

POTENTIATION OF THE REAGINIC (IgE) ANTIBODY RESPONSE TO OVALBUMIN IN THE GUINEA PIG WITH A SOLUBLE METABOLIC PRODUCT FROM *ASCARIS SUUM*¹

BERT E. STROMBERG²

From the Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

A soluble antigen, the ACF antigen, obtained from cultures of larvae of *Ascaris suum* was able to potentiate the reaginic response to an unrelated antigen in the guinea pig. When the ACF antigen was administered at the same time or 4 days after ovalbumin, there was a weak primary response and a strong statistically significant secondary IgE response, compared with animals immunized only with ovalbumin. This potentiated response was not observed when the ACF antigen was presented 10 days before or 8 or 12 days after the ovalbumin.

Helminth infections have the unique ability to not only elicit a parasite-specific IgE response (1), but also to elevate the IgE responses to unrelated antigens. Orr and Blair (2) observed that if they induced a reaginic response to egg albumin or conalbumin in rats and then infected the rats with *Nippostrongylus brasiliensis*, the level of circulating IgE was greatly increased, often by 100 times or more. In subsequent studies they observed that this "potentiated reagin response" was transitory, and the levels were dependent upon the titers at the time of helminth infection (3, 4). Jarrett and Stewart (5) extended this work by demonstrating the ability of *N. brasiliensis* to potentiate the rat IgE response to two other antigens, keyhole limpet hemocyanin and house dust extract. Although the IgE levels are enhanced by *N. brasiliensis* infection, it appears that the IgG and IgM antibody levels are not altered (6, 7).

Other helminths have also been reported to potentiate the IgE response to nonrelated antigens. Jarrett (8) demonstrated that rats that had been immunized against ovalbumin (OA)³ and were then infected with the trematode *Fasciola hepatica* would produce an enhanced IgE response to the OA. The nematode *Ascaris suum* has also been shown to potentiate the reaginic response to OA in the guinea pig (9), although attempts

at potentiation by using rat and mouse models were unsuccessful (10, 11).

It has been observed that only a living parasite infection will induce a potentiated IgE response. Attempts to potentiate with extracts of disrupted adult worms of *N. brasiliensis* (3) were unsuccessful, as were the daily injections of the excretory-secretory products of adult worms maintained *in vitro* (12). This report demonstrates the ability of a soluble product obtained from developing larvae of *A. suum* to potentiate the reaginic response to an unrelated antigen, OA.

MATERIALS AND METHODS

Animals. Female Hartley strain guinea pigs (500 to 600 g) were used throughout this study. Animals were purchased from Skippack Farms (Skippack, Pa.) and maintained in our animal facility, where they were fed water and guinea pig chow ad libitum.

Antigens. Guinea pigs were immunized with OA (5 × crystallized, Calbiochem, San Diego, Calif.), precipitated in an alum gel (13). Each animal received 1 mg of OA in 10 mg AlK(OH)₂. In later experiments some animals received 10 µg of OA in 10 mg of the AlK(OH)₂.

The soluble metabolic product, *Ascaris* culture fluid (ACF) antigen, was obtained by culturing third-stage larvae of *A. suum* through the third moult to the fourth stage in a chemically defined, low m.w. culture medium as previously described (14). Briefly, third-stage larvae recovered from the lungs of rabbits 9 days after oral infection were cultured in medium 199 with Earle's modified salts supplemented with glucose, glycyl-histidyl-lysine, penicillin, and streptomycin and maintained in an atmosphere of N₂:O₂:CO₂ (90:5:5) for seven days at 37°C. Fourth-stage larvae were removed by filtration, and the medium was washed and concentrated (× 20) by diafiltration in an Amicon filter (DE-52; Amicon Inc. Lexington, Mass.) with a YM10 membrane (10,000 m.w. exclusion limit). The ACF antigen had a protein concentration of 310 µg/ml by the Lowry method and 88 µg/ml carbohydrate when using the Anthrone technique (15).

Experimental design. Groups of 16 guinea pigs were immunized i.p. with 1.0 mg or 10 µg of OA in 10 mg of AlK(OH)₂ administered on Day 0 and Day 28. In order to determine if the ACF antigen would potentiate the IgE response to OA, the groups were also immunized with 31 µg of ACF antigen, i.p., on Day -10, 0, 4, 8, or 12, and again 28 days later.

Blood (2 ml) was obtained by cardiac puncture, and the serum was collected and stored at -20°C until used. Three or four animals were bled on Days 0, 1, 4, 6, 8, 11, 15, 18, 21, 25, and 28 after each OA immunization.

The IgE response was determined by using the passive cu-

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² Present address: Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota 55108

³ Abbreviations used in this paper: ACF, *Ascaris* culture fluid; OA, ovalbumin; PCA, passive cutaneous anaphylaxis; IHA, indirect hemagglutination.

taneous anaphylaxis (PCA) test (16, 17). Dermal sites were sensitized with a 0.1-ml injection of varying dilutions (1:10, 1:50, 1:100, 1:500, 1:1000, 1:5000 and 1:10,000) of antiserum in at least two animals. After a long latent period (6 days), the guinea pigs were challenged i.v. with 1 mg OA and 2.5% Evans Blue dye in 1 ml of 0.15 M NaCl (saline). Thirty minutes after challenge, skin reactions were evaluated by measuring the diameter and intensity (on a scale of 0 to 4+) of the PCA response. These responses were also evaluated for their thermoability by heating the sera at 56°C for 4 hr. Titers were determined by the highest dilution able to elicit a positive reaction, a response greater than 5 mm and 2+ intensity. Sera were considered negative if they were unable to provoke a reaction at a 1/10 dilution. Titers were determined for each animal and the mean was calculated for each sample day on log-converted data. Statistical significance was calculated on log-converted data by using Student's *t* test.

The presence of IgG1 was also determined for some experiments by utilizing a short latent period (6 hr) PCA reaction with serum that had been heated to 56°C for 4 to 6 hr before titration. Hemagglutinating antibodies were also evaluated in some experiments with the indirect hemagglutination (IHA) test (18) using the microtiter system (Cooke Engineering Co., Alexandria, Va.). The indicator system consisted of sheep red blood cells coupled with tannic acid to OA (25 µg/ml). The mean daily titers and statistical significance were determined as described above.

Some of these experiments (Days 0, 4, and 8) were also evaluated for their reaginic response to the ACF antigen by utilizing the techniques described above and challenging the PCA-sensitized animals with 124 µg of ACF antigen and 2.5% Evans blue dye in 1 ml of saline.

RESULTS

These experiments have demonstrated that the ACF antigen is able to induce a potentiated IgE response to the nonrelated antigen, OA. Figure 1 presents the reaginic response to OA when the animals were immunized with OA (1.0 mg) or OA (1.0 mg) and ACF (31 µg) at the same time (Day 0). The response to OA alone demonstrated very little primary reaginic response and a slightly increased response on secondary stimulation. Guinea pigs immunized with ACF only demonstrated no IgE titer to OA. The animals that received both OA and ACF (Day 0) produced a low level primary response (1:15 titer) and on secondary stimulation produced a maximum mean titer of 1:5000 on Day 9. The elevated responses on Days 6 through 11

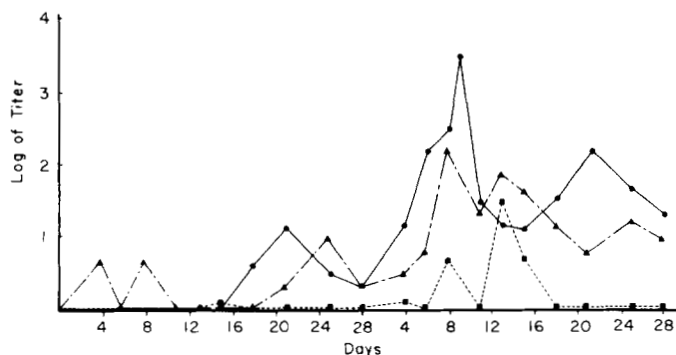


Figure 1. The IgE response of guinea pigs to OA, when given alone, or when the animals also received ACF at the same time (Day 0), or 4 days after OA administration (Day 4). The animals were immunized with OA on Day 0 and 28. Each data point is the mean of at least four animals.

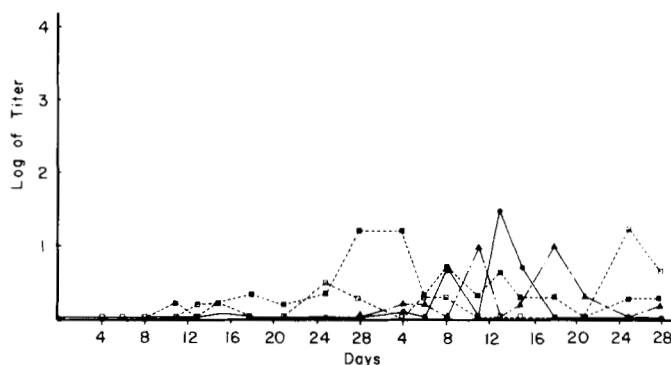


Figure 2. The IgE response to OA in guinea pigs that also received ACF 8 days after (Day 8), 12 days after (Day 12), or 10 days before (Day -10), OA administration. The controls were animals that received OA alone. Each data point is the mean of four animals.

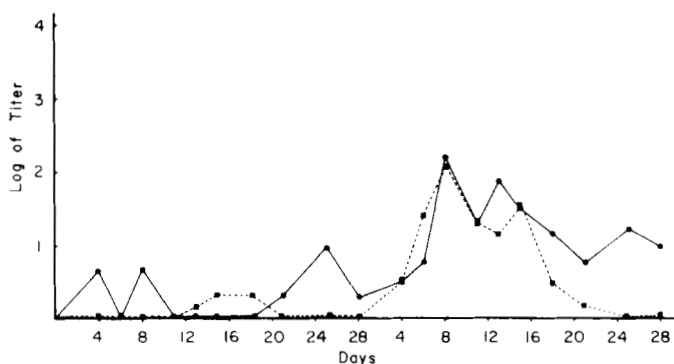


Figure 3. A comparison of the IgE response to OA in guinea pigs when the animals were given 1 mg of OA, or 10 µg of OA, on Day 0 and 28. Each animal also received the ACF antigen at the same time of OA administration. Each data point is the mean of four animals.

did not show much variation between animals but were significantly different from the OA control group. When the soluble antigen was given 4 days after the OA (Day 4), the reaginic response was similar yet somewhat reduced when compared with the Day 0 group (Fig. 1). The peak secondary response (Days 6 through 11) also demonstrated statistically significant differences when compared with the OA control.

When the ACF antigen was presented 8 or 12 days after the OA, both the primary and secondary responses were variable and did not differ significantly from the OA control group at any time, Figure 2. The IgE response elicited when the ACF was administered 10 days before the OA (Day -10) was quite variable and also showed no difference for the OA control (Fig. 2). This response did not demonstrate any effect by the administration of ACF.

The Day 0 and Day 4 experiments were repeated with 10 µg of OA in 10 mg of alum. The results of these experiments demonstrated that this 100-fold decrease in antigen concentration did not significantly alter the host response, however, it was somewhat reduced when compared with the 1-mg experiments (Fig. 3). The IgE responses in all groups were greatly reduced (to 1:10 or 1:50) when heated to 56°C for 4 hr.

The hemagglutinating responses did not show any enhanced response resulting from the ACF immunization, regardless of time of administration.

Figure 4 graphically illustrates the IgG1 responses for the control group and Day 0 and Day 8 potentiated groups. The Day -10, 0, and 4 groups all demonstrated similar responses

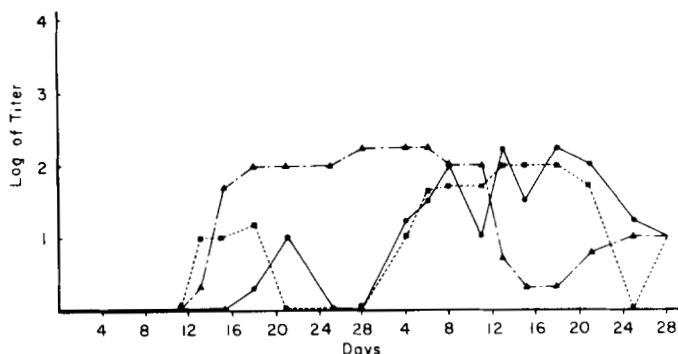


Figure 4. The IgG₁ response of guinea pigs to OA, when given OA alone (●—●, control), or with ACF given at the same time as OA (■—■, Day 0) or with the ACF given 8 days after the OA (▲—▲, Day 8). All animals were immunized with OA on Day 0 and 28. Each data point is the mean of three or four animals.

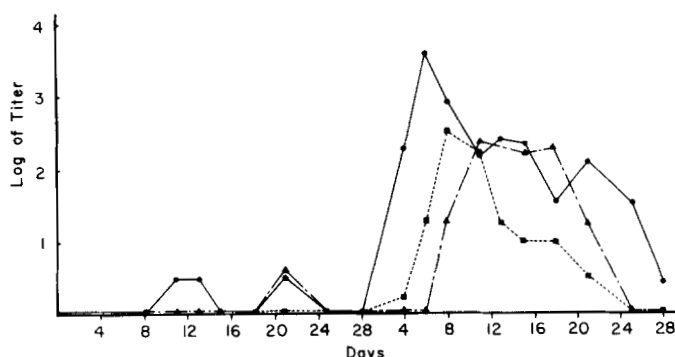


Figure 5. The IgE response to ACF in guinea pigs that received ACF alone (●—●), ACF and OA at the same time (■—■), or ACF 4 days after OA (▲—▲). The animals were immunized on Day 0 and 28. Each data point is the mean of at least four animals.

that did not differ significantly from the controls. However, the Day 8 and Day 12 groups had similar responses that differed from the controls. These two groups produced a strong IgG response about Day 15 of the primary bleedings and remained high until about the 11th day of the secondary bleedings, after which they remained low (1:10).

The IgE response to the ACF antigen showed little effect in relation to the time of OA presentation, but rather demonstrated little or no primary response and a secondary response that peaked 6 to 8 days after the ACF administration. These responses were somewhat lower than when the ACF antigen is presented alone. Figure 5 demonstrates the reagenic responses to ACF when the animals were immunized with ACF only, at the same time as OA and 4 days after the OA immunization.

DISCUSSION

The results reported have demonstrated that it is possible to induce IgE antibodies to OA in the guinea pig as well as to potentiate this reagenic response with a soluble, helminth-derived product.

It has been shown by many authors that an active helminth infection will potentiate the reagenic response to several non-related antigens (2, 5, 8, 9). It has also been accepted that IgE antibody potentiation requires the presence of living worms. Orr *et al.* (3) attempted to potentiate the reagenic response to OA using a soluble product obtained by homogenizing and sonicating adult worms of *N. brasiliensis*. They administered

this adult extract orally, i.p., or intramuscularly 10 days post OA immunization and were unable to potentiate the response. Jarrett (12) was also unsuccessful in stimulating an enhanced IgE antibody response with excretory-secretory products of adult *N. brasiliensis* obtained by *in vitro* maintenance. These metabolic products were administered in large quantities for several days and were shown to contain the allergen of *N. brasiliensis*. However, Jarrett and Stewart (19) were able to potentiate the reagenic response with radiation-attenuated infective larvae, which would not develop to adults, as well as with transplanted adult worms. Their data indicate the potentiator is most likely a product of both larvae and adults.

Our ability to potentiate the IgE antibody response with 31 µg of ACF demonstrated that an ongoing parasite infection was not necessary for reagenic potentiation. This study indicated that it is more probably a matter of having the correct parasite product in an appropriate concentration. The ACF is an excretory-secretory product that is produced when the third larval stage moults to the fourth stage. It is glycoprotein with an estimated m.w. of 67,000 (15). The ACF was able to induce a protective immune response to a challenge *A. suum* infection (15) and it was able to induce a very strong (1:50,000) IgE antibody response (17, 20). Animals immunized with control culture medium (containing no larvae) did not demonstrate any enhanced IgE production. We have also observed that the ACF is neither mitogenic nor cytotoxic to normal guinea pig lymphocytes when included in the culture medium (unpublished observations). The ACF appears to be similar to the IgE-potentiating factor described by Suemura and Ishizaka (12), which is a soluble product of T cells.

Realizing that the 1 mg of OA was a massive dose of antigen, we repeated the first two experiments (Day 0 and 4) using 10 µg to OA in the same quantity of alum. The results demonstrated that the responses were similar but somewhat reduced. Therefore, the low dose was not evaluated for the other time sequences.

This study also demonstrated that the time that the soluble potentiator (ACF) was administered in relation to the OA immunization was important. The ability of ACF to enhance the IgE response to OA when they were given at the same time or shortly thereafter (Day 4) might indicate that an ongoing IgE response was not necessary, but rather that the potentiating activity may start with the IgE response. When the ACF was given 8 or 12 days after the OA, it was unable to potentiate the reagenic response, perhaps the IgE response was no longer sensitive to the action of ACF. The ACF was also unable to potentiate the IgE response when given 10 days before the OA. Kojima and Ovary (23) reported that a *N. brasiliensis* infection given 5 to 14 days before immunization with the antigen was able to potentiate the reagenic response in the mouse.

It was shown that the ACF was capable of eliciting its own IgE response. When administered with OA or at times shortly before or after OA, the response was reduced. Observations indicated that the peak ACF response was delayed by 2 days when given in conjunction with OA immunization. The reason for this occurrence is not known.

When the ACF was given before, at the same time, or shortly after (Day -10, 0 or 4) the OA, there was no potentiation of the IgG₁ response as reported by Block *et al.* (7). However, when the ACF was given 8 or 12 days after the OA, there was a potentiated response. All groups demonstrated responses similar to the controls when evaluated with the IHA test. These results are in agreement with the findings of Jarrett *et al.* (6) and Block *et al.* (7).

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