BACKCROSS STUDIES ON THE GENETICS OF RESISTANCE TO MALARIA IN MICE

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IT IS well known that studies on experimental malaria in animals have yielded valuable information on the applied chemotherapy of this disease in man. Less appreciated is the fact that the Plasmodium organism can be a valuable research tool in fundamental studies no less important than work on other microorganisms. Use of Plasmodium berghei and Plasmodium gallinaceum in this sense has yielded basic information and has stimulated interest in (1) the mode of action of drugs in infections and parasitic diseases other than malaria, (2) the mechanisms of drug resistance and the relationship between molecular structure and activity, (3) the interrelationships between the metabolism of parasitic and infectious diseases and cancer, (4) the endocrine aspects of erythropoiesis, (5) the nature of the physiologic alteration following X-radiation, (6) the genetics of the invading organism, and (7) the genetics of host resistance. The following report concerns itself with survival of mice after malarial infection, and suggests that in this species resistance is probably largely genetically controlled.

Differences in the duration of survival after experimental infection with Plasmodium berghei have been demonstrated in certain inbred strains of mice and their F1 hybrids. In 48 series utilizing 496 mice, C57 BL/6 and C57 L animals survived significantly longer than all other strains tested. In 38 series utilizing 385 mice, F1 hybrids of C57 BL/6 or of C57 L mice as one parent, with one exception, survived significantly longer than other tested strains or hybrids. The solitary exception was the mutual F1 hybrid, C57 BL/6 × C57 L F1, which was less resistant than either parent. These results, summarized below (table 1), suggested that multiple genetic factors were probably effecting resistance to malaria. Additional data now reported on other hybrids, on backcrosses of the (C57 BL/6 × DBA/2) F1 hybrid to either parent, and on the F2 generation, collected concurrently with the previously reported studies, support the thesis for the genetic basis of resistance to malaria in mice. (GREENBERG, NADEL, and COATNEY 1953 and 1954; NADEL, GREENBERG, and COATNEY 1954; NADEL, GREENBERG, JAY, and COATNEY 1954).

METHODS

All animals were bred and weaned at the Animal Production Section, Laboratory Aids Branch of the National Institutes of Health; the subscripts JN have been

1 Part of this material appeared as an abstract in the Proceedings of the 51st annual meeting of the American Society of Pathologists and Bacteriologists in April 8-10, 1954, in Philadelphia, Pa.
2 Laboratory of Pathology, National Cancer Institute.
3 Laboratory of Tropical Diseases, National Microbiological Institute.
4 Laboratory Aids Branch, National Institutes of Health.
5 Public Health Service, Department of Health, Education, and Welfare.
TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Mean survival (days)</th>
<th>Standard error</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Backcross and F2 animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 DBA/2*</td>
<td>70</td>
<td>9.9</td>
<td>0.46</td>
<td>14.82</td>
</tr>
<tr>
<td>2 C57 BL/6*</td>
<td>51</td>
<td>17.6</td>
<td>0.64</td>
<td>20.88</td>
</tr>
<tr>
<td>3 (C57 BL/6 × DBA/2)F1*</td>
<td>44</td>
<td>21.2</td>
<td>0.45</td>
<td>8.88</td>
</tr>
<tr>
<td>4 (C57 BL/6 × DBA/2)F2</td>
<td>174</td>
<td>17.1</td>
<td>0.54</td>
<td>49.84</td>
</tr>
<tr>
<td>5 (C57 BL/6 × DBA/2)F1 × DBA/2</td>
<td>169</td>
<td>13.8</td>
<td>0.48</td>
<td>38.94</td>
</tr>
<tr>
<td>6 (C57 BL/6 × DBA/2)F1 × C57 BL/6</td>
<td>81</td>
<td>20.0</td>
<td>0.93</td>
<td>70.05</td>
</tr>
<tr>
<td>F1 hybrids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (STR/N × A/LN)F1</td>
<td>165</td>
<td>18.2</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>8 (STR/N × C57 BL/6)F1</td>
<td>103</td>
<td>24.5</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>9 (STR/N × DBA/2)F1</td>
<td>135</td>
<td>17.9</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>10 (C57 L × DBA/2)F1</td>
<td>140</td>
<td>19.3</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

Statistical evaluation for significance (P < 0.01) showed that; Groups 1 and 5 are different from all other groups and each other; group 2 is different from 1, 3, and 6; group 3 is different from 1, 2, 4, and 5; there is no difference between groups 2 and 4 or between 3 and 6; group 8 was significantly different from groups 2, 4, 7, 9 and 10; there was no difference amongst groups 7, 9 and 10.

* Data previously reported but presented for comparative purposes (GREENBERG, NADEL, and COATNEY 1953 and 1954).

For comparative purposes, the following is a summary of survival data on all inbred strains and hybrids previously reported (GREENBERG, NADEL, and COATNEY 1953 and 1954):

- F1 hybrids: (C57 BL/6 × C57 L) (11.3); (BALB/c × A/LN) (13.2); (C57 BL/6 × C57 BR/cd) (15.1); (A/LN × DBA/2) (15.1); (BALB/c × C57 BR/cd) (15.1); (C57 BL/6 × A/LN) (16.4). (C57 L × A/LN) (19.3); (C57 BL/6 × A/LN) (22.7).
- Inbred strains: A/LN (8.4); SWR (8.8); C57 BR/cd (9.7); C58/LN (10.4); STR/N (10.5); BRSUNT/N (10.7); C57 BR/cd (12.3); BALB/c (12.5); RIL (AKR/Lw) (14.4); C57 L (15.0).

deleted from the nomenclature used in the test. For the sake of brevity (C57 BL/6 × DBA/2) F1 animals are referred to as BDF1, while the F2 hybrids of this mating (C57 BL/6 × DBA/2) × (C57 BL/6 × DBA/2) are referred to as BDF2.

Survival studies on 3 groups of animals are reported. These include backcross studies on BDF1 × C57 BL/6, and BDF1 × DBA/2 and reciprocal matings, studies on the BDF2 and reciprocals, and studies on the F1 hybrids of female STR/N and male A/LN, C57 BL/6 or DBA/2 mice, as well as the C57 L × DBA/2 F1 hybrid.

The Kasapa strain of Plasmodium berghei was used. The strain was maintained by weekly passage of infected blood through non-inbred Swiss mice. The Swiss mice served as donors of infected blood for all the experimental mice. Heparinized infected heart blood was diluted with physiological saline and each mouse received through a tail vein approximately 1,000,000 parasitized erythrocytes, in 0.1 ml of inoculum. Blood smears were examined on the third or fourth day after inoculation to establish the presence of the infection.

Mice of both sexes and approximately two months old (plus or minus 1 week) were used. Four to eight groups of mice infected on the same day with the
same inoculum formed a single series, and in each series 5 Swiss mice were included. Each hybrid or backcross was represented by 4 to 24 individuals in from 4 to 13 series.

RESULTS

All of the animals died with malaria between the 5th and 36th day after inoculation. In all groups there were deaths as early as the 5th or 6th day.

**Group I. Backcross study.** Offspring resulting from the resistant parent strain (C57 BL/6, 17.60 ± 0.64 days) and its more resistant hybrid (BDF₁, 21.23 ± 0.45 days) lived longer than offspring of the same hybrid and its less resistant parent (DBA/2, 9.90 ± 0.46 days). This difference in survival, 20.0 ± 0.93 days for BDF₁ × C57 BL/6 mice versus 13.8 ± 0.48 days for BDF₁ × DBA/2 mice is significant (fig. 1 and table 1).

**Group II. F₂ group.** No significant difference was detected between the survival of BDF₂ or DBF₂ animals. The overall average survival of the F₂ group was 17.1 ± 0.54 days. (fig. 1 and table 1).

**Group III. New hybrids.** Heterosis was noted in each of the four previously untested F₁ hybrids as seen in table 1. The longest lived F₁ hybrid, (STR/N × C57 BL/6) F₁, lived 24.5 ± 0.44 days after experimental injection with *Plasmodium berghei*. No significant difference was noted in the survival of (STR/N × A/LN)F₁ hybrids (18.2 ± 0.58) in comparison with (STR/N × DBA/2) F₁ hybrids (17.9 ± 0.47).

DISCUSSION

Additional evidence has been presented to support the thesis of a genetic basis of resistance to malaria in mice. Including this report, we have now studied survival after infection with *Plasmodium berghei* in over 2000 mice from 12 inbred strains and
from 13 different F1 hybrid crosses of these strains, as well as the backcrosses and F2 hybrids from one of the more resistant hybrids (BDF1). The 12 inbred strains have been found to fall into a short, intermediate, or a long survival group, with further subdivision probably present in the intermediate group. Of the 13 different kinds of F1 hybrids tested, 10 have exhibited significant heterosis in respect to resistance to malaria; two hybrids, (BALB/c × A/LN) F1 and (C57 BL/6 × C57 BR/cd) F1, showed no significant heterosis but proved more resistant than one of their parents; and one hybrid (C57 BL/6 × C57 L) F1 was significantly less vigorous than either parent. It has been suggested that in classical studies on inbred corn, increased vigor (heterosis) in a hybrid is to be expected where many different genes are involved, while decreased vigor occurs in F1 hybrids resulting from the mating of closely related parents (Gowen 1952). The analogy between increased vigor in corn and increased resistance to malaria is reinforced by the decrease in resistance observed in the (C57 BL/6 × C57 L) F1 animals, hybrids of closely related parent strains. Further studies on closely related strains and their hybrids would be helpful in deciding whether a smaller number of gene differences in the parents makes for decreased resistance to malaria in the F1 hybrid as is implied in our results.

The results obtained with the BDF1 and BDF2 hybrids of C57 BL/6 and DBA/2 mice, and with the backcrosses of the BDF1 to each of the parents, would seem to support the genetic basis of resistance to malaria. The BDF1 hybrids exhibited heterosis, while the BDF2 exhibited none. The latter survived no longer than the longer-lived parent (fig. 1 and table 1). The backcross of the BDF1 to DBA/2 was significantly shorter-lived than the C57 BL/6 parent, the BDF1, the BDF2 hybrids, and the backcrosses to the C57 BL/6 parent, but was however, longer-lived than the DBA/2 parents. The backcross to C57 BL/6 was longer-lived than the C57 BL/6 parents and behaved very much like the BDF1 hybrids. Since the maternal parents in both backcrosses were the same, one can safely eliminate ordinary maternal influences as a major factor in the differences in survival of the backcross mice. In addition, in our studies, coat color appeared to be of no significance in the survival of mice of either the backcrosses or BDF2 hybrids.

The wide range of survival time in all groups studied, and the lack of good correlation between individual extreme survival time within a group, with the mean survival of the group, prevent a clear-cut genetic analysis of the data.6 When all our survival data were combined, deaths appeared to have occurred in 2 distinct waves with one peak on the 6th and the other on the 21st day after inoculation (figure 2). Referring back to our individual strain and hybrid data, it appeared that most short survival mice, (e.g., A/LN, DBA/2) died in the first wave of deaths while almost all of the long survival mice (e.g., (STR/N × C57 BL/6) F1 hybrids) died in the second wave. With increase in survival time there appeared to be a shift in deaths from the first to the second wave. However, even in the longest-lived hybrids, a small proportion of the mice died early. While this is probably an oversimplification, it might be said that the mean survival data we have been collecting

* Dr. John W. Gowen, Professor of Genetics, Iowa State College, helpfully pointed out that the possibility of a one-gene-difference with a sizable amount of variation due to environmental circumstances should also be considered.
are an expression of the proportion of the mice which survive the early wave of deaths.

The wide range of death observed in all series suggests that extragenetical as well as genetical factors may be implicated in death from malaria in mice. Nonetheless the consistent reproducibility of the mean survival data for each strain and hybrid is striking and significant. The same is true of the backcross studies. If extragenetical factors are also involved, their superimposition on a stable gene background may still yield complicated but reproducible patterns for each inbred strain and hybrid. In addition to the strain differences observed, the evidence for a genetic basis for increased survival resides in the demonstration of (1) heterosis, (2) significantly increased survival in the backcrosses to the more resistant parent, and (3) increased variance in the F2 and backcross animals in comparison to that of the parent strains or the F1 hybrids (table 1).

Although survival time after experimental infection was the criterion applied in classifying strains for their resistance to malaria, other measurements may prove to be of additional value in further defining the nature of the strain differences. We found that survival reflected in the simplest measurable and reproducible manner the overall ability of a strain to resist the physiological insults of malarial parasitization. Indeed, without recorded survival data, no knowledge of strain differences would have been apparent. Total parasitemia itself gave no inkling that significant strain differences existed. In our earlier study on DBA/2, C57 BL/6 and their BDF1 hybrids, average survival was 9.9, 17.6 and 21.2 days, respectively, yet the rate of increase of
total parasitemia was approximately the same for all three groups (Greenberg, Nadel, and Coatney 1953). However, the diphasic nature of all combined survival data awakened new interest in the slight notch observed in the earlier reported total parasitemia curve for the BDF₁ hybrids, and suggested that attention be given to a more detailed parasitemia analysis. A preliminary study has indicated that early deaths are associated with a high degree of infection of mature erythrocytes, while late deaths were associated with a high degree of infection of young erythrocytes, coupled with a gradual increase in the proportion of young erythrocytes in the total erythrocyte population. In most of the longer lived animals, the infection of mature erythrocytes was checked when a relatively small percentage of them had been infected. However, the correlation between survival and maximal degree of invasion of mature erythrocytes may not, from these preliminary studies, be a completely direct one. In eight strains studied, the order of decreasing parasitemia of mature erythrocytes on the sixth day after experimental infection has been found to be (1) non-inbred Swiss (40%), (2) DBA/2 (33%), (3) C₃H (25%), (4) C₅₇ BL/6 (17%), (5) A/LN (15%), (6) BALB/C (13%), (7) STR (7%), and (8) BRSUNT (2%). This does not directly correlate with the order of increasing survival presented in the footnote in table 1. Survival-time though a reflection of presumably many factors, is a better reflection of overall host resistance.

SUMMARY

A comparison was made of the differences in the duration of survival after experimental infection with Plasmodium berghei in 12 inbred strains of mice and in F₁ hybrids involving 13 combinations of these strains. In addition, the survival of backcross mice of the (C₅₇ BL/6 × DBA/2) F₁ hybrid was compared to that of their short-survival (DBA/2) and their long-survival (C₅₇ BL/6) parents, as well as to the (C₅₇ BL/6 × DBA/2) F₂ hybrids. Data based on over 2000 mice indicate that hybrids of C₅₇ BL/6 or of C₅₇ L parents exhibited the longest mean survival time. With the exception of the (C₅₇ BL/6 × C₅₇ L) F₁ hybrid, and the (C₅₇ BL/6 × C₅₇ BR/cd) F₁ hybrid, all other hybrids of these parents exhibited heterosis in respect to increased survival after malaria infection. The presence of (1) heterosis, (2) greater variance in the F₂ and in the backcross animals in comparison to the variance in the parents and F₁ hybrids, and (3) the significant increased survival of the backcross to the long-survival parent in comparison to that of the backcross to the short-survival parent indicate that multiple genetic factors may be involved. However, although multiple genes may influence the differences in survival, extra-genetic factors are probably also present. The presence of, as yet undetermined, non-genetic factors is indicated by (1) the wide range in the time of death exhibited by mice in all series from short or long survival mice, and (2) the presence of a bimodal curve of death based on a compilation of the survival data of all mice tested without regard to strain background. Subsequent re-evaluation of the data on the individual strains and hybrids indicated that the peak of deaths in the short-survival mice coincided with the early peak on the overall bimodal curve, while the peak of death in the long-survival mice coincided with that of the late peak in the overall bimodal curve.
LITERATURE CITED


