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Inbred mouse strain resistance to *Mycobacterium lepraemurium* follows the *Ity/Lsh* pattern

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Summary. Inbred mouse strains and their F_1 hybrids infected intravenously with *Mycobacterium lepraemurium* showed different mean survival times (MST). BALB/c and C57BL mice were particularly susceptible, whereas C3H, CBA and DBA/2 mice were relatively resistant. Resistance as judged by MST was dominant in the F_1 hybrids. A similar ranking order was obtained by comparing the doubling time of the bacillus in the bone marrow, the increase in spleen weight between 4 and 12 weeks after infection, and the pathology of the liver during infection. The general pattern suggests that mouse resistance to *M. lepraemurium* is, at least in part, controlled by a gene with the same strain distribution as the genes for resistance to *Salmonella typhimurium* (*Ity*^{r}) and *Leishmania donovani* (*Lsh*^{r}) and the gene controlling resistance to *Mycobacterium bovis* BCG (*Bcg*). *Ity*, *Lsh* and *Bcg* are all known to be on chromosome 1, suggesting a centre controlling reactions to intracellular infections.

INTRODUCTION

Among a variety of inbred strains of mice the distribution of resistance to *Salmonella typhimurium* infection is identical to that of resistance to *Leishmania donovani* (Plant & Glynn, 1974; Bradley, 1974). The demonstration (Plant & Glynn, 1979; Bradley, Taylor, Blackwell, Evans & Freeman, 1979) that a major gene controlling resistance to *S. typhimurium*, *Ity*, and another for innate resistance to *L. donovani*, *Lsh*, were both situated on mouse chromosome 1, suggested that a region on this chromosome might be responsible for the control of a group of intracellular infections. This was made more likely with the demonstration (Forget, Skamene, Gros, Miailhe & Turcotte, 1981) that the *in vivo* multiplication of *Mycobacterium bovis* BCG was easily controlled by the salmonella- and leishmania-resistant strains C3H/HeCr, A/J and DBA/2, but much less so by the sensitive strains B10.A., C57BL/6 and BALB/c. A gene *Bcg* has been mapped to Chromosome 1 (Gros, Skamene & Forget, 1981; Skamene, Gros, Forget, Kongshavn, St Charles & Taylor, 1982).

Below we present data indicating that another intracellular infection, mouse leprosy, may also be controlled, at least in part, by the same chromosomal region.

The rodent leprosy bacillus, *Mycobacterium lepraemurium*, is an obligate intracellular parasite which causes a generalized infection of macrophages throughout the reticuloendothelial system. Once the infection becomes systemic, it is progressive and fatal whatever the strain of mouse. Resistance therefore is only a relative term which needs careful definition and cannot be expected to have exactly the same meaning

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as when applied to the other infections already mentioned. Nor is it surprising that there is disagreement in the literature (Closs & Lovik 1980) concerning the precise definition of *M. lepraemurium* susceptible and resistant strains, an essential requirement for the proper analysis of the expression of genes for resistance. The confusion arises largely because of the discrepancies between resistance as judged from survival times and resistance judged from the observed pathology of the infection. Differences in doses and routes of infection used by individual experimenters also make comparisons difficult.

MATERIALS AND METHODS

Bacteria

The Douglas strain of *Mycobacterium lepraemurium* used was maintained by regular passage through female CBA mice. Suspensions of live bacilli were prepared as required, by the method of Brown & Krenzien (1976), from heavily infected livers and spleens of mice infected intravenously 5–6 months before.

Inbred mice

The strains used, A/J, BALB/c, CBA/Ca, C3H/He, C57BL, DBA/2 and the congenic strains B10.D2 new and B10.D2 old line were all from stocks bred in the St Mary's Hospital Medical School Animal Department.

The F_1 hybrids (BALB/c × CBA), (BALB/c × C3H), BALB/c × C57BL), (CBA × C57BL) and (C57BL × DBA/2) were bred specially from the inbred strains. The strain given first in each hybrid is that of the female parent.

Survival times

Groups of between twenty-six and sixty-one female mice of the inbred strains, nineteen and thirty female mice of the hybrids and thirty-eight females of each of the congenic strains were injected intravenously, each mouse receiving 0·2 ml saline containing 10^7 freshly harvested *M. lepraemurium* bacilli.

Cages were inspected regularly and deaths due to infection recorded.

Bacterial growth rates in vivo

Groups of twenty mice were injected intravenously with 10^9 *M. lepraemurium*. Four mice from each strain were killed on days 1, 18, 28, 43 and 57 after infection. The number of acid fast bacilli (AFB) present in the bone marrow was determined by homogenizing the femoral bone marrow plugs of each mouse in saline and counting the number of AFB in spot smears of known dilutions as described by Brown & Krenzien (1976).

Growth curves of log AFB counted against time were approximately linear. Regression coefficients were calculated by the method of least squares and the doubling time of the organisms in each strain calculated from the formula: doubling time (days) = $[\log_{10}{}^2 (t_2 - t_1)]/(\log AFB_2 - \log AFB_1)$, where AFB_1 and $_2$ are the counts at times t_1 and t_2 (days).

Increase in spleen weight

Groups of two–four mice given 10^8 *M. lepraemurium* intravenously were killed at 4 and 12 weeks and the spleen weights recorded.

Histopathology

Mice given 10^8 *M. lepraemurium* were killed at six–eight weeks. The livers were fixed in Carnoy's solution, sectioned and stained with Haematoxylin and Eosin or Ziehl-Neelsen. Sternums were treated similarly but in addition were decalcified.

RESULTS

Mortality experiment

The survival times of different mouse strains given 10^7 *M. lepraemurium* intravenously (i.v.) are shown in Table 1.

DBA/2 survived the longest with a mean time to death (MTD) of 200 days and BALB/c the shortest,

Table 1. Survival time of inbred mouse strains given 10^7 *M. lepraemurium* intravenously

Ity/Lsh genotype	Strain	No. tested	Mean survival time (days ± SD)
rr	DBA/2	32	$195^a \pm 25$
rr	CBA	61	$154^b \pm 16$
rr	C3H	26	$147^c \pm 8$
	all *Ity*^{r}	119	$163^d \pm 26$
ss	C57BL	33	$130^e \pm 13$
ss	BALB/c	48	$115^f \pm 4{\cdot}5$
	all *Ity*^{s}	81	$121^g \pm 11$

Comparisons of pairs by *t* test give: ab, ce, dg, ef $P < 0{\cdot}001$; bc $P < 0{\cdot}05$.

MTD 115 days. The salmonella-leishmania- resistant strains DBA/2, C3H and CBA all survived significantly longer than the salmonella- leishmania- sensitive strains C57BL and BALB/c ($P<0{\cdot}001$).

As expected the congenic strains B10.D2 new and old lines behaved like C57BL (Table 2). The B10.D2 old line which only differs from the new in the lack of the complement component C5, was slightly more susceptible, but the difference was not statistically significant.

The survival times of the hybrid strains all suggest that resistance, as measured by survival time, is dominant to susceptibility as it is with salmonella and leishmania infections (Table 2). The (CBA × C57BL), (C57BL × DBA/2) and the (BALB/c × C3H) F_1 hybrids are each slightly but significantly more resistant than their respective resistant parents suggesting the possibility of complementation. Similarly the survival time of the (BALB/c × C57BL) F_1 hybrid is the same as C57BL and significantly longer than BALB/c.

Table 2. Survival time of congenic and F_1 hybrid strains given 10^7 *M. lepraemurium* intravenously.

Ity/Lsh genotype	Strain	No. tested	Mean survival time (days ± SD)
ss	B10.D2 new line	34	130[h] ± 17
ss	B10.D2 old line	39	125[i] ± 9
sr	(C57BL × DBA/2) F_1	19	208[j] ± 11
rs	(CBA × C57BL) F_1	29	189[k] ± 13
sr	(BALB/c × C3H) F_1	21	160[l] ± 5
sr	(BALB/c × CBA) F_1	21	153[m] ± 8
ss	(BALB/c × C57BL) F_1	19	136[n] ± 5

Comparisons of pairs (including Table 1) by *t* tests give: he, hi, ie, not significant; ja $P<0{\cdot}05$; je $P<0{\cdot}001$; kb, ke, lc, lf, $P<0{\cdot}001$; mb not significant; mf $P<0{\cdot}001$; ne not significant; nf $P<0{\cdot}001$; nm $P<0{\cdot}001$

Table 3. Doubling time of *M. lepraemurium* in bone marrow

Ity/Lsh genotype	Strain	Doubling time* (days)
rr	C3H	6·0
rr	CBA	4·4
rr	A	4·1
ss	C57BL	3·6
ss	BALB/c	2·7
sr	(C57BL × CBA)F_1	4·0

* Calculated from slopes of regression lines of viable counts at 1, 18, 23 and 43 days after i.v.i. 10^9 bacteria.

Examination of the regression lines by Bartlett's test showed heterogeneity of variance. Detailed comparison by analysis of covariance of all possible pairs of regression lines showed significant differences in slope ($P<0{\cdot}01$) except between A, CBA, C57BL and (C57BL × CBA) F_1 where the differences were not significant.

In vivo multiplication of *M. lepraemurium*

Table 3 gives the doubling times for *M. lepraemurium* based on viable counts on bone marrow over a period of 6 weeks. The appearance of the marrow at 7 weeks is shown in Fig. 1. Note that the challenge dose was 10^9 i.v.i., i.e. 100-fold that used for the mortality experiment. This may well have blurred the results somewhat. Nevertheless, the general ranking is clear and supports the conclusions drawn from the survival data. The doubling time in the (CBA × C57BL) F_1 hybrid is the same as in the CBA parent not slower.

The doubling times are shorter than those usually given (Brown & Krenzien 1976). Since the times are net, i.e. the result of multiplication plus killing, they suggest that little killing occurs in the marrow.

Spleen weights

Among the parental lines the increase in spleen weight in the resistant strains is significantly smaller than in the sensitive, although there are considerable individual differences (Table 4). For example, among the resistant strains C3H shows twice the increase of DBA/2, while among the sensitive strains the increase in BALB/c is one and one half times that of C57BL.

Of the congenic strains B10.D2 new line is close to C57BL. B10.D2 old line shows a smaller increase, but with only two mice tested the significance is uncertain.

The hybrid strains show increases in between that of their parents with no evidence of dominance.

Histopathology

A detailed description of the pathology of *M. lepraemurium* infection in the various mouse strains will be given elsewhere. However, a preliminary comparison of the livers of the infected mice after 7 weeks infection showed profound differences between resistant and

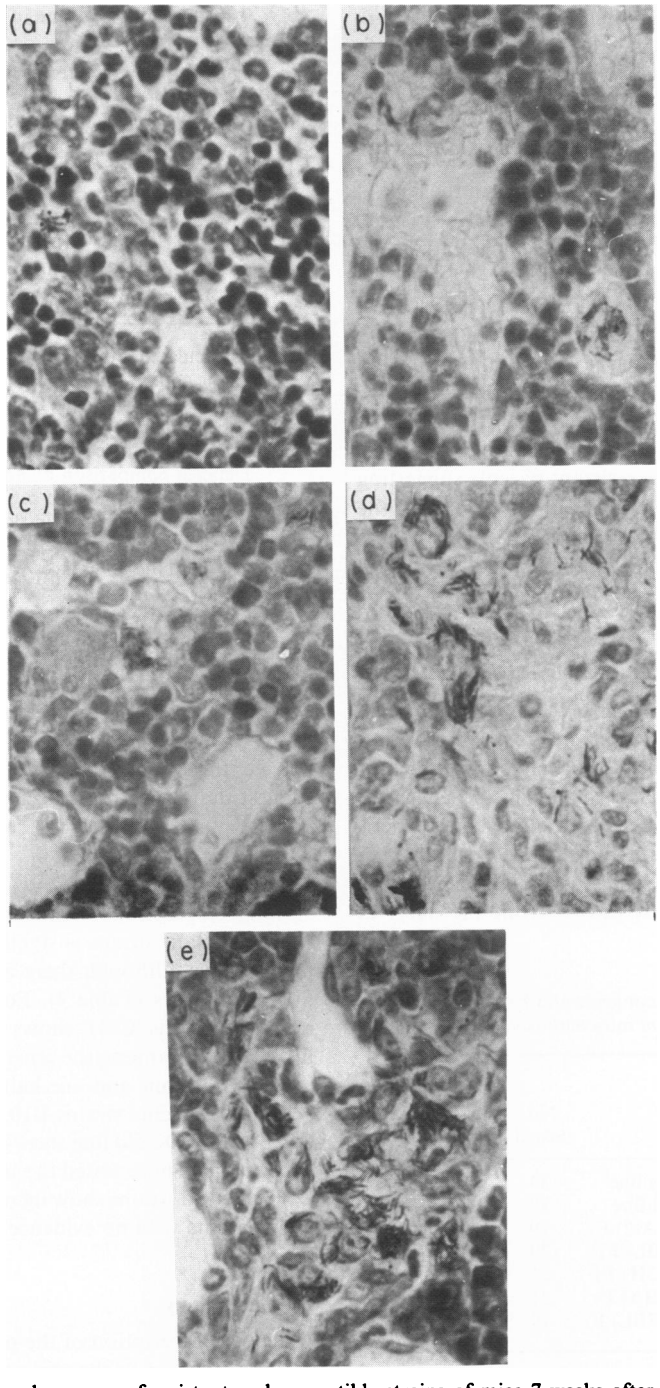

Figure 1. Sections of sternal marrow of resistant and susceptible strains of mice 7 weeks after 10^9 *M. lepraemurium* i.v.i. Ziehl-Neelsen stain for acid-fast bacilli (AFB). There are relatively few AFB in strains DBA/2 (a), CBA (b), and C3H (c) compared with C57BL (d) and BALB/c (e). (Magnification × 250.)

Table 4. Increases in spleen weights in infected mice

Ity/Lsh genotype	Strain	No. tested	Increase in spleen weight between 4 and 12 weeks
rr	DBA/2	4	124[a]
rr	CBA	4	183[b]
rr	C3H/He	4	245[c]
	All *Ity*^{r}		184[d] ± 72
ss	C57BL	4	406[e]
ss	BALB/c	4	628[f]
	All *Ity*^{s}		517[g] ± 134
sr	(BALB/c × CBA) F_1	4	305[m]
rs	(CBA × C57BL) F_1	4	321[k]
ss	(BALB/c × C57BL) F_1	2	459
ss	B10. D2 new line	2	448
ss	B10. D2 old line	2	312

Comparisons of pairs by t tests give; dg, mf, $P < 0{\cdot}001$; ce, ef, $P < 0{\cdot}01$; ac, bc, mb, kb, ke, $P < 0{\cdot}05$; ab not significant.

sensitive strains in the number and size of granulomas (Fig. 2). In BALB/c mice which again appeared to be the most susceptible, liver granulomas are composed mostly of macrophages with very few lymphocytes at the periphery. Acid fast stain shows masses of bacilli within the granuloma macrophages and clumps of bacilli within Kupffer cells. C57BL mice showed the largest number of granulomas in the study. They were composed of a mixture of epithelioid cells and macrophages and a large number of lymphocytes. Acid fast bacilli were fewer in number inside the granuloma macrophages and were relatively rare in Kupffer cells.

The liver granulomas in DBA/2, CBA and C3H mice were smaller than in BALB/c and C57BL mice and the proportion of lymphocytes was higher. Acid fast bacilli were present in smaller numbers overall and were more localized in clumps. Examination of livers at other times suggests that lesions develop more slowly in the resistant than in the sensitive strains.

DISCUSSION

When resistance of mice to *M. lepraemurium* is defined by their survival time after intravenous infection, mouse strains can be ranked in an order similar to that obtained by comparing *M. lepraemurium* doubling times in the bone marrow and the spleen weight increases between 4 and 12 weeks after infection. The biggest increases occur in the least resistant mice. Mouse strains resistant to *S. typhimurium* and *L. donovani*, ie *Ity*^{r}, *Lsh*^{r} are found at the resistant end of the lepraemurium scale and *Ity*^{s}, *Lsh*^{s} strains at the sensitive end. However, the detailed ranking differs. For example, of the salmonella-resistant strains DBA/2 is the least resistant to *S. typhimurium*, but the most resistant to *M. lepraemurium*.

Nevertheless, the general pattern strongly suggests that mouse resistance to *M. lepraemurium* is, at least in part, controlled by a gene with the same strain distribution as *Ity*^{r} and *Lsh*^{r} (Plant & Glynn 1979, Bradley *et al.*, 1979) and the *Bcg* gene controlling resistance to BCG (Forget *et al.*, 1981, Gros *et al.*, 1981). Moreover the hybrid strains show that as in the other three infections resistance to *M. lepraemurium* is dominant.

Whatever mechanism is controlled by the *M. lepraemurium* resistance gene, it is less effective than the corresponding system dealing with salmonella infection, since all the *M. lepraemurium* mice eventually die. Nevertheless the two groups of strains respectively resistant and susceptible to salmonella, leishmania and BCG infection show a clear and significant difference in resistance to *M. lepraemurium*. However, nearly all strains remain separate from their neighbours. The findings indicate that many genes are involved in resistance, blurring but not completely hiding the effects of the gene postulated here as equivalent to *Ity/Lsh/Bcg*.

The relatively large infecting doses (10^{7-9} i.v.i.) used also blur the distinction between resistant and sensitive strains. We do not know whether the intravenous injection of very small numbers of viable bacilli would reveal an ability to sterilize infection in some strains as has been reported for subcutaneous infections (Alexander & Curtis, 1979). Previous experience with this infection (Brown & Krenzien, 1976) suggests that it would not. An estimated inoculation of one–ten viable organisms produced bone marrow counts of 10^7 in 23 weeks, from which level of infection mice would certainly die. Nevertheless, the use of such small inocula and an adaptation of the counting method proposed by Brown & Krenzien (1976) may be of value in assessing the susceptibility of mouse strains. It is interesting that small intravenous doses of BCG separate mouse strains into resistant and susceptible groups on the basis of the spleen count at 28 days (Forget *et al.*, 1981). However, the characteristics of the two mycobacterial infections are different. BCG is

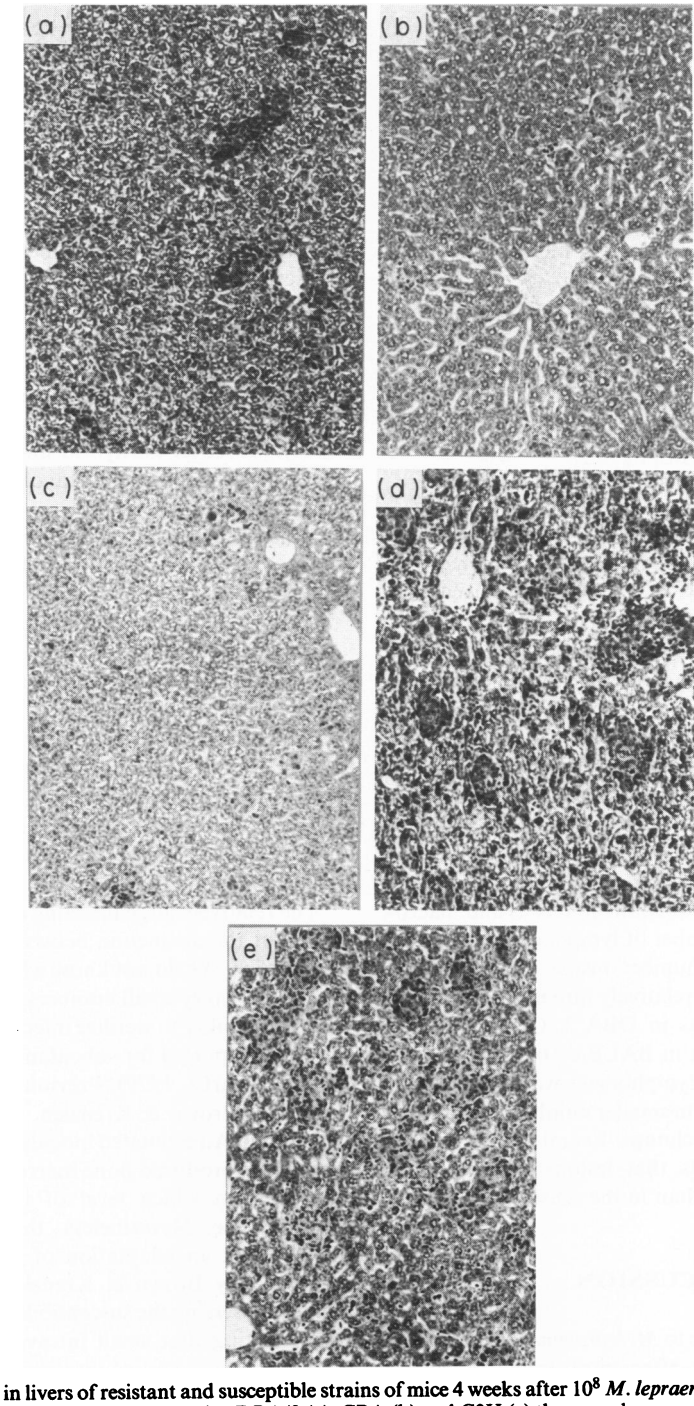

Figure 2. Granulomas in livers of resistant and susceptible strains of mice 4 weeks after 10^8 *M. lepraemurium* i.v.i. Haematoxylin and Eosin stain. (Magnification × 40.) In strains DBA/2 (a), CBA (b) and C3H (c) the granulomas are smaller and less frequent than in strains C57BL (d) and BALB/c (e). The liver tissue is essentially intact in (a), (b) and (c), but the normal pattern is distorted in (d) and (e).

avirulent for mice unless massive doses, in excess of 10^9 viable organisms are given intravenously.

Our data on the hybrids mean survival times also provide some more specific indications that several genes are involved. Complementation, though probably by different genes, has also been described for resistance to salmonella infection by Plant & Glynn (1980).

Where we have used the same strains our results agree with those of Lefford *et al.* (1977) who also gave *M. lepraemurium* intravenously and classed DBA/2, C3H/HeJ and A/J as resistant, C57BL/6 and BALB/c as susceptible on the basis of 50% survival times. Different results were reported by others (Closs & Haugen 1974; Lagrange & Hurtrel, 1979; Alexander & Curtis, 1979) who classed C57BL as resistant and C3H and BALB/c as susceptible. This view was based on footpad inoculation and interpretation of lesion histology in terms of cell-mediated immune responses. Our histological findings in the liver are compatible with the results of Closs & Haugen (1974) but show the importance of looking over a long time period.

Possible effects due to the use of different C3H sublines need to be excluded but would not explain the results in other strains. There has been much speculation on possible immunological reasons for the experimental differences (Lefford *et al.*, 1977, Lagrange & Closs, 1979), but further work is needed to disentangle several simultaneous complex processes. However it is no longer safe or adequate to use just C57BL and C3H as representative 'susceptible and resistant' strains in *M. lepraemurium* research.

Our object here is to show that whatever else is going on, one aspect of resistance to *M. lepraemurium* closely resembles in its strain distribution resistance to *S. typhimurium*, *L. donovani* and *M. bovis BCG*. This gives further support to the suggestion (Plant & Glynn, 1979) that a group of genes on mouse chromosome 1 may control resistance to certain intracellular infections. In the four so far described the common factor most likely to be relevant is that all the causative agents pass some time inside macrophages. It is worth noting, however, that infection due to *Listeria monocytogenes* or Rickettsiae have different patterns of resistance in mice (Cheers & McKenzie 1978; Skamene, Kongshavn & Sachs, 1979; Groves & Osterman, 1978).

Plant & Glynn (1974) suggested that *Ity* controlled the development of cell-mediated immunity to *S. typhimurium*. Hormaeche (1979) postulated that it controlled early growth of salmonella in macrophages. The two views are not incompatible. Cell-mediated immunity is induced very early and could affect bacterial growth well before it was detectable by other means such as delayed hypersensitivity. Equally the way in which macrophages process and present antigens may affect the development of cell-mediated immunity perhaps by altering the balance of stimulation of effector and suppressor T cells.

It is not yet known whether *Ity* and *Lsh* are closely linked genes or are actually identical. There is no good evidence that they are separate (Plant, Blackwell, O'Brien, Bradley & Glynn, 1982). Formal proof that a gene controlling resistance to *M. lepraemurium* exists and is located on chromosome 1 is still to come.

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