

Effect of *Ascaris suum* and other adjuvants on the potentiation of the IgE response in guinea-pigs

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Summary. The potentiation effect of various adjuvants on the production of guinea-pig IgE was investigated using Freund's complete and incomplete adjuvant, the lipopolysaccharide of *Escherichia coli* and *Salmonella typhosa*, *Bordetella pertussis*, and the nematodes *Nippostrongylus brasiliensis* and *Ascaris suum*. While all the antigens had a variable effect on the potential of the IgG1 response, only infection with *A. suum* resulted in an enhanced IgE response to the antigen, egg albumin. Maximum potentiation occurred when primary immunization and nematode infection were accomplished simultaneously.

INTRODUCTION

Two classes of anaphylactic antibody have been identified in the guinea-pig, IgG1 and IgE (Catty & Fraser, 1972; Ovary, Benacerraf & Block, 1963). Although both of these antibodies mediate immediate-type anaphylactic reactions, they can be easily distinguished on the basis of heat lability and tissue fixation latency for the passive cutaneous anaphylaxis (PCA) reaction (Parish, 1970). While IgG1 is readily obtained by conventional immunization and separation procedures (Oliveira, Osler, Siraganian & Sandberg, 1970), IgE is

not, except when guinea-pigs are immunized with live parasites such as *Trichinella spiralis* (Catty & Fraser, 1972) or *Ascaris suum* (Dobson, Morseth & Soulsby, 1971). Moderate enhancement of the IgE response has been reported by Perini & Mota (1973) using lipopolysaccharide as an adjuvant. Multiple injections with hapten-carrier conjugates in alum gel have also resulted in higher titre IgE anti-hapten activity, but the immunization schedule requires months of repeated antigen challenges (Levine, Chang & Vaz, 1971).

The purpose of this study was to investigate the possibility that routinely used adjuvants or parasitic infection could be used to enhance the guinea-pig IgE response to a non-parasite antigen using a relatively short immunization schedule.

MATERIALS AND METHODS

Animals

Male Hartley strain outbred guinea-pigs (Dutchland Farms, Denver, Pennsylvania) weighing 280–320 g were used for all immunization procedures. Group size varied from five to ten animals. Guinea-pigs weighing 350–450 g were used for PCA evaluation of sera.

Antigens

For use in primary immunizations, egg albumin (EA) 5× crystallized (Calbiochem, San Diego, Calif.) was used either as a solution in phosphate buffered saline (PBS), 0.2 M, pH 7.2, or precipitated with alum gel

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prepared from $\text{AlK}(\text{SO}_4)_2$ (Williams & Chase, 1974).

Adult worm antigen *Ascaris suum* (gift of Dr Bert Stromberg, University of Pennsylvania, The School of Veterinary Medicine) was prepared from 600 mg of lyophilized adult stage *A. suum* added to 60 ml of distilled water. The suspension was homogenized with a tissue grinder and allowed to stand for 2 h at 5°, centrifuged at 650 *g* for 15 min; and the supernatant filtered through a Gelman pre-filter, and a 0.45 µm membrane filter under vacuum. The final protein concentration was approximately 1 mg/ml. From 0.5 to 0.7 ml of this preparation in 0.5% Evans blue dye (Matheson Coleman and Bell, Norwood, Ohio) was used to challenge sensitized animals for the PCA reaction.

Immunizations and bleeding

Primary immunization of guinea-pigs was accomplished by injecting either 10 µg EA in PBS or 10 µg EA in 10 mg alum intraperitoneally in a volume of 1 ml.

Freund's complete adjuvant (CFA) and Freund's incomplete adjuvant (ICFA) (Colorado Serum Co., Denver, Colorado) were emulsified with EA and injected subcutaneously in both axillary regions of guinea-pigs.

Other adjuvants were given before, at the same time, or after primary immunization with EA or EA in alum as outlined in the Results. These adjuvants were administered as follows: bacterial lipopolysaccharides (LPS) of *Escherichia coli* and *Salmonella typhosa* (Difco Labs, Detroit, Michigan) were injected intraperitoneally in concentrations ranging from 0.1 µg/kg to 20 µg/kg in 0.15 M saline. Heat-killed *Bordetella pertussis* (Park, Davis and Co., Detroit, Michigan) 15×10^9 organisms were injected intraperitoneally. *Nippostrongylus brasiliensis*, maintained in our laboratory according to Standen (1963), was injected as the infective larval stage, 3000 larvae subcutaneously in the groin, or as lyophilized larvae, 10 µg intraperitoneally. Embryonated eggs of *A. suum* 2×10^4 (Dobson *et al.*, 1971) were injected subcutaneously in the groin.

Animals were lightly anaesthetized with ether and bled by cardiac puncture. The serum was separated from whole blood and stored at -20°. IgE activity has remained stable in frozen serum for up to 13 months.

Criteria for identification of antibody class

The PCA reaction (Ovary, 1964) was used for qualitative and quantitative evaluation of all test sera. A combination of heat inactivation at 56° for 4 h and a

latent period of 8 days were used to identify IgE antibody in the PCA reaction. IgG1 was identified in heat inactivated sera using a 3 h latent period PCA (Parish, 1970; Perini & Mota, 1973).

RESULTS

Guinea-pigs receiving 10 µg of soluble EA i.p. in PBS did not produce any detectable IgE or IgG1 anti-EA in serum samples taken on days 10 and 30 post immunization. When EA (10 µg) was precipitated with alum (10 mg) and administered i.p. to guinea-pigs, moderate IgE and IgG1 anti-EA were detected on day 11 with PCA reciprocal titres of 50 and 100 respectively (Table 1).

In an attempt to confirm the findings of Perini & Mota (1973), bacterial lipopolysaccharide (LPS) from *E. coli* and *S. typhosa* (0.1–20 µg/kg) were injected i.p. either 24 h prior, 24 h or 48 h after injection of 10 µg EA, and the animals were bled on day 10 and day 30. Very low titre IgG1 activity was observed only in sera from animals that received a supplementary injection of *S. typhosa* LPS; no IgE antibody activity was detected in any of the sera. Several other adjuvants were used in an attempt to elicit IgE responses. In most instances, only variable titres of IgG1 without detectable IgE activity were observed (Table 1). Animals

Table 1. Summary of effect of adjuvants on the potentiation of IgE and IgG1 anti-EA in guinea-pigs

Antigen	Adjuvant	Maximum titre	
		IgE	IgG1
EA	None	< 5	< 5
EA + Alum	None	50	100
EA	LPS (<i>E. coli</i>)	< 5	< 5
EA	LPS (<i>S. typhosa</i>)	< 5	20
EA	CFA	< 5	320
EA + Alum	<i>Bordetella pertussis</i>	< 5	40
EA + Alum	<i>Nippostrongylus brasiliensis</i> (live)	< 5	640
EA + Alum	<i>Nippostrongylus brasiliensis</i> (lyophilized)	< 5	50
EA + Alum	<i>Ascaris suum</i>	400	400

Titres are the reciprocal of the highest dilution giving a positive PCA reaction in guinea-pigs according to the criteria outlined in the Materials and Methods. Maximum titre is the highest observed with pooled anti-sera from all experiments.

receiving EA emulsified in CFA produced high titre IgG anti-EA detectable 14 and 21 days after immunization. *Bordetella pertussis* was administered to guinea-pigs 24 h after EA in alum and only minimal IgG activity was detected on day 12. Infective larvae of *N. brasiliensis* injected subcutaneously in the groin region of guinea-pigs 1 or 14 days after the animals had received EA in alum, substantially potentiated only an IgG response. Animals that received 10 µg of lyophilized *N. brasiliensis* in place of living larvae, did not demonstrate any enhanced IgE or IgG antibody response. Animals were bled every other day beginning on day 4 through day 28. While live larvae potentiated the IgG response, lyophilized larvae did not have any demonstrable effect on the response to EA.

Infection of immunized guinea-pigs with *A. suum* was successful in potentiating an IgE response. Embryonated eggs of *A. suum* (2×10^4) were injected subcutaneously in the groin of guinea-pigs 7 days

before, simultaneously with, 24 h after or 7 days after EA in alum. A potentiation in the IgE anti-EA response was detected with all immunization procedures using *A. suum* as adjuvant. Individual animals were sequentially bled in order to determine time sequence of their response. The results of several experiments are summarized in Fig. 1. Although each of the procedures was successful in potentiating IgE antibody, the maximum effect was observed when ascaris infection was given on the same day as immunization. The highest IgE titre observed was 1/400 (average 1/160); IgG responses were also elevated. Only when *A. suum*

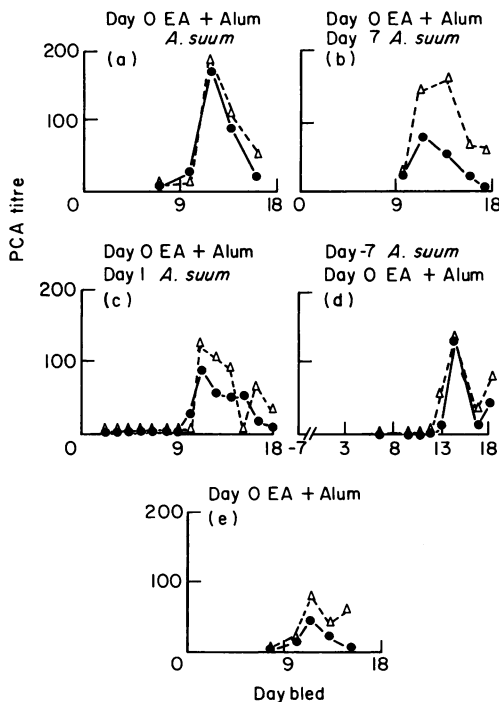


Figure 1. Comparison of IgE v. IgG anti-EA production in varied immunization procedures with *Ascaris suum* as adjuvant. ●, IgE; △, IgG. PCA titres shown are the average reciprocals of end-point titre dilutions of antisera of all experiments. (a), (b) average of four experiments; (c) average of eight experiments; (d) average of two experiments; (e) average of three experiments.

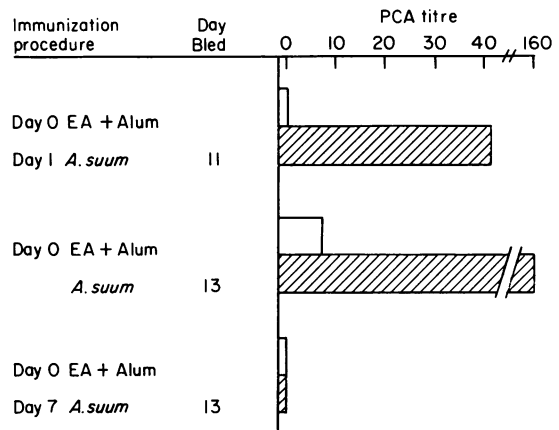


Figure 2. Comparison of anti-*Ascaris suum* antibody activity observed in three immunization procedures. Open bars, IgE; hatched bars, IgG.

infection was initiated 7 days after primary immunization was there a separation of the potentiation of IgE and IgG responses; but, in this instance, the potentiation of IgG was greater than that of IgE.

All animals did not demonstrate potentiation of IgE. When serum from individual animals was examined, it was found that approximately 70% of the animals responded with high IgE titres. The remaining animals did not respond at all.

A limited number of serum samples were also examined for anti-ascaris activity. No significant potentiation of IgE anti-ascaris was detected. An enhanced IgG response was detected in serum from animals infected simultaneously and one day after immunization with EA. When immunized animals were infected on day 7, no anti-ascaris activity was observed (Fig. 2).

DISCUSSION

Conventional adjuvants evaluated in this study either failed to potentiate an IgE response or only potentiated the IgG response. We were not able to confirm the observation of Perini & Mota (1973) that the B-cell mitogen, LPS, is capable of enhancing the IgE response; a different strain of guinea-pig was used, however, and it has been previously demonstrated that strains of guinea-pig differ in their immune response (Ellman, Inman & Green, 1971).

The rat, which is the definitive host for *N. brasiliensis* will demonstrate a potentiated IgE response to a non-parasite antigen when infection takes place during the ongoing IgE response (Ohman & Block, 1972). The guinea-pig is an abnormal host for *N. brasiliensis* in which no parasite development takes place (Lindquist, 1949; Parker, 1961). In this study, it did not demonstrate a potentiated IgE response to EA suggesting that perhaps some parasite development is required in the host animal. This assumption is supported by the findings of Ogilvie (1967) with *N. brasiliensis* and Bradbury, Percy & Strejan (1974) with *A. suum* where neither extract was able to potentiate the IgE response in rats.

Similarly, the guinea-pig is an abnormal host for *A. suum*; development from the infective egg to the third larval stage does take place, however. When embryonated eggs of *A. suum* are administered subcutaneously most eggs are reported to remain viable at the injection site and probably produce some excretory-secretory products while a few may hatch, develop and migrate to the lung (Soulsby, 1957). Perhaps it is this development which is necessary to induce the potentiated reaginic response to EA. Soulsby (1973) originally suggested that the excretory-secretory products might be responsible for the anti-ascaris IgE response in guinea-pigs. This concept is supported by the recent evidence of Stromberg & Soulsby (1978) where a substance, SD antigen, isolated from *in vitro* culture of *A. suum* larvae was able to induce a strong IgE response as well as to potentiate a reaginic response to EA. The SD antigen is an excretory-secretory product of third-stage larvae which develop through the third moult to the fourth larval stage (Stromberg, personal communication). It is possible that this represents at least one of the substances generated *in vivo* in the present study which is responsible for the potentiated IgE response. Further study with live *A. suum* infection and with SD purified antigen from live *in vitro* populations of *A. suum* may

elucidate the mechanism by which this parasite potentiates an IgE immune response.

Several investigators have demonstrated that an immunization with *A. suum* extracts (Hamaoka, Newburger, Katz & Benacerraf, 1974) or *N. brasiliensis* infection (Kojima & Ovary, 1976; Ishizaka, Urban, Takatsu & Ishizaka, 1976), are capable of initiating a carrier effect, presumably by stimulation of helper T cells. Regardless of the adjuvant used in this study, animals primed with EA in alum by itself yield a modest IgE response indicating successful priming; whereas EA alone did not elicit any detectable immune response. Only infection with eggs of *A. suum* was capable of potentiating the response of EA in alum-primed animals perhaps by providing additional helper function. The proposed helper stimulus was not apparently specific for IgE since the IgG response also was potentiated. That the response always demonstrates a sharp peak may be due to the transient nature of the stimulus provided by *A. suum* and thus only transient helper function. Alternatively, it could be speculated that the function of *A. suum* is to release the IgE from a constant state of suppression. We think the latter possibility is unlikely but further studies would be necessary to rule this out.

As might have been expected, there was also a minimal anti-ascaris response. Only IgG1 was detectable in appreciable amounts, however. It is still possible that IgE anti-ascaris activity may occur at a time much later than the peak potentiated IgE anti-EA response.

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