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SHORT COMMUNICATION

Genetic control of susceptibility to pulmonary infection with Chlamydia pneumoniae in the mouse

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A mouse model was used to study the genetic control of differential host response to pulmonary infection with Chlamydia pneumoniae. The A/J and C57BL/6 strains show differential response to intranasal infection with respect to their ability to clear pulmonary bacterial load and the extent of lung pathology developed by 2 weeks post infection. The genetic basis of this interstrain difference was studied by whole-genome scan in an informative [A/J × C57BL/6J] F2 cross using the pulmonary microbial load as a phenotypic readout of host response. We detected a highly significant linkage (LOD score = 11.5) on chromosome 17 that overlaps with the major histocompatibility (MHC) locus. This quantitative trait locus (QTL) accounts for ~30% of the phenotypic variance with B6 alleles conferring susceptibility and inherited in a recessive fashion. Significant linkage was also detected to chromosome 5 in female mice, while chromosome 6 showed suggestive linkage in male mice, pointing to additional complexity in the genetic control of the difference in susceptibility observed in A/J and C57BL/6J.
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Introduction

Chlamydia pneumoniae is a member of the highly prevalent *Chlamydia* genus, obligate intracellular bacterial pathogens that are responsible for a wide spectrum of pathologies. Species of *Chlamydia* cause a variety of acute and chronic infections in humans, with pathology attributable to repeated infection and chlamydial persistence. The widespread *C. pneumoniae* has a seroprevalence of over 50% among adults and is the causative agent of a variety of upper and lower respiratory diseases, such as bronchitis, pneumonia and sinusitis, and has been implicated in adult-onset asthma.[1,2] Although respiratory disease is the most common manifestation of this infection, additional associations with chronic conditions, such as heart disease[3] and Alzheimer's disease[4] have been noted. These diseases are thought to be mediated by detrimental host responses, which may be enhanced or co-induced by the bacteria.

All *Chlamydia* species are capable of inducing a strong cytokine response in the host, and innate immunity is critical for controlling bacterial replication in the acute phase of infection. Mutant mice bearing loss-of-function mutations in IFNγ, iNOS or IL-12 develop increased bacterial load in *C. pneumonia* infection models (reviewed in Rottenberg *et al.*[5]) and underline the importance of the IFNγ-mediated host response. While Th1-type cytokines limit chlamydial replication, they may also contribute to pathological changes in the lung.[6] CD4+ and CD8+ T cells are important in both innate and adaptive immune mechanisms, which are responsible for clearance of chlamydial infection and protection against reinfection.[7] For example, depletion of CD8+ T cells *in vivo* impairs clearance during primary infection and abolishes protective immunity against *C. pneumoniae* in mice,[8] while CD8−/− mice demonstrate enhanced susceptibility to infection due to a decreased Th1 response.[7] CD4+ T cells also play a protective role in pulmonary infection,[9] but have been shown to be more critical in models of genital chlamydial infection.[10] In humans, prior exposure does not seem to provide adequate protective immunity and may in fact augment the severity of pathology (repeated infections).[11]

As with other infectious agents, exposure to *Chlamydia* species does not result in clinical symptoms in all individuals, suggesting a contribution of genetic background. We have previously reported on a respiratory model of *C. pneumoniae* infection in mouse that reveals discrete phenotypes amenable to quantitative measures of disease susceptibility.[12,13] Using this model, response to infection with *Chlamydia* species varies widely in inbred strains of mice. When infected with *C. pneumoniae*, C57BL/6J (B6) mice show a robust inflammatory response characterized by a strong Th1-driven response, including high levels of IFNγ and IL-12.[13,14] However, the consequences of this early inflammation are concomitant to greater lung disease and pathology in this strain,

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compared to A/J. The extent of chlamydial lung disease can be estimated by an increase in total lung weight, an indicator of cellular infiltration to the site of infection. B6 mice exhibit substantial lung weight increases post infection.[15] On the other hand, the A/J strain mounts a rather poor inflammatory response immediately following infection and presents with higher chlamydial burden in the lung at early time points. Nevertheless, A/J mice are able to efficiently clear the infection without developing major lung pathology. We have used genome-wide linkage in an informative population of [A/J × B6] F2 mice to investigate the genetic basis of this interstrain variation in response to *C. pneumoniae* infection.

Results and discussion

We infected A/J and C57BL/6 (B6) female mice with an intranasal challenge of 5×10^7 *C. pneumoniae* and measured the increase in lung weight (indicator of disease) and chlamydial load in the lung at day 15 post infection. Previous kinetic studies in these two strains showed that day 15 falls within the curative phase of the infection; thus, assessing microbial load at that time provides a phenotypic readout of protective host response. The pulmonary chlamydial load was assessed by quantitative PCR using DNA primers for the *C. pneumoniae* genome, according to a protocol we have previously described.[16] Results in Figure 1 ($\log_{10}$ *C. pneumoniae* per mg lung tissue) show that at day 15, B6 mice have a 5- to 10-fold higher chlamydial burden in the lung than A/J mice, with this difference being more prominent in male (Figure 1a) than female mice (Figure 1b). To analyze the possible contribution of genetic factors to this phase of the infection, ~200 [A/J × B6] F2 mice were similarly infected and phenotyped for pulmonary parameters 15 days post infection. In this experiment, the F2 mice showed values ranging from very low ($\log_{10}$ ~1–1.5; threshold of detection) to very high chlamydial load in the lung ($\log_{10}$ ~4.5), with a normal distribution that is suggestive of a complex genetic trait (Figure 1).

Some F2 mice were found to have no *C. pneumoniae* present in the lung; this could result from sterilization of the lung by productive immune response or incomplete delivery of the infectious inoculum or a combination of both. To take this into account, separate linkage analyses were carried out with or without these mice included. We analyzed all F2 mice using the two-part parametric model described by Broman[17] to account for the non-normal distribution of the phenotype with a peak at the minimum phenotype (*Cp*/mg lung = 0). This approach combines the analysis of the binary trait (clearance vs nonclearance) with the quantitative trait of mice presenting with a measurable chlamydial load.[17] Overall results from this two-part model were similar to those presented below, with binary trait linkage contributing little to the combined LOD score.

The continuous and normally distributed values of chlamydial load in the [A/J × B6] F2 population with measurable *Cp* in the lung, suggested a complex trait and were amenable to standard quantitative trait locus (QTL) analysis. Therefore, the genotype of 154 F2 mice was determined for 140 informative polymorphisms of the microsatellite type chosen from the Broad Institute

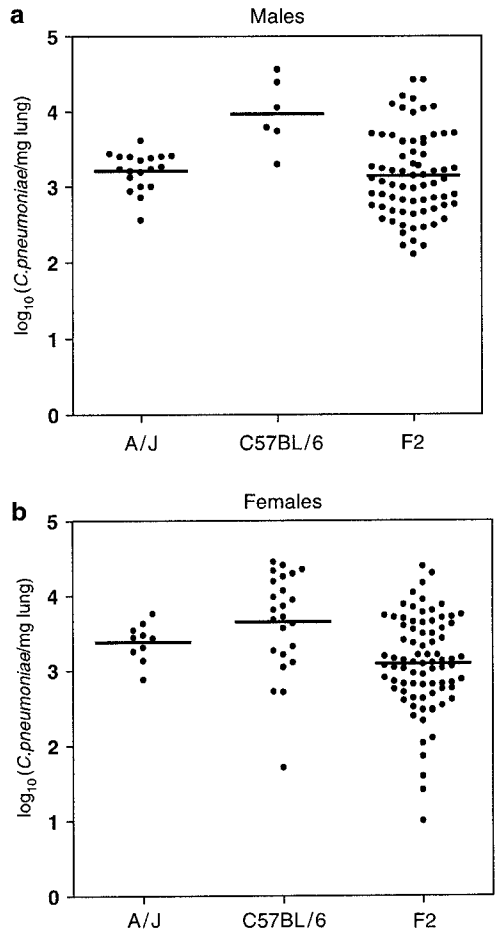

Figure 1 Distribution of chlamydial load in the lungs of A/J, C57BL/6J and [A/J × B6] F2 mice. [A/J × B6] F2 mice bred at McGill University and A/J and B6 control mice (Jackson Labs., Bar Harbor, ME, USA) were infected intranasally following the procedure described previously[12] with 5×10^7 *Cp* organisms at 10–16 weeks of age. *Chlamydia pneumoniae* strain CDC/CWL-029 (ATCC VR-1310) was prepared as described,[30] using Buffalo Green Monkey Kidney cells (Diagnostic Hybrids Inc., Athens, OH, USA) as host cells and purifying elementary bodies by sedimentation and centrifugation.[30] The level of bacteria in the lung was measured by quantitative PCR using *Chlamydia*-specific 23S rRNA as described[16] at 15 days post infection in all mice. The distribution of measurable *Cp* organisms in F2 mice is shown for males (**a**) and females (**b**) separately, in comparison to parental strains from the same infection. The log of *Chlamydia* organisms is plotted per milligram of lung tissue. Each dot represents a mouse. All animal protocols were approved by the Animal Care and Use Committee following NIH guidelines.

(http://www.broad.mit.edu/cgi-bin/mouse/index), at a spacing of ~10 cM across the entire genome. Initially, single marker regression analysis was performed (Table 1) using Map Manager QTX[18] to reveal a number of significant linkages. Markers mapping to chromosome 17 were associated with high significance, with the strongest linkage to *D17Mit62* (LOD = 11.5; $\chi^2 = 53.1$; $P < 0.00001$). Additional suggestive linkages were obtained for chromosomes 6 and 5, with LOD scores of 2.6 and 2.7, respectively. We further analyzed the data set using the R/QTL package[19] and found the chromosome 17 QTL to be significant when analyzing male and

Table 1 Single marker regression analysis of response to *Chlamydia pneumoniae* infection in [A/J × B6] F2 mice

STS marker	*Position*		*Free regression model*[a]			*Males only*		*Females only*	
	cM	*Mbases*	χ^2	*LOD*	*P-value*	*LOD*[b]	*P-value*	*LOD*[b]	*P-value*
D5Mit97	74	137.3	12.5	2.72	0.002	NS	NS	3.85	0.0001
D6Mit274	20.5	48.6	11.6	2.52	0.003	2.89	0.0013	NS	NS
D6Mit188	32.5	75.4	11.9	2.58	0.002	1.56	0.027	NS	NS
D6Mit284	37.5	92.6	10.8	2.34	0.005	1.63	0.023	NS	NS
D17Mit164	4.1	3.9	27.6	6.0	$<10^{-6}$	6.28	$<10^{-6}$	1.33	0.05
D17Mit101	16.4	28.9	46.9	10.2	$<10^{-6}$	8.37	$<10^{-6}$	3.17	0.0007
D17Mit62	17.4	33.3	53.1	11.5	$<10^{-6}$	8.5	$<10^{-6}$	4.11	8×10^{-5}
D17Mit66	24.5	47.2	48.5	10.5	$<10^{-6}$	6.33	$<10^{-6}$	4.70	2×10^{-5}
D17Mit139	30.2	52.1	29.3	6.37	$<10^{-6}$	5.13	1×10^{-5}	1.96	0.011
D17Mit193	41.5	67.4	19.2	4.17	7.0×10^{5}	2.91	0.0012	1.63	0.023

[a]Linkage analysis was performed using single marker regression in the Map Manager QTX program using no model constraints.
[b]NS = nonsignificant linkage ($P > 0.05$).

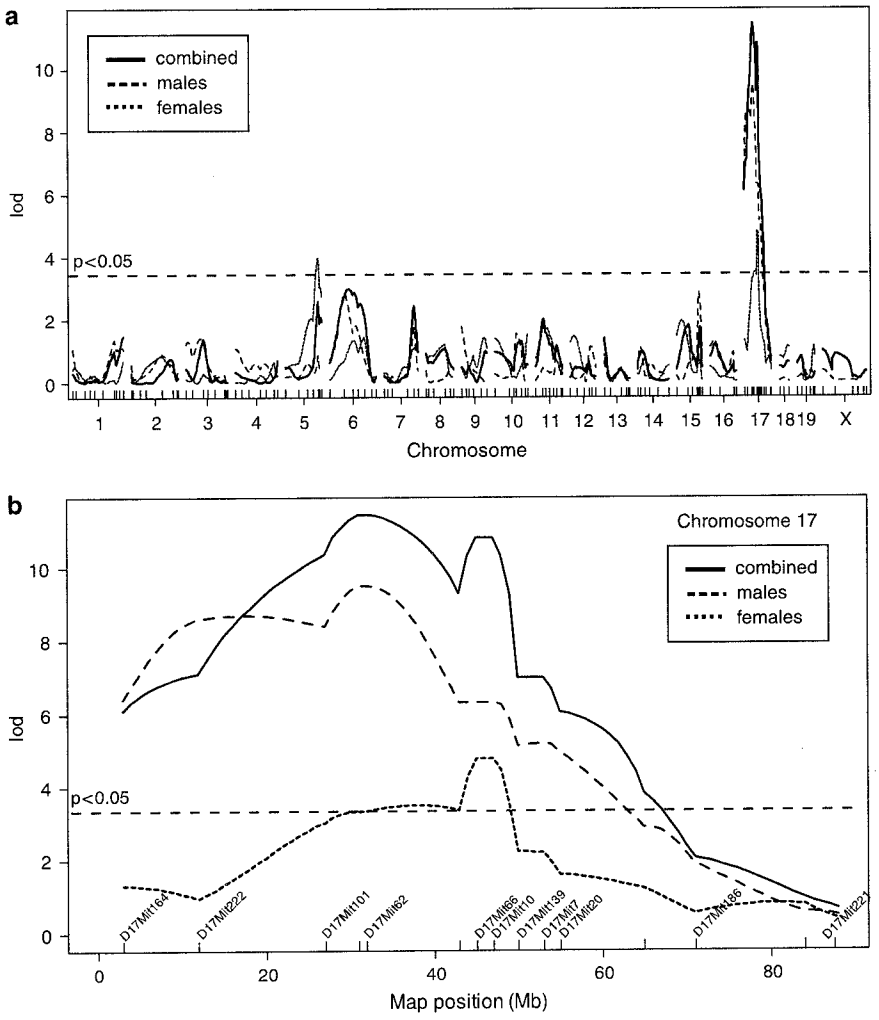

Figure 2 Quantitative trait linkage analysis of *Chlamydia pneumoniae* load in informative [A/J × B6] F2 mice. Regression interval mapping (Haley–Knott method) was performed using R/QTL on the whole genome for all informative F2 mice (full line) and on male and female mice separately (dashed and dotted lines, respectively), using log transformed *Chlamydia* genomes per milligram lung as a quantitative trait. Resulting LOD scores were plotted for all chromosomes (**a**) and for chromosome 17 separately (**b**). Permutation testing (500 tests) revealed the genome-wide significance threshold for $P = 0.05$ at LOD score = 3.5 (marked as a dashed line in panels a and b). F2 mice were genotyped using standard methods[31] for 140 informative microsatellite markers chosen across all 20 chromosomes at a spacing of 10–20 cM. QTL, quantitative trait locus.

female mice, separately, but with much stronger linkage in male mice (Figure 2a). This locus was found to control nearly 30% (29% at *D17Mit62*) of the phenotypic variance in this quantitative trait. Interval mapping using Haley–Knott regression (1 cM steps) of chromosome 17 identified two highly significant linkage peaks that exceed the significance threshold established for genome-wide significance of $P<0.05$ (Figure 2b). LOD score thresholds for significance were determined for this data set by permutation testing (500 tests), with significant LOD scores of 4.28 and 3.5 corresponding to a 99 and 95% genome-wide confidence, respectively. Analysis of female mice alone revealed significant linkage on chromosome 5, with a peak LOD score of 3.85 (*D5Mit97*), whereas this locus was not found to be significant in male mice (Figure 2a). A two-dimensional genome scan was performed in R/QTL with a two-QTL model to test for epistasis in male, female and all mice combined. No major interacting loci were uncovered, while the chromosome 5 and 17 contributions were additive in female mice (data not shown).

Segregation of [A × B6] F2 mice according to their genotype at *D17Mit62* shows that individuals homozygous for B6 alleles carry a 10-fold higher chlamydial load in the lungs, when compared to mice homozygous for the A allele (Figure 3). The chlamydial load in heterozygous (AB) mice is similar to AA homozygous mice, suggesting that the B6 allele-associated 'susceptibility' is inherited in a recessive manner. The strong effect of *D17Mit62* alleles on pulmonary microbial load was similar in male (Figure 3a) and female F2 mice (Figure 3b). To further delineate the genetic interval, F2 mice showing recombination events within the chromosome 17 locus were examined for chlamydial load (Figure 4). Although the number of mice in each group is too small to show any significant differences, it appears that homozygosity for B6 alleles at *D17Mit62* is required for elevated bacterial loads, with little additive effect of allelic combination (b/b, h and a/a) at adjacently distal markers (Figure 4, left panel). Likewise, heterozygosity (h) or homozygosity (a/a) for A/J-derived resistance alleles at *D17Mit62* is associated with lower chlamydial loads, irrespective of allelic combination at adjacently proximal or distal markers (Figure 4, right panel). Although a larger number of recombinants will be required to validate this finding, these observations would position the major gene effect in the immediate vicinity of *D17Mit62*.

Infection by *C. pneumoniae* results in both acute and serious chronic conditions that are highly prevalent in the human population. Differential susceptibility to disease onset and progression, stemming largely from persistence or multiple infections by the pathogen, suggests an important role for host genetics. Mouse models of respiratory infection by *C. pneumoniae* show high variability between inbred strains in terms of chlamydial load in the lung and consequent lung pathology.[13] We investigated the chlamydial load in the lung at day 15 post infection, a time point at which mice from both parental strains are in the process of eliminating the infection. We chose a late time point to investigate the mechanisms underlying differential clearance rates, which in the A/J and B6 strains reflect major differences in lung pathology and disease. As the majority of disease resulting from *Chlamydia* species in

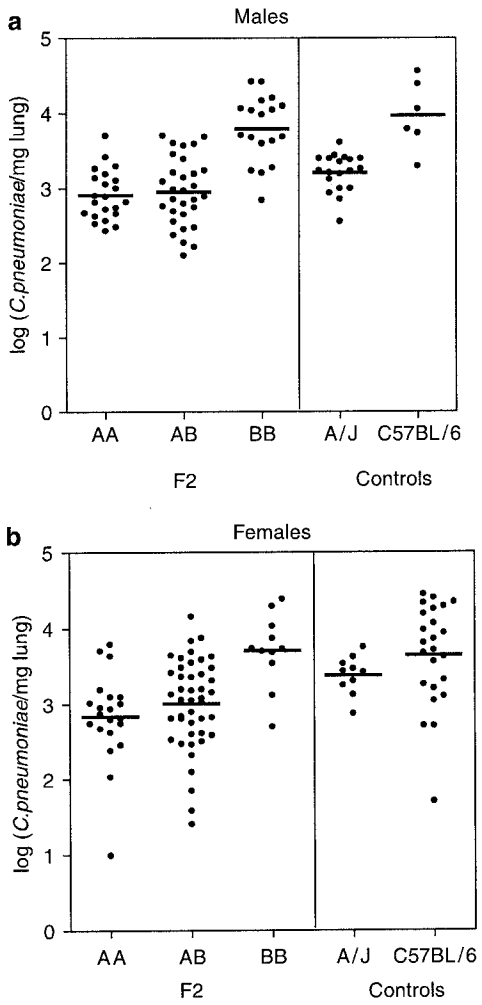

Figure 3 Effect of allelic combination at D17Mit62 on pulmonary chlamydial load in [A/J × B6] F2 mice. Levels of *Chlamydia pneumoniae* in the lung were measured at day 15 post infection in F2 mice (as described in Figure 1). Individual mice were segregated according to their respective genotype at the chromosome 17 marker, D17Mit62, and the log (*Cp*/mg lung) was plotted in comparison to the controls for male (**a**) and female mice (**b**). Genotypes are represented as AA = A/J, AB = heterozygous and BB = B6, and each dot represents a mouse.

humans is the consequence of chronic or persistent infections, this ability to efficiently eliminate the bacteria without the induction of immunopathology at the site of infection may be of great importance. The major histocompatibility (MHC) region encompasses approximately 10 Mbases (from *Kifc1* to *H2-M2*)[20] and has been linked to differential susceptibility for numerous autoimmune and infectious diseases. The genes mapping to this region include the MHC class II and class I molecules, which are important for the host in the discrimination between self and nonself, and T-cell development and adaptive immunity. In addition, a variety of non-*H2* genes that are critical in host response are also found in the MHC region, including members of the complement cascade, TNFα and LTα, and proteins (TAP1 and TAP2) involved in antigen processing.[21] The high concentration of polymorphic genes affecting

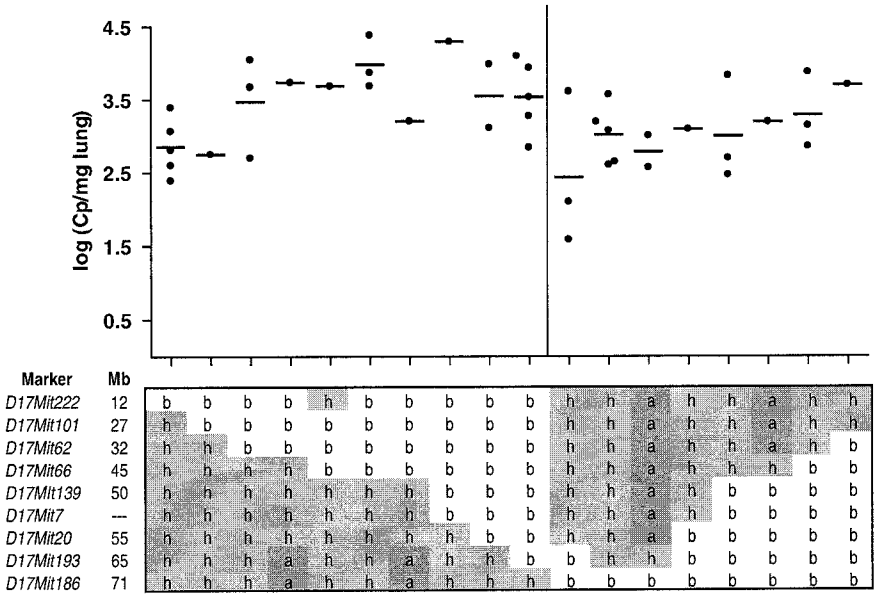

Figure 4 Effect of recombinant chromosome 17 haplotypes on pulmonary chlamydial load in [A/J × B6] F2 mice. F2 mice were grouped according to their haplotype at chromosome 17, and only those showing recombinant chromosomes are plotted. Levels of *Chlamydia pneumoniae* in the lung were measured at day 15 post infection in F2 mice (as described in Figure 1), and the log of the chlamydial load is shown for each mouse in each group, represented as a dot. The haplotypes were ordered according to location of the recombination event and coded by allele; dark gray = A/J, gray = heterozygous and white = B6 alleles.

host innate and acquired immune responses in this region of the human and mouse genomes has associated this region with numerous disease traits.[20] These polymorphisms define well-described *H2* haplotypes in the various inbred strains of mice, including *H2a* (carried by A/J) and *H2b* (carried by C57BL/6). More specifically, the importance of *H2* genes in contributing to outcome following infection by chlamydial species (*C. trachomatis*, *C. muridarum* and *C. pneumoniae*), in both humans and mice, has been reported. Studies in human populations have shown association between the HLA-A31 molecule and increased risk of pelvic inflammatory disease,[22] while HLA haplotypes also appear to predict recurrent *C. trachomatis* infections in adolescents.[23] Polymorphisms in the *TNFα* gene have been associated with scarring trachoma[24] and, more recently, risk of ocular *C. trachomatis* sequelae in a Gambian population.[25] In a mouse model of genital tract infection, MHC class II−/− mice fail to resolve the infection compared to controls, with lower levels of serum antibodies.[26] Recently, Qiu *et al.*[27] used reciprocal *H2* congenic mice on B6 and C3H/HeN backgrounds to show dependence of chlamydial replication on the MHC in a mouse model of pneumonitis (*C. muridarum*) lung infection. The authors found that B6 mice carrying the C3H *H2* haplotype (*H2k*) were more susceptible than B6 controls; however, C3H mice carrying the B6 *H2* haplotype (*H2b*) were equally susceptible as C3H control mice, despite displaying cytokine profiles similar to B6. This reveals that non-MHC elements may also contribute to chlamydial replication and pathology in this model. Our study represents the first whole-genome mapping study of *C. pneumoniae* susceptibility and underlines the key role of the mouse MHC in mediating bacterial clearance. The region immediately surrounding the most significantly linked marker in our analysis (*D17Mit62*) contains the *H2* genes themselves; *Daxx*, a gene involved in apoptosis; *Adamts10*, a disintegrin/metalloprotease involved in tissue remodeling; complement C4 and C2; *TNFα* and *LTα*, as well as the lymphocyte antigen 6 complex. Prior linkage mapping studies by other groups have focused on early infection time points and mapped several key loci on chromosomes 2, 3 and 11 controlling this response in a model of *C. trachomatis* infection.[28] Further characterization of these loci identified the genes *Igtp* and *Irgb10*, encoding p47 GTPases on chromosome 11 as key components of host response.[29] However, these particular studies did not find linkage to chromosome 17. Substantial differences in the model, particularly the time point of interest and the site of infection, as well as selection of both mouse strains and *Chlamydia* species fully explain the disparity in the linkage findings.

The identification of genetic factors affecting host response to infection with *C. pneumoniae* will lead to a greater understanding of the nature of chronic and persistent chlamydial infections that have been linked to important conditions such as artherosclerosis, as well as offering insight into host–pathogen interactions in this complex disease.

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