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Antibody-Dependent Adherence and Damage to the Young Adult Worms of *Angiostrongylus cantonensis* by Rat Eosinophils *in Vitro*

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Introduction

Angiostrongylus cantonensis is a neurotropic and pulmonary parasite in rats. The parasite occasionally infects man, causing a condition known as eosinophilic meningoencephalitis (Alicata and Jindrak, 1970). When infective third-stage larvae are eaten by rats, they migrate to and develop in the rat brains for 26–29 days after infection, when they move to the pulmonary arteries, mature, mate and lay eggs. The infected rats usually acquire protective immunity to the parasite (Lim *et al.*, 1965; Yoshimura *et al.*, 1979a, b; Au and Ko, 1979a; Yong and Dobson, 1982a), although the mechanism(s) of the protective immunity and the major sites of worm destruction remain to be determined (Au and Ko, 1979a). It has been recently established that the protective immunity could be transferred a certain extent to naive rats by intraperitoneal injections of serum (Yong and Dobson, 1982b) or lymph node cells (Yong and Dobson, 1982c) from immune rats. Specific worm stage(s) vulnerable to the host protective immunity has to be determined, however.

On the other hand, peripheral eosinophilia occurs in infected rats 6–7 weeks postinfection, when oviposition by female worms or hatching of the first-stage larvae usually occurs in the lungs (Au and Ko, 1979a, b). Although the possible role of the eosinophilia in *A. cantonensis* infection in rats has not been identified, it has been revealed that eosinophils would play an important role in killing of young adult worms surgically transferred into the pulmonary arteries of nonpermissive hosts (Yoshimura *et al.*, 1980, 1983).

In an attempt to gain an insight into the possible effector role of the eosinophils in *A. cantonensis* infection in rats and nonpermissive hosts as well, we investigated the ability of the cells to adhere to and cause damage to the parasite *in vitro*.

Materials and Methods

Parasite: The life cycle of a Hawaiian strain of *A. cantonensis* has been main-

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tained in our laboratory through Wistar rats and *Biomphalaria glabrata* snails. The adult worms of a Japanese sheep liver fluke (*Fasciola* sp.) were collected from the bile ducts of a cow at a local slaughterhouse, and used for obtaining intrauterine eggs for miracidial hatching. *Lymnea ollula* snails exposed to the miracidia were maintained in an aquarium, from the wall of which the metacercariae were harvested 2–3 months later.

Animals and infection with A. cantonensis *and* Fasciola *sp.*: Male outbred Wistar rats weighing 250–300 g and inbred F344 rats weighing 200 g were utilized. The experimental infection of rats with *A. cantonensis* was performed as described elsewhere (Yoshimura and Soulsby, 1976). Rats were also given *Fasciola* sp. metacercariae orally.

Antisera: Sera were obtained at appropriate intervals from F344 rats infected with 60 third-stage larvae of *A. cantonensis* and also from Wistar rats infected with 30 metacercariae of *Fasciola* sp. as described previously (Yoshimura and Soulsby, 1976), and stored at −80 C until use.

Serology: Phosphate buffered saline (pH 7.2) extracts of *A. cantonensis* and *Fasciola* sp. adult worms were prepared by the method of Yoshimura and Soulsby (1976). Gel diffusion for assessing precipitin titers of sera from rats infected with *A. cantonensis* or *Fasciola* sp. was carried out as described elsewhere (Ouchterlony and Nillsson, 1973). Forty-eight hr-passive cutaneous anaphylaxis (PCA) reaction to assess reaginic antibody was performed as previously described (Yoshimura *et al.*, 1979b).

Preparation of intracranial worms: Weeks 2 and 3 intracranial worms of *A. cantonensis* were harvested from the subarachnoid spaces of rats infected with 160–180 third-stage larvae, as described previously (Yoshimura *et al.*, 1980), and collected in RPMI 1640 (Nissui Seiyaku, Tokyo) supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin.

Preparation of eosinophil-rich peritoneal exudate cells (PEC): Five-week *A. cantonensis* (60 third-stage larvae/rat)-infected F344 rats were intraperitoneally injected with 10 ml of 0.9% saline 48 hr prior to cell harvest. The rats were killed with ether, the abdominal skin cut open and 50 ml of TC 199 (Nissui Seiyaku, Tokyo) supplemented with heparin (5 units/ml) injected into the peritoneal cavity. After gentle massage for 1 min, the fluid and cells were then withdrawn using a Pasteur pipette. Cells obtained from 2–3 rats were suspended in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml) (10% FCS/RPMI), and incubated in plastic tissue culture dish (Falcon 3002, Oxnard, Ca.) for 2 hr at 37 C in 5% CO_2 in air to remove the adherent cells. Then, the dish was gently agitated and the supernatant removed and centrifuged at 1500 rpm for 10 min. Following removal of the supernatant, the number of cells and eosinophils remaining in the sediments was determined by Turk's stain and Discombe's stain respectively using a hemocytometer. Differential cell counts of the non-adherent cells were also made on Giemsa stained smear preparations. These cell preparations (PEC) (N=22) contained a mean % eosinophil count of 64.6±9.5, neutrophil count of 5.0±3.3, lymphocyte count of 11.0±3.2, macrophage count of 17.0±7.1, and mast cell count of 2.4±2.8.

In vitro *cell adherence assay*: Eight to 10 worms of Weeks-2 or 3 intracranial *A. cantonensis* were added to 0.2 ml of PEC suspension, 0.2 ml of heat-inactivated (hi) infected rat serum (IRS) or hi-normal rat serum (NRS), or hi-RPMI 1640, and 0.2 ml of 10% FCS/RPMI in Nunc multidish wells (Cat. No. 143982, Inter Med, Denmark) and incubated at 37 C in 5% CO_2 in air. The third-stage larvae for cell ad-

herence assay were harvested from infected *B. glabrata* snails by digestion with artificial gastric juice and separated by a slight modification of the method of Oshima (1976). For 3rd-stage larvae, 0.05 ml of 10% FCS/RPMI containing 30–40 larvae was added to 0.05 ml of PEC suspension plus 0.05 ml of IRS or NRS in microslide culture chambers (Bellco Glass, Vineland, NJ.), and incubated as described above.

At the termination of incubation, all worms of each well were transferred to a glass slide with a small amount of 0.9% saline solution for light microscopic examination. Cell attachment to the parasite was quantitated by assessing cellular adherence to the exterior surface of the worms, and scored as follows: (a) 4+, total worm surface was covered with cells; (b) 3+, 60–90% of the surface covered; (c) 2+, 30–60% of the surface covered; (d) 1+, 10–30% of the surface covered; and (e) 0, less than 10% of the surface covered.

A preliminary study on PEC:worm (Week 3 worms) ratio, using IRS obtained from 8-week infected rats and 2 hr-incubation system, indicated that significant cell adherence occurred at a ratio of 100,000:1 (mean score ± SD = 2.3±0.7) or 200,000:1 (2.6±0.8) but not at a ratio of 20,000:1 (0.9±0.8). The ratio of 100,000:1 was thus employed throughout this study. A ratio of 20,000:1 was, however, employed for the assay of the 3rd-stage larvae, since their size is extremely small as compared with that of the Week 3 worms.

The time course of PEC adherence to Week 3 worms in the presence of IRS was followed up to 48 hr after incubation. A peak cell adherence (score=2.8±0.5, N=90) was noted at 2 hr of incubation and gradually decreased to control (NRS or RPMI 1640) levels at 48 hr. The evaluation of cell adherence in the present study was thus performed at 2 hr following incubation.

In order to determine the end titer for cell adherence, a serum pool from 8-week infected rats was used; the study indicated that a 1:384 dilution was still capable of inducing significant cell adherence to Week 3 worms. The final serum dilution of 1:24 was thus utilized throughout this study.

Column chromatography: Two ml of IRS obtained from 8-week infected rats were gel-filtrated on a Sephadex G-200 column (25×720 mm) as described previously (Yoshimura and Yamagishi, 1976), and fractions obtained were concentrated using a collodion bag (Sartorius) and then used for cell adherence assays.

Inhibition of cell adherence by Protein A: Protein A (*Staphylococcus aureus*) (Pharmacia) was dissolved in RPMI 1640 to 2 mg/ml. 0.2 mg of Protein A was then added to each well containing IRS or its IgG fraction, PEC suspension, and Week 3 worms.

Transmission electron microscopy: After their incubation with PEC and IRS, or NRS for 2 or 20 hr, the Week 3 worms were washed several times in 0.9% saline and then fixed for electron microscopy in 2.0% cold glutaraldehyde in 0.1 M phosphate buffer, and post-fixed in 2.0% osmium tetroxide; the specimens were then dehydrated and embedded in epon 812. The preparation of ultrathin sections, staining with uranyl acetate and lead citrate, and examination with a Hitachi H-500 electron microscope were carried out as described elsewhere (Yoshimura *et al.*, 1983).

In order to determine the localization of antibody on the worm surface, Week 3 worms incubated in IRS or NRS plus PEC for 1 hr at 37 C were washed five times in 0.9% saline and re-incubated for an additional hour in a ferritin-conjugated IgG fraction rabbit anti-rat IgG (1:5 dilution) (Cappel Laboratories, Dawningtown, Pa.). The worms were then washed several

times in saline, fixed in glutaraldehyde and processed as described above.

Scanning electron microscopy: Worms incubated with PEC and NRS or IRS for 2 hr were washed five times in 0.9% saline and fixed as described for transmission electron microscopy. The worms were dehydrated in ethanol and dried in a Hitachi HCP-1 critical point dryer. Specimens were then coated with gold-palladium in an ion coater (Type IB-3, Eiko Engineering, Tokai-mura, Ibaragi-ken) and examined in a Hitachi S-450 scanning electron microscope.

Pulmonary arterial transfer of worms: In order to determine whether cell adherence reactions to Week 3 worms *in vitro* affect their subsequent survival *in vivo*, the worms incubated with PEC and IRS, or NRS (or RPMI 1640) for 20 hr were washed several times in 0.9% saline and surgically transferred into the pulmonary arteries of naive recipient rats by the method of Yoshimura *et al.* (1979b). The rats were necropsied 24 days posttransfer for determining worm recovery.

Statistical analysis: Mean cell adherence scores and mean worm recovery rates were calculated on individual groups and statistical differences in these values between groups were assessed by Student's t-test.

Results

Specific antibody-dependent adherence of PEC to Weeks 2 or 3 worms

An initial experiment was attempted to determine when PEC adherence-promoting antibody to Week 3 worms appears in sera of rats infected with *A. cantonensis*. Fig. 1 indicates that this antibody could not be detected in sera from rats 2 weeks following infection but appeared by 4 weeks and then being consistently present throughout the course of the observations.

The antibody-dependent PEC adherence to the third-stage larvae could not be observed even when they were incubated for 20 hr in the presence of either IRS (obtained from 2-, 4-, and 8-week infected rats) or NRS. The PEC adherence, however, occurred on Week 2 intracranial worms in the presence of IRS (obtained from 4-, 8- and 12-week infected rats) and their cell adherence scores were comparable to those of Week 3 worms. The data are thus suggestive of the stage specificity of the antibody-dependent PEC adherence.

In an attempt to determine the antibody specificity of this PEC adherence, sera from 8-, 10-, and 12-week *Fasciola* sp. infected rats were used for assessing their capability of promoting PEC adherence to Week 3 worms. These sera, however,

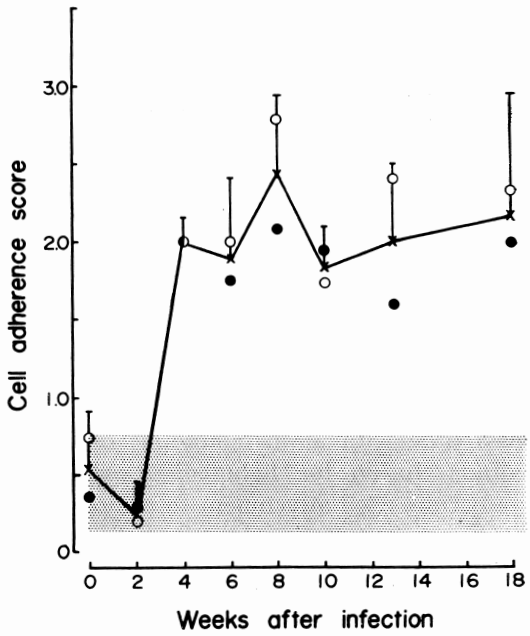


Fig. 1 Time course of the production of antibody capable of promoting *in vitro* cell adherence to Week 3 intracranial worms in rats infected with *A. cantonensis*. The values were obtained from three independent experiments. ●, mean score for male worms; ○, mean score for female worms; × with vertical bar, mean ± SD for male plus female worms. Shaded area denotes mean score ± SD for worms incubated only with eosinophil-rich peritoneal exudate cells in RPMI 1640.

Table 1 Opsonizing effect of infected rat serum on the *in vitro* cell adherence reactions to Week 3 intracranial *Angiostrongylus cantonensis*

1st incubation	Washing in RPMI	2nd incubation added with fresh	Cell adherence score*		
			Male Worms	Female worms	Mean
IRS+Worms	Yes	PEC	2.1±1.0 (41)	3.0±0.9 (41)	2.6±1.1[a] (82)
IRS+Worms	No	PEC	3.5±0.6 (33)	3.8±0.4 (42)	3.7±0.5[b] (75)
None	None	IRS+Worms+PEC	3.5±0.6 (41)	3.7±0.5 (42)	3.6±0.6[c] (83)
RPMI+Worms	No	PEC	0.5±0.6 (43)	0.5±0.6 (42)	0.5±0.6[d] (85)
IRS+PEC	Yes	Worms	0.8±0.6 (43)	1.0±0.7 (42)	0.9±0.7[e] (85)
IRS+PEC	No	Worms	3.5±0.7 (41)	3.5±0.6 (40)	3.5±0.6[f] (81)

IRS=8-week infected rat serum. PEC=Eosinophil-rich peritoneal exudate cells. =RPMI 1640.
* Mean±SD (N) of duplicate assays in three independent experiments.
a, b, c and f differed significantly from d. Differences between a and e, and between d and e, were also significant ($p<0.001$).

Table 2 Immunoglobulin class involved in the *in vitro* cell adherence reactions to Week 3 intracranial *Angiostrongylus cantonensis*

	Cell adherence score*		
	Male worms	Female worms	Mean
Gel filtration			
IgM	1.1±0.8 (29)	1.5±1.2 (32)	1.3±1.1[a] (61)
IgG	2.9±1.1 (29)	2.4+0.7 (32)	2.6±0.9[b] (61)
Albumin	1.0±0.7 (30)	1.0±1.0 (32)	1.0±0.8[c] (62)
IRS	3.6±0.6 (30)	2.9±1.0 (33)	3.3±0.9[d] (63)
PBS	0.4±0.6 (29)	0.4±0.6 (30)	0.4±0.6 (59)
RPMI 1640	0.4±0.6 (30)	0.4±0.7 (30)	0.4±0.6 (60)
Inhibition by Protein A (PA)			
IgG	2.1±1.0 (30)	3.0±1.2 (30)	2.5±1.2[e] (60)
IgG+PA	1.4±0.9 (30)	1.5±1.1 (30)	1.5±1.0[f] (60)
IRS	2.8±0.8 (30)	2.8±0.8 (30)	2.8±0.8[g] (60)
IRS+PA	2.3±1.0 (29)	2.6±0.7 (31)	2.5±0.8[h] (60)
NRS	0.5±0.5 (15)	0.5±0.6 (15)	0.5±0.6 (30)
RPMI 1640	0.4±0.6 (23)	0.3±0.5 (38)	0.4±0.5 (61)

IRS=8-week infected rat serum. PBS=Phosphate buffered saline (pH. 7.2) used for eluate of gel filtration.
* Mean±SD (N) of duplicate assays in two independent experiments.
d differed significantly from a, b, and c. Other statistical differences: a vs b $p<0.001$; b vs c $P<0.001$; e vs f $p<0.001$; g vs h $p<0.05$.

failed to induce PEC adherence, in spite of the presence of anti-*Fasciola* sp. antibody (precipitin titer=1:32).

The opsonic or cytophilic nature of the antibodies was further studied by incubation of IRS or RPMI 1640 with Week 3 worms, or with PEC for 2 hr at 37 C. The worms and cells were then washed three times in large volumes of RPMI 1640. Fresh PEC were added to the preincubated worms whereas fresh worms were added to the preincubated PEC. Both preparations were incubated in the absence of any added rat serum for additional 2 hr. As

a control, the group of simultaneous incubation of worms and PEC in the presence of IRS was also provided. Table 1 indicates that significant PEC adherence (score =2.6) was observed when preincubated worms were used, whereas only slight PEC adherence (0.9) was observed with the use of preincubated PEC.

Determination of antibody class involved in the PEC adherence

A serum pool from 8-week infected rats was fractionated by Sephadex G-200 gel filtration. Table 2 indicates that 7S IgG fraction (score=2.6) contained most of the activity present in the whole IRS (3.3), although a slight activity was noted in both 19S IgM and albumin fractions, the activities of which were probably due to the tailing contamination of IgG. An additional approach to identify the class of immunoglobulin involved in the PEC adherence reaction was made by assessing the inhibitory effect of Protein A on antibody-cell reaction, since this agent is known to bind to the Fc portion of rat IgG (Kronvall *et al.*, 1970). Table 2 shows that the addition of Protein A to 7S IgG fraction or whole IRS caused a significant depression of the adherence reaction. These data suggest that the mechanism of PEC adherence is mediated through the Fc portion of anti-*A. cantonensis* IgG.

Immunoelectron microscopic study using ferritin-conjugated rabbit anti-rat IgG demonstrated that the label was found on the surface of the Week 3 worms at the sites of eosinophil-parasite contact (Fig. 2). To the contrary, no label could be found on the surface of fresh parasite immediately after recovery from the rat brains.

In order to examine the possible involvement of IgE antibody in the adherence reaction, PCA positive serum samples from rats taken 6 and 8 weeks after infection were heated at 56 C for 2 hr and resulting PCA negative sera were tested for adherence-promoting activity. PCA negative sera were then found to be still capable of inducing PEC adherence reaction.

Damage to Week 3 worms by PEC adherence reaction

As the PEC adherence did not affect the survival of worms during the period of incubation, the *in vitro* toxic effect of the PEC adherence on Week 3 worms was confirmed by assessing their *in vivo* survival, when they were transferred into the lungs of naive recipient rats. Table 3 shows that the survival of both male (Exp. I) and female (Exp. II) worms incubated with PEC plus IRS was significantly less than that of those worms incubated with IRS alone, or with PEC plus RPMI 1640 or with PEC plus NRS. This is indicative of the possibility that the antibody-

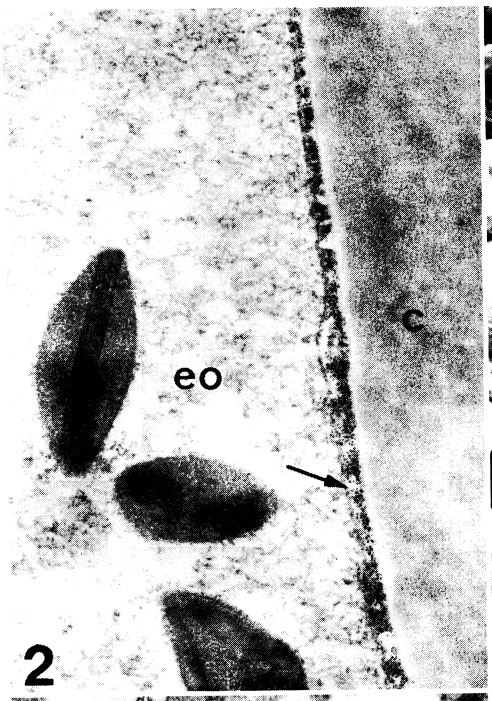
Explanation of Figures

Fig. 2 Transmission electron micrograph showing interaction between Week 3 intracranial worm and an eosinophil (eo). Ferritin particles (arrow) are seen on the cuticular (c) surface of the parasite (× 45,000).

Fig. 3 Scanning electron micrograph showing the attachment of many cells to the surface of Week 3 intracranial worm. Note flattened cells and pseudopodia extending from the cells to cover the worm surface (×2,160).

Fig. 4 Transmission electron micrograph showing an eosinophil (eo) adhering to the cuticle (c) of Week 3 intracranial worm. Note electron dense material (arrow) between the parasite and cell surface. Large cytoplasmic vacuoles (arrowhead) containing dense, coarsely granular materials are seen in the eosinophil (×25,200).

Fig. 5 Transmission electron micrograph showing an eosinophil (eo) adhering to the cuticle (c) of Week 3 intracranial worm after 20 hr in culture. Note ragged losses of granule cores (arrow) (×12,600).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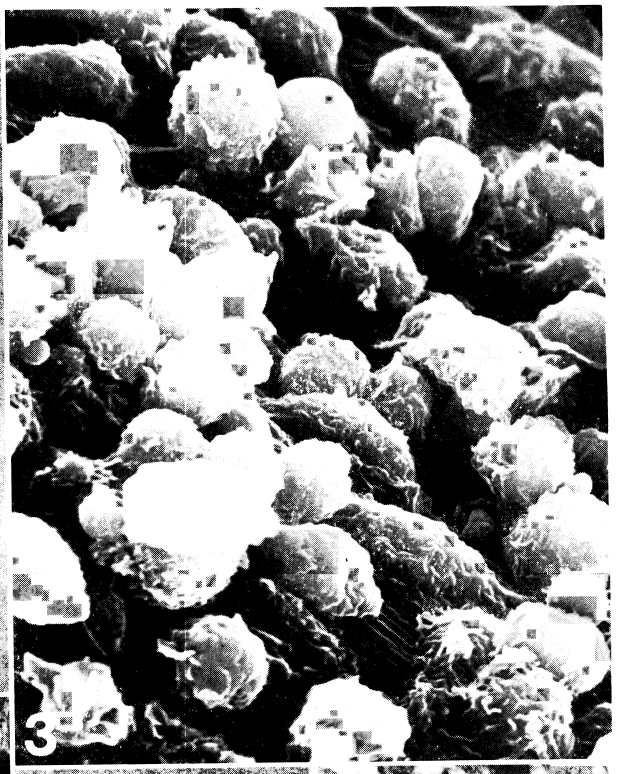
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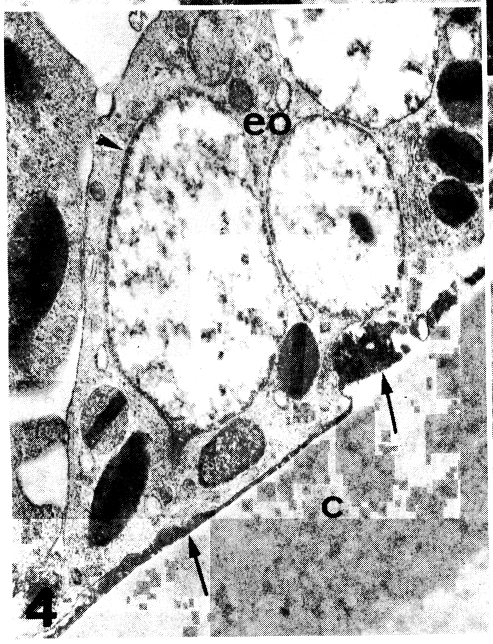
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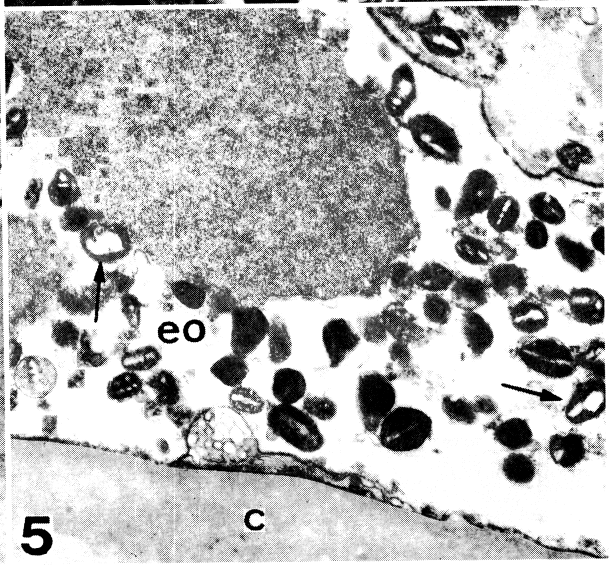
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Table 3 The survival of Week 3 intracranial *Angiostrongylus cantonensis* incubated with eosinophil-rich peritoneal exudate cells plus infected rat or normal rat serum, when transferred into the pulmonary arteries of naive recipient rats

Worms incubated with	Exp. I			Exp. II		
	No. of rats implanted	% Worm recovery*		No. of rats implanted	% Worm recovery*	
		Male	Female		Male	Female
PEC+IRS	18	39.5±24.0[a]	32.9±33.1	11	53.5±28.9	40.0±23.9[d]
PEC+NRS	—	—	—	9	50.8±28.9	54.5±21.2[e]
IRS	15	58.2±20.9[b]	25.9±28.0	—	—	—
PEC+RPMI 1640	14	72.9±27.2[c]	25.2±24.9	8	62.5±32.0	59.3±22.2[f]

Experiments I and II were carried out in quintuplicate and triplicate respectively.

PEC=Eosinophil-rich peritoneal exudate cells. IRS=8-week infected rat serum. NRS=Normal rat serum.

* Mean±SD. Statistical differences: a vs b $p<0.05$; a vs c $p<0.001$; d vs e $p<0.02$; d vs f $p<0.001$.

dependent PEC adherence caused damages to the worms, leading to less worm survival *in vivo*.

Electron microscopy of PEC-parasite interaction

Scanning electron microscopy indicates that, when the Week 3 worms were incubated with PEC in the presence of IRS for 2 hr, the surface of the worms was attached with numerous cells, some of which were flattened and extended pseudopodia over the surface of the parasite (Fig. 3). With transmission electron microscopy, most of the cells adhering to the surface of the worms were found to be eosinophils, and some extracellular electron dense material was found deposited at the sites of eosinophil-parasite contact (Fig. 4). It is also of interest to note that the eosinophil included large cytoplasmic vacuoles containing electron dense, coarsely granular materials (Fig. 4). At 20 hr after incubation, some degenerating eosinophils with ragged losses of granule cores were found attached to the surface of the worms (Fig. 5).

Discussion

Although the association of eosinophilia with helminth infections has been well known, the mechanism of eosinophilia and the functional role of the cells have only recently begun to be elucidated. The evaluation of eosinophilia in parasitic infections was stimulated by *in vivo* information that the acquired resistance of mice to *Schistosoma mansoni* was diminished by depletion of eosinophils in these mice with monospecific anti-eosinophil serum (Mahmoud *et al.*, 1975) and also by an additional *in vitro* evidence that the eosinophil could be an effector cell capable of inducing antibody-dependent damage to schistosomula of *S. mansoni* (Butterworth *et al.*, 1975). Following these pilot studies, IgG antibody-dependent eosinophil adherence to and killing of the newborn larvae of *Trichinella spiralis* (Kazura and Grove, 1978; Kazura and Aikawa, 1980) and of the microfilariae of *Dipetalonema viteae* (Rudin *et al.*, 1980) and *Onchocerca volvulus* (Greene *et al.*, 1981) have been identified.

The current study clearly indicates that specific IgG antibody-dependent eosinophil adherence occurs on Weeks 2–3 intracranial worms of *A. cantonensis* but not on the third-stage larvae, and also that the cell adherence caused marked damages to the worms as confirmed by worm transfer experiments. Although the *in vivo* sig-

nificance of this *in vitro* PEC adherence is still unclear, the finding is suggestive of the possibility that the eosinophil would play an important role in protective immunity in *A. cantonensis* infections in rats (Lim *et al.*, 1965; Yoshimura *et al.*, 1979a, b; Au and Ko, 1979a; Yong and Dobson, 1982a). Our data show that in at least *in vitro* systems, Weeks 2 or 3 intracranial worms were vulnerable to the antibody-dependent eosinophilic attack. Although no data are available as to the *in vivo* vulnerability of these particular stages of worms, the current data may provide an insight that the infective third-stage larvae would possibly be refractory to immunological killing mechanisms *in vivo*.

Additionally, the present results may serve as an indirect evidence of potential cytotoxic action of the eosinophil to *A. cantonensis* in nonpermissive hosts. Consistent with this supposition is the observation that the eosinophil would be closely associated with the killing mechanism of young adult worms surgically transplanted into the pulmonary arteries of the nonpermissive hosts (Yoshimura *et al.*, 1980, 1983).

The present work revealed that the eosinophil adherence could be mediated by IgG class antibody, the opsonic (but not cytophilic) action of which was responsible for the adherence reaction (Table 1). When one considers that both eosinophils (PEC) and intracranial worms were harvested from infected rats, it seems likely that the cells and parasites might have been sensitized with specific IgG antibody *in vivo*. It may be impossible, therefore, to deny the involvement of the cytophilic effect of the IgG antibody in the present PEC adherence. This assumption may be supported by the fact that slight cell adherence (score=0.5) occurred even in the absence of IRS (Table 1).

Cytotoxic factor(s) of eosinophils to Week 3 young adult worms remains to be determined. Electron microscopic evidences showing the ragged losses of granule cores and the presence of large cytoplasmic vacuoles containing lysosomal granules are, however, suggestive of the killing mechanisms by the eosinophils. It has been well established that major basic protein (MBP) in granule core is highly cytotoxic to schistosomula of *S. mansoni* (Butterworth *et al.*, 1979), the newborn larvae of *T. spiralis* (Wassom and Gleich, 1979), tumor cells (Butterworth *et al.*, 1979) and various normal cells (Gleich *et al.*, 1979). Ultrastructural evidences indicating similar losses of granule cores have been reported in those cells adhering to the larvae of *T. spiralis* in the presence of immune serum (Perrudet-Badoux *et al.*, 1978) and adhering to the microfilariae in the lymph nodes of onchocerciasis volvulus patients (Rácz *et al.*, 1982), and those cells in the ileum of Crohn's disease (Dvorak, 1980), in all cases of which cytotoxic action of MBP has been suggested to be involved. The involvement of lysosomal enzymes from granule matrices, e.g., peroxidase (Jong *et al.*, 1981; Kazura *et al.*, 1981; Buys *et al.*, 1981) cannot be also excluded, since the formation of the cytoplasmic vacuoles in the eosinophils adhering to the cuticular surface of *A. cantonensis*, and the deposit of electron-dense, secretion material between the eosinophil and the parasite, are consistent with those observations in the cells attached to schistosomula of *S. mansoni* and the newborn larvae of *T. spiralis in vitro* (McLaren *et al.*, 1977; McLaren *et al.*, 1978; Kazura and Aikawa, 1980).

In addition to the IgG antibody-dependent eosinophil adherence, IgE-dependent adherence and damage to *S. mansoni* schistosomula (Capron *et al.*, 1981), and complement-dependent eosinophil adherence to and killing of *S. mansoni* (Rahalho-Pinto *et al.*, 1978; McLaren and Rahalho-Pinto, 1979; Anwar *et al.*, 1979; McKean *et al.*, 1981) have been also evaluated. In

the present investigation, IgE antibody-dependent cell adherence could be excluded, but the presence of complement-dependent adherence and damage to *A. cantonensis* remains to be evaluated, since all the sera tested in this study were heat-inactivated.

When *A. cantonensis* infects nonpermissive hosts such as mice and guinea pigs, the parasite migrates to and develops in the brain for about 20 days after infection but dies there without moving to the lungs (Yamashita *et al.*, 1975). The mechanism of this worm death is still unclear, but it is of interest to note that marked eosinophilia can be usually observed in the cerebrospinal fluid of human angiostrongyliasis cantonensis patients (Alicata and Jindrak, 1970). In addition to their regulatory functions of allergic inflammations (Gleich, 1977; Kay, 1979), therefore, eosinophils may have more crucial roles in killing *A. cantonensis* in the brains of the nonpermissive hosts than in those of the rat.

Summary

Eosinophil-rich rat peritoneal exudate cells (PEC) containing 65±9.5% eosinophils were harvested from 5-week *Angiostrongylus cantonensis* infected F344 rats. In the presence of heat-inactivated serum from infected rats, PEC adhered to 2–3-week old intracranial worms but not to the third-stage larvae of *A. cantonensis*. This PEC adherence could not be induced by anti-*Fasciola* sp. rat serum, being suggestive of specific antibody dependence. The PEC adherence was also dependent on the opsonic nature of IgG antibody that appeared by 4 weeks after *A. cantonensis* infection and persisted until at least 18 weeks. Immunoelectron microscopic study indicated that the IgG antibody was localized at the areas of cell-parasite contact on the surface of the worms. Damages to the worms were demonstrated by the reduction of worm recovery rates when those worms with adherent PEC were surgically transferred into the pulmonary arteries of naive recipient rats. These data suggest that the antibody-dependent eosinophil adherence would possibly be associated with the protective immunity to *A. cantonensis* infection in rats.

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In vitro における広東住血線虫幼若成虫へのラット好酸球の抗体依存性附着と傷害作用について

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広東住血線虫 (*Angiostrongylus cantonensis*) 感染ラットの腹腔から好酸球含量の高い腹腔滲出細胞 (PEC) を採取し，これを感染ラット血清の存在下で，本虫の第3期幼虫，ならびに感染後2～3週のラットの脳クモ膜下から採取した虫体に作用させた．その結果，特異抗体の存在下で，PEC は2～3週齢の脳内幼若虫には附着するが，第3期幼虫には附着しないことが明らかとなった．また，この PEC の虫体への附着は肝蛭感染ラット血清では誘導することができず，特異抗体依存性であることを示唆している．PEC の附着を誘導する抗体は IgG クラスのものであり，IgM はこれに関与しない．また，その作用は主として IgG のオプソニン効果によるものである．特異 IgG 抗体がこの PEC 附着を誘導することは免疫電顕法によっても確認された．PEC の附着した虫体をラットの肺動脈へ移植したところ，その生着率は対照より有意に低く，*in vitro* での好酸球の附着が虫体を傷害することが示唆された．