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The specificity of the antibody response to internal antigens of *Ascaris*: heterogeneity in infected humans, and MHC (H-2) control of the repertoire in mice

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SUMMARY

Children from an area of Africa endemic for the large roundworm of humans, *Ascaris lumbricoides*, were found to vary considerably in the specificity of their serum IgG response to the internal antigens of the parasite. This was particularly noticeable for responses to a 14-kD protein (ABA-1) of the parasite that has previously been shown to be the subject of a strong IgE antibody response in infected animals. The possibility that this heterogeneity in immune repertoire has a genetic basis was explored in inbred mice infected with *Ascaris suum*. This showed that no strain responded to all the potential antigens, that the recognition profiles of strains bearing independent haplotypes were unique, and only H-2-indentical strains had responses of similar specificities. Major histocompatability complex (MHC) restriction was confirmed using H-2-congenic animals on BALB and B10 backgrounds, which responded according to their H-2 haplotype. It is likely, therefore, that it is the MHC which controls the repertoire to *Ascaris* antigens in infected people. If this is so, then there will be implications for immunopathology associated with ascariasis, and possibly also for resistance and susceptibility to infection.

Keywords *Ascaris lumbricoides* *Ascaris suum* parasitic nematodes MHC H-2 restriction ABA-1

INTRODUCTION

People infected with parasitic nematodes vary considerably in the specificity of their antibody responses to the parasites concerned (Pritchard *et al.*, 1986; Almond *et al.*, 1986; Maizels *et al.*, 1987; Pinder, Dupont & Egwang, 1988; Roach *et al.*, 1988). This heterogeneity has relevance not only to serological assays for both circulating antibody and antigen, but also for the identification of people who are predisposed to high or low levels of infection and/or to particular pathologies. The possible causes of variability are legion and include infection history, parasite load, immunological tolerance, exposure to cross-reacting infections, and the genetic constitution of the host. Whatever the case might be, it is important to understand the cause of heterogeneity in the expressed repertoire, how it arises, and what it means.

We have previously found that infected humans and experimental animals vary in their antibody responses to the secreted antigens of the tissue-invasive stages of the nematode *Ascaris* (Kennedy *et al.*, 1987a; Haswell-Elkins *et al.*, 1989; Tomlinson *et al.*, 1989; Kennedy, 1989). In mice, the patterns of responses are under precise control of the H-2 complex, which argues strongly that HLA restriction might operate in infected humans.

In this paper, we report on an analysis of the specificity of the antibody response to the internal antigens of *Ascaris*. The justification for concentrating on this compartment as opposed to surface or secreted antigens is that it has been, and remains, the most frequently used for serological work on the infection in humans. Moreover, it contains large quantities of potent allergens (Jarrett & Miller, 1982), and has, therefore, relevance to the hypersensitivity responses which can be life-threatening in ascariasis (Ogilvie & Savigny, 1982). Such reactions are a feature of nematode infections in general and, because of the availability of its allergens in relative bulk, *Ascaris* presents a particularly amenable model for the study of hypersensitivity responses in the context of infection.

We show that infected people vary considerably in their responses to internal antigens of *Ascaris*. The possibility of a genetic basis for this was explored in mice, in which the specificity of the antibody response was found to be MHC-restricted.

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MATERIALS AND METHODS

Inbred and congenic mice

All animals were purchased from Olac, Bicester, UK, with the

Table 1. The major histocompatibility complex (H-2) type of the strains of mice used

Strain	H-2 haplotype
C57B10/ScSn (B10)	b
BALB/B	b
DBA2	d
BALB/c	d
B10.D2	d
CBA/Ca	k
C3H/He	k
BALB/k	k
B10.BR	k
NIH	q
B10.G	q
SJL	s
B10.S	s

exception of NIH and BALB/c strains, which were bred within the Wellcome Laboratories for Experimental Parasitology from OLAC stock, and CBA/Ca, which were obtained from the National Institute for Medical Research, London, UK. Infections or immunizations were begun when the animals were 8 weeks of age. The strains of mice used are given in Table 1, along with their H-2 haplotypes.

Internal antigens of Ascaris

The perienteric fluid (ABF) of adults of *Ascaris lumbricoides* from children in Nigeria, or *Ascaris suum* from pigs in Scotland, was prepared and stored as described previously (Kennedy *et al.*, 1987b). The purified 14-kD antigen was prepared from ABF by immunoaffinity chromatography, as described previously (Tomlinson *et al.*, 1989), and has been named ABA-1 (McGibbon *et al.*, 1990; Christie *et al.*, 1990).

Antisera

Mice were infected orally with 2000 infective eggs of *A. suum* at 28-day intervals, and bled 14 days after the last of three infections. The reason for choosing tertiary infections was that recognition profiles of secreted antigens of the parasite do not appear to change with further infections (Tomlinson *et al.*, 1989). For direct immunization with parasite protein, mice were given 20 μg of purified 14-kD antigen, or 100 μg of ABF, in Freund's complete adjuvant in three sites on the back, and this was repeated 7 days later. They were boosted 28 days after that with the same quantity of protein in Freund's incomplete adjuvant, and bled 14 days later.

Radioiodination and immunoprecipitation

ABF was dialysed against phosphate-buffered saline (PBS) and labelled with ^{125}I using Iodo-Gen, as described previously (Kennedy & Qureshi, 1986). The isotope was obtained from Amersham International, Amersham, UK and the iodination reagent from Pierce Chemical Co., Rockford, IL (product no. 28600). Immunoprecipitation was performed using a *Staphyloccus aureus*-based assay, in which iodinated culture medium was incubated with serum overnight at 4°C, and IgG immune complexes captured on heat-killed, formalin-fixed bacteria (Pansorbin Standardised 507861; Calbiochem-Behring, La Jolla, CA). When a broader isotype specificity for the assay was required, 2·5 μl of rabbit anti-human or mouse Ig (whole molecule) were added to the reaction mixture. The radioactivity in the washed pellets was counted in a LKB gamma counter and prepared for SDS–PAGE.

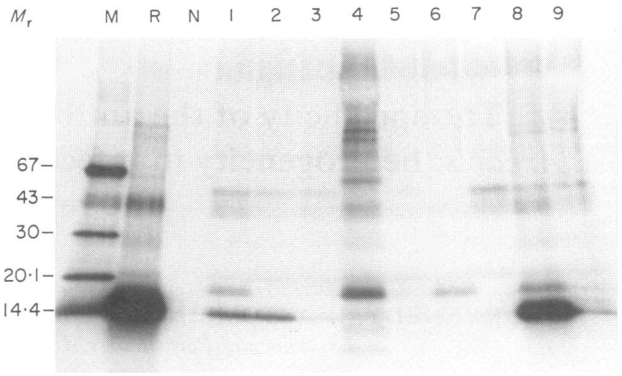

Fig. 1. Heterogeneity in the specificity of the antibody response of humans infected with *Ascaris lumbricoides*. Body fluid from adult *A. lumbricoides* parasites (ABF) was labelled with ^{125}I and immunoprecipitated with serum from an uninfected European (N), and from children living in an endemic area of Nigeria (1–9). The immunoprecipitates were analysed by gradient SDS–PAGE, along with a sample of the iodinated antigen (R). (M) was loaded with iodinated standard marker proteins (Pharmacia 17-0446-01), and their relative molecular masses (M_r) as given by the manufacturers are indicated in KD.

Polyacrylamide gel electrophoresis (SDS–PAGE)

This was carried out in a Pharmacia (Uppsala, Sweden) GE-2/4 LS slab gel apparatus according to the manufacturers instructions. Gradient gels (5–25%) consisted of 120 mm separating gel and 15 mm stack gel, and were 0·7 mm thick. Samples were prepared according to published methods (Kennedy & Qureshi, 1986), with 5% β-2-mercaptoethanol or 1 mg/ml iodoacetamide for reducing and non-reducing conditions, respectively. Following electrophoresis, gels were fixed in 25% methanol, 10% acetic acid, 1% glycerol, then dried. Autoradiographs were exposed to flashed Fuji RX film at −70°C. Apparent molecular masses were estimated with reference to standard marker proteins (Pharmacia 17-0446-01), and are given in kD.

RESULTS

Heterogeneity in the antigen recognition of humans infected with Ascaris

Radio-iodinated ABF from *A. lumbricoides* parasites collected in Nigeria was immunoprecipitated with serum from people living in the same area, and analysed by SDS–PAGE and autoradiography (Fig. 1). This revealed a remarkable degree of variation between individuals in the antigens to which they responded. This assay was protein A-mediated, and, therefore, restricted to IgG antibody. However, modification of the assay in order to take other isotypes into account made no difference to the recognition profiles (not shown). All of the people were infected with adult parasites at the time of sampling, with the exception of subjects 5 and 6. Serum from the latter two

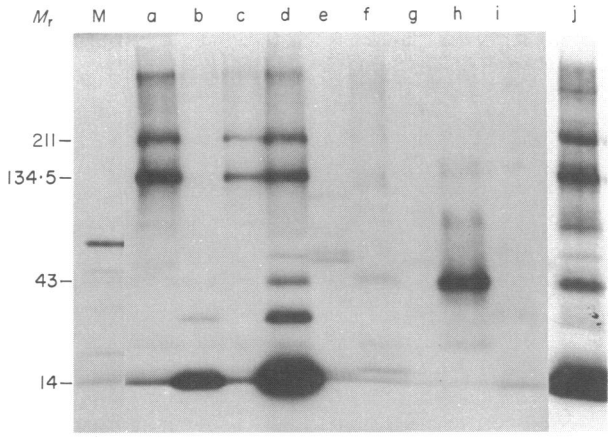

Fig. 2. Differences between the antigen recognition profiles of inbred strains of mice infected with *Ascaris suum*. Blood was sampled 14 days after the last of three infections with the parasite, and pools made between serum from at least three individuals per strain. The immunoprecipitates were analysed by gradient SDS–PAGE and were: infected BALB/c (a), BALB/c immunized with purified 14 kD in adjuvant (b), infected DBA/2 (c), infected SJL (d), infected CBA/Ca (e), infected C57BL/10ScSn (B10) (f), normal BALB/c (g), infected NIH (h), infected C3H/He (i), and BALB/c immunized with whole ABF in adjuvant (j). Marker proteins were loaded onto (M), and the M_r of the major antigen components are as indicated.

precipitated less radioactivity than did some of the others, but our survey is at present too limited for us to attempt any correlation between antibody level and infection status. The variation in responsiveness to the 14-kD component was particularly notable since it is a proven allergen (Tomlinson *et al.*, 1989; Christie *et al.*, 1990; McGibbon *et al.*, 1990).

Differential responsiveness by inbred strains of mice

In order to establish whether or not the above effect could have a genetic basis, various strains of inbred mice were infected on three occasions, and the specificity of their antibody responses analysed as above (Fig. 2). For this we used infections with, and antigens from, the porcine homologue, *Ascaris suum*, which is very similar antigenically and morphologically to *A. lumbricoides*, and our previous findings argue for close similarity between their antigens (Kennedy *et al.*, 1987b, 1989). Moreover, the SDS-PAGE profiles of their body fluid proteins are very similar and, from our limited experience, there is as much variation between the two species as within them (not shown).

No strain was found to respond to all the components of ABF, and the recognition patterns varied considerably. Inclusion of rabbit anti-mouse Ig in the reaction mixture increased the intensity but not the pattern of antigen recognition. It is unlikely, then, that restricted recognition was the result of an Ig isotype bias. There was similarity between the recognition profiles of H-2-identical strains (see Table 1 for H-2 haplotypes of the strains used), but this was to some extent obscured by differences in the levels of antibody response. The MHC might, therefore, control the specificity of response and background genes its level. Only H-2^s mice (here, SJL, Fig 2d) have been found to respond to the 14-kD antigen in the past (Kennedy *et al.*, 1987a; Tomlinson *et al.*, 1989), except when immunized with the protein in adjuvant. In this case, BALB/c will respond (Fig. 2b).

In Fig. 2b there is a ladder of bands of relative mobilities (M_r) 13·7, 28·1 and (faintly) 41·1. We take this to arise from decreasingly low levels of the 14-kD antigen in monomeric, dimeric and trimeric, etc., aggregations. In Fig. 2d, the series continues with additional bands at 56·1 and 71·3 kD. We also find this to be true for immunoprecipitations of purified 14 kD (not shown). This effect occurs in samples that have been boiled before loading onto the gel, so the effect is unlikely to be a reflection of the natural behaviour of the protein but rather that small quantities of spontaneously formed aggregates become visible when large quantities of the radiolabelled protein are present.

There is some confusion in the literature about the molecular mass of the molecule which we have estimated as 14 kD. Other workers have sized it at M_r of several kD less, although this might be explained in one case at least by a homogenization which was performed in the absence of proteinase inhibitors (Greenspon *et al.*, 1986). The disparities possibly lie in variations in SDS–PAGE procedures, since its size has been estimated as approximately 10 kD under different conditions, and by HPLC in the presence of SDS (McGibbon *et al.*, 1990; unpublished observations). In our case, the 14 kD has consistently run at 14 kD, or slightly below, in our 5–25% acrylamide gradient gels. However, in homogeneous 15% acrylamide gels which have been electrophoresed longer than usual, and under reducing conditions, we find that the protein can run at 11–12 kD (not shown). The ladder of dimers, trimers, etc., does provide a further means of estimating the monomer's mass, which we calculate as $14{\cdot}01 \pm 0{\cdot}33$ kD, calculated from the mobilities of all the bands in the ladder. Its M_r is, therefore, operationally defined, and we await its full sequence for its definitive mass.

We found it difficult to eliminate the small degree of non-specific precipitation of the 14 kD, and contamination of adjacent tracks by small quantities of it. This is compounded by the need to expose autoradiographs sufficiently to reveal the higher molecular weight species, but it left it difficult to establish exactly which strains responded. For reasons yet unclear, this is a persistent problem with immunoprecipitates using mouse sera, but the situation is clearer with the use of purified protein (Fig. 3). This illustrates the responses of the same strains as used for the experiment shown in Fig. 2. Again, responses to the 14 kD molecule were only elicited in the H-2^s strain (track e), unless the antigen was presented in adjuvant (track c). We only illustrate the response elicited by adjuvant immunization of BALB/c mice in Fig. 3, but anti-14 kD responses can also be produced in CBA, C57BL/10ScSn (B10) and NIH mice, none of which is H-2^s. In B 10.S and SJL, which are H-2^s, adjuvant immunization elicits the expected strong anti-14 kD response. The responses of selected F_1 hybrid strains were also characterized, and the results are summarized in Table 2.

H-2 restriction of responses

Similarity between the response profiles of H-2-identical strains is not necessarily proof of MHC control of antigen recognition, because several such strains are related, and common background genes could have been at work. The response profiles of H-2 congenic animals were, therefore, examined on the BALB/c and B10 backgrounds (Fig. 4A, B). These provided more compelling evidence for H-2 control. For example, only H-2^b strains responded to the M_r 15·5-kD protein (see also Fig. 2).

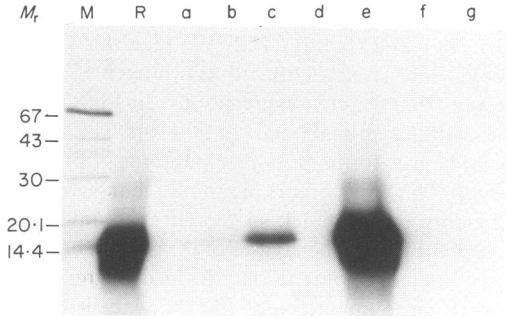

Fig. 3. Recognition of purified 14-kD antigen by various strains of mice. Radio-iodinated 14 kD (R) was immunoprecipitated with sera obtained as detailed for Fig. 2. The immunoprecipitates were loaded as follows: normal BALB/c (a), infected BALB/c (b), BALB/c immunized with the 14 kD in adjuvant (c), infected CBA (d), infected SJL (e), infected B10 (f), and infected NIH (g). (M) was loaded with iodinated marker proteins as used above, and their M_r as given by the manufacturer are indicated.

Table 2. Recognition of the 14-kD antigen (ABA-1) by various strains of inbred mice and their F1 hybrids

Strain	H-2 haplotype	Anti-14-kD response
C57B 10/ScSn (B10)	b	—
BALB/c	d	—
B10.D2	d	—
CBA/Ca	k	—
SJL	s	+++
B10.S	s	+
B10 × B10.S	b,s	+
B10·D2 × B10.S	d,s	+
SJL × B10.D2	s,d	++
BALB/c × SJL	d,s	+++
CBA/Ca × SJL	k,s	+++
SJL × B10	s,b	++

The animals were infected with *Ascaris* as described in the Materials and Methods and pooled serum from each strain or hybrid was immunoprecipitated with ^{125}I-labelled purified 14-kD antigen in the protein A-based assay. Immunoprecipitates were run out on SDS–PAGE gels, and the intensity of the resultant bands scored according to intensity.

H-2^d animals had similar profiles, although they differed in intensity, but a consistently strong response to the 211 and 134·5 kD molecules was evident, as it was for this haplotype in Fig. 2.

The consistency of response between H-2 identical strains appeared to falter for H-2^k strains (Fig. 2e and i, Fig. 4c and h). This could be more apparent than real due to the faintness of the response mounted by H-2^k animals in general, but there does appear to be some genuine differences. If these are real, then the effect might be a reflection of the influence of background gene products on tolerance in these animals (see Discussion).

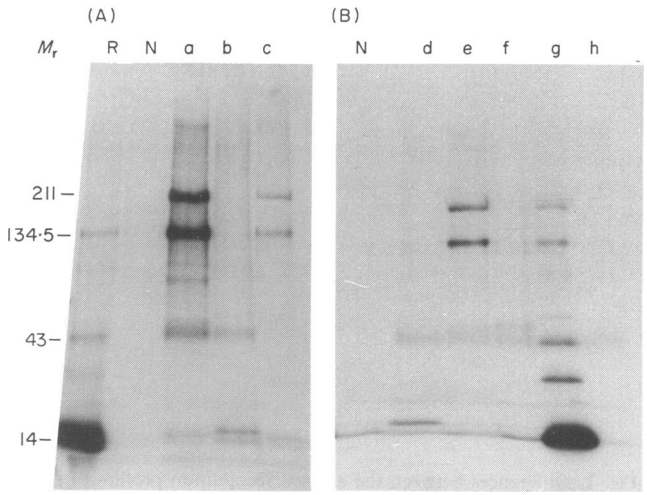

Fig. 4. MHC restriction of the antibody response to *Ascaris* internal antigens. H-2 congenic strains of mice on the BALB (a) and B10 (b) genetic backgrounds were infected with *A. suum* and their sera were immunoprecipitated with radio-iodinated ABF (R). (A) Immunoprecipitate with normal BALB/c serum (N), infected BALB/c (a), infected BALB/B (b), and infected BALB/K (c). (B) Immunoprecipitate with normal B10 serum (N), infected B10 (d), infected B10.D2 (e), infected B10.G (f), infected B10.S (g), and infected B10.BR (h). (R) was loaded with the reference antigen, and the M_r of the major antigen components are as indicated.

As before, the SDS–PAGE analysis of the immunoprecipitates were poor at discriminating the anti-14 kD response. An analysis using purified 14-kD antigen was again performed to clarify this (Table 2). This showed that only those inbred strains or hybrids bearing an H-2^s allele responded to the protein. There was also an indication that the genetic background affected the level of the response against the antigen in that responses tended to be low in B10 congenics or their hybrids.

DISCUSSION

A feature of intestinal nematodiases of humans, including ascariasis, is the overdispersion of the parasite population among its hosts (Hliang, 1985; Anderson, 1986; Elkins, Haswell-Elkins & Anderson, 1986), and the apparent predisposition of some individuals to heavy or light infections (Haswell-Elkins, Elkins & Anderson, 1987). The basis for this is unknown, but the genetic constitution of the host is clearly an important potential influence. Epidemiological work on the relationship between MHC and infection status is still in its infancy, but there are preliminary indications that certain HLA alleles are influential (Bundy, 1988). Experimental work in rodents has served to reinforce this possibility in that animals with certain haplotypes are more susceptible to infection with the nematode *Trichinella spiralis*, this effect being particularly noticeable when parasite levels are low (Wassom *et al.*, 1984). This has been taken to considerable detail, and susceptibility to the infection has been correlated to expression of the class II I-E molecule (Wassom, Krco & David, 1987). If it transpires that the MHC is indeed influential in humans, then it is essential to understand the mechanism involved. An obvious candidate at the moment would be the control of the immune repertoire.

MHC-linked immune responses (Ir) genes were originally characterized in experimental systems employing adjuvant-assisted immunization with antigens irrelevant to infections, such as keyhole limpet haemocyanin, sperm whale myoglobulin and insulin (Klein, 1986). There are, however, few if any examples of such effects being manifest against the components of organisms as antigenically elaborate as nematodes. The fact that H-2-restricted antigen recognition can be demonstrated for *Ascaris* infection is, therefore, noteworthy, particularly since it applies to large molecules which presumably comprise more than one potential epitope. The effect could be due to the presence of repeating epitopes, as occurs in certain antigens of the malaria parasite *Plasmodium falciparum* (Kemp *et al.*, 1986). However, for the latter, this is thought to be a result of selection under immune pressure (Anders, 1986), which is unlikely to apply to the internal components of parasitic nematodes, unless they are precursors of exo-antigens.

Whatever the cause of heterogeneous antigen recognition between individual humans, it is clear that certain people do not respond to the most abundant protein constituent of the parasite, the 14-kD molecule. A possible cause of this is that different degrees of exposure to the antigen might occur from individual to individual. This is probably not the explanation because the protein is apparently also released by tissue-invasive stages of the parasite (Kennedy & Qureshi, 1986; Kennedy *et al.*, 1987b), and responses to other internal components occur in people who do not happen to respond to the 14-kD molecule. It would seem likely, therefore, that the cause of heterogeneity is intrinsic to the host. The fact that people do not respond evenly to parasite components has implications for serodiagnosis, particularly when one protein is present in large relative amounts. In serodiagnostic applications, then, modified antigen preparations might be required.

Differential responses to the 14-kD antigen has immediate implications for immunopathology in that it is an established allergen (ABA-1; Christie *et al.*, 1990; McGibbon *et al.*, 1990). The specificity of the IgE antibody response to it is under MHC control, but non-MHC genes control its level (Tomlinson *et al.*, 1989). The most obvious hypersensitivity in ascariasis is Loeffler's syndrome, which is a pulmonary hypersensitivity to migrating juvenile forms of the parasite (Ogilvie & de Savigny, 1982). There is, moreover, one report of lethal intestinal anaphylactic responses following anthelmintic treatment for the infection (Odunjo, 1970).

Responses to internal components are presumably stimulated when parasites disintegrate and their antigens are released. The most likely occasion for this would be during the tissue migration of larvae from the intestine to the lungs. The release of internal antigens could occur either following the death of the parasites, or during the moulting process which all nematodes have to undergo during their growth and development. Whatever the case, it is possible that people having an elevated response to endoantigens are those in whose tissues the larvae are being killed. Whether differences in level of response to internal components would be useful to gain information on immune status will, however, depend heavily on genetic factors in the control of antigen recognition.

MHC control of the anti-*Ascaris* repertoire is remarkably consistent between strains of H-2 identical mice when screened against the secreted antigens of infective (Kennedy *et al.*, 1987a) or lung-stage larvae (Tomlinson *et al.*, 1989). It was surprising, therefore, to find that the H-2^k strains varied in their antigen recognition profiles when screened against parasite body fluid (Figs 2 and 4). We cannot be confident of this fact as yet because the level of response mounted by these strains was uniformly low and antigen recognition profiles were difficult to discriminate. If the differences are real, however, it could be due to variations in the isotype balance in antibody responses between the strains, or the effects of background genes which differ between the H-2^k strains used here. This is reminiscent of background gene influences on the immune repertoire in *Ascaris*-infected F_1 hybrids (Tomlinson *et al.*, 1989). Such effects have been recorded frequently in immunogenetics, and would be predicted by the cross-tolerance hypothesis in the regulation of Ir gene effects (Schwartz, 1978; Matzinger, 1981). This states that the specificity of an individual's immune response is governed by the MHC alleles they bear and tolerance to their own tissues. The set of self-molecules to which tolerance operates is, however, itself determined by the MHC genes carried. This would have implications for the study of antibody repertoires in outbred human populations, in that people bearing an allele of HLA which has the potential to permit a response to particular *Ascaris* antigens need not necessarily respond in the expected way.

Finally, we have not yet established which are the influential loci in determining the repertoire to *Ascaris* infection in mice, but it would be anticipated that they would lie in the class II region. If so, then the response to the 14-kD must be I-A-restricted because the H-2^s haplotype is I-E-defective (Murphy *et al.*, 1980; Jones, Murphy & McDevitt, 1981). The recombinant strains necessary to confirm this I-A restriction are not available to us at the moment, but an alternative might be to use anti-I-A antibodies as inhibitors in lymphocyte proliferation assays. Of pertinence here is our finding that a similar MHC restriction of responses to the antigen of a parasitic nematode of both mice and humans, *T. spiralis*. In this case, we do have evidence from H-2 recombinants that the response is controlled from the H-2 class II region (M. W. Kennedy and A. E. McIntosh, unpublished data). This could in turn have a bearing on resistance and susceptibility to nematode infections, as mentioned above, since the H-2 has been firmly established as contributing to the immune control of *T. spiralis* infections (Wassom *et al.*, 1984, 1987). It has to be added, however, that non-H-2 genes can be equally or more important to the outcome of infection (Wakelin, 1985, 1988), but the evidence to date firmly places MHC-associated effects as important elements in the integrated mechanisms involved in immune control of parasitic nematodes.

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