Immunology 1981 **43** 787

Genetic control of immunity to *Trichinella spiralis*. Donor bone marrow cells determine responses to infection in mouse radiation chimaeras

D. WAKELIN & ANNE M. DONACHIE *Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden, Glasgow, Scotland*

Accepted for publication 26 February 1981

Summary. Radiation chimaeras, prepared from NIH (rapid-responder) mice or from the F_1 progeny of a cross between H-2 compatible B10.G (slow-responder) and NIH mice, were tested for their ability to respond to infection with the intestinal nematode parasite *Trichinella spiralis*. Mice reconstituted with bone marrow (BM) from NIH donors showed the rapid response characteristic of this strain, i.e. expelled worms from the intestine before day 12 of infection; those given BM from B10.G mice showed a slow expulsion pattern, losing worms after day 12. There was no evidence that the environment of the recipient exerted any influence on the ability of the BM cells to express the response characteristic of the donor. When chimaeras were given immune mesenteric lymph node cells (IMLNC) from infected NIH donors there was successful adoptive transfer of immunity, resulting in an accelerated loss of worms. As before, the time course of the accelerated response was determined by the genotype of the BM used. These results confirm that genetic control of the process of worm expulsion is expressed at the level of a bone marrow-derived cell population and is independent of lymphocyte responsiveness. They further show that the factors involved are an inherent property of the cells concerned. The possibility that these cells are myeloid in nature is discussed.

Correspondence: Dr D. Wakelin, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Bearsden, Glasgow G61 1QH, Scotland.

0019-2805/81/0800-0787$02.00

INTRODUCTION

Although parasitic organisms elicit complex immune responses in their hosts, it is nevertheless possible to demonstrate distinct, genetically-determined differences in resistance to infection, both between particular strains of a host species and between individuals within a genetically heterogeneous population (Wakelin, 1978). A number of recent studies, principally with bacterial and protozoan parasites, have shown that such differences in resistance may operate at the level of non-specific effectors, such as macrophages, as well as at the level of immune responses (Blackwell, Freeman & Bradley, 1980; Bradley, 1977; Hormaeche, 1979; Howard, Hale & Liew, 1980; Kongshavn, Sadarangani & Skamene, 1980).

Variation in resistance to parasitic worms has been demonstrated in several host–parasite relationships but analysed in relatively few (Mitchell, 1979). A convenient model for such analysis is provided by the nematode *Trichinella spiralis* in mice. The responses which regulate infection have been extensively studied and there are clear-cut differences between inbred strains of mice in the two major parameters by which infections are assessed, namely (a) the duration of the intestinal phase which follows oral administration of infective larvae, and (b) the number of larvae which

become encysted in the muscles of the host after release by the adult worms in the intestine. Work in this laboratory has shown that the first parameter is influenced by genes which are not linked to the major histocompatibility complex (H-2) (Wakelin, 1980); the second parameter is influenced by both non-H-2 and H-2-linked genes (Wassom, David & Gleich, 1979).

There is evidence to suggest that the adult worms of *T. spiralis* are removed from the intestine by a complex T cell-mediated, inflammatory response (spontaneous cure). In mice characterized as rapid-responders, spontaneous cure is effective within 12 days; in slow-responders the response does not operate until after this time (Wakelin, 1980). By using reciprocal adoptive transfers between H-2 compatible mice showing opposite response patterns, it has been possible to show that the delay in spontaneous cure in slow-responders does not arise from a delayed immunological response. Cells capable of transferring an accelerated spontaneous cure appear in the mesenteric nodes of such mice as early as they do in rapid-responder mice (Wakelin & Donachie, 1980). It was therefore suggested that strains may differ in their abilities to translate the immunological response to infection into an effective inflammatory response, and that this difference may be a property of the non-lymphoid, bone marrow-derived cells concerned. This hypothesis has been tested using adoptive transfer experiments in radiation chimaeras. The results show that the response characteristics of a chimaeric recipient are determined by the status of the bone marrow donor used and not by that of the lymphocyte donor.

MATERIALS AND METHODS

Mice

Inbred NIH (H-2^q) were obtained from Hacking & Churchill Ltd, and the B10 congenic strain B10.G (H-2^q) from Olac 1976 Ltd. NIH respond rapidly to *T. spiralis*, B10.G are slow responders. Hybrid mice (B10.G × NIH F_1) were bred in the laboratory. Mice were used in groups of five or six when 6–8 weeks old; only males were used as donors and recipients.

Parasite

The strain of *T. spiralis* and the methods used in maintenance, infection and recovery have been described previously (Wakelin & Lloyd, 1976; Wakelin & Wilson, 1977). Unless stated otherwise control and recipient mice were infected with approximately 300 larvae.

Cell suspensions

Bone marrow (BM) and immune mesenteric lymph node cell (IMLNC) suspensions were prepared by standard techniques and injected intravenously. Donors of IMLNC were infected with 300 larvae 8 days before collection of cells; donors of BM were uninfected. Transfer of IMLNC and infection with *T. spiralis* were carried out on the same day.

Preparation of chimaeras

Mice were irradiated at 850 rad using a 500 Ci ^{60}Cobalt source (output 1050 rad/min) and reconstituted within 6 hr by injection of 1×10^7 BM. Irradiated mice were given antibiotic in their drinking water.

Anti-sheep red blood cell response

Mice were immunized by intraperitoneal injection of 0·25 ml of a 4% suspension of sheep red blood cells (SRBC; Flow Laboratories) in saline. Antibody titres were measured by a standard microtitre haemagglutination technique.

Experimental design

Adoptive transfer of immunity is possible in both NIH and B10.G mice (Wakelin & Donachie, 1980). Cell recipients show an accelerated spontaneous cure, with worm loss commencing 2 or 3 days earlier than in controls. The relative difference in response pattern between the strains is maintained under these conditions and is not affected by the source of IMLNC used, i.e. whether they originate from homologous or heterologous donors. Loss of worms from cell recipients before day 8 is characteristic of NIH mice, loss between day 8 and day 12 of B10.G. In the experiments described below mice were killed at day 7 or 8 and, where possible, at day 11 or 12 in order to categorize the response pattern shown.

Statistics

Comparison of mean worm recoveries from control and recipient mice was made using Student's t test. A probability of $P > 0{\cdot}05$ was considered non-significant.

RESULTS

The ability to respond to *T. spiralis* is suppressed by irradiation, and complete recovery, even from sublethal doses, may take several weeks (unpublished data). A number of preliminary tests showed that a

normal response to adoptively-transferred IMLNC was present by 12 weeks after irradiation and reconstitution and this was therefore taken as the minimum time for recovery in the experimental work.

Adoptive transfer into NIH mice reconstituted with NIH or B10.G bone marrow

Two experiments were carried out. In the first, mice reconstituted for 13 weeks were given 3×10^7 IMLNC from NIH donors and killed 8 days after infection. in the second, mice were given 2×10^7 cells after 12 weeks recovery and killed 8 and 12 days after infection (Table 1).

The results at day 8 were similar in both experiments in that adoptive transfer resulted in an accelerated loss of worms from control recipients and from recipients previously reconstituted with BM from NIH donors. There was no loss at this time from mice reconstituted with BM from B10.G mice, although loss had occurred by day 12 (Experiment 2).

Adoptive transfer into (B10.G × NIH) F_1 mice reconstituted with parental bone marrow

Although the mice used in the above experiments had apparently become completely reconstituted it was noticed, following the preparation of the chimaeras, that some of the mice given BM from B10.G donors failed to reconstitute satisfactorily and had to be excluded. For this reason it was considered necessary to examine the effects of adoptive transfer into chimaeras prepared from (B10.G × NIH) F_1 mice and to confirm the completeness of recovery by measuring antibody response to injected SRBC.

It had been shown in previous work (Wakelin, 1980) that the F_1 progeny of a cross between rapid- and slow-responder mice (B10 × NIH) behaved as NIH when infected with *T. spiralis*, i.e. rapid-responsiveness was inherited as a dominant characteristic. This result was confirmed in an initial experiment using female progeny from the B10.G × NIH cross (Fig. 1). The male progeny were used in the adoptive-transfer experiment and were given 3×10^7 IMLNC from NIH donors after 12–16 weeks reconstitution with BM from one or other of the parental strains. Untreated F_1 were used as infection controls and the activity of the IMLNC was monitored in NIH mice. SRBC were injected 2 days before infection. Because the F_1 mice were comparatively old (18–24) weeks) at the time of infection, and as spontaneous cure occurs earlier in older mice, kills were made on days 7 and 11 after infection (Table 2).

The IMLNC used transferred a high level of immunity both in reconstituted F_1 mice and in the NIH used to monitor their activity (day 8 mean worm counts in control NIH = 137·8, in cell recipients = 14·1). However, loss of worms by day 7

Table 1. Adoptive transfer of immunity to *T. spiralis* in control (unirradiated) and chimaeric NIH mice

	Number of worms recovered					
	Expt 1		Expt 2			
	Day 8		Day 8		Day 12	
Group	Mean	SD	Mean	SD	Mean	SD
Control—no cells	102·3	30·2	139·4	15·6	ND	
Control + IMLNC	46·9*	26·9	50·5*	16·7	ND	
ϟ + NIH BM	ND		118·4	12·1	1·0†	
ϟ + NIH BM + IMLNC	40·1	26·8	49·3*	24·4	ND	
ϟ + B10.G BM + IMLNC	118·9	18·0	118·3	3·8	30·2	26·0

Recipients given 3×10^7 (experiment 1) or 2×10^7 (experiment 2) IMLNC from NIH donors. All mice infected with approximately 300 larvae on day 0.

* Mean significantly different from corresponding group not given IMLNC.

† One mouse.

ϟ , Chimaeric mice exposed to irradiation.

ND, not done.

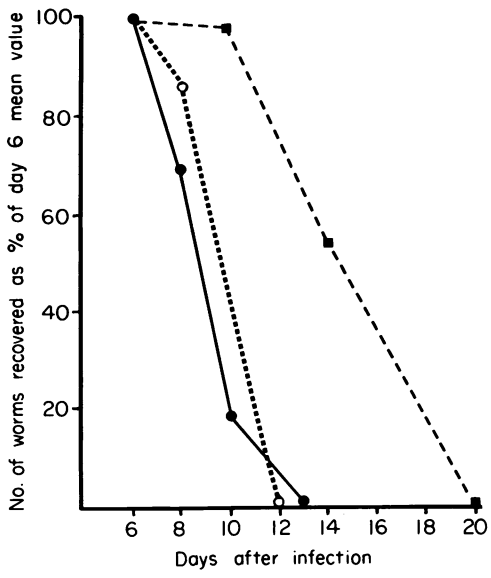

Figure 1. Course of infection with *T. spiralis* in (B10.G × NIH) ♀ F_1 mice (●——●). Also shown are curves representing the course of infection in parental NIH (o- - - -o) and B10.G mice (■- - -■).

occurred only in the F_1 recipients that had been reconstituted with NIH BM; F_1 recipients given BM from B10.G donors did show an accelerated worm expulsion but this was not evident until day 11. The levels of anti-SRBC antibodies recorded on day 7 indicate that reconstitution had been successful to an equivalent degree in mice given NIH or B10.G BM.

DISCUSSION

In mice that have been lethally irradiated and reconstituted with BM from H-2 compatible donors there is virtually complete replacement of haemopoietic, myeloid and lymphoid tissues by cells of the donor type (Korngold & Sprent, 1978; Onoe, Fernandes & Good, 1980). When fully recovered the cellular and humoral immune responses of the chimaeras are equivalent to those of the donors. In some circumstances the presence of mature T cells in compatible BM may give rise to chronic graft-versus-host disease, but this is relatively uncommon and occurred in only two out of eleven donor–recipient combinations tested by Korngold & Sprent (1978). Although there was some evidence in the present work that reconstitution with B10.G BM was somewhat less successful in NIH than in F_1 mice, the majority of chimaeras prepared survived well, showed no evidence of any GVH disease and regained normal levels of humoral responsiveness (Table 2).

Chimaeric mice have been used on a number of occasions to identify the cells through which genetically determined resistance or susceptibility to infec-

Table 2. Adoptive transfer of immunity to *T. spiralis* in chimaeric (B10.G × NIH) F_1 mice.

Group	Number of worms recovered			
	Day 7		Day 11	
	Mean	SD	Mean	SD
Control F_1—no cells	137·3	25·8	ND	
♀+NIH BM	146·3	17·6	13·5	22·3
♀+NIH BM+IMLNC	28·8*	22·7	ND	
♀+B10.G BM	143·0	40·0	121·2	47·7
♀+B10.G BM+IMLNC	133·5	21·6	10·7*	11·1

Recipients given 3×10^7 IMLNC from NIH donors. All mice infected with approximately 300 larvae on day 0. Four-tenth millilitres of 2·5% SRBC injected day −2.

Anti-SRBC titres ($\log_2$) in mice killed on day 7

Control F_1
♀+NIH BM } 11·0
♀+B10.G BM }

* Mean significantly different from corresponding group not given IMLNC.

ND, not done.

tion is expressed. For example, in mice infected with the intracellular protozoan *Leishmania tropica* the innate susceptibility of chimaeras is determined by the genotype of the bone marrow donor and it is considered that the genetic control is expressed at the level of the macrophage (Howard *et al.*, 1980). In contrast, susceptibility to *Listeria monocytogenes*, though similarly expressed at the level of the macrophage, is dependent upon the genotype of the recipient, i.e. there is extrinsic regulation of macrophage responsiveness (Kongshavn, Sadarangani & Skamewe, 1980).

In the experiments described here it was clear that the responses of radiation chimaeras to *T. spiralis* were determined directly by the genotype of the BM donor; the ability of the BM cells to participate in rapid or slow responses arose from an inherent characteristic of the cells and was not influenced by the environment of the recipient. In B10.G × NIH F_1 mice reconstituted with NIH BM the course of a primary infection followed that characteristic of the rapid-responder strain; in mice reconstituted from B10.G donors the course followed that of the slow-responder strain (Table 2). Adoptive transfer of IMLNC into such mice did accelerate the process of spontaneous cure, but in the case of mice given B10.G BM the time of the accelerated response was characteristic of the B10.G strain, rather than of the rapid-responder NIH strain from which the IMLNC were taken. A similar picture was seen in chimaeras made from NIH mice (Table 1).

These results confirm and amplify the hypothesis that the genetic control of worm expulsion is expressed at the level of a non-lymphoid cell population whose presence is necessary for the generation of the intestinal inflammatory responses that bring about spontaneous cure (Wakelin & Donachie, 1980). It is known that IMLNC alone cannot bring about worm expulsion. In irradiated recipients immunity is expressed only in anti-worm effects such as reduced growth and fecundity, and worm expulsion requires the additional presence of BM-derived cells (Wakelin & Wilson, 1980). The IMLNC involved are T cells (Wakelin & Wilson, 1979) and, as B cells seem to play little part in the expulsive response, it would seem probable that the necessary BM-derived cells are myeloid in nature, although their identity is, as yet, unknown. A variety of inflammatory cells invade the intestinal mucosa during the course of infection and it is likely that this infiltration is largely immunologically (and specifically T-cell) mediated; certainly a number of the changes associated with infection are reduced or absent in T-deprived hosts (Ruitenberg & Elgersma, 1976; Ruitenberg, Elgersma, Kruizinga & Leenstra, 1977; Walls, Carter, Leuchars & Davies, 1973). If this is the case then the point of genetic control may lie in the response of myeloid cells, or their precursors, to T-cell factors generated during the early stages of infection.

ACKNOWLEDGMENTS

This work was supported by Medical Research Council project grants G977/65/T and G80/0058/T.

REFERENCES

BLACKWELL J., FREEMAN J. & BRADLEY D. (1980) Influence of H-2 complex on acquired resistance to *Leishmania donovani* infection in mice. *Nature (Lond.)*, **283,** 72.

BRADLEY D.J. (1977) Regulation of *Leishmania* populations within the host. II Genetic control of acute susceptibility of mice to *Leishmania donovani* infection. *Clin. exp. Immunol.* **30,** 130.

HORMAECHE C.E. (1979) The natural resistance of radiation chimeras to *Salmonella typhimurium* C5. *Immunology* **37,** 329.

HOWARD J.G., HALE C. & LIEW F.Y. (1980) Genetically-determined susceptibility to *Leishmania tropica* infection is expressed by haemopoietic donor cells in mouse radiation chimaeras. *Nature (Lond.)*, **288,** 161.

KONGSHAVN P.A.L., SADARANGANI C. & SKAMEWE E. (1980) Genetically determined differences in antibacterial activity of macrophages are expressed in the environment in which the macrophage precursors mature. *Cell. Immunol.* **53,** 341.

KORNGOLD R. & SPRENT J. (1978) Lethal graft-versus-host disease after bone marrow transplantation across minor histocompatibility barriers in mice. *J. exp. Med.* **148,** 1687.

MITCHELL G.F. (1979) Responses to infection with metazoan and protozoan parasites in mice. *Adv. Immunol.* **28,** 451.

ONOE K., FERNANDES G. & GOOD R.A. (1980) Humoral and cell-mediated immune responses in fully allogeneic bone marrow chimera in mice. *J. exp. Med.* **151,** 115.

RUITENBERG E.J. & ELGERSMA A. (1976) Absence of intestinal mast cell response in congenitally athymic mice during *Trichinella spiralis* infection. *Nature (Lond.)*, **264,** 258.

RUITENBERG E.J., ELGERSMA A., KRUIZINGA W. & LEENSTRA F. (1977) *Trichinella spiralis* infection in congenitally athymic (nude) mice. *Immunology*, **33,** 881.

WAKELIN D. (1978) Genetic control of susceptibility and resistance to parasitic infection. *Adv. Parasitol.* **16,** 219.

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. & LLOYD M. (1976) Immunity to primary and challenge infections of *Trichinella spiralis* in mice: a re-examination of conventional parameters. *Parasitology*, **72,** 173.

WAKELIN D. & WILSON M.M. (1977) Transfer of immunity to *Trichinella spiralis* in the mouse with mesenteric lymph node cells: time of appearance of effective cells in donors and expression of immunity in recipients. *Parasitology*, **74,** 215.

WAKELIN D. & WILSON M.M. (1979) T and B cells in the transfer of immunity against *Trichinella spiralis* in mice. *Immunology*, **37,** 103.

WAKELIN D. & WILSON M.M. (1980) Immunity to *Trichinella spiralis* in irradiated mice. *Int. J. Parasitol.* **10,** 37.

WALLS R.S., CARTER R.L., LEUCHARS E. & DAVIES A.J.S. (1973) The immunopathology of trichiniasis in T-cell deficient mice. *Clin. exp. Immunol.* **13,** 231.

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.