Supporting Online Material for

Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals

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Supporting Online Material

Materials and Methods

Host and parasite

We used five strains of inbred mice: A/J, C57BL/6J, CBA/Ca, DBA/1 and NIH (Harlan, UK). Strains were chosen based on previous work (*1, 2*) to include both relatively resistant and non-resistant strains. All mice were 9-10 weeks old at the start of the experiment. We used three different parasite clones, denoted AS_{11849}, AJ_{4777} and DK_{104}. Clones were selected based on previous studies (*3, 4*), to maximize variation in infection intensity.

Setup and sampling

Each mouse was infected with one of the three parasite clones or left uninfected. The inoculation dose was 10^5 parasites. Inoculations were performed as described by de Roode et al. (*3*). The experiment was performed in three experimental blocks. In total the experiment comprised 152 mice (N=29-32 of each strain).

We weighed mice on an electronic balance and took blood samples from the tail before inoculation and then daily for days 5-15 post inoculation (p.i.) to measure infection intensity and RBC density. We use maximum parasite density (no. of parasites/μl blood) as a measure of infection intensity. Another common measure of infection intensity in the malaria literature is the maximum proportion of infected RBC. These two measures are strongly correlated (r=0.87 in the present data set) and analyses

based on parasite density and proportion infected RBC yield the same conclusions. We measured RBC density using flow cytometry (Beckman Coulter) and estimated the proportion of infected RBC by microscopy; parasite density was calculated by multiplying these values.

Data set and statistical analyses

We analyzed the data by means of mixed linear models. Mouse strain and parasite clone were treated as fixed effects, while experimental block and its interactions with strain and clone were treated as random effects. The significance of random effects was assessed by log-likelihood ratio test (*5*). Non-significant random effects were excluded from the model at $P>0.25$. Analyses were performed with PROC MIXED in SAS 9.1 (*6*), using the Satterthwaite approximation of denominator df of fixed effects.

In analyses testing for variation in tolerance, we used log (minimum RBC density) or log(minimum weight) as dependent variables, and log (pre inoculation value) as covariate (if statistically significant). The variables were log-transformed because we wanted to test for proportionate changes in minimum weight and RBC density with increasing infection intensity.

If the relationship between disease severity (here minimum RBC density and minimum weight) and infection intensity is nonlinear, but only linear terms are included in the statistical model, this may give rise to spurious variation in tolerance (*7*). We therefore tested for non-linear relationships by including quadratic terms in the models. Slopes were estimated with Z-transformed data (i.e., mean=0, s.d.=1).

Twenty five per cent of the infected mice died or were euthanized, all between day 10 and 14. The mortality presents a potential problem for the analysis of tolerance because in mice that died, minimum weight and RBC density often occurred on the day of death. To ensure that the results were not biased by mice that died before reaching even lower values, unambiguous minima were obtained by including in the analyses of tolerance only mice which had survived long enough for their RBC density/weight to begin to increase again (N=129 for minimum RBC and N=123 for minimum weight) [(for the sake of completeness, we also the present analyses based on all mice in the supporting online text (see below)]. However, analyses of resistance were based on all mice, because mice that died had in all cases passed the peak parasite density.

Supporting text

The inclusion of clone in the statistical models

In the analyses of tolerance above we assume that the severity of disease induced by a particular parasite genotype (the RBC or weight loss it causes) is a direct consequence of its infection intensity, and that there is no difference in per parasite virulence between clones. The same assumption is made in previous studies of tolerance in plants [which have used parasites of unknown genetic composition, e.g. refs (*8-10*)]. However, the per parasite virulence could differ between parasite genotypes. We therefore repeated the analysis of tolerance including also the factor clone and its interactions (in this analysis we excluded uninfected mice; thus, the factor clone has 3 levels: DK, AS or AJ; N=96 and 90 for minimum RBC density and minimum weight, respectively). In the case of minimum RBC density, there were significant effects of both clone [$F(2, 76)=92.9$,

$P<0.0001$] and strain×clone [$F(8, 76)=3.61$, $P=0.0013$]. However, the tolerance term (strain×infection intensity) remains significant when controlling for these effects [$F(4, 76)=4.75$, $P=0.0018$]. Also in the case of minimum weight there was an effect of clone [$F(2, 77)=29.5$, $P<0.0001$], but again the strain×infection intensity term remained significant [$F(4, 77)=2.69$, $P=0.037$]. Thus, variation for tolerance is not confounded by clonal variation in per parasite virulence. This analysis also shows that the variation for tolerance we report is not arising as some artefactual consequence of including the uninfected mice.

The use of parasite intensity measures other than peak density

Variation in infection intensity may not be fully captured by peak density. For example, the rate at which the infection intensity declines after the peak may also affect anaemia and weight loss. If mouse strains differ with respect to such infection dynamics, this may result in spurious variation for tolerance. Therefore, we repeated the analyses of tolerance using the total number of parasites present in an infection as measure of infection intensity. For these analyses, we selected mice that survived at least 3 days post peak and calculated total densities by summing the daily densities (the generation time for the asexual stage of *P. chabaudi* is 24h) from day 2 pre peak up to and including day 3 post peak (N=112 for minimum RBC density and N=99 for minimum weight). Analyses of both minimum RBC density and minimum weight using this measure of infection intensity yielded the same conclusions as the analyses with peak density above (min RBC density: initial RBC density: $F(1, 101)=9.63$, $P=0.002$; total parasite density: $F(1, 99.2)=192$, $P<0.0001$; strain: $F(4, 99.5)=0.59$, $P=0.67$; density×strain: $F(4, 99.3)=6.76$,

$P<0.0001$; experimental block: $\chi^2=27.9$, $P<0.0001$; block×strain: $P>0.25$; minimum weight: initial weight: $F(1, 83)=105$, P<0.0001, strain: $F(4, 83)=7.80$, $P<0.0001$; density, linear term: $F(1, 83)=70.8$, $P<0.0001$; density, quadratic term: $F(1, 83)=35.8$, $P<0.0001$; density×strain: $F(4, 83)=4.23$, $P=0.004$; strain×density^2: $F(4, 83)=2.54$, $P=0.046$); experimental block and strain×block: $P>0.25$. Thus, there is no reason to suspect that the strain-by-infection intensity interactions are particular to the measure of parasite burden.

Analyses based on all mice

As described in the Materials and Methods above, the main analyses of tolerance (fig 2) are based on a subset of data. Specifically, we excluded mice whose RBC density and/or weight did not start to rise before they died. However, the exclusion of these mice could possibly bias the results, if mice that died before reaching minimum RBC density/weight are not random with respect to tolerance. We therefore also performed analyses based on all mice (N=152). These analyses yielded the same conclusions as the analyses presented in fig 2: Minimum RBC density: Strain: $F(4, 140)=0.26$, $P=0.90$; peak parasite density: $F(1, 140)=147.4$, $P<0.0001$; strain×density: $F(4, 140)=5.61$, $P=0.0003$; experimental block: $\chi^2=19.1$, $P<0.0001$. Initial RBC density ($P=0.49$), parasite density^2 ($P=0.20$) and block × strain ($P>0.25$) were not significant and therefore excluded from the model. Minimum weight: initial weight: $F(1, 140)=177.0$, $P<0.0001$; strain: $F(4, 139)=1.92$, $P=0.11$; peak parasite density: $F(1,140)=3.54$, $P=0.062$; density^2: $F(1, 140)=25.0$, $P<0.0001$; strain×density: $F(4, 138)=3.99$, $P=0.0043$; experimental block: $\chi^2=22.2$, $P<0.0001$. Strain×density^2 ($P=0.44$) and block×strain ($P>0.25$) were not significant and therefore excluded from the model.

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Fig S1 Råberg et al.

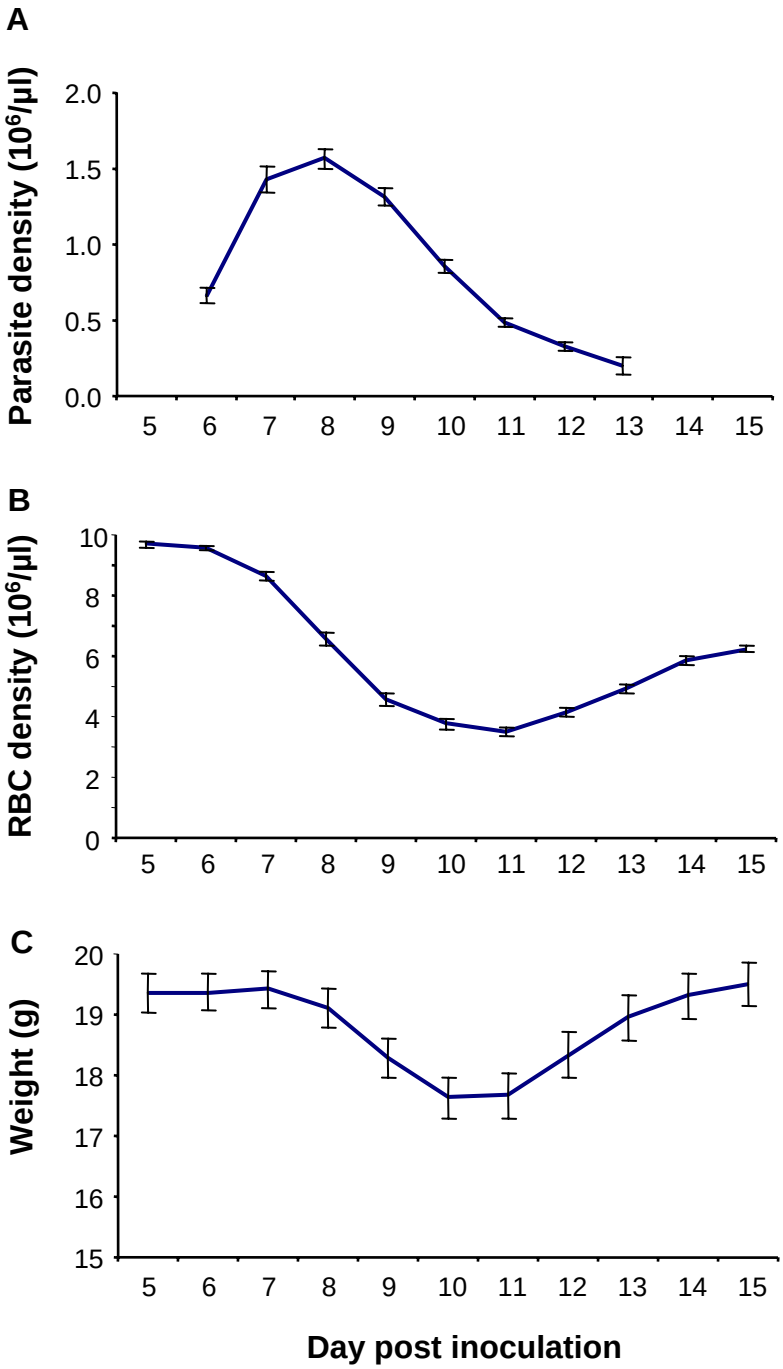

Fig 1S. Dynamics of infection across all mouse strains and parasite clones. (**A)** Parasite (mean±s.e.) density over time. (**B**) RBC (mean±s.e.) density over time. (**C**) Weight (mean±s.e.) over time.