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Potential of IgE Responses to Third-Party Antigens Mediated by *Ascaris suum* Soluble Products

Key Words

Ascaris
IgE
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Abstract

A reductive approach was used to examine the potentiation of IgE responses by nematode infection. *Ascaris* homogenized extract, *Ascaris* pseudocoelomic (body) fluid (ABF) and purified *Ascaris* allergen (ABA) were tested for their ability to act as protein carriers and as mediators of potentiated IgE responses to third-party (ovalbumin; OVA) responses. All three nematode products were excellent protein carriers for the hapten dinitrophenol and showed significantly better activity in this respect than OVA. Neither ABF nor ABA enhanced the level of the IgE response to the third-party antigen but both prolonged the response markedly. ABF, but not ABA, induced high levels of total circulating IgE when given at the same time as OVA with alum. The data suggest that the enhancement and prolongation of IgE responses by nematodes may be two separate but related activities.

Introduction

Helminth infection in animals and humans causes a rapid and sustained rise in circulating IgE both to parasite and non-parasite antigens. This phenomenon has generated considerable interest in the context of the regulation of IgE responses [1] and in the context of host protection against parasitic disease. The mechanism by which helminths induce this response is unclear although the recent experimentation into the role of interleukin 4 (IL-4) in IgE regulation [2] clearly suggests that IL-4 is involved at some point. Treatment of *Nippostrongylus brasiliensis*-infected mice with antibodies to IL-4, for example, ablates the IgE response in these animals [3]. Attempts to address the initial stimulatory events induced by nematodes that result in this IL-4-mediated effect have been sparse. For example, a soluble extract prepared from homogenized whole *Ascaris*

is a commonly used carrier for the generation of IgE responses to haptens such as dinitrophenol [DNP; 4-6], but there is no understanding of which molecules in this *Ascaris* extract are acting as potent IgE-inducing carriers [7] or how this effect is mediated. Our recent isolation and HPLC purification of the major *Ascaris* allergen (ABA) [8], originally described by Hussain et al. [9] and Strejan and Surlan [10], suggested to us that the use of *Ascaris* body fluid components, particularly ABA, might enable us to approach this question with more clearly defined tools than crude extracts or infections. Thus, in the experimentation described below, we compare soluble extracts of homogenized *Ascaris* (AE), isolated pseudocoelomic (body) fluid of *Ascaris* (ABF) and the purified body fluid allergen (ABA) to assess their activity as protein carriers for DNP, as stimulators of IgE activity to a third-party antigen (ovalbumin; OVA) and as stimulators of generalized IgE po-

tentiation. We restricted our experimentation to primary responses since our main interest is in the early phase of the generation of the response. OVA was chosen as a third-party antigen since, when given with alum, it yields a significant yet relatively short-term primary IgE response itself [11, 12] and as such allows the discrimination of heightened IgE responses and lengthened responses to third-party antigens by the *Ascaris* products. Stromberg [13, 14] has also attempted to address this question, in guinea pigs, using supernatants from cultured *Ascaris*. Culture supernatants, however, have the potential for contamination of the supernatants with bacterial endotoxins with non-specific B cell stimulatory activity.

In this study, we demonstrate that ABF, AE and ABA were all excellent protein carriers for DNP for the purpose of generating IgE responses. In addition, neither ABF nor ABA increased primary IgE responses to OVA-alum but both were able to maintain the high levels of IgE induced by OVA-alum long after the IgE levels had markedly fallen in the animals receiving the third-party antigens alone. ABF, but not ABA, were capable of stimulating a significant increase in total circulating IgE. These results suggest that the IgE amplification effect of helminth infection and the ability of helminths to maintain high IgE levels to third-party antigens could be two separate, but related activities.

Materials and Methods

Animals

Female Balb/c mice (6–8 weeks) were obtained from Charles River and fed and watered ad libitum. Male Sprague-Dawley rats (200–250 g) were obtained from the same source and maintained in the same manner.

Ascaris Preparations

The preparation of *Ascaris* products was as described previously [8]. Briefly, adult, live, *Ascaris suum* were obtained from an abattoir and washed extensively with sterile PBS. For AE, young female and male worms were homogenized, on ice, in a tissue homogenizer at high speed. The resulting homogenate was spun at 800 g for 20 min to remove coarse debris, 18,000 g to remove fine debris and then at 100,000 g for 1 h (4 °C) to remove suspended particulates. The soluble extract was then sterilized by passage through a 0.2 µm filter. ABF was collected by severing the tip of the tail of adult worms and collecting the effluent. This fluid was spun as above to remove particles. The fluid was then concentrated 10 × with a Centricon 10 (Amicon) concentrator (10 kD cut-off point) and diluted with sterile PBS back to the original concentration. This procedure was performed twice, as an alternative to dialysis, to remove small peptides and the 6 to 8 kD mast cell degranulatory factor from the fluid. The ABF was then sterile-filtered as above. An aliquot of this ABF was loaded onto a TSK 3000 (Beckman) column and chromatographed using a Beckman System Gold HPLC. The ABA peak was collected manually to ensure

maximum purity. All preparations were tested for protein concentration by the modified Lowry technique. When used for inoculations, the amount of ABA is adjusted to reflect the amount of ABA present in the ABF, taking into account that approximately 60% of the ABF protein is ABA.

Immunizations

OVA (grade VI; Sigma), AE, ABF and ABA were conjugated with 2,4-dinitrophenyl sulphonic acid (DNP) essentially after Eisen et al. [15]. Free DNP was removed from the preparations first by column chromatography over Sephadex G25-150 (Pharmacia) and subsequently by concentration and dilution using Centricon concentration as above. The following conjugates resulted: DNP-OVA (3.5×10^{-5} mol DNP/mg OVA); DNP-ABF (6.3×10^{-4} mol DNP/mg ABF); DNP-ABA (2.2×10^{-4} mol DNP/mg ABA); DNP-AE (1.43×10^{-4} mol DNP/mg AE). OVA was absorbed to alum (Sigma) at a ratio of 50 µg to 20 mg alum essentially as described [16]. Mice were injected subcutaneously or intraperitoneally without anesthetic with agent, vehicle or saline in a volume of 0.2 ml.

Determination of Antibody Levels

Serum samples from individual mice (6 mice/group) were pooled to control for mouse-to-mouse variation within groups. Total IgE was analyzed by capture ELISA essentially as described [16] using goat anti-mouse isotype-specific IgE (Nordic) as the first antibody and a similar antibody labelled with horseradish peroxidase as a second antibody. Development was initiated with *o*-phenylenediamine and H_2O_2 , stopped with 2.5 M H_2SO_4 and the plates were read at 492 nm on an automated ELISA reader (BioRad). The standard curve for total IgE was generated from 10 dilutions of purified mouse IgE (Nordic). Antigen-specific IgE was measured using 48-hour PCA essentially as described by Ovary [17]. Doubling dilutions were used to establish titres. Duplicates of all PCA reactions never varied by more than one dilution. PCA was used for measuring antigen-specific IgE (rather than total IgE) since this has been shown to be more sensitive and specific. Unlike the IgE subtypes, reagents for antigen-specific IgE determination are less readily available and, on the whole, are less reliable than PCA quantification. Antigen-specific IgG1 and IgG2 were measured using horseradish peroxidase-labelled isotype-specific goat anti-mouse antibodies on a standard ELISA [16]. IgG1 and IgG2a titres reflect those dilutions giving > 0.1 absorbance units on ELISA. Because of the inherent variations in serum antibody levels, data are presented as individual experiments which are representative of multiple repeats rather than as means ± SD of a number of experiments. This manner of presentation yields more information than expressing multiple experiments as a 'change from baseline'. In all experiments, any significant change in one experiment was seen as a significant change in all repeats.

Results

Ascaris Products as Protein Carriers for the Hapten DNP

It has previously been shown [7] that AE are superior protein carriers for the generation of IgE responses to haptens. In these experiments, homogenized AE was used as a positive control for the generation of significant IgE

levels directed to DNP. The results shown in figure 1 are from a representative experiment of 5. They illustrate that the use of ABF or ABA as protein carriers generated an anti-DNP response that was both significant and long lasting. The response using OVA as a protein carrier was significant only at day 14; by day 30 after inoculation, the response had fallen to near background levels. A similar result was seen using BSA as a protein carrier (data not shown). The *Ascaris* products, on the other hand, when used as protein carriers, generated IgE responses which showed no signs of significant decrease even at day 30 after inoculation. The best response was consistently found with ABF but, surprisingly, the HPLC-purified allergen (ABA) also consistently gave significant anti-DNP IgE levels of similar duration of slightly less intensity.

Ascaris Products as Stimulators of Third-Party Responses to OVA

Because nematode infection is known to have an effect on IgE responses against third-party antigens given during the infection [18, 19], we assessed whether this effect could be reproduced with worm products. The third-party antigen, OVA absorbed to alum, was given intraperitoneally within 10 min of a subcutaneous injection, on the back, with ABF or ABA suspended in a mineral oil vehicle. The mineral oil vehicle was used to prevent rapid elimination and degradation of the *Ascaris* proteins. Vehicle alone and saline controls were also performed. Antigen-specific IgE responses by PCA were measured on day 21 after inoculation. The results shown in figure 2 are representative of three experiments. They show clearly that ABF and ABA given at a distant site are capable of influencing the outcome, with respect to circulating anti-OVA IgE, of an intraperitoneal inoculation of OVA-alum. The vehicle in which the products are suspended had no effect on the anti-OVA IgE responses but ABF and ABA yielded much higher IgE responses than control at day 21 after inoculation. To assess whether this response truly represented a heightened IgE response to OVA, the experiment was repeated but this time anti-OVA IgE was sampled at days 10, 14, 21 and 32 after inoculation. The results shown in figure 3, which are representative of 4 experiments, show that early in the response (day 10) there is no difference in the levels of anti-OVA IgE between the OVA + vehicle group and the group receiving the additional subcutaneous inoculation with ABA. ABF inoculation consistently induced slightly, but not significantly, higher responses to OVA. In contrast, as early as day 14, the levels of anti-OVA IgE in the group given OVA-alum with a subcutaneous inoculation of the vehicle alone began to drop. Both groups

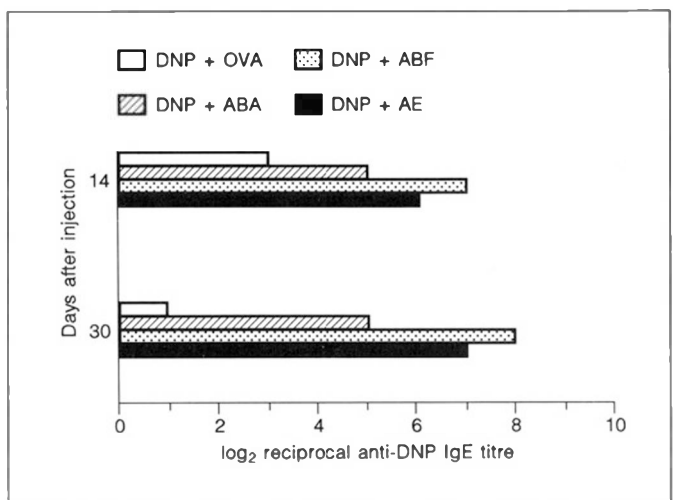


Fig. 1. IgE anti-DNP responses induced by *Ascaris* products as hapten carriers.

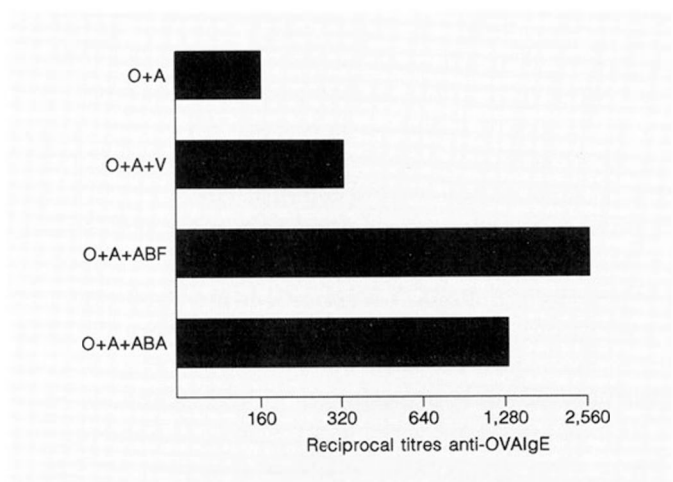


Fig. 2. Induction of IgE to a third-party antigen (OVA) on day 21 after inoculation. O + A = OVA + alum given intraperitoneally; O + A + V = as O + A but accompanied by a subcutaneous injection at a distant site with the vehicle; O + A + ABF = as O + A + V but with ABF added to the vehicle; O + A + ABA = as O + A + V but with ABA added to the vehicle.

receiving the nematode products retained high levels of IgE. This trend continued throughout the length of the experiment with anti-OVA IgE levels in the group inoculated with the vehicle alone dropping to titres of 1/128 by day 32 whilst ABA and ABF induced levels of 1/2,048 and 1/8,192, respectively, at this time. These data suggest that the *Ascaris* products do not markedly increase the IgE response to OVA but that they are capable of dramatically prolonging it.

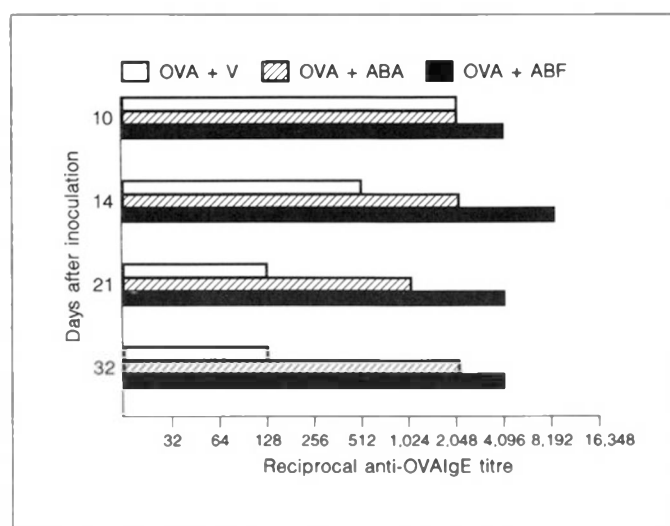


Fig. 3. Time course of induction of IgE to a third-party antigen (OVA). O + V = OVA + alum intraperitoneally, vehicle given subcutaneously at a distant site; O + ABF = as O + V but with ABF added to the vehicle; O + ABA = as O + V but with ABA added to the vehicle.

In another series of experiments, serum was taken from mice (6 mice/group) treated as above with OVA-alum and injected subcutaneously, as previously, with saline, vehicle or ABF. Serum taken from these groups was tested for the antigen-specific anti-OVA IgG1 and IgG2 response. The results shown in figure 4 are representative of 3 experiments and clearly demonstrate that anti-OVA IgG1 levels, but not IgG2 levels, are affected by ABF treatment.

Because nematode infection has been demonstrated to increase, not simply prolong, IgE responses to non nematode antigens [19], we assessed the total IgE in groups (6 mice/group) of animals which had been given OVA + alum intraperitoneally as above or additional inoculations with ABF or ABA subcutaneously at a distant site as above. The results shown in figure 5 (representative of 5 experiments) reveal that ABF was capable of inducing a marked increase in total IgE. In contrast, ABA was not capable of inducing such an increase in total IgE. This lack of ability of ABA to induce increased levels of total IgE is not due simply to a dose effect since the concentration of ABA in the inoculum was adjusted to match the amount of ABA present in the ABF inoculum. Thus, ABA is equally capable at prolonging responses to third-party antigens (given in a manner as to induce IgE) when compared to ABF, but not capable of stimulating a generalized non-specific IgE response.

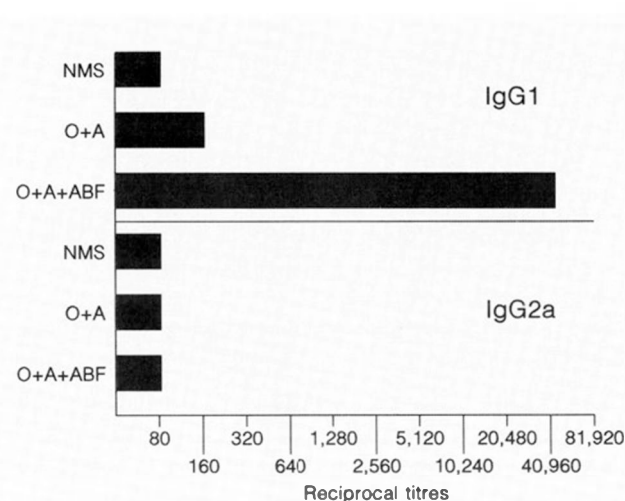


Fig. 4. IgG1 and IgG2a levels, at day 21, to a third-party (OVA) antigen in the presence (O + A + ABF) or absence (O + A + V) of *Ascaris* products.

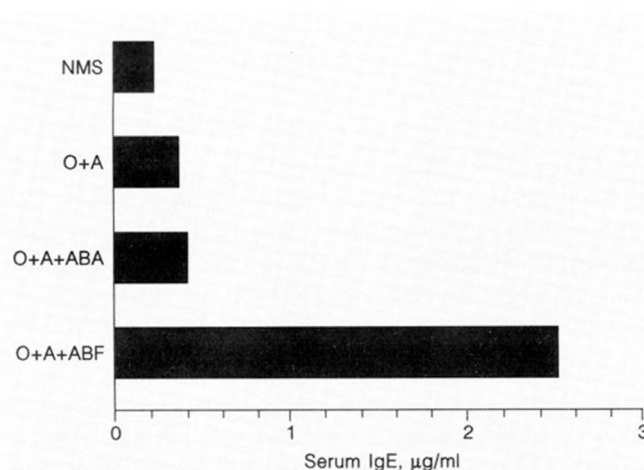


Fig. 5. Total circulating IgE in response to inoculation with *Ascaris* products. NMS = Normal mouse serum; O + A = serum from animals inoculated intraperitoneally with OVA + alum; O + A + ABA = as O + A but mice were also given a subcutaneous injection of ABA; O + A + ABF = as O + A but mice were given a subcutaneous injection with ABF.

Discussion

Nematode infection has been used extensively as an approach to understanding IgE regulation [1, 20]. Early studies such as those by Orr and Blair [21] revealed that *Nip-*

postrongylus infection induced generalized IgE responses with much of this response directed to non-parasite antigens. More recent studies [20] have shown IgE plasma cell levels in the mesenteric lymph nodes of *Trichinella spiralis*-infected mice rising from undetectable levels to 33% by 15 days after infection. The mechanisms behind this potentiation of the immune response are unresolved, but it is clear that both IgE and IgG1 are affected by nematode infection [22]; a link that clearly implicates the TH2 T helper cell [23] and the IL-4 pathway.

Any attempt to address the mechanisms by which nematodes regulate the IL-4 pathway and the molecules involved depends on a reductive approach, i.e. what is the minimal element which is capable of inducing this effect? In terms of nematode infection this approach had been initiated mainly with *Ascaris*. Early studies [7] showed that binding DNP to an AE yielded much higher anti-DNP IgE antibodies than linking it to other protein moieties. AE, however, is an extremely complex mixture of proteins, lipids and polysaccharides. In this study we have taken the reductive approach and used the ABF, a much less complex mixture, and the HPLC-purified ABA as carriers for DNP. The experimentation above shows that both of the *Ascaris* products are superior to OVA in the induction of anti-DNP IgE. The response with both products is significantly prolonged. Both of these *Ascaris* products gave similar results to AE. This suggests that the ABA protein is capable of activating the same systems for IgE production and/or maintenance as the complex body fluid and indeed, by extrapolation, of infection.

Induction of non-specific IgE by nematode infection is not, however, directly addressed by hapten-carrier experimentation. To address this issue directly, potentiation of responses to a third-party antigen, OVA, was performed. Previous work has shown that infection of rats with *Nippostrongylus* [24] or of guinea pigs with *Ascaris* [18] yielded potentiated responses to OVA when tested 14–21 days after OVA inoculation. In this study we used the ABF and the ABA as *Ascaris* products. There is evidence in the literature [25, 26] of high molecular weight molecules in *Ascaris* homogenized extracts which interfere, in the mouse system, with the generation of IgE responses. This material, probably derived from the cuticle, is not present in the body fluid, the excretory/secretory products of *Ascaris* or, obviously, the purified allergen [pers. observations].

When examined at day 21, we found increased levels of anti-OVA IgE in animals given ABF or ABA subcutaneously on the same day as the intraperitoneal inoculation of OVA + alum. We found, however, that if the response was examined in more detail, the data revealed that there was

no increase in the magnitude of the IgE anti-OVA response early in the experiment, but that the nematode products markedly prolonged the IgE response. Thus, if IgE responses were monitored late (at day 21 for example), there was an *apparent* potentiation since the IgE response induced by the OVA alone had dropped markedly by this time, whereas in the animals treated with the nematode products, the levels had remained high. Since both OVA [12] and alum [24] preferentially induce IgE responses, these data suggest that both the *Ascaris* products were able to maintain a high, ongoing response but were not capable of markedly increasing this response.

We were concerned with the generation and maintenance of primary IgE responses and thus we did not examine the effects of nematode products on the secondary response. Because of the maintenance of a high primary response, it is reasonable to assume that a potentiation may be seen in the secondary response as in some other systems [14]. These data are consistent with the findings of Jarrett et al. [24] that *Nippostrongylus* infection did not result in high anti-OVA responses in rats, on day 22 after inoculation, if OVA was not given with a strong IgE adjuvant. Given the data above, it is likely that if the study of Jarrett et al. was structured in a different manner such that earlier time points could be examined, the apparent potentiation of the response by *Nippostrongylus* might have been found to be a prolongation of the initial peak of the anti-OVA IgE response induced, in this case, by OVA-pertussis inoculation.

In the final section we addressed the question of whether *Ascaris* products were capable of inducing the increases in total IgE seen in nematode infection. ABF boosted circulating IgE from 0.2 to 2 µg/ml by day 21 after inoculation. Mice receiving OVA alone, the mineral oil vehicle or ABA did not show this increase in circulating IgE. Although this increase is considerably lower than that shown to be induced by *Nippostrongylus* infection [up to 20 µg/ml by day 14 after infection; 3], it is a marked increase considering it is in response to a single injection and the fact that there have been questions regarding the ability of *Ascaris* products to stimulate IgE responses in mice [25, 26].

These data taken together indicate that the body fluid of *Ascaris*, ABF, is capable of inducing a generalized non-specific IgE response, maintaining an ongoing IgE response to a third-party antigen and acting as an efficient protein carrier for the generation of anti-hapten IgE. The body fluid allergen, ABA, however is only capable of the latter two of these activities. This suggests the possibility that the IgE potentiation by nematode infection could be

bi-partite. One nematode-derived factor may be involved in the non-specific stimulation of B cells and another factor may stimulate, or mimic IL-4 activity. Our current in vitro experimentation explores this possibility in more depth.

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