Immunology 1994 **82** 261–267

Vaccination against the nematode *Trichinella spiralis* in high- and low-responder mice. Effects of different adjuvants upon protective immunity and immune responsiveness

K. ROBINSON, T. BELLABY & D. WAKELIN *MRC Experimental Parasitology Research Group, Department of Life Science, University of Nottingham, Nottingham*

SUMMARY

Groups of NIH and C57BL/10 (B10) mice were vaccinated subcutaneously with excretory/secretory material from the nematode parasite *Trichinella spiralis* using a variety of different adjuvants, i.e. complete Freund's adjuvant (CFA), 'TiterMax', alum and ISCOMs. ISCOMs were also given orally. NIH mice, known to be rapid responders to *T. spiralis*, expelled their worms earlier than usual with vaccination. The slow-responder B10 mice did not expel worms any earlier, no matter which adjuvant was used. The antibody isotype response profiles following vaccination with the four adjuvants did not differ significantly; however NIH mice generally produced higher levels of antibody than B10 and CFA induced the highest overall response. NIH cells yielded stimulation indices far in excess of the B10 following *in vitro* stimulation with *T. spiralis* antigen. Secretion of interleukin-5 (IL-5) and interferon-γ (IFN-γ) by these cells followed similar trends, i.e. higher levels from NIH than B10. A high IL-5 level in the NIH strain was accompanied by low-level IFN-γ production following infection, whereas the IFN-γ response was not observed in B10 supernatants. This study shows that vaccination using these adjuvants did not appear to modify the immune response qualitatively, but the magnitude of the response was affected greatly.

INTRODUCTION

Expulsion of adult *Trichinella spiralis* worms from the intestine is thought to be mediated by a T-cell-dependent inflammatory response.[1] Data concerning the association between T-helper (Th) subset activity and expulsion of *Trichinella* infection are conflicting.[2] Both Zhu and Bell[3] and Pond *et al.*[4] reported that, in terms of cytokine production, a high-responder mouse strain (AKR) produced a higher level of Th1 subset activity than the low-responder B10.BR strain, whose response was Th2 dominated.[5] These results suggested that responsiveness to *T. spiralis* infection is heavily influenced by activation of the Th1 subset. Grencis *et al.*,[6] working with NIH and B10.G mice which express high- and low-response phenotypes respectively, found no strain differences in cytokine profiles (Th2-associated cytokines predominated) and postulated that protective immunity is mediated by a Th2 type response in both high- and low-responder strains. Kelly *et al.*[7] reported a compartmentalization of Th1 and Th2 cell subsets to different lymphoid organs during infection in B10.Q mice.

A previous immunization study[8] showed that rapid-responder NIH mice expelled an infection earlier when vaccinated with muscle larval homogenate antigen in complete Freund's adjuvant (CFA) but the expulsion kinetics of slow-responder C57BL/10 (B10) mice were unchanged. This result indicated that CFA immunization had failed to influence the genetically determined pattern of responsiveness.

Much has been published concerning the immunomodulatory properties of adjuvants.[9,10] It has been shown that the use of different adjuvants in a vaccine can determine the type of immune response elicited in terms of antibody isotype and cellular activity. The use of particular adjuvants may therefore have considerable potential for increasing levels of resistance to infections where specific components of the immune response may need to be boosted selectively. In this study, *T. spiralis* antigen preparations were mixed with adjuvants from four main groups: water-in-oil emulsions, e.g. CFA; oil-in-water emulsions and pluronics, e.g. 'TiterMax'; mineral gels, e.g. alum; saponins, e.g. immunostimulatory complexes (ISCOMs). The basic mechanisms of adjuvanticity of these substances are all different and give rise to a range of immune response profiles.[10] It was anticipated that adjuvants yielding responses appropriate to the *T. spiralis* expulsion process should be effective in accelerating worm loss from vaccinated mice. If the slower expulsion of slow-responder mouse strains such as B10

Received 9 November 1993; revised 15 February 1994; accepted 16 February 1994.

Abbreviations: ES, excretory/secretory antigen; CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant; ISCOM, immunostimulatory complex; MLNC, mesenteric lymph node cells; SC, spleen cells; SI, stimulation index.

Correspondence: Dr K. Robinson, MRC Experimental Parasitology Research Group, Dept. of Life Science, University of Nottingham, Nottingham NG7 2RD, U.K.

is explained in part by an inefficient immune response, vaccination with an appropriate adjuvant should lead to accelerated worm loss.

MATERIALS AND METHODS

Mice

Specific pathogen-free (SPF) female NIH and C57BL/10 mice were purchased from Harlan Olac (Bicester, U.K.). Outbred CFLP mice were bred in the Department of Life Science, University of Nottingham. Mice were used at 6–8 weeks of age.

Parasite

Trichinella spiralis was maintained in CFLP mice immunosuppressed by exposure to 450 Rads of gamma radiation from a cobalt source before infection. Methods for infection and parasite recovery were as described previously.[11] Mice were infected orally with 300 muscle larvae.

Antigen

Excretory/secretory antigen (ES) products were obtained by an overnight incubation at 37° of freshly isolated muscle larvae in sterile RPMI-1640 with 500 U/ml penicillin, 500 μg/ml streptomycin and 2·5 μg/ml Fungizone (Gibco Ltd, Paisley, U.K.). Culture medium was filtered through a 0·2-μm sterile filter and dialysed overnight at 4° against distilled water. Muscle larvae were homogenized as described previously.[12] Both the ES and homogenate preparations were freeze dried and stored with desiccant at −40°.

Preparation of material for immunisation

A solution of 2 mg/ml ES in phosphate-buffered saline (PBS) was emulsified in CFA (Sigma, Poole, U.K.) or in 'TiterMax' (Stratech Scientific Ltd, Luton, U.K.) to give a final concentration of 100 μg ES per 0·1 ml. ES was adsorbed to aluminium potassium sulphate according to a modified method of Sanchez *et al.*[13] The procedure yielded 100 μg ES and 23·3 mg alum per 0·1 ml immunizing dose. ISCOMs containing *T. spiralis* muscle larvae homogenate were kindly prepared by Dr A. McI. Mowat from the Immunology Department of Glasgow University (Glasgow, U.K.). Two doses containing 10 μg antigen protein were given at 7-day intervals for subcutaneous vaccination. Two 45-μg doses were given for oral vaccination.

Experimental protocol

Groups of five mice were immunized subcutaneously with 100 μg ES plus adjuvant or phosphate-buffered saline (PBS) plus adjuvant on day −14. They were injected with 100 μg ES in PBS or PBS alone on day −7 before oral infection with 300 *T. spiralis* muscle larvae on day 0. Mice were killed on days 6 and 8 (NIH) or 7 and 12 (B10) post-infection. After killing, adult worms were recovered using a modified Baermann technique[14] and counted. Representative time–course graphs of worm expulsion in unvaccinated mice of both strains are shown in Fig. 1. Loss of worms begins by day 8 in NIH mice and after day 11 in B10 mice. In comparing the worm burdens of vaccinated, adjuvant control and non-vaccinated mice the numbers of worms recovered on day 8 (NIH) or 12 (B10) are expressed as a percentage of the day 6 or day 7 count respectively. For immunological studies, NIH mice were killed on day 8, B10 on day 9. At the point of killing, serum samples, mesenteric lymph nodes (MLN) and spleens were removed.

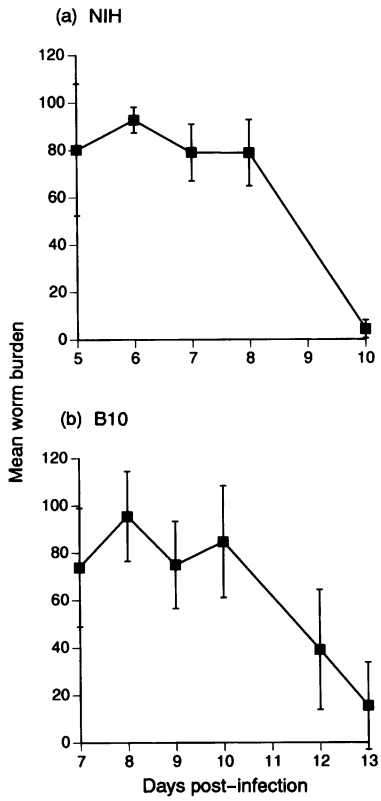

Figure 1. Time–course of expulsion of *T. spiralis* adult worms from (a) NIH and (b) B10 mice. Mean worm burdens for groups of five mice, infected orally on day 0 with 300 muscle larvae, are shown at several time-points. Error bars represent standard deviations.

Enzyme-linked immunosorbent assay (ELISA)

Levels of specific IgG1, IgG2a, IgG2b, IgG3 and IgM in serum samples diluted to 1/50 were determined using a method based on that of Bolas-Fernandez and Wakelin.[15]

Lymphocyte proliferation assay

Two hundred microlitre aliquots of spleen cell (SC) and MLN cell (MLNC) suspensions at 5×10^6 cells/ml in RPMI-1640 medium (Gibco) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin and $7{\cdot}5 \times 10^{-5}$ M monothioglycerol (BDH Chemicals Ltd, Poole, U.K.) were cultured in 96-well flat-bottomed tissue culture trays (Nunc, Life Technologies Ltd, Paisley, U.K.). Concanavalin A (Con A; 5 μg/ml) (Sigma) or 50 μg/ml ES antigen was added. Cellular proliferation was followed by [^{3}H]thymidine incorporation.

Preparation of MLN and spleen cells supernatants for cytokine assays

MLNC and SC were cultured as described above, with 5 μg/ml Con A or 50 μg/ml ES. After 24 and 48 hr incubation, supernatants were removed and centrifuged at 10,000 rpm in a microfuge for 1 min to pellet any cells. The sterile supernatants were stored at −80° until required.

Cytokine analyses

Interleukin-5 (IL-5) and interferon-γ (IFN-γ) levels in MLNC and SC supernatants were measured by sandwich ELISA as described previously.[16,17] IL-5 assays were carried out using TRFK-5 and biotinylated TRFK-4 monoclonal antibodies (Pharmingen, San Diego, CA). IFN-γ assays employed the antibodies R46A2 and biotinylated XMG1.2 (Pharmingen). Cytokines were quantified by reference to calibrated standard supernatants kindly donated by Dr R. K. Grencis (University of Manchester, Manchester, U.K.).

Statistics

ELISA optical density readings of tested sera and worm burdens from different treatment groups were compared using the Mann–Whitney U-test. $P \geqslant 0{\cdot}05$ was considered to be non-significant.

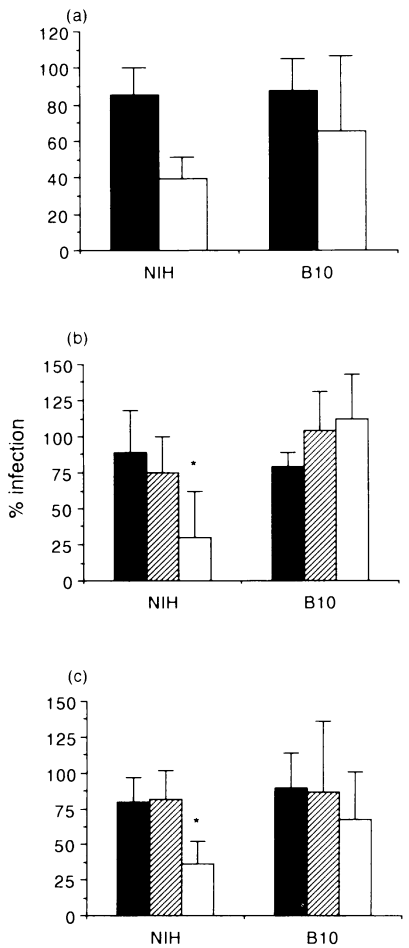

Figure 2. Expulsion of *T. spiralis* adult worms from groups of NIH and B10 mice treated in three different ways: (1) infection with 300 muscle larvae, (2) vaccination with PBS and adjuvant before infection, (3) vaccination with ES antigen plus adjuvant before infection. The adjuvants used are (a) CFA, (b) 'TiterMax' and (c) potassium alum. Mean worm burdens on day 8 (NIH) and day 12 (B10) post-infection are expressed as percentages of the day 6 (NIH) and day 7 (B10) mean burdens. (■) No vaccination given; (▨) PBS + adjuvant vaccination; (□) ES + adjuvant vaccination. Standard deviations are shown. *Worm burdens from vaccinated groups significantly lower ($P < 0{\cdot}05$) than those from non-vaccinated groups. An adjuvant control vaccination group was not included in the CFA experiment.

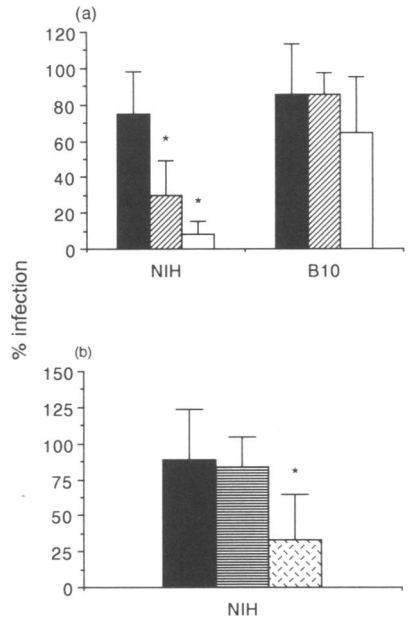

Figure 3. Expulsion of *T. spiralis* adult worms from mice vaccinated subcutaneously and orally with ISCOMs containing muscle larval homogenate antigen. (a) Comparison of worm expulsion from NIH and B10 mice vaccinated subcutaneously with 10 μg ISCOMs or 100 μg muscle larval homogenate in CFA with non-vaccinated mice. (■) No vaccination given; (○) ISCOM vaccination; (□) homogenate + CFA vaccination. (b) The effect of vaccinating NIH mice orally (45 μg dose) or subcutaneously (10 μg dose) with ISCOMs on worm expulsion. (■) No vaccination given; (▨) oral vaccination; (▧) subcutaneous vaccination. Mean worm burdens on day 8 (NIH) and 12 (B10) post-infection are expressed as percentages of the day 6 (NIH) and day 7 (B10) mean burdens. Standard deviations are shown. *Worm burdens from vaccinated groups that are significantly lower ($P < 0{\cdot}05$) than those of non-vaccinated groups.

RESULTS

Worm expulsion responses following vaccination with antigen in four adjuvants

Complete Freund's adjuvant. Vaccination using CFA and incomplete Freund's adjuvant (IFA) has been carried out previously.[8,18,19] This experiment was therefore essentially confirmatory, providing a baseline for comparison with results obtained using other adjuvants. Worm burdens were compared in vaccinated and non-vaccinated mice only. The numbers of worms recovered after infection showed no significant differences between the groups of B10 mice, but the vaccinated NIH mice had significantly fewer worms than the non-vaccinated group ($P < 0{\cdot}025$) (see Fig. 2a).

'TiterMax' adjuvant and potassium alum. A similar experimental design was used to test the effects of Hunter's 'TiterMax' adjuvant and those of potassium alum, but additional adjuvant control groups were also included. With both adjuvants, the numbers of worms recovered from the groups of B10 mice did not differ significantly, whereas the ES-vaccinated groups of

NIH mice had fewer worms than the corresponding controls (TiterMax, $P < 0{\cdot}025$; alum, $P < 0{\cdot}005$) (Fig. 2b and c). Like the previous experiment, NIH mice could be made to expel their worms earlier as a result of vaccination with ES in adjuvant while no significant effect was seen with the B10 strain. No significant effect as a result of vaccination with adjuvant alone was detected in either strain.

Immunostimulatory complexes. A comparison of subcutaneous vaccination with ISCOMs (10 μg protein/dose) and 100 μg larval homogenate in CFA on the expulsion of a *T. spiralis* infection was carried out. In the case of B10, vaccination with homogenate antigen in neither ISCOMs nor CFA had any effect (Fig. 3a). NIH mice vaccinated with homogenate in CFA had significantly lower worm burdens on day 8 post-infection than non-vaccinated mice ($P < 0{\cdot}005$). Those vaccinated with ISCOMs also had significantly fewer worms ($P = 0{\cdot}01$), but vaccination with CFA was more effective in accelerating the expulsion of adult worms from the gut ($P < 0{\cdot}01$).

ISCOMs have been successfully used in oral vaccines[20] with a wide variety of antigenic molecules. The generation of a local immune response in the gut by a vaccine may be more effective for the expulsion of a parasitic infection than a systemic response. An experiment was therefore designed to compare the efficacy of oral and subcutaneous vaccination with *T. spiralis* ISCOMs in NIH mice (Fig. 3b). There was no significant reduction in the worm burdens of mice vaccinated orally compared with those not vaccinated. Animals vaccinated subcutaneously had significantly fewer worms ($P = 0{\cdot}01$).

Anti-*T. spiralis* antibody isotypes raised by vaccination using different adjuvants

From the data shown in Fig. 4, it is clear that the principal isotype induced by vaccination was IgG1. Prior vaccination appeared to boost the antibody response to an infection in both strains of mice for all adjuvants, except where B10 mice were vaccinated using alum. In this case there was little or no antibody response when vaccinated or when subsequently infected. The IgG1 and IgG2b antibody levels obtained when mice were infected after vaccination using alum were

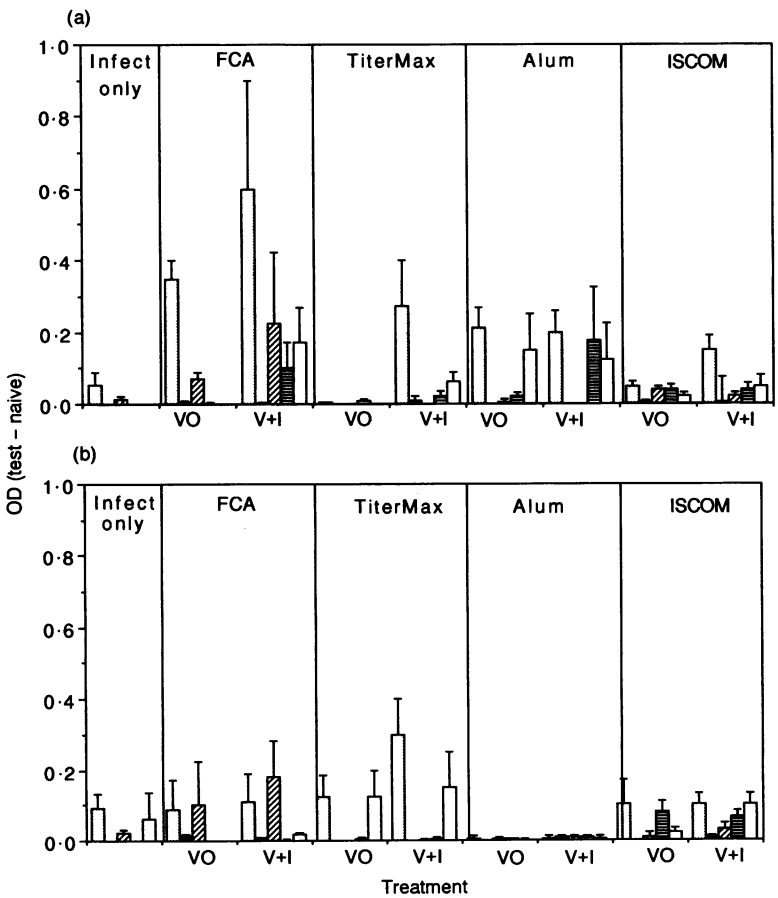

Figure 4. Serum *T. spiralis*-specific antibody levels of five isotypes in (a) NIH and (b) B10 mice vaccinated with ES and various adjuvants determined by ELISA. The levels of IgG1 (○), IgG2a (○), IgG2b (○), IgG3 (○) and IgM (□) are illustrated by the difference in optical density between wells containing test serum and naive serum. The bars represent a mean ± SD of the levels from five mice. Mice were vaccinated with 100 μg ES in CFA, 'TiterMax' and alum or 45 μg ISCOMs and boosted 7 days later. Serum collection from animals that were vaccinated only (VO), was carried out 2 weeks post-boost. Vaccinated and infected groups (V + I) received an oral infection of 300 muscle larvae 8 (NIH) or 9 (B10) days before serum collection and 7 days post-boost. The first column shows data for mice that had been infected only.

significantly lower ($P = 0{\cdot}004$ for IgG1, $P = 0{\cdot}016$ for IgG2b) than obtained from those infected only.

In general, NIH mice produced higher levels of antibody than the B10 strain. Although isotype profiles were not affected by the use of adjuvants, the degree of responsiveness induced was. CFA induced the highest antibody responses in NIH mice, while 'TiterMax' induced the highest response in the B10 strain.

Cellular responses of NIH and B10 mice to vaccination and infection—a comparison of CFA and alum adjuvants

The results of proliferation assays following *in vitro* stimulation with ES are shown in Table 1. Only stimulation indices (SI) greater than 2·0 were considered to indicate that cellular proliferation had occurred. In both strains, SI less than 2·0 were obtained using naive MLNC and SC proving the ES extract to be non-mitogenic. Each batch of cells was also stimulated with Con A and found to give high proliferative responses (data not shown).

MLNC and SC from infected NIH mice produced good proliferative responses. Cell cultures from mice vaccinated with ES and CFA or alum exhibited enhanced proliferation, but those of vaccinated and infected mice yielded even higher SI. Vaccination with ES therefore enhanced the immune response to infection. Cells from infected B10 mice showed no proliferation. SI from mice vaccinated with ES and CFA and then infected were higher than SI from mice that were merely vaccinated. The SI values were low in comparison with those of NIH cell cultures. Cells from mice vaccinated with ES and alum proliferated at low levels in response to ES, giving similar SI to those of cells from vaccinated and infected mice.

Cytokine production from antigen-stimulated spleen and lymph node cells of vaccinated mice

Table 2 illustrates the levels of IL-5 and IFN-γ secreted by these cell cultures. MLNC from infected NIH mice produced IL-5 and low levels of IFN-γ while SC produced more of both cytokines. Cells from infected B10 produced markedly lower cytokine levels. No IFN-γ was detected in MLNC or SC supernatants. B10 mice vaccinated with ES plus CFA also produced much lower levels of IL-5 than the NIH strain and no IFN-γ was detected. When these mice were infected the concentration of IL-5 secreted by MLNC increased to a level comparable to those seen in NIH. No IL-5 was detected in supernatants from SC cultures and no IFN-γ was detected in either strain.

No significant cytokine responses were detected from alum-vaccinated NIH mice in comparison to results for CFA vaccination. When subsequently infected, as with CFA, the concentrations of IL-5 increased. Cells from alum-vaccinated B10 mice produced no detectable cytokine responses. When an infection was given the levels of IL-5 increased, but the response was poor in comparison with NIH mice.

Table 1. SI* obtained when MLNC and SC from treated NIH and B10 mice were stimulated *in vitro* with 50 μg/ml *T. spiralis* ES

	MLNC		SC	
Treatment group	NIH	B10	NIH	B10
Infection only	5·9	1·9	7·9	2·0
Naive	1·6	1·7	1·5	1·5
Vacc: ES + CFA	12·4	4·7	5·3	1·3
Vacc: ES + CFA, infection	14·5	6·4	12·1	3·5
Vacc: ES + alum	12·0	3·4	14·0	2·5
Vacc: ES + alum infection	17·4	2·0	18·0	2·9

NIH and B10 mice were vaccinated with 100 μg ES plus adjuvant and boosted at 7-day intervals before infection with 300 *T. spiralis* muscle larvae. NIH mice were killed on day 8 post-infection, B10 on day 9. MLNC and SC were cultured at 5×10^6/ml. Proliferation was measured by [^{3}H]thymidine incorporation.

$$*SI = \frac{\text{mean c.p.m. stimulated cells}}{\text{mean c.p.m. unstimulated cells}}.$$

Table 2. U/ml of IL-5 and IFN-γ secreted by MLNC and SC from treated NIH and B10 mice, stimulated *in vitro* with *T. spiralis* ES

	NIH (U/ml)		B10 (U/ml)	
Treatment group	IL-5	IFN-γ	IL-5	IFN-γ
MLNC				
Infection only	230	—	14	—
Naive	—	—	—	—
Vacc: ES + CFA	27	7·3	6·8	—
Vacc: ES + CFA, infection	530	—	315	—
Vacc: ES + alum	—	—	—	—
Vacc: ES + alum, infection	340	—	52	—
SC				
Infection only	490	48	60	—
Naive	—	—	—	—
Vacc: ES + CFA	10	7·0	7·6	—
Vacc: ES + CFA, infection	270	—	—	—
Vacc: ES + alum	—	7·6	—	—
Vacc: ES + alum, infection	500	—	210	—

NIH and B10 mice, vaccinated with 100 μg ES plus adjuvant and infected with 300 *T. spiralis* muscle larvae, were killed on days 8 and 9 post-infection respectively. MLNC and SC were cultured with 50 μg/ml ES for 24 hr before supernatants were removed and tested for IL-5 and IFN-γ by sandwich ELISA. The limit of sensitivity of these assays was 6·0 U/ml for IL-5 and 3·1 U/ml for IFN-γ. (—) Concentration below the limit of sensitivity.

DISCUSSION

Vaccination is known to influence patterns of epitope recognition as well as profiles of cellular and antibody responses[21] and may therefore overcome genetically determined low responsiveness. Given the differential effects of the various classes of adjuvants, it might be expected that the success of vaccination in altering genetically determined patterns of response would be critically determined by the adjuvant used. This aspect has been considered, using a mouse model, by vaccinating rapid- and slow-responder mouse strains against *T. spiralis* infection using ES and homogenate antigen with a variety of adjuvants and the degree of accelerated worm expulsion from the intestine as criteria.

Rapid-responder strains have been successfully protected by immunization with antigen preparations of *T. spiralis*,[8,12,18] but the slow-responder B10 strain has been very difficult to immunze. Wakelin *et al.*[8] reported that B10 mice did not show accelerated expulsion of an infection when previously injected with larval homogenate antigen in CFA.

One reason for the failure of vaccination in this strain could be failure to elicit an appropriate T-cell response. Successful vaccination of B10 mice might therefore be possible with the use of other adjuvants. However it was not possible to vaccinate this strain against *T. spiralis* by giving antigens in CFA, 'TiterMax', alum or ISCOMs. In contrast, NIH mice could be successfully vaccinated using any of the adjuvants. Immunization was unsuccessful when ISCOMs were given orally but this was probably due to the dosage being too low and/or too few doses being given.

It is interesting to compare the immune responses elicited in the two strains with antigens presented in different adjuvants. No real differences in antibody isotype profiles could be found, IgG1 being the predominant isotype for all vaccinations, both before and after infection and with all adjuvants used (see Fig. 4). CFA has been shown to elicit both IgG2a and IgG1,[21] potassium alum to elicit IgG1[22] and non-ionic block polymer surfactants such as 'TiterMax', IgG2a.[23] Our results, however, show an absence of IgG2a in the isotype profiles of vaccinated mice of both strains. It is possible that parasite antigens are potent immunomodulators and dominated the response profiles through Th subset induction.

A surprising observation was the isotype response of alum-vaccinated B10 mice. As alum is thought to skew immune responses towards a Th2 IgG1-type profile, it was expected to be an excellent adjuvant for enhancing immunity to *T. spiralis*. However, immunization using alum induced accelerated worm expulsion only in NIH mice and the IgG1 and IgG2b responses of this strain appeared to be depressed (IgG1, $P = 0{\cdot}004$; IgG2b, $P = 0{\cdot}016$).

Some differences were observed in the cytokine responses induced by CFA and alum vaccinations of NIH and B10 mice. Grun and Maurer[22] showed that vaccination with alum could induce Th2-type responses, whereas CFA induced both Th1- and Th2-type responses. Our findings agreed with this, in that MLNC and SC from CFA-vaccinated NIH mice secreted IL-5 and IFN-γ (see Table 2). SC from alum-vaccinated NIH mice, however, secreted equivalent levels of IFN-γ in the absence of IL-5. Neither of these cytokines was found in NIH MLNC supernatants from alum-vaccinated mice and no IFN-γ was detected in B10 supernatants. The alum vaccinations were shown to have primed both mouse strains, because IL-5 levels in vaccinated-challenged mice were raised well above those of non-vaccinated controls,

It is clear that there are host strain-dependent differences in response to vaccination. Similar antibody isotype profiles were observed for each type of vaccination and infection, but higher antibody levels were produced by NIH mice (see Fig. 4). Similar patterns of SI were obtained from MLNC and SC of NIH and B10 mice cultured with ES although NIH SI were two- to six-fold higher. MLNC and SC from mice treated in various ways yielded similar patterns of IL-5 production, the NIH secreting higher levels than the B10. However, B10 supernatants contained no detectable IFN-γ. Grencis *et al.*[6] found Th2-type cell activity in rapid- and slow-responder mice, agreeing with our data, but they also found similar cytokine levels for both response phenotypes. Our data agree with Pond *et al.*[5] in respect of the rapid-responder strain producing higher levels of Th1-type cytokine (IFN-γ in our case) than the slow-responder strain. It has been suggested[2,24] that the time taken to raise an immune response is much greater for the B10 strain, allowing adult worms to remain in the gut for longer periods. In the present study, immune responses were related at similar points on expulsion curves for these strains. These points may not correlate to the equivalent points on immune response curves. It is therefore possible that the B10 strain cannot generate a rapid response to infection, but slowly builds up immunity to a critical level when expulsion can then occur.

The results presented here show that there is a complex interaction between host strain, adjuvant response and induction of protective immunity.

ACKNOWLEDGMENTS

This work was supported by grant G9029310 from the Medical Research Council.

REFERENCES

1. Wakelin D. & Denham D.A. (1983) The immune response. In: *Trichinella and Trichinosis* (ed. W. C. Campbell), pp. 265–308. Plenum Press, New York.
2. Wakelin D. (1993) Allergic inflammation as a hypothesis for the expulsion of worms from tissues. *Parasitol. Today*, **9**, 115.
3. Zhu D. & Bell R.G. (1989) IL-2 production, IL-2 receptor expression and IL-2 responsiveness of spleen and MLN cells from inbred mice infected with *Trichinella spiralis*. *J. Immunol.* **142**, 3262.
4. Pond L., Wassom D.L. & Hayes C.E. (1989) Evidence for differential induction of helper T cell subsets during *Trichinella spiralis* infection. *J. Immunol.* **143**, 4232.
5. Pond L., Wassom D.L. & Hayes C.E. (1992) Influence of resistant and susceptible genotype, IL-1, and lymphoid organ on *Trichinella spiralis*-induced cytokine secretion. *J. Immunol.* **149**, 957.
6. Grencis R.K., Hultner L. & Else K.J. (1991) Host protective immunity to *Trichinella spiralis* in mice: activation of Th subsets and lymphokine secretion in mice expressing different response phenotypes. *Immunology*, **74**, 329.
7. Kelly E.A.B., Cruz E.S., Hauda K.M. & Wassom D.L. (1991) IFN-γ- and IL-5-producing cells compartmentalize to different lymphoid organs in *Trichinella spiralis*-infected mice. *J. Immunol.* **147**, 306.
8. Wakelin D., Mitchell L.A., Donachie A.M. & Grencis R.K. (1986) Genetic control of immunity to *Trichinella spiralis* in mice. Response of rapid- and slow-responder strains to immunization with parasite antigens. *Parasite Immunol.* **8**, 159.
9. Bomford R. (1990) Immunomodulation by adjuvants. *S.G.M. Symp.* **45**, 143.
10. Bomford R. (1989) Adjuvants for anti-parasite vaccines. *Parasitol. Today*, **5**, 41.
11. Wakelin D. & Lloyd M. (1976) Immunity to primary and challenge infections of *Trichinella spiralis* in mice: a re-examination of conventional parameters. *Parasitology*, **72**, 173.
12. Lee T.D.G., Grencis R.K. & Wakelin D. (1982) Specific cross immunity between *Trichinella spiralis* and *Trichuris muris*: immunization with heterologous infections and antigens, and transfer of immunity with heterologous immune mesenteric lymph node cells. *Parasitology*, **84**, 381.
13. Sanchez D., Ionescu-Matiu I., Dreesman G.R., Kramp W., Six I.R., Hollinger F.B. & Melnick J.L. (1980) Humoral and cellular

immunity to hepatitis B virus-derived antigens: comparative activity of Freund's complete adjuvant, alum, and liposomes. *Infect. Immun.* **30,** 728.

14. Wakelin D. & Wilson M. (1977) Transfer of immunity to *Trichinella spiralis* in the mouse with mesenteric lymph node cells: the time of appearance of effective cells in donors and expression of immunity to recipients. *Parasitology*, **74,** 215.
15. Bolas-Fernandez F. & Wakelin D. (1989) Infectivity of *Trichinella* isolates in mice is determined by host immune responsiveness. *Parasitology*, **99,** 83.
16. Mosmann T.R. & Fong T.A.T. (1990) Specific assays for cytokine production by T cells. *J. immunol. Meth.* **116,** 151.
17. Else K.J. & Grencis R.K. (1991) Cellular immune responses to the murine nematode parasite *Trichuris muris*. I. Differential cytokine production during acute or chronic infection. *Immunology*, **72,** 508.
18. Silberstein D.S. & Despommier D.D. (1984) Antigens from *Trichinella spiralis* that induce a protective response in the mouse. *J. Immunol.* **132,** 898.
19. Jarvis L.M. & Pritchard D.I. (1992) An evaluation of the role of carbohydrate epitopes in immunity to *Trichinella spiralis*. *Parasite Immunol.* **14,** 489.
20. Mowat A.M. & Donachie A.M. (1991) ISCOMS—a novel strategy for mucosal immunization? *Immunol. Today*, **12,** 383.
21. Kenney J.S., Hughes B.W., Masada M.P. & Allison A.C. (1989) Influence of adjuvants on the quantity, affinity, isotype and epitope specificity of murine antibodies. *J. immunol. Meth.* **121,** 157.
22. Grun J.L. & Maurer P.H. (1989) Different T helper subsets elicited utilizing two different adjuvant vehicles: the role of endogenous interleukin 1 in proliferative responses. *Cell. Immunol.* **121,** 134.
23. Audibert F.M. & Lise L.D. (1993) Adjuvants: current status, clinical perspectives and future prospects. *Immunol. Today*, **14,** 281.
24. Crook K. & Wakelin D. (1994) Induction of T lymphocyte subsets and levels of interleukin-2 and interleukin-3 after infection with *Trichinella spiralis* are similar in mice of high- and low-responder phenotypes. *Int. J. Parasitol.* **24,** 119.