Monoclonal IgA antibody-mediated expulsion of *Trichinella* from the intestine of mice

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(*Received 6 August 2002; revised 10 December 2002; accepted 10 December 2002*)

SUMMARY

To assess the potential role of IgA antibody in expulsion of the nematode of the genus *Trichinella* from the intestine, a panel of IgA monoclonal antibodies (mAbs) were produced from the mesenteric lymph node cells from BALB/c mice orally vaccinated with irradiated muscle larvae of *Trichinella britovi*. One IgA mAb, HUSM-Tb1, formed immunoprecipitates on the surface of live muscle larvae, and by immunohistochemistry reacted with their stichocytes and cuticular surface, but not with those tissues of the adult stage or newborn larvae. Intraperitoneal injection of BALB/c mice with this mAb 5 h before challenge conferred a high level of protection (more than 95%) against *T. britovi* infection, when 2·0 mg of specific IgA/20 g body weight was given to a mouse. The same treatment produced a similar effect in SCID mice lacking functional T- and B-cells, indicating no requirement of synergistc T-cell factors for the effect. Passive transfer of the mAb at the time of challenge or later showed less or no effect upon worm expulsion. It is concluded that the mucosal IgA response, when adequately induced, can impede the establishment of infective *Trichinella* parasites in the mouse intestine.

Key words: *Trichinella*, IgA, monoclonal antibody, passive transfer, expulsion.

INTRODUCTION

In the last decade, it has become increasingly evident that multiple independent effector mechanisms are involved in worm expulsion of intestinal nematodes, depending on host and parasite factors, or combination of both (Bell, 1998; Else & Finkelman, 1998). The significance of the antibody response for worm expulsion, however, is not clearly defined except for a few laboratory models of intestinal nematode infection such as the 'rapid expulsion' of *Trichinella* spp. by infected rats (Bell & McGregor, 1979; Appleton & McGregor, 1984, 1985, 1987) and expulsion of the same parasite by vaccinated mice (Jarvis & Pritchard, 1992; Robinson, Bellaby & Wakelin, 1995*a*). Particularly, little notice has been given to IgA antibody secreted at the mucosal surface for protective immunity to *Trichinella* spp. or any other intestinal nematodes (Bell, 1998; Else & Finkelman, 1998). Only 1 direct demonstration of IgA for mucosal immunity to nematode infection comes, hitherto, from a successful passive transfer of immunity to *Trichuris muris* infection in mice by IgA monoclonal antibodies (mAbs) (Roach *et al*. 1991).

Oral inoculation of ultraviolet (UV)-irradiated muscle larvae of *Trichinella britovi* confers remarkable protective immunity to a homologous challenge infection in mice (Nakayama *et al*. 1998). Using this model, we have produced a panel of mAbs to determine the possible role of IgA antibody for mucosal immunity to *Trichinella* infection in mice. As shown in this study, it is clearly demonstrated that passive transfer of monoclonal IgA antibody without synergistc T-cell help confers remarkable protective immunity to *T. britovi* infection in a dose-dependent manner.

MATERIALS AND METHODS

Animals

Mice of an inbred (BALB/c) and an outbred (ICR) strains were purchased from a commercial breeder (Japan CLEA, Meguro, Tokyo, Japan) and kept in the Institute for Animal Experiments, Hirosaki University School of Medicine. SCID mice (C.B-17/Icr-*scid* Jcl) lacking functional T- and B-cells were bred in The Central Institute for Experimental Animals, Kawasaki, Japan. They were housed in plastic boxes with commercial pellets (MF; Oriental Yeast Co. Ltd, Tokyo, Japan) and water *ad libitum*. All animal experiments followed the Guidelines on Animal Experimentation as set out by Hirosaki University.

Parasite

T. britovi originated from a Asiatic black bear, *Ursus thibetanus japonicus*, shot in Aomori prefecture, Japan in 1974 (Pozio *et al*. 1996), and thereafter

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Parasitology (2003), **126**, 591–598.
DOI: 10.1017/S003118200300310X Printed in the United Kingdom

maintained in our laboratory using ICR mice and Mongolian gerbils, *Meriones unguiculatus*. To obtain infective muscle larvae, a routine tissue digestion of infected muscles using physiological saline containing 0·8% pepsin and 0·8% HCl was performed at 37 °C for 3–4 h. Digested material was passed through 125 μm metal mesh, and washed several times. Parasite antigens used for immunological assays were prepared from muscle larvae washed thoroughly.

Attenuation of parasites and infection

Short wave-length UV (254 nm) irradiation of freed muscle larvae at a dose of 5 mJ/cm^2 was carried out as described previously (Nakayama *et al*. 1996). Oral inoculation of irradiated or naive larvae was carried out using a metal gastric sonde, and the mean of administered larvae was calculated from counts of 6 random aliquots processed similarly.

Hybridoma production

To collect the mesenteric lymph nodes (MLN) cells for fusion with the mouse myeloma cell line of X-63, three protocols for immunization of BALB/c mice were carried out as follows. (A) Female 12- to 18-week-old mice were orally inoculated with 300 muscle larvae, and similarly reinfected 42 days later. (B) Female 6-week-old mice were orally inoculated with 1000 irradiated muscle larvae, and repeatedly inoculated with the same number of irradiated larvae at 24, 31 and 46 days after the primary inoculation. (C) Mice immunized as described in (B) were orally challenged with 300 muscle larvae at 9th day after the last immunization. Four days after the last oral inoculation, the MLN were carefully removed from 5 mice of each protocol, and used for fusion with murine myeloma cells. Briefly, lymph node cells and myeloma cells were fused at a ratio of 5 : 1 using 50% polyethylene glycol (mol. wt. 1300–1600; Sigma Chemical Co., St Louis, MO, USA). The cells were plated on flat-bottomed 24-well plates at 2×10^6 total cells/well. Splenocytes from normal BALB/c mice were used as feeder cells. Hybridoma cells were selected with HAT medium (Sigma Chemical Co.) from the day of fusion. Screening for antibody production was performed using the enzyme-linked immunosorbent assay (ELISA) of culture supernatants. Immunohistochemically determined clones of interest were subcloned twice by limiting dilution.

Incubation of cultured larvae with mAb

Muscle larvae were repeatedly washed in sterile PBS, and suspended in RPMI 1640 medium (Nissui Pharmaceutical Co., Sugamo, Tokyo, Japan) supplemented with 0·3% L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 0·25 μg/ml amphotericin B, and 10% heat-inactivated fetal bovine serum (Filtron, Export Dve, Brooklyn, Australia). The suspension was placed in a 24-well culture plate, and the same volume of culture supernatant containing mAb was added to each well. Plates were incubated under sterile conditions of 5% CO_2 at 37 °C, and the reaction was checked every 12 h under an inverted microscope. Similarly, 7-day-old *T. britovi* adults were collected from the intestine of infected ICR mice, washed thoroughly, and incubated with mAbs.

Antibody preparation for passive transfer

Clones producing mAb were injected i.p. into pristane-primed BALB/c mice to produce ascitic fluids. Ascitic fluids were centrifuged and precipitated in 33% ammonium sulfate solution to remove contaminated IgG. After ultracentrifugation at 6000 ***g***, the supernatant was diluted in 0·15 M Dulbecco's (−) phosphate-buffered saline (PBS), pH 7·6, and applied to a stirred ultrafiltration cell (model 8200; Amicon division, W. R. Grace & Co., Beverly, MA, USA) equipped with YM100 DIAFLO® ultrafiltration membrane (cutting point 100 kDa; Amicon division). After repeated ultrafiltrations, the remnant was sterilized by filtration. A part of the material was applied to a gel filtration using Sephadex®-G-200 (Pharmacia Biotec AB, Uppsala, Sweden), and fractions of IgA at high purity were collected, and analysed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) using 15% gel under reducing conditions to determine the purity. Using a fraction of IgA at the highest purity as a standard, the concentration of specific IgA in the semi-purified ascitic material was determined by ELISA. These semi-purified mAbs were adjusted at 4·0 mg/ml in sterilized PBS, and kept at −80 °C until use.

Parasite antigens

Muscle larvae washed several times in PBS were immersed in 50 mM Tris buffer, pH 8·2, containing 1% Triton X-100, 150 mM NaCl, 10 mM EDTA and 0·5 mM phenylmethylsulfonyl fluoride. After 3 cycles of freezing and thawing, the samples were subjected to intermittent ultrasonication for 5 min. Supernatant was collected by centrifugation at 6000 ***g*** for 30 min, and the protein concentration was determined using Bio-Rad protein assay solution (Bio-Rad Lab., Richmond, CA, USA).

ELISA and Western blotting

The assay was carried out according to a standard procedure, and elimination of the carbohydrate epitopes of crude antigens was by periodate treatment as described previously (Sato *et al*. 1996). Secondary antibodies used for antibody screenings

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Table 1. Results of screenings for antibodies produced in the hybridoma culture

Immunization protocol for BALB/c mice*	Total number of wells	Number of wells prominent for†		
		IgA	IgG	IgM
Normal–normal	120	20	12	18
Irradiated–irradiated	96	3	3	3
Irradiated–normal	120	42	4	4

* For details, see Materials and Methods section.
† Almost all positive wells had prominence of a single class of antibody, although multiple hybridoma colonies grew in all wells.

of cultures and other tests were peroxidase-conjugated, affinity-purified goat antibody to mouse IgA (α) absorbed with solid phase human serum and mouse IgG and IgM (ZYMED, San Francisco, CA, USA), peroxidase-conjugated goat F(ab′)$_2$ fragment to mouse IgG (Fc) (Organon Teknika Corp., Durham, NC, USA), and peroxidase-conjugated goat F(ab′)$_2$ fragment to mouse IgM (μ) (American Qualex, La Mirada, CA, USA). For immunoblotting, parasite antigens and pre-stained protein molecular weight standards (Life Technologies, Inc., Gaithersburg, MD, USA) were separated by SDS–PAGE using 12·5% and 15% gels under reducing conditions, and transferred on nitrocellulose membranes. To block non-specific binding sites, the membrane was incubated for 60 min with PBS containing 3% skim milk. Primary antibody was applied as culture supernatant, and secondary antibody was applied at a 1:500 dilution. To assess non-specific reactions, control strips were similarly prepared, but without using the primary antibody. Bound antibody was detected using colour development by 3, 3′-diaminobenzidine.

Immunohistochemistry

Cryostat sections of 6 μm thickness were cut from muscles of a gerbil infected with *T. britovi*, and processed as described previously (Sato, Inaba & Kamiya, 1997).

Injection of mAbs, collection of blood and faeces, and worm recovery

Mice were injected i.p. with 0·5 ml of PBS containing selected doses of IgA mAb before or after the infection. To estimate the fate of injected IgA mAb, blood from the orbital venous plexus and faeces from the animal bedding were collected at certain intervals. Faeces were dried at 37 °C, and kept at −20 °C until use. Faeces were dissolved in PBS containing 0·05% (v/v) Tween 20® and 0·1% sodium azide at a ratio of 1 mg/10 μl, centrifuged, and the supernatant

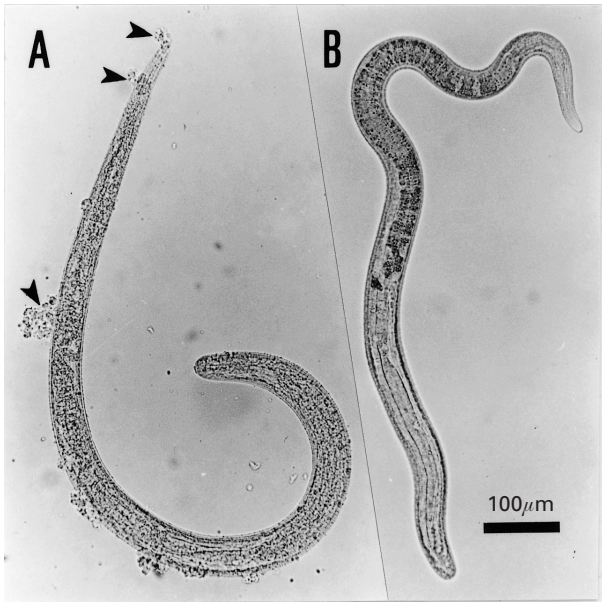

Fig. 1. Reactivity of IgA mAbs (A, HUSM-Tb1; B, HUSM-Tb2) with live *Trichinella britovi* muscle larvae at 24 h of incubation. Note circumlarval precipitation (arrow heads) by mAb HUSM-Tb1 (A), but not mAb HUSM-Tb2 (B). Granularity evident in a worm shown in (A) is an artifact during worm immobilization by heat.

was used for ELISA. Serum samples at a dilution of 1:50 were similarly examined. Worm recovery from the small intestine was made on day 7 after infection as described previously (Nakayama *et al*. 1996).

Statistics

Data were expressed as mean ± S.D. Differences between 2 groups were examined for significance using the Student's *t*-test, unless otherwise stated. A *P* value less than 0·05 denoted statistical significance.

RESULTS

Characteristics of two IgA mAbs specific to T. britovi *muscle larvae*

Screenings of antibody production in the hybridoma culture detected prominence of IgA-producing hybridomas originating from mice immunized repeatedly with irradiated larvae and challenged with normal ones (Table 1). By the aid of immunohistochemistry and Western blotting, hybridomas producing antibodies of the same characteristic were eliminated before subcloning. Incubation of muscle larvae in culture supernatants from subcloned hybridomas detected 1 IgA-producing hybridoma that originated from mice immunized repeatedly with irradiated larvae and challenged with normal ones; only this mAb (HUSM-Tb1) reacted with muscle larvae to form circumlarval immunoprecipitates (Fig. 1). Incubation of 7-day-old adults

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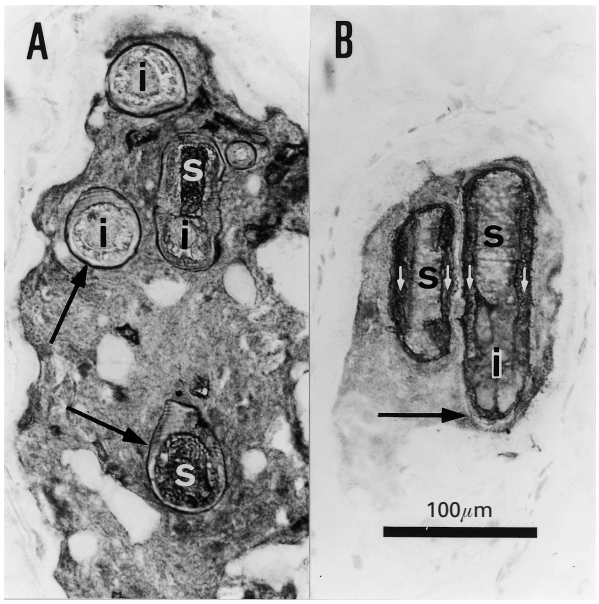

Fig. 2. Immunohistochemical reactivity of IgA mAbs (A, HUSM-Tb1; B, HUSM-Tb2) with encapsulated muscle larvae. Note strong positive reactions with stichocytes (s) and cuticular surface (big arrows), but not intestinal cells (i) by mAb HUSM-Tb1, whereas mAb HUSM-Tb2 reacted with the muscle layer (small arrows), but not stichocytes (s), intestine (i) or cuticular surface (big arrow).

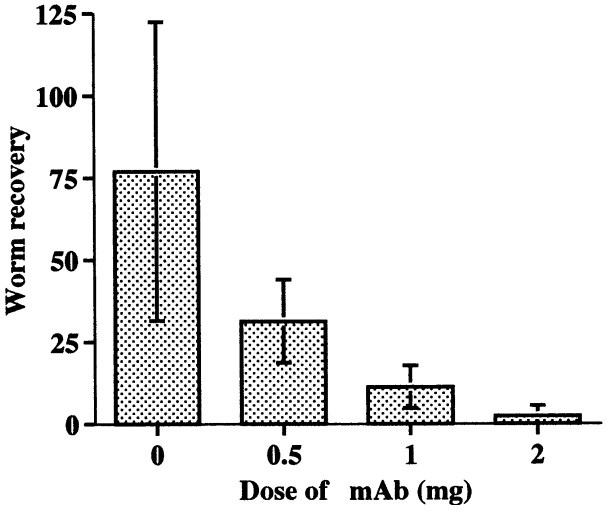

Fig. 3. Dose-dependent protective effect of passively-transfered IgA mAb HUSM-Tb1. Female 5-week-old BALB/c mice were injected i.p. with the mAb, and infected orally with 183±7 *Trichinella britovi* muscle larvae 5 h later. Uninjected mice ($n=4$), and injected mice ($n=3$/group). There is a good negative correlation ($r_s=-0{\cdot}896$) between mAb dose and worm recovery by Spearman's correlation coefficient test by ranks ($P<0{\cdot}002$).

or newborn larvae released from them with this mAb produced no detectable changes on their surfaces. By immunohistochemistry, the mAb reacted with stichocytes and the cuticular surface of muscle larvae (Fig. 2). Nurse cells, but not unparasitized

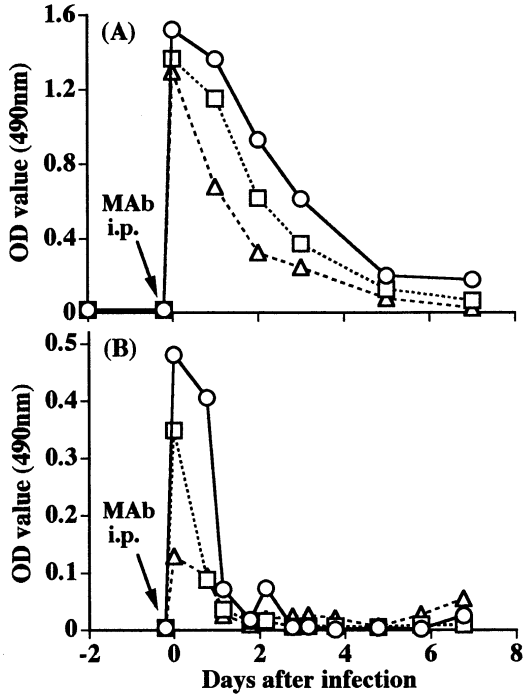

Fig. 4. Temporal changes of injected IgA mAb in serum (A) and faeces (B) of the recipient mice used in the experiment described in Fig. 3.

mouse muscles, reacted similarly with the mAb, suggesting an excretory/secretory (E/S) characteristic of reactive antigens. One IgA mAb (HUSM-Tb2) was arbitrarily selected from hybridoma stocks originating from mice infected twice with normal larvae. This mAb did not form circumlarval precipitation by *in vitro* culture, and reacted immunohistochemically with the muscle layer of larvae (Figs 1 and 2). Both mAbs reacted with antigens resistant to periodate treatment that eliminated carbohydrate epitopes, and gave broad smear-type reactions in Western blotting (data not shown).

Effects of in vivo *administration of IgA mAbs on worm recovery*

Semi-purified IgA mAb of HUSM-Tb1 of different doses was injected i.p., and inoculated orally with *T. britovi* muscle larvae 5 h later (Fig. 3). This experiment clearly demonstrated dose-dependent reduction in worm recovery from treated mice. Similarly, levels of injected IgA in the serum and faecal excretion were detectable and correlated with injected amounts of the mAb (Fig. 4). At infection time (5 h after injection), the level of IgA in serum and faeces showed peaks, and became undetectable within a few days or 1 day, respectively.

To clarify the developmental stage of intestinal *T. britovi* which was the target of mAb HUSM-Tb1, mAb was injected on days 0 (infection time), 1, 2, or 4 after infection (Fig. 5). Reduced worm recovery was noted only in mice injected on the day of infection. There was no effect of mAb on worm growth,

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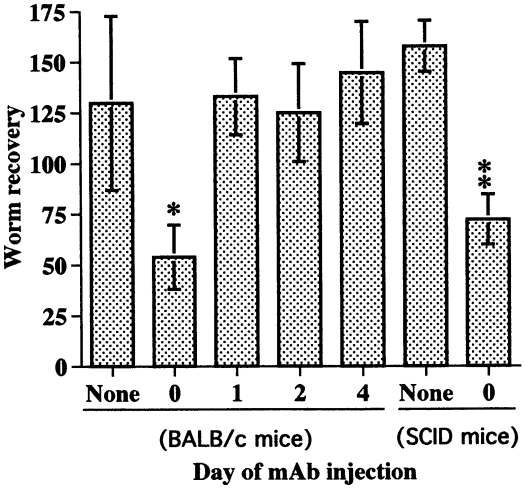

Fig. 5. Effect of the time of IgA mAb injection on worm recovery. Female 5-week-old BALB/c (4/group) and SCID mice (4/group) were infected orally with 217±13 *Trichinella britovi* muscle larvae, and injected i.p. with mAb HUSM-Tb1 immediately (day 0) or on days 1–4 after the infection. $^{*}P<0{\cdot}05$ or $^{**}P<0{\cdot}001$, compared with control, infected mice of each strain.

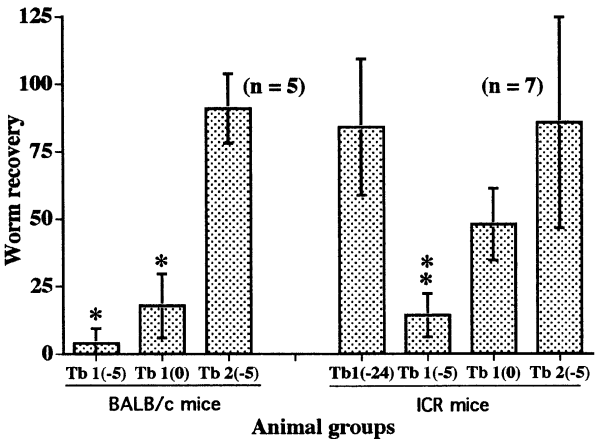

Fig. 6. Effect of the time of IgA mAb injection on worm recovery. Female 5-week-old BALB/c (19·5±1·7 g of body weights, 4/group unless otherwise mentioned) and ICR mice (27·7±2·3 g of body weights; 5/group unless otherwise mentioned) were injected i.p. with mAbs HUSM-Tb1 or HUSM-Tb2 at different times, and then infected orally with 171±32 *Trichinella britovi* muscle larvae. Animal group is expressed by 'IgA mAb injected (the time in hours of injection relative to the time of infection)'. $^{*}P<0{\cdot}001$ or $^{**}P<0{\cdot}003$, compared with HUSM-Tb2-treated control mice of each strain.

evaluated by worm length of adult males and females collected from mice with mAb injection on days 0, 1, 2, and 4 after infection. Furthermore, injection of mAb HUSM-Tb1 5 h before infection showed greater reduction of worm recovery than treatment at the time of infection (Fig. 6). Injection of 2 mg mAb of HUSM-Tb1 5 h before infection in BALB/c and ICR mice attained 95·6% and 83·4% reduction of worm recovery, respectively, compared with mice

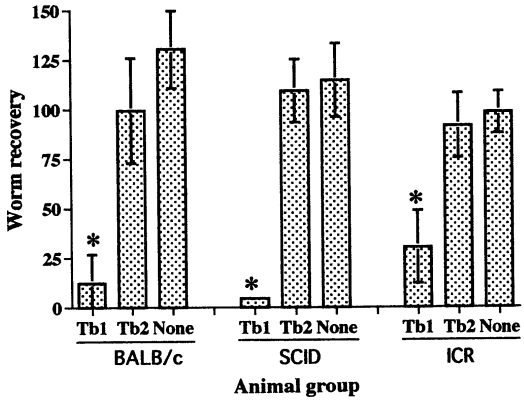

Fig. 7. IgA mAb-mediated expulsion of *Trichinella britovi* from the intestine of 3 strains of mice. Seven-week-old BALB/c (21·1±3·1 g of body weights, 4 females and 2 males/group), female SCID (18·1±1·9 g of body weights, 4 females/group), and ICR mice (29·7±4·3 g of body weights, 2 females and 3 males/group) were injected i.p. with 2 mg of mAbs HUSM-Tb1 and Tb2, or none, and infected orally with 184±29 *T. britovi* muscle larvae 5 h later. $^{*}P<0{\cdot}001$, compared with control infected mice of each strain.

injected with mAb HUSM-Tb2. When 3 strains of mice including SCID mice were subjected to the same schedule of injection, BALB/c, SCID and ICR mice attained 90·5% (97·5% when the average was calculated from 4 mice with less than 21 g of body weight), 96·1% and 69·1% reduction of worm recovery, respectively (Fig. 7). When the same dose of IgA mAb HUSM-Tb1 was injected to mice, the major factor that determined the worm recovery seemed to be the body weight of animals used in the experiment.

DISCUSSION

The 'rapid expulsion' of *Trichinella* spp. from rats is a well characterized phenomenon, and is primarily mediated by humoral responses. Passive transfer experiments using either protective serum absorbed with subclass-specific antibody (Appleton & McGregor, 1987) or mAbs (Appleton, Schain & McGregor, 1988; Ahmad *et al.* 1991; Ahmad, Wang & Bell, 1991; Carlisle, McGregor & Appleton, 1991*a*, *b*; Bell *et al.* 1992) provide evidence that IgM, IgG1, IgG2a, IgG2c and IgE isotypes play a role in this protective immunity. In contrast, mice do not show the 'rapid expulsion', but 'associative expulsion' (Alizadeh & Wakelin, 1982; Bell, 1992). In this host, passive transfer of protective rat IgG mAbs to mice did not lead to rapid expulsion, whereas transfer of mouse immune serum to intestinally-primed rats did result in rapid expulsion (Bell, 1992). Furthermore, passive transfer of serum generated in mice by parenteral vaccination using E/S products with an adjuvant conferred significant protection upon recipient mice, although vaccination

https://doi.org/10.1017/S003118200300310X Published online by Cambridge University Press

itself protects limited strains of donor mice (Jarvis & Pritchard, 1992; Robinson *et al.* 1995*a*). In *Trichinella* infection, previous studies have suggested a strong temporal correlation between mucosal IgA production and a reduction in fecundity and size of adult worms, but not the rate of expulsion, in challenge infections following natural infection or oral vaccination (deVos, Danell & Dick, 1992; deVos & Dick, 1993; Robinson, Bellaby & Wakelin, 1995*b*). To demonstrate directly a possible role of mucosal IgA in protective immunity to *Trichinella* infection, we have employed a strategy of production and passive transfer of murine IgA mAb to the recipient mice.

In this study, 3 immunization protocols were used to isolate hybridoma clones from MLN cells, and the results of the primary screening for produced antibody in each culture well suggest a preponderance of IgA-producing cells in the MLN in mice receiving irradiated and challenged normal parasites, compared with mice receiving only normal ones. Because most orally-inoculated muscle larvae irradiated by 5 mJ/cm^2 UV are expelled from the intestine within 24 h (Nakayama *et al.* 1996) and the rapid loss of larval cuticular antigens of *Trichinella* spp. occurs during the first 1–2 days of its enteral life (Philipp *et al.* 1981), it is speculated that this immunization protocol might efficiently induce the humoral immune response to the muscle larvae. The IgA monoclonal antibody HUSM-Tb1 is one of those produced by this procedure, and shows a specificity to stichocytes and cuticular surface of the muscle larvae, but not adults or newborn larvae. An epitope shared by these two structures of *Trichinella* spp. was frequently documented in mAbs which can successfully mediate the 'rapid expulsion' in rats (Grencis *et al.* 1986; Capó, Silberstein & Despommier, 1986; McLaren, Ortega-Pierres & Parkhouse, 1987; Denkers *et al.* 1990; Bell *et al.* 1992).

The highest effect (over 95% protection) in the recipient mice was achieved when the recipient was injected i.p. with an adequate dose of IgA mAb HUSM-Tb1 prior to oral challenge infection. Injections of the mAb around the infection time or later were less effective than at 5 h before oral inoculation. These observations might be associated with the strict specificity of the mAb to the infective larvae and temporal kinetics of injected IgA in the body of recipients, i.e., the level of injected mAb in the faeces reached a peak within several hours and became marginally detectable at 24 h. Accordingly, the intestinal IgA concentration was elevated soon after the intraperitoneal injection of the recipient with IgA mAb, and kept at an effective level for a short time. The narrow window of efficacy suggests that the rejection process by passively transferred IgA mAb HUSM-Tb1 is due to its ability to impede the establishment and maintenance of larvae in their epithelial niche. It has been speculated that this is the process which leads to the 'rapid expulsion' in rats (Bell, McGregor & Despommier, 1979; Carlisle *et al.* 1990, 1991*a*, *b*).

Distinct from the 'rapid expulsion' in adult rats, but similar to that in suckling rats, passive transfer of protective IgA mAb to mice did not need any synergistic action of T-cells or T-cell products such as IL-4 (Ramaswamy, Goodman & Bell, 1994; Ramaswamy, Hakimi & Bell, 1994; Bell, 1998), because the recipient SCID mice as well as immunocompetent mice expelled the inoculated parasites without any other treatment. This is ascribed to the fact that IgA antibody is a typical mucosa-defensive isotype and continuously transported via the mucosal epithelium to the intestinal lumen even under normal conditions. This speculation was supported by our unpublished data that an adequate dose of this protective IgA mAb also worked in adult rats, as in mice, without any other manipulation.

Although this study does not define the principal process of expulsion of *T. britovi* from mice vaccinated orally with irradiated muscle larvae, it is possible that a mucosal IgA response plays a host-protective role to challenge infections. The role of secretory IgA in neutralization of viral (Taylor & Dimmock, 1985; Renegar & Small, 1991), bacterial (Pierce, Cray & Sircar, 1978; Lycke, Bromander & Holmgren, 1989), and protozoan infections (Underdown *et al.* 1988; Leyva *et al.* 1992; Enriquez & Riggs, 1998) is clearly established. As shown in this study, the protective role of secretory IgA is found also in a mucosal-dwelling nematode infection.

This work was supported in part by Grants-in-Aid (Nos. 13670240 and 13575039) from the Japan Society for the Promotion of Science. We would like to acknowledge the Japan Health Science Foundation for a scholarship to H. Sato, that enabled valuable discussion with Professor John R. Kusel, IBLS, University of Glasgow.

REFERENCES

AHMAD, A., WANG, C. H. & BELL, R. G. (1991). A role for IgE in intestinal immunity: expression of rapid expulsion of *Trichinella spiralis* in rats transfused with IgE and thoracic duct lymphocytes. *Journal for Immunology* **146**, 3563–3570.

AHMAD, A., BELL, R. G., WANG, C. H. & SACUTO, F. R. (1991). Characterization of the thoracic duct T-helper cells that co-mediate, with antibody, the rapid expulsion of *Trichinella spiralis* in adult rats. *Parasite Immunology* **13**, 147–159.

ALIZADEH, H. & WAKELIN, D. (1982). Comparison of rapid expulsion of *Trichinella spiralis* in mice and rats. *International Journal for Parasitology* **12**, 65–73.

APPLETON, J. A. & McGREGOR, D. D. (1984). Rapid expulsion of *Trichinella spiralis* in suckling rats. *Science* **226**, 70–72.

APPLETON, J. A. & McGREGOR, D. D. (1985). Life-phase specific induction and expression of rapid expulsion in rats suckling *Trichinella spiralis*-infected dams. *Immunology* **55**, 225–232.

APPLETON, J. A. & McGREGOR, D. D. (1987). Characterization of the immune mediator of rapid expulsion of *Trichinella spiralis* in suckling rats. *Immunology* **62**, 477–484.

APPLETON, J. A., SCHAIN, L. R. & McGREGOR, D. D. (1988). Rapid expulsion of *Trichinella spiralis* in suckling rats: mediation by monoclonal antibodies. *Immunology* **65**, 487–492.

BELL, R. G. (1992). *Trichinella spiralis*: evidence that mice do not express rapid expulsion. *Experimental Parasitology* **74**, 417–430.

BELL, R. G. (1998). The generation and expression of immunity to *Trichinella spiralis* in laboratory rodents. *Advances in Parasitology* **41**, 149–217.

BELL, R. G. & McGREGOR, D. D. (1979). *Trichinella spiralis*: role of different life cycle phases in induction, maintenance, and expression of rapid expulsion in rats. *Experimental Parasitology* **48**, 51–60.

BELL, R. G., McGREGOR, D. D. & DESPOMMIER, D. D. (1979). *Trichinella spiralis*: mediation of the intestinal component of protective immunity in the rat by multiple, phase-specific, antiparasitic responses. *Experimental Parasitology* **47**, 140–157.

BELL, R. G., APPLETON, J. A., NEGRAO-CORREA, D. A. & ADAMS, L. S. (1992). Rapid expulsion of *Trichinella spiralis* in adult rats mediated by monoclonal antibodies of distinct IgG isotypes. *Immunology* **75**, 520–527.

CAPÓ, V., SILBERSTEIN, D. & DESPOMMIER, D. D. (1986). Immunocytolocalization of two protection-inducing antigens of *Trichinella spiralis* during its enteral phase in immune and non-immune mice. *Journal of Parasitology* **72**, 931–938.

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**, 126–132.

CARLISLE, M. S., McGREGOR, D. D. & APPLETON, J. A. (1991*a*). Intestinal mucus entrapment of *Trichinella spiralis* larvae induced by specific antibodies. *Immunology* **74**, 546–551.

CARLISLE, M. S., McGREGOR, D. D. & APPLETON, J. A. (1991*b*). The role of the antibody Fc region in rapid expulsion of *Trichinella spiralis* in suckling rats. *Immunology* **74**, 552–558.

DENKERS, E. Y., WASSOM, D. L., KRCO, J. & HAYES, C. E. (1990). The mouse antibody response to *Trichinella spiralis* defines a single, immunodominant epitope shared by multiple antigens. *Journal of Immunology* **144**, 3152–3159.

DEVOS, T. & DICK, T. A. (1993). *Trichinella spiralis*: the effect of oral immunization and the adjuvancy of cholera toxin on the mucosal and systemic immune response to mice. *Experimental Parasitology* **76**, 182–191.

DEVOS, T., DANELL, G. & DICK, T. A. (1992). *Trichinella spiralis*: dose dependence and kinetics of the mucosal immune response in mice. *Experimental Parasitology* **75**, 99–111.

ELSE, K. J. & FINKELMAN, F. D. (1998). Intestinal nematode parasites, cytokines and effector mechanisms. *International Journal for Parasitology* **28**, 1145–1158.

ENRIQUEZ, F. J. & RIGGS, M. W. (1998). Role of immunoglobulin A monoclonal antibodies against P23 in controlling murine *Cryptosporidium parvum* infection. *Infection and Immunity* **66**, 4469–4473.

GRENCIS, R. K., CRAWFORD, C., PRITCHARD, D. I., BEHNKE, J. M. & WAKELIN, D. (1986). Immunization of mice with surface antigens from muscle larvae of *Trichinella spiralis*. *Parasite Immunology* **8**, 587–596.

JARVIS, L. M. & PRITCHARD, D. I. (1992). An evaluation of the role of carbohydrate epitopes in immunity to *Trichinella spiralis*. *Parasite Immunology* **14**, 489–501.

LEYVA, O., RICO, G., RAMOS, F., MORAN, P., MELENDRO, E. I. & ZIMENEZ, C. (1992). Inhibition of adhesion process mediated by anti-*Entamoeba histolytica* specific monoclonal IgA antibodies. *Archives of Medical Research* **23**, 227–229.

LYCKE, N., BROMANDER, A. & HOLMGREN, J. (1989). Role of local IgA antitoxin-producing cells for intestinal protection against cholera challenge. *International Archives of Allergy and Applied Immunology* **88**, 273–279.

McLAREN, D. J., ORTEGA-PIERRES, G. & PARKHOUSE, R. M. E. (1987). *Trichinella spiralis*: immunocytochemical localization of surface and intracellular antigens using monoclonal antibody probes. *Parasitology* **94**, 101–114.

NAKAYAMA, H., INABA, T., NARGIS, M., CHISTY, M. M., ITO, M. & KAMIYA, H. (1998). Immunization of laboratory animals with ultraviolet-attenuated larvae against homologous challenge infection with *Trichinella britovi*. *Southeast Asian Journal of Tropical Medicine and Hygiene* **29**, 563–566.

NAKAYAMA, H., INABA, T., OZAKI, T. & KAMIYA, H. (1996). Effect of ultraviolet irradiation on the development of *Trichinella spiralis*. *Japanese Journal of Parasitology* **45**, 181–184.

PHILIPP, M., TAYLOR, P. M., PARKHOUSE, R. M. E. & OGILVIE, B. M. (1981). Immune response to stage-specific surface antigens of the parasite nematode *Trichinella spiralis*. *Journal of Experimental Medicine* **154**, 210–215.

PIERCE, N. F., CRAY, W. C. & SIRCAR, B. K. (1978). Induction of a mucosal antitoxin response and its role in immunity to experimental canine cholera. *Infection and Immunity* **21**, 185–193.

POZIO, E., LA ROSA, G., YAMAGUTI, T. & SAITO, S. (1996). *Trichinella britovi* from Japan. *Journal of Parasitology* **82**, 847–849.

RAMASWAMY, K., GOODMAN, R. E. & BELL, R. G. (1994). Cytokine profile of protective anti-*Trichinella spiralis* $CD4^+$ $OX22^-$ and non-protective $CD4^+$ $OX22^+$ thoracic duct cells in rats: secretion of IL-4 alone does not determine protective capacity. *Parasite Immunology* **16**, 435–445.

RAMASWAMY, K., HAKIMI, J. & BELL, R. G. (1994). Evidence for an interleukin 4-inducible immunoglobulin E uptake and transport mechanism in the intestine. *Journal of Experimental Medicine* **180**, 1793–1803.

RENEGAR, K. B. & SMALL, P. A. (1991). Passive transfer of local immunity to influenza virus infection by IgA antibody. *Journal of Immunology* **146**, 1972–1978.

ROACH, T. I. A., ELSE, K. J., WAKELIN, D., McLAREN, D. J. & GRENCIS, R. K. (1991). *Trichuris muris*: antigen recognition and transfer of immunity in mice by IgA monoclonal antibodies. *Parasite Immunology* **13**, 1–12.

ROBINSON, K., BELLABY, T. & WAKELIN, D. (1995*a*). Immunity to *Trichinella spiralis* transferred by serum form vaccinated mice not protected by immunization. *Parasite Immunology* **17**, 85–90.

https://doi.org/10.1017/S003118200300310X Published online by Cambridge University Press

ROBINSON, K., BELLABY, T. & WAKELIN, D. (1995*b*). Oral and parenteral vaccination against *Trichinella spiralis* infections in high- and low-responder mice. *International Journal for Parasitology* **25**, 989–992.

SATO, H., INABA, T. & KAMIYA, H. (1997). Production of murine monoclonal antibodies to guinea pig leukocytes and immunohistochemistry of guinea pig skin exposed to *Schistosoma mansoni*. *Hybridoma* **16**, 529–536.

SATO, H., KAMIYA, H., GRAUERT, M.-R., STERN, D., ALTAMIRANO, Z., PERDOMO, R., CARMONA, C., CARBÓ, A., ALVAREZ, C., MONTI, J., SAKAI, H., OKU, Y. & KAMIYA, M. (1996). Comparison of serodiagnostic tests and ultrasonography for cystic hydatidosis in an epidemiological study of rural Uruguay. *Journal of Parasitology* **82**, 852–854.

TAYLOR, H. P. & DIMMOCK, N. J. (1985). Mechanism of neutralization of influenza virus by secretory IgA is different from that of monomeric IgA or IgG. *Journal of Experimental Medicine* **161**, 198–209.

UNDERDOWN, B. J., SKEA, D. L., LONEY, G. M. & SNIDER, D. P. (1988). Murine giardiasis and mucosal immunity: a model for the study of immunity to intestinal protozoan parasites. *Monographs in Allergy* **24**, 287–296.

https://doi.org/10.1017/S003118200300310X Published online by Cambridge University Press