

MHC RESTRICTION OF THE ANTIBODY REPERTOIRE TO SECRETORY ANTIGENS, AND A MAJOR ALLERGEN, OF THE NEMATODE PARASITE *Ascaris*¹

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Humans vary considerably in the antigen specificity of their immune responses to parasitic nematodes, and in the infection loads of individuals living in the same environment. The possibility that the former has a genetic basis operating through repertoire control of the immune system was investigated using infection of mice with the nematode *Ascaris*. The specificity of the antibody response was examined using excretory/secretory (ES) materials of the parasite as target Ag. No strain of mouse was found to recognize all of the potentially antigenic components of ES, and the Ag recognition patterns varied considerably from strain to strain. Using H-2 congenic mice on both the BALB and B10 backgrounds, it was established that the antigen recognition patterns were MHC-determined. Focusing on one particular component of ES, of M_r 14,000, only H-2^s strains responded in IgG. This MHC restriction of the repertoire was confined to infection, and broke down under adjuvant-assisted immunization with the purified protein. The M_r 14,000 molecule was also found to be a potent allergen in a passive cutaneous anaphylaxis assay, and the IgE response to it was also restricted to H-2^s. This haplotype was, however, a low IgE responder on the SJL background. There is, therefore, MHC control of the specificity of the immune response to this molecule, but non-MHC control of the amplitude of the IgE antibody response to it. Hybrids between responder and nonresponder strains (BALB/c × SJL) F_1 , responded to the M_r 14,000, but their responses to other ES components could not be predicted from the response patterns of parental strains. For example, the BALB/c parent responded to a 118-kDa component, but the SJL parent and the F_1 progeny did not. Moreover, the response to a 41-kDa Ag was substantially down-regulated in the F_1 , whereas both parental strains responded vigorously. This new model system, therefore, has implications for MHC control of responses to the allergens of pathogens, and for the complex immunoregulation in heterozygotes in the context of infection.

Ascaris lumbricoides is a cosmopolitan intestinal nematode parasite of man, infecting more than a billion people worldwide (1). The adult worms are among the largest of human parasites, are known to cause nutritional disturbances, and can cause death through intestinal perforation, rupture, or blockage. Although both the prevalence and intensity of infection with *Ascaris* tend to peak in the first two decades of life (2), there is little evidence of sterile immunity in humans, and individuals living in endemic areas exhibit considerable variability in their parasite burdens (3–5). This overdispersion of the parasite population could have a variety and combination of causes, including genetic, behavioral, and sociologic differences in the hosts. Insufficient information is available to implicate any particular genetic basis, but it would seem pertinent to consider the role of the immune system, and genetic control thereof, in any host susceptibility to infection.

It could be argued that the search for relevant genetic determinants that affect the immunobiology of ascariasis would be strategically best aimed at host-parasite interface components. These comprise the materials released by the parasites, or presented on their surface. Here we have focused on in vitro-released ES³ preparations. ES is the target of substantial antibody responses in infection with several nematode species, and has received considerable attention recently as sources of protective and serodiagnostic antigens (6). We have used the products of the pig parasite *Ascaris suum*, which is more readily available than the human form, but the two species are sufficiently homologous antigenically to justify *A. suum* as a model for the human parasite (7).

Ascaris presents an unrivalled opportunity to study immune responses to the allergens of transmissible disease agents. It is renowned for the potency of its allergens (6), and hypersensitivity responses are an important aspect of the morbidity and mortality associated with the disease. This is particularly true of reactions to the tissue-invasive larval stages of the parasite, pulmonary hypersensitivity to which is life threatening (6, 8). This fact provides further justification for focusing on ES because this Ag compartment has been reported to contain potent allergens as well as IgE-potentiating factors (9–11).

We have previously described a model system in mice in which genetic control of the antibody repertoire to ES

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³ Abbreviations used in this paper: ES, excretory/secretory Ag; ABF, adult *Ascaris* worm body fluid; PCA, passive cutaneous anaphylaxis.

Ag of the parasite could be investigated (12). Inbred mice exhibit antibody responses that recognize only a subset of the potentially antigenic components of ES, and the repertoire varies considerably between strains. Here we show that this selectivity is H-2 restricted. Moreover, the data show that there is a complex regulation of responsiveness to individual Ag in F₁ animals, which cannot be predicted from the recognition profiles of parental strains. Finally, of relevance to immunopathology of infections in general, this H-2 control of Ag recognition extends to a major allergen of the parasite, presenting a model for the MHC control of responses to allergens in general.

MATERIALS AND METHODS

Inbred and congenic mice. These were purchased from OLAC (1976) Ltd., Bicester, UK, and were used at 8 wk of age. F₁ hybrids were bred within the Wellcome Laboratories for Experimental Parasitology from stock obtained from OLAC. NIH, CBA/Ca, and BALB/c mice were also bred on site or obtained from the National Institute for Medical Research, London, UK. The strains of mice used are given in Table I along with their H-2 haplotypes.

Parasites and their in vitro-released materials. *A. suum* lung-stage larval forms were recovered from rabbits infected 7 days previously, and cultured in vitro, following previous methods (7, 13). Nematodes moult in a programmed manner during their life cycles, and the lung stages of *Ascaris* are in transition between the third and fourth larval forms, and hence abbreviated L3/4. Parasites were cultured in the following protein-free medium: RPMI 1640, Dutch modification (GIBCO, Paisley, UK; product no. 041-02409), containing 1 mM sodium pyruvate, 400 ng/ml glycyl-L-histidyl-L-lysine (Sigma G 1887, Sigma Chemical Co., St. Louis, MO), 16 μ M glutathione (reduced, Sigma 4251), 1 mg/ml glucose, 100 IU/ml penicillin, and 100 μ g/ml streptomycin. The cultures were maintained at 37°C in 5% CO₂/air. Medium was harvested weekly, filtered through a 0.22- μ m, low protein-retention filter (Millex GV SLGV025BS; Millipore S.A., Molsheim, France), concentrated about 20 times using centrifugal ultrafiltration with a nominal m.w. cut-off of 10,000 (Centricon 10; Amicon, Danvers, MA), dialyzed against phosphate buffered saline pH 7.2 and stored at -70°C until used. These cultures can be maintained for about 14 days, and the percentage of dead or moribund organisms kept low by positive selection of living larvae by migration through a cotton wool plug when numbers of immotile larvae approached 5%.

Antisera. Antiserum to *A. suum* was raised in rabbits (Sandy Lop; Hyline Rabbits Ltd., Northwich, Cheshire, UK) infected orally every 28 days with 3000 embryonated eggs, and serum collected 14 days after the last of four infections. Mice were orally infected with 2000 viable eggs of *A. suum* at 28-day intervals, bled by cardiac puncture on day 28 of a primary infection and day 14 of subsequent infections. Serum was aliquoted and stored at -20°C until used, and freeze/thawing was kept to a minimum. Antiserum to the 14-kDa molecule was raised in rabbits from material purified as follows. An emulsion of 0.3 ml of CFA containing 100 μ g of protein was injected into one thigh muscle of rabbits, followed 7 days later by a repeat dose in the opposite thigh. The rabbit was boosted with 100 μ g

protein of IFA in multiple subcutaneous sites on the back, and bled 6, 8, and 10 days later. Serum prepared from this stage of immunization was used for affinity purification, since later boosts resulted in antibody of too high affinity.

14 kDa Ag. This molecule is secreted by the parasite (7, 13), and it is abundant in the peritenteric fluid of adult worms (13, 14), from which source the purified protein was prepared. Two methods were used. First, ABF was separated on 1.5-mm thick 5 to 25% gradient SDS-PAGE gels, with the samples unboiled and laced with radiolabeled ABF material. The gels were vacuum dried at 60°C, and the 14-kDa band excised using an autoradiograph of the gels as template. Protein was recovered from the gel matrix by electroelution in a Bio-Rad (Richmond, CA) electroelutor (product no. 165-2976), SDS removed from the protein sample by dialysis against 10% methanol at 4°C over 48 h using 2000 m.w. cut-off membrane (Sigma D 2272), followed by extensive dialysis against PBS, and concentrated by ultrafiltration. This preparation was used to immunize rabbits to provide antibody for immunoaffinity chromatography. Protein A-purified rabbit anti-14-kDa antibody was bound to Affigel 10 (Bio-Rad, product no. 153-6046), and used to prepare an affinity column. ABF was applied to the column, followed by washing with 0.1 M 3-[N-Morpholino]propane-sulfonic acid (Sigma M-9381) buffer, pH 7.5, and the 14-kDa protein was eluted with 0.2 M, pH 2.3, glycine/HCl buffer.

Radiolodination and immunoprecipitation of secreted Ag. Culture medium was dialyzed against PBS and labeled with ¹²⁵I using IODO-GEN as previously described (13). The isotope was obtained from Amersham International, and the iodination reagent from Pierce Chemical Co., Rockford, IL. Immunoprecipitation was performed using a *Staphylococcus aureus*-based assay (13) in which iodinated culture medium, or purified 14-kDa molecule, 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 required, the assay was adapted to detect antibody from a wider spectrum of Ig isotypes by including antibody to mouse γ -globulins in the reaction mixture (sheep anti-mouse γ -globulins; Scottish Antibody Production Unit, Carlisle, Lanarkshire; product no. S021-220). The radioactivity in the washed pellets was counted in a γ -counter and prepared for SDS-PAGE. The level of precipitation is expressed as the amount of ¹²⁵I cpm precipitated by antibody as a percentage of the counts precipitable by TCA.

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 to 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 (13), with 5% β -2-ME or 1 mg/ml iodoacetamide for reducing and nonreducing conditions, respectively. After electrophoresis, gels were fixed in 25% methanol, 10% acetic acid, 1% glycerol, then dried. Autoradiographs were exposed at -70°C with flashed Fuji RX film. Apparent molecular masses were estimated by reference to standard marker proteins (Pharmacia 17-0446-01), and are given in kDa.

PCA. Inbred Wistar rats were shaved on the back, and 100- μ l samples of test serum, or dilutions thereof, injected intradermally. Then 24 h later, the rats were given an i.v. injection of 50 μ g Ag with 5 mg Evans Blue (BDH, Poole, Dorset, UK; product no. 24115 2L) in PBS, and the diameters of the reaction spots were measured 30 min later. Normal serum controls were used on every occasion in each rat, and every assay point was carried out in duplicate on separate animals. Mouse-rat heterologous PCA carried out as used here is known to be specific for IgE antibody (15).

RESULTS

***Ascaris* ES materials and their antigenicity.** The parasites used for the generation of ES Ag were derived from rabbits, and it was first necessary to establish which components of ES are parasite derived, and which from the host. Figure 1 presents an immunoprecipitation experiment using serum from infected rabbits. This established that all the discernible labeled components were antigenic, although we had previously found that ES is contaminated with rabbit serum albumin, which coruns on SDS-PAGE with a parasite Ag of 67kDa (13). Treatment with 2-ME showed that there are slight shifts in the mobility of certain components with reduction, e.g., the M_r 41-kDa molecule, and the components of M_r 225 and 410 kDa are disulfide-linked dimers or heterodimers.

TABLE I
H-2 haplotypes of strains of mice used

Strain	H-2 Haplotype
C57B10 (B10)	b
BALB/b	b
DBA ₂	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

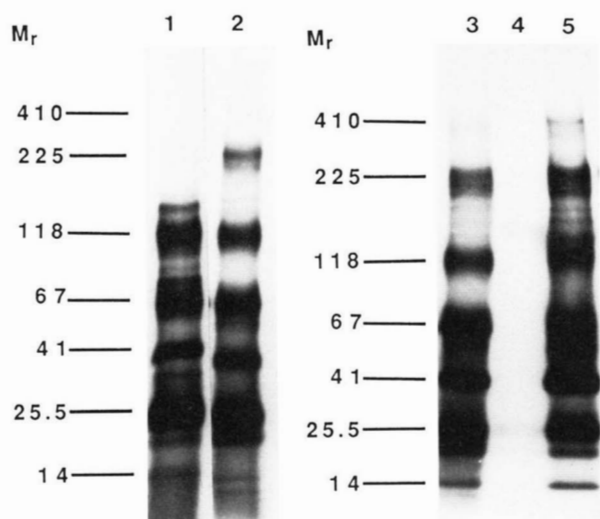


Figure 1. The secreted Ag of lung-stage larvae of *A. suum*. ^{125}I -labeled ES³ was analyzed on 5 to 25% gradient SDS-PAGE gels under reducing (lane 1) and nonreducing conditions (2), respectively. In a separate experiment, iodinated ES (3) was immunoprecipitated in a protein A-based assay with normal rabbit serum (4), and serum from rabbits infected three times with *A. suum* (5). Relative mobilities of the major ES components (M_r) are given in kDa as calculated from a calibration curve constructed using standard proteins as detailed in *Materials and Methods*.

This means that if only one subunit of a heterodimer were to be bound by antibody, then analysis of immunoprecipitates under reducing conditions would lead to the erroneous identification of two gene products as antigenic. In all the following experiments, therefore, immunoprecipitates were analyzed under nonreducing conditions.

There is considerable heterogeneity in the specificity of antibody responses of humans infected with *Ascaris* (16), which is particularly noticeable for the 14-kDa Ag. This variability could be due to intensity or frequency of infection, exposure to cross-reactive organisms, and/or the genetic constitution of the people themselves. Cross-reactions are known to occur (14), but we will concentrate here on the possibility of a genetic basis for the effect, using mice as the genetically defined host.

H-2 restricted recognition of *Ascaris* ES Ag in mice. An initial characterization of inbred mouse strains with independent H-2 haplotypes was reported previously (12). This showed that no strain recognized all the components of the ES products which were potentially antigenic, even in the context of repeated infections, and only H-2^s strains recognized the 14-kDa molecule. However, our findings indicate that nonresponder strains to the 14-kDa Ag, e.g., BALB/c, respond if presented with the Ag in adjuvant (Fig. 2). The repertoire restriction might, therefore, only operate in the context of infection.

H-2 restriction was tested using mice on the BALB/c and B10 backgrounds along with some of the original inbred strains. When H-2^k animals were compared on three different backgrounds, the recognition profiles were indistinguishable (Fig. 3). The same applied to H-2^d congenics (Fig. 4), although the primary response patterns differed. This inferred that background genes could affect the kinetics of development of the responses, but not the mature recognition profiles. The sequential development of Ag recognition was the slowest in DBA/2 mice, but additional infections always resulted in identical responses between all the H-2^d strains tested.

In the converse experiment, the H-2 haplotype was varied on the same (B10 or BALB) backgrounds. In this case, each strain responded according to its MHC type, examples of which are given in Figures 5 and 6.

Although we do not show it here, the recognition profiles did not alter with increasing infection dose, and we have found no significant variation between individuals within a strain. Also, there were no differences between the specificities of responses induced in mice infected orally with infective eggs or i.p. with liberated infective larvae. Finally, the number of Ag recognized did not increase with further infections (up to six infections), and adapting the protein A immunoprecipitation assay to take into account other Ig isotypes did not alter the recognition profiles in any of the above, or subsequent, analyses.

Unexpected response profiles of F_1 hybrids. Infected humans, in contrast to rabbits, do not respond to all the Ag released by the parasite (16). If this were due to MHC restriction, then it is unlikely to be in the context of homozygosity along the MHC. To model the heterozygous condition we used F_1 hybrid mice, and selected a cross between a responder and a nonresponder for the 14-kDa Ag (BALB/c $\times$ SLJ) F_1 . The response patterns of the parental and hybrid animals are given in Figure 7, showing that the F_1 response was not merely a combination of the parental recognition patterns. The 14-kDa Ag, which was recognized by the H-2^s, but not the H-2^d, parent was precipitated by serum from infected F_1 . In contrast, the F_1 failed to respond to the 118-kDa Ag, unlike its H-2^s parent. The most unexpected finding, however, was the lack of recognition of the 41-kDa molecule by the F_1 , in contrast to both parental strains. In some experiments, there was a faint band in the 41 kDa region of the F_1 lanes. If this does indicate recognition by the F_1 , then there was considerable down-regulation, and in some replicate experiments, no precipitation of molecules in the 41-kDa region was detectable. A consensus from three different experiments is given in Figure 8. The nonrecognition by the F_1 of some of the ES components recognized by the parents could be due to a cross-tolerance with self. This can be tested in hybrids bearing the same H-2 alleles as the above hybrid, but on a genetic background which is known not to impede responsiveness. The B10 background fits this criterion (Figs. 3, 4, and 5), and a consensus for the response profile of (B10.D2 $\times$ B10.S) F_1 hybrid is also given in Figure 8. This hybrid mounted a response to the 41, 118, and the 225 kDa, unlike the (BALB/c $\times$ SJL) F_1 which bears identical H-2 alleles.

A number of other hybrid combinations have been examined, and in all cases only those bearing the H-2^s allele were found to respond to the 14 kDa by immunoprecipitation and SDS-PAGE analysis. The levels of the

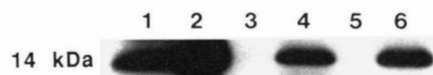


Figure 2. Difference between the antibody response to the 14-kDa Ag in infection and adjuvant-assisted immunization. Affinity purified 14-kDa protein was radiolabeled (lane 2) and immunoprecipitated with serum from normal mice (3), BALB/c mice immunized with 14 kDa in CFA (4), and multiply *Ascaris*-infected BALB/c (5) or SJL (6) mice. Lane 1 was loaded with a 14.4-kDa marker protein, α -lactalbumin. Immunoprecipitates were analyzed on 5 to 25% gradient SDS-PAGE gels, and only the 14-kDa region is shown here.

Figure 3. Recognition profiles of H-2^k strains of mice in response to *Ascaris* infection. Radiiodinated ES (reference Ag, lane R) was immunoprecipitated with normal serum from each strain (lanes a), and serum taken from mice 28 days after infection (lanes b), and 14 days after the last of three infections (lanes c). Track M was loaded with iodinated marker proteins of M_r 14.4, 20.1, 30, 43, and 67 kDa.

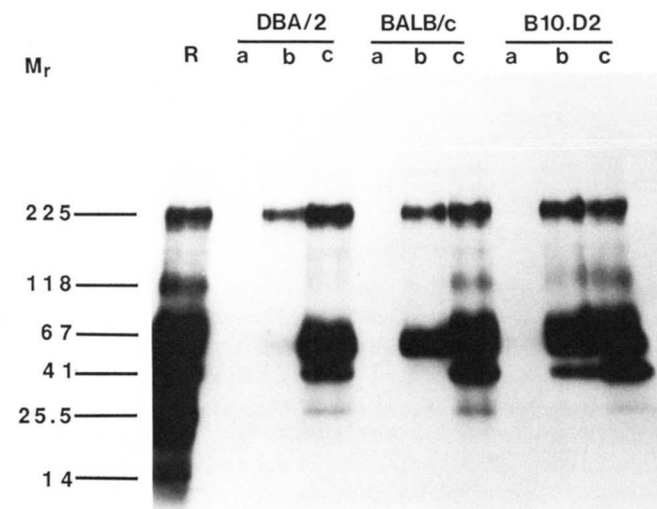
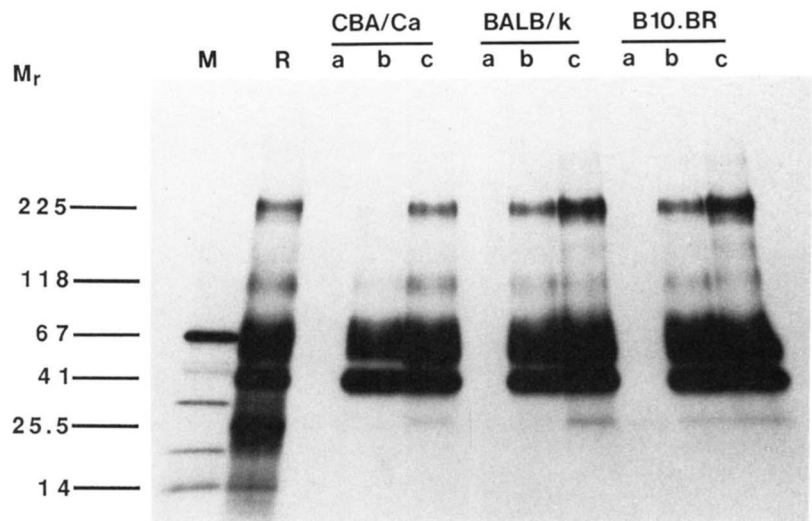


Figure 4. Recognition profiles of H-2^d strains of mice in response to *Ascaris* infection. Radiiodinated ES (lane R) was immunoprecipitated with normal serum from each strain (lanes a), and serum taken from mice 28 days after primary infection (lanes b), and 14 days after the last of three infections (lanes c).

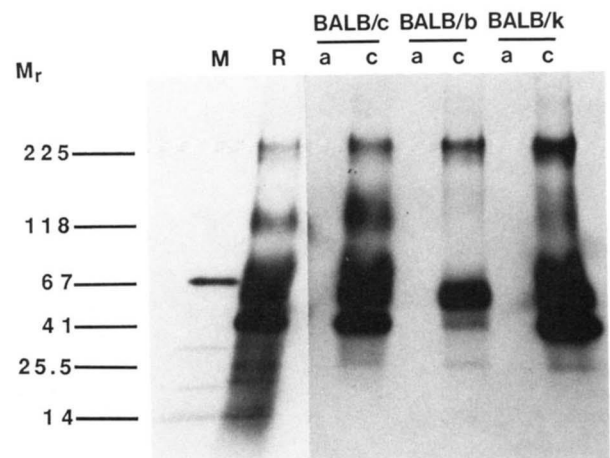


Figure 6. Recognition profiles of mice of differing H-2 haplotype, but identical genetic background (BALB). Radiiodinated ES (lane R) was immunoprecipitated with normal serum from each strain (lanes a) and serum from mice infected three times with *A. suum* (lanes c). M, marker proteins.



Figure 5. Recognition profiles of mice of differing H-2 haplotype, but identical genetic background (B10). Radiiodinated ES (lane R) was immunoprecipitated with normal serum from each strain (lanes a) and serum from mice infected three times with *A. suum* (lanes c). M, marker proteins.

responses do, however, vary, as shown in Figure 9. As before, only H-2^s-bearing inbred strains or hybrids mounted a response to the 14 kDa, with the SJL being the highest responder, and B10 congenics and hybrids being low responders. This behavior of the B10 strains contrasts with what we have found to be their high responder status in their IgE response to *Ascaris* whole somatic Ag (data not shown). It is also worthy of note that the (BALB/c $\times$ CBA)_{F1} hybrid exhibits no response to the 14 kDa, even though BALB/c and CBA hybrids with SJL mice are the highest responders among the hybrids tested. This serves to further emphasize the absence of response in non-H-2^s animals, regardless of genetic background.

MHC-restricted responses to major allergen. The ES of the lung-stage of *Ascaris* has been shown to contain potent allergens (9, 11), as have other *Ascaris* Ag preparations (17, 18). In the light of H-2-restricted responses to this Ag set, it was important to establish whether the restriction extended to reaginic antibody. This was tested in a heterologous PCA using mouse serum assayed in the rat, and affinity purified 14-kDa protein as eliciting allergen. Table II shows the results of an experiment using tertiary infection sera from a panel of H-2 congeneric

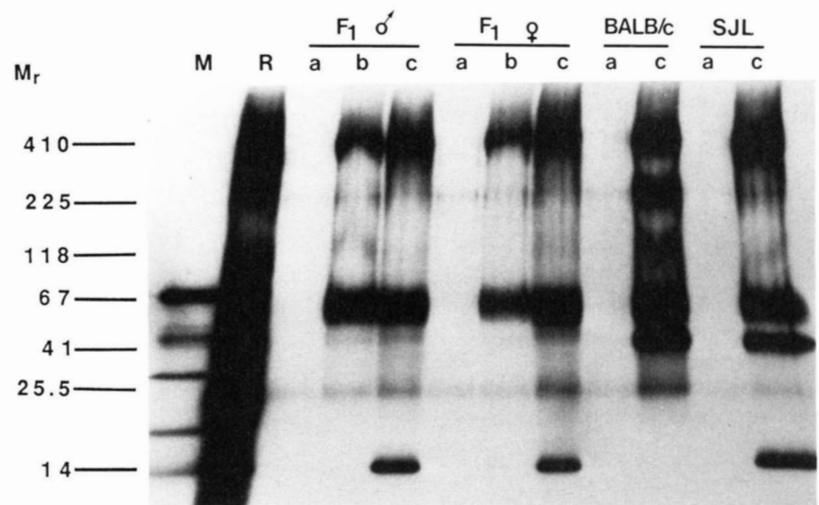


Figure 7. Unexpected recognition profiles of F_1 hybrids. Radioiodinated ES (lane R) was immunoprecipitated with serum from normal mice of each inbred strain and hybrids as indicated (lanes a), serum from mice infected once (lanes b), and three times with *A. suum* (lanes c). The sera were pooled from infected BALB/c (male) or SJL (female) parents, and the F_1 lanes show typical results from individual F_1 animals. M, marker proteins.

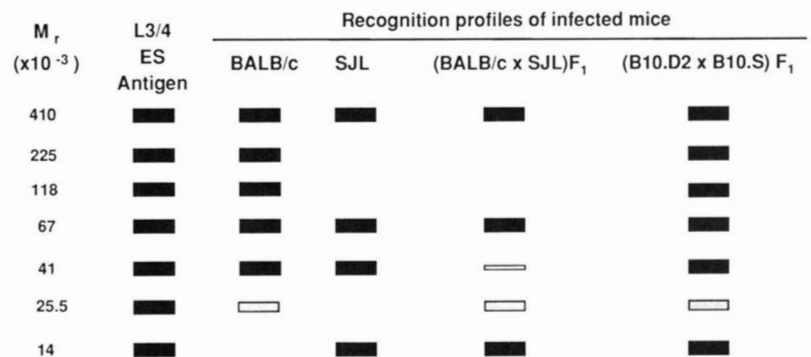


Figure 8. Consensus diagram to show the responses of F_1 hybrids to ES Ag, constructed from three separate experiments. The density of tone and width of the bands represent strength of recognition of each component.

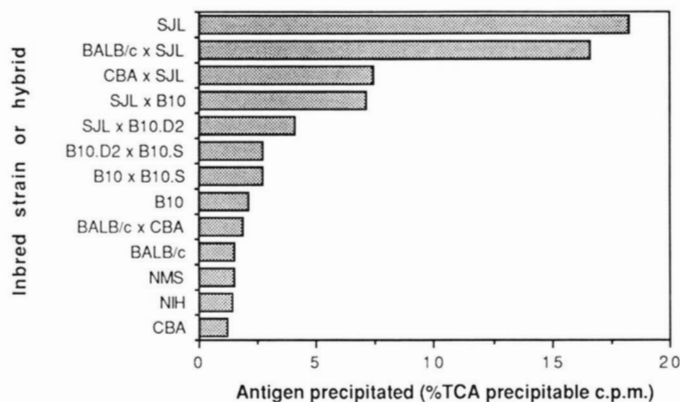


Figure 9. Genetic control of the level of response to the 14-kDa Ag of *Ascaris*. The hybrid and parental strains indicated were infected four times with the parasite, serum sampled at 14 days after the final infection and assayed for IgG antibody to the 14-kDa Ag in radioimmunoprecipitation assay. The response is expressed as the amount of input ^{125}I cpm precipitated as a percent of the TCA-precipitable cpm. NMS, normal mouse serum.

strains. The data showed that only H-2^s mice produced IgE antibody to the 14 kDa. To eliminate the possibility that the IgE response to *Ascaris* Ag was limiting in non-H-2^s strains, the experiment was also performed using whole ABF, from which the 14 kDa was purified. Because the 14 kDa is only one of many proteins present in ABF (13, 14), the amount of ABF given in challenge was tenfold the amount given as purified 14 kDa. The data show that all the congenics produced IgE antibody to *Ascaris* Ag during infection, and underscores the H-2 restriction of the anti-14 kDa IgE response.

When SJL (H-2^s) mouse serum was used in the PCA

Strain of Mouse	H-2 Haplotype	PCA Titer	
		Purified 14 kDa	ABF
B10	b	0	256
B10.D2	d	0	256
B10.BR	k	0	≥512
B10.G	q	0	256
B10.S	s	128	512
SJL	s	0	16

^a PCA³ was carried out using inbred WLEP rats as detailed in *Materials and Methods*. Rats were each given 50 μg protein of 14-kDa and 500- μg protein of ABF as challenge antigens. The mouse sera used were pooled from at least six animals per group, 14 days after the last of three oral infections with *A. suum*.

assay, it produced no reaction to the 14 kDa, and remarkably little to ABF. In the case of the former, no reaction was detectable at any dose of Ag up to 250 μg of purified 14-kDa per rat. SJL mice are known to be modest producers of IgE antibody, and the related SJA/9 strain is IgE defective (19–22).

DISCUSSION

Ascaris is one of the most abundant and widespread multicellular parasites of man, and infected individuals are highly heterogeneous in their infection loads, hypersensitivity responses, and immune recognition of parasite Ag (16). An understanding of the genetic determinants of these phenomena is important for ascariasis in particular, but it also has implications for the immunobiology of nematode infections in general, in which hypersensitivity responses are a prominent feature.

This new model system for investigating genetic control

of immune responses to ascariasis has revealed MHC-restricted responses to individual Ag species presented by this antigenically complex parasite. Moreover, the restriction is expressed against the materials secreted by the parasite, and is, therefore, likely to have direct relevance to the immunobiology of the infection. Although not previously described for nematodes, a similar effect has been demonstrated for a member of another group of multicellular parasites, the schistosomes (23). For these, the MHC restriction was detected using *in vitro* translated whole worm mRNA as target Ag. These products would not have been fully processed in terms of posttranslational modifications, e.g., glycosylation, and their relevance to the fully processed Ag presented by the parasite is not known. Also, total parasite mRNA translates would have encoded all the proteins being synthesized by the parasite at a given time, and it would be difficult to establish which of these are elements of the host-parasite interface.

One of the remarkable features of the *Ascaris* system is that restricted recognition of whole molecules is demonstrable at all. This was surprising in the light of the occurrence of common epitopes on distinct ES molecules of the closely related, and antigenically cross-reactive, nematode, *Toxocara canis*. In that case, mAb analysis has shown that certain epitopes are common to all the major components of ES, and that these determinants are probably carbohydrate in nature (24, 25). For *Ascaris*, then, such common epitopes might not exist, or else the polyclonal antibody response does not have these specificities as a significant feature.

It would be premature to assign a mechanism for the MHC restriction observed here, but there is evidence for different processes involved in recognition or nonrecognition of the different constituents of ES. Starting with the 14 kDa, the principal possibilities would be as follows. First, the 14 kDa, or peptides thereof, fail to associate appropriately with class II MHC molecules, except those of the H-2^s haplotype. Second, Ag + MHC mimics self in all but H-2^s, and the immune system is tolerant to that specificity. This would not explain the responder status of (BALB/c × SJL)_{F1}. Third, the TCR repertoire might not encompass the 14 kDa specificity. This possibility is not consistent with the fact that only H-2^s congenics respond to the protein, because the TCR genes ought to be identical between congenics on the B10 or the BALB backgrounds. Fourth, immunoregulatory pathways might be involved. In this respect, I-E has been implicated in the induction of suppressor circuits effective against responses permissible in the context of I-A (26, 27). This could explain the responsiveness of the IE⁻ H-2^s haplotype to the 14-kDa Ag, but would not be consistent with the non-responsiveness of H-2^b and H-2^q strains, which are also IE⁻ (28, 29). The responder phenotype of H-2^d × H-2^s hybrid mice also argues against a role for I-E-linked suppression, because they would express I-E^d (as well as E_B^s). If I-E-linked suppression does occur for some of the H-2 haplotypes used here, it cannot be generalized. Finally, because only the H-2^s congenics respond on a B10 background, cross-tolerance (30, 31) to non-MHC molecules is also unlikely to explain the effect. The strongest possibility, therefore, would seem to be that restriction of the anti-14-kDa response acts through association of processed Ag only with I-A^s. It would, how-

ever, remain to explain how adjuvant-assisted immunization overrides the block in an otherwise nonresponder strain. This could be due to differences in Ag processing and presentation between the two routes of exposure; it is thought, for example, that antigen presentation can vary from tissue to tissue (32).

The regulation of the response to the 118- and 225-kDa molecules appears to be quite different from that to the 14 kDa; the BALB/c responded to these ES components, but both the SJL parent and the (BALB/c × SJL)_{F1} did not. This pattern of inheritance has been frequently noted in immunogenetics, and has been rationalized within the cross-tolerance hypothesis (30, 31), whereby Ag plus MHC mimics self in a hybrid's genetic background. It would be predicted, therefore, that an F₁ bearing the same H-2 alleles as the above hybrid might respond to the 118 and 225 kDa if the genetic background were different. We already knew that certain B10 mice responded to these molecules (Figs. 3, 4, and 5), and the idea was, therefore, testable in a (B10.D2 × B10.S)_{F1} cross (Fig. 8). The H-2 of the hybrid might have still affected tolerance specificities, but it nevertheless mounted a response to the molecules in question. It is likely, therefore, that the failure of responsiveness to these antigens in the (BALB/c × SJL)_{F1} is due to cross-tolerance with self components in the SJL background.

The dominant and recessive inheritance of responses to the 14- and 118-kDa Ag, respectively, might be encompassed by current immunologic theory, but the response pattern to the 41-kDa molecule is more demanding of explanation. This component was the subject of a response in both the SJL and BALB/c parents, but the response to it was substantially down-regulated in their F₁. Two possibilities arise. First, each parent might respond, or be tolerant, to separate epitopes on the molecule. In the F₁, therefore, lack of responsiveness to the whole molecule could be due to tolerance to both epitopes. This would essentially be a two-way extension of cross tolerance, and such a situation has been reported (33). One could predict, therefore, that H-2^{bx} hybrids might respond strongly on the B10 background, which is permissive in this respect (Figs. 3, 4, and 5). The (B10.D2 × B10.S)_{F1} hybrid was useful in this respect, and responded to the 41 kDa vigorously, again implicating cross-tolerance with background gene products.

The simplest explanation would, however, be that there are two different species of components of 41 kDa secreted by the parasite, and that tolerance/nonresponsiveness to each occurs independently in the F₁. This point demands investigation, and we are currently pursuing this by biochemical means. Our investigations so far, however, would argue against this possibility.

The above analyses were based on an IgG-specific immunoassay, and, given the renowned allergenic activity of *Ascaris* products, it was important to establish whether the H-2 restriction extended to reaginic antibody production. For this we focused on the 14-kDa Ag, and assayed the IgE antibody response to the purified protein by PCA. This showed that the specific IgE response was H-2-restricted, and that the recognition of the molecule by mice of different haplotypes was identical to that when screened for IgG antibody by protein A-mediated immunoprecipitation. This has implications for the regulation of IgE responses in general, because there is some evi-

dence that human hypersensitivity to certain environmental allergens is MHC associated (34–36), and HLA DR loci have been shown to be restriction elements for T cell clones in proliferative responses to purified allergens (37).

Although the MHC is clearly in control of the antibody repertoire to the 14 kDa, there are also non-H-2 genes in control of the amplitude of the IgE response to this Ag; H-2^s mice on both the SJL and the B10 backgrounds responded vigorously to the purified protein in IgG, but only the latter produced an IgE response to it. This would be consistent with the known paucity of IgE responsiveness of SJL mice, and the related SJA/9 strain is IgE defective (19–22). We are, therefore, presented with an experimental system with which to investigate two different aspects of the IgE response to the Ag of a pathogen.

The *Ascaris*/mouse system also presents a novel system for the study of the genetic control of responses to allergens in general. The system has an advantage over many other experimental allergen systems in that it does not require resorting to the use of adjuvant to elicit allergen-specific IgE antibody responses. This point is worthy of emphasis because, as we show here, adjuvant can elicit responses which are unlike those elicited by exposure through a natural route. The MHC control of the antibody repertoire to *Ascaris* allergens might, therefore, provide a valuable model in which responses can be studied in the context of exposure to allergens in a biologically relevant way.

Is MHC restriction of Ag/allergen recognition relevant to immunity in nematode infections? Insufficient data are available to answer this, but there are already clear indications of MHC involvement. The strongest experimental evidence comes from work on *Trichinella spiralis* infection in the mouse, in which H-2 haplotype has been shown to influence the duration and level of the infection at both the intestinal and muscle-penetrative stages (38, 39). Moreover, expression of the I-E^k molecule is associated with susceptibility to infection (40), which brings to mind the I-E^k-restricted suppressor cells which can inhibit I-A-restricted responses (26, 27). Returning to ascariasis, recent epidemiologic research on humans living in an endemic area for the disease has revealed an association between certain HLA alleles and infection or non-infection with the parasite (41). Finally, there is a consensus that IgE antibody responses are involved in effective immune control of nematode infections (42). Our findings on MHC restriction of responses to parasite allergens might, therefore, provide a framework to relate MHC control of the specificity of the immune response directly to one of the effector mechanisms operative against multicellular parasites.

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REFERENCES

1. World Health Organisation. 1981. Intestinal protozoan and helminthic infections. *WHO Tech. Rep. Ser.* 666.
2. Anderson, R. M. 1986. The population dynamics and epidemiology of intestinal nematode infections. *Trans. R. Soc. Trop. Med. Hyg.* 80:686.
3. Hlilang, T. 1985. *Ascaris lumbricoides* infections in Burma. In *Ascariasis and Its Public Health Significance*. D. W. T. Crompton, M. C. Nesheim, and Z. Pawlowski, eds. Taylor and Francis, London, p. 83.
4. Elkins, D., M. Haswell-Elkins, and R. M. Anderson. 1986. The epidemiology and control of intestinal helminths in the Pulicat Lake region of southern India. I. Study design and pre- and post-treatment observations on *Ascaris lumbricoides* infection. *Trans. R. Soc. Trop. Med. Hyg.* 80:774.
5. Haswell-Elkins, M. R., D. B. Elkins, and R. M. Anderson. 1987. Evidence for predisposition in humans to infection with *Ascaris*, hookworm, *Enterobius*, and *Trichuris* in a South Indian fishing community. *Parasitology* 95:323.
6. Ogilvie, B. M., and D. de Savigny. 1982. Immune response to nematodes. In *Immunology of Parasitic Infections*. S. Cohen, and K. S. Warren, eds. Blackwell Scientific Publications, Oxford, UK, p. 715.
7. Kennedy, M. W., F. Gureshi, M. Haswell-Elkins, and D. B. Elkins. 1987. Homology and heterology between the secreted antigens of the parasitic larval stages of *Ascaris lumbricoides* and *Ascaris suum*. *Clin. Exp. Immunol.* 67:20.
8. Coles, G. C. 1985. Allergy and immunopathology of ascariasis. In *Ascariasis and Its Public Health Significance*. D. W. T. Crompton, M. C. Nesheim, and Z. S. Pawlowski, eds. Taylor and Francis, London, p. 167.
9. Stromberg, B. E. 1979. IgE and IgG1 antibody production by a soluble product of *Ascaris suum* in the guinea pig. *Immunology* 38:489.
10. Stromberg, B. E. 1980. Potentiation of the reaginic (IgE) antibody response to ovalbumin in the guinea pig with a soluble metabolic product of *Ascaris suum*. *J. Immunol.* 125:833.
11. Urban, J. F., H. Alizadeh, and R. D. Romanovski. 1988. *Ascaris suum*: development of intestinal immunity to infective second-stage larvae in swine. *Exp. Parasitol.* 66:66.
12. Kennedy, M. W., A. M. S. Gordon, L. A. Tomlinson, and F. Gureshi. 1986. Genetic (major histocompatibility complex?) control of the antibody repertoire to the secreted antigens of *Ascaris*. *Parasite Immunol.* 9:269.
13. Kennedy, M. W., and F. Gureshi. 1986. Stage-specific secreted antigens of the parasitic larval stages of the nematode *Ascaris*. *Immunology* 58:515.
14. Kennedy, M. W., F. Gureshi, E. M. Fraser, M. R. Haswell-Elkins, D. B. Elkins, and H. V. Smith. 1989. Antigenic relationships between the surface-exposed, secreted and somatic materials of the nematode parasites *Ascaris lumbricoides*, *Ascaris suum*, and *Toxocara canis*. *Clin. Exp. Immunol.* 75:493.
15. Ovary, Z. 1986. Passive cutaneous anaphylaxis. In *Handbook of Experimental Immunology*, 4th Ed. Vol. 1. D. M. Weir, L. A. Herzenberg, C. Blackwell, and L. A. Herzenberg, eds. Blackwell Scientific Publications, Oxford, UK, p. 33.1.
16. Kennedy, M. W. 1989. Genetic control of the immune repertoire in nematode infections. *Parasitology Today*. In press.
17. O'Donnell, I. J., and G. F. Mitchell. 1978. An investigation of the allergens of *Ascaris lumbricoides* using a radioallergosorbent test (RAST) and sera of naturally infected humans: comparison with an allergen for mice identified by a passive cutaneous anaphylaxis test. *Aust. J. Biol. Sci.* 31:459.
18. Tsuji, M., T. Hayashi, S. Yamamoto, Y. Sakata, and T. Toshida. 1977. IgE-type antibodies to *Ascaris suum* in man. *Int. Arch. Allergy Appl. Immunol.* 55:78.
19. Azuma, M., T. Hirano, H. Miyajima, N. Watanabe, H. Yagita, S. Enomoto, S. Furusawa, Z. Ovary, T. Kinashi, T. Honjo, and K. Okumura. 1987. Regulation of murine IgE production in SJA/9 and nude mice: potentiation of IgE production by recombinant interleukin 4. *J. Immunol.* 139:2538.
20. Watanabe, N., S. Kojima, and Z. Ovary. 1976. Suppression of IgE antibody production in SJL mice. I. Nonspecific suppressor T cells. *J. Exp. Med.* 143:833.
21. Kumagai, Y., T. Hirano, N. Watanabe, K. Okumura, and Z. Ovary. 1983. Studies on IgE production in mice. I. Spontaneous suppression of IgE production in SJA/9 mice. *Immunogenetics* 18:147.
22. Katona, I. M., J. F. Urban, and F. D. Finkelman. 1985. B cells that simultaneously express surface IgM and IgE in *Nippostrongylus brasiliensis*-infected SJA/9 mice do not provide evidence for isotype switching without gene deletion. *Proc. Natl. Acad. Sci. USA* 139:2538.
23. Kee, K. C., D. W. Taylor, J. S. Cordingley, A. E. Butterworth, and A. J. Munro. 1986. Genetic influence on the antibody response to antigens of *Schistosoma mansoni* in chronically infected mice. *Parasite Immunol.* 8:565.
24. Kennedy, M. W., R. M. Maizels, M. Meghji, L. Young, F. Gureshi, and H. V. Smith. 1987. Species-specific and common epitopes on the secreted and surface antigens of *Toxocara canis* and *Toxocara cati* infective larvae. *Parasite Immunol.* 9:407.
25. Maizels, R. M., M. W. Kennedy, M. Meghji, B. D. Robertson, and H. V. Smith. 1987. Shared carbohydrate epitopes on distinct surface and secreted antigens of the parasitic nematode *Toxocara canis*. *J. Immunol.* 139:207.
26. Baxevanis, C. N., Z. A. Nagy, and J. Klein. 1981. A novel type of T-T cell interaction removes the requirement for I-B region in the H-2 complex. *Proc. Natl. Acad. Sci. USA* 78:3809.
27. Oliveira, D. B. G., N. Blackwell, A. E. Virchis, and R. A. Axelrod.

1985. T helper and T suppressor cells are restricted by the A and E molecules, respectively, in the F antigen system. *Immunogenetics* 22:169.
28. **Murphy, D. B., P. P. Jones, M. R. Loken, and H. O. McDevitt.** 1980. Interaction between I region loci influences the expression of a cell surface Ia antigen. *Proc. Natl. Acad. Sci. USA* 77:5404.
 29. **Jones, P. P., D. B. Murphy, and H. O. McDevitt.** 1981. Variable synthesis and expression of E_a and A_e (E_g) Ia polypeptide chains in mice of different H-2 haplotypes. *Immunogenetics* 12:321.
 30. **Schwartz, R. H.** 1978. A clonal deletion model for Ir gene control of immune response. *Scand. J. Immunol.* 7:3.
 31. **Matzinger, P.** 1981. A one-receptor view of T-cell behaviour. *Nature* 292:497.
 32. **Marrack, P., and J. Kappler.** 1988. The T-cell repertoire for antigen and MHC. *Immunol. Today* 9:308.
 33. **Gasser, D. L., and D. C. Shreffler.** 1974. Genetic control of the immune response in mice: V minor genes involved in the response to H-Y and Ea-1 antigens. *Immunogenetics* 1:133.
 34. **Marsh, D. G., S. H. Hsu, M. Roebber E. Ehrlich-Kautzky, L. R. Freidhoff, D. A. Meyers, M. K. Pollard, and W. B. Bias.** 1982. HLA-Dw2: a genetic marker for human immune response to short ragweed allergen Ra5. I. Response resulting primarily from natural antigenic exposure. *J. Exp. Med.* 155:1439.
 35. **Frick, O. L.** 1987. Predisposing factors in the development of allergy. In *Allergy: A Textbook*. M. H. Lessof, T. H. Lee, and D. M. Kemeny, eds. John Wiley, New York, p. 347.
 36. **Thomson, G.** 1986. Human HLA genetics and disease associations. In *Handbook of Experimental Immunology*, Vol. 3. D. M. Weir, ed. Blackwell Scientific Publications, Oxford, UK, p. 102.1.
 37. **O'Hehir, R. E., D. D. Eckels, A. J. Frew, A. B. Kay, and J. R. Lamb.** 1988. MHC class II restriction specificity of cloned human T cells reactive with *Dermatophagoides farinae* (house dust mite). *Immunology* 64:627.
 38. **Wakelin, D., and A. M. Donachie.** 1983. Genetic control of immunity to *Trichinella spiralis*: influence of H-2-linked genes on immunity to the intestinal phase of infection. *Immunology* 48:343.
 39. **Wassom, D. L., D. Wakelin, B. O. Brooks, C. J. Krco, and C. S. David.** 1984. Genetic control of immunity to *Trichinella spiralis* infections in mice. Hypothesis to explain the role of H-2 genes in primary and challenge infections. *Immunology* 51:625.
 40. **Wassom, D. L., C. J. Krco, and C. S. David.** 1987. I-E expression and susceptibility to parasite infection. *Immunol. Today* 8:39.
 41. **Bundy, D. A. P.** 1988. Population ecology of intestinal helminth infections in human communities. *Philos. Trans. R. Soc. London (Biol.)* 321:405.
 42. **Miller, H. R. P.** 1984. The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals. *Vet. Immunol. Immunopathol.* 6:167.