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The role of mucus in antibody-mediated rapid expulsion of *Trichinella spiralis* in suckling rats

M. S. CARLISLE, D. D. MCGREGOR & J. A. APPLETON *James A. Baker Institute for Animal Health, Department of Microbiology, Immunology and Parasitology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York, U.S.A.*

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SUMMARY

Rat pups suckling dams parasitized by *Trichinella spiralis* express rapid expulsion, a protective response that is associated with the entrapment of infectious muscle larvae in intestinal mucus. Immunofluorescent studies revealed that antibodies were bound to the surfaces of the entrapped larvae. Mucus binding and rapid expulsion occurred in normal pups dosed with larvae coated with antibodies prepared from infected rat serum. Subsequent experiments revealed that entrapped larvae escaped from mucus after 2 hr *in vitro* incubation in saline. Escape correlated with the loss of the surface-bound antibodies, suggesting that mucus entrapment was reversible and dependent on antibody coating. Finally, when protective antibodies were injected 1, 2 or 6 hr after larvae were administered to pups, the parasites were forced to leave their epithelial niche and became enveloped in mucus. The above findings suggest that mucus trapping of *T. spiralis* larvae is dependent upon the coating of larvae by antibody, but that trapping is reversible, and is not in itself the pivotal event in rapid expulsion. The primary mechanism of rapid expulsion appears to be antibody-mediated inhibition of processes required for the parasite to maintain itself in the epithelium.

INTRODUCTION

Rat pups suckling *Trichinella spiralis*-infected dams are immune to challenge with the parasite (Culbertson, 1943; Appleton & McGregor, 1984). This immunity is conspicuously expressed in the rapid expulsion (RE) of 75–99% of an oral challenge dose of *T. spiralis* muscle larvae (ML) within 24 hr. RE can be conferred upon suckling rats by passive transfer of polyclonal serum antibodies or monoclonal antibodies (mAb) of the IgG1 or IgG2c subclasses (Appleton, Schain & McGregor, 1988). The antibodies are protective when administered orally or i.p. prior to infection with the parasite (Appleton & McGregor, 1987).

The above findings identified antibody as the specific mediator of RE in infant rats; however, they failed to reveal whether antibody directly impairs the capacity of infectious ML to colonize intestinal epithelium or whether other, non-specific factors have a role in the expulsion process. Mucus in particular has been suggested as a co-factor in the rejection of larvae. Significant numbers of *T. spiralis* larvae have been observed entrapped in intestinal mucus from immune rats within minutes of oral challenge with infectious ML (Lee & Ogilvie, 1982; Appleton & McGregor, 1984; Bell, Adams & Ogden, 1984; Carlisle *et al.*, 1988). Mucus and antibodies have been implicated in the expulsion of another parasitic nematode (Miller, Huntley & Wallace, 1981; Miller & Huntley, 1982).

In the experiments reported here, the relationship between mucus and antibody in the expulsion of *T. spiralis* was investigated. The results indicate that although mucus may function in concert with antibody to prevent parasite invasion, mucus trapping in itself is not the mechanism of RE. A more likely explanation of the rejection process is the ability of antibody to impede the establishment and maintenance of larvae in their epithelial niche.

Abbreviations: BBS, borate-buffered saline; BSA, bovine serum albumin; ddH_2O, double distilled water; DPBS, Dulbecco's phosphate-buffered saline; ESA, excretory-secretory antigens; FITC, fluorescein isothiocyanate; Ig, immunoglobulin; i.p., intraperitoneal; mAb, monoclonal antibody; ML, muscle larvae; 0·85% NaCl-AB, 0·85% NaCl containing 200 IU penicillin, 200 μg streptomycin and 100 μg gentamycin per ml; PBS, phosphate-buffered saline; RE, rapid expulsion.

Correspondence: Dr J. A. Appleton, James A. Baker Institute for Animal Health, Dept. of Microbiology, Immunology and Parasitology, New York State College of Veterinary Medicine, Ithaca, NY 14853, U.S.A.

MATERIALS AND METHODS

Rats

Female AO rats (produced in the James A. Baker vivarium) were maintained and bred between 8 and 12 weeks of age as described previously (Appleton & McGregor, 1985b). Fourteen-day-old rat pups were used for all experiments.

Parasite

All procedures relating to *T. spiralis* maintenance and rat infection have been described previously (Appleton & McGregor, 1984, 1985a, b).

Antibodies

Anti-*T. spiralis* rat mAb have been described elsewhere (Appleton *et al.*, 1988). mAb 9E (IgG2c) and mAb 9D (IgG1), used in experiments reported here, were concentrated from hybridoma-induced nude mouse ascites by one of the following methods. (i) Precipitation using 40% saturated $(NH_4)_2 SO_4$. Precipitates were washed once with 45% saturated $(NH_4)_2 SO_4$ and dialysed against borate-buffered saline (BBS), pH 8·3. (iii) Affinity chromatography on a column bearing mouse monoclonal anti-rat kappa chain (MARK-1; Bazin, Cormont & DeClercq, 1984; kindly provided by Dr H. Bazin, University of Luvain, Brussels, Belgium), as described elsewhere (Appleton *et al.*, 1988). Either purification method yielded antibody of comparable binding activity per mg protein, as measured in ELISA and by absorbance at 280 nm. The ELISA employed excretory-secretory antigens (ESA) of ML and has been described previously (Appleton *et al.*, 1988).

T. spiralis-infected rat serum was obtained from adult AO rats dosed with 1000 ML. ESA-specific antibodies were measured in individual sera collected 7 weeks post-infection. Rats with serum levels of ESA-specific antibodies equal to or greater than those of sera previously documented to be protective were anaesthetized and exsanguinated by cardiac puncture 9–12 weeks post-infection. Sera were pooled. Normal rat serum was collected by cardiac puncture (as above) from uninfected adult AO rats. Sera were heat inactivated at 56° for 30 min. Immunoglobulin was precipitated with 40% saturated $(NH_4)_2 SO_4$ and processed as described above. Antibodies were stored at −20°.

Immunofluorescent staining of mucus-entrapped worms

Embedding mucus-entrapped worms. Mucus was harvested from rat pups that were (i) injected three times, i.p., with *T. spiralis*-infected rat serum Ig (30 mg total) during 18 hr prior to challenge; or (ii) reared by dams previously infected with 1000 ML. Pups were dosed with 200 ML and killed 30 min later. The small intestines were removed, and the contents flushed with 0·85% NaCl into test tubes packed in ice. The contents of 10 intestines were pooled and centrifuged at 280 ***g*** for 2 min. The pellet was transferred to a gelatin capsule (Eli Lilly Company, Indianapolis, IN). Embedding medium (OCT, Miles Scientific, Naperville, IL) was added to the capsule, then the capsule was placed in an aluminum boat filled with the same medium. The boat was immersed in isopentane and then frozen in liquid nitrogen. Ten-micrometer sections were cut on an American Optical Cry O-cut II microtome (American Optical Company, Buffalo, NY). Cut sections on glass were immersed in acetone for 10 min at 4° and were stored at −80°.

Negative and positive control sections were prepared by incubating approximately 40,000 ML in 1 ml Ig (20 mg/ml) from normal or *T. spiralis*-infected rats. One millilitre of each larval/antibody mixture was rocked gently at room temperature for 40 min. The larvae were pelleted by centrifuging at 280 ***g*** and the supernate aspirated. Larvae were suspended in cold (4°) 0·85% NaCl, centrifuged as before, and this washing procedure repeated. Washed, pelleted larvae were embedded as described above.

Staining of sections. Sections were thawed, covered with 10% normal goat serum (Pel-Freez Biologicals, Rogers, AR) in phosphate-buffered saline (PBS), pH 7·2, and incubated for 18 min at room temperature. Sections were drained of normal goat serum and covered with fluorescein-conjugated goat anti-rat Ig (Fisher Scientific, Rochester, NY), prepared in PBS containing 4 mg/ml rhodamine-conjugated bovine serum albumin (BSA; St Louis, MO). After 30 min at room temperature sections were washed three times in PBS for 5 min, dipped into ddH_2O and then mounted in gelvatol medium, pH 7·2 (Johnson *et al.*, 1982), and coverslipped. Sections were examined with a Leitz ortholux fluorescence microscope (Ernst Leitz, Wetzlar, FRG), fitted with a 490 nm filter and photographed using a Leitz orthomat W camera and 400 ASA Kodak Ektachrome film.

Treatment of larvae with specific antibody prior to infecting rats

Approximately 40,000 infectious ML were incubated for 30 min at room temperature with gentle rocking in a 1·5 ml polypropylene microcentrifuge tube in 1 ml of 0·85% NaCl containing 20 mg *T. spiralis*-infected rat serum Ig or normal rat serum Ig. Subsequently, larvae were processed in one of two ways. First, larvae and Ig were diluted to 400 ML/ml in a solution of 0·6% nutrient broth and 2% gelatin (final Ig concentration of 200 μg/ml). Pups were dosed with 200 ML suspended in the diluted Ig solution. Second, antibody-treated larvae were suspended in 500 ml of cold 0·85% NaCl and centrifuged 2 min at 280 ***g***. The supernatant was aspirated and washing was repeated twice. Larvae were suspended in 0·6% nutrient broth and 2% gelatin at a concentration of 400 ML/ml. Pups were dosed with 200 ML.

Pups were killed at 30 min post-challenge by cervical dislocation. The small intestines were immediately clamped *in situ* at four sites to yield segments comprising the proximal 0–5%, the proximal 6–52% and the distal 53–100% of the small intestine. Each intestinal segment was flushed with saline to collect the luminal contents. These contents were examined for free larvae and mucus-associated worms, as described previously (Appleton & McGregor, 1984). The segments were opened and incubated at 37° for 5 hr in 0·85% NaCl-AB to allow worms to migrate from the tissue. Litter mates were killed 24 hr after challenge and their intact intestines were flushed, opened and incubated as above. Worms that migrated from intestinal tissue during the 5 hr incubation were counted to assess intestinal worm burden. These numbers were used as an index of the immune status of the pup, as rapid expulsion is complete by 24 hr (Appleton & McGregor, 1985a). Parasites were counted using a dissecting microscope.

In vitro *escape of larvae from mucus*

Pups suckling *T. spiralis*-infected dams were challenged with 200 ML and killed 30 min later. The luminal contents of the entire small intestine of individual pups were collected (as described above), in a volume of 5 ml, into a test tube on ice. The intestinal contents were transferred to 100 × 20 mm polystyrene tissue culture dishes (Becton-Dickinson and Company, Lincoln Park, NJ) with grids etched onto the bottom surface. Each dish contained 20 ml of 0·85% NaCl-AB. The dishes were incubated at 37°. The number of muscle larvae free in each dish and trapped in mucus were counted (as described above) at the start of incubation (0 hr) and at 2-hr intervals.

Immunofluorescent staining of worms free from mucus

Collection of larvae free from mucus. The subjects of these experiments were pups suckling either *T. spiralis*-infected dams or normal dams that had received 80 mg *T. spiralis*-specific rat serum Ig intravenously 2 days previously. *In vitro* escape of larvae from mucus was assessed as described above. After 4 hr and 6 hr, free worms were collected from each sample and placed on ice.

Three sets of staining controls were prepared. One set was prepared by incubating challenge larvae in 0·85% NaCl-AB at 37° for 6 hr. Another set was prepared by obtaining freshly digested ML just before immunofluorescent staining. The third set was prepared by challenging normal pups with 200 ML. The pups were killed 30 min after challenge and their small intestines were flushed with saline, opened, then incubated at 37° for 5 hr in 0·85% NaCl-AB. Worms that migrated from the intestines during 5 hr incubation were collected and placed on ice.

Staining of larvae. Recovered larvae were placed in 12 × 75 mm polystyrene test tubes (Becton-Dickinson), and incubated with fluorescein-conjugated goat anti-rat IgG (heavy and light chain specific; Organon-Teknika-Cappel, Malvern, PA) prepared in PBS containing 4 mg/ml rhodamine conjugated-BSA and 10% normal goat serum. Worms were incubated 30 min on ice, washed twice with 2 ml cold PBS, transferred to a glass slide in a small volume of PBS and covered with gelvatol mounting medium, pH 7·2, and coverslipped.

The presence of larval surface antigens was assessed by incubating larvae in *T. spiralis*-infected rat serum Ig for 30 min on ice followed by two washes with 2 ml cold PBS prior to adding the fluorescein conjugate, as above. Stained ML were examined and photographed as described above.

In vitro *shedding of antibody by ML*

An ELISA using intact ML in suspension as antigen was performed as described previously (Appleton & McGregor, 1985b), with the following variations. Briefly, 160 ML were incubated with infected rat or normal serum Ig (fivefold serial dilutions tested in triplicate). The ML were washed twice with 0·1% gelatin-DPBS and incubated for 0 min, 30 min, 1 hr, 2 hr, 4 hr or 6 hr at 37°. Shed antibodies were washed away with two changes of 0·1% gelatin-DPBS before addition of peroxidase-conjugated goat anti-rat IgG (heavy and light chain specific; Organon-Teknika-Cappel). Substrate addition and completion of the procedure was performed as described previously (Appleton & McGregor, 1985b).

Transfer of antibody after parasite establishment

Rat pups were injected i.p. with 30 mg *T. spiralis*-infected or normal rat serum Ig, or with 2·5 mg *T. spiralis*-specific IgG2c or IgG1 mAb. Antibodies were administered 1 hr prior to challenge with 200 ML, or at various intervals up to 30 hr post-challenge. Established parasites were recovered from intestines of pups 48 hr post-challenge and counted as described above. In one experiment, uninjected littermates were killed at the time of antibody administration in order to document the number of parasites established at that time. In another experiment, mucus entrapment of parasites was assessed 30 min post-challenge in half the pups that received antibody 1 hr prior to challenge. Of pups given antibody 4 hr or 6 hr post-challenge, half were killed 1 hr later. The small intestines from these pups were flushed with saline to collect the luminal contents. The number of mucus-entrapped, free, and established larvae were counted as described above.

RESULTS

Antibody binding to surface of mucus-entrapped worms

Fluorescent antibody staining revealed antibody bound to the surface of mucus-entrapped ML harvested from the intestines of infant rats passively immunized by i.p. injection of specific

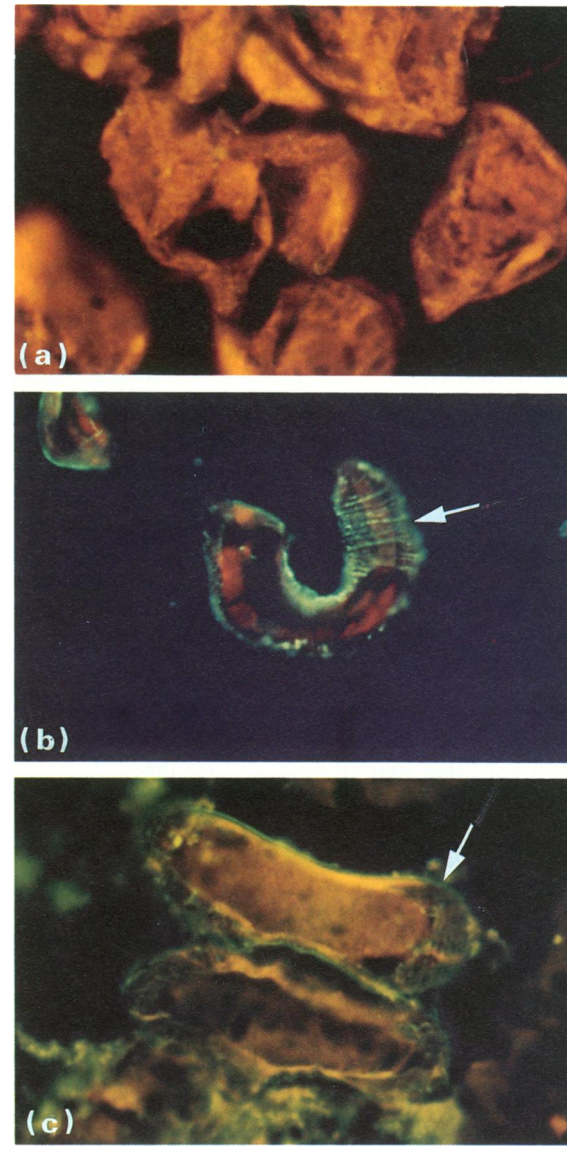

Figure 1. Immunofluorescent staining of *T. spiralis* larvae with fluorescein-conjugated goat-anti rat Ig. Rhodamine-conjugated BSA was applied as a counterstain. Fresh ML were embedded and sections incubated with (a) normal rat serum Ig or (b) *T. spiralis*-infected rat serum Ig prior to staining with FITC-conjugated anti-rat Ig. (c) Mucus was collected from the small intestines of pups that had been injected three times i.p. with *T. spiralis*-infected rat serum Ig (30 mg total) during 18 hr prior to challenge with 200 ML. Mucus was embedded, sectioned and sections stained with FITC-anti rat Ig. Original magnifications: (a and c) × 400; (b) × 250. Arrows: rat antibody highlights striae of larval cuticle.

Table 1. Effect of *in vitro* treatment with specific antibodies on establishment of larvae

Treatment of larvae	Localization of larvae at 30 min post-challenge									Intestinal parasite burden at 24 hr post-challenge†
	Proximal 0–5%*			Proximal 6–52%			Distal 53–100%			
	Lumen	Mucus	Epithelium†	Lumen	Mucus	Epithelium	Lumen	Mucus	Epithelium	
Exp. 1 Muscle larvae not washed after incubation with antibody										
Infected rat serum Ig	0±0	0±0	2± 1‡	0±0‡	5±6	3± 3‡	0±0‡	45±33‡	15±10	54±13‡
Normal rat serum Ig	1±2	0±0	33±15	7±3	1±1	49±23	3±3	0±1	10± 7	100±16
Exp. 2 Muscle larvae washed after incubation with antibody										
Infected rat serum Ig	2±3	0±1	19±16	5±7	4±3‡	19± 8‡	1±1	16±15‡	10±13	83±27‡
Normal rat serum Ig	4±3	0±0	51±39	9±6	0±0	68±40	2±3	0± 0	6± 5	141±39

* Percentage of intestinal length.

† Larvae migrating into saline from tissue (see text).

‡ Significantly different from normal serum Ig recipients ($P<0{\cdot}01$) by Student's or Cochran *t*-test in each treatment group; $n=6$ pups.

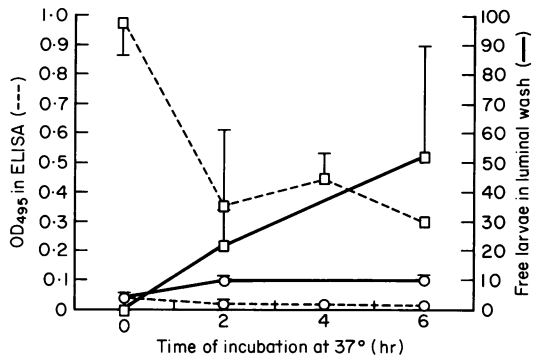

Figure 2. Correlation of larval escape from mucus with shedding of specific antibody *in vitro*. Larval escape from mucus: 14-day-old pups suckling *T. spiralis*-infected dams were challenged with 200 larvae and groups of three pups were killed at 30 min post-challenge. The contents of the intestinal lumen were flushed out with 0·85% NaCl. Mucus aggregates were incubated in 0·85% NaCl at 37° for 0, 2 or 6 hr. Larvae present in the saline, free of mucus, were counted before and after incubation. Mucus was compressed between microscope slides to count larvae remaining entrapped in mucus. (□——□) Mucus from immune pups; (○——○) mucus from normal pups. Shedding of specific antibody *in vitro*: *T. spiralis* larvae were incubated in a 1:250 dilution of infected rat serum Ig (□— —□) or normal rat serum Ig (○— — —○), washed, then incubated for 0, 2, 4 or 6 hr at 37°. A goat anti-rat IgG peroxidase-conjugate was then applied to detect the levels of specific antibody present on the surface of the worm.

antibodies (Fig. 1). A similar distribution of antibody was observed on the surface of mucus-entrapped ML harvested from the intestines of pups suckling *T. spiralis*-infected dams (not shown). Although larvae appeared to be coated with antibody, striae on the parasite surface (Kim & Ledbetter, 1980) were highlighted with fluorescence.

Antibody coating of ML prior to challenge

The presence of antibody on the surface of ML trapped in intestinal mucus indicated that during RE, larvae may contact antibody in the lumen and immediately become entrapped. Because accurate measurement of luminal antibodies is wrought with difficulties, we sought to determine whether larvae coated with antibody *in vitro* could penetrate the epithelium. We also tested whether delivery of additional antibody to the lumen along with antibody-coated larvae would enhance RE. When pups were challenged with antibody-coated ML suspended in specific antibodies, there was a 46% reduction in the number of larvae established in the intestinal epithelium 24 hr later. At 30 min post-challenge, mucus entrapment of ML was greatest in the distal 53–100% of the small intestine. Washing larvae free of antibody prior to challenge had no significant effect on mucus entrapment or RE (Table 1, Exps 1 and 2).

Specific antibody and *in vitro* escape of larvae from mucus

When examining larvae entrapped in mucus, we noted that parasites were active, often giving the impression that they were trying to free themselves from mucus. This suggested that mucus entrapment might be reversible, that is, that antibody-coated larvae may shed antibody (or immune complexes) thereby freeing themselves from mucus during passage through the intestine. Once free, the larvae might establish themselves in the epithelium. To test this proposition, we measured the escape of ML from immune mucus *in vitro*. For these experiments, mucus was harvested from pups suckling *T. spiralis*-infected dams or from pups suckling a normal dam that had been injected intravenously with *T. spiralis*-specific rat serum Ig. After 2 hr incubation in saline at 37°, some ML freed themselves from mucus; however, by 6 hr a significant number of ML had escaped (Fig. 2). In separate experiments, when antibodies and fresh larvae were added to mucus that had been incubated for 6 hr at 37°, such mucus was capable of entrapping larvae (not shown). Escape coincided with antibody shedding, as indicated by the diminished binding of anti-rat IgG to escaped larvae. Although all larvae escaped from mucus were coated with antibodies, the amount of antibody detected on the surface was less than that detected on larvae that had been free in the lumen of immune rats at the time of luminal content collection (Fig. 3). This finding was further supported by quantitative ELISA results that revealed that the amount of antibody bound to the surface of *T. spiralis* ML was diminished after 2 hr *in vitro* incubation (Fig. 2).

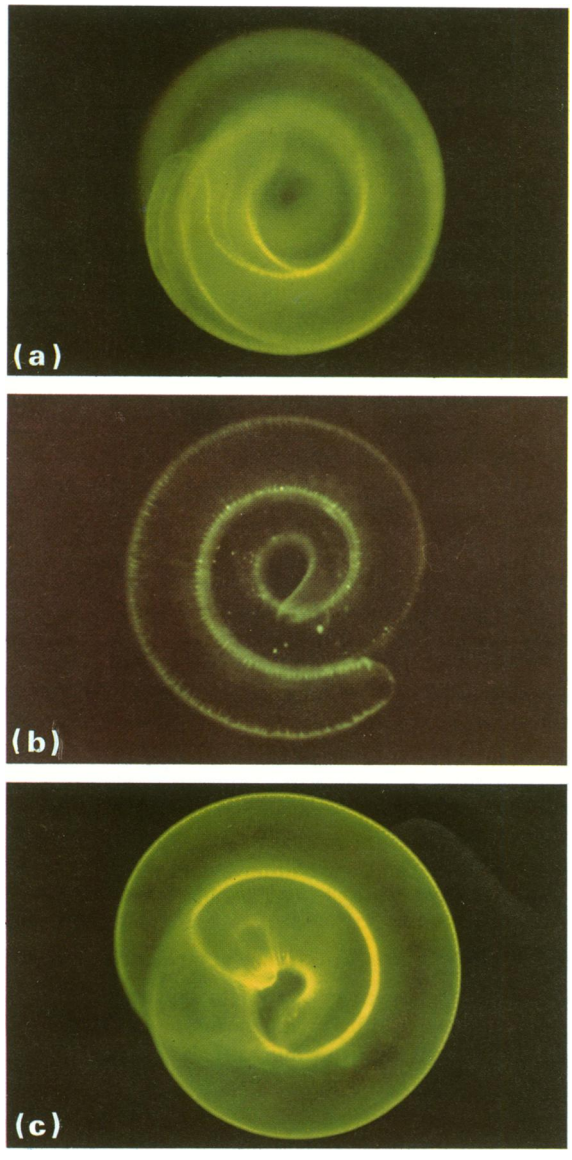

Figure 3. Immunofluorescent staining of larvae escaped from mucus *in vitro*. Intestinal contents from pups reared by dams previously infected with 1000 ML were collected as described (see text). Larva free within intestinal contents at time of harvest (a) and larva free from mucus after 6 hr incubation *in vitro* (b), stained with FITC-anti-rat IgG. In order to document the presence of antigen on the larval surface, other larvae escaped from mucus (as above) were incubated with *T. spiralis*-infected rat serum Ig prior to addition of the conjugate (c). Original magnification: (a–c) × 250.

Table 2. Protective effect of antibodies administered before and after parasite challenge

Time of antibody delivery relative to challenge	Intestinal parasite burden 48 hr post-challenge* Pups treated with antibodies from: Normal rats	Hybridoma 9E	Hybridoma 9D	Infected rats
Exp. 1				
1 h prior	77 ± 3		37 ± 6†	
6 hr post	99 ± 19		46 ± 18†	
30 hr post	98 ± 9		109 ± 17	
Exp. 2				
1 hr prior	115 ± 12	1 ± 1‡		24 ± 36‡
6 hr post	108 ± 14	30 ± 25‡		83 ± 33
30 hr post	121 ± 20	97 ± 18		131 ± 20
Exp. 3				
1 hr prior	101 ± 10	8 ± 7†		
4 hr post	115 ± 28	20 ± 10†		
8 hr post	93 ± 17	61 ± 22		
16 hr post	102 ± 28	88 ± 5		
25 hr post	110 ± 31	90 ± 12		

* Larvae migrating into saline from tissue.

† Significantly lower than normal rat serum Ig recipients ($P = < 0{\cdot}05$) by Student's *t*-test, $n = 3$ pups.

‡ Significantly lower than normal rat serum Ig recipients ($P = < 0{\cdot}01$) by studentized range test; $n = 3$ pups.

Transfer of antibody after parasite establishment

The entrapment of antibody-coated larvae in mucus suggested that antibody present in the intestinal lumen prevented parasite establishment. However, the finding that mucus entrapment was reversible, as well as previous findings that circulating antibodies are protective (Appleton & McGregor, 1984), implied that luminal antibodies and mucus entrapment may not in themselves be sufficient to secure RE. Therefore, the effect of giving protective antibodies systemically after larvae had penetrated the intestinal epithelium was investigated.

When *T. spiralis*-infected rat serum Ig was administered to pups 1 hr prior to challenge with ML, there were 79% fewer worms established at 48 hr post-challenge, compared to pups given normal rat serum Ig. Monoclonal antibody 9E provided 92–99% protection and mAb 9D provided 67% protection (Table 2). Antibodies delivered at 4 hr and 6 hr post-challenge were also protective, although protection was more variable. Antibodies injected 30 hr post-challenge had no protective effect (Tables 2, 3, 4).

Table 3 shows the number of larvae in the intestinal epithelium of normal, uninjected littermates of pups treated with mAb 9E at 6 hr and 30 hr post-challenge. At the time of injection, the parasite burden of uninjected pups was comparable to that of control pups at 48 hr, indicating that parasite establishment was complete at this time. In contrast, the parasite burden at 48 hr in mAb 9E-treated pups was dramatically reduced, indicating that larvae in the epithelium at 6 hr were susceptible to protective antibodies.

Table 4 shows that pups given mAb 9D, either 4 hr or 6 hr post-challenge, rejected the parasite and that the expelled larvae became entrapped in mucus.

DISCUSSION

Mucus functions as a lubricant and barrier on mucosal surfaces (Florey, 1962; Silberberg & Meyer, 1982). The adhesive properties of mucus ensure that it remains in contact with the epithelium (Allen *et al.*, 1982). *T. spiralis* larvae must traverse intestinal mucus in order to penetrate the epithelium. The finding that larvae become associated with mucus during RE (Lee & Ogilvie, 1982; Appleton & McGregor, 1984; Bell *et al.*,

Table 3. Effect of monoclonal antibody 9E on established, intestinal *T. spiralis*. Intestinal parasite burden at the time of antibody delivery was quantified in uninjected littermates of treated pups

Treatment of pups		Intestinal parasite burden*	
Time of antibody delivery relative to challenge	Antibody	At antibody delivery time	At 48 hr post-challenge
1 hr prior	9E	—	3±3†
	Normal rat serum Ig	—	85±7
6 hr post	9E	90±13	12±10†‡
	Normal rat serum Ig	97±2	81±17
30 hr post	9E	106±5	101±11
	Normal rat serum Ig	103±7	114±18

* Larvae migrating into saline from tissue.

† Significantly lower than normal serum Ig recipients ($P = <0{\cdot}01$) by Student's or Cochran *t*-test. $n = 3$ pups.

‡ Significantly lower than parasite burden in littermates at injection time, ($P = <0{\cdot}001$) by Student's or Cochran *t*-test.

Table 4. Mucus entrapment of larvae in intestines of pups treated with antibody 9 before and after parasite challenge

Treatment of pups			
Time of antibody delivery relative to challenge	Antibody	Mucus entrapment*	Intestinal parasite burden at 48 hr post-challenge†
1 hr prior	9D	17±16	34±23‡
	Normal rat serum Ig	1± 1	104±20
4 hr post	9D	12± 8	49± 7
	Normal rat serum Ig	2± 3	110±32
6 hr post	9D	13±15	53±28
	Normal rat serum Ig	0± 1	127±57

* Larvae entrapped in intestinal mucus recovered from luminal contents.

† Larvae migrating into saline from tissue.

‡ Significantly lower than normal rat serum Ig recipients ($P = <0{\cdot}05$) by Student's or Cochran *t*-test; $n = 3$ pups.

1984; Carlisle *et al.*, 1988) suggests that mucus acts in concert with antibody to form an antigen-specific protective barrier. Examples of this type of mechanism include the accelerated removal of BSA by antibodies and mucus in the small intestines of orally immunized rats (Pang, Walker & Bloch, 1981). It has also been reported that the mobility of human spermatozoa is significantly impaired when the spermatozoa are treated with anti-sperm antibodies from infertile men (Aitken *et al.*, 1988) or women (Price & Boettcher, 1979). Impaired mobility is thought to be partially responsible for the inability of antibody-coated spermatozoa to penetrate cervical mucus (Jager *et al.*, 1981).

As for a defensive role for mucus in animals infected with *T. spiralis*, two possibilities come to mind. First, the parasite associates with mucus that has an affinity for antibodies that bind specifically to worms. The effect is to retain worms in the intestinal lumen. Second, antibody compromises the mobility or metabolism of larvae. In this case, their subsequent association with mucus might facilitate transit of the affected larvae through the intestine. The former mechanism involves a role for mucus in preventing the establishment of infectious larvae, whereas the latter implies a non-specific role whereby mucus serves as a vehicle for the transport of larvae that have been functionally impaired by antibody. The distinction is important for it bears on the question of whether antibody impedes the biology of the parasite or merely acts as a ligand in retaining functionally competent larvae within the lumen of the gut.

Several lines of evidence suggest that antibody acts directly on *T. spiralis* larvae in ways that prevent the larvae from maintaining their foothold in intestinal epithelium. Earlier experiments showed that RE is expressed for at least 24 hr after the oral delivery of antibodies to infant rats (Appleton & McGregor, 1984). In addition, it has been reported that antibodies injected i.p. are highly protective against oral challenge with the parasite (Culbertson, 1942; Appleton & McGregor, 1984, 1987). These results indicate that it is not necessary to continuously deliver antibodies to the lumen in order for the antibodies to realize their protective function. Indeed, all four rat IgG subclasses are transported rapidly from the lumen to the blood by Fc receptors on the enterocytes of suckling rats (Peppard, Jackson & MacKenzie, 1985). This transport is remarkably efficient, as the Fc receptors are of high affinity and rapid turnover (Mackenzie, Morris & Morris, 1983). Consequently, milk-derived IgG is rapidly concentrated in the blood of suckling rats. Thus, if IgG were to protect pups by binding larvae before they enter the epithelium, then the antibodies would have to associate specifically with intestinal mucus in order to escape the high affinity Fc receptors of the epithelium. Alternatively, antibodies would have to bind to larvae in the stomach. To our knowledge, antibody binding to mucus has not been documented for IgG in suckling rats or any other species. It is possible that larvae may contact antibody in the relatively hospitable environment of the stomach of suckling rats. Upon entering the intestine, such larvae would become entrapped in mucus.

The most compelling evidence that larvae contact antibody in the epithelium comes from the experiments reported here in which antibody was injected systemically after parasites were established in the epithelium. The expulsion of such larvae by circulating antibodies indicates that antibodies can attack the parasite in its intestinal niche. Thus, the critical event underlying RE appears to be the expulsion of larvae from the epithelium. It is speculated that if larvae shed antibodies (as we have shown they can do *in vitro*) during their transit through the intestine and regain the capacity to penetrate the epithelium, they may encounter antibody in this location and be expelled once again.

How long after epithelial penetration are *T. spiralis* larvae subject to expulsion? It was found that polyclonal or monoclonal antibodies were protective when delivered as long as 4–8 hr after oral infection. By comparison, antibody had no beneficial effect whatsoever when given 30 hr post-challenge. There is a correlation between parasite surface antigen expression described by others (Phillip, Parkhouse & Ogilvie, 1980; Parkhouse & Clark, 1983; Parkhouse & Ortega-Pierres, 1984) and the level of protection observed here at the specified times post-

infection. Within 6–12 hr post-infection, L1 larvae molt to the L2 stage (reviewed by Despommier, 1983). With each molt, the surface antigens change both quantitatively and qualitatively. The surface antigens of L1 larvae are targeted by mAb able to mediate RE (Appleton *et al.*, 1988). Our experiments revealed that antibodies able to mediate RE were less effective if delivered after 8 hr, approximately the time of the first molt when many surface antigens are lost. Adult *T. spiralis* worms are present in the intestine from 31 hr post-infection (Despommier, 1983). Such worms bear surface antigens that are distinct from those of the larval stages (Phillip *et al.*, 1980). This correlates with the observation (M. S. Carlisle and J. A. Appleton, unpublished data) that protective rat mAb 9D and mAb 9E bind to ML but not to adult worms and with earlier findings that adult worms are not affected by the immunity conveyed by parasitized rat dams to their pups (Appleton & McGregor, 1985b).

In conclusion, the mechanism whereby *T. spiralis* larvae are rapidly expelled from the suckling rat intestine involves expulsion of L1 larvae from the epithelium. The expulsion is mediated by surface antigen-reactive antibodies. Larvae expelled from the epithelium become entrapped in intestinal mucus. This entrapment may be reversible. Although mucus entrapment of larvae may occur prior to their initial entry into the epithelium, it is postulated that the primary mechanism in rapid expulsion of *T. spiralis* larvae is the expulsion of larvae from intestinal epithelium by antibodies that are present in blood.

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