Multigenic control of *Listeria monocytogenes* susceptibility in mice

We have used a novel quantitative trait locus model to study the genetics of survival of F2 progeny of susceptible BALB/cByJ and resistant C57BL/6ByJ mice that have been infected with *Listeria monocytogenes*. This allowed us to map modifiers of *L. monocytogenes* susceptibility to chromosomes 5 and 13.

L. monocytogenes, a Gram-positive bacterium, causes a wide range of diseases, from localized enteritis to systemic infections in both immunocompromised and immunocompetent individuals[1,2]. Human genetic linkage analysis of susceptibility to infectious diseases like listeriosis is difficult due to the rarity of related individuals with known phenotype status[3]. In contrast, inbred mouse strains have several described mendelian and polygenic differences in susceptibility to listeriosis[4,5] and are easier to use in genetic experiments. Despite this, the genetic basis for differences in mouse susceptibility has been identified in only one case[6]. Therefore, we chose to revisit the issue of mapping *L. monocytogenes* sensitivity using the C57BL/6ByJ and BALB/cByJ mouse strains[7].

Our experiments established that $2–5\times10^4$ colony forming units (c.f.u.) of 10403s (ref. 8) *L. monocytogenes* injected intravenously causes death of BALB/cByJ (C) animals within 72 hours, whereas all C57BL/6ByJ (B) mice survive indefinitely. Our histologic examination of liver (Fig. 1) and spleen (data not shown) of infected parental strains revealed differences in the influx of innate immune cells (Web Methods). Liver from both strains contained similar large aggregates of neutrophils at early stages of infection (Fig. 1*a*,*d*). In the resistant strain, however, the influx of macrophages ultimately led to formation of granulomas (Fig. 1*b*,*c*) and recovery of the animals, whereas susceptible animals formed rare granulomas and died with confluent neutrophilic abscesses (Fig. 1*e*).

Our analysis of the course of *L. monocytogenes* infection in 116 age-matched female BALB/cByJ×C57BL/6ByJ F2 (CB6F2/ByJ) mice revealed a complex phenotypic segregation pattern. The CB6F2/ByJ animals that died as a result of infection displayed a wide range of survival times with a mean of 106 hours. In addition, a large proportion of the progeny survived past the 240-hour time point and were considered to be recovered (Web Fig. A).

This unusual phenotype distribution effectively precluded use of traditional quantitative trait loci (QTL) mapping tools, which require normally distributed values[9]. An alternative nonparametric approach, in which trait values are replaced with ranks, is also not ideal to analyze our data because many individuals have identical trait values[10]. To overcome these problems, we analyzed the survival time data using a novel single-QTL model (Web Methods). In this model, a mouse with genotype g (CC, CB or BB) has probability p_g of surviving the infection. If the mouse does die, its log survival time y is normally distributed with mean μ_g and standard deviation σ (independent of g). To map the trait, we calculated separate lod scores for p_g (lod(p)) and μ_g (lod(μ)), and a combined score for both parameters (lod(p,μ)) in 1-cM steps along a genetic map of the cross determined by a set of linked polymorphic markers[11]. Our analysis of the cross using this method revealed two loci at which the lod scores were substantially above the empirically determined genome-wide 5% threshold of significance (Fig. 2*a*). The chromosome 5 locus near *D5Mit357* demonstrated significant effects on the probability of survival (p_g) and combined (p,μ) traits, whereas the chromosome 13 locus near *D13Mit147* had a substantial influence on all three traits (Fig. 2*a*).

To verify the significance and location of these loci, we analyzed a second cross consisting of 84 mixed-sex CB6F2/ByJ animals. Our analysis of the second cross confirmed the results of the first. The previously identified region on chromosome 5 had a lod(p,μ) score of 3.16 at *D5Mit338*, confirming our *a priori* hypothesis about the role of this region. The peak lod(p,μ) on chromosome 13 was 9.5 at a slightly different position than in the first cross; however, the 95% confidence intervals from the first and the second cross overlap (Fig. 2*b*). The differences in the magnitude of lod scores observed in the two crosses can be attributed, among other factors, to the inherent variability of the experiments involving infecting and phenotyping live animals.

The general trends of the effect of the identified loci can be discerned by

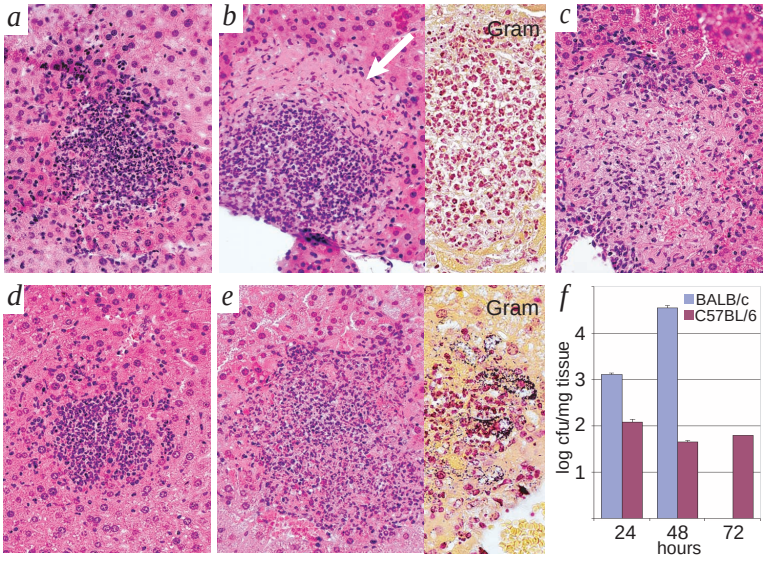
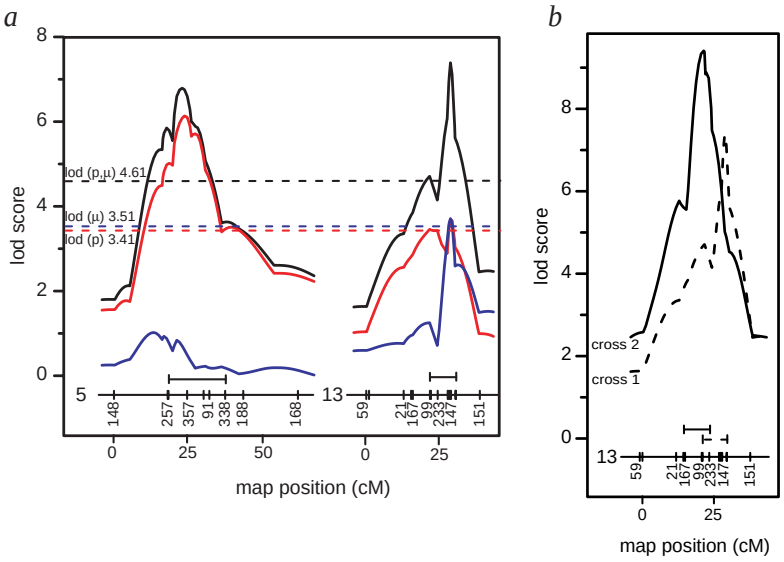


Fig. 1 Progression of listeriosis. *a*–*e*, Liver sections stained with hematoxylin and eosin. At 24 h post-infection, abundant acute inflammations with neutrophil-rich abscesses are present in both C57BL/6ByJ (***a***) and BALB/cByJ (***d***) strains. By 48 h, the acute inflammatory response is unchecked in the susceptible BALB/cByJ strain (***e***). The resistant C57BL/6ByJ strain (***b***) shows incipient granuloma formation, which is indicated by the surrounding mononuclear cells forming a pale-staining zone around the dark-staining neutrophils (arrow). A Gram stain at 48 h revealed abundant Gram-positive rods in liver from susceptible mice (***e***), whereas none are seen in liver from resistant mice (***b***). At 72 h, all C57BL/6ByJ animals (***c***) have well-formed granulomas in their livers. All susceptible mice have succumbed to infection by this time. The histology of C57BL/6ByJ liver is phenotypically similar to that previously observed in C57BL/10 liver, except that we found granulomas forming over a shorter time frame[14]. In contrast to previous studies, however, the BALB/cByJ neutrophilic abscesses are identical in quality, but not quantity, to those seen in the resistant strain[14]. ***f***, Differences in viable *L. monocytogenes* recovered from homogenized livers of infected animals.



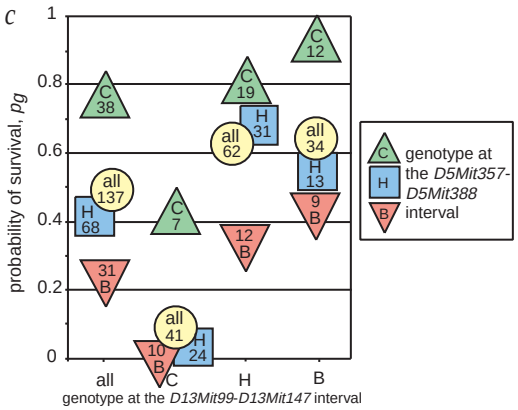


Fig. 2 Mapping results. ***a***, Interval mapping of the *L. monocytogenes* susceptibility trait used seven parameters (p_{CC}, p_{CB}, p_{BB}, μ_{CC}, μ_{CB}, μ_{BB} and σ) to calculate three lod scores: lod(p) (red), to test the hypothesis $p_{CC} = p_{CB} = p_{BB}$; lod(μ) (blue line), to test the hypothesis $\mu_{CC} = \mu_{CB} = \mu_{BB}$; and lod(p,μ) (black), to test the combined hypothesis that both the p_g and μ_g were equal. Markers from the MIT set[11] are identified by their numbers for each respective chromosome. Horizontal lines indicate empirically determined significance thresholds. ***b***, Comparison of the interval mapping results from cross 1 and cross 2. Horizontal lines indicate 95% confidence intervals for each of the crosses. ***c***, Cumulative effect of identified loci on probability of survival p_g. Column "All" on the *x* axis of the graph and "All" circles illustrate the effect of loci on p_g without regard for the genotype at the other locus. Only animals nonrecombinant in the respective intervals were selected for calculation of probabilities of survival. Numbers inside the symbols indicate the number of animals of that genotype.

studying the data in Fig. 2*c*. For example, when only the genotype at the *D13Mit99–D13Mit147* region is considered (Fig. 2*c*, circles), the C57BL/6ByJ (B) allele appears to have a dominant effect, increasing the probability that a mouse survives (p_g). At the *D5Mit357–D5Mit338* region, the allele from the sensitive BALB/cByJ (C) strain actually increased the proportion of mice surviving (Fig. 2*c*, triangles). Animals that are homozygous for both susceptibility alleles uniformly succumbed to the infection, with a very short average survival time μ_g of 90 hours. Conversely, 11 of 12 animals that were homozygous for both resistance alleles survived indefinitely. These data support the contention that these two loci have major effects on the outcome of *L. monocytogenes* infection. F2 animals homozygous for both grandparental alleles, however, do not faithfully recapitulate the grandparental phenotype (Fig. 2*c*), indicating the existence of additional undetected QTL.

We have developed a new genetic analysis method useful for identification of mouse genes that influence susceptibility to listeriosis. In the future, it may be possible to test the role of their human orthologs on susceptibility to infectious agents[12]. Nevertheless, characterization of mouse *L. monocytogenes* susceptibility genes will benefit our understanding of infectious pathogenesis, regardless of the existence of functional variation in their human orthologs[13].

Note: supplementary information is available on the Nature Genetics web site (http://genetics.nature.com/supplementary_info).

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