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H-2 (I-A) control of the antibody repertoire to secreted antigens of *Trichinella spiralis* in infection and its relevance to resistance and susceptibility

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SUMMARY

Humans infected with the parasitic nematode *Trichinella spiralis* vary in the specificity of their antibody responses to the antigens of the parasite. The possibility that such host variation in antigen recognition has a genetic basis was examined in infected inbred mice whose antigen recognition profiles were characterized by immunoprecipitation of biosynthetically labelled secreted materials of adult parasites and SDS–PAGE. The strains varied considerably in repertoire and none produced detectable antibody to all the potential antigens. Using a panel of H-2 congenic and recombinant strains it was established that the repertoire was determined by the major histocompatibility complex (MHC), the I-A region in particular. Other factors, such as level of infection and variation between individuals, affected antigen recognition profiles, but this was always within limits imposed by the MHC. Lastly, an attempt to correlate antibody repertoire with relative susceptibility or resistance to *T. spiralis* failed to reveal any clear association. This also applied to the AKR/J and AKR-Fv-1^b strains, which are H-2-identical but differ in a non-MHC susceptibility locus. These findings would argue, therefore, that the I-A region controls the antibody repertoire in this nematode infection but that the repertoire overall has little influence on the efficiency with which the infections are controlled by the immune system. Should this also apply for other nematode infections, then antigen recognition profiles of infected individual humans and domestic animals might not, therefore, be useful indicators of relative resistance or susceptibility to infection.

INTRODUCTION

The major histocompatibility complex (MHC) or closely linked genes are known to influence levels of resistance or susceptibility of inbred mouse strains to parasitic nematodes, although non-MHC genes are also strongly influential.[1] One possible mechanism for MHC-restricted effects would be through its control of the antibody repertoire. There is a consensus, however, that the antibody response to parasitic nematodes plays a lesser role in immune expulsion than does the cellular response, this being particularly so for the subject of this paper, *Trichinella spiralis*.[2] We have nevertheless concentrated on antibody in this paper because it is the most accessible manifestation of the acquired immune response and serological markers for resistance or susceptibility would be of more practical use in predicting the outcome of infection than the assay of T-cell responses. This would be particularly so, for example, in the screening of large populations of humans living in areas endemic for parasitic nematodes.

More is known about the genetics of resistance and susceptibility to *T. spiralis* than for any other helminth parasite, and the response phenotypes of numerous strains of mice have now been characterized.[1] Relative resistance is quantified by measuring the time taken for adult parasites to be immunologically expelled from the intestine or by measuring the accumulation of larvae (derived from the adult females) in the striated musculature.[3,4]

We concentrate here on the intestinal stage and the antibody response to the antigens secreted by adult worms. We reason that those exoantigens are likely to be important in the expulsion of *Trichinella* because immune attack on them could counteract their putative role in nutrition, tissue penetration and in countering the immune response itself. The specificity of the antibody responses of a range of inbred, H-2 congenic, and recombinant mice were examined and the I-A region of the H-2

Abbreviations: ES, excretory–secretory antigens of adult *Trichinella spiralis*; MHC, major histocompatibility complex; MW, molecular weight; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis.

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complex was found to control the antibody repertoire. Moreover, there was no clear evidence that the antigen recognition patterns of different strains correlated with their relative resistance or susceptibility to the infection.

MATERIALS AND METHODS

Mice, parasite infections and serum collections

BALB/c and NIH mice were bred at the Wellcome Laboratories for Experimental Parasitology from stock originally obtained from Harlan Olac, Bicester, Oxon, U.K. All other strains were obtained from Halan Olac and were from barrier- or isolator-reared colonies. Mice were 8–10 weeks old before use.

Infective larvae of *Trichinella spiralis*, suspended in 0·1% agar solution, were given orally in gavage. Unless otherwise stated, mice were infected with 125 larvae on three occasions, 28 days apart, and blood was sampled 14 days after the last of three infections. Serum was pooled from at least three mice per group, taking equal quantities of serum from each mouse.

AKR/J and AKR-Fv1^b mice were bred at the University of Wisconsin and infected at 8 weeks of age, receiving three infections of 150 larvae at 21-day intervals. Animals were bled 14 days after the last of the three infections and serum from individual animals was sent to Glasgow for analysis. The parasite used for these infections was of a different strain from that used in Glasgow, but both isolates were derived from infected pigs and their immunobiological characteristics are comparable.

Biosynthetic labelling of parasite in vitro*-released materials (ES)*

Adult parasites were obtained by the Baermann technique from the intestines of mice infected with 500 larvae 5 or 6 days previously. They were harvested 30 min after removal from the mice, washed several times by sedimentation under unit gravity in culture medium and placed into a methionine-free version of Earle's Minimal Essential Medium (GIBCO Ltd, Paisley, Renfrewshire, U.K.; cat. no. 041-01900) containing the following additives: 2·4 mM L-glutamine, 1 mg/ml glucose, 400 ng/ml glycyl-1-hisidyl-1-lysine (Sigma, Poole, Dorset, U.K.; cat. no. G1887), 40 μg/ml glutathione, non-essential amino acids (1 ml of 100 × concentrate to 100 ml of medium; 043-01140H; Gibco), 5 μg/ml Amphotericin B (Fungizone; Flow Laboratories; Irvine, Ayrshire, U.K.; cat. no. 16-723-46), 100 IU/ml penicillin, 100 μg/ml streptomycin, 25 μg/ml gentamycin sulphate (Sigma; G 7632) and one tablet each of Cephalexin and VCNT (Mast Laboratories, Bootle, Merseyside, U.K.; cat. nos MS 10 and MS6). [^{35}S]methionine (Amersham International, Amersham, Bucks, U.K.; cat. no. SJ1015) was added to the medium to give a final activity of 1·85 MBq per ml, and the parasites cultured overnight at 37° *in vitro* under 5% CO_2/air mixture. Culture supernatant was concentrated approximately 10 times by centrifugal ultrafiltration using Centriprep 10 devices (Amicon, Upper Mill, Glous, U.K.; cat. no. 4304). Unincorporated isotope was depleted by repeated cycles of concentration and replenishment with phosphate-buffered saline (PBS), pH 7·2, until the concentrate was colourless.

Immunoprecipitation and SDS–PAGE

Immunoprecipitation was carried out using approximately 100,000 c.p.m. of biosynthetically labelled ES concentrate in 5–20 μl per duplicate assay tubes. Immune complexes were captured with protein A-coated *Staphylococcus aureus* bacteria (Pansorbin Standardised 507861; Calbiochem-Behring, La Jolla CA) and analysed by 5–25% gradient SDS–PAGE, as described previously.[5] The assay was adapted when necessary to accommodate Ig isotypes which do not bind protein A by the addition of sheep antibody to mouse γ-globulins (S021-220; Scottish Antibody Production Unit, Carluke, Lanarkshire, U.K.). The molecular weight standard proteins used in the SDS–PAGE were obtained from Pharmacia (Uppsala, Sweden; cat. no. 17-0446-01) and were radio-iodinated for use. After electrophoresis, gels were impregnanted with scintillant ('Amplify'; Amersham International; cat. no. NAMP 100) before vacuum drying at 80° and exposure to pre-flashed Fuji RX X-ray film.

All the SDS–PAGE analysis was caried out under non-reducing conditions. This was to avoid the confusion that can arise from immune recognition of disulphide linked heterodimers; if only one member of such a complex were bound by antibody, reducing SDS–PAGE of an immunoprecipitate would lead to more than one band on the fluorograph.

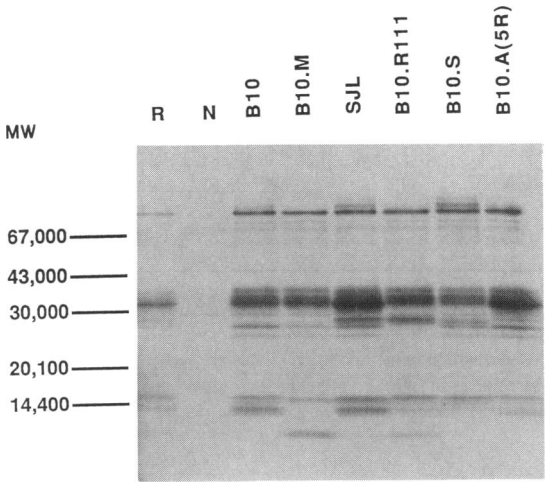

Figure 1. H-2 control of the antibody repertoire in *Trichinella spiralis* infection. Mice were infected with 125 larvae of the parasite on three occasions and blood was sampled 14 days after the last infection. [^{35}S]methionine-labelled secreted materials of adult parasites (R) was reacted with normal serum (N) or serum from infected mice of the strains indicated and immune complexes captured on protein A-coated *Staphylococcus aureus* bacterial particles before analysis by gradient SDS–PAGE and fluorography. Sera were pooled from three mice per strain. The H-2 haplotypes of the strains used are given in Tables 1 and 2. The positions and relative mobilities (MW) of iodinated marker proteins (Pharmacia 17-0446-01) are as indicated.

RESULTS

In most of the following experiments mice were infected with relatively low numbers (125 or 150) of *T. spiralis* infective larvae. This infection level was selected because MHC-controlled genetic effects on the immune response are most readily observed in this dose range.[3] The 21–28-day intervals between infections allowed the inflammation resulting from a previous self-curing infection to resolve before reinfection. This, for secondary infections at least, permits the establishment of mature adult parasites before the onset of immune expulsion.[6]

The antigen used in all experiments was biosynthetically labelled secreted products of adult worms. This is likely to

Table 1. H-2 alleles of the recombinant strains of mice used

Strain	Haplotype	K	A_β	A_α	E_β	E_α	S	D	L
A.TL	tl	s	k	k	k	k	k	d	d
B10.A	a	k	k	k	k	k	d	d	d
B10.AQR	yl	q	k	k	k	k	d	d	d
B10.A(4R)	h4	k	k	k	(k/b	b)	b	b	b
B10.A(5R)	i5	b	b	b	b/k	k	d	d	d

Taken from the listings of refs 7 and 8. All of the above strains have antigen recognition patterns similar to H-2^k strains (Fig. 2), except B10.A(5R), which exhibited an H-2^b-like repertoire. Parentheses represent instances in which a strain is defective in the expression of functional I-E heterodimers on the cell surface.[9-11]

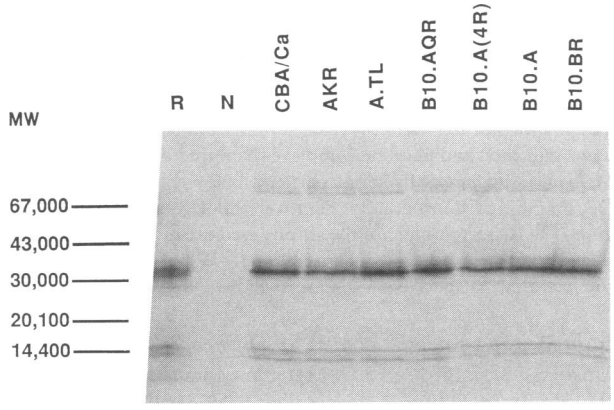

Figure 2. Role of I-A^k in repertoire selection. H-2^k and recombinant strains were infected with *T. spiralis* and their sera analysed as for Fig. 1. The H-2 haplotype compositions of the strains used are given in Table 2. Mice were infected with 125 larvae on three occasions and bled 14 days after the last infection. R, [^{35}S]methionine-labelled secreted antigen used in the immunoprecipitations. N, normal serum. The positions and relative mobilities (MW) of iodinated marker proteins are as indicated.

include some material synthesized by larvae released by the female worms during the culture period, probably as a minor contaminant.

MHC-restriction of the antibody repertoire and role of the I-A region

The antigen recognition profiles of a range of mouse strains were characterized and are illustrated in Fig. 1. Some of the parasite's ES components were recognized by all strains, but responses to other molecular species, particularly those in the low MW range, varied considerably between strains. The immunoprecipitation profiles were not affected when the *Staphylococcus aureus* particles were precoated with anti-mouse Ig to accommodate isotypes which do not bind to a protein A (not shown). Also, the profiles were found to be similar between sera taken 21 days after a primary infection, 14 days after secondary and 14 days after a tertiary infection (not shown). The intensity of banding was, however, better defined after booster infections and tertiary infections were used for the following analyses.

Although all are not illustrated in Fig. 1, the antigen recognition profiles of C57BL10 (=B10), B10.A, B10.BR,

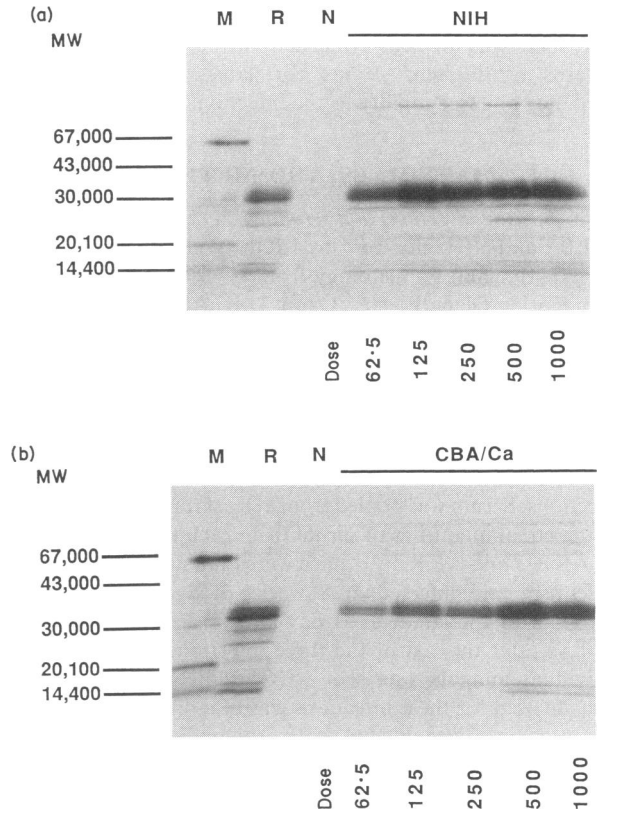

Figure 3. Effect of size of infection on antigen recognition. NIH (a) and CBA (b) mice were infected with the indicated doses on three occasions and serum sampled 14 days after the last infection. R, [^{35}S]methionine-labelled secreted antigen used in the immunoprecipitations. N, normal mouse serum. M, marker proteins.

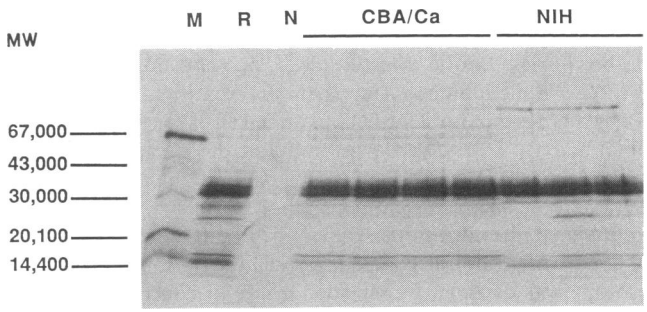

Figure 4. Heterogeneity in antigen recognition among members of inbred strains. CBA and NIH mice were infected with 500 *T. spiralis* infective larvae on three occasions and serum was sampled from individual animals 14 days after the last infection. Immunoprecipitation and SDS-PAGE analysis as for Figure 1. N, normal mouse serum. R, ^{35}S-methionine labelled secreted antigen used in the immunoprecipitations. M, marker proteins.

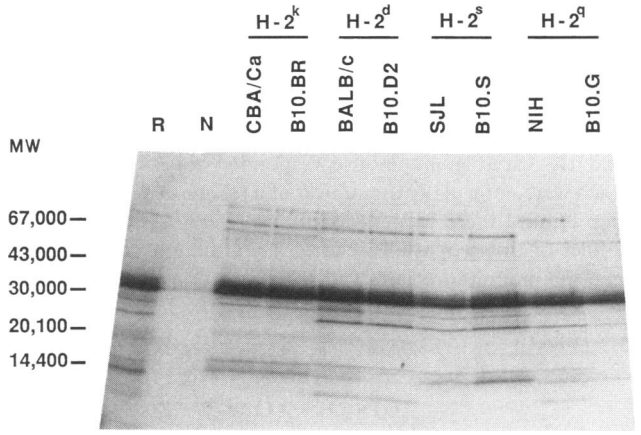

Figure 5. Comparison between fast and slow reponder strains of mice with common H-2 haplotypes. Mice were infected with 125 infective larvae of *T. spiralis* on three occasions and blood was sampled 14 days after the last infection. Immunoprecipitation and SDS–PAGE analysis as for Fig. 1. R, [^{35}S]methionine-labelled secreted antigen used in the immunoprecipitations. N, normal mouse serum.

Table 2. The resistance and susceptibility to *Trichinella spiralis* infection of mice used and their H-2 haplotypes

Strain	H-2	Ranking		
		Wassom	Bell	Wakelin
B10.A	a	—	Weak	—
B10 (=C57BL/10)	b	67	Weak	Slow
BALB/c	d	57	Intermediate	Slow
B10.D2	d	—	Weak	Slow
B10.M	f	—	—	—
AKR	k	62	Intermediate	Slow
AKR-Fv-1^b	k	—	—	—
B10.BR	k	104	Weak	Slow
CBA/Ca	k	—	—	—
CBA/J	k	93	Intermediate	Slow
B10.G	q	—	—	Slow
NIH	q	—	—	Rapid
B10.R111(71NS)	r	—	—	
B10.S	s	64	Weak	Slow
SJL	s	64	—	Rapid

Compiled from refs. 1 and 12. The rankings given were derived as follows: Wassom-recoveries of muscle stage larvae from mice infected with 200 infective stages expressed, as a percentage of the mean number which accumulate in C3HeBFeJ mice. Bell—survival time of adult parasites in the intestine from infections with 400–500 larvae; strong, < 14 days; intermediate, 14–15 days; weak, > 18 days. Wakelin—survival time of adult parasites in the intestine from infections with 300–400 larvae; rapid, < 12 days; slow, > 12 days. The strains characterized by different workers were not necessarily from the same source. Table adapted from ref. 1.

B10.G, B10.S, B10.D2, B10.M, B10.R111(7NS), B10.A(5R), BALB/c, CBA/Ca, NIH and SJL strains were analysed. Only strains bearing the same H-2 genes were found to have similar repertoires, albeit with slight differences in banding intensities.

This initial screening also indicated that the I-A region was central to the control of the antibody repertoire in that animals bearing H-2^a (B10.A) and H-2^k (B10.BR) were similar, as were those of H-2^b (B10) and H-2^{i5} [B10.A(5R)] (Fig. 1). To confirm this, H-2 recombinant strains bearing only I-A^k in common were compared (Table 1 and Fig. 2). Thus, those strains expressing the k allele at I-A shared the recognition profile of H-2^k mice even when non-k alleles were expressed at other loci. For example, so long as I-A^k was expressed, the recognition profile was essentially unchanged when b or d alleles were expressed at the S, D or L loci (A.TL, B10.A, B10.A(4R), B10.AQR) or when s or q alleles were expressed at the H-2K locus (A.TL and B10.AQR). In addition, B10.A(4R) mice, which express I-A^k but not I-E, shared the H-2^k recognition pattern. Finally, the fact that the B10.A(4R) profile differed from that of B10.A(5R) mice (the latter resembled the pattern for H-2^b mice) confirmed that the repertoires were strongly influenced by the $A_\beta A_\alpha$ genes.

Dose–response and differences in repertoire between individual mice

In spite of the consistency of I-A^k strains dealt with above, there were a few slight disparities concerning immunoprecipitation of the higher MW molecules. This may have been due to differences in the response of individual mice or to variations in the level of antigenic stimulus through variability in the number of parasites which established in the intestine. These possibilities were examined in a dose–response experiment and by analysing the repertoires of individual animals.

Mice of two strains, CBA and NIH, were split into groups which received a particular dose of larvae on three occasions and were bled 14 days after the final dose. Figure 3 shows that some additional ES components were precipitated by sera from mice infected at the higher levels, but that this still did not result in the percipitation of all the potential antigens.

Confirming attention to the dose level of 500 larvae, the responses of individual mice of the two strains were then analysed after three infections (Fig. 4). This showed that the responses of the CBA mice were essentially uniform but that the NIH mice exhibited some variability. One individual of the latter strain responded strongly to a component of between MW 20,000 and 30,000, which elicited a response in NIH mice given only 125 larvae in other experiments (e.g. Fig. 5 below). There might, therefore, be some individuals within this inbred strain which are predisposed to responsiveness to that ES component, or there may be a stochastic element in the selection of the expressed repertoire.

Both the level of antigen exposure and individual variations between infected animals can, therefore, influence the repertoire, but this appeared only to operate within limits imposed by the MHC alleles carried.

Differences between H-2-identical susceptible and resistant strains

One of the prime objectives of this study was to find out if there is a correlation between the genetically controlled antibody

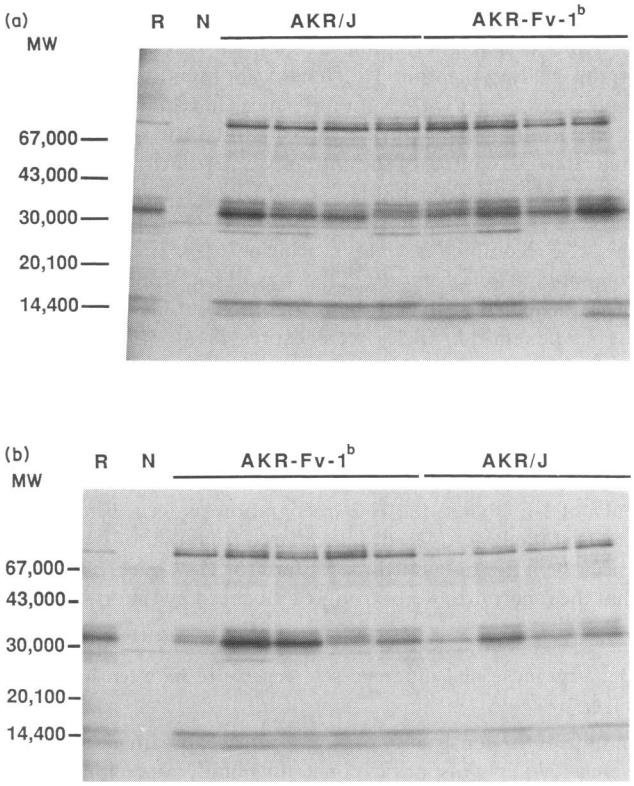

Figure 6. Comparison between strains of mice which are congenic for a non-H-2-linked susceptibility locus. The responses of individual mice are given. AKR/J are relatively resistant to *T. spiralis* infection and AKR-Fv-1^b susceptible. Both strains are H-2^k. Immunoprecipitation and SDS–PAGE analysis as for Fig. 1. R, [^{35}S]methionine-labelled secreted antigen used in the immunoprecipitations. N, normal mouse serum.

repertoire and susceptibility or resistance to the infection. Two ways of approaching this would be to examine the repertoire of H-2-identical strains which differ in genetic background and relative resistance to infection, or to examine mice which are congenic for a non-H-2 susceptibility locus.

In the first of these approaches, we examined the antigen recognition profiles of pairs of H-2-identical strains (Fig. 5). The pairs which are of most relevance here are the H-2^s strains SJL and B10.S and the H-2^q strains NIH and B10.G, the first of each pair being rapid responders to the intestinal phase of the infection and the second being slow (Table 2). In both cases, the slow responders produced little antibody to a high MW component but were otherwise identical to the fast responders of each pair. Responses to this entity could not, however, be directly related to resistance since the susceptible H-2^k and H-2^d strains also produced antibody to it.

In the second approach, we analyse the repertoire of a pair of congenic strains which differed at the Fv-1 locus on chromosome 4. AKR/J mice are relatively resistant and AKR-Fv-1^b mice are highly susceptible to *T. spiralis*, as measured by the number of larvae which accumulate in the striated musculature.[4] This is not strictly comparable with susceptibility, as defined by the survival time of the intestinal phase of infection, but a relationship will probably exist between the duration of the intestinal phase and the accumulation of larvae in the musculature.

This experiment was carried out using an infection of 150 larvae per mouse and serum was taken 14 days after the last of three infections given 21 days apart. The response profiles of individual mice are illustrated in Fig. 6. There was some heterogeneity between individual mice, but each strain was similarly variable; no mouse responded to ES components, which were not usually the subject of a response in H-2^k animals (Fig. 2), and there was essentially no overall difference in the profiles of the two strains. Moreover, although not illustrated here, the immunoprecipitation profiles of pooled sera from the two strains were identical.

DISCUSSION

MHC control of the antibody repertoire to infection with parasitic helminths in mice has been shown in infection with the trematode *Schistosoma mansoni*[13] and the nematodes *Ascaris suum*[14–16] and *Brugia malayi*.[17] It could be argued that none of these parasite species is natural to the mouse and that the MHC-restricted recognition is artifactual or atypical of definitie host–parasite combinations. Indeed, MHC effects on the antibody response were observed, but considered subordinate, in a study of primary infections with *Trichuris muris* in the mouse (a natural host–parasite combination), prompting the prediction that the clarity of MHC control in *Ascaris* infection is probably rare.[18] Stronger evidence for MHC control has, however, now appeared for the former parasite[19] and the present results on *T. spiralis* further emphasize that the genetic control observed is likely to be a general phenomenon. This is further supported by our recent findings that there is also MHC control of the antibody repertoire to ES antigens of *Nippostrongylus brasiliensis* in the rat, which is also a natural combination.[20]

Individual infected humans are known to vary in the specificity of their antibody responses to infection with several species of nematode (reviewed in ref. 21), including *T. spiralis*,[22] and attempts to relate this to pathology and/or immunity to infection has occupied considerable research effort. It might be, however, that differential responsiveness is merely a consequence of HLA polymorphism and is otherwise incidental. On the other hand, MHC control of the repertoire might relate to other consequences of infection with nematodes, such as hypersensitivity responses. An example of this has already arisen in the MHC control of the response to a major allergen of *Ascaris*.[15,16] The specificity of the IgE response might, therefore, influence the severity of type I hypersensitivity responses, particularly if some parasite allergens are present in proportionately large amounts.

Our present experiments with *T. spiralis* infection in H-2 congenic and recombinant mice indicated that the class II I-A region controls the repertoire and negates a role for the closely linked polymorphic loci of the S region.[23] This role for I-A is also reflected in our findings on I-A control of the IgE response to the ABA-1 allergen of *Ascaris*.[24]

Two possible mechanisms for the Ir gene effects observed here are the inability of certain class II molecules to present processed peptides of a particular antigen[25,26] and/or cross-tolerance with self-components leading to deletion of a particular specificity from the T-cell repertoire.[27–29] Cross-tolerance could, for example, be the cause of the slight differences in the

responses of H-2-identical strains with disparate genetic backgrounds (Fig. 5), but this seemed to apply to only a very few ES components.

Two other factors were also found to affect the antibody repertoire to *T. spiralis* infection. The first was the level of infection; increasing the dose of *T. spiralis* led to an increase in the number of ES components to which a response was detectable. In no case, however, did this result in recognition of all potential ES antigens by any strain tested. The second factor was variation between individual mice. The extent of this appeared to vary from strain to strain but the heterogeneity within a strain was usually less than that which occurred between strains. The possible causes of this heterogeneity are manifold, including heterogeneity in T-cell epitope recognition between members of an inbred strain[30] and residual heterozygosity at non-MHC loci,[31] which would then affect the immune repertoire through cross-tolerance.[28,28]

One of the prime objectives of this investigation was to find out whether or not there is a correlation between the genetically controlled antibody repertoire and susceptibility or resistance to the infection. One way of approaching this is to examine the repertoire of H-2 identical strains which differ in genetic background and response phenotype, or to examine mice which are congenic for a susceptibility locus. A comparison between H-2-identical strains which have previously been characterized as slow or fast responders to *T. spiralis* showed that the latter strains tended to respond to a high MW ES components in contrast to slow responders (Fig. 5). This could, however, merely be an effect of the rate of response of the strains concerned rather than a cause of susceptibility, particularly since the molecules concerned were subject to a response by susceptible strains of other haplotypes.

An alternative test of the influence of antibody repertoire on the outcome of infection was provided by the analysis of the AKR/J and the AKR-Fv-1^b strains, which had been previously characterized as susceptible and resistant, respectively, to the muscle stage of *T. spiralis*. Resistance to the muscle stage might not be strictly comparable to the rate of immune expulsion of the intestinal stage, but there is some agreement in ranking of other strains which have been characterized by both methods.[1] This presumably reflects the fact that the longer the parasite survives in the intestine, the greater will be the consequent burden of muscle larvae.

The AKR/J and the AKR-Fv-1^b strains are both H-2^k, but the former has a segment of C57BL/6 DNA on chromosome 4 which is marked by the Fv-1^b allele. Unlike the parental AKR strain, the T cells of AKR-Fv-1^b mice express I-J^k.[32] Our comparison of the antigen recognition patterns of the two strains showed that, whilst there was a degree of individual variation, there was essentially no overall difference between the strains. It is hence unlikely that the disparities between the two strains in their immune control of the parasite can be attributed to differences in the specificity of their antibody responses. On the other hand, we have not examined the isotype of the antibody elicited in any detail. The present analysis was confined to protein A-binding antibody but we found no difference in the antigen recognition profiles when the immunoprecipitation assay was adapted to take account of other isotypes. This might not, however, have detected IgE and IgA antibody because of their low concentrations in serum. These isotypes cannot be ignored because they are thought to affect gastro-intestinal nematoides or to be involved in immune expulsion mechanisms.[33,34]

If MHC control of the antibody repertoire cannot explain resistance/susceptibility phenotypes, it must then be asked whether the T-cell repertoire is influential. T cells certainly have a critical role in adoptive transfer of immunity to *T. spiralis*[35] and *T. spiralis*-specific T-cell lines are protective *in vivo*.[36] If their repertoire is important, then those of B and T cells must be different. The idea of a dichotomy of B- and T-cell repertoires originated from studies on artificial amino acid co-polymers or phylogenetically related proteins, usually in the context of adjuvant-assisted immunization (reviewed in refs 8 and 37). Whether B- and T-cell epitopes on antigens of infective agents are mutually exclusive is a matter of debate. Work on *Plasmodium falciparum* and *Mycobacterium leprae*, for example, would indicate that T and B cells may recognize distinct epitopes.[38,39] On the other hand, detailed work on the haemagglutinin of influenza virus has revealed a considerable degree of commonality between T- and B-cell epitopes recognized by MHC-identical individuals[40] and there may not, therefore, be any fundamental difference between B- and T-cell epitopes. If so, then MHC control of neither the B nor the T-cell repertoire might be the reason for the H-2 effects on resistance/susceptibility to *T. spiralis*.

T cells are, however, central to the control of lymphokine release, and this is likely to be crucial. It is not clear, however, how the MHC could control this directly, although regulatory genes which are closely linked to MHC class II loci could be involved. The MHC-associated effects might, therefore, operate through control of lymphokine release which might, in turn, affect, for instance, the predominance of a particular T-cell effector mechanism or Ig isotype in the antibody response to infection.[41]

The relevance of repertoire control will always be difficult to settle in conventional inbred experimental animals because it could be argued that the repertoire *is* influential but that its effects are swamped by the effects of non-MHC genes. The use of I-A or I-E transgenic animals could be of considerable value in this regard. We are nevertheless left for the moment with the conclusion that analysis of immune repertoire will probably be of little value in the attempt to predict the relative resistance to nematode infection in humans or domestic animals.

In the Introduction to this paper we justified focusing on the ES materials of the parasite because of their putative role in immunity. ES antigens from a number of nematodes have been found to contain protective antigens, but these are not necessarily the crucial ones in resistance or susceptibility to *T. spiralis*. The other source of exoantigens which has received considerable attention, and which is demonstrably protective against *T. spiralis*, is surface antigens,[42] although it is not clear to what degree surface and ES materials are mutually exclusive in this species. Differences in the reactivity of fast and slow responder strains have been shown in the past for surface-associated antigens which can be radio-iodinated *in situ*,[43] but this was reflected in the rate at which antibody to discrete components became detectable rather than absolute responsiveneses or non-responsiveness to them. Moreover, only two strains of mice were examined in that study, so differential antigen recognition might have been missed. We are currently re-examining the genetic control of recognition of surface antigens using the sera employed in the present study.

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