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Regulation of *Leishmania* populations within the host

II. GENETIC CONTROL OF ACUTE SUSCEPTIBILITY OF MICE TO *LEISHMANIA DONOVANI* INFECTION

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SUMMARY

The acute growth rates of *Leishmania donovani* populations in twenty-five inbred mouse strains fall into two distinct groups: the susceptible (S) and the resistant (R). Hybrids within either category resemble their parents in susceptibility. Hybrids between categories are moderately resistant. Back-crossing of F_1 hybrids to R and S parents, and interbreeding the F_1 generation, give susceptibility ratios consistent with single gene control of acute susceptibility to visceral leishmaniasis. The distribution of this character among inbred mouse strains does not correspond to any well-studied gene nor does it appear to be linked to the H2 histocompatibility locus.

INTRODUCTION

We have shown (Bradley & Kirkley, 1977) that different mouse strains vary in their responses to *Leishmania donovani*, an intracellular parasite of the mononuclear phagocytes. Parasite numbers in the liver were followed after intravenous infection of mice. It was suggested that the course of infection could be divided into two parts. There was variation in the acute growth rate of the parasite population during the first 3 weeks, and in the subsequent fate of the parasites.

Here we consider the acute phase (the first 2–3 weeks) in detail. The seven mouse strains previously observed tended to show one of two immediate growth patterns: either parasite numbers increased less than 4-fold between days 1 and 15, or the increase exceeded 50-fold. The literature also suggests a wide variation in the growth rate of *L. donovani* in mice (Actor, 1960). We here examine the acute growth rate of the parasites in twenty-five inbred mouse strains, and still find that only two patterns are observed. We have carried out breeding experiments and conclude that a single gene or linkage group controls acute susceptibility to *Leishmania donovani* in the mouse.

MATERIALS AND METHODS

Mice. Inbred mouse strains in a specific pathogen-free state were obtained from the Medical Research Council Laboratory Animals Centre, Carshalton, U.K., through the kindness of Dr M. Festing, and are listed in Table 2.

He also provided six F_1 hybrids between strains and some outbred lines. Additional mice of five strains were obtained from the Jackson Laboratory, Bar Harbor, Maine, through Dr B. A. Taylor. PO and Ash mice were from the Sir William Dunn School of Pathology colony, and the breeding experiments described in the Results section were carried out there from Carshalton stock.

Parasites. The L82 strain of Ethiopian *Leishmania donovani* was used. Details of its history and mode of preparation have been described (Bradley & Kirkley, 1977). The fourteenth and sixteenth hamster passages were used in the main experiments, in May and October 1972, and 10^7 amastigotes were injected intravenously into the mice in 0·2 ml of Medium 199 containing 5% foetal calf serum. A small group of mice was killed on the day following injection, and the remainder were killed at or near day 15 after injection.

Preparation and counting of slides. This was carried out as previously described (Bradley & Kirkley, 1977) on liver imprints and the counts expressed as Leishman-Donovani units (LDU) per liver (Actor, 1960). All counts were performed 'blind'.

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RESULTS

Acute growth rates in various mouse strains

Three mice of each strain tested were injected with 10^7 parasites and killed 15 days later. Mice of three strains were killed on day 1. Liver parasite counts on the first day showed no significant difference between a highly susceptible (PO), a resistant (CBA) and a highly resistant (Ash) strain (Table 1). The mean count was 31·5 LDU, close to the 35 LDU expected if 70% of the inoculum had localized in the liver and no appreciable multiplication had yet occurred (Stauber, 1958). Three mice from each of

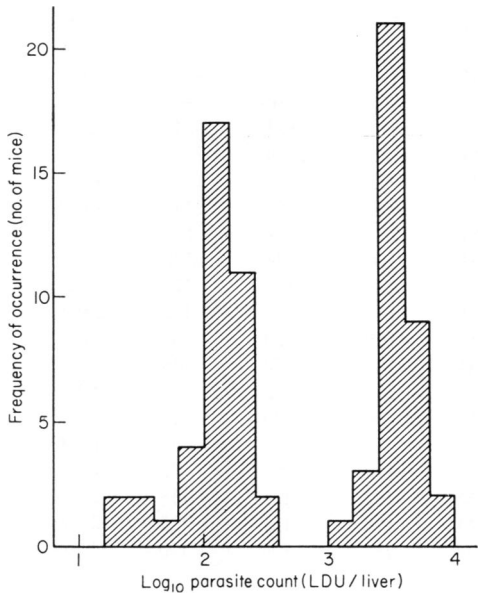

FIG. 1. Histogram of the distribution of parasite counts in seventy-five mice of twenty-five inbred strains 14–16 days after infection with *L. donovani*. The counts, in LDU per liver, are set along a logarithmic scale.

twenty-five inbred strains were killed on day 15. When the parasite counts per liver from the seventy-five mice were plotted as a histogram, two well-marked peaks were apparent (Fig. 1). The results by strain are in Table 2. The means and variances are related by an equation of the type:

$$F = s^2 = a\bar{x}^b.$$

Regression of the log variance on the log mean gave a value for b of 1·86 and a highly significant correlation ($r = 0{\cdot}90$). This indicates (Taylor, 1961) that logarithmic transformation of the counts is appropriate to normalize the distributions and render the variance independent of the mean. Following transformation the regression became flat and insignificant. The mean logarithmic counts and their standard errors

TABLE 1. Parasite numbers in the liver 24 h after infection with 10^7 amastigotes intravenously, in the experiment on many mouse strains

Mouse strain	No. of mice	Mean count (LDU)	Mean log count (LDU)	Log count (s.d.)
PO	4	34·5	1·514	0·167
CBA	4	26·8	1·422	0·129
Ash	3	34·0	1·499	0·211
Total	11	31·5	1·474	0·156

TABLE 2. Parasite numbers in livers of mice from twenty-five inbred strains 14–16 days after infection with 10^7 amastigotes

Mouse strain	Sex	Counts of parasites in LDU/liver at 14–16 days			Mean log count	Log count(s.d.)	Increase (ratio to day 1 level) from day 1 to days 14–16
BALB/c	F	3699	3616	3000	3·534	0·050	115·1
CE	F	4035	3467	2646	3·523	0·093	111·9
C57BL	M	4263	3476	3353	3·565	0·056	123·3
C57BL/10 Sc Sn	F	6006	4891	4718	3·714	0·057	173·8
BIO.A	M	5659	3476	3310	3·605	0·129	135·2
BIO.BR	M	3597	3556	2015	3·470	0·144	99·1
BIO.D2-new	M	8350	6372	4509	3·793	0·134	208·4
BIO.LP-*a*	M	5066	2540	1445	3·423	0·273	88·9
DBA/1	M	5476	3474	3241	3·597	0·124	132·7
ICFW	M	3882	2236	2220	3·428	0·139	90·0
NMRI	F	2905	2892	2861	3·460	0·003	96·8
NZW	M	5096	3891	3455	3·612	0·087	137·4
A	M	160	157	129	2·170	0·052	5·0
AKR	M	158	154	138	2·175	0·031	5·0
A2G	M	278	198	175	2·328	0·104	7·1
CBA/Ca	M	148	116	112	2·095	0·066	4·2
CBA/H-T6	M	165	130	60	2·037	0·230	3·7
C3H/He	M	181	170	145	2·217	0·050	5·5
C3H/He-*mg*	M	201	196	142	2·249	0·084	6·0
C57BR/cd	M	36	20	17	1·363	0·171	0·8
C57L	M	87	73	37	1·790	0·196	2·1
DBA/2	F	110	93	78	1·967	0·075	3·1
F/ST	M	202	121	118	2·153	0·132	4·8
NZB	M	198	151	123	2·189	0·104	5·2
129Rr/J	M	252	196	130	2·269	0·145	6·2
Hybrid strain							
BALB/C × C57BL/10 Sc Sn	M	4899	3684	2750	3·565	0·125	123·3
C57BL/10 Sc Sn × DBA/1	M	8995	8055	6366	3·888	0·077	259·4
BALB/c × DBA/2	F	3312	2640	2544	3·449	0·062	94·4
C3H/He × DBA/2	M	217	131	90	2·136	0·192	4·6
NZB × NZW	M	491	450	290	2·602	0·123	13·4

are depicted in Fig. 2. Clearly, the inbred mouse strains separate into two main categories of parasite load which do not overlap at all. One group of thirteen strains, subsequently called resistant (R), have a less than 8-fold increase in the liver, while the multiplication of parasites in the other twelve susceptible (S) strains exceeds 80-fold (Table 3). The highest individual count per liver in the R group was 278 LDU. The doubling time varied from 2 days in the S mice to 6 days in the resistant ones, assuming a steady multiplication from day 1.

Among the five F_1 hybrid strains tested (Table 2), three crosses were between two susceptible strains, and in all cases the hybrids were highly susceptible. The single hybrid between two resistant strains was resistant. The remaining group of F_1 mice, hybrid between resistant NZB and susceptible NZW mice, showed an intermediate parasite growth rate, with a 13·4-fold increase.

Outbred mice gave more variable counts. LAC G was highly susceptible and LAC A of mixed susceptibility. The mice here recorded as HR (Fig. 2) were a mixed stock of truly hairless mice crossed with NMRI, and furred stock heterozygous for the *hr* gene used in this experiment, whereas hairless individuals were used in the preceding study (Bradley & Kirkley, 1977).

The inference drawn from these results was that there are two categories of inbred mice, R and S, suggesting that genetic control of acute susceptibility of *L. donovani* in the mouse is relatively simple. All these findings were consistent with a single gene or linkage group controlling the greater part of

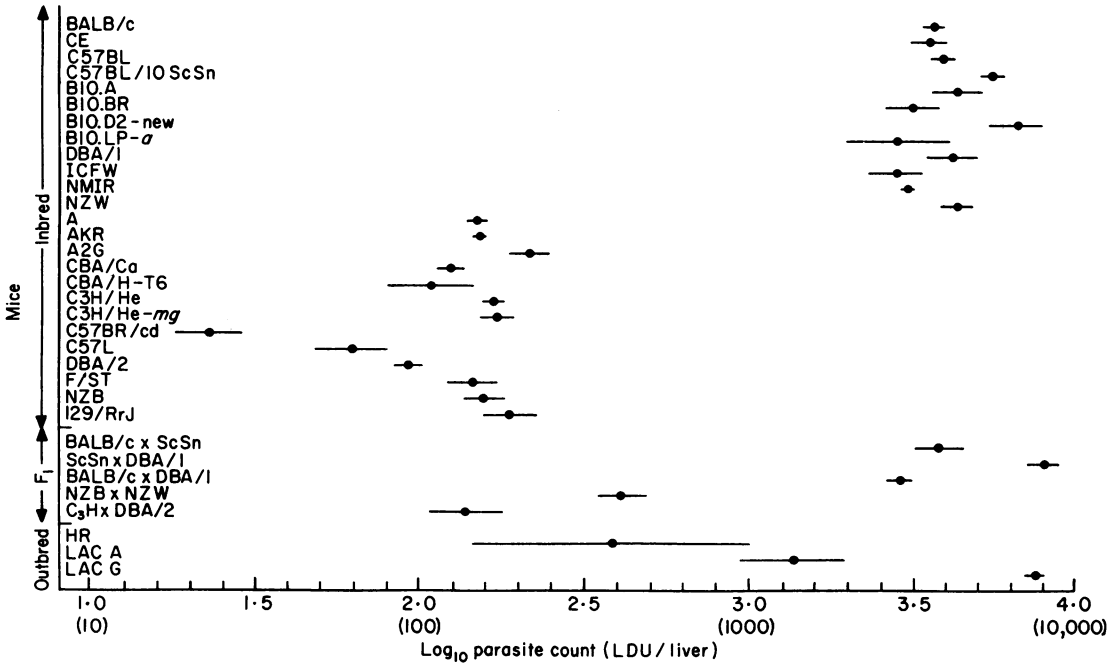

FIG. 2. Liver parasite burdens in groups of three mice, 14–16 days after infection with *L. donovani*, of inbred hybrid and outbred mouse strains. Each point indicates the logarithmic mean, and the bar extends one standard error on each side.

acute susceptibility to leishmaniasis, since there were no intermediates between R and S in inbred strains and a hybrid F_1 between them supported an intermediate rate of parasite growth. To test the hypothesis of single gene control, the susceptibility of F_2 and back-cross mice was determined.

Reproducibility and environmental factors

To determine how far the results obtained so far might depend on environmental factors, similar growth experiments were repeated with inbred mice from the Jackson laboratory, Bar Harbor, using five mouse strains as close as possible genetically to five of the twenty-five inbred lines used already. The results (Table 4) show that not only did the mice fall into the same categories, three resistant and two susceptible, regardless of the place of origin of the strain, but the ranking of the strains in each category was the same. Using logarithmically transformed counts there was a high degree of correlation ($r = 0{\cdot}98$) and the slope of the regression lines was not significantly different from 1·0 ($p = 0{\cdot}7$). Thus experiments using mice of the same genetic strains but of different origin and carried out at different times gave concordant results.

TABLE 3. Mean increases in parasite numbers from day 1 to day 15 in livers of mice in each of three resistance categories

Category	No. of mice	Mean log of counts (LDU)	Increase from day 1 (ratio to day 1 level)
Susceptible (S)	39	3·560	121·9
Hybrid (S×R)	3	2·602	13·4
Resistant (R)	36	2·077	5·0

TABLE 4. Parasite growth in genetically similar mouse strains from different environments. Mean logarithmic parasite counts (LDU) in mouse liver 15 days after infection with 10^7 amastigotes intravenously. Three mice in each group

Mouse strain	Mouse breeding place	
	MRCLAC,* Surrey, U.K.	Jackson laboratory, Maine, U.S.A.
DBA/1	3·60	4·00
C57BL	3·57	3·78
C3H	2·22	2·66
DBA/2	1·97	2·53
C57L	1·79	2·06

* Medical Research Council Laboratory Animals Centre.

In other experiments CBA mice from two other laboratories and C3H mice from one other have been used and results were again concordant, whilst acute growth rates have been repeatedly determined in descendants of NMRI and C3H mice over 18 months without change of resistance category.

Breeding experiments

Mice resistant to leishmaniasis, of strain C3H/He-*mg*, were crossed with highly susceptible female NMRI inbred mice. F_1 progeny were back-crossed to each parental line and were also interbred to give the F_2 generation. These groups of mice were all given 10^7 amastigotes of *L. donovani* intravenously on the same day and were killed on days 14–16 after infection. Counts of the liver parasites were made. Small groups of the F_1 and parental lines were included in the experiment as controls and a baseline group of mice was killed on day 1. In a separate experiment more F_1 mice were compared with the resistant parents for parasite growth over 15 days.

The F_1 mice were found to be slightly more susceptible than C3H parents (Table 5), confirming the result of the R × S cross in the multiple strain experiment, with the F_1 load 2·4 times that of the R mice, as compared with 2·68 times in the NZB × NZW cross.

The results of the main experiment are set out in Fig. 3, which shows that the logarithmically transformed counts for the F_2 and back-cross to susceptible mouse populations fall into two distinct categories which do not overlap. The right-hand peaks in the histogram are identical in position with the mean counts from susceptible NMRI mice, while the remaining mice have similar counts to inbred resistant strains and F_1 hybrids. 22% of the F_2 and 38% of the back-cross to susceptible mice are highly susceptible, not significantly different from the 25% and 50%, respectively, expected on the basis of single gene control (Tables 6 and 7). The proportion of susceptible mice is thus consistent with a single gene controlling susceptibility.

TABLE 5. Parasite numbers in resistant and F_1 (resistant × susceptible) hybrid mice 15 days after infection

Mouse strain	No. of mice	Sex	Mean log count	Log count (s.d.)	Mean log sexes continued	
C3H	3	M	2·192	0·205		
C3H	3	F	2·192	0·153	2·192	
C3H × NMRI FI	4	M	2·593	0·137		$P < 0·001$
C3H × NMRI FI	3	F	2·537	0·046	2·569	

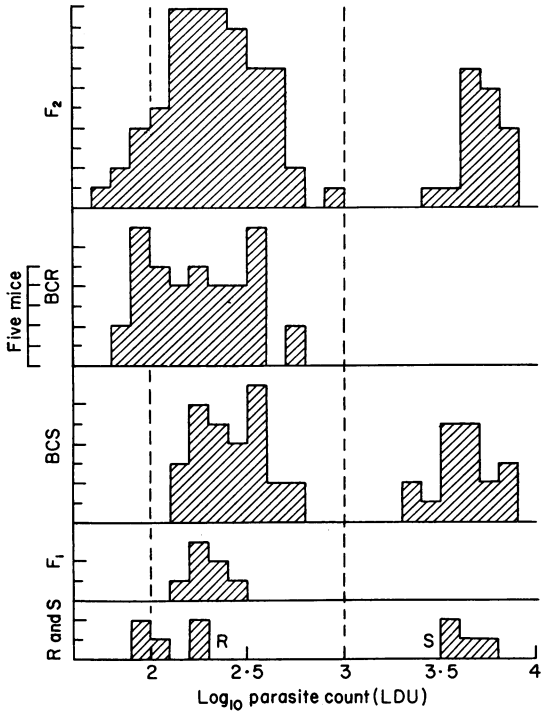

FIG. 3. Histogram of the distribution of parasite counts in the livers of mice in the breeding experiment for the F_2 generation, back-crosses to each parental strain (BCS, back-cross to susceptible strain; BCR, back-cross to resistant strain), and small groups of resistant (R), susceptible (S) and F_1 control mice.

In this experiment, there is evidence of a small sex difference in parasite counts, shown by all three groups and both high and low count categories (Table 8). The difference overall is between 0·09 and 0·15 log LDU depending on which groups are included in the calculation. The mean logs of the susceptible groups in F_2, back-cross to S and S control are very similar, as are their variances, and the mean log count for the F_1 hybrids is very close to the mean log for the more resistant mice in the back-cross to susceptible group (Table 8).

Evaluation of the more resistant cluster of mice in the F_2 generation and the whole group of back-crosses to resistant parents is more difficult as the distributions of counts for resistant and F_1 mice tend to

TABLE 6. Parasite numbers in F_2 and back-cross mice from breeding experiment, to show numbers of mice in various resistance categories

Mice examined	F_2	BCS*	BCR†
No. of Mice examined	87	47	40
Mice with count > 1000 LDU	19	18	0
Mice with count ⩽ 1000 LDU	68	29	40
Mice with 159–1000 LDU	46	26	22
Mice with 1–158 LDU	22	3	18
No. of male mice	44	23	20
Male mice with count > 1000 LDU	8	8	0
No. of female mice	43	24	20
Female mice with count > 1000 LDU	11	10	0

* Back-cross to susceptible strain.
† Back-cross to resistant strain

TABLE 7. Observed percentage distribution of mice between three resistance groups (susceptible are quite distinct; intermediate and resistant tend to merge) compared with expectation on the hypothesis of single gene control

Mouse		Resistant	Intermediate	Susceptible
F_2	Expected	25	50	25
	Observed	25	53	22
BCS*	Expected	0	50	50
	Observed	6	55	38
BCR†	Expected	50	50	0
	Observed	45	55	0

* Back-cross to susceptible strain.
† Back-cross to resistant strain.

overlap. The pattern has been analysed in three ways: (a) assume on the basis of data on parental and F_1 strains in Fig. 3 and elsewhere that the expected 'trough' of counts between resistant and F_1 strains in that experiment is at $\log_{10}$ LDU of 2·2 (158·5 LDU). The distribution of counts is given in Table 7. (b) Use independent estimates from several experiments for the mean log and its standard deviation for the R and F_1 populations. Generate the expected distributions of log counts on the single gene hypothesis and compare it with the observed counts for the F_2 and back-cross to R (Fig. 4). The observed counts have been corrected for the sex difference described above. There is seen to be close agreement between observed and expected counts. (c) Generate the expected distribution in the F_2 generation (one truly resistant phenotype to two F_1 phenotypes) by combining the back-cross to resistant (expected one resistant to one F_1 type) with half the number of counts from the resistant portion of the back-cross to susceptible (expected to be all F_1). This is done in Fig. 5.

By all three methods of analysis the counts observed are seen to fit closely to those predicted on the basis of Mendelian inheritance of a single gene or linkage group controlling susceptibility to *L. donovani* infection in the mouse liver.

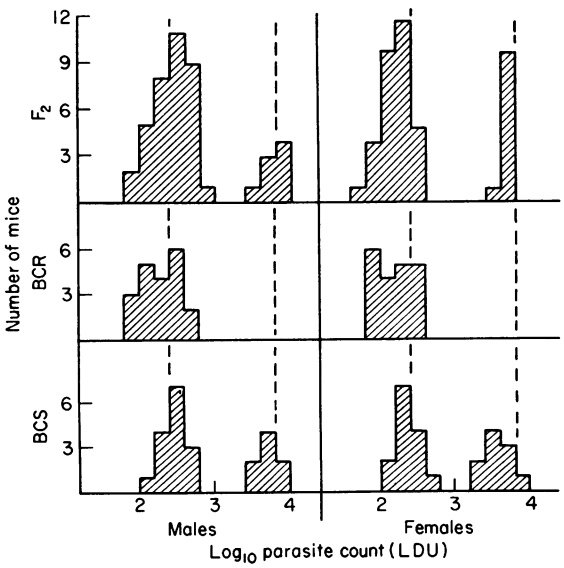

FIG. 4. Histogram of observed parasite counts (solid lines) in F_2 and back-cross mice 14–16 days after infection compared with predictions on a single gene hypothesis (broken line), using independent estimates of mean and standard deviation of log counts for resistant and heterozygous populations (BCS and BCR as in Fig. 3). The separate groups of susceptible mice are omitted from the diagrams.

TABLE 8. Characteristics of the groups of parasite counts in breeding experiments (corresponding to the separate peaks in Figs 3 and 4)

	High count groups					Medium count groups (similar to F_1)		
	F_2 generation		Back-cross to susceptible			Back-cross to susceptible		
Characteristics	Males	Females	Males	Females	Susceptible females	Males	Females	F_1 generation females
No. of mice	8	11	8	10	4	15	14	7
Mean log of counts	3·752	3·670	3·685	3·570	3·612	2·448	2·375	2·290
Variance of log counts	0·015	0·004	0·014	0·025	0·008	0·031	0·028	0·009
Difference between sex means	0·082		0·115		—	0·073		—
Overall mean log	3·70		3·62		3·61	2·41		2·29

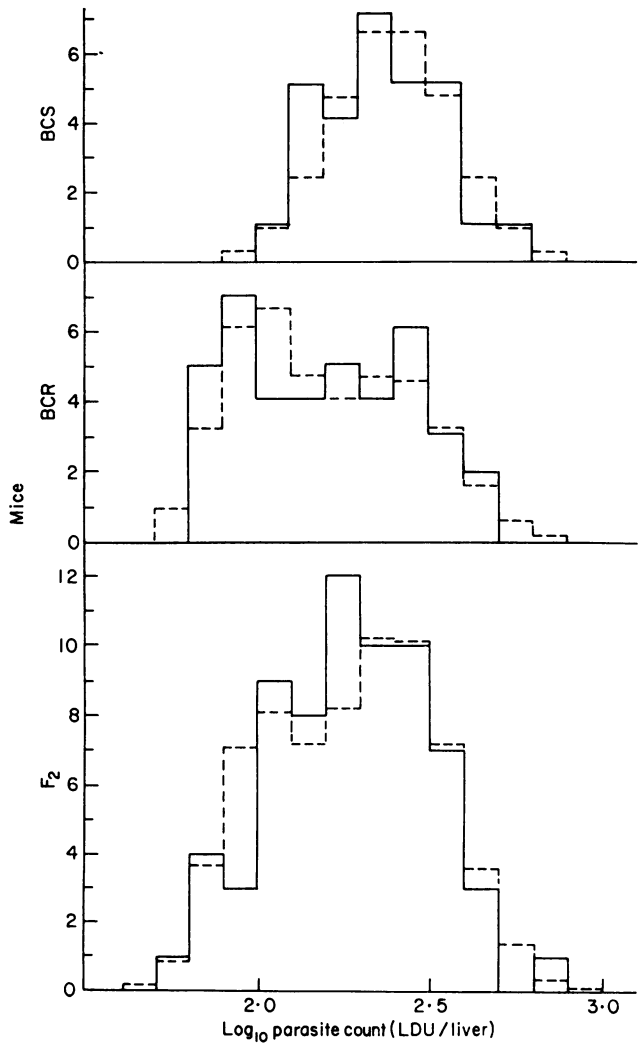

FIG. 5. Histogram of observed lower group of parasite counts in the F_2 generation (solid lines) compared with predictions on a single gene hypothesis using the back-cross results to estimate the distributions of resistant and F_1 population counts (broken line). BCS and BCR as in Fig. 3. Ratios expected: BCS (R:S, 1:1); BCR (RR:RS, 1:1); F_2 (RR:RS, 1:2).

DISCUSSION

These experiments clearly indicate the nature of the genetic control of susceptibility to leishmaniasis and also provide a few hints as to the nature of the genetic action.

The experiment, using multiple inbred mouse strains, confirms the suggestion that there are few acute resistance categories into which mouse strains fit (Bradley & Kirkley, 1977). The complete absence of log counts between 2·4 and 3·4 after 15 days (Table 2), or with an increase between 8-fold and 80-fold, is the chief finding. This could result from very simple genetic control of resistance, or from a simple biochemical common path affected by many different genes. The four hybrids between concordant strains, whose offspring maintain the parental susceptibility or resistance, give no evidence of complementation, thus favouring a simple genetic control (Table 2). The intermediate susceptibility of the F_1 hybrid suggests that, if a single gene be involved, resistance is incompletely dominant.

In the breeding experiment the results are highly consistent with predictions on a single gene (or tight linkage group) hypothesis (Fig. 3). In particular, the reappearance in the F_2 generation of the large gap between the 22% of susceptible mice with a mean log count of 3·7 and the remaining mice, all with a log count under 3·0, and a similar result with the back-cross to susceptible mice, is all strongly against control of resistance by multiple unlinked genes. The relatively small variance of the counts of the susceptible mice is further evidence. The agreement between data and predictions on the hypothesis of single gene (or tight linkage group) control of resistance is close (Figs 4 and 5, Tables 6 and 8). The evidence is so far wholly consistent with single gene control and against more complicated genetic explanations.

The multiple strain experiment could be explained if some strains had another pre-existing infection that affected susceptibility to leishmaniasis. This could not apply to the breeding experiment, and the mice were of specific pathogen-free origin and samples were specifically screened, after splenectomy, for *Eperythrozoon coccoides* which is known to disturb immune responses to protozoa (Peters, 1965). The close concordance of results from genetically similar mice bred in England and the U.S.A. is further evidence against an environmental explanation of strain differences.

Though the evidence is good that one gene (or tight linkage group) dominates the acute growth rate of *Leishmania* in the mice studied, other genes also have an effect. Within the resistant mouse strains of the multiple strain experiment, C57BR and C57L were particularly resistant and gave counts significantly (at the 0·2% and 5% levels, respectively) different from C3H, a 'typical' resistant strain. Furthermore, C57L was more resistant than C3H in the mice from the U.S.A., which is somewhat against an environmental explanation for the differences observed; also C57BR and C57L are more closely related to each other genetically than to other strains. We have already (Bradley & Kirkley, 1977) shown variation between susceptible strains in the time at which parasite numbers begin to fall, and that this may correspond in time with an increase in spleen weight. In C57L the spleen is already larger than in most other strains at 2 weeks of infection, so that the low count could be a combination of slow rise and early fall. However, the spleen weight in C57BR remains low, and there are no significant differences in counts between the various inbred susceptible strains at 2 weeks, so this appears to be an inadequate explanation.

If we accept that one gene or tight group controls the great part of acute susceptibility to leishmaniasis, do these experiments give any hints as to the identity or mode of action of the gene? The pattern of susceptibility among the twenty-five strains does not appear to fit the distribution of any well-known gene. In particular, we have considered the major histocompatibility loci, the loci concerned with susceptibility to viruses, and those concerned with the enzyme systems of phagocytic cells. The various alleles at H1, H2 and H3 seem to be distributed fairly randomly between the resistant and susceptible mice, and in particular the H2 alleles a, b, k and d are found in at least one strain belonging to each category, as is also true of H1a, b, c and H3a and b. Such evidence gives no information concerning linked genes such as Ir–I, for which breeding experiments would be required.

Additional evidence against the relevance of the H2 locus to acute leishmaniasis susceptibility is provided by the B10 series of lines (B10.A, B10.BR, B10.D2-new, B10.LP-a), which are almost congenic

with C57BL/10 Sc Sn except for a major histocompatibility difference. Some donors are strains resistant to leishmaniasis. Nevertheless, none of these lines is significantly different from C57BL/10 in susceptibility. Not only does this suggest that for those lines H2 is irrelevant to acute resistance, but also, as Ir–I is so closely linked to H2, it may suggest, less definitely, that Ir–I is not concerned with leishmaniasis resistance early in the infection.

The distribution of low levels of glucuronidase (Morrow, Greenspan & Carroll, 1950) and catalase (Rechcigl and Heston, 1963) between mouse strains does not correspond to leishmaniasis susceptibility.

Situations where a single gene largely controls susceptibility to infection have previously been described for virus infections (Bang & Warwick, 1960; Fenner, 1972), but insufficient strains were examined for comparison with leishmaniasis susceptibility.

On the basis of these experiments we conclude that the greater part of acute susceptibility or resistance to leishmaniasis is determined by a single gene (or tight linkage group), that this is not identical with the major histocompatibility loci H1, H2 or H3, nor is there any evidence of its identity with any gene already characterized. We have provisionally given it the symbol *Lsh* and are attempting to map it.

There are several studies showing variable susceptibility to bacterial infection in different mouse strains, although single gene control has not been shown. Four strains of mice included in our study had been examined (Pierce-Chase, Fauve & Dubos, 1964) for susceptibility to *Corynebacterium kutsheri* and one was susceptible. The reactions of all four were completely opposite to their leishmanial susceptibility. Activation of latent corynebacterial infections by pseudotuberculosis in mice corresponded to leishmaniasis susceptibility in three strains (Fauve *et al.*, 1964). Susceptibility of mice to mammalian tuberculosis in four mouse strains gave results consistent with leishmaniasis in them: in order of decreasing tuberculosis resistance they were A, C3H, C57BL and DBA/1, as for leishmaniasis, but they did not comprise two distinct groups (Pierce-Chase, Dubos & Middlebrook, 1947), and also differences between strains were not apparent until after 2 weeks from infection.

Genetic resistance to *Salmonella typhimurium* has been extensively studied, mostly using specifically bred mouse lines. Two studies using standard strains gave differing results. Using intraperitoneal infections of the Keller strain, C57BL/6J, BALB/cJ and C3H/HeJ were killed by one to ten bacteria, DBA/2J was killed more slowly and considered of intermediate susceptibility and A/J was resistant, but with an LD_{50} under 10^4 *Salmonellae* (Robson & Vas, 1972). These findings only partly resembled leishmaniasis susceptibility. However, Plant & Glynn (1974), using *S. typhimurium* strain C5 injected subcutaneously, found that on the basis of LD_{50} determinations, strains BALB/c, C57BL and B10.D2-new were killed by under fifty bacteria whilst DBA/2, C3H/He, A/J and CBA required inocula exceeding 2×10^5. Our series includes the seven strains studied by Plant & Glynn (1974), and there is precise correspondence between resistance to *S. typhimurium* and to *L. donovani*. This may not be a chance finding. As C57B1 and B10.D2 are almost congenic except at the H2 locus they cannot be considered independent, so there are concordant results on six separate mouse strains for the two infections. The likelihood of this arising by chance is 1:64 and possibly we may be dealing with the same genetic mechanism, though if so, we would not agree with Plant & Glynn (1974) that the Ir–I gene is involved. However, there are many biological differences between *Salmonella* administered subcutaneously and intravenous *L. donovani*. Although most responses to the cutaneous infection *L. tropica* vary between strains, they do not correspond to variations in the visceral infection.

The question then arises as to why it is so unusual to find simple genetic control of resistance to infection above the virus level. It was not found in the immensely thorough studies of Lurie (1964) on tuberculosis or of Webster (1933) or Gowen (1960) on *Salmonella typhimurium*. Leishmaniasis may be atypical; there are three methodological aspects which may be relevant, however.

Firstly, earlier workers have begun, usually of necessity, from outbred stock and deliberately selected for the extremes of susceptibility and resistance. This will tend to accumulate together all the genes favouring resistance, even if relatively minor in effect, so that in breeding experiments it may be difficult to pick out the more important genes. In a survey of the type we were able to carry out using inbred strains not pre-selected for the character under study, any single important gene is more likely to stand out against a 'noise' of randomly scattered less important ones.

Secondly, the mouse leishmaniasis system has two characteristics favourable to genetic analysis. The infection appears to fall into two phases (Bradley and Kirkley, 1977). During the first two weeks it appears that 'innate' or 'natural' immunity predominates, whilst subsequent events are governed largely by acquired immunity (Bradley, unpublished). The infection is but slowly pathogenic and, therefore, determinants of pathology are not apparent in the earlier stages of infection. Therefore, in mouse leishmaniasis there is a separation in time of events more often only separable by experimental manipulation. The chronic nature of leishmanial infection, which makes so many types of experiment tedious, facilitates genetic analysis of the process.

Thirdly, the selection of variables in earlier work has been such as to compound rather than dissect the genetic mechanisms. Lurie (1964) used several complex measures of rabbit tuberculosis whilst Gowen (1960), in his extensive studies of mouse typhoid, used the proportion of mice surviving to 21 days from infection, a complex variable affected by parasite growth rate, immune responses and pathogenic processes.

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REFERENCES

ACTOR, P. (1960) Protein and vitamin intake and visceral leishmaniasis in the mouse. *Exp. Parasit.* **10**, 1.

BANG, F.B. & WARWICK, A. (1960) Mouse macrophages as host cells for the mouse hepatitis virus and the genetic basis of their susceptibility. *Proc. nat. Acad. Sci. (Wash.)*, **46**, 1065.

BRADLEY, D.J. & KIRKLEY, J. (1977) Regulation of *Leishmania* populations within the host. I. The variable course of *Leishmania donovani* infections in mice. *Clin. exp. Immunol.* **30**, 119.

FAUVE, R.M., PIERCE-CHASE, C.H. & DUBOS, R. (1964) Corynebacterial pseudotuberculosis in mice. II. Activation of natural and experimental latent infections. *J. exp. Med.* **120**, 282.

FENNER, F. (1972) Genetic aspects of viral diseases of animals. *Prog. med. Genet.* **8**, 1.

GOWEN, J.W. (1960) Genetic effects in nonspecific resistance to infectious diseases. *Bact. Rev.* **24**, 192.

LURIE, M.B. (1964) *Resistance to Tuberculosis: Experimental Studies in Native and Acquired Defensive Mechanisms.* Harvard University Press, Cambridge, Massachusetts.

MORROW, A.G., GREENSPAN, E.M. & CARROLL, D.M. (1950) Comparative studies of liver glucoronidase activity in inbred mice. *J. nat. Cancer Inst.* **10**, 1199.

PETERS, W. (1965) Competitive relationship between *Eperythrozoon coccoides* and *Plasmodium berghei* in the mouse. *Exp. Parasit.* **16**, 158.

PIERCE-CHASE, C.H., DUBOS, R.J. & MIDDLEBROOK, G. (1947) Infection of mice with mammalian tubercle bacilli grown in Tween-albumin liquid medium. *J. exp. Med.* **86**, 159.

PIERCE-CHASE, C.H., FAUVE, R.M. & DUBOS, R. (1964) Corynebacterial pseudotuberculosis in mice, I. Comparative susceptibility of mouse strains to experimental infection with *Corynebacterium kutscheri*. *J. exp. Med.* **120**, 267.

PLANT, J. & GLYNN, A.A. (1974) Natural resistance to *Salmonella* infection, delayed hypersensitivity and Ir genes in different strains of mice. *Nature (Lond.)*, **248**, 345.

RECHCIGL, M. & HESTON, W.E. (1963) Tissue catalase activity in several C57BL substrains and in other strains of inbred mice. *J. nat. Cancer Inst.* **30**, 855.

ROBSON, H.G. & VAS, S.I. (1972) Resistance of inbred mice to *Salmonella typhimurium*. *J. infect. Dis.* **126**, 378.

STAUBER, L.A. (1958) Host resistance to the Khartoum strain of *Leishmania donovani*. *Rice Inst. Pamph.* **45**, 80.

TAYLOR, L.R. (1961) Aggregation, variance and the mean. *Nature (Lond.)*, **189**, 732.

WEBSTER, T. (1933) Inherited and acquired factors in resistance to infection. *J. exp. Med.* **57**, 793.