Immunology 1984 **51** 625

Genetic control of immunity to *Trichinella spiralis* infections of mice. Hypothesis to explain the role of H-2 genes in primary and challenge infections

D. L. WASSOM, D. WAKELIN*, B. O. BROOKS, C. J. KRCO† & C. S. DAVID† *Department of Preventive Medicine, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York, * Department of Zoology, University of Nottingham, University Park, Nottingham, and † Department of Immunology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota, U.S.A.*

Accepted for publication 16 September 1983

Summary. H-2 congenic strains of mice were compared for their ability to expel *T. spiralis* infections from the small intestine and for their ability to limit the reproduction of adult female worms. B10.M mice ($H\text{-}2^f$) expelled both primary and challenge infections more quickly than did the strains B10.Q($H\text{-}2^q$) and B10.BR($H\text{-}2^k$). During a primary infection, expulsion of worms from B10.M mice began before Day 9 post-infection and worm counts differed significantly ($P < \cdot 05$ Student's *t*-test) from counts in B10.BR mice on each of Days 12 and 15. B10.Q mice expelled worms more rapidly than B10.BR but worm counts did not differ significantly until Day 15. Whereas B10.M mice responded most quickly to expel worms from the gut, B10.Q mice were most effective in limiting worm reproduction. Female worms harvested from B10.Q mice and cultured for 24 hr *in vitro* produced significantly fewer newborn larvae than did worms from B10.M or B10.BR mice. Worms from B10.M mice were less fecund than worms from B10.BR, but this difference was not apparent before Day 9 post-infection, and worms from B10.M were always more fecund than worms from B10.Q. Challenge infections 21 days following a priming dose of 200 *T. spiralis* muscle larvae were rejected very quickly. B10.M mice expelled 65% of their worms during the first 24 h. By Day 6 after challenge, B10.M mice had expelled 84% of their worms; B10.Q and B10.BR expelled 75% and 37% respectively. These results suggest that a rapid expulsion response may be expressed in many different strains of mice depending on how the mice are immunized and the size of the infecting dose. Fecundity of female worms 6 days following a challenge infection was reduced for all strains tested when compared to primary infection controls; however, worms from B10.Q mice were less fecund than worms from B10.M or B10.BR. Results of these experiments demonstrate that H-2 genes play an important role in controlling the immune response which expels worms from the gut and the response which limits worm reproduction. These H-2-controlled differences are expressed during both primary and challenge infections. As the present results conflict somewhat with results published elsewhere, we have proposed a new hypothesis to explain the data collected in our laboratories thus far. According to this hypothesis, the anti-adult response, the anti-fecundity response, and the rapid expulsion response are under independent genetic control and influenced by the interacting products of both H-2 and non-H-2 genes.

Correspondence: Dr D. L. Wassom, Dept. of Preventive Medicine, NYS College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, U.S.A.

INTRODUCTION

Several years ago, studying *Trichinella spiralis* infections in H-2 congenic strains of mice, we demonstrated

for the first time that genes within the major histocompatibility complex (MHC) influence susceptibility to infection with a parasitic helminth (Wassom, David & Gleich, 1979). We have since mapped two H-2 genes which influence the immune response to *T. spiralis* infection. (Wassom *et al.*, 1983a.) These H-2 genes, together with genes mapping outside of the H-2 complex, control the relative degrees of susceptibility or resistance expressed by a given strain of mouse. Although it has been shown that non-H-2 genes influence the rate at which worms are expelled from the gut and the rate at which newborn larvae are produced by female worms (Wakelin, 1980; Wakelin & Donachie, 1980, 1981, 1983; Bell & McGregor 1980; Bell, McGregor & Adams, 1982a,b; Wassom, Brooks & Cypess, 1983b), the stages in the parasite's life history which are influenced by H-2 genes have been only partially defined (Wakelin & Donachie, 1983). In the present report we examine the influence of H-2 genes on the kinetics of worm expulsion and on the ability of female worms to reproduce, and propose an hypothesis to explain the data now available on genetic control in this system.

MATERIALS AND METHODS

Experimental animals

H-2 congenic male mice 5–8 weeks of age, were used in all experiments. Mice were bred in the colony of DLW at Cornell University.

Mice were infected with 150–200 muscle larvae of *T. spiralis* exactly as described by Wassom *et al.* (1983b). Counts of worms in the small intestine and assays for fecundity of female worms were as described previously (Wassom *et al.*, 1983b).

RESULTS

The stain B10.BR($H\text{-}2^k$) is known to be more susceptible to infection with *T. spiralis* than are the strains B10.Q($H\text{-}2^q$) and B10.M($H\text{-}2^f$) when total body larval counts are used as an index of susceptibility (Wassom *et al.*, 1979). These three strains of mice share the C57BL/10 genetic background and differ only at alleles expressed at H-2 gene loci. At least 24 mice of each of the above strains were infected with *T. spiralis* on Day 0. Six, 9, 12 and 15 days after infection, six mice of each strain were killed and the numbers of adult worms in the small intestine counted. Results of two independent experiments are summarized in Fig. 1. Differences in worm counts were not apparent on Day 6 of a primary infection. By Day 9 however, B10.M mice ($H\text{-}2^f$) had begun to expel their infections. Worm counts for B10.M mice were significantly lower than counts for B10.BR on Days 12 and 15 after infection. B10.Q mice expelled their infections at a rate comparable to B10.BR during the early stages of infection but had significantly fewer worms present on

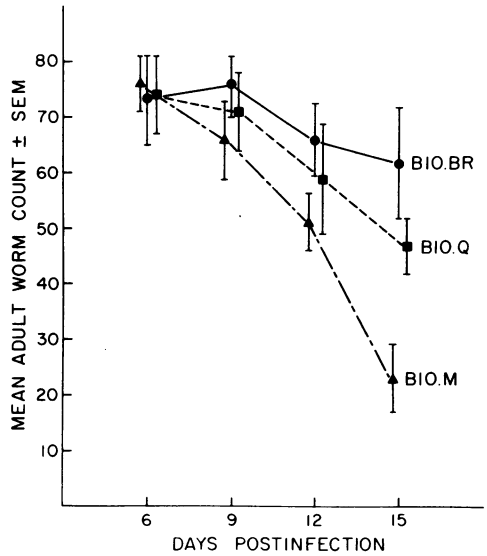

Figure 1. Kinetics of worm expulsion during a primary infection with *T. spiralis*. B10.BR (●); B10.Q (■), B10.M (▲).

Day 15. In separate experiments (data not shown), it was established that mice of all three strains completely expel their infections prior to Day 21.

There were no differences among the strains in the proportion of female to male worms present in the small intestine. Female worms comprised approximately two-thirds of all worms present on Days 6–12 of infection and dropped slightly to approximately 60% on Day 15 (Table 1). The number of newborn larvae produced by the female worms was also determined. Worms taken from B10.Q mice were always less fecund than worms from the other strains (Fig. 2). After Day 6, worms from B10.M mice were less fecund than worms from B10.BR.

An interesting way to evaluate the biological impact of *T. spiralis* infection on the host is to estimate the number of newborn larvae which gain access to the host's system during the course of the infection. This

Table 1. Female worms expressed as a percentage of total worm count in the small intestine on Days 6, 9, 12 and 15 after infection

Strain	Percent ± SEM			
	Day 6	Day 9	Day 12	Day 15
B10.BR	67±1·4	76±1·5	65±3·2	59±1·8
B10.M	66±2·2	68±1·8	60±2·3	55±2·2
B10.Q	65±1·1	65±2·1	66±3·0	61±3·4

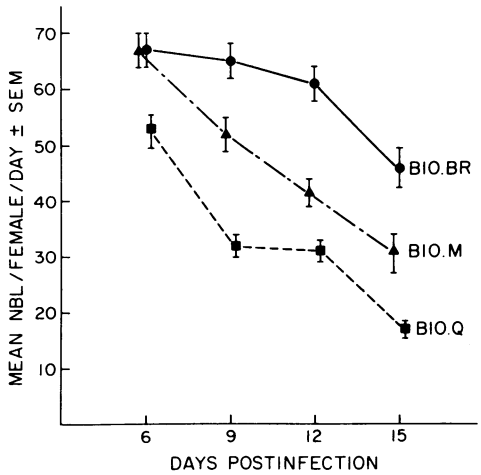

Figure 2. Number of *T. spiralis* newborn larvae produced per day by female worms harvested from the small intestine of B10.BR (●), B10.Q (■), or B10.M (▲) mice.

parameter is a product of the length of time that adult worms are present in the small intestine and the number of newborn larvae that they release while they are there. We have devised such an estimate by multiplying the average number of female worms in the gut on each of Days 6, 9, 12 and 15 after infection by the corresponding number of newborn larvae produced by each female worm. Results are shown in Fig. 3. The area under the curve for each strain estimates the total number of newborn larvae produced between Days 6 and 15. The actual number of newborn larvae produced were 14,014, 9,569, and 6,593 for the B10.BR, B10.M, and B10.Q, respectively. It is clear from such an analysis that the combined influence of genes which regulate the expulsion of worms from the gut and genes which control the antifecundity response, may in large part account for the H-2-mediated differences in larval burdens demonstrated in our earlier experiments. It is not yet known if H-2 genes influence the ability of the host to limit the migration of NBL from the gut to the muscle.

The above experiments confirm that H-2 genes have a marked influence on the immune response which expels worms from the gut during a primary infection. These experiments also establish that H-2 genes participate in the response which limits the reproduction of female worms. To discover whether or not H-2 genes participate in the host's response to challenge infections, B10.BR, B10.Q and B10.M mice were infected with 200 infective larvae of *T. spiralis* and reinfected along with previously uninfected controls 21 days later. This protocol was chosen as mice of all three strains expel worms from a primary infection prior to Day 21, and thus should be primed for challenge but no longer expelling primary infections. In initial experiments, adult counts were performed on Days 6, 9 and 12 following the challenge infection. Fecundity assays were performed only on Day 6 after

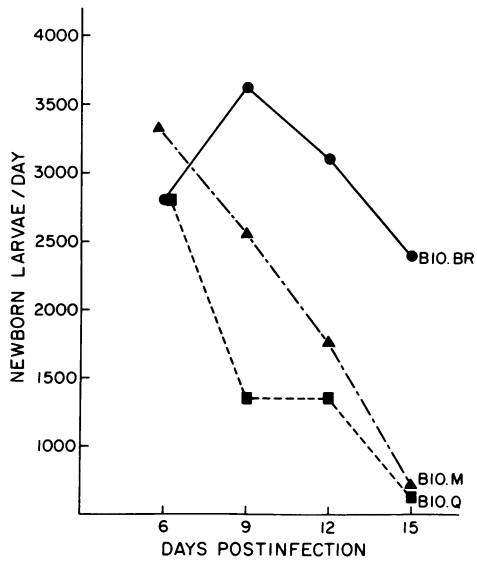

Figure 3. Total numbers of *T. spiralis* newborn larvae produced between Days 6 and 15 after infection. B10.BR (●), B10.Q (■), B10.M (▲). Values were obtained by multiplying the average number of female worms in the small intestine of each strain by the average number of newborn larvae produced by individual female worms. Error bars are not shown as these values were obtained by multiplying one mean by another. An estimate of the variability around these mean values can be obtained by consulting Figs 1 and 2 and Table 1.

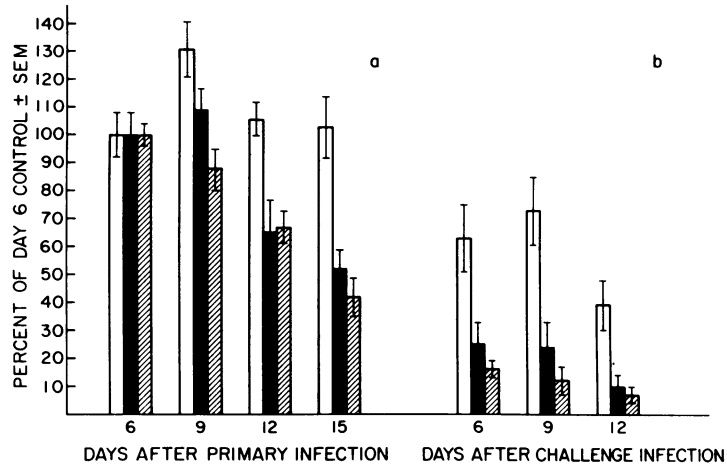

Figure 4. Kinetics of worm expulsion during challenge infections with *T. spiralis*. (a) Number of worms remaining in the small intestine of control animals receiving a primary infection with *T. spiralis*; numbers are expressed as a percentage of the Day 6 experimental group for each strain. (b) Number of worms remaining in the small intestine of mice reinfected with *T. spiralis* 21 days following a primary infection of 200 muscle larvae; numbers are expressed as a percentage of the Day 6 experimental control group for each strain. (□) B10.BR, (■) B10.Q, (■) B10.M.

challenge as insufficient worms were available for comparison on Days 9 and 12. Results are shown in Fig. 4 and in Table 2. Expulsion of worms from control mice receiving a primary infection is shown in Fig. 4a. These mice expelled the infection in a pattern similar to that shown in Fig. 1. In contrast, reinfected mice expelled a significant proportion of their worms before Day 6 (Fig. 4b); B10.Q and B10.M mice expelled 75% and 84% of their infections respectively while low responder B10.BR mice expelled 37% of their worms prior to Day 6. In the reinfected mice, worms present on Day 6 were then expelled in a pattern similar to that shown for a primary infection but more quickly; in both primary and challenge infections, B10.M and B10.Q mice expelled worms more quickly than did B10.BR (Fig. 4a,b; Student's *t*-test $P < 0{\cdot}05$).

Table 2. Fecundity of female *T. spiralis* worms taken from mice 6 days after a challenge infection

Strain	Percent reduction in newborn larvae*	*P*†
B10.BR	41	$<0{\cdot}05$
B10.M	54	$<0{\cdot}005$
B10.Q	76	$<0{\cdot}005$

* When compared to non-immune controls.
† Student's *t*-test.

The influence of H-2 genes on the fecundity of female worms during a challenge infection is shown in Table 2. Female worms from B10.Q mice produced fewer newborn larvae than did worms from the other two strains. Reinfected mice of all three strains produced significantly fewer newborn larvae than did primary infection controls.

Although Bell *et al.* (1982a) reported that C57BL/10 mice exhibit only a weak rapid expulsion response (significant loss of worms within first 24 h after a challenge infection), it was necessary to determine whether the reduction in worm burdens seen 6 days after challenge was in fact due to rapid expulsion occuring with the first 24 h. Mice were infected with 200 muscle larvae of *T. spiralis*, reinfected after 21 days, and worms counted 24 or 72 h later. In these experiments, worms failing to migrate out of the small intestine during 5 hr of incubation in saline (37°) were counted after digesting the gut in 1% acid-pepsin for 2 hr at 37°. B10.Q and B10.M mice eliminated 57 and 65% of their infection within the first 24 hr after challenge (Table 3). Counts for B10.Q and B10.M were significantly lower ($P < 0{\cdot}05$) than counts for B10.BR (27% reduction). Because these strains differ only at H-2 gene loci, H-2 genes must influence this rapid expulsion response. It is also apparent that under appropriate conditions of immunization, mice expressing the C57BL/10 genetic background are capable of eliciting a rapid expulsion response com-

Table 3. Number of *T. spiralis* in the small intestine of mice 24 and 72 hr after a challenge (immune) or primary (non-immune) infection with 200 muscle larvae

	Intestinal worm counts ± SEM					
	24 hr			72 hr		
Strain	Count	% Reduction*	*P*	Count	% Reduction*	*P*
B10.BR						
non-immune	71 ± 6·9	27%	NS	73 ± 6·7	42%	< 0·025
immune	52 ± 7·3			42 ± 6·4		
B10.Q						
non-immune	68 ± 4·7	57%	< 0·005	78 ± 8·4	87%	< 0·005
immune	29 ± 7·4			10 ± 5·0		
B10.M						
non-immune	68 ± 5·5	65%	< 0·005	76 ± 3·23	71%	< 0·005
immune	24 ± 4·3			22 ± 3·42		

* When compared to non-immune controls.

parable to that reported by Bell *et al.* (1982a) for NFR/N and NFS/N mice.

DISCUSSION

The results of these experiments are somewhat at variance with the earlier reports of Wakelin (1980), Wakelin & Donachie (1980, 1981) and Bell *et al.* (1980, 1982a,b) concerning the relative importance of H-2 genes in controlling immunity to the intestinal phase of *T. spiralis* infections. The conclusion drawn from the above referenced studies was that regulation of intestinal immunity to *T. spiralis* was primarily by non-H-2 genes; mice expressing the C57BL/10 genetic background were all identified as slow responders. In the present experiments, C57BL/10 mice infected with 150–200 muscle larvae expelled worms more quickly than would have been predicted from these earlier studies. However, recent experiments in our laboratory have shown that worms establishing after infection with 100 or 200 *T. spiralis* larvae are expelled more rapidly than worms establishing after infection with 400 or 600 larvae. Additionally, lymphocyte responsiveness to *Trichinella* antigens is depressed in mice receiving the larger infective doses (Wassom *et al.*, submitted for publication). Therefore, we believe that the slower rates of expulsion reported for *T. spiralis* infections in C57BL/10 mice may be directly related to the fact that the experiments of Wakelin (1980), Wakelin & Donachie (1980, 1981, 1983) and Bell *et al.* (1980, 1982a,b) utilized infective doses that ranged two-fold higher than the doses reported here.

Both Wakelin (1980) and Bell *et al.* (1980, 1982a) reported that B10 mice were slow to expel challenge infections from the gut, and again it was concluded that H-2 genes play only a minor role in regulating the secondary anti-*Trichinella* response. Additionally, Bell *et al.* (1982a) in a genetic analysis of rapid expulsion among inbred strains of mice, concluded that the rapid expulsion response against *T. spiralis* is controlled by an autosomal dominant gene not linked to the H-2 complex.

However, our results, using lower infective doses in challenge infections of H-2 congenic strains of mice, support the conclusion that H-2 genes do play a significant role in controlling the expulsion of adult worms from challenge infections and in controlling rapid expulsion. The susceptible *H-2*^{k} mice expelled 27% of a challenge infection during the first 24 h while the resistant *H-2*^{q} and *H-2*^{f} mice expelled 57% and 65%, respectively. We suggest, based on our findings, that a rapid expulsion response can be expressed in many strains of mice and that the expression is influenced by both H-2 and non-H-2 genes.

Results of our experiments indicate that the H-2 genes which influence the anti-fecundity response may act independently from those which influence expulsion of worms from the gut. For example, B10.Q mice inhibit worm reproduction as early as Day 6 after

infection, several days before the onset of worm expulsion. Although it could be argued that the anti-fecundity response is an early manifestation of the same response which expels the worms a few days later, this argument fails to explain why B10.M mice, which respond most rapidly to expel worms from the gut, are less proficient than B10.Q mice at limiting worm fecundity.

Based on the data now available, we propose that the anti-fecundity, the anti-adult, and the rapid expulsion responses are under independent genetic control and regulated by the interacting products of H-2 and non-H-2 genes. Such H-2–non-H-2 interactions have been shown to occur in other experimental systems. For example, Cutting *et al.* (1981) demonstrated that complementing MHC and non-MHC linked genes control the ability of inbred chickens to resist avian sarcoma virus-induced tumors, and Munro, Taussig & Archer (1976) showed that H-2–non-H-2 complementation occurs in the antibody response of mice to [(T,G)-A--L].

The postulated interaction of H-2 and non-H-2 genes in the generation of immunity to *T. spiralis* would explain many of the observations made thus far by researchers studying this host–parasite system. If, for example, independent genes control the ability of effector cell populations to respond to H-2-encoded stimuli, strains sharing common H-2 alleles may well exhibit different phenotypic responses to infection. Additionally, if H-2 genes control the kinetics of lymphocyte responsiveness as first suggested by Wakelin & Donachie (1980), then H-2 alleles associated with a rapid response would transmit signals to relevant effector cells more quickly than would alleles associated with a slow response.

Recent experiments of Wakelin & Donachie (1983) showed that H-2D-end genes may influence the expulsion of *T. spiralis* from the small intestine. B10.T(6R) mice ($K^qI^qD^d$) were slow to expel worms when compared to B10.G($K^qI^qD^q$). More importantly, lymph node cells from infected B10.T(6R) mice were capable of transferring immunity to B10.G mice, but immunity could not be adoptively transferred to B10.T(6R) using immune cells from homologous donors nor with cells from B10.G mice. This implied that an additional component of the immune response, not functional in B10.T(6R) mice, was required in order to generate an optimal response against the parasite. These results support those of Wassom and coworkers (Wassom, David & Gleich, 1980; Wassom *et al.*, 1983a) who demonstrated that at least two H-2 genes influence immunity to *T. spiralis* in the mouse. One gene maps to the A_β locus (I-A) and is designated Ts-1. The other gene maps to the D end of the H-2 complex between the S and D loci and is designated Ts-2. B10.T(6R) mice and other strains expressing the $Ts\text{-}2^d$ allele are more susceptible to *T. spiralis* infection than are otherwise resistant strains expressing haplotypes of independent origin.

Based on the results of Wakelin & Donachie (1983) and Wassom *et al.* (1983a), we propose that the $Ts\text{-}2^d$ allele is associated with a defect in the second of two cell populations which must interact prior to optimal expression of the expulsion response. Thus, B10.T(6R) mice and other strains expressing the $Ts\text{-}2^d$-associated defect may lack the ability to respond optimally themselves yet possess a population of cells which will mediate immunity when transferred to appropriate recipients. Such an hypothesis is not without a precedent. Strassman *et al.* (1980), using a protocol similar to that used by Wakelin & Donachie (1983), showed that T–T cell interaction is required in the optimal expression of delayed type hypersensitivity (DTH) to [(T,G)-A--L]. In the latter system, non-responders to [(T,G)-A--L] possess $Ly\text{-}1^+$ T cells capable of adoptively transferring responsiveness to (responder × non-responder) F_1 mice but not to syngeneic non-responder recipients. The genetic defect in these non-responder mice occurred in a second population of T cells which express the $Ly\text{-}123^+$ phenotype. The similarity between the *Trichinella* and [(T,G)-A--L] systems is strengthened by our recent finding that the *in vitro* proliferation response of explanted lymph node cells from mice primed with *T. spiralis* antigen requires two populations of T cells in order to generate an optimal response. As was shown in the [(T,G)-A--L] system, one population expresses the $Ly\text{-}1^+$ phenotype and the other $Ly\text{-}123^+$ (Krco, David & Wassom, 1982).

We have proposed an hypothesis suggesting that H-2 and non-H-2 genes interact in the generation of a functional immune response to *T. spiralis*. Although this hypothesis is compatible with data collected thus far, it is supported only by circumstantial evidence and is certainly not the only hypothesis that could be formulated to explain these data. The strength of our hypothesis is that the immunological tools necessary to properly test it are available and the H-2 genes which influence the host response to *T. spiralis* have been precisely mapped. Experiments to characterize the cellular interactions which occur in *Trichinella*-infected mice can therefore be conducted using strains of mice which differ measurably in response to

infection but which differ only in the genes that control this response. To complement these *in vivo* studies, we have produced and characterized *Trichinella*-specific helper T cell clones from both resistant and susceptible strains of mice and used monoclonal antibodies to identify the genes which control antigen recognition and macrophage–T cell interactions (Krco *et al.*, 1983). Monoclonal antibodies specific for a number of *T. spiralis* antigens have also been produced. The *in vitro* study of cloned T cells and the use of monoclonal anti-*T. spiralis* antibody may provide valuable new information regarding how the host's immune system recognizes, processes, and responds to functional parasite antigens. It is hoped that continued study of this host–parasite system may help to provide definitive answers to the elusive question of how immunity to parasitic helminths is controlled and effected.

ACKNOWLEDGMENTS

This work was supported by NIH grants AI 17079 and AI 14764.

REFERENCES

BELL R.G., MCGREGOR D.D. (1980) Variation in anti-Trichinella responsiveness in inbred mouse strains. In: *Genetic Control of Natural Resistance to Infection and Malignancy* (eds E. Skamene, P. A. L. Kongshavn and M. Landy), p. 67. Academic Press, New York.

BELL R.G., MCGREGOR D.D. & ADAMS L.S. (1982a) *Trichinella spiralis*: characterization and strain distribution of rapid expulsion in inbred mice. *Exp. Parasit.* **53,** 301.

BELL R.G., MCGREGOR D.D. & ADAMS L.S. (1982b) *Trichinella spiralis*: genetic basis for differential expression of phase-specific intestinal immunity in inbred mice. *Exp. Parasit.* **53,** 315.

CUTTING J.A., WATANABE D.H., STREBEL F.R. & MCBRIDE R.A. (1981) Complementing MHC- and non-MHC linked genes and resistance to avain sarcoma virus-induced tumors in inbred lines of chickens. *J. Immunogenet.* **8,** 215.

KRCO C.J., DAVID C.S. & WASSOM D.L. (1982) Characterization of an *in vitro* proliferation response to solubilized *Trichinella spiralis* antigens: role of Ia antigens and Ly-1$^+$ T cells. *Cell. Immunol.* **68,** 359.

KRCO C.J., WASSOM D.L., ABRAMSON E.J. & DAVID C.S. (1983) Cloned T cells recognize *Trichinella spiralis* antigen in association with an E^k_β E^k_α restriction element. *Immunogenetics*, (in press).

MUNRO A., TAUSSIG M. & ARCHER J. (1976) I-region products and cell interactions: Contribution of non H-2 genes to acceptor and factor for (T,G)-A--L. In: *Ir Genes and Ia Antigens* (ed. H. O. McDevitt), p. 487. Academic Press Inc., New York.

STRASSMANN G., ESHHAR Z. & MOZES E. (1980) Genetic regulation of delayed-type hypersensitivity responses to poly(LTyr, LGlu)-poly(DL Ala)--poly(LLys). II. Evidence for a T-T cell collaboration in delayed-type hypersensitivity responses and for a T-cell defect at the efferent phase in nonresponder *H-2*^{k} mice. *J. exp. Med.* **151,** 628.

WAKELIN D. (1980) Genetic control of immunity to parasites. Infection with *Trichinella spiralis* in inbred and congenic mice showing rapid and slow responses to infection. *Parasite Immunol.* **2,** 85.

WAKELIN D. & DONACHIE A.M. (1980) Genetic control of immunity to parasites: adoptive transfer of immunity between inbred strains of mice characterized by rapid and slow immune expulsion of *Trichinella spiralis*. *Parasite Immunol.* **2,** 249.

WAKELIN D. & DONACHIE A.M. (1981) Genetic control of immunity to *Trichinella spiralis*. Donor bone marrow cells determine responses to infection in mouse radiation chimaeras. *Immunology*, **43,** 787.

WAKELIN D. & DONACHIE A.M. (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.

WASSOM D.L., BROOKS B.O., BABISH J.G. & DAVID C.S. (1983a) A gene mapping between the S and D regions of the H-2 complex influences resistance to *Trichinella spiralis* infections of mice. *J. Immunogenet.* **10,** 371.

WASSOM D.L., BROOKS B.O. & CYPESS R.H. (1983b) *Trichinella spiralis*: Role of non-H-2 genes in resistance to primary infection. *Exp. Parasit.* **55,** 153.

WASSOM D.L., DAVID C.S. & GLEICH G.J. (1979) Genes within the major histocompatibility complex influence susceptibility to *Trichinella spiralis* in the mouse. *Immunogenetics*, **9,** 491.

WASSOM D.L., DAVID C.S. & GLEICH G.J. (1980) MHC-linked genetic control of the immune response to parasites: *Trichinella spiralis* in the mouse. In: *Genetic Control of Natural Resistance to Infection and Malignancy* (eds E. Skamene, P. A. L. Kongshavn and M. Landy), p. 75. Academic Press Inc., New York.