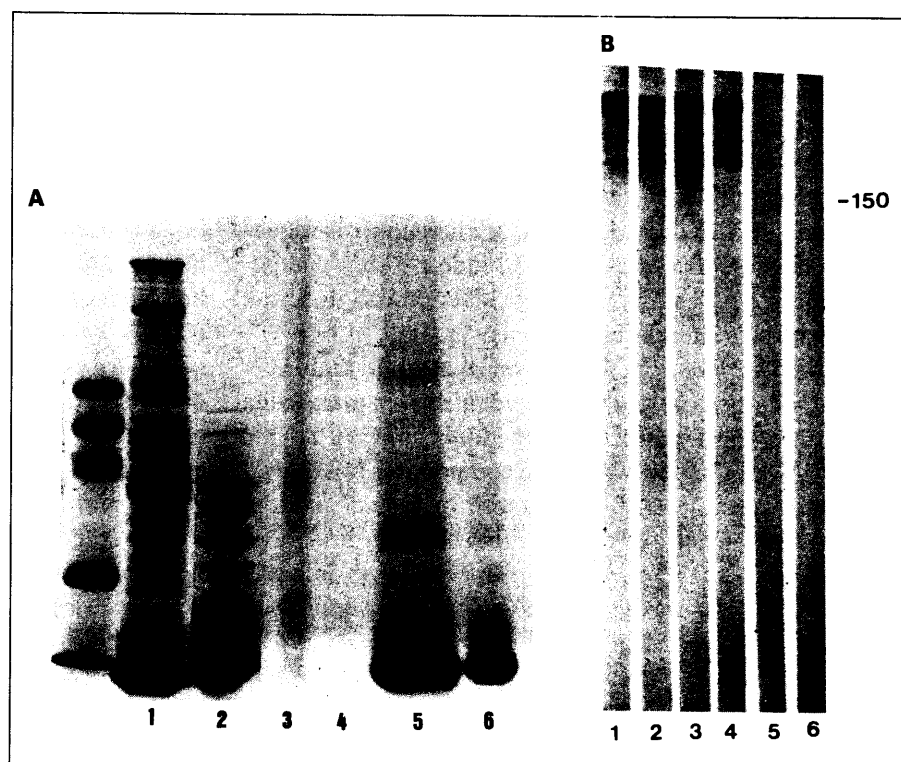
Immunoblots of these materials were then performed using a rabbit anti-peak I antiserum. The results (fig. 5B) showed at least 3 bands above 150,000 daltons in the unfractionated samples and in peaks I and I-D. In contrast, nothing was detected in peaks III and III-D.

In order to demonstrate the biological activity of the antigens recognized by immunoblotting, we performed an affinity chromatography, using anti-peak I antibodies as ligands. The effluent and eluate fractions obtained from the column were assessed for the ability to suppress an anti-OA or to induce an anti-ASC antibody response. Figure 6 shows that there was no alteration in the anti-OA response in the group injected with OA and unbound material. On the other hand, the anti-ASC response in this group was enhanced when compared to the group injected with the unfractionated extract. When the animals received OA and the fraction recovered by elution at basic pH, they displayed a diminished anti-OA response and no IgE antibodies to ASC were detected in this group.

Discussion

The studies described here showed that the suppressive components present in the *A. suum* extract and responsible for the previously reported damping of IgE antibody production in mice can be isolated from its immunogenic components by gel filtration chromatography. They were detected in the first peak eluted from a column of Sephadex G-200 or Sephacryl S-200 and had an apparent MW of

Fig. 5. A SDS-PAGE patterns of whole extract (lane 1), delipidated extract (lane 2), peak I (lane 3), peak I-D (lane 4), peak III (lane 5) and peak III-D (lane 6) in a 6–16% gradient gel. The MW markers (extreme left) included mouse IgG (150,000), BSA (67,000), OA (43,000), trypsinogen (24,000) and lysozyme (14,000). **B** Immunoblotting of the same preparations reacted with rabbit anti-peak I serum at 1:50 dilution.



530,000 daltons. However, since the major peak of carbohydrates was obtained in the same fractions, it is possible that this MW determined by an empirical method in this study may not represent a true value [22]. The biological activity of these components could be demonstrated not only in a heterologous but also in the homologous system. Thus, the anti-ASC antibody response obtained with an immunogenic fraction (eluted in peak III) was diminished upon concomitant injection of a suppressive fraction (eluted in peak I). Besides, when the suppressive components were removed by anti-peak I antibodies an enhanced anti-ASC IgE response was elicited in mice immunized with the non-reactive components. These results could therefore explain the low titres of anti-ASC IgE antibodies obtained with the whole extract [11, 13] and may indicate a predominance of the suppressive effect in the unfractionated material. To exclude the possibility that the suppression of specific IgE antibody response determined by PCA reactions was due to receptor occupancy on mast cells by non-specific IgE immunoglobulins, we performed some ELISA titrations. Plasmas of OA- or OA + ASC-immunized mice were used in the assays. As expected, the results previously obtained were confirmed for both anti-OA IgE and IgG antibodies (data not shown).

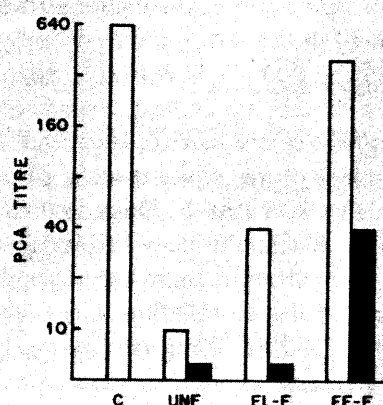


Fig. 6. Biological activity of extract fractions obtained by affinity chromatography against anti-peak I antibodies as ligands. Anti-OA (□) and anti-ASC (■) IgE antibody titres were determined on day 21 in mice immunized with OA (C) or OA plus the unfractionated extract (UNF), the effluent (EF-F) or the elution (EL-F) fractions.

Allergens from *A. suum* perienteric fluid and body extracts have been isolated by gel filtration and identified as low-MW components (14,000–45,000) in rats, rabbits and man [10, 31–33]. As we demonstrated here, similar components seem to be allergenic in the mouse too. Besides, we showed that the first peak eluted from the chromatography column, which did not induce or elicit anaphylactic responses in these models, contained indeed other components, which could suppress the production of IgE to the own as well as to unrelated antigens.

It should be mentioned, however, that some high-MW (360,000) allergens have also been identified in man, using sera from naturally infected individuals [34, 35]. At the present, it is not known why these components fail to trigger a more prominent IgE response in animal models. This is probably not due to the employment of unnatural hosts, since it was shown that in *N. brasiliensis*-infected rats (natural host) the allergenic activity was also associated with low-MW allergens [35].

We have recently observed [36] that lipoproteins isolated from the whole extract did not have any suppressive activity in the IgE antibody response to OA. In the present work, the lipoprotein-free extract was fractionated and the results showed that the small amount of proteins obtained in the first eluted peak was sufficient to induce the same suppression as the unfractionated material applied to the column.

Precipitation of lipoproteins, on the other hand, resulted in alteration of the banding pattern in SDS-PAGE, with loss of stained bands above MW 67,000. The absence of these bands as well as bands in peaks I and I-D, which displayed suppressive activity in vivo, suggested that we were facing a problem of low concentration of polypeptides not detectable by SDS-PAGE. This was further confirmed in immunoblots where at least 3 bands above MV 150,000 could be recognized in these same preparations with anti-peak I antibodies. In addition, when these antibodies were used as ligands in affinity chromatography, we obtained an effluent fraction devoid of any suppressive effect, which was recovered in the elution fraction. Part of the reactive components, however, may have been lost in the purification steps, leading to a less pronounced suppression of the OA response when compared to the whole extract. As mentioned before, anti-ASC IgE antibodies were only induced by the effluent but not by the elution fraction. These results demonstrated, therefore, that the suppressive components could also be isolated from the immunogenic ones through IgG antibodies raised against peak I, which did not cross-react with peak III.

Regarding the possible mechanisms underlying the

suppressive phenomenon described here, we demonstrated that distinct regulatory mechanisms are involved in the suppression of IgE and IgG isotypes [16].

As it is well known by now, several cytokines regulate the switching and expression of specific immunoglobulin isotypes [37, 38]. Cytokines do also present cross-inhibitory effects that may account for reciprocal regulation of different helper T cell clones [39]. Finkelman et al. [40, 41] have demonstrated in in vivo studies on isotype selection, using *N. brasiliensis*-infected mice, that interleukin-4 is required for generation and maintenance of antigen-specific and polyclonal IgE responses. In addition, interferon- γ inhibits both IgE and IgG1 responses and induces IgG2a production.

Based on these findings we may, therefore, suppose that the preferential stimulation of one or more cytokines by the suppressive components of the extract would interfere with the response towards the immunogenic components as well as unrelated antigens.

Further studies required to elucidate the proper role of cytokines in the mechanisms responsible for the extract-induced suppression of humoral immune responses are now underway.

Acknowledgments

M.F.M.S. was in receipt of a fellowship from CNPq (303347/87-2) and FAPESP (84/2754-8).

References

- Jarrett EEE, Miller HRP: Production and activities of IgE in helminth infections. *Prog Allergy* 1982;31:178-233.
- Barriga OO: Immunomodulation by nematodes: A review. *Vet Parasitol* 1984;14:299-320.
- Ishizaka K: Regulation of immunoglobulin E biosynthesis. *Adv Immunol* 1989;47:1-43.
- Weizger I, Patterson R, Pruzanski JD: *Ascaris* hypersensitivity in the rhesus monkey. I. A model for the study of immediate type hypersensitivity in the primate. *J Allergy* 1968;91:14-22.
- Dobson C, Morseth DJ, Soulsby EJJ: Immunoglobulin E-type antibodies induced by *Ascaris suum* infections in guinea pigs. *J Immunol* 1971;106:128-133.
- Bradbury SM, Percy DH, Strejan GH: Immunology of *Ascaris suum* infection. I. Production of reaginic antibodies to worm components in rats. *Int Arch Allergy Appl Immunol* 1974;46:498-511.
- Crandall CA: *Ascaris suum*: Homocytotropic antibody responses in mice. *Exp Parasitol* 1976;39:69-73.
- Strejan J, Campbell DH: Hypersensitivity to *Ascaris* antigens. I. Skin-sensitizing activity of serum fractions from guinea pigs sensitized to crude extracts. *Immunology* 1967;98:893-898.
- Okumura K, Tada T: Regulation of homocytotropic antibody formation in the rat. III. Effect of thymectomy and splenectomy. *J Immunol* 1971;106:1019-1025.
- Hussain R, Bradbury SM, Strejan F: Hypersensitivity to *Ascaris* antigens. VIII. Characterization of a highly purified allergen. *J Immunol* 1973;111:260-268.
- Macedo MS, Braga F, Mota I: Control of IgE and IgG1 antibody production in mice. *Cell Immunol* 1976;23:342-355.
- Stromberg BE: IgE and IgG1 antibody production by a soluble product of *Ascaris suum* in the guinea pig. *Immunology* 1979;38:489-496.
- Macedo MS, Catty D: Effect of X-irradiation on the homocytotropic and agglutinating antibody production in mice. *Immunology* 1977;33:611-619.
- Macedo MS, Mota I: Antigenic competition in IgE antibody production. I. Establishment of parameters involved in primary and secondary responses. *Immunology* 1980;40:701-707.
- Soares MFM, Macedo MS, Mota I: Antigenic competition in IgE, IgG1 and IgG2 antibody production in the mouse. *Int Arch Allergy Appl Immunol* 1985;78:449-451.
- 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;20:203-211.
- Macedo MS, Mota I: Antigenic competition in IgE antibody production. II. Effect of cyclophosphamide. *Immunology* 1982;47:67-74.
- Soares MFM, Macedo MS, Perini A, Mota I: Antigenic competition in IgE antibody production. III. Suppressive effect on Th and B cells. *Int Arch Allergy Appl Immunol* 1986;79:196-201.
- Soares MFM, Oliveira EB, Mota I, Macedo MS: Suppression of IgE antibody production by *Ascaris suum* extract: Characterization of suppressive component(s). *Braz J Med Biol Res* 1988;21:527-529.
- Lowry OH, Rosebrough NJ, Farr AC, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-275.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F: Colorimetric methods for determination of sugars and related substances. *Anal Chem* 1956;28:350-356.
- Andrews P: Estimation of molecular size and molecular weights of biological compounds by gel filtration; in Glick D (ed): *Methods of Biochemical Analysis*. New York, Wiley, 1970, pp 1-53.
- Gidz LI: Ultracentrifugal separation of lipoproteins; in Williams CA, Chase MW (eds): *Methods in Immunology and Immunochemistry*. New York, American Press, 1968, pp 302-310.
- Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-685.
- Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* 1979;76:4350-4356.
- Johnstone A, Thorpe R: Purification of immunoglobulins, constituent chains and fragments; in Johnstone A, Thorpe R (eds): *Immunochemistry in Practice*. London, Blackwell, 1982, pp 41-75.
- Mota I, Wong D: Homologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sci* 1969;8:813-820.
- 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;117:1622-1628.
- 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.
- Ambler J, Doe JE, Gemmel DK, Roberts JA, Orr TSC: Biological techniques for studying the allergenic components of nematodes. I. Detection of allergenic components in *Ascaris suum* extracts. *J Immunol Methods* 1972;1:317-324.
- Hoggarth-Scott RS: The molecular weight range of nematode allergens. *Immunology* 1967;13:535-541.
- Ambler J, Miller JN, Johnson P, Orr TSC: Characterization of an allergen extracted from *Ascaris suum*: Determination of the molecular weight, isoelectric point, amino acid and carbohydrate content of the native allergen. *Immunochemistry* 1973;10:815-820.
- Tsugi M, Hayashi T, Yamamoto S, Sakata Y, Toshida T: IgE type antibodies to *Ascaris* antigens in man. *Int Arch Allergy Appl Immunol* 1977;55:78-81.
- Donnel IJO, Mitchell GF: An investigation of the allergens of *Ascaris lumbricoides* using a radioallergosorbent test (RAST) and sera of naturally infected humans: Comparison with an allergen for mice identified by a passive cutaneous anaphylaxis test. *Aust J Biol Sci* 1978;31:459-487.
- Mc William AS, Stewart GA, Turner KL: An immunochemical investigation of the allergens from *Ascaris suum* perienteric fluid. *Int Arch Allergy Appl Immunol* 1987;82:125-132.
- Soares MFM, Mota I, Macedo MS: Further characterization of *Ascaris suum* component(s) with suppressive activity on the IgE antibody response. *Braz J Med Biol Res* 1990;23:589-592.
- Clifford MS, Finkelman FD, Paul WE: Regulation of IgG1 and IgE production by interleukin 4. *Immunol Rev* 1988;102:51-75.
- Coffman RL, Savelkoul HFJ, Lebman DA: Cytokine regulation of immunoglobulin isotype switching and expression. *Semin Immunol* 1989;1:55-63.
- Mosmann TR, Coffman RL: Heterogeneity of cytokine secretion patterns and functions of IL helper T cells. *Adv Immunol* 1989;46:111-147.
- Finkelman FD, Katona IM, Urban JF, Holmes J, Ohara J, Tung AS, Sample JG, Paul WE: IL-4 required to generate and sustain in vivo IgE responses. *J Immunol* 1988;141:2335-2341.
- Finkelman FD, Holmes J, Katona IM, Urban JF Jr, Beckmann MM, Park LS, Schooley KA, Coffman RL, Mosmann TR, Paul WE: Lymphokine control of in vivo immunoglobulin isotype selection. *Annu Rev Immunol* 1990;8:303-333.