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# Murine Major Histocompatibility Complex and Immune Response to *Eimeria falciformis*

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**The genetics of the immune response to *Eimeria falciformis* were investigated in three inbred and six congenic strains of mice. There were significant differences among strains in oocyst production and age-related mortality from parasitic infection. Genes within the *H-2* complex and also non-*H-2* genes share in the immune response to eimerian infection.**

Protozoan parasites with complex life cycles, such as *Leishmania* sp., are under polygenic control (1). Coccidian parasites are also complex, and host resistance to the parasites is also most likely controlled by multiple genes (4, 11). The use of inbred and congenic (highly inbred strains differing genetically only at histocompatibility loci) strains of mice provides a model system for understanding genetically determined immunity to coccidian parasites. The intracellular parasite genus *Eimeria* is highly species and strain specific. Klesius and Hinds (7) infected eight inbred strains of mice with *Eimeria ferrisi*. Susceptibility to the parasite varied with different doses of inoculated oocysts in different strains. They crossed strain C57BL/6 with the resistant mouse strains CBA, C3H/Anf, and DBA/2 and found that $F_1$ mice were more resistant. Thus, resistance was a dominant genetic expression. Some $F_1$ hybrids were more resistant than either of the parent strains, suggesting that location of the gene for resistance could be determined with congenic mice. Rose et al. (10) studied the susceptibility to *E. vermiformis* in seven recombinant strains of BALB/c × C57BL/6 mice and found that resistance to this parasite was not associated with the *H-2* locus. Unfortunately, their use of recombinant strains was not the best choice for study of the *H-2* complex, since their strains differed not only at the *H-2* locus but also at many other gene loci (6). Clare et al. (2) found significant differences among B genotypes of chickens infected with *E. tenella*. The relationship of the genetics of immunity to coccidian infection can be best studied by using congenic strains of mice. In this study, we report on the influence of host genetic background and age on the susceptibility and immune response to coccidian infection with *E. falciformis*.

**Animals.** Mice of three inbred strains (BALB/c, C57BL/10, and C3H/He) of three age groups (28, 42, and 108 days old) and six adult congenic strains differing only at their *H-2* loci (B10.PL, B10.BR, B10.D2, C57/10, B10.A, and B10.M) were purchased from Jackson Laboratory, Bar Harbor, Maine. Before experimental infection, mice were checked for the presence of helminth eggs or oocysts by a fecal flotation technique.

TABLE 1. Mean total oocyst production, ages, and death rate of three inbred strains of mice infected with *E. falciformis*

| Mouse strain | *H-2* haplotype | Age (days) | No. of mice infected | No. of surviving mice | Death rate (%) | Mean total oocysts ($10^6$) (range)[a] |
|---|---|---|---|---|---|---|
| BALB/c | *d* | 28 | 10 | 4 | 60 | 17.6 (14.72–20.51) |
| | | 42 | 10 | 9 | 10 | 17.0 (15.01–18.93) |
| | | 108 | 10 | 10 | 0 | 14.7 (13.65–15.79) |
| C57BL/10 | *b* | 28 | 9 | 2 | 78 | 33.6 ± 3.22 (SE) |
| | | 42 | 9 | 4 | 56 | 27.7 (17.37–38.10) |
| | | 108 | 10 | 10 | 0 | 28.7 (27.03–30.40) |
| C3H/He | *k* | 28 | 10 | 0 | 100 | |
| | | 42 | 10 | 2 | 80 | 11.5 ± 0.10 (SE) |
| | | 108 | 10 | 6 | 40 | 7.2 (5.44–8.93) |

[a] Confidence limits of 95% used only with sample sizes of four or more.

**Parasite and infection.** Oocysts of *E. falciformis* were provided by C. A. Speer (Veterinary Research Laboratory, Montana State University, Bozeman). Oocysts were separated from feces by sieving and sugar flotation, sporulated, and then stored in aqueous 2.5% $K_2Cr_2O_7$ solution at 5°C for less than 2 months before use. Since multiple doses are more immunogenic than a single inoculation (5), we infected the mice in experiment 1 by intubation three times as follows: on day 0, each received 500 sporulated oocysts; on day 2, each received 1,000 oocysts; and on day 4, each received 1,500 oocysts. In experiment 2, the mice were 70 days old. Each mouse was inoculated with 500, 800, and 800 oocysts on days 0, 2, and 4, respectively. Assessment of oocyst production began on day 7 postinoculation after the first inocula-

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TABLE 2. Mean total oocyst production of six congenic strains of mice infected with *E. falciformis*

| Mouse strain | *H-2* haplotype | No. of mice infected | No. of surviving mice | Mean total oocysts ($10^6$) (range)[a] |
|---|---|---|---|---|
| B10.PL | *u* | 9 | 7 | 13.6 (10.17–17.02) |
| B10.BR | *k* | 10 | 7 | 22.8 (16.06–28.59) |
| B10.D2 | *d* | 10 | 8 | 19.3 (16.82–21.67) |
| C57BL/10 | *b* | 10 | 10 | 29.9 (26.60–33.28) |
| B10.A | *a* | 10 | 10 | 29.2 (23.65–34.76) |
| B10.M | *f* | 10 | 10 | 20.0 (16.10–23.87) |

[a] Confidence limits, 95%.

tion. Feces were collected at 2-day intervals in pans containing 2.5% $K_2Cr_2O_7$. Collections continued until the end of patency (15 to 16 days postinoculation), and oocysts were counted by the McMaster technique (8).

**Oocyst production.** Two experiments were conducted. In experiment 1, the susceptibility to *E. falciformis* of three inbred mouse strains of three ages (BALB/c, C57BL/10, and C3H/He) was determined. The data are summarized in Table 1. Oocyst production was analyzed with a three-way analysis of variance by using fecal collection date, age, and strain as the three variables. The oocyst production in different strains was significantly different ($P < 0.001$). Our data confirm that BALB/c mice can be classed as resistant hosts, whereas C57BL/6 and our C57BL/10 mice were susceptible strains (9). There was no difference among age groups ($P > 0.05$).

**Death rate.** During the patent period (9 to 12 days postinoculation), many mice died from the infection. The cumulative mortality data were rank transformed and then analyzed by three-way analysis of variance with time after infection, age, and strain as the variables. The death rate was significantly different among strains ($P < 0.001$) and also significant among age groups ($P < 0.001$) (Table 1). The low oocyst production and high mortality of C3H/He mice suggests that the gene(s) controlling resistance is not the same as that for oocyst production. The effect of host age on mortality from coccidiosis is controversial (3). Our data clearly support the premise that young animals are more susceptible than older ones.

**Congenic mice.** In experiment 2, there were significant differences in oocyst production among strains of congenic mice (two-way analysis of variance, $P < 0.001$) (Table 2). Therefore, we conclude that genes within the *H-2* complex play a role in the susceptibility of mice to *E. falciformis* infection. Non-*H-2* genes also share in the immune response to eimerian infection. Oocyst production from paired strains of the same haplotype, but with different genetic backgrounds (BALB/c and B10.D2 [haplotype *d*] and C3H/He and B10.BR [haplotype *k*]) was significantly different ($P < 0.05$ for *d*, $P < 0.001$ for *k*). There was no difference ($P > 0.05$) from the other strains in oocyst production in C57BL/10 mice (Fig. 1).

The nature of the resistance to coccidian infection reflects the complexity of the host-parasite interaction. Differences in susceptibility in strains of mice (judged by oocyst production, age, and death rate) demonstrate that at least one of the genes for resistance to *E. falciformis* is linked to the *H-2* complex. Paired haplotype experiments also showed that non-*H-2* genes share in the immune response. More intensive study of the genetic control of resistance to eimerian infection is needed with congenic mice of known genetic background.

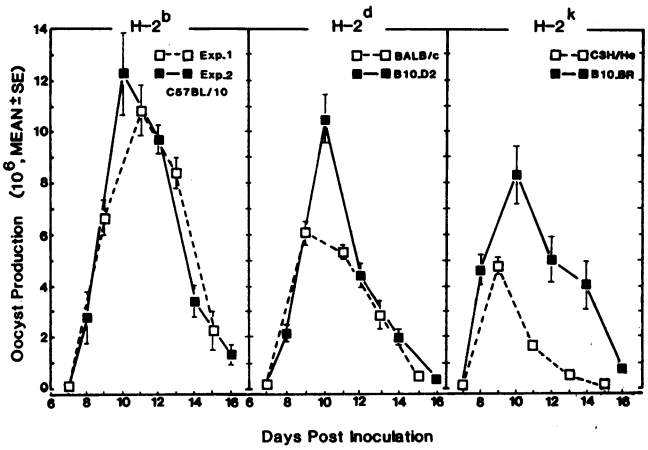


FIG. 1. Comparison of oocyst production from paired strains of mice with the same haplotype infected with *E. falciformis*. Bars above and below symbols indicate standard error.

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