

The course of malaria in mice: MHC effects, but no general MHC heterozygote advantage in single-strain infections

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Summary

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 (F₂ 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). Moreover, 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). Indeed, 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 (APANUS *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*. F₂ 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 between 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 post infection (*p.i.*), and minimal blood cell counts and body weights were reached on average at day 10 and 11

p.i., respectively. At that stage, the mice had lost on average 2.2% (± 0.7 SE) of their initial body weight and 58.1% (± 2.3) of 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 (the time x MHC term in Table 1): the homozygous $H-2^b$ type seemed to clear their parasites faster than $H-2^{ab}$ followed by $H-2^a$ (Fig. 1a). The response of the heterozygous $H-2^{ab}$ was between the two homozygous genotypes (Fig. 1a, 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* (clone x time term in Table 1; Fig. 1c). This corresponds to a similar pattern in the time course of the blood cell counts (clone x time in Table 1; Fig. 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 x 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 susceptibility (Fig. 1). Nor were heterozygotes more resistant than what would be expected from the average of the two homozygotes, i.e. the apparent resistance of 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 (Fig. 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 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 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; HILL *et al.* 1992) is an oft-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 vigour (FINN 1963; TARIN *et al.* 2004). Maternal effects could explain why some congenic strains produce different olfactory signals in parental strains but not in F₂ 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 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 hosts' 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 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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Table 1 The effect of host MHC, parasite clone and host gender on the time course of disease symptoms. 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 variable: parasitemia (6 measurements from day 4-14), blood cell counts (11 measurements from day 1-22), and body weight (19 measurements from day 4-22) given as differences to the weight at day 0. For within-subject analyses we used the multivariate F-tests or Wilk’s lambda (when a factor had more than two levels as in “MHC”).

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
<i>Plasmodium</i> 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, 96	<0.0001	0.1	1, 94	0.77
MHC x clone	0.8	2, 94	0.43	0.5	2, 94	0.61	0.3	2, 94	0.75
MHC x gender	0.9	2, 94	0.39	1.2	2, 94	0.32	0.2	2, 94	0.82
Clone x gender	10.1	1, 94	0.002	9.5	1, 94	0.003	0.4	1, 94	0.52
MHC x clone x 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 x MHC	2.4	10, 180	0.01	1.2	20, 170	0.26	1.0	36, 154	0.46
Time x clone	6.7	5, 90	<0.0001	2.9	10, 85	0.003	1.7	18, 77	0.05
Time x gender	1.5	5, 90	0.20	1.9	10, 85	0.06	6.2	18, 77	<0.0001
Time x MHC x clone	0.4	10, 180	0.92	0.6	20, 170	0.94	1.2	36, 154	0.27
Time x MHC x gender	0.7	10, 180	0.72	1.0	20, 170	0.46	0.8	36, 154	0.78
Time x clone x gender	1.1	5, 90	0.38	1.7	10, 85	0.10	1.9	18, 77	0.03
Time x MHC x clone x gender	1.4	10, 180	0.18	0.7	20, 170	0.86	0.8	36, 154	0.72

Figure Legends

Fig. 1 The effects of MHC and parasite clone on the course of disease symptoms. The figures give 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), $H-2^b$ (black circles), and $H-2^{ab}$ (grey circles), or clone *AS* (open diamonds) and clone *CW* (closed diamonds). See Supplementary material for details about the experimental procedure, and Table 1 for statistics.

Fig. 2 The effect of the $H-2^{ab}$ genotype *versus* the average effects of the respective homozygote MHC types during infection with *P. chabaudi*. To test whether the heterozygotes (grey 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 figure gives the average of 10 randomly reduced samples. The heterozygosity effect in the repeated measures ANOVAs (average of 10 runs \pm SE): $F_{1,76} = 6.0 \pm 0.62$, $p = 0.026 \pm 0.007$. The heterozygosity effect averaged over all days was also significant when tested with the method of linear contrasts on the non-reduced 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: $t_{93} = 2.63$, $p = 0.01$).

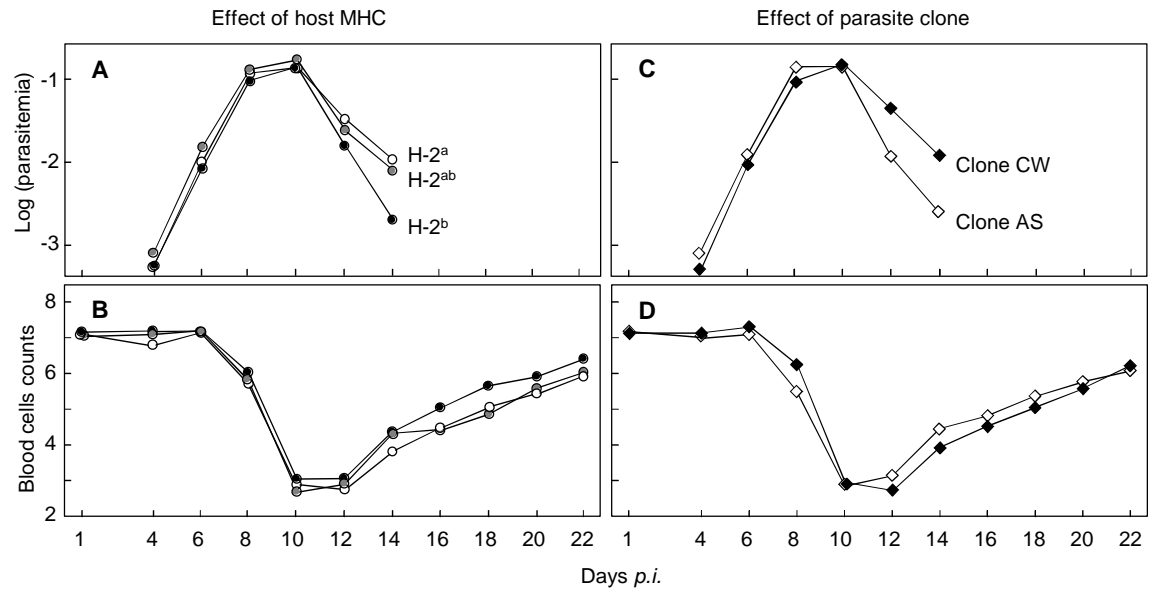


Figure 1
 (Wedekind, Walker & Little)

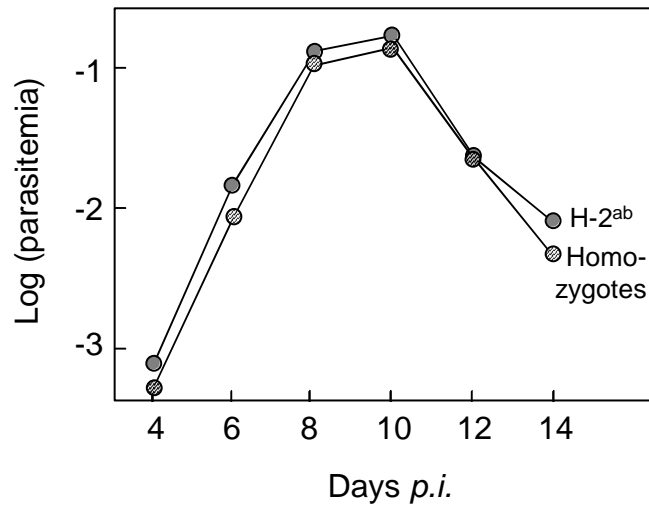


Figure 2
(Wedekind, Walker & Little)