

Eosinophilic inflammation and airway hyper-responsiveness are profoundly inhibited by a helminth (*Ascaris suum*) extract in a murine model of asthma

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Summary

Background The increase of atopic disorders in developed countries has been associated with the decline of infectious diseases, including helminthic infections. We have already demonstrated that adult worm extracts from *Ascaris suum* (ASC) suppress the IgE antibody production against unrelated antigens.

Objective Here we investigated the influence of ASC on the development of pulmonary eosinophilic inflammation in a murine model of asthma.

Methods Heat-coagulated egg white alone (EWI) or mixed with ASC (EWI + ASC) was implanted subcutaneously in B10.A or C57BL/6 mice, and 14 days later they were challenged intratracheally with OVA or exposed to aerosolized OVA for 4 days.

Results The suppressive effect of ASC was demonstrated on the accumulation of cells into airways, with reduction of eosinophil numbers and of eosinophil peroxidase activity in EWI + ASC-immunized mice. This effect correlated with a marked reduction of IL-5 and IL-4 levels in the BAL from C57BL/6 and B10.A mice, respectively, and of eotaxin in BAL and lung tissue from both strains. OVA-specific IgG1 and IgE levels were also impaired in serum and BAL from these mice. Airway hyper-reactivity to methacholine was obtained in B10.A mice sensitized with EWI, but the respiratory mechanical parameters returned to normal levels in EWI + ASC-immunized mice.

Conclusion These results indicate that ASC has a profound inhibitory effect on lung inflammation and hyper-responsiveness and that suppression of IL-5 or IL-4 and of eotaxin contributes to this effect.

Keywords asthma, eosinophil, airway hyper-responsiveness, cytokine, chemokine, eosinophil peroxidase, IgE

Submitted 26 February 2002; revised 30 April 2002; accepted 11 July 2002

Introduction

The pathogenesis of asthma has been directly associated with eosinophilic inflammation of the airways. Among eosinophil-active cytokines, IL-5 was shown to be the primary determinant of eosinophil differentiation, activation, and survival [1]. The importance of IL-5 in the development of antigen-induced eosinophilia was supported by Foster and colleagues [2] using genetically deficient mice. Mice in whom the IL-5 gene was disrupted did not develop aero-allergen-induced pulmonary eosinophilia and reconstitution of these mice with IL-5 completely restored their response. Furthermore, IL-5 and eotaxin have been shown to cooperate in the orchestration of eosinophil accumulation in tissues, because IL-5 increases responsiveness to eotaxin [3] and regulates the expression of CCR3 receptors for eotaxin in eosinophils [4].

Epidemiological surveys reveal that the increase in the cases of asthma has frequently been accompanied by the decrease of infections acquired in the childhood. Shirakawa and colleagues

[5] followed Japanese schoolchildren and observed a strong inverse association between delayed-type hypersensitivity to bacille Calmette-Guérin (BCG) and atopy. Positive tuberculin responses predicted a lower incidence of asthma, lower serum IgE levels, and cytokine profiles biased towards a type-1 profile. The prevalence of *Ascaris lumbricoides* infections has also declined in the Japanese population over the past 50 years, while the prevalence of atopic disorders has increased [6]. Comparative studies of populations with varying degrees of helminthic infection have indicated that infection of low intensity may potentiate [7, 8], and heavy infection suppress, allergic reactivity [9–11]. Lynch and colleagues [12] reported that anti-helminthic treatment of a group of heavily infected children in a slum area in Venezuela resulted in a decrease in the initially elevated total serum IgE levels and an increase in skin test reactivity and specific IgE antibody levels against environmental allergens.

Cytokines derived from the Th2 response induced by helminths have a crucial role in the immunomodulatory ability of these parasites [13, 14]. For example, we have shown that an *Ascaris suum* body extract (ASC) contains high molecular weight protein components which display suppressive effects on the Th1 and Th2 responses to ovalbumin (OVA), decreasing delayed-type hypersensitivity reactions and the levels of IgM,

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IgG2a, IgG1 and IgE antibodies [15–17]. The utilization of monoclonal antibodies to neutralize IL-4 and IL-10 confirmed the participation of these cytokines in the suppression of the OVA-specific Th1 response [18]. Recently, Yasdanbakhsh and colleagues [19] suggested that an anti-inflammatory cytokine network established during chronic helminth infections and mediated by IL-10 and TGF- β might be responsible for the lower prevalence of allergic diseases in infected populations.

Due to the experimental and ethical limitations of studies with humans, several models of lung hypersensitivity have been developed in animals, in order to reproduce the whole net of inflammatory events that take place in the lung. For the induction of lung inflammation, most experimental protocols consist of immunizations with adjuvant and multiple antigenic boosters. Pereira de Siqueira and colleagues [20] developed a new protocol of immunization with a subcutaneous implant of heat-coagulated hen's egg white that induces a typical late-phase reaction (LPR) with a prominent eosinophilic infiltrate. It consisted of immunizing mice once only and challenging them intratracheally with OVA after two weeks.

The aim of the present study was to verify whether ASC could suppress eosinophilic lung inflammation and hyper-responsiveness in this murine model of asthma. For this, ASC was added to egg white solution before heat coagulation. In some experiments the animals received an aerosol, instead of intratracheal, challenge for 4 consecutive days. Since the genetic background determines the extent of IgE production and development of allergic reactions, we tested five mouse strains for the induction of LPR following implant of egg white fragment. The B10.A and C57BL/6 mice that gave the strongest reactions were chosen for the experiments reported here. The effect of ASC on accumulation of cells into airways, cytokine, chemokine and antibody production, as well as airway hyper-reactivity was analysed in these mice.

Materials and methods

Animals

Seven to eight-week-old male B10.A or C57BL/6 mice, and three-month-old male Wistar rats were obtained from a colony at the Institute of Biomedical Sciences, University of São Paulo, Brazil. The animals were maintained at the animal house facilities of the Department of Immunology, under specific pathogen-free conditions.

Preparations of antigens

Pasteurized and lyophilized hen's egg white (ITO Avicultura, São Paulo, Brazil) was prepared at 100 mg/mL in PBS, and 30 μ L (3 mg) were placed in a microwave oven for 2 min. The solidified egg white was dehydrated in ethanol for 24 h and hydrated again in PBS for 2 h before implantation in the upper dorsal region of a mouse.

Ascaris suum extract (ASC) was prepared as previously described [16] and 30 μ L of reconstituted ASC containing 3 mg of lyophilized extract (1.5 mg of protein) were added to the 30 μ L of egg white solution before heat coagulation.

Immunization and challenge protocols

Egg white implant (EWI): heat-coagulated hen's egg white alone (EWI) or mixed with ASC (EWI + ASC) was implanted

into the subcutaneous tissue of the dorsal region of B10.A or C57BL/6 mice.

Antigen challenge: 14 days later animals received 600 μ g of grade II ovalbumin (OVA) by intratracheal instillation or were exposed daily for 4 days to 1% aerosolized OVA (2 mL) for 20 min. The aerosol was delivered into an adapted chamber by an ultrasonic nebulizer (ICEL US-800, São Paulo, Brazil). Groups of non-sensitized mice were also challenged in one way or the other.

Death: groups of mice were killed 1, 2, 3 or 7 days after intratracheal challenge and 6, 24 or 48 h after the last aerosol challenge (day 17 of immunization).

Bronchoalveolar lavage (BAL)

Mice were killed by an overdose of chloral hydrate and the lungs were washed with 10 mL of saline with 10 mM ethylenediaminetetraacetic acid (EDTA) through the pulmonary artery. The tracheas were cannulated and the airway lumen washed with 6 $\times$ 0.5 mL of Hank's balanced salt solution (HBSS) or HBSS + EDTA (for chemokine quantification). The resulting BAL fluids were immediately centrifuged at 100 g, 4 °C, for 10 min. The supernatants were stored for cytokine, chemokine and antibody determination. The cell pellets were resuspended in 1 mL of HBSS for cell counts and (eosinophil peroxidase (EPO) assay. Total cell counts were performed in a haemocytometer and differential cell counts in cytocentrifuge preparations stained with Wright-Giemsa. The lungs were then removed, weighed and homogenized to give a 5% (wt/vol) suspension in HBSS and centrifuged (1500 g, 10 min). After lysis of red blood cells, the pellet was homogenized in HBSS + 0.5% HTAB (hexadecyltrimethylammonium bromide) for EPO assay or +10 mM EDTA for chemokine quantification.

EPO assay

EPO activity was measured in BAL and lung tissue according to the method of Strath and colleagues [21]. The suspension of BAL cells or the lung tissue was frozen/thawed three times using liquid nitrogen. After centrifugation at 4 °C, for 10 min, the supernatant was serially diluted and placed in a 96-well plate (75 mL/well) followed by the addition of 150 mL of substrate (1.5 mM OPD and 6.6 mM H₂O₂ in 0.05 M Tris-HCl, pH 8.0). After 30 min at room temperature, the reaction was stopped by the addition of 75 mL of 0.2 M citric acid and absorbance of the samples was determined at 492 nm.

Histochemical procedures for identification of cyanide-resistant EPO activity in lung frozen tissue followed the technique described by Tibério and colleagues [22]. Slides were counterstained in haematoxylin (Sigma, St Louis, MO, USA) and mounted with permount (Fisher Scientific Co., Pittsburgh, PA, USA).

Quantification of cytokines and chemokines

Cytokines and chemokines were measured in BAL fluid by specific two-site sandwich ELISA, using the following mAb: for IL-4, BVD-1D11 and biotinylated BVD6-24G2; for IL-5, TRFK5 and biotinylated TRFK4; for IFN- γ , XMG 1.2 and biotinylated AN18; for IL-10, 2A5 and biotinylated SXC-1; for eotaxin, AF420NA and biotinylated BAF420 and for MCP-1, 10F7 and biotinylated 5D3-F7. Binding of biotinylated monoclonal antibodies was detected using

streptoavidin-biotinylated horseradish peroxidase complex (Amersham Int., Amersham, UK) and 2–2'-azino-bis(3-ethylbenz-thiazoline- 6-sulphonic acid) or O-phenylenediamine (OPD; Sigma) in 0.1 M citrate buffer containing hydrogen peroxide. Samples were quantified by comparison with standard curves of recombinant mouse cytokines or chemokines. Each figure is representative of at least three experiments with similar results.

Titration of IgG1 anti-OVA by ELISA

Blood samples were obtained 14 days after immunization and 2 days after aerosol challenge by retro-orbital bleeding. Plasma were tested for IgG1 antibodies using OVA-coated 96-well plates and biotinylated goat anti-mouse IgG1 antiserum. The reactions were developed with streptoavidin-peroxidase conjugate (Sigma), OPD and H₂O₂ and the plates were read at 450 nm on an automated ELISA reader (Dynatech, MR 5000). Titration curves were carried out for all samples and the results are shown as the mean $\pm$ SEM absorbance at various sera dilutions for six samples/group. Titration of IgG1 antibodies in the BAL fluid obtained 2 days after challenge followed the same protocol.

Passive cutaneous anaphylaxis

Since the protocol of immunization employed induces very high amounts of IgG1 compared to IgE [23] and the former competes with the latter for antigen binding in sandwich ELISA, the IgE antibody response was measured by passive cutaneous anaphylaxis (PCA) as described by Mota and Wong [24]. PCA reactions were performed in rats, using a sensitization period of 18–24 h and OVA in Evan's blue solution for challenge. The PCA titre was expressed as the reciprocal of the highest dilution of pooled sera or BAL fluid that gave a lesion of more than 5 mm in diameter in triplicate tests. The detection threshold of the technique was established at 1 : 5 dilution.

Measurement of airway responsiveness

Conductance of the respiratory system (Grs) and dynamic compliance (Cr_s) were monitored in anaesthetized, tracheostomized, and ventilated mice in a Harvard 386 apparatus. Different doses of methacholine (3–10 000 μ g/kg) were administered via a syringe catheter tied into the jugular vein and data were stored at 30 s, 1, 2, 3 and 4 min after agonist injection. Whole body plethysmograph pressure (from which changes in lung volume were derived) and tracheal pressure were transduced by differential pressure transducers. The outputs were amplified and transferred to a computer in which 16 consecutive breaths were stored to average one data-point. The Grs and Cr_s peak response to each agonist dose was expressed as percentage of baseline value obtained before the next dose of agonist. The dose of methacholine required to reduce Grs to 60% (ED₆₀%), and Cr_s to 70% (ED₇₀%) of their control value was estimated from the log dose-response curve and chosen as our data-point.

Statistical analysis

Two-way analysis of variance followed by multiple comparisons using Tukey method [25] was employed to compare antibody levels, cytokine and chemokine concentrations and mechanical data. Differences between EPO levels and cell numbers in the several groups were analysed by the Mann–Whitney test. Differences were considered statistically significant at $P < 0.05$. The SPSS statistical package (Release 8.0, Standard Version, 1997) was used.

Results

Suppression of allergen-induced airway eosinophilia in ASC-immunized mice

The effect of ASC on the recruitment of cells to the airway lumen was evaluated in B10.A and C57BL/6 mice immunized with EWI or EWI + ASC and intratracheally challenged with OVA on day 14. Figure 1 shows that in both strains the total number of cells recovered in BAL fluid from EWI-immunized mice 7 days after challenge was about 70-fold higher than the number recovered from non-immunized mice that were only challenged. Mobilization of eosinophils accounted for 50% of these cells. In the EWI + ASC group, however, the total amount of cells was significantly reduced as well as the influx of eosinophils. A similar effect was observed when groups of mice immunized in the same way received an aerosol challenge of antigen, although the cell migration at the peak of response (24–48 h after the last allergen inhalation) was less vigorous (data not shown).

In the lung tissue, the accumulation of eosinophils was evaluated by the levels of EPO (Fig. 2). The high EPO activity found in homogenates of lung tissue from EWI-immunized mice were in agreement with an intense lung eosinophilia, which was also greatly diminished in EWI + ASC-immunized mice, as indicated by the lower EPO activity detected in their lung tissue. In addition, the EPO activity in BAL confirmed the results obtained with differential cell counts. A reduction of EPO activity was observed in lysates of BAL cells from EWI + ASC groups of both strains of mice compared with EWI groups.

Inhibition of IL-4 and IL-5 levels in BAL fluid

IL-4 and IL-5 are the main cytokines involved in eosinophilic inflammation [26] and were therefore measured in BAL fluid at different time-points after intratracheal challenge of sensitized mice. Figure 3 shows that both cytokines were detected in EWI groups only on days 2 and 3, but not on day 1 (data not shown) or 7 following challenge. Interestingly, whereas IL-4 was produced by most of B10.A mice (Fig. 3a), IL-5 was detected in the

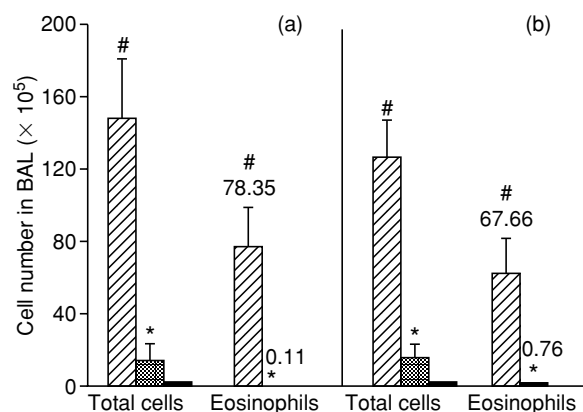


Fig. 1. Effect of ASC on the airway inflammation induced by EWI. B10.A (a) or C57BL/6 (b) mice were immunized with EWI (striped column) or EWI + ASC (dotted column), and 14 days later they were intratracheally challenged with OVA. Non-immunized mice challenged with OVA only were included as controls (black column). Seven days after challenge, BAL fluid was collected and total cell and eosinophil numbers were analysed. The results represent the mean $\pm$ SEM for 6 animals/group. * $P < 0.05$ compared with EWI group; # $P < 0.05$ compared with control group.

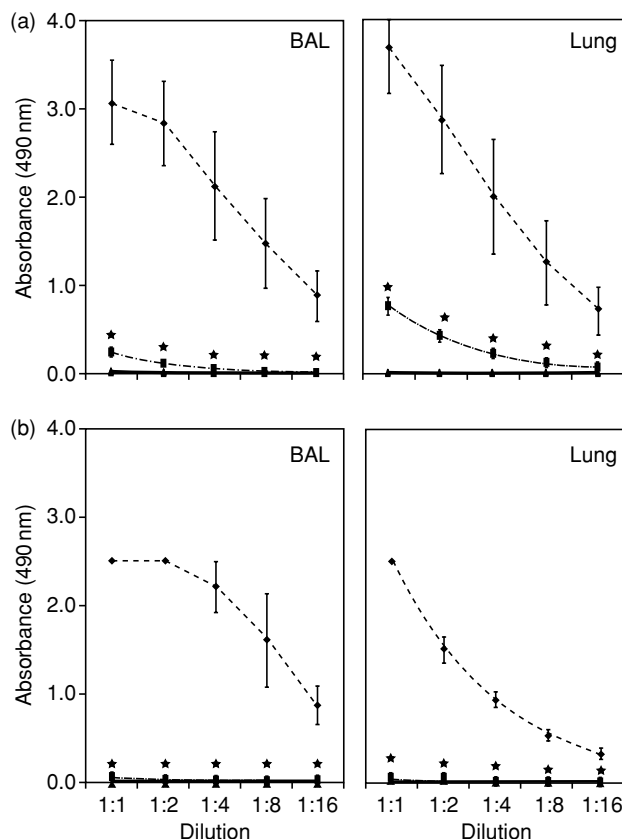


Fig. 2. Measurement of EPO activity in lung tissue and in BAL cells. B10.A (a) or C57BL/6 (b) mice were immunized with EWI (dotted line) or EWI + ASC (dashed line), and 14 days later they were intratracheally challenged with OVA. Non-immunized mice challenged with OVA only were included as controls (full line). Seven days after challenge, BAL cells and lung tissue were collected for EPO measurement. The results represent the mean $\pm$ SEM absorbance for six animals/group. * $P < 0.05$ compared with EWI group.

BAL of the majority of C57BL/6 mice (Fig. 3d). EWI + ASC-immunized mice did not produce any IL-5 and only two out of 6–7 animals had detectable levels of IL-4 in both strains.

The decrease in IL-4 and IL-5 production in the latter groups was not compensated by an increase in IFN- γ levels. The amount of this cytokine remained below the detection threshold (< 1.56 ng/mL) in both strains of mice in all time-points analysed. IL-10 was also not detected in BAL after OVA challenge (< 3.12 U/mL).

Inhibition of eotaxin expression during the development of OVA-induced lung eosinophilia

Although we were unable to detect cytokines in BAL fluid from any group of EWI-sensitized, aerosol challenged mice (data not shown), the presence of chemokines in BAL fluid and lung tissue was noticed from 6 to 48 h after the last aerosol challenge. Figure 4 shows the concentrations of eotaxin found in lung tissue 6 h (highest levels) and in BAL 48 h after challenge. In the BAL fluid, a significant difference between EWI-immunized and non-immunized mice was observed only in the C57BL/6, but not in the B10.A strain. B10.A mice, however, had augmented levels of eotaxin at 24 h after antigen challenge (data not shown). The results depicted in Fig. 4 also reveal that the

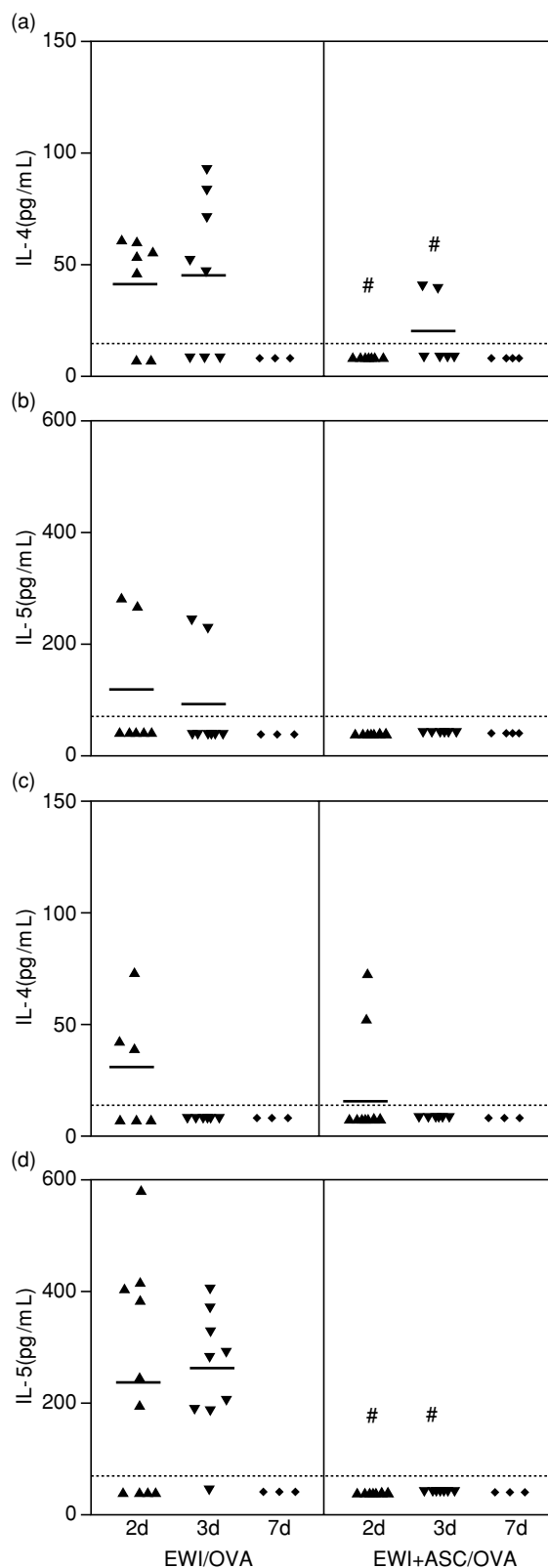


Fig. 3. Effect of ASC on IL-4 and IL-5 levels in BAL fluid. B10.A (a and b) or C57BL/6 (c and d) mice were immunized with EWI or EWI + ASC, and 14 days later they were intratracheally challenged with OVA. Two, three, and seven days after challenge, BAL fluid was collected for individual cytokine measurement by ELISA (horizontal bars, mean values). Dotted lines represent the detection limit of the assays (16 pg/mL for IL-4 and 78 pg/mL for IL-5). # $P < 0.05$ compared with EWI/OVA group.

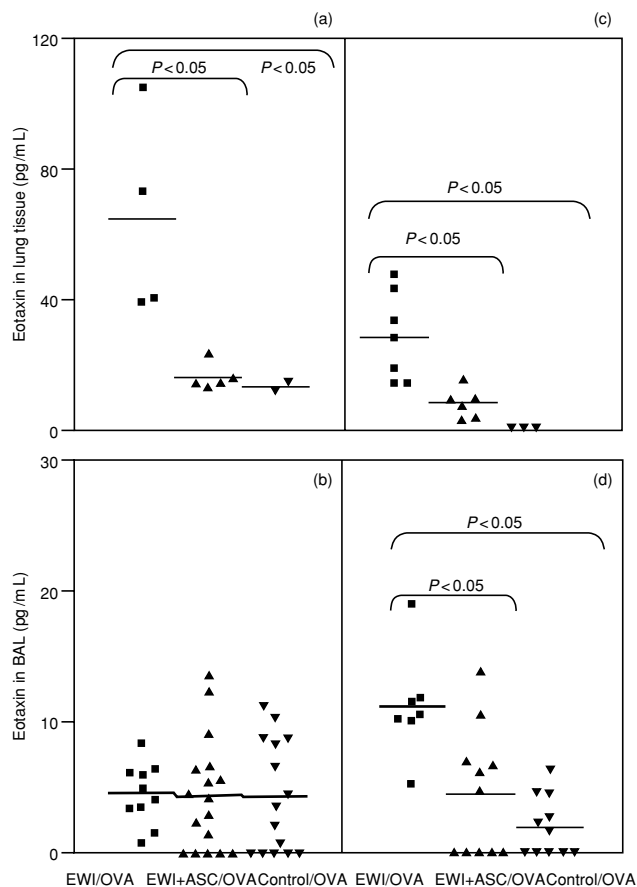


Fig. 4. Measurement of eotaxin in lung tissue and in BAL fluid. B10.A (a and b) or C57BL/6 (c and d) mice were immunized with EWI or EWI + ASC, and 14 days later they were challenged with aerosolized OVA for 4 consecutive days. Non-immunized mice challenged with OVA only were included as controls. The results express individual eotaxin amounts in lung tissue 6 h after the last challenge and in BAL fluid after 48 h (horizontal bars, mean values).

presence of ASC in the implants markedly inhibited eotaxin expression in lung tissue of both strains of mice and in airways of C57BL/6 mice. Similarly, the suppressive effect of ASC was evident on the eotaxin levels found in BAL fluid of B10.A mice at 24 h (data not shown).

Monocyte chemoattractant protein 1 (MCP-1) was also quantified but its concentration was similar among sensitized and non-sensitized groups (data not shown).

Suppressive effect of ASC on IgE and IgG1 antibody production

There is much evidence supporting a role for IgE antibodies not only in the immediate-phase of allergic reactions, but also during the ensuing late-phase reaction that is frequently accompanied by increased airway responsiveness [27]. Therefore, we assessed the production of murine anaphylactic antibodies (IgE and IgG1) in EWI- and EWI + ASC-immunized mice. As it can be observed in Figs 5a and b, the primary IgG1 response represented by the serum levels of OVA-specific antibodies on day 14 of immunization was suppressed in the EWI + ASC group compared with the EWI group. Although there was no difference between these groups in the secondary antibody response

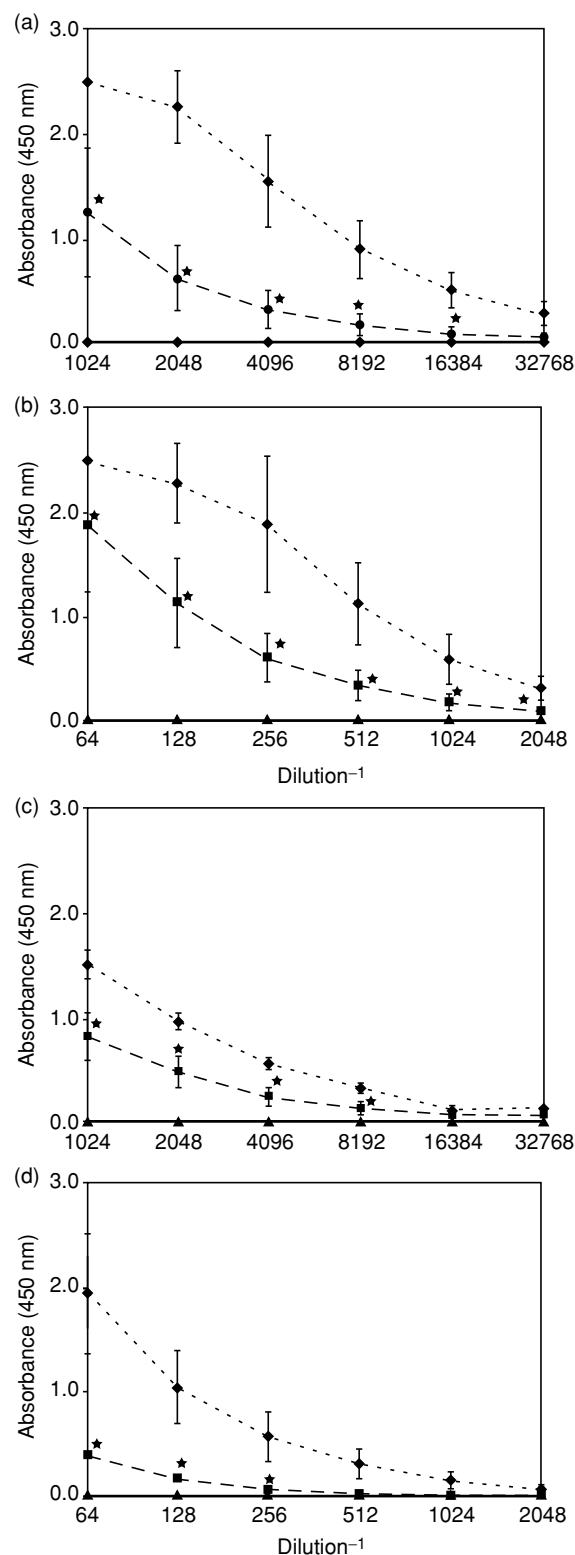


Fig. 5. Effect of ASC on OVA-specific IgG1 levels in serum and BAL fluid. B10.A (a and b) or C57BL/6 (c and d) mice were immunized with EWI (dotted line) or EWI + ASC (dashed line), and 14 days later they were bled and challenged with aerosolized OVA for 4 consecutive days. Non-immunized mice challenged with OVA were also bled before challenge and included as controls (full line). Forty-eight hours after the last aerosol challenge BAL fluid was collected for IgG1 determination by ELISA. The results in serum (a and c) and BAL fluid (b and d) represent the mean $\pm$ SEM absorbance for six animals/group. * $P < 0.05$ compared to EWI group.

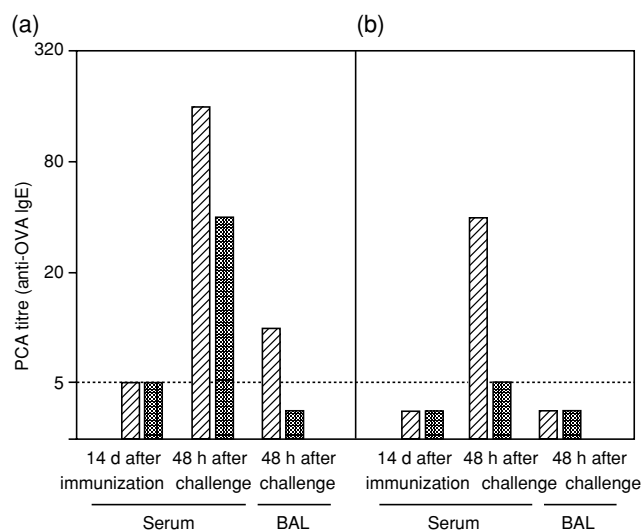


Fig. 6. Effect of ASC on OVA-specific IgE levels in serum and BAL fluid. B10.A (a) or C57BL/6 (b) mice were immunized with EWI (striped column) or EWI + ASC (dotted column), and 14 days later they were bled and challenged with aerosolized OVA for 4 consecutive days. Forty-eight hours after the last aerosol challenge they were bled again and BAL fluid was collected for IgE determination by PCA. The PCA titre represents the highest dilution of pooled sera or BAL fluid that gave a positive reaction (diameter > 5 mm).

measured in serum 48 h postallergen inhalations (data not shown), the levels of IgG1 antibodies found in BAL fluid were still significantly different at this same time-point (Figs 5c and d). Of note and consistently with the IL-4 data presented above, B10.A mice possessed significantly more OVA-specific IgG1 antibodies in serum and BAL fluid than C57BL/6 mice (Fig. 5).

The IgE antibody response (Fig. 6) rose in serum only after allergen challenge, as previously demonstrated [23], but the PCA titres were lower in EWI + ASC-immunized mice as compared to EWI-immunized mice of both strains. Again, B10.A mice produced more OVA-specific IgE antibodies than C57BL/6 mice. These antibodies were also detected in BAL fluid only of B10.A mice and when they received EWI-but not EWI + ASC-implants (Fig. 6a).

Airway hyper-reactivity is abolished in ASC-immunized mice

To determine the effect of ASC on the development of respiratory hyper-reactivity a dose-response curve to methacholine was evaluated *in vivo* 48 h after the last aerosol challenge (the intratracheal challenge was not suitable for these experiments because of technical limitations). Values of Crs, which represents distal bronchioli and lung tissue, and Grs, which represents airway mechanical data, were obtained by volume, airflow and pressure data. The results in Table 1 are expressed as log of the dose of methacholine that reduced to 70 and 60% the baseline values of Crs and Grs, respectively. In B10.A mice, allergen challenge induced in EWI-immunized mice a significant reduction in Crs and Grs compared with the control group, indicating a high level of airways and lung tissue reactivity to the administered agonist. This respiratory hyper-reactivity to methacholine was abolished in mice immunized with EWI + ASC, which displayed Crs and Grs values similar to those of non-immunized mice. Eosinophils were also quantified

Table 1. Effect of ASC on lung hyper-reactivity development in EWI-immunized mice after the aerosol challenge

B10.A	ED60%Grs (Conductance)	ED70%Crs (Compliance)
EWI/OVA	0.91 ± 0.18#	1.08 ± 0.15#
EWI + ASC/OVA	2.13 ± 0.27*	1.94 ± 0.11*
Control/OVA	2.38 ± 0.42	2.33 ± 0.12

B10.A mice were immunized with EWI or EWI + ASC and challenged after 14 days with aerosolized OVA for 4 consecutive days. Non-immunized mice challenged in the same way were used as controls (Control/OVA). Forty-eight hours after the last challenge, lung hyper-reactivity to methacholine was evaluated. Dose-response curves to methacholine were obtained by the intravenous administration of 3–10 000 µg/kg. The conductance of the respiratory system (Grs) and dynamic compliance (Crs) at each dose of methacholine were expressed as a percentage of the baseline value. The dose of methacholine required to reduce Grs to 60% (ED60%), and Crs to 70% (ED70%) of their control values was estimated from the log dose-peak response curve. **P* < 0.05 compared with EWI/OVA group; #*P* < 0.05 compared with Control/OVA group.

in the lung tissue from these mice using histochemical procedures for the identification of cyanide-resistant EPO activity. A lower number of EPO-positive cells was found among 300 inflammatory cells in the EWI + ASC group compared with the EWI group (4.25 ± 0.65 vs. 35.33 ± 1.91). Airway hyper-responsiveness (AHR) was also evaluated in C57BL/6 mice but neither EWI nor EWI + ASC group differed from the control group in regard to both respiratory mechanical parameters (data not shown).

Discussion

Allergic asthma is a chronic disease with a complex and multifactorial aetiology. In the immediate phase, bronchoconstriction is initiated by pharmacological mediators released by mast cells that are activated by allergen-specific IgE antibodies in the presence of allergen. The late-phase reaction is associated with increase in bronchial reactivity and a chronic and persistent obstruction provoked by a severe eosinophilic inflammation in the lung. In this study we demonstrated that the extract from a helminth (*Ascaris suum*) could suppress the eosinophilic inflammation and airway hyper-reactivity induced by immunization with EWI and challenge with OVA. The results showed a striking decrease in eosinophil influx into the airway lumen and low levels of EPO activity in the lung tissue from EWI + ASC-immunized animals. The suppressive effect of ASC was similar in C57BL/6 and B10.A mice regardless of the way and route of antigen challenge.

The suppression of eosinophilic inflammation in C57BL/6 mice immunized with EWI + ASC seemed to be mostly due to the reduction of IL-5 and eotaxin levels, which are selective eosinophil chemoattractants [28], and were detected in EWI-immunized mice in significant amounts. The importance of IL-5 in eosinophil recruitment into the airways and development of AHR has been emphasized in different sensitization protocols [2, 29, 30]. In B10.A mice that received EWI + ASC, the reduction of eosinophil influx was accompanied, more clearly, by a decrease of IL-4 levels compared with EWI-immunized mice. The role of IL-4 in allergic sensitization and eosinophil

accumulation was also demonstrated in IL-4-deficient mice [31, 32]. In addition, it is known that IL-4 induces the expression of adhesion molecules for eosinophils in the endothelium [33] and eotaxin synthesis by lung epithelial cells [34].

Eotaxin, the pivotal chemokine in eosinophil recruitment, was also diminished in B10.A lung tissue from EWI + ASC-compared to EWI-immunized mice soon after aerosol challenge and in BAL fluid after 24 h, corresponding to the time-point in which the number of eosinophils found in BAL from EWI-immunized mice was highest (data not shown). Hence, reduction of both IL-4 and eotaxin might have led to impairment of eosinophilic inflammation in B10.A mice.

IL-10 and IFN- γ were not detected neither in B10.A or C57BL/6 BAL fluid nor in supernatants from draining lymph node cells restimulated *in vitro* with OVA at 14 or 21 days after immunization (data not shown). Therefore, the cytokine profile obtained in response to EWI was characterized by IL-4 and IL-5 dominance in B10.A and C57BL/6 mice, respectively.

As expected from previous reports [35, 36], this cytokine profile was associated with a larger production of IgE and IgG1 antibodies in B10.A than in C57BL/6 mice. The ability of passively transferred IgE and IgG1 antibodies to elicit not only immediate hypersensitivity reactions, but also AHR after limited airway exposure to antigen, was reported by Oshiba and colleagues [37]. The development of eosinophilic inflammation and AHR can occur in IgE-deficient mice [38], but this may result from allergen-specific IgG1 production. The same, however, can also be obtained in B cell-deficient mice, which do not produce any immunoglobulin, provided that systemic sensitization with adjuvant and repeated airway challenges are used [30], instead of airway sensitization [29]. A comparative analysis of all these reports led to the conclusion that IgE and IgG1 may be necessary for inducing AHR in models of sensitization that mobilize substantially lower numbers of eosinophils into the airways [30].

In our model, we used the aerosol challenge regimen, which resulted in about 4-fold less eosinophils in BAL fluid at the peak of the response compared with the intratracheal challenge, in both mouse strains that were submitted to measurement of respiratory mechanical parameters. In the B10.A strain, that produced high amounts of IgE and IgG1 antibody, lung hyper-responsiveness was demonstrated by the reduced values of Crs and Grs in response to methacholine in EWI-immunized mice. In contrast, EWI + ASC-immunized B10.A mice, that produced low levels of antibody, had Crs and Grs values similar to those of non-immunized mice, indicating a profound suppressive effect of ASC on AHR in this strain and a positive correlation with the inhibition of antibody production. C57BL/6 EWI-immunized mice did not show enhanced airway and distal lung tissue reactivity, which could be due to their lower IgE and IgG1 antibody levels. However, it is also known that this strain has decreased basal Crs and Grs compared to other mouse strains [39, 40], indicating that its genetic background has a critical influence on the pulmonary function.

Zhang and colleagues [41] also reported that C57BL/6 mice repeatedly sensitized with OVA differed from nonsensitized mice in Crs, but no significant difference was observed in regard to Grs. In addition, they found that OVA-immunized C57BL/6 mice had markedly decreased AHR, IL-4 transcript levels and OVA-specific IgE and IgG1 antibodies compared with BALB/c mice, despite similar degrees of eosinophilia in these two strains.

Consistent with this study, our results show that EWI-immunized C57BL/6 mice had decreased AHR, IL-4 and antibody levels compared with B10.A mice, though similar eosinophil numbers were found in BAL from these strains after OVA challenge.

It should be mentioned at this point that ASC suppressive components can be isolated from the allergenic ones by gel filtration chromatography [16, 42]. When these allergenic components are added to EWI, there is no change in the eosinophilic inflammation elicited by the OVA challenge (M. F. Macedo-Soares, Butantan Institute, personal communication), indicating that antigenic competition is not involved in the inhibition of eosinophil infiltration by ASC. In addition, ASC suppresses the IgE antibody production against OVA even when they are injected at separate sites [43], excluding any biochemical effect of the extract on EWI. Although a strong IgE antibody response can be obtained when the allergenic ASC components are injected with an adjuvant like alum, intratracheal instillation of ASC in EWI + ASC-immunized mice did not lead to an influx of eosinophils into the lung (data not shown). As discussed previously [42], these results reinforce the notion that the action of the suppressive components is prevalent in the whole extract.

Recently, Wang and colleagues [44] reported that mice infected with the helminth *Strongyloides stercoralis* prior to sensitization with OVA show a potentiated systemic Th2 response and yet a simultaneously suppressed IgE response and eotaxin levels in the lungs after intratracheal challenge with OVA. The data presented here demonstrate that adult worms of the helminth *Ascaris suum* also possess immunosuppressive components with the capacity to inhibit lung inflammation and hyper-reactivity. Therefore, we propose that the expression of allergic reactivity in heavily helminth-infected populations might be modulated by these components.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Brazilian Research Council (CNPq). C. Lima was in receipt of a predoctoral fellowship from FAPESP (99/12056-1).

References

- 1 Cara DC, Negrão-Correa D, Teixeira MM. Mechanisms underlying eosinophil trafficking and their relevance in vivo. *Histol Histopathol* 2000; 15:899–920.
- 2 Foster PS, Hogan SP, Ramsay A, Matthaei KI, Young G. IL-5 deficiency abolishes eosinophilia, airway hyper-reactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996; 183:195–201.
- 3 Mould AW, Matthaei KI, Young IG, Foster PS. Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J Clin Invest* 1997; 99:1064–71.
- 4 Lee Tiffany H, Alkhatib G, Combadiere C, Berger E, Murphy PM. CC chemokine receptors 1 and 3 are differentially regulated by IL-5 during maturation of eosinophilic HL-60 cells. *J Immunol* 1998; 160:1385–92.
- 5 Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997; 275:77–9.

- 6 Mao X-Q, Sun D-J, Miyoshi A et al. The link between helminthic infection and atopy. *Parasitol Today* 2000; 16:186–8.
- 7 Joubert JR, Van Schalkwyk DJ, Turner KJ. *Ascaris lumbricoides* and the human immunogenic response: enhanced IgE-mediated reactivity to common inhaled allergens. *S Afr Med J* 1980; 57:409–12.
- 8 Lynch NR, Di Prisco-Fuenmayor MC. High allergic reactivity in a tropical environment. *Clin Allergy* 1984; 14:233–40.
- 9 Kaplan JE, Larrick JW, Yost JA. Hyper-immunoglobulinemia E in the Waorani, an isolated Amerindian population. *Am J Trop Med Hyg* 1980; 29:1012–7.
- 10 Lynch NR, Lopez RI, Isturiz G et al. Allergic reactivity and helminthic infection in Amerindians of the Amazon basin. *Int Arch Allergy Appl Immunol* 1983; 72:369–72.
- 11 Lynch NR, López RI, Di Prisco-Fuenmayor MC et al. Allergic reactivity and socioeconomic level in a tropical environment. *Clin Allergy* 1987; 17:199–207.
- 12 Lynch NR, Hagel I, Perez M, Di Prisco M, Lopez R, Alvarez N. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. *J Allergy Clin Immunol* 1993; 92:404–11.
- 13 Boros DL, Whitfield JR. Endogenous IL-10 regulates IFN- γ and IL-5 cytokine production and the granulomatous response in *Schistosoma mansoni* infected mice. *Immunology* 1998; 94:481–7.
- 14 Mac'Donald AS, Maizels RM, Lawrence RA, Dransfield I, Allen JE. Requirement for *in vivo* production of IL-4, but not IL-10, in the induction of proliferative suppression by filarial parasites. *J Immunol* 1998; 160:1304–12.
- 15 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; 21:527–9.
- 16 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.
- 17 Ferreira AP, Faquim ES, Abrahamsohn IA, Macedo MS. Immunization with *Ascaris suum* extract impairs T cell functions in mice. *Cell Immunol* 1995; 162:202–10.
- 18 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; 47:10–8.
- 19 Yazdanbakhsh M, van den Biggelaar A, Maizels RM. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. *Trends Immunol* 2001; 22:372–7.
- 20 Pereira de Siqueira AL, Russo M et al. A new murine model of pulmonary eosinophilic hypersensitivity: contribution to experimental asthma. *J Allergy Clin Immunol* 1997; 100:383–8.
- 21 Strath M, Warren DJ, Sanderson CJ. Detection of eosinophils using an eosinophil peroxidase assay. Its use as an assay for eosinophil differentiation factors. *J Immunol Meth* 1985; 83:209–15.
- 22 Tibério IFLC, Turco GMG, Leick-Maldonado EA et al. Effects of neurokinin depletion on airway inflammation induced by chronic antigen exposure. *Am J Resp Crit Care Med* 1997; 155:1739–47.
- 23 Russo M, Jancar S, Pereira de Siqueira AL et al. Prevention of lung eosinophilia inflammation by oral tolerance. *Immunol Lett* 1998; 61:15–23.
- 24 Mota I, Wong D. Homologous passive cutaneous anaphylactic activity of mouse antiserum during the course of immunization. *Life Sci* 1969; 8:813–20.
- 25 Zar JH. *Biostatistical Analysis*. New Jersey: Prentice Hall 1984.
- 26 Foster PS. Allergic networks regulating eosinophilia. *Am J Resp Cell Mol Biol* 1999; 21:451–4.
- 27 Zweiman B. The late-phase reaction. role of IgE, its receptor and cytokines. *Curr Opin Immunol* 1993; 5:950–5.
- 28 Collins PD, Marleau S, Griffiths-Johnson DA, Jose PJ, Williams TJ. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J Exp Med* 1995; 182:1169–74.
- 29 Hamelmann E, Vella AT, Oshiba A, Marrack P, Kapler JW, Gelfand EW. Allergic airway sensitization induces T-cell activation but not airway hyperresponsiveness in B-cell deficient mice. *Proc Natl Acad Sci USA* 1997; 94:1350–5.
- 30 Hamelmann E, Takeda K, Schwarze J, Vella AT, Irvin CG, Gelfand EW. Development of eosinophilic airway inflammation and airway hyperresponsiveness requires interleukin-5 but not immunoglobulin E or B lymphocytes. *Am J Resp Cell Mol Biol* 1999; 21:480–9.
- 31 Brusselle G, Kips J, Joos G, Bluethmann H, Pauwels R. Allergen-induced airway inflammation and bronchial responsiveness in wild-type and interleukin-4-deficient mice. *Am J Resp Cell Mol Biol* 1995; 12:254–9.
- 32 Coyle AJ, Le Gros G, Bertrand C et al. Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Resp Cell Mol Biol* 1995; 13:54–9.
- 33 Schleimer RP, Sterbinsky S, Kaiser S. IL-4 induced adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. *J Immunol* 1992; 148:1086–92.
- 34 Mochizuki M, Bartels J, Mallet AI, Christophers E, Schröder JM. IL-4 induces eotaxin: a possible mechanism of selective eosinophil recruitment in helminth infection and atopy. *J Immunol* 1998; 160:60–8.
- 35 Coffman RL, Seymour BW, Lebman DA et al. The role of helper T cell products in mouse B cell differentiation and isotype regulation. *Immunol Rev* 1988; 102:5–28.
- 36 Coffman RL, Lebman DA, Rothman P. Mechanism and regulation of immunoglobulin isotype switching. *Adv Immunol* 1993; 54:229–70.
- 37 Oshiba A, Hamelmann E, Takeda K et al. Passive transfer of immediate hypersensitivity and airway hyperresponsiveness by allergen-specific immunoglobulin (Ig)E and IgG1 in mice. *J Clin Invest* 1996; 97:1398–408.
- 38 Mehlhop PD, van de Rijn M, Goldberg AB et al. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc Natl Acad Sci USA* 1997; 94:1344–9.
- 39 Konno S, Adachi M, Matsuura T et al. Bronchial reactivity to methacoline and serotonin in six inbred mouse strains. *Arerugi* 1993; 42:42–7.
- 40 De Sanctis GT, Merchant M, Beier DR et al. Quantitative locus analysis of airway hyperresponsiveness in A/J and C57BL/6 mice. *Nature Genet* 1995; 11:150–4.
- 41 Zhang Y, Lamm WJE, Albert RK, Chi EY, Henderson Jr WR, Lewis DB. Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. *Am J Resp Crit Care Med* 1997; 155:661–9.
- 42 Macedo-Soares MF, Mota I, Macedo MS. Isolation of *Ascaris suum* components which suppress IgE antibody responses. *Int Arch Allergy Appl Immunol* 1992; 97:37–43.
- 43 Macedo-Soares MF, Macedo MS, Mota I. Antigenic competition in IgE, IgG1 and IgG2 antibody production in mice. *Int Arch Allergy Appl Immunol* 1985; 78:449–51.
- 44 Wang C-C, Nolan TJ, Schad GA, Abraham D. Infection in mice with the helminth *Strongyloides stercoralis* suppresses pulmonary allergic responses to ovalbumin. *Clin Exp Allergy* 2001; 31:495–503.