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Note

The Course of Malaria in Mice: Major Histocompatibility Complex (MHC) Effects, but No General MHC Heterozygote Advantage in Single-Strain Infections

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ABSTRACT

A general MHC-heterozygote advantage in parasite-infected organisms is often assumed, although there is little experimental evidence for this. We tested the response of MHC-congenic mice (F_2 segregants) to malaria and found the course of infection to be significantly influenced by MHC haplotype, parasite strain, and host gender. However, the MHC heterozygotes did worse than expected from the average response of the homozygotes.

WHEN fighting infection, MHC heterozygotes are often expected to be superior to both their respective homozygotes (*i.e.*, exhibit overdominance) because they can present a wider range of antigens to T lymphocytes (Doherty and Zinkernagel 1975). Indeed, MHC heterozygotes normally show slower disease progression and/or more rapid clearance of an infection than the average of homozygotes, further providing a population-level advantage of heterozygosity (*e.g.*, Thursz *et al.* 1997; Carrington *et al.* 1999), and MHC heterozygotes are typically overrepresented in vertebrate populations (*e.g.*, Hedrick and Thomson 1983; Black and Hedrick 1997). However, in population studies it is often unclear whether the observed heterozygote advantage is due to overdominance, to dominance of resistance, or explained only by the specific allele frequencies in a host population (Lipsitch *et al.* 2003). Allele-specific measures are therefore necessary to explain the kind of heterozygote advantage that is observed (Apanius *et al.* 1997; Penn *et al.* 2002; McClelland *et al.* 2003).

We studied MHC-congenic mice during experimental exposure to two clones of *Plasmodium chabaudi*. F_2 segregants were used as hosts ($N_{total} = 107$) to compare different homozygotes and the respective heterozygotes and to control for possible maternal effects or differences in the background genetics among congenic strains (see below). We used a fully factorial experimental design to examine the separate and combined impact of host MHC (*H-2*^{a}, *H-2*^{ab}, *H-2*^{b}), host gender (two sexes), and parasite clone ("*AS*" and "*CW*"; Beale *et al.* 1978). Variation in age was minimized by synchronized breeding. When age was included as a covariate in the statistical models, the results did not change qualitatively and significant *P*-values tended to drop slightly (results not shown). Body weights and blood cell densities around exposure (day 0 and 1, respectively) were not significantly different among the experimental groups, except that, as expected (Suckow *et al.* 2000), males were initially heavier than females ($t = 11.0$, $P < 0.0001$) and had lower blood cell densities ($t = 3.0$, $P = 0.004$). We tracked the time course of the disease through daily weight measurements and in repeated parasitemia counts and blood cell counts. The resulting repeated-measures analyses of variances (ANOVAs) are summarized in Table 1 and discussed below. We then used these findings to specifically compare the performance of MHC heterozygotes to the average performance of the homozygotes.

Consistent with previous studies (Taylor *et al.* 1998; Mackinnon and Read 1999), mean parasitemia rose dramatically during the first 10 days postinfection (p.i.), and minimal blood cell counts and body weights on average were reached at day 10 and 11 p.i., respectively. At that stage, the mice on average had lost 2.2% (±0.7 SE) of their initial body weight and 58.1% (±2.3) of

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TABLE 1

The effect of host MHC, parasite clone, and host gender on the time course of disease symptoms

	Disease symptom								
	Parasitemia			Blood cell counts			Body weight change		
	F	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
	Between subjects								
Host MHC	9.6	2, 94	0.0002	5.2	2, 94	0.007	1.2	2, 94	0.29
Plasmodium clone	5.9	1, 94	0.017	0.1	1, 94	0.79	3.4	1, 94	0.07
Host gender	0.02	1, 94	0.89	27.8	1, 94	<0.0001	0.1	1, 94	0.77
MHC × clone	0.8	2, 94	0.43	0.5	2, 94	0.61	0.3	2, 94	0.75
MHC × gender	0.9	2, 94	0.39	1.2	2, 94	0.32	0.2	2, 94	0.82
Clone × gender	10.1	1, 94	0.002	9.5	1, 94	0.003	0.4	1, 94	0.52
MHC × clone × gender	0.7	2, 94	0.49	1.2	2, 94	0.31	1.2	2, 94	0.31
	Within subjects (repeated measurements on individual mice)								
Time	296.7	5, 90	<0.0001	166.1	10, 85	<0.0001	33.1	18, 77	<0.0001
Time × MHC	2.4	10, 180	0.01	1.2	20, 170	0.26	1.0	36, 154	0.46
Time × clone	6.7	5, 90	<0.0001	2.9	10, 85	0.003	1.7	18, 77	0.05
Time × gender	1.5	5, 90	0.20	1.9	10, 85	0.06	6.2	18, 77	<0.0001
Time × MHC × clone	0.4	10, 180	0.92	0.6	20, 170	0.94	1.2	36, 154	0.27
Time × MHC × gender	0.7	10, 180	0.72	1.0	20, 170	0.46	0.8	36, 154	0.78
Time × clone × gender	1.1	5, 90	0.38	1.7	10, 85	0.10	1.9	18, 77	0.03
Time × MHC × clone × gender	1.4	10, 180	0.18	0.7	20, 170	0.86	0.8	36, 154	0.72

The experiment was designed for a fully factorial repeated-measures analysis of variance (ANOVA), incorporating the fixed-effect factors "host MHC," "host gender," and "Plasmodium clone" with repeated measures of the following dependent variables: parasitemia (6 measurements from day 4 to day 14), blood cell counts (11 measurements from day 1 to day 22), and body weight (19 measurements from day 4 to day 22), given as differences from the weight at day 0. For within-subject analyses, we used the multivariate *F*-tests or Wilk's λ (when a factor had more than two levels as in "MHC").

their initial red blood cells. All mice survived the acute phase of the infection and recovered as parasitemia declined over the next few days.

The host's MHC genotype had a strong effect on parasitemia and on blood cell counts, but did not significantly affect body weight change (Table 1). The homozygous *H-2*^{a} appeared to be more susceptible than the homozygous *H-2*^{b} type, confirming previous findings comparing inbred lines but where background genetics and maternal effects were not fully controlled (Wunderlich *et al.* 1988). MHC genotype also influenced the time course of parasitemia (time × MHC in Table 1): the homozygous *H-2*^{b} type seemed to clear its parasites faster than *H-2*^{ab}, followed by *H-2*^{a} (Figure 1A). The response of the heterozygous *H-2*^{ab} was between the two homozygous genotypes (Figure 1, A and B; see below).

We found differences between the parasite clones that are consistent with previous studies (Mackinnon and Read 1999). Clone *CW* reached overall higher parasitemia (Table 1), but clone *AS* had its peak parasitemia earlier than clone *CW* (time × clone in Table 1; Figure 1C). This corresponds to a similar pattern in the time course of the blood cell counts (time × clone in Table 1; Figure 1D). The two clones caused different disease patterns not only because of intrinsic clone-specific characteristics but also depending on host characteristics: the two sexes react differently to the parasite clones (significant clone × gender interaction, Table 1). Gender-specific virulence is common in vertebrates (Zuk and McKean 1996), but here we show an interaction between gender and parasite clone. Specifically, clone *CW* reached higher parasitemia than clone *AS* only in female hosts, while in male hosts, the two clones differed in the time course of their parasitemia, with clone *AS* reaching its peak parasitemia earlier but also disappearing earlier than clone *CW* (data not shown). However, there was no significant interaction between parasite clone and MHC genotype in any of the traits (Table 1). This suggests that the two clones are similar in at least some of their MHC-presented peptides, such that, for a given parasite clone, virulence is relatively stable across host MHC types.

The MHC heterozygotes in our study were neither superior nor inferior to the respective homozygotes; *i.e.*, we found no evidence for overdominance of resistance or susceptibilty (Figure 1). Nor were heterozygotes more resistant than what would be expected from the average of the two homozygotes; *i.e.*, the apparent resistance of

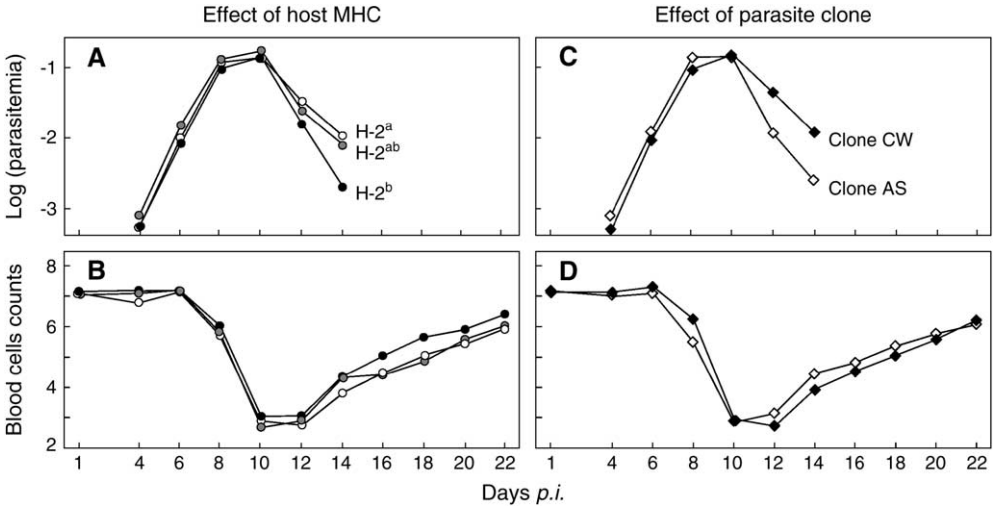

FIGURE 1.—The effects of MHC and parasite clone on the course of disease symptoms. The mean parasitemia ($\log_{10}$ transformed) and the mean blood cell number ($\times 10^9$)/ml blood for MHC genotypes *H-2*^{a} (open circles), *H-2*^{b} (solid circles), and *H-2*^{ab} (shaded circles), or clone *AS* (open diamonds) and clone *CW* (solid diamonds) are given. See supplementary material at http://www.genetics.org/supplemental/ for details about the experimental procedure and Table 1 for statistics.

the *H-2*^{b} genotype was not dominant. Indeed, contrary to what is commonly assumed (see references above), the MHC heterozygotes in our study reached significantly higher parasitemias than the average of the two homozygotes (Figure 2). This pattern cannot be explained by differences in starting conditions, as all genotypes were similar in age, weight, gender, and parasite clone (P always > 0.25). A number of factors may explain cases of poor heterozygote performance, including that MHC heterozygosity has an impact on T-cell receptor repertoire selection (ROBEY and FOWLKES 1994; VUKUSIC *et al.* 1995) or that parasite-generated T-cell antagonism, which is known to reduce the efficacy of T-cell-mediated immunity in malaria (GILBERT *et al.* 1998), has an even more pronounced effect in heterozygotes than in homozygotes. Whatever the physiological explanation, we can conclude that the widely assumed and sometimes supported MHC heterozygosity advantage is only a rule with exceptions. It remains, however, unclear whether this conclusion is true for both class I and class II genes (the congenic lines that we used vary over the entire MHC region). Differences in the recognition system or in gene dose effects between class I and class II genes (DORF *et al.* 1979; MOORE *et al.* 1980) could potentially influence antigen recognition under homozygous or heterozygous conditions.

The link between MHC and human malaria (HILL *et al.* 1991, 1992) is an often cited example of the influence of MHC genes on the course of a disease. However, for humans, such links cannot be studied under controlled experimental conditions. Other studies used inbred mouse strains that were congenic with respect to the MHC (WUNDERLICH *et al.* 1988; BAGOT *et al.* 2002; CIGEL *et al.* 2003), but they did not control for a number of potentially confounding effects. For example, mother's age is known to affect offspring size, number, and general vigor (FINN 1963; TARIN *et al.* 2004). Maternal effects could explain why some congenic strains produce different olfactory signals in parental strains but not in F_2 segregants (CARROLL *et al.* 2002) or why congenic strains sometimes differ in behavior (HEIMRICH *et al.* 1988). In addition, MHC-congenic lines may differ with respect to the mutation load on their background genes, as there is at least one example of different mortalities during early development (WEDEKIND *et al.* 1996). Variation in maternal effects or mutation load could interact with pathogen susceptibilities (CARROLL and POTTS

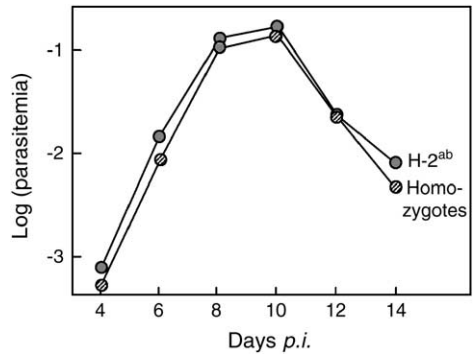

FIGURE 2.—The effect of the *H-2*^{ab} genotype *vs.* the average effects of the respective homozygote MHC types during infection with *P. chabaudi.* To test whether the heterozygotes (shaded circles) were as susceptible as the average of the two homozygotes (striped circles), we first equalized the sample size of the homozygous variants before pooling them. We did that by randomly reducing the larger group to the sample size of the smaller group. We then calculated the repeated-measures ANOVAs (fixed factors: heterozygosity, gender, and parasite clone) with the reduced sample size. The average of 10 randomly reduced samples is given. The heterozygosity effect in the repeated-measures ANOVAs (average of 10 runs ± SE) is $F_{1,76} = 6.0 \pm 0.62$, $P = 0.026 \pm 0.007$. The heterozygosity effect averaged over all days was also significant when tested with the method of linear contrasts on the nonreduced sample size (*i.e.*, using weights of 0.5 for each of the homozygotes and −1 for the heterozygotes and using the between-mouse variance as the residual: $t_{93} = 2.63$, $P = 0.01$).

2001). Thus, for studies that aim to isolate the effects of particular loci, it is critical to use breeding designs that randomize all background effects (Carroll and Potts 2001; Wolfer *et al.* 2002; Wedekind *et al.* 2004).

In conclusion, we found that, when tested under rigorous experimental conditions, variation in the MHC can have a significant effect on the course of Plasmodium infection, but that MHC heterozygote advantage through overdominance or dominance of resistance cannot be assumed. It remains unclear whether and how our finding is related to the fact that Plasmodium is a comparatively large organism with a presumably large antigen repertoire. However, recent studies on pathogens with a presumably smaller antigen repertoire (Theiler's virus and Salmonella) confirm that MHC heterozygote advantage cannot generally be assumed in the case of single-clone infections (Penn *et al.* 2002; McClelland *et al.* 2003). Future studies on malaria might incorporate a wider range of parasite clones and host H2 genotypes or might focus on the effects of multiple-clone infections where the diversity of parasite antigens confronting the MHC may change this result. In the case of Plasmodium, genetically variable infections are harder to clear and are sometimes more virulent than single-clone infections (Taylor *et al.* 1998; De Roode *et al.* 2003) and thus may lead to different host-parasite interactions.

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LITERATURE CITED

Apanius, V., D. Penn, P. R. Slev, L. R. Ruff and W. K. Potts, 1997 The nature of selection on the major histocompatibility complex. Crit. Rev. Immunol. **17:** 179–224.

Bagot, S., M. I. Boubou, S. Campino, C. Behrschmidt, O. Gorgette *et al.*, 2002 Susceptibility to experimental cerebral malaria induced by *Plasmodium berghei* ANKA in inbred mouse strains recently derived from wild stock. Infect. Immun. **70:** 2049–2056.

Beale, G. H., R. Carter and D. Walliker, 1978 Genetics, pp. 213–245 in *Rodent Malaria*, edited by R. Killick-Kendrick and W. Peters. Academic Press, London.

Black, F. L., and P. W. Hedrick, 1997 Strong balancing selection at HLA loci: evidence from segregation in South Amerindian families. Proc. Natl. Acad. Sci. USA **94:** 12452–12456.

Carrington, M., G. W. Nelson, M. P. Martin, T. Kissner, D. Vlahov *et al.*, 1999 HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science **283:** 1748–1752.

Carroll, L. S., and W. K. Potts, 2001 Accumulated background variation among H2 mutant congenic strains: elimination through PCR-based genotyping of F-2 segregants. J. Immunol. Methods **257:** 137–143.

Carroll, L. S., D. J. Penn and W. K. Potts, 2002 Discrimination of MHC-derived odors by untrained mice is consistent with divergence in peptide-binding region residues. Proc. Natl. Acad. Sci. USA **99:** 2187–2192.

Cigel, F., J. Batchelder, J. M. Burns, D. Yanez, H. van der Heyde *et al.*, 2003 Immunity to blood-stage murine malarial parasites is MHC class II dependent. Immunol. Lett. **89:** 243–249.

De Roode, J. C., A. F. Read, B. H. K. Chan and M. J. Mackinnon, 2003 Rodent malaria parasites suffer from the presence of conspecific clones in three-clone *Plasmodium chabaudi* infections. Parasitology **127:** 411–418.

Doherty, P. C., and R. M. Zinkernagel, 1975 Enhanced immunological surveillance in mice heterozygous at H-2 gene complex. Nature **256:** 50–52.

Dorf, M. E., J. H. Stimpfling and B. Benacerraf, 1979 Gene dose effects in Ir gene-controlled systems. J. Immunol. **123:** 269–271.

Finn, C. A., 1963 Reproductive capacity and litter size in mice: effect of age and environment. J. Reprod. Fertil. **6:** 205.

Gilbert, S. C., M. Plebanski, S. Gupta, J. Morris, M. J. Cox *et al.*, 1998 Association of malaria parasite population structure, HLA, and immunological antagonism. Science **279:** 1173–1177.

Hedrick, P. W., and G. Thomson, 1983 Evidence for balancing selection at Hla. Genetics **104:** 449–456.

Heimrich, B., H. Schwegler, W. E. Crusio and W. Buselmaier, 1988 Substrain divergence in C3h inbred mice. Behav. Genet. **18:** 671–674.

Hill, A. V. S., C. E. M. Allsopp, D. Kwiatkowski, N. M. Anstey, P. Twumasi *et al.*, 1991 Common West African HLA antigens are associated with protection from severe malaria. Nature **352:** 595–600.

Hill, A. V. S., J. Elvin, A. C. Willis, M. Aidoo, C. E. M. Allsopp *et al.*, 1992 Molecular analysis of the association of HLA-B53 and resistance to severe malaria. Nature **360:** 434–439.

Lipsitch, M., C. T. Bergstrom and R. Antia, 2003 Effect of human leukocyte antigen heterozygosity on infectious disease outcome: the need for allele-specific measures. BMC Med. Genet. **4:** 2.

Mackinnon, M. J., and A. F. Read, 1999 Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. Evolution **53:** 689–703.

McClelland, E. E., D. J. Penn and W. K. Potts, 2003 Major histocompatibility complex heterozygote superiority during coinfection. Infect. Immun. **71:** 2079–2086.

Moore, M. J., D. E. Singer and R. M. Williams, 1980 Linkage of severity of experimental allergic encephalomyelitis to the rat major histocompatibility locus. J. Immunol. **124:** 1815–1820.

Penn, D. J., K. Damjanovich and W. K. Potts, 2002 MHC heterozygosity confers a selective advantage against multiple-strain infections. Proc. Natl. Acad. Sci. USA **99:** 11260–11264.

Robey, E., and B. J. Fowlkes, 1994 Selective events in T-cell development. Annu. Rev. Immunol. **12:** 675–705.

Suckow, M. A., P. Danneman and C. Brayton, 2000 *The Laboratory Mouse.* CRC Press, London.

Tarin, J. J., V. Gomez-Piquer, F. Rausell, C. Hermenegildo and A. Cano, 2004 Effect of delayed breeding on the reproductive performance of female mice. Reprod. Fertil. Dev. **16:** 373–378.

Taylor, L. H., M. J. Mackinnon and A. F. Read, 1998 Virulence of mixed-clone and single-clone infections of the rodent malaria *Plasmodium chabaudi*. Evolution **52:** 583–591.

Thursz, M. R., H. C. Thomas, B. M. Greenwood and A. V. S. Hill, 1997 Heterozygote advantage for HLA class-II type in hepatitis B virus infection. Nat. Genet. **17:** 11–12.

Vukusic, B., L. Poplonski, L. Phillips, J. Pawling, T. Delovitch *et al.*, 1995 Both MHC and background gene heterozygosity alter T cell receptor repertoire selection in an antigen-specific response. Mol. Immunol. **32:** 1355–1367.

Wedekind, C., M. Chapuisat, E. Macas and T. Rülicke, 1996 Nonrandom fertilization in mice correlates with the MHC and something else. Heredity **77:** 400–409.

Wedekind, C., M. Walker, J. Portmann, B. Cenni, R. Müller *et al.*, 2004 MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in whitefish. J. Evol. Biol. **17:** 11–18.

Wolfer, D. P., W. E. Crusio and H. P. Lipp, 2002 Knockout mice: simple solutions to the problems of genetic background and flanking genes. Trends Neurosci. **25:** 336–340.

Wunderlich, F., H. Mossmann, M. Helwig and G. Schillinger, 1988 Resistance to *Plasmodium chabaudi* in B-10 mice: influence of the H-2-complex and testosterone. Infect. Immun. **56:** 2400–2406.

Zuk, M., and K. A. McKean, 1996 Sex differences in parasite infections: patterns and processes. Int. J. Parasitol. **26:** 1009–1024.

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