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Transfer of innate resistance and susceptibility to *Leishmania donovani* infection in mouse radiation bone marrow chimaeras

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Summary. Reciprocal radiation bone marrow chimaeras were made between H-2-compatible strains of mice innately resistant or susceptible to visceral leishmaniasis. In initial experiments, susceptibility but not resistance to *Leishmania donovani* could be transferred with donor bone marrow into irradiated recipients. In subsequent experiments it was possible to transfer both resistance and susceptibility. This was achieved either by selecting more radiosensitive mouse strains as susceptible recipients, or alternatively by increasing the irradiation dose for the susceptible recipients used in the initial experiments. Using the higher irradiation dose, successful transfer of resistance and susceptibility between congenic mice carrying the Lsh^r and Lsh^s alleles on the more radioresistant B10 genetic background provided firm evidence that the results obtained in this study were specifically related to expression of the *Lsh* gene. We conclude that *Lsh* gene-controlled resistance and susceptibility to *L. donovani* is determined by bone marrow-derived cells. The cell type(s) involved is likely to be of the macrophage lineage.

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INTRODUCTION

The response of inbred mice to *Leishmania donovani* infection can be divided into two phases each under distinct genetic control (Bradley & Kirkley, 1977; Blackwell, Freeman & Bradley, 1980). During the first 2 weeks of infection, the multiplication of amastigotes in macrophages of liver and spleen is controlled by a single autosomal gene, *Lsh* (Bradley, 1977). This gene segregates for incompletely dominant resistant (Lsh^r) and recessive susceptible (Lsh^s) alleles and maps to chromosome 1 (Bradley *et al.*, 1979).

In addition to *Lsh*, several other murine innate resistance genes have been described which regulate the initial intracellular growth of certain protozoa, bacteria and viruses (reviewed by Rosenstreich, Weinblatt & O'Brien, 1982). Of particular interest with respect to *Lsh* are the genes *Ity* and *Bcg* which control early multiplication of *Salmonella typhimurium* and *Mycobacterium bovis* respectively (Plant & Glynn, 1976; Skamene, 1982). Both of these bacteria survive and multiply in macrophages of liver and spleen as do *L. donovani* amastigotes. *Ity* and *Bcg* are also located on chromosome 1 and, on the basis of formal linkage analysis, the three genes are thought to be identical (Plant *et al.*, 1982).

The molecular mechanism by which genes mediate innate resistance to phylogenetically unrelated organisms remains unknown. However, more information exists on their cellular location of action. Thus,

agents which alter or diminish T lymphocyte activity have no effect on *Lsh/Ity* expression (Bradley & Kirkley, 1972; O'Brien & Metcalf, 1982; Ulczak & Blackwell, 1983; Crocker, Blackwell & Bradley, 1984), whilst selective inactivation of tissue macrophages with silica lowered resistance to *S. typhimurium* infections (O'Brien, Scher & Formal, 1979). Hormaeche (1979) was able to transfer the early growth rate phenotype of *S. typhimurium* into lethally irradiated mice reconstituted with appropriate bone marrow from resistant or susceptible donors (radiation bone marrow chimaeras). Mature radiation bone marrow chimaeras possess macrophages as well as other haematopoietic elements of donor origin (Virolainen, 1968). Hence, the combined results from the several laboratories suggest that *Lsh*, *Ity* and *Bcg* gene activities are expressed within macrophages.

In the present paper we have extended this work by studying transfer of innate resistance and susceptibility to *L. donovani* in reciprocal radiation bone marrow chimaeras. We have found that both resistance and susceptibility can be transferred with donor bone marrow, although conditions for successful transfer of resistance are more stringent.

MATERIALS AND METHODS

Mice

A/J, CBA/Ca, C3H/He-mg, DBA/2 strains (*Lsh*^{r}) and C57Bl/10ScSn (B10), B10.D2/n, B10.A, B10.BR, BALB/B and BALB/K strains (*Lsh*^{s}) were purchased from OLAC (1976) Ltd. The C57L strain (*Lsh*^{r}) was bred in our laboratory from breeding pairs originally obtained from the Jackson Laboratory, Bar Harbor, ME. Congenic mice carrying the *Lsh*^{r} allele on a B10 genetic background (B10.*Lsh*^{r}) were bred by one of us (JB) at the LSHTM in the following way. The *Lsh*^{r} allele from strain C57L was backcrossed onto the B10(*Lsh*^{s}) genetic background and was made homozygous after 10 generations of backcrossing. Examination of the linked loci *Idh-1* and *In*, for which the two original strains carried different alleles, indicated that these had converted to the B10 genetic background alleles. The congenic strain also types as *Ity*^{r} (Janet Plant, personal communication) and *Bcg*^{r} (Emil Skamene, personal communication). In the present experiments, F1 hybrids of B10 (*Lsh*^{s}) and B10.*Lsh*^{r} mice were used in place of B10.*Lsh*^{r} mice since these are phenotypically as resistant as homozygous B10.*Lsh*^{r} mice and could be produced in larger numbers more rapidly. Female mice at 8–10 weeks of age were used in all experiments.

Parasites

Mice were infected with 10^6 or 10^7 amastigotes of the L82 Ethiopian strain of *L. donovani* which has been maintained in our laboratory by continuous hamster passage since 1972. Details of amastigote extraction and enumeration of liver parasite burdens are described elsewhere (Bradley & Kirkley, 1977).

Preparation of radiation bone marrow chimaeras

Mice were exposed to appropriate lethal doses of whole body X-irradiation for each strain at a dose rate of 108 rads/min from a Marconi X-ray machine. On the same day, irradiated mice were reconstituted with bone marrow from syngeneic or H-2-compatible allogeneic donors. Bone marrow cells were extracted from both femora of each healthy donor with RPMI medium supplemented with 5% foetal calf serum and antibiotics using a 25 gauge needle. After viability had been assessed by trypan blue exclusion, 10^7 viable nucleated cells were injected intravenously into irradiated recipients. Reconstituted mice were immediately supplied with neomycin (20 μg/litre) and polymyxin (200 U/litre) in their drinking water. This was replaced daily for 3 weeks. Chimaeras were allowed to mature for 12 weeks before being used in experiments.

RESULTS

In all experiments normal and chimaeric mice were killed 15 days after intravenous infection with 10^6 or 10^7 *L. donovani* amastigotes. By this time, maximal differences in liver parasite burdens are found between innately resistant and susceptible mice (Bradley & Kirkley, 1977).

Transfer of susceptibility but not resistance

Our initial chimaeras were made from the *Lsh*^{r} and *Lsh*^{s} H-2-compatible combinations shown in Table 1. In all cases susceptibility was readily transferred into resistant hosts, whereas susceptible mice reconstituted with resistant bone marrow consistently retained the recipient phenotype. Figure 1 shows the results of one of these combinations where chimaeras were made reciprocally between A/J (*Lsh*^{r}) and B10.A (*Lsh*^{s}) strains. A/J mice reconstituted with B10.A bone marrow became susceptible, yet B10.A recipients of

Table 1. Experiments in which resistance could not be transferred into susceptible recipients.

Recipient strain	H-2 haplotype	Dose of irradiation (rads)	Transfer of resistance	Transfer of susceptibility
Lsh^r DBA/2	d	800	+	+
Lsh^s B10.D2	d	900	−	+
Lsh^r C3H/HeN	k	850	+	+
Lsh^s B10.BR	k	900	−	+
Lsh^r A/J	a	850	+	+
Lsh^s B10.A	a	900	−	+

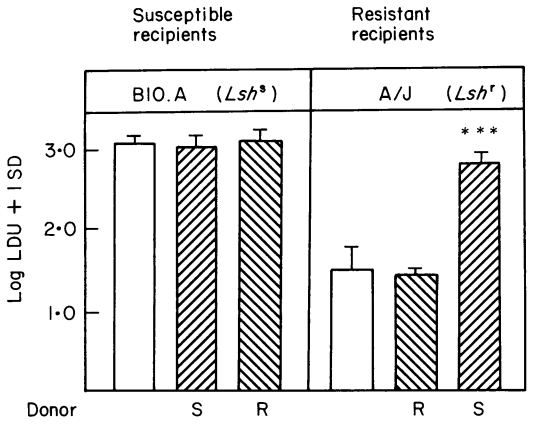

Figure 1. Transfer of susceptibility but not resistance. *L. donovani* infections (LDU) measured in the livers of normal and chimaeric mice on day 15 after intravenous infection with 10^7 amastigotes. The *left-hand panel* shows the mean LDU values +1 SD for control B10.A (*Lsh*^{s}) mice or B10.A mice exposed to 900 rads X-irradiation and reconstituted with bone marrow from syngeneic or H-2-compatible A/J (*Lsh*^{r}) mice. The *right-hand panel* shows mean LDU values in control A/J (*Lsh*^{r}) mice or A/J mice exposed to 850 rads and reconstituted with bone marrow from syngeneic or H-2-compatible B10.A (*Lsh*^{s}) mice. (▨) Recipients of *Lsh*^{r} bone marrow; (■) recipients of *Lsh*^{s} bone marrow; (□) controls. ***$P < 0{\cdot}001$ (Student's unpaired *t*-test).

A/J bone marrow were as susceptible as control B10.A mice or B10.A mice reconstituted with syngeneic bone marrow. In each case, the susceptible recipient was a mouse of the B10 congenic series which had received 900 rads.

Reciprocal transfer of both resistance and susceptibility

The previous results suggested that the difficulty in transferring the resistant phenotype might be due to incomplete chimaerism in those susceptible recipients. To test this possibility we adopted two approaches. Firstly, mouse strains on a BALB genetic background were used as susceptible recipients. BALB mice are known to be relatively sensitive to the effects of lethal irradiation (Grahn & Hamilton, 1957). Secondly, we increased the dose of irradiation to 950 rads from that used previously (900 rads) for making chimaeras from susceptible mice on B10 genetic backgrounds. Table 2 shows the strain combinations and doses used in these experiments. In all cases it was possible to demonstrate significant transfer of both resistance and susceptibility into resistant and susceptible recipients respectively (Fig. 2). In two of these combinations, both using C57L (*Lsh*^{r}) strains as recipients of bone marrow from either BALB/B or B10 (*Lsh*^{s}) donors, the transfer of susceptibility was only partial (Fig. 2b, c). To determine whether this was due to incomplete chimaerism or the effects of background genes possessed by this *Lsh*^{r} strain, C57L mice were reconstituted with B10 (*Lsh*^{s}) bone marrow following exposure to 950 rads instead of 850 rads used previously (Table 2). In the same experiment, chimaeras were made reciprocally between B10 (*Lsh*^{s}) and congenic resistant [B10 (*Lsh*^{s}) × B10.*Lsh*^{r}]F_1 mice. This enabled a comparison of the extent to which susceptibility was transferred in C57L and congenic mice under identical experimental conditions (i.e. after irradiation with 950 rads).

Figure 3 shows that reconstitution with B10 bone marrow conferred greater susceptibility in F_1 mice than it did in C57L mice ($P < 0{\cdot}01$). In addition, control C57L mice were more resistant than control F_1 mice ($P < 0{\cdot}01$). Parallel infection of homozygous congenic B10.*Lsh*^{r} mice showed that the lowered resistance of F_1 mice over C57L mice was not related to heterozygosity of F_1 mice at the *Lsh* locus (log

Table 2. Experiments in which both resistance and susceptibility could be transferred into susceptible and resistant recipients respectively

	Recipient strain	H-2 haplotype	Dose of irradiation (rads)	Transfer of resistance	Transfer of susceptibility
Lsh^r	CBA/Ca	k	850	+	+
Lsh^s	BALB/K	k	750	+	+
Lsh^r	C57L	b	800	+	+
Lsh^s	BALB/B	b	750	+	+
Lsh^r	CBA/Ca	k	850	+	+
Lsh^s	B10.BR	k	950	+	+
Lsh^r	C57L	b	800	+	+
Lsh^s	B10	b	950	+	+

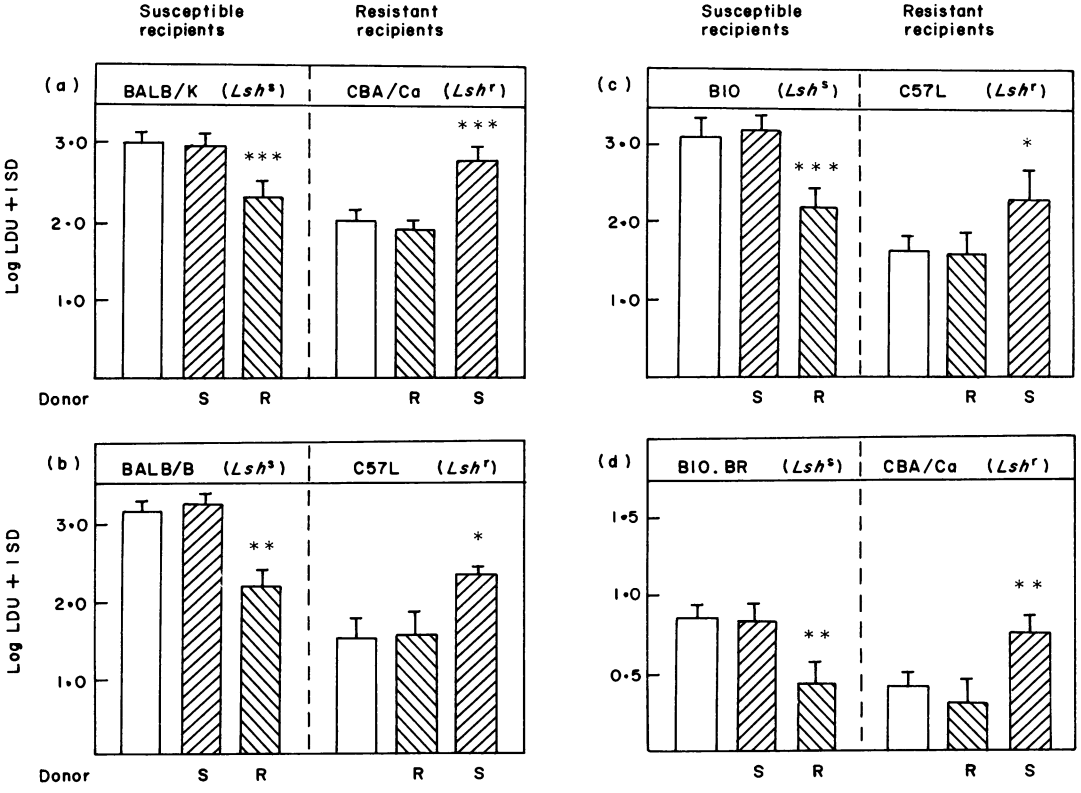

Figure 2. Transfer of resistance and susceptibility. *L. donovani* infections (LDU) measured in the livers of normal and chimaeric mice on day 15 after infection with 10^7 (a–c) or 10^6 (d) amastigotes. In all boxes, *left-hand panels* show mean LDU values + 1 SD for control *Lsh*^{s} mice and for *Lsh*^{s} mice exposed to 950 rads X-irradiation and reconstituted with bone marrow from syngeneic or H-2-compatible *Lsh*^{r} mice. *Right-hand panels* show mean LDU values + 1 SD for control *Lsh*^{r} mice and for *Lsh*^{r} mice exposed to 900 (CBA/Ca) or 800 (C57L) rads and reconstituted with bone marrow from syngeneic or H-2-compatible *Lsh*^{s} mice. (▨) Recipients of *Lsh*^{r} bone marrow; (■) recipients of *Lsh*^{s} bone marrow; (□) controls. $^*P<0{\cdot}01$, $^{**}P<0{\cdot}005$, $^{***}P<0{\cdot}001$ (Student's unpaired *t*-test).

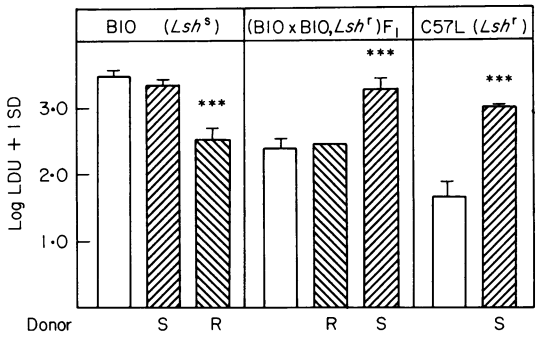

Figure 3. Transfer of resistance and susceptibility between congenic mice. *L. donovani* infections (LDU) measured in the livers of normal and chimaeric mice on day 15 after infection with 10^7 amastigotes. The *left-hand panel* shows mean LDU values +1 SD for control B10 (*Lsh*^{s}) mice and for B10 (*Lsh*^{s}) mice exposed to 950 rads X-irradiation and reconstituted with bone marrow from syngeneic or [B10 (*Lsh*^{s}) × B10.*Lsh*^{r}]F_1 mice. The *centre panel* shows mean LDU values +1 SD for control [B10 (*Lsh*^{s}) × B10.*Lsh*^{r}]F_1 mice and for F_1 mice irradiated and reconstituted with bone marrow from syngeneic or B10 (*Lsh*^{s}) mice. The *right-hand panel* shows mean LDU values +1 SD for control C57L (*Lsh*^{r}) mice and for C57L mice irradiated and reconstituted with bone marrow from B10 (*Lsh*^{s}) mice. (■) Recipients of *Lsh*^{r} bone marrow; (▨) recipients of *Lsh*^{s} bone marrow; (□) controls. ***$P < 0{\cdot}001$ (Student's unpaired *t*-test).

LDU+1 SD for $F_1 = 2{\cdot}40 + 0{\cdot}01$ and for B10.*Lsh*$^r = 2{\cdot}49 + 0{\cdot}13$). Thus, the increased resistance of C57L mice may be related to the effects of background genes.

DISCUSSION

The results presented here demonstrate that the cell type(s) mediating innate resistance and susceptibility to *L. donovani* are of bone marrow origin. It was of interest that while we were consistently able to transfer the susceptible phenotype into resistant recipients with bone marrow from susceptible donors, the reciprocal transfer of resistance took place in only five of eight experiments (Tables 1, 2). Successful transfer of resistance appeared to depend on two factors; the recipient mouse strain and the dose of irradiation. Thus, the relatively radiosensitive susceptible mouse strains BALB/B and BALB/K readily became more *Leishmania*-resistant with appropriate donor bone marrow (Fig. 2a, b), whilst the more radioresistant B10 strains remained susceptible to leishmaniasis (Table 1) unless the dose of irradiation was increased (Fig. 2c, d). These results may be explained by the presence in the chimaeras of a residual population of parasite-susceptible macrophages of recipient origin. These could support extensive parasite proliferation and consequently obscure any effect of the transferred resistant macrophages. Our data suggested that it was only possible to demonstrate transfer of resistance if the numbers of susceptible macrophages were decreased below some threshold as may have occurred following adequate levels of irradiation. Similarly, Hormaeche, Brock & Pettifor (1980) have demonstrated with *S. typhimurium* infections that mixed chimaeras possessing equal numbers of resistant and susceptible haematopoietic cells are phenotypically susceptible. It was therefore of interest that, in the present study, we were unable to transfer complete susceptibility into one particular *Lsh*^{r} strain (C57L; Fig. 2b, c). This contrasts with the other *Lsh*^{r} strains tested, all of which became fully susceptible after reconstitution with bone marrow from various *Lsh*^{s} donors. Our experiments with the F_1 congenic mice suggested that this decreased susceptibility of reconstituted C57L mice could be attributed to the effects of 'environmental' resistance genes possessed by this strain, and not to incomplete chimaerism. This conclusion is also supported by the observation that in the control groups, C57L mice were more resistant than the congenic mice. In addition, the successful transfer of resistance and susceptibility between the congenic F_1 (*Lsh*^{r}) and B10 (*Lsh*^{s}) mice provided firm evidence that the results obtained in this study were specifically related to expression of the *Lsh* gene, since these mice are identical except at this and closely related loci (Blackwell *et al.*, in preparation).

Besides the *S. typhimurium* model, other macrophage infections have been studied with radiation bone marrow chimaeras. Overall resistance and susceptibility to *Leishmania tropica* was transferred reciprocally between H-2-compatible resistant (B10) and susceptible (BALB) strains of mice (Howard, Hale & Liew, 1980). By contrast, innate resistance to *Listeria monocytogenes* could not be transferred with donor bone marrow. Radiation bone marrow chimaeras made reciprocally between resistant (B10.A) and susceptible (A/J) strains consistently retained the recipient phenotype suggesting that resistance to *L. monocytogenes* is a property of the host environment (Kongshavn, Sadarangani & Skamene, 1980). To exclude the possibility of incomplete chimaerism in these experiments, the authors proved that the reci-

pients possessed lymphocytes and NK cells of donor type. Nevertheless, it was not possible to prove that the cells naturally infected by *Listeria* (liver and spleen macrophages) had also been transferred.

These examples illustrate how radiation bone marrow chimaeras are a useful means of determining the level at which natural resistance genes are expressed *in vivo*. In the present study, we have demonstrated that natural resistance to *L. donovani* is determined by cells of haematopoietic origin. Since there is accumulating evidence that lymphocytes are not necessary for expression of the *Lsh/Ity* gene (Bradley & Kirkley, 1972; Hormaeche *et al.*, 1983; O'Brien & Metcalf, 1982), our results are consistent with the view that natural resistance to *L. donovani*, in contrast to *L. monocytogenes*, is an autonomous function of liver macrophages. Direct evidence in support of this view has recently been obtained in our laboratory by experiments demonstrating that isolated liver macrophages express *Lsh* gene activity *in vitro* (Crocker, Blackwell & Bradley, 1984, and in preparation). Future studies aimed at elucidating the mechanism of action of *Lsh* will undoubtedly focus on the macrophage as a potential source of valuable information.

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