Immunology 1991 **74** 552–558

The role of the antibody Fc region in 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, College of Veterinary Medicine, Ithaca, New York, U.S.A.*

Accepted for publication 3 July 1991

SUMMARY

When an IgG2c monoclonal antibody specific for *Trichinella spiralis* muscle stage larvae was cleaved with pepsin to yield $F(ab')_2$ fragments, the latter retained their capacity to cause mucus entrapment and rapid expulsion of larvae from the intestines of suckling rats. When fed to pups, the $F(ab')_2$ fragments of this antibody and the $F(ab')_2$ fragments of a similarly prepared IgG2a antibody caused mucus entrapment of muscle larvae (ML), demonstrating that trapping is not dependent upon the Fc region of the antibody molecule. Despite the fact that these two antibodies had similar specificities and that their $F(ab')_2$ fragments caused larval entrapment in mucus, $F(ab')_2$ fragments of the IgG2a antibody failed to protect rat pups. Fragments of the IgG2c antibody caused rapid expulsion when injected into pups, but the distribution of larvae was dramatically different from when the fragments were delivered orally. These results indicate that entrapment of *T. spiralis* in mucus is not in itself the cause of the expulsion. The more likely possibility is that antibody impedes a function of *Trichinella spiralis* that is related to the capacity of the parasite to reside in its epithelial niche.

INTRODUCTION

Rapid expulsion of *Trichinella spiralis* muscle larvae (ML) by suckling rats is mediated by antibodies that are transferred to pups in their dam's milk.[1,2] Absorption of protective serum immunoglobulins with subclass-specific antibodies demonstrated that IgG1 has a major role in the expulsion process.[3] Additional studies with monoclonal antibodies (mAb) have shown that protection could also be effected by antibodies of the IgM, IgG2b and IgG2c subclasses, whereas a single antibody of the IgG2a subclass conferred little if any protection.[4,5]

A role for mucus trapping in the host's immune defence against micro-organisms has been suggested for *T. spiralis*[2,6,7] as well as other intestinal-dwelling parasites[8-11] and bacteria,[12,13] but the mechanism by which such antibodies mediate entrapment is unknown. Results from experiments performed with abbreviated *T. spiralis* infections in adult rats led Bell *et al.* to conclude that mucus entrapment was not essential to parasite expulsion.[14] The experiments reported here were designed to determine the contribution of the antibody Fc to the processes of mucus entrapment and RE of *T. spiralis* in infant rats. The results indicate that the $F(ab')_2$ fragments of a protective mAb can mediate these two phenomena. In addition, the results support our previous conclusion that although mucus entrapment may contribute to RE, it is neither essential nor adequate for the expression of that immunity.

Abbreviations: ESA, excretory/secretory antigens; gmbw, grams per body weight; H & L, heavy and light chains; Ig, immunoglobulin; i.p., intraperitoneal; IRS, infected rat serum; mAb, monoclonal antibodies; ML, muscle larvae; MW, molecular weight; NRS, normal rat serum; RE, rapid expulsion.

Correspondence: Dr M. S. Carlisle, James A. Baker Institute for Animal Health, Dept. of Microbiology, Immunology and Parasitology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, U.S.A.

MATERIALS AND METHODS

Rats

Female AO rats produced in the James A. Baker Institute vivarium were maintained and bred between 8 and 12 weeks of age, as described previously.[15] Fourteen-day-old suckling rat pups were used for all experiments.

Parasite

Procedures relating to *T. spiralis* maintenance and rat infection have been described elsewhere.[2,16]

Antibodies

T. spiralis infected rat serum (IRS) Ig and normal rat serum (NRS) Ig were produced as described previously.[6] The rat mAb 9E (IgG2c) and 18H (IgG2a) used in the experiments reported here have been described by Appleton *et al.*[4] The mAb were concentrated from hybridoma-induced nude mouse ascites by salt precipitation using 33% (mAb 9E) or 40% (mAb 18H) saturated $(NH_4)_2SO_4$. Precipitates were washed once with 45% saturated $(NH_4)_2SO_4$ and dialysed against borate-buffered saline (BBS), pH 8·3. The rat mAb 305 (IgM) was produced as

described elsewhere.[5] The mAb was concentrated from hybridoma-induced rat ascites with 40% saturated $(NH_4)_2SO_4$, as described above.

Pepsin digestion of mAb 9E and purification of F(ab′)$_2$ fragments
Pepsin digestion was performed according to Rousseaux, Rousseaux-Prevost & Bazin[17] with several modifications. Briefly, 130 mg mAb 9E (10 mg/ml prepared as described above) were incubated for 4 hr at 37° with 1% or 2% (w/w) of chromatographically purified pepsin (Sigma Chemical Company, St Louis, MO) in 0·1 M Na-acetate buffer, pH 4·5. The pH of the digestion mixture was then raised to 8·0 and the cleaved antibody was placed on ice. Products were separated at 4° on either Sephacryl S-200 HR or S-300 HR (Pharmacia LKB Biotechnology, Piscataway, NJ; column dimensions 2·5 × 85 cm) in 0·01 M Na-phosphate, 0·15 M NaCl, pH 7·4. Five-millilitre fractions were collected (FRAC-100, Pharmacia LKB Biotechnology). Samples from every third fraction were resolved in SDS–PAGE (8% resolving gel with a 3% stacking gel), and stained with silver.[18] Pre-stained molecular weight standards (Diversified Biotech, Newton Centre, MA) were run in parallel.

PAGE analysis of the unfractionated, digestion mixture revealed that a substantial amount of mAb 9E had not been cleaved by pepsin. Therefore, the mixture was subjected to gel filtration and fractions containing proteins of 96,000–120,000 MW were pooled and concentrated using 45% saturated $(NH_4)_2SO_4$. Samples of the pooled fractions were resolved in SDS–PAGE and stained with silver or Western blotted.[19] Blotted proteins were developed with goat anti-rat IgG (Fc) peroxidase or with goat anti-rat IgG (H & L) peroxidase (Organon Teknika-Cappel, Malvern, PA).[4]

Monoclonal antibody 18H (IgG2a) and NRS Ig were digested with 1% pepsin, following the procedure described for IgG2a.[17] SDS–PAGE analysis revealed complete cleavage of the mAb 18H. Accordingly, the digested proteins were not fractionated but were stored at −20° until used.

Protection experiments
Concentrated F(ab′)$_2$ fragments, intact mAb or NRS Ig were given orally or i.p. (2·5 mg/20 gmbw) to pups suckling normal dams. Six pups each were injected or orally dosed with each preparation. Pups were challenged with 200 ML 1 hr later. The number of ML entrapped in intestinal mucus was assessed 30 min post-challenge by flushing out the luminal contents and counting free and mucus-associated larvae. Thereafter, the intestines were opened and incubated in 0·85% NaCl for 5 hr at 37°. Worms that migrated into the saline were counted. In one experiment, the luminal contents were first collected and then the intestines were digested with 1% pepsin/HCl at 37° for 1 hr with agitation. Worms present in the digest were counted. Littermates were killed 24 or 48 hr post-challenge to determine the number of larvae remaining in the intestine (saline incubation procedure). In some experiments, blood was collected from pups during necropsy.

Quantification of antibody in pup serum
Levels of F(ab′)$_2$-9E or intact 9E in sera pooled from pups within each group were measured by ELISA. The assay employed ESA and was performed as described elsewhere[4] using peroxidase-conjugated, goat anti-rat IgG (H&L). The concentration of F(ab′)$_2$-9E or intact 9E present in the pooled pup sera was calculated using a standard curve of the appropriate antibody preparation.

Intraperitoneal and oral transfer of mAb 305
Fourteen-day-old pups were given 2·5 mg mAb 305, 30 mg NRS Ig or IRS Ig in three divided doses over 18 hr prior to challenge. (This was our routine procedure until it was modified to one dose, 1 hr prior to challenge, as described above.) Six pups were injected i.p. or dosed orally with each antibody. Pups were challenged with 200 ML. Mucus entrapment of ML and intestinal worm burden at 24 hr were assessed as described above.

RESULTS

Cleavage of the 9E mAb by pepsin

Pepsin cleavage of mAb 9E in two separate experiments yielded fragments of different sizes. Experiments 1 and 2 (Tables 1 and 2, Fig. 1) were performed with a product of approximately 110,000 MW. These fragments failed to stain in Western blots with an IgG Fc-specific reagent (not shown) but reacted strongly with anti-IgG (H & L). Other experiments (Tables 3, 5 and 6) were performed with fragments prepared by the same method, but which in SDS–PAGE were 97,000 MW (not shown). Fragments resulting from the two digestions were indistinguishable in terms of their protective capacity and mucus entrapment of ML (see below).

Mucus entrapment of larvae in the intestines of pups immunized with F(ab′)$_2$-9E

F(ab′)$_2$-9E had the ability to entrap *T. spiralis* ML in mucus which rivalled that of the native antibody, but only when the fragments were given orally. Table 1 shows that larvae were effectively trapped in mucus and expelled from the intestine when the 9E mAb or its F(ab′)$_2$ were fed to infant rats. The intact antibody had similar effects when delivered i.p.; however, F(ab′)$_2$-9E fragments administered by this route failed to cause significant larval entrapment at 30 min post-challenge. Although the systemically injected fragments conferred only a relatively modest level of protection, as judged by the intestinal parasite burden after 24 hr, they efficiently impeded the *in vitro* migration of larvae from the intestinal epithelium into saline 30 min post-challenge. Similar results were obtained in subsequent experiments using the same digestion products (Table 2) or the similarly prepared but smaller fragments of mAb 9E that were prepared on a different occasion (Tables 3, 5 and 6).

Thirty minutes post-challenge larvae were present but immobile in the epithelium of pups passively immunized by i.p. injection of F(ab′)$_2$-9E. This was revealed by collecting intestines from immunized littermates and digesting the tissue in 1% pepsin/HCl. Comparing numbers of larvae recovered by two methods in Table 2 shows that larvae were present in the epithelium of immunized pups, but were unable to migrate into saline.

The concentrations of F(ab′)$_2$-9E or intact 9E in the sera of passively immunized pups was determined by ELISA. The results of two separate experiments are plotted in Fig. 1, where it can be seen that when administered orally the intact antibodies were transported rapidly and in moderate concentration to the

Table 1. Recovery of larvae from intestines of pups passively immunized with $F(ab')_2$ of mAb 9E

Treatment of pups		Intestinal larvae 30 min post-challenge			Intestinal parasite burden† 24 hr post-challenge
Route of antibody delivery	Antibody*	Lumen: Free	Lumen: In mucus	Epithelium†	
Oral	NRS Ig	1±2	1±1	57±19	90±27
	mAb 9E	0±0	58±27‡	0±1‡	16±15‡
	$F(ab')_2$-9E	0±0	30±18‡	1±1‡	42±15‡
Intraperitoneal	NRS Ig	2±3	1±1	31±16	83±14
	mAb 9E	0±0	49±17‡	4±6‡	1±1‡
	$F(ab')_2$-9E	0±0	5±3	1±2‡	39±8‡

* Dose = 2·5 mg protein per 20 gmbw.

† Larvae migrating into saline from tissue.

‡ Significantly different from NRS Ig recipients (by either route of delivery) ($P = < 0{\cdot}05$) by studentized range test for equal sample sizes. Pooled data from two separate experiments; $n = 6$ pups per treatment group.

Table 2. Recovery of larvae from intestines of pups passively immunized i.p. with $F(ab')_2$ of mAb 9E

Treatment of pups* (Antibody)	Intestinal larvae 30 min post-challenge: Lumen: Free	Lumen: Mucus	Epithelium: 5 hr migration†	Epithelium: Digest‡	Intestinal parasite burden† 48 hr post-challenge
NRS Ig	1±2	1±2	60±22	86±40	115±16
$F(ab')_2$-9E	0±0	5±5	17±14§	80±20	74±21§
mAb 9E	0±0	58±31§	5±8§	16±25	0±1§

* Pups were injected i.p. or dosed orally with 2·5 mg protein per 20 gmbw.

† Larvae migrating into saline from intestinal tissue.

‡ Larvae recovered from littermates by digestion of intestines in 1% pepsin/HCl.

§ Significantly different from NRS Ig recipients as well as all groups treated 30 hr post-challenge (oral or i.p. route of delivery) ($P = < 0{\cdot}05$) by studentized range test for equal sample sizes. $n = 3$ pups per treatment group.

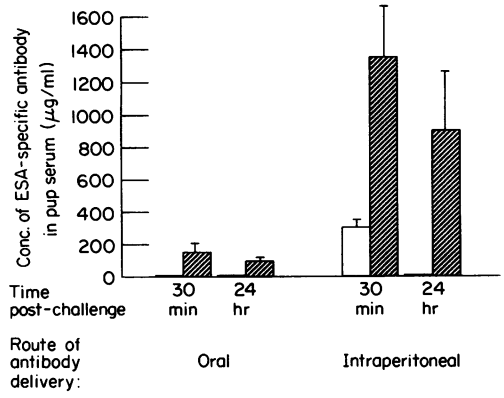

Figure 1. Concentration (μg/ml) of mAb 9E (■) and $F(ab')_2$-9E (□) in pooled pup sera. The values for pooled sera from two separate experiments were averaged and the inter-experiment variation graphed.

blood. When given by the same route, $F(ab')_2$-9E achieved only a very low level (7 μg/ml) in the blood at 30 min and 24 hr. Entirely different results were obtained when the same antibody or its fragments were delivered i.p. In these circumstances, the intact antibody achieved estimated serum concentrations at 1300 μg/ml and 904 μg/ml at 30 min and 24 hr, respectively. After intraperitoneal injection, $F(ab')_2$-9E attained a blood level of 300 μg/ml at 30 min but fell rapidly to a low level (6 μg/ml) at 24 hr post-challenge.

Mucus entrapment of larvae in the intestines of pups treated with 18H or $F(ab')_2$-18H

In these experiments, the 18H mAb demonstrated the unusual property of being able to entrap *T. spiralis* ML in mucus, although the mAb had no significant protective value. We

Table 3. Recovery of larvae from intestines of pups passively immunized with F(ab′)$_2$ of mAb 18H

Treatment of pups		Intestinal larvae 30 min post-challenge			Intestinal parasite burden† 48 hr post-challenge
		Lumen			
Route of antibody delivery	Antibody*	Free	In mucus	Epithelium†	
Oral	NRS Ig	1±1	0±1	94±27	126±19
	F(ab′)$_2$-NRS	0±1	0±0	100±6	117±8
	mAb 18H	1±1	43±17‡	1±1‡	104±33
	F(ab′)$_2$-18H	0±0	106±38‡	4±2‡	123±30
	mAb 9E	0±0	92±26‡	0±0‡	25±4‡
	F(ab′)$_2$-9E	0±0	84±19‡	4±2‡	43±5‡
Intraperitoneal	NRS Ig	0±0	0±0	60±28	112±17
	F(ab′)$_2$-NRS	0±0	0±0	119±6	132±11
	mAb 18H	0±0	5±3	54±24	88±11
	F(ab′)$_2$-18H	0±1	4±4	73±34	83±16
	mAb 9E	0±0	37±7‡	12±12	22±13‡
	F(ab′)$_2$-9E	0±0	0±1	1±1‡	30±7‡

* Dose = 2·5 mg protein per 20 gmbw.

† Larvae migrating into saline from tissue.

‡ Significantly different from NRS Ig recipients (oral or i.p. route of delivery) ($P = < 0{\cdot}05$) by studentized range test for equal sample sizes. $n = 3$ pups per treatment group.

Table 4. Recovery of larvae from intestines of pups immunized with polymeric mAb 305

Treatment of pups		Intestinal larvae 30 min post-challenge			Intestinal parasite burden† 24 hr post-challenge
		Lumen			
Route of antibody delivery	Antibody*	Free	In mucus	Epithelium†	
Oral	NRS Ig	4±4	0±0	58±10	121±25
	IRS Ig	0±0	33±19	14±15‡	3±3‡
	305	0±0	35±22	17±14‡	67±9‡
Intraperitoneal	NRS Ig	0±0	0±0	70±17	92±4
	IRS Ig	0±0	80±13‡	1±2‡	1±1‡
	305	0±0	31±12	17±19‡	58±7‡

* Dose = 30 mg polyclonal antibody or 2·5 mg mAb 305 in three divided doses over 18 hr prior to challenge.

† Larvae migrating into saline from tissue.

‡ Significantly different from NRS Ig recipients (oral or i.p. route of delivery) ($P = < 0{\cdot}05$) by studentized range test for equal sample sizes. $n = 3$ pups per treatment group.

considered the possibility that the antibody's lack of protective capacity might be related in some way to a property of its heavy chain. To test this proposition, the 18H mAb was cleaved by pepsin and then compared with the native antibody for its capacity to entrap larvae and protect recipient rats. The pepsin-generated fragments were sized by SDS–PAGE at 102,000 MW (not shown). In protection experiments, the 18H mAb and F(ab′)$_2$-18H were given orally or injected i.p. Normal rat serum Ig, the 9E mAb and its similarly prepared fragments served as controls.

Table 3 shows that significant mucus entrapment of larvae occurred in recipients that were immunized orally with either the intact 18H mAb or F(ab′)$_2$-18H. Few larvae migrated from the intestinal tissue of these rats 30 min post-challenge. When the same antibodies were delivered i.p., mucus entrapment was trivial and substantial numbers of larvae established themselves

Table 5. Mucus entrapment of larvae in intestines of pups injected i.p. and dosed orally with $F(ab')_2$ of mAb 9E

Treatment of pups			Intestinal larvae 30 min post-challenge			Intestinal parasite burden* 24 hr post-challenge
			Lumen			
Route of antibody delivery	Dose (mg) per 20 gmbw	Antibody	Free	In mucus	Epithelium*	
Oral+ Intraperitoneal	2·5	NRS Ig	0±0	0±0	51±19	141±28
		mAb 9E	0±0	67±14†	1±1†	0±1†
		$F(ab')_2$-9E	0±0	100±17†	0±0†	4±4‡
Oral	2·5	NRS Ig	1±1	0±0	64±18	126±19
		mAb 9E	0±0	92±17†	0±0†	14±8†
		$F(ab')_2$-9E	0±0	88±40†	0±0†	26±13†
Intraperitoneal	2·5	NRS Ig	1±1	0±1	58±20	134±6
		mAb 9E	0±0	83±20†	0±1†	1±1†
		$F(ab')_2$-9E	0±0	0±1	1±1†	26±5†
Oral	5	$F(ab')_2$-9E	1±1	80+14†	1±2†	50±2†
Intraperitoneal	5	$F(ab')_2$-9E	0±0	15±15	0±0†	2±2†

* Larvae migrating into saline from tissue.

† Significantly different from NRS Ig recipients (oral or i.p. route of delivery) ($P = <0·05$) by studentized range test for equal sample sizes. $n = 3$ pups per treatment group.

‡ Significantly less than $F(ab')_2$-9E, 5 mg/20 gmbw delivered orally ($P = <0·05$) by studentized range test for equal sample sizes. $n = 3$ pups per treatment group.

Table 6. Protective effect of $F(ab')_2$ of mAb 9E administered before and after parasite challenge

Treatment of pups*		Intestinal parasite burden in rats†		
		Time of antibody delivery relative to challenge		
Route of antibody delivery	Antibody	−1 hr	+4 hr	+30 hr
Oral	NRS Ig	126±19	107±19	127±27
	mAb 9E	25±4‡	38±17‡	131±19
	$F(ab')_2$-9E	43±5‡	79±9‡§	116±26
Intraperitoneal	NRS Ig	112±17	96±8	107±10
	mAb 9E	22±13‡	25±6‡	122±33
	$F(ab')_2$-9E	30±7‡	63±3‡§	103±15

* Pups were injected i.p. or dosed orally with 2·5 mg protein per 20 gmbw.

† Larvae migrating into saline from tissue.

‡ Significantly different from NRS Ig recipients as well as all groups treated 30 hr post-challenge (oral or i.p. route of delivery) ($P = <0·05$) by studentized range test for equal sample sizes. $n = 3$ pups per treatment group.

§ Significantly greater than mAb 9E recipients (4 hr post challenge) and mAb 9E and $F(ab')_2$-9E (1 hr prior challenge) (for oral or i.p. route of delivery) ($P = <0·05$) by studentized range test for equal sample sizes. $n = 3$ pups per treatment group.

in the intestinal epithelium. Despite the differences in early establishment of larvae, neither oral nor i.p. immunization with 18H or $F(ab')_2$-18H was protective.

Mucus entrapment and rapid expulsion of larvae from the intestines of pups passively immunized with mAb 305

We further investigated the role of the Fc and the influence of antibody size in protection and mucus entrapment using an IgM mAb, 305. Size and subclass may cause this antibody to be distributed in rat tissues differently from the IgG subclasses. Table 4 shows that when administered by either route, mAb 305 was moderately effective in causing larval entrapment as well as protection.

Protective effect of combined systemic and oral delivery of antibodies

To further investigate the relationship between protection and the route by which antibodies were administered, an experiment was performed in which the 9E mAb or its pepsin-cleaved fragments were given separately or together by the oral or i.p. routes. Table 5 shows that combined administration was far more effective than when the antibodies were given by either route alone. The difference was most conspicuous when antibody fragments were used. Thus rats passively immunized with $F(ab')_2$-9E orally and i.p. enjoyed far greater immunity than did pups immunized by an equivalent dose of antibody given by either route alone. Doubling the dose of $F(ab')_2$-9E failed to improve the level of protection conferred on orally immunized pups, whereas doubling the i.p. dose improved protection dramatically. The results in Table 5 also substantiate a finding

revealed in earlier experiments, namely that the entrapment of larvae occurs in rats treated orally or intraperitoneally with the native antibody whereas the trapping mediated by pepsin-cleaved fragments of the same antibody occurs only when the fragments are given orally.

Protective effect of F(ab′)$_2$-9E administered before or after parasite challenge

Experiments reported previously demonstrated that protective antibodies can mediate rapid expulsion of *T. spiralis* larvae even when the antibodies are derived *after* the larvae have established themselves in intestinal epithelium.[6] Therefore the question arose whether fragments of antibody lacking the Fc have a similar capacity and can exercise their protective power when confined to the lumen of the gut. To answer that question mAb 9E or F(ab′)$_2$-9E were administered orally or i.p. to pups either 1 hr before or 4 or 30 hr after recipient pups were challenged orally with infectious larvae. Table 6 indicates that the intact antibody conferred similar protection when given by either route 1 hr before or 4 hr after challenge. By comparison, the protective power of F(ab′)$_2$-9E was significantly less when the fragments were delivered by either route 4 hr post-challenge. Neither intact antibody nor its fragments conferred any protection whatsoever when administered 30 hr post-challenge.

DISCUSSION

The experiments reported here were undertaken with two purposes in mind. First, we wished to test further the hypothesis that the action of antibody to effect mucus entrapment of *T. spiralis* larvae is dependent upon the Fc region of the antibody molecule. Parallel experiments[5] revealed that three of four IgG subclasses could mediate mucus entrapment. We sought to determine whether IgG2a failed to cause entrapment because of some characteristic of the Fc. Second, we wished to know whether the Fc had a role in the capacity of antibodies to expel parasites from the intestine.

Experiments using pepsin-generated fragments of the 9E mAb (an IgG2c) revealed that the Fc was not required for mucus entrapment. F(ab′)$_2$-9E efficiently entrapped *T. spiralis* larvae both *in vitro*[5] and *in vivo*, when delivered *per os* (Table 1). Moreover, an IgM mAb, 305, had a modest but nonetheless demonstrable capacity to entrap larvae. Yet another mAb, 18H (an IgG2a), and its F(ab′)$_2$ caused entrapment under similar circumstances. This result was unexpected, because separate experiments showed that entrapment does not occur when the 18H mAb is transferred serially to suckling rats by passively immunized dams.[5] This apparent contradiction is unexplained. Despite similar mucus entrapment by fragments of 18H and 9E given *per os*, 18H was not protective while 9E was highly protective. Since the entrapment process is reversible *in vitro*,[6] it is plausible that larvae originally present in mucus later escape from mucus and establish themselves in intestinal epithelium. However that may be, it is evident that antibody-induced entrapment of larvae in mucus can occur in circumstances where the antibodies concerned have little if any protective value.

In contrast, we found that when injected i.p., F(ab′)$_2$ fragments of 9E did not cause mucus entrapment. This result suggests that F(ab′)$_2$ fragments of 9E did not reach the lumen. Intact 9E does reach the lumen, as evidenced by the finding that mucus from rats injected i.p. with whole antibody entrapped larvae *in vitro*.[5] Thus, efficient transfer of the 9E mAb to the lumen depended upon the Fc. Like F(ab′)$_2$-9E, the F(ab′)$_2$-18H did not cause mucus entrapment when injected i.p. Neither antibody preparation prevented initial establishment of the parasite in the epithelium, and when evaluated 24 hr post-challenge F(ab′)$_2$-18H-treated rats were not protected. However, established larvae in F(ab′)$_2$-9E-treated rats were unable to migrate from tissue into saline during our standard parasite recovery procedure, and could only by recovered by digesting intestinal tissue with pepsin. Despite their ability to establish in F(ab′)$_2$-9E-treated rats, these compromised larvae were eventually expelled from the intestine. We have recovered similarly compromised larvae in weaned rats injected i.p. with intact 9E mAb. However, unlike suckling rats, larvae in weaned rats are not expelled (O. Otubu, M. S. Carlisle and J. A. Appleton, unpublished observations). The mechanism of this intriguing antibody-mediated compromise remains unsolved.

The 9E mAb which is protective and the 18H mAb which has little if any protective capacity, share antigen specificities. The two antibodies bind the same four larval surface antigens and a family of ES proteins (J. A. Appleton and L. Usack, unpublished data).[4] It is plausible, although not proven, that 9E interacts with its target antigen(s) in such a way that it causes a functional compromise of the parasite. 18H may bind to the same target molecule(s), but in a way that it does not impede that function.

Unlike native antibody, pepsin-generated antibody fragments fail to cross the intestinal epithelium to enter the blood.[20] That phenomenon was exploited in the present study to demonstrate that orally administered F(ab′)$_2$-9E can protect recipient rats despite the fact that it is confined to the intestinal lumen. Such antibody fragments were also effective in expelling larvae when antibody was delivered after infection, indicating that the established parasite was accessible to luminal antibodies. Another significant finding was that antibody fragments delivered orally were limited in their protective capacity, while fragments delivered i.p. were more protective at a higher dose. Taken together the results suggest that the luminal compromise of larvae via entrapment in mucus (or other mechanisms) provides some defence against infection with *T. spiralis*, but that circulating antibodies which expel the parasite from intestinal epithelium are more potent.

Three conclusions can be drawn from these findings. First, protective antibody can impede the colonization of intestinal epithelium by infectious larvae, particularly when antibodies are present in the lumen of the gut. Mucus entrapment of larvae occurs at this time, but is not adequate in itself to mediate protection. Second, the presence of protective antibody in the lumen not only impedes the penetration of larvae but can also promote the expulsion of larvae that have established themselves in the epithelium. Finally, mucus entrapment and protective activities of antibodies are not Fc dependent.

ACKNOWLEDGMENTS

We thank Ms A. Hesser for preparation of this manuscript and Ms Lynn Usack for technical assistance with the production of monoclonal antibodies.

REFERENCES

1. CULBERTSON J.T. (1943) Natural transmission of immunity against

Trichinella spiralis from mother rats to their offspring. *J. Parasitol.* **29,** 114.

2. Appleton J.A. & McGregor D.D. (1984) Rapid expulsion of *Trichinella spiralis* in suckling rats. *Science*, **226,** 70.
3. Appleton J.A. & McGregor D.D. (1987) Characterization of the immune mediator of rapid expulsion of *Trichinella spiralis* in suckling rats. *Immunology*, **62,** 477.
4. Appleton J.A., Schain L.R. & McGregor D.D. (1988) Rapid expulsion in suckling rats: Mediation by monoclonal antibodies. *Immunology*, **65,** 487.
5. Carlisle M.S., McGregor D.D. & Appleton J.A. Mucus entrapment of *Trichinella spiralis* larvae induced by specific antibodies. *Immunology*, **74,** 546.
6. Carlisle M.S., McGregor D.D. & Appleton J.A. (1990) The role of mucus in antibody-mediated rapid expulsion of *Trichinella spiralis* in suckling rats. *Immunology*, **70,** 126.
7. Lee G.B. & Ogilvie B.M. (1982) The intestinal mucus layer in *Trichinella spiralis*-infected rats. In: *Recent Advances in Mucosal Immunity* (eds W. Strober, L. A. Hanson and K. W. Sell), p. 319. Raven Press, NY.
8. Miller H.R.P., Huntley J.F. & Wallace G.R. (1981) Immune exclusion and mucus trapping during the rapid expulsion of *Nippostrongylus brasiliensis* from primed rats. *Immunology*, **44,** 419.
9. Miller H.R.P. & Huntley J.F. (1982) Protection against nematodes by intestinal mucus. In: *Mucus in Health and Disease* Volume II (eds E. Chantler, J. Elder and M. Elstein), p. 243. Plenum Press, New York.
10. Miller H.R.P., Jackson F., Newlands G. & Appleyard W.T. (1983) Immune exclusion, a mechanism of protection against the ovine nematode *Haemonchus contortus*. *Res. Vet. Sci.* **35,** 357.
11. Douglass T.G. & Speer C.A. (1985) Effect of intestinal contents from normal and immunized mice on sporozoites of *Eimeria falciformis*. *J. Protozool.* **32,** 156.
12. Schrank G.D. & Verwey W.F. (1976) Distribution of cholera organisms in experimental *Vibrio cholerae* infections: proposed mechanisms of pathogenesis and antibacterial immunity. *Infect. Immun.* **13,** 195.
13. McSweegan E., Burr D.H. & Walker R.I. (1987) Intestinal mucus gel and secretory antibody are barriers to *Campylobacter jejuni* adherence to INT 407 cells. *Infect. Immun.* **55,** 1431.
14. Bell R.G., Adams L.S. & Ogden W.R. (1984) Intestinal mucus trapping in the rapid expulsion of *Trichinella spiralis* by rats: induction and expression analyzed by quantitative worm recovery. *Infect. Immun.* **45,** 267.
15. Appleton J.A. & McGregor D.D. (1985a) Life-phase specific induction and expression of rapid expulsion in rats suckling *Trichinella spiralis*-infected dams. *Immunology*, **55,** 225.
16. Appleton J.A. & McGregor D.D. (1985b) Induction and expression of rapid expulsion in suckling rats. In: *Proceedings of the Sixth International Conference on Trichinellosis* (ed C. W. Kim), p. 58. State University of New York Press, New York.
17. Rousseaux J., Rousseaux-Prevost R. & Bazin H. (1983) Optimal conditions for the preparation of Fab and $F(ab')_2$ fragments from monoclonal IgG of different rat IgG subclasses. *J. Immunol. Meth.* **64,** 141.
18. Wray W., Boulikas T., Wray V.T. & Hancock R. (1981) Silver staining of proteins in polyacrylamide gels. *Anal. Biochem.* **118,** 197.
19. Burnette W.N. (1981) Western blotting: electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* **112,** 195.
20. Borthistle B.K., Kubo R.T., Brown W.R. & Grey H. (1977) Studies on receptors for IgG on epithelial cells of the rat intestine. *J. Immunol.* **119,** 471.