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IgE and IgG1 antibody production by a soluble product of *Ascaris suum* in the guinea-pig

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Summary. Third-stage larvae of *Ascaris suum* cultured to the fourth stage in a chemically defined culture medium produced a substance, the 'ACF antigen', which was allergenic in the guinea-pig. When three different concentrations (3·1, 31 and 62 μg) of the ACF antigen were given intraperitoneally, only the highest concentration induced a primary IgE specific antibody response (1:100 titre) as determined with the passive cutaneous anaphylaxis reaction. Upon secondary exposure all concentrations demonstrated a strong IgE response (1:50,000 peak titre) with very little IgG1 activity (1:100). The secondary IgE responses began to rise on the fourth day, peaked on the sixth day and returned to relatively low levels by the fourteenth day (1:100). The intramuscular administration of the ACF antigen did not induce the extremely high titres of IgE as found with the intraperitoneal injection, but rather a low level response (1:500 peak) which did not differ greatly from the IgG1 response.

INTRODUCTION

Helminths have been shown to have a unique ability to induce reagin-mediated hypersensitivity in man and most experimental animals (Sadun, 1972). Perhaps one of the most studied allergenic parasites is *Ascaris* spp., which is able to induce high levels of IgE in a variety of laboratory animals (Hogarth-Scott, 1967; Strejan & Campbell, 1968; McAninich & Patterson, 1970; Dobson, Morseth & Soulsby, 1971a). Several investigators were able to provoke a passive cutaneous anaphylactic (PCA) IgE reaction in animals sensitized with an *Ascaris* infection using saline extracts or the perienteric fluid of adult ascarids (Hogarth-Scott, 1967; Dobson *et al.*, 1971a; Ambler, Doe, Gemmell, Roberts & Orr, 1972; Hussain, Strejan & Campbell, 1972; Mitchell, 1976; Kuo & Yoo, 1977). More recently, two antigens which were able to provoke a reaginic response were isolated and characterized from saline extracts of adult *A. suum* (Ambler, Miller, Johnson & Orr, 1973; Hussain, Bradbury & Strejan, 1973); only one, however, was able to induce an IgE response in the rat and that required the use of alum as an adjuvant (Hussain *et al.*, 1972).

This paper reports another *Ascaris* allergen, the *Ascaris* culture fluid (ACF) antigen, obtained from developing larvae of *A. suum* maintained in a chemically defined culture medium. This antigen is able to induce high titres of IgE when given i.p. without the use of adjuvants or potentiators. It is also reported that the route of administration is important in the induction of an IgE response.

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MATERIALS AND METHODS

Animals

Female Hartley strain guinea-pigs (500–600 g) were used throughout this study and female New Zealand white rabbits (3 kg) were used to provide larvae of *A. suum* for culture. All animals were purchased locally (Skippack Farms, Skippack, Pa) and maintained in our animal house where they were fed a commercial preparation and water *ad libitum*.

Production of ACF antigen

The ACF antigen was produced by third-stage larvae of *A. suum* when cultured *in vitro* through the third moult to the fourth stage in a chemically defined culture medium as previously described (Stromberg, Khoury & Soulsby, 1977). Briefly, third stage larvae recovered from the lungs of rabbits 7 days after oral infection were cultured in medium 199, supplemented with glucose, glycyl-histidyl-lysine, penicillin, and streptomycin and maintained in an atmosphere of N_2:O_2:CO_2 (90:5:5) for 7 days at 37°. After 7 days in culture, greater than 95% of the larvae had moulted to the fourth stage and less than one per cent had died. 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.) using a YM 10 membrane (10,000 molecular weight exclusion limit). The ACF antigen had a protein concentration of 310 μg/ml as assessed by the method of Lowry, Rosebrough, Farr & Randall (1951) and a carbohydrate concentration of 22 μg/ml as determined by the Anthrone technique (Kabat & Mayer, 1961).

In these experiments, groups of sixteen guinea-pigs were immunized i.p. with 3·1, 31 or 62 μg of ACF antigen or i.m. with 31 μg of ACF antigen, on day 0 and were boosted in a similar manner 28 days later.

Blood (4 ml) was obtained by cardiac puncture and the serum was collected and stored at −20° until used. Relays of four animals were bled on alternate days for a period of 24 days after each immunization.

Passive cutaneous anaphylaxis (PCA) test

PCA reactions were used to detect and quantify the homocytotropic antibody response (Ovary, 1964). 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, 1:10,000, 1:50,000) of antiserum. After a short (6 h) or long (6 day) latent period the guinea-pigs were challenged with 124 μg of ACF antigen 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 the intensity of the PCA response. Titres were determined by the highest dilution able to elicit a positive reaction. Sera were considered negative if no PCA reaction was obtained at a dilution of 1:10. Titres were determined for each animal and the mean determined for each sample day on log converted data. Statistical significance was calculated on the log converted data using the Student's *t* test.

The presence of IgG1 was assessed by a short latent period PCA reaction with serum which had been heated to 56° for 4–6 h. IgE was judged to be present by a positive skin test in a 6 day latent period PCA with serum which also demonstrated thermolability (Mota & Perini, 1970; Dobson *et al.*, 1971a; Dobson, Rockey & Soulsby, 1971b; Perini & Mota, 1972).

RESULTS

Intraperitoneal immunization

The IgE and IgG1 antibody responses to immunization with 3·1, 31 or 62 μg of ACF antigen are presented in Fig. 1 and Tables 1 and 2.

There was very little IgE response to the 3·1 and 31 μg doses of ACF antigen (two of sixteen and one of sixteen animals responding, respectively). Likewise there were few animals demonstrating a low level primary IgG1 response (one of sixteen and six of sixteen animals responding). There was, however, a statistically significant IgE response to the 62 μg dose of antigen with all the animals responding. There were no animals which responded with a primary IgG1 titre to the 62 μg dose.

Upon second exposure to the ACF antigen there was a very strong and rapid IgE response to all three concentrations of antigen. The 3·1 μg of antigen elicited the strongest mean response (1:7937) by the sixth day with all animals responding and this response had dropped to relatively low levels by the eighth day. When animals were immunized a second time with 31 μg of antigen all animals responded rapidly but the titres were lower (mean of 1:3344) than seen with the 3·1 μg dose and took longer to return to low levels. Animals immunized with the highest dose of antigen also demonstrated a strong IgE response (mean of 1:3684) by the sixth day with all animals responding; the titres, however, remained relatively high (mean of 1:1357) until the fourteenth day.

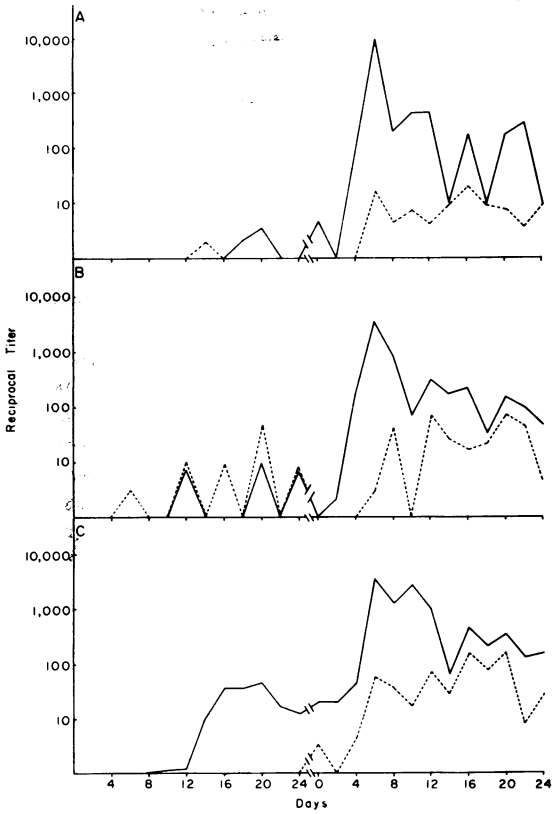

Figure 1. The IgE (——) and IgG1 (– – –) antibody response to i.p. immunization with 3·1 μg (Fig. 1A), 31 μg (Fig. 1B) and 62 μg (Fig. 1C) of ACF antigen. Animals were immunized on day 0 and boosted on day 28 (0) and bled on alternate days for a period of 24 days. Each data point is the mean for at least four animals.

The 31 μg group were immunized a third time with all animals responding with IgE titres of 1:10,000 or greater on day 6 and 8. The IgE titres returned to relatively low levels by the tenth day and were not followed beyond day 12.

The secondary IgG1 response for all three concentrations of ACF antigen was relatively low throughout the sampling period as seen in Table 2. All animals responded with low titres (1:171 maximum).

Intramuscular immunization

As seen in Fig. 2, the i.m. presentation of ACF antigen did not induce a substantial primary IgE response, with only three of sixteen animals responding. Eleven of sixteen animals produced a primary IgG1 response but the maximum titre was only 1:100. There was a stronger secondary IgG1 response with titres above 1:100 between days 6 and 14 and all animals responded. This was a stronger and more prolonged IgG1 response than the 31 μg i.p. group. The secondary IgE responses were considerably stronger than the primary responses but significantly less than those induced by i.p. injection. All animals responded to produce a peak titre of 1:500 on day 8 with relatively low titres thereafter.

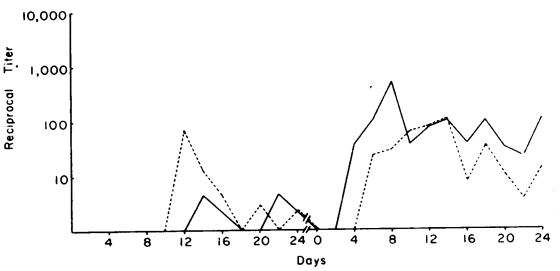

Figure 2. The IgE (——) and IgG1 (– – –) antibody response to intramuscular immunization with 31 μg of ACF antigen. Animals were immunized on day 0 and boosted on day 28 (0) and bled on alternate days for a period of 24 days. Each data point is the mean for at least four animals.

DISCUSSION

This report describes a new allergen, the ACF antigen, from *A. suum* and is unlike most of the previously reported helminth allergens in that it is an excretory–secretory product collected from developing larvae. As third-stage larvae develop and moult to the fourth stage, *in vitro*, they produce a substance, the ACF antigen. This antigen is able to provoke an active cutaneous anaphylactic reaction in guinea-pigs immunized against *A. suum* as well as to induce a protective immune response to a challenge infection in guinea-pigs when given i.m. in Freund's complete adjuvant. The ACF antigen has been identified as a single substance on gel filtration and electrophoresis and as a single precipitin band when reacted with specific antisera in gel diffusion and immunoelectrophoresis (Stromberg, 1979).

Ambler, Croft, Doe, Gemmell, Miller & Orr (1973) described an allergen in the excretory–secretory products of adult *A. suum* when maintained overnight in saline. This substance was similar to their Allergen A which had been isolated from saline extract of adult *A. suum* (Ambler *et al.*, 1972). The majority of *Ascaris* allergens described in the literature have been extracts of adult worms (Strejan & Campbell, 1968; McAninch

Table 1. IgE* response to ACF antigen

Day	Route of immunization and antigen dose			
	i.p. 3·1†	i.p. 31‡	i.p. 62§	i.m. 31¶
Primary response				
0	0±0·0	0±0·0	0±0·0	0±0·0
2	0±0·0	0±0·0	0±0·0	0±0·0
4	0±0·0	0±0·0	0±0·0	0±0·0
6	0±0·0	0±0·0	0±0·0	0±0·0
8	0±0·0	0±0·0	0±0·0	0±0·0
10	0±0·0	0±0·0	2±2·0	0±0·0
12	0±0·0	7±7·0	5±2·2	0±0·0
14	0±0·0	0±0·0	10±0·0	5±2·2
16	2±2·0	0±0·0	37±7·4	2±2·0
18	2±2·0	0±0·0	37±7·4	0±0·0
20	4±3·6	10±0·0	46±4·6	0±0·0
22	0±0·0	0±0·0	17±6·1	5±2·2
24	0±0·0	7±7·0	14±3·7	2±2·0
Secondary response				
0	5±2·2	0±0·0	22±4·6	0±0·0
2	0±0·0	2±2·0	22±4·6	0±0·0
4	100±0·0	171±4·2	46±7·6	37±2·0
6	7937±1·3	3344±2·8	3684±2·0	100±0·0
8	215±2·2	841±1·2	1357±2·0	500±0·0
10	464±2·2	71±2·2	2924±1·7	37±6·4
12	464±2·2	334±1·5	1710±1·7	71±1·4
14	100±0·0	178±1·8	63±7·9	100±0·0
16	171±2·5	224±2·0	464±2·2	37±2·0
18	100±0·0	32±4·4	215±2·2	100±0·0
20	171±1·7	150±1·9	368±2·0	32±3·2
22	292±1·7	106±1·7	136±2·0	22±2·2
24	100±0·0	50±0·0	171±1·7	100±0·0

* Mean IgE titre ± SEM as determined for at least four animals each sample day post-immunization by conversion to logarithms.
† Immunization with 3·1 μg ACF antigen i.p.
‡ Immunization with 31 μg ACF antigen i.p.
§ Immunization with 62 μg ACF antigen i.p.
¶ Immunization with 31 μg ACF antigen i.m.

Table 2. IgG1* response to ACF antigen

Day	Route of immunization and antigen dose			
	i.p. 3·1†	i.p. 31‡	i.p. 62§	i.m. 31¶
Primary response				
0	0±0·0	0±0·0	0±0·0	0±0·0
2	0±0·0	0±0·0	0±0·0	0±0·0
4	0±0·0	0±0·0	0±0·0	0±0·0
6	0±0·0	3±3·0	0±0·0	0±0·0
8	0±0·0	0±0·0	0±0·0	0±0·0
10	0±0·0	0±0·0	0±0·0	0±0·0
12	0±0·0	10±10·0	0±0·0	71±1·4
14	2±2·0	0±0·0	0±0·0	14±3·7
16	0±0·0	10±10·0	0±0·0	5±2·2
18	0±0·0	0±0·0	0±0·0	0±0·0
20	0±0·0	50±0·0	0±0·0	3±3·0
22	0±0·0	0±0·0	0±0·0	0±0·0
24	0±0·0	7±7·0	0±0·0	2±2·0
Secondary response				
0	0±0·0	0±0·0	4±3·7	0±0·0
2	0±0·0	0±0·0	0±0·0	0±0·0
4	0±0·0	0±0·0	5±4·6	0±0·0
6	17±1·7	3±3·9	108±2·2	122±2·2
8	5±4·6	40±1·6	37±6·4	129±1·7
10	8±3·1	0±0·0	17±4·2	163±3·1
12	5±4·6	71±2·2	79±3·9	171±1·4
14	10±3·8	27±1·8	29±6·1	100±0·0
16	22±2·1	18±1·8	171±1·7	8±3·1
18	10±3·8	22±1·6	79±3·1	37±2·0
20	8±3·1	71±1·2	171±1·7	10±0·0
22	4±3·7	47±1·7	8±7·9	4±3·7
24	10±0·0	5±1·9	29±6·1	14±3·7

* Mean IgG1 titre ± SEM as determined for at least four animals each sample day post-immunization by conversion to logarithms.
† Immunization with 3·1 μg ACF antigen i.p.
‡ Immunization with 31 μg ACF antigen i.p.
§ Immunization with 62 μg ACF antigen i.p.
¶ Immunization with 31 μg ACF antigen i.m.

& Patterson, 1970; Hussain *et al.*, 1972) or the perienteric fluid of adult worms (Hogarth-Scott, 1967; Kuo & Yoo, 1977). Only two ascarid allergens have been isolated and characterized, Allergen A (Ambler *et al.*, 1973; Ambler, Miller & Orr, 1974a, b) and Asc-1 (Hussain *et al.*, 1972; Hussain, Bradbury & Strejan, 1973) and both were isolated from extracts of adult worms.

The guinea-pig has been shown to produce at least two different types of homocytotropic antibodies (Block, 1968). The IgG1 type described by Ovary, Benaceraf & Block (1963), and the IgE type was found in animals infected with *Trichinella spiralis* (Catty, 1969) or *A. suum* (Dobson *et al.*, 1971a, b). Levine, Chang & Vaz (1971) also described reagin production in the guinea-pig in response to repeated injection of minute doses of benzylpenicilloyl-bovine gammaglobulin. Perini & Mota (1972) described a third homocytotropic antibody, IgG1b, in the guinea-pig which is similar to IgE (heat and mercaptoethanol sensitive) but it persists in the skin for less than 1 week. To determine if our IgE may have been IgG1b, we ran 10

or 12 day latent period PCA tests for all our peak response sera often without loss of titre or commonly with the titre reduced to the next lower dilution.

A significant primary IgE response was elicited by the i.p. presentation of 62 μg of the ACF antigen, when compared with the lower doses (3·1 and 31 μg), which induced low and sporadic primary responses. This demonstrates that the primary response is dependent on antigen dose. Similar findings were reported by Strejan & Surlan (1977) where increasing doses of Asc-1 induced higher titre IgE responses as well as increasing the number of animals responding.

All three concentrations of the ACF antigen induced high titres of IgE on secondary stimulation. This response is very strong and rapid, beginning on the fourth day, peaking on the sixth day (1:7937 for 3·1 μg) and returning to relatively low levels by the tenth day for the lower concentrations and being somewhat more prolonged with the 62 μg dose. This is also similar to the findings of Strejan & Surlan (1977) except that these titres are considerably higher (1:50,000 *v.* 1:1600). Few investigators have reported such high titres which were so consistently produced in all animals (on secondary immunization).

Other investigators have also reported high titres of IgE. Dobson *et al.* (1971a) reported a titre of 1:5000 of IgE-like antibody in *A. suum* -immunized guinea-pigs when given second-stage larvae of *A. suum* intravenously, while Khoury (personal communication) reported titres of 1:10,000 in orally infected guinea-pigs which had been immunized against *A. suum*. Jarrett, Haig & Bazin (1976) demonstrated parasite-specific IgE titres of 1:4096 in rats given a secondary infection of *Nippostrongylus brasiliensis*. Jarrett & Stewart (1974) demonstrated high titres (1:32,768) to ovalbumin given i.p. with *Bordetella pertussis* adjuvant in the Hooded Lister rat.

The induction of high levels of specific IgE with the ACF antigen did not require the use of adjuvants or immunopotentiators. Most studies utilizing soluble antigens employed adjuvants such as alum or *B. pertussis* to induce a predominantly IgE response (Strejan & Campbell, 1968; Mota & Perini, 1970; Jarrett & Stewart, 1974; Mitchell, 1976) but little is known of the roles of these adjuvants (Mota, 1964; Clasen, Munoz & Bergman, 1970; Levine & Vaz, 1970; Prouvost-Danon, Stiffel, Mouton & Biozzi, 1971; Tada, Okumura, Ochiai & Iwasa, 1972). In this way the ACF antigen in the guinea-pig may serve as a model for the study of IgE production.

This study has also demonstrated the importance of the route of presentation in inducing a reaginic response in the guinea-pig. When 31 μg of ACF antigen were given i.m. there was a minimal response of 1:500 after secondary stimulation compared to 1:3344 for the same dose given i.p. It is attractive to speculate about the potential of different routes and the type of response elicited but studies in other species demonstrate great variability. Jarrett, Haig, McDougal & McNulty (1976) demonstrated that primary and booster (secondary and tertiary) IgE responses were elicited to ovalbumin in the rat when given *per os* or subcutaneously with *B. pertussis*; while several investigators have demonstrated that the oral presentation of antigen in mice inhibited IgE production (Andre, Hermans, Vaerman & Cambiasco, 1975; David, 1975; Vaz, Maia, Hanson & Lynch, 1977).

The ability of the ACF antigen to induce high levels of specific IgE may indicate what occurs naturally in the immunized host. After oral infection with eggs of *A. suum*, larvae hatch and develop through a moult where they may produce the ACF antigen which induces high levels of IgE and resistance to re-infection. The importance of IgE and IgG1 antibody was indicated by its ability to transfer protection in Strain 2 guinea-pigs (Khoury, Stromberg & Soulsby, 1977).

Further studies are required to demonstrate the importance of the ACF antigen and its ability to induce high specific IgE levels in leading to a better understanding of the IgE response in guinea-pigs and its involvement in the protective immune response against *A. suum*. Studies are underway comparing the ACF antigen with Asc-1 and evaluating the ability of Asc-1 to induce IgE antibody when presented i.p.

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REFERENCES

AMBLER J., MILLER J.N. & ORR T.S.C. (1974a) Some properties of *Ascaris suum* allergen A. *Int. Archs Allergy*, **46**, 427.

AMBLER J., MILLER J.N. & ORR T.S.C. (1974b) Ultraviolet light absorption and fluorescence properties of an

allergen extracted from *Ascaris suum*. *Int.Archs Allergy*, **47**, 351.

AMBLER J., MILLER J.N., JOHNSON P. & ORR, T.S.C. (1973) Characterization of an allergen extracted from *Ascaris suum*. Determination of molecular weight, isoelectric point, amino acid and carbohydrate content of the native allergen. *Immunochemistry*, **10**, 815.

AMBLER J., DOE J.E., GEMMELL D.K., ROBERTS J.A. & ORR T.S.C. (1972) Biological techniques for studying the allergenic components of nematodes. I. Detection of allergenic components in *Ascaris suum* extracts. *J. Immunol. Meth.* **1**, 317.

AMBLER J., CROFT A.R., DOE J.E., GEMMELL D.K., MILLER J.N. & ORR T.S.C. (1973) Biological techniques for studying the allergenic components of nematodes. II. The characterization of the allergen released by *Ascaris suum* maintained in saline. *J. Immunol. Meth.* **2**, 315.

ANDRE C., HERMANS J.F., VAERMAN J.P. & CAMBIASO C.L. (1975) A mechanism for the induction of immunological tolerance by antigen feeding: antigen–antibody complexes. *J. exp. Med.* **142**, 1509.

BLOCK K.J. (1968) The antibody in anaphylaxis. In: *Cellular and Humoral Mechanisms in Anaphylaxis and Allergy* (Ed. by H. Z. Mouat) p. 1. S. Karger, Basel.

CATTY D. (1969) The immunology of nematode infectious trichinosis in guinea pigs as a model. *Monogr. Allergy*, **5**, 11.

CLAUSEN C.R., MUNOZ J. & BERGMAN R.K. (1970) A reaginic type of antibody stimulated by extract of *Bordetella pertussis* inbred strains of mice. *J. Immunol.* **104**, 312.

DAVID M.F. (1975) Prevention of homocytotropic antibody formation and anaphylaxis in rats by prefeeding antigen. *J. Allergy Clin. Immunol.* **55**, 135 (Abstr.).

DOBSON C., MORSETH D.J. & SOULSBY E.J.L. (1971a) Immunoglobulin E-type antibodies induced by *Ascaris suum* infection in guinea pigs. *J. Immunol.* **106**, 128.

DOBSON C., ROCKEY J. & SOULSBY E.J.L. (1971b) Immunoglobulin E antibodies in guinea pigs: characterization of monomeric and polymeric components. *J. Immunol.* **107**, 1431.

HOGARTH-SCOTT R.S. (1967) The molecular weight range of nematode allergens. *Immunology*, **13**, 535.

HUSSAIN R., STREJAN G. & CAMPBELL D.H. (1972) Hypersensitivity to *Ascaris* allergens. VII. Isolation and partial characterization of an allergen. *J. Immunol.* **109**, 638.

HUSSAIN R., BRADBURY S.M. & STREJAN G. (1973) Hypersensitivity to *Ascaris* antigens. VIII. Characterization of a highly purified allergen. *J. Immunol.* **111**, 260.

JARRETT E.E.E. & STEWART D.C. (1974) Rat IgE production. I. Effect of dose of antigen on primary and secondary reaginic antibody responses. *Immunology*, **27**, 365.

JARRENT E.E.E., HAIG D.M. & BAZIN H. (1976) Time course studies on rat IgE production in *N. brasiliensis* infection. *Clin. exp. Immunol.* **24**, 346.

JARRETT E.E.E., HAIG D.M., MCDOUGALL W. & MCNULTY E. (1976) Rat IgE production. II. Primary and booster reaginic antibody responses following intradermal or oral immunization. *Immunology*, **30**, 671.

KABAT E.A. & MAYER M.M. (1961) *Experimental Immunochemistry*, p. 528. Charles C. Thomas, Springfield, Illinois.

KHOURY P.B., STROMBERG B.E. & SOULSBY E.J.L. (1977) Immune mechanisms to *Ascaris suum* in inbred guinea-pigs. I. Passive transfer of immunity by cells or serum. *Immunology*, **32**, 405.

KUO C.Y. & YOO T.J. (1977) A new allergen from the perienteric fluid of *Ascaris suum* with respect to charges. *Int. Archs Allergy Appl. Immun.* **54**, 308.

LEVINE B.B. & VAZ N.M. (1970) Effect of combinations of inbred strain, antigen dose on immune responsiveness and reagin production. A potential mouse model for immune aspects of human atopic allergy. *Int. Archs Allergy Appl. Immun.* **39**, 156.

LEVINE B.B., CHANG H. & VAZ N.M. (1971) The production of hapten-specific reaginic antibodies in the guinea pig. *J. Immunol.* **106**, 29.

LOWRY O.H., ROSEBROUGH N.H., FARR A.L. & RANDALL R.J. (1951) Protein measurement with the Folin phenol-reagent. *J. Biochem.* **193**, 265.

MCANINCH J.R. & PATTERSON R. (1970) Reagin-like antibody formation in rabbits during heterologous antibody response to *Ascaris* antigens. *Immunology*, **18**, 91.

MITCHELL G.F. (1976) Studies on immune responses to parasite antigens in mice. II. Aspects of the T cell dependence of circulating reagin production to *Ascaris suum* antigens. *Int. Archs Allergy Appl. Immun.* **52**, 64.

MOTA I. (1964) The mechanism of anaphylaxis. I. Production and biological properties of mast cell sensitizing antibodies. *Immunology*, **7**, 681.

MOTA I. & PERINI A. (1970) A heat labile maccaptoethanol susceptible homocytotropic antibody in the guinea pig. *Life. Sci.* **9**, 923.

OVARY Z. (1964) Passive cutaneous anaphylaxis. *In: Immunological Methods: CIOMS Symposium* (Ed. by J. F. Ackroyd), p. 259. Blackwell Scientific Publications, Oxford.

OVARY Z., BENACERAF B. & BLOCK K.J. (1963) Properties of guinea-pig 7S antibodies. II. Identificational antibodies involved in passive cutaneous and systemic anaphylaxis. *J. exp. Med.* **117**, 951.

PERINI A. & MOTA I. (1972) Heterogeneity of guinea pig homocytotropic antibodies. *Immunology*, **22**, 915.

PROUVOST-DANON A., STIFFEL C., MOUTON D. & BIOZZI G. (1971) Anaphylactic antibodies in mice genetically selected for antibody production. *Immunology*, **20**, 25.

SADUN E.H. (1972) Homocytotropic antibody response to parasitic infections. In: *Immunity to Animal Parasites* (Ed. by E. J. L. Soulsby), p. 97. Academic Press, New York.

STREJAN G. & CAMPBELL D.G. (1968) Hypersensitivity to *Ascaris* allergens. IV. Production of homocytotropic antibodies in the rat. *J. Immunol.* **101**, 628.

STREJAN G.H. & SURLAN D. (1977) Reaginic antibody production to *Ascaris suum* allergen, Asc-1. I. The function of glutaraldehyde-polymerized antigen in the induction of reaginic (IgE) antibodies in the rat. *Int. Archs Allergy*, **54**, 487.

STROMBERG B.E. (1978) The isolation and partial characterization of a protective antigen from developing larvae of *Ascaris suum*. *Int. J. Parasit.* **9**. (In press.)

STROMBERG B.E., KHOURY P.B. & SOULSBY E.J.L. (1977) Development of larvae of *Ascaris suum* from the third to fourth stage in a chemically defined medium. *Int. J. Parasitol.* **7**, 149.

TADA T., OKUMURA K., OCHIAI T. & IWASA S. (1972) Effect of

lymphocytosis-promoting factor of *Bordetella pertussis* on the immune response. II. Adjuvant effect for the production of reaginic antibody in the rat. *Int. Archs Allergy Appl. Immun.* **43,** 207.

VAZ N.M., MAIA L.C.S., HANSON D.G. & LYNCH J.M. (1977) Inhibition of homocytotropic antibody responses in adult inbred mice by previous feeding of the specific antigen. *J. Allergy clin. Immunol.* **60,** 110.