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Mapping immunodominant antigens and H-2-linked antibody responses in mice urogenitally infected with *Chlamydia muridarum*

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Abstract

To identify immunodominant antigens and MHC-restricted antibody responses, seven different strains of mice were intravaginally infected with *Chlamydia muridarum* and compared for antibody responses to 257 *C. muridarum* proteins. The 7 strains of mice recognized a total of 109 proteins as antigens, of which, 5 antigens (TC0660, TC0727, TC0828, TC0726 & TC0268) were each recognized by 60% or more mice from each mouse strain and thus designated as immunodominant antigens. Furthermore, antibody responses to 19 other antigens displayed strong associations with mouse H-2 haplotypes, including 6 antigens (TC0480, TC0912, TC0229, TCA04, TC0289 & TC0892) whose antibody responses were linked to H-2^b, 8 (TC0035, TC0387, TC0052, TC0781, TC0373, TC0117, TC0066 & TC0396) to H-2^d and 5 (TC0512, TC0177, TC0589, TC0794 & TC0596) to H-2^k haplotypes respectively. Interestingly, H-2^b was negatively associated with antibody responses to most of the antigens that were positively linked to H-2^d or H-2^k haplotypes. These results by mapping *C. trachomatis* antigens commonly recognized by mice with different strain background and H-2 genes and revealing antigen association with H-2 haplotypes have provided important information for developing chlamydial subunit vaccines and understanding chlamydial pathogenesis.

Keywords

Chlamydia muridarum; Immunodominant antigens; H-2-restricted antibody responses; Mouse urogenital tract infection

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1. Introduction

Chlamydia trachomatis is a major cause of sexually transmitted bacterial infection. The acute lower genital tract infection can lead to long lasting inflammatory pathologies in the upper genital tracts in some women, resulting in complications such as pelvic inflammatory diseases and infertility [1-3]. Despite extensive research efforts in understanding *C. trachomatis* pathogenic mechanisms, it is still not clear how exactly *C. trachomatis* infection ascends to the upper genital tract and why some women are more susceptible to the development of upper genital tract complications than the others. Both host genetics and responses to *C. trachomatis* infection and infection conditions may contribute to *C. trachomatis* pathogenicity. *C. trachomatis*-associated reactive arthritis has been linked to HLA-A27 [4, 5]. Women carrying certain alleles of DQ were found to be more susceptible to upper genital infection and infertility [6, 7]. Besides these host immunogenetics associations, *C. trachomatis*-infected cells are known to produce inflammatory cytokines [8, 9], which may contribute to Chlamydia-induced inflammatory damages in the upper genital tract [10-12]. However, information on the antigen basis of *C. trachomatis* pathogenesis is still limited. In addition, there is still no licensed *C. trachomatis* vaccine due to lack of knowledge on immunodominant protective antigens [13].

Chlamydial intracellular infection starts with the invasion of an epithelial cell with an infectious elementary body (EB) via Chlamydia-induced endocytosis that requires the EB organisms to inject preexisting proteins into epithelial cells [14-18]. The internalized EB rapidly differentiates into a noninfectious but metabolically active reticulate body (RB). The RB makes new proteins not only for the organism multiplication but also for secretion into inclusion lumen [19], inclusion membrane [20, 21] and host cell cytosol [22-27]. After replication, the progeny RBs differentiate back into EBs for spreading to near-by cells. At the individual organism levels, the bi-phasic developmental cycle is highly asynchronous and protein expression profiles also vary considerably [28]. Although it is difficult to directly detect chlamydial protein expression during natural infection, host antibody responses to chlamydial antigens has been used to indirectly monitor the expression of immunogenic chlamydial proteins in animals and humans [29-31].

Intravaginal infection of mice with *Chlamydia muridarum* organisms (also known as the mouse biovar of *C. trachomatis*) has been used as an animal model for studying *C. trachomatis* pathogenesis and screening for vaccine antigens [12, 32-38]. In the current study, we compared 7 different inbred strains of mice for their antibody responses to 257 *C. muridarum* antigens. Among the 109 antigens recognized by the 7 strains of mice, 5 were immunodominantly recognized by all 7 strains. Furthermore, antibody responses to 19 antigens displayed associations with mouse H-2 haplotypes. Mapping chlamydial antigens commonly recognized by mice with different strain background and H-2 genes and revealing antigens to which antibody responses are associated with H-2 haplotypes should benefit the development of chlamydial subunit vaccines and understanding chlamydial pathogenesis.

2. Materials and Methods

2.1. Mouse urogenital tract infection and vaginal live organism shedding

Chlamydia muridarum (also called MoPn) Nigg strain organisms were grown in HeLa cells (ATCC, Manassas, VA 20108), purified and titrated as described previously [12]. Aliquots of live organisms were stored at -80°C till use. The following female mice were purchased at the age of 6 to 7 weeks old from The Jackson Laboratory (Bar Harbor, Maine) and Charles River (Wilmington, MA): C57BL/6J (H-2^b), C57BL/10J (H-2^b), Balb/cJ (H-2^d), DBA/2J (H-2^d), C3H/HeN (H-2^k), CBA/J (H-2^k) and A/J (H-2^a). Each mouse was infected

intravaginally with 2×10^4 IFUs of live *C. muridarum* organisms in 20 μ l of SPG (sucrose-phosphate-glutamate buffer consisting of 218mM sucrose, 3.76mM KH₂PO₄, 7.1mM K₂HPO₄, 4.9mM glutamate, pH 7.2). Five days prior to infection, each mouse was injected with 2.5mg Depo-provera (Pharmacia Upjohn, Kalamazoo, MI) subcutaneously to increase mouse susceptibility to *C. muridarum* infection. Vaginal swabs were taken once every week after the intravaginal infection for monitoring live organism shedding. Each swab was soaked in 0.5ml of SPG and vortexed with glass beads and the chlamydial organisms released into the supernatants were titrated on HeLa cell monolayers in duplicates as described previously [36]. Briefly, the serially diluted swab samples were inoculated onto HeLa cell monolayers grown on coverslips. After incubation for 24 hours in the presence of 2 μ g/ml cycloheximide, the cultures were processed for immunofluorescence assay as described below. The inclusions were counted under a fluorescence microscope and 5 random fields were counted per coverslip. For coverslips with less than one IFU per field, the entire coverslips were counted. Coverslips showing obvious cytotoxicity of HeLa cells were excluded. The total number of IFUs per swab was calculated based on the number of IFUs per field, number of fields per coverslip, dilution factors and inoculation and total sample volumes. An average was taken from the serially diluted and duplicate samples for any given swab. The calculated total number of IFUs/swab was converted into log₁₀ and the log₁₀ IFUs were used to calculate mean and standard deviation at each time point. All mice were sacrificed 54 days after infection for collecting sera for antibody measurements as described below.

2.2. Immunofluorescence assay

The Immunofluorescence assay was carried out as described previously [39]. Briefly, the infected cells were fixed with 2% paraformaldehyde dissolved in PBS for 30 min at room temperature, followed by permeabilization with 2% saponin (Sigma) for 1h. After washing and blocking, the cell samples were subjected to chemical and immune labeling: for quantitating live organisms from vaginal swab samples, the infected cells were labeled with Hoechst (blue, Sigma) for visualizing DNA and a rabbit anti-chlamydial chaperon cofactor antibody (unpublished data) plus a goat anti-rabbit IgG conjugated with Cy2 (green; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for visualizing chlamydial inclusions. The inclusions were counted under an Olympus AX-70 fluorescence microscope equipped with multiple filter sets (Olympus, Melville, NY) as described above; For titrating anti-*C. muridarum* antibody titers in mouse serum samples, the infected cell samples were also labeled with the DNA dye Hoechst. However, the co-labeling was with the serially diluted mouse serum samples plus a goat anti-mouse IgG conjugated with Cy2 (Jackson ImmunoResearch Laboratories). The antibody titer for a given mouse antiserum sample was determined as the highest dilution of the antiserum that still gave positive inclusion staining.

2.3. Prokaryotic expression of chlamydial fusion proteins

A total of 257 open reading frames (ORFs) from *C. muridarum* genome (NCBI protein accession# AAF39550, <http://www.ncbi.nlm.nih.gov/sites/entrez>; ref: [40]) were cloned into pGEX vectors (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) as described previously [41]. All ORFs were expressed as fusion proteins with glutathione-s-transferase (GST) fused to the N-terminus of the chlamydial proteins. The 257 ORFs include genes whose orthologs from *C. trachomatis* that were found to be immunogenic in humans [30, 31, 42, 43], genes encoded by the plasmid or regulated by plasmid [44, 45] and genes unique to *C. muridarum* comparing to the *C. trachomatis* genome. Expression of the fusion proteins was induced with isopropyl-beta-D-thiogalactoside (IPTG; Invitrogen, Carlsbad, CA) and the fusion proteins were extracted by lysing the bacteria via sonication in a Triton-X100 lysis buffer (1% TritonX-100, 1mM PMSF, 75 units/ml of Aprotinin, 20 mM Leupeptin and 1.6 mM Pepstatin) as described previously [30, 31]. After a high-speed centrifugation to remove

debris, the fusion protein-containing supernatants were either directly added to glutathione-coated microplates for measuring their reactivity with mouse sera in an ELISA as described below or further purified using glutathione-conjugated agarose beads (Pharmacia).

2.4. Enzyme-Linked ImmunoSorbent Assay (ELISA)

To detect the reactivity of antisera from 35 mice (5 from each of the 7 strains) with the 257 chlamydial fusion proteins, a protein array ELISA was used as described elsewhere [31, 42, 46]. Briefly, bacterial lysates containing the GST fusion proteins were directly added to the 96 well microplates pre-coated with glutathione (Pierce, Rockford, IL) to allow GST to interact with glutathione. After washing to remove excess fusion proteins and blocking with 2.5% nonfat milk (in phosphate-buffered solution), individual mouse serum samples after the appropriate dilutions were applied to the microplates. The serum antibody binding to antigens was detected with a goat anti-mouse IgG conjugated with HRP (Jackson ImmunoResearch Laboratories) in combination with the soluble substrate 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS; Sigma) and quantitated by reading the absorbance (OD) at 405 nm using a microplate reader (Molecular Devices Corporation, Sunnyvale, CA). To reduce background binding, all mouse serum samples were pre-absorbed with lysates made from XL-1blue bacteria expressing GST alone.

2.5. Statistical analysis

Student *t* test was used for analyzing quantitative data such as binding intensity while the Fisher's Exact test (<http://www.danielsoper.com/statcalc/calc29.aspx>) for analyzing qualitative data such as binding frequency.

3. Results

3.1. Mapping immunodominant antigens recognized by mice with different background and H-2 haplotypes

A total of 35 female mice in 7 different strains (with 5 mice in each strain) were intravaginally infected with *C. muridarum*. All 35 mice regardless of strains were productively infected although different strains displayed slightly different susceptibility (Table 1). After each mouse was inoculated with 20,000 IFUs of live organisms, vaginal swabs collected from most mice on day 7 after infection contained more than 20,000 live organisms. The high numbers of live organism shedding continued on day 14. By day 28, most mice resolved infection (data not shown). Sera were collected from all mice on day 54 and titrated for anti-*C. muridarum* antibodies in an immunofluorescence assay. All 7 strains of mice developed significant titers of antibodies against *C. muridarum* organisms. There was no statistically significant difference in the overall antibody titers between the 7 groups of mice.

The antisera were then reacted with a total of 257 *C. muridarum* GST fusion proteins for profiling the antigen specificities of each mouse antiserum. These fusion proteins were selected because of the following reasons: (A) their orthologs from *C. trachomatis* have been found to be immunogenic during *C. trachomatis* infection in humans [30, 31, 42]; (B) they are encoded by the plasmid or plasmid-regulated ORFs [44] and the plasmid has been shown to contribute to chlamydial pathogenesis [45, 47]; (C) ORFs that are unique to *C. muridarum* comparing to the *C. trachomatis* genome; and (D) additional hypothetical ORFs. When the 257 *C. muridarum* fusion proteins were reacted with each of the 35 antisera, a total of 109 proteins were recognized by at least one mouse antiserum, suggesting that ~42% of the analyzed proteins (Fig. 1) may be expressed and immunogenic during *C. muridarum* infection in mice. This conclusion is based on the assumption that the antigen-antibody interactions detected in the fusion protein arrays assays were not caused by cross-reactivity.

That's to say, the antibodies reactive with a given antigen were induced by the same antigen during mouse infection. Among the 7 strains of mice, C3H recognized most antigens (with a total of 70), followed by A/J (50), Balb/c (38), CBA (36), DBA/2 (32), C57BL/10 (30) and C58BL/6 (28). A total of 5 antigens were recognized by 60% or more mice from each of the 7 strains. It turned out that these 5 antigens were each recognized by >90% of mice when all 35 mice were considered. Thus, they were designated as immunodominant antigens. These are TC0660 (ortholog of CT381 in *C. trachomatis*, ABC transporter), TC0727 (ortholog of CT443, outer membrane complex protein B or OmcB), TC0828 (ortholog of CT541, peptidyl-prolyl cis-trans isomerase Mip), TC0726 (ortholog of CT442, 15 Kda cysteine-rich outer membrane protein or CRP), TC0268 (ortholog of CT875, hypothetical protein). The orthologs of these antigens from *C. trachomatis* were recognized by 50% or more women who were urogenitally infected with *C. trachomatis* [31].

3.2. Identification of H-2-linked antibody responses to *C. muridarum* antigens

We next compared the antigen specificities of the antibody responses between the 7 strains of mice. Although only 5 antigens were recognized by 60% or more mice from each of all 7 strains (see above), a total of 41 antigens were each recognized by 60% or more mice from any given strain (Fig. 2A). To identify antigens against which antibody responses were likely linked to a given H-2 haplotype, antigens were resorted based on their recognition frequency of 60% or greater from strains of mice that share the same H-2 haplotypes. For example, an antigen had to be recognized by 60% or more mice from both C57BL/6J (H-2^b) and C57BL/10J (H-2^b) strains to be considered as a H-2^b-linked antigen. A total of 19 antigens were preferentially recognized by strains of mice that share the same H-2 haplotype but not mice with different H-2 haplotypes (Fig. 2B & C).

TC0480, TC0912, TC0229, TCA04, TC0289 & TC0892 were all significantly recognized by both C57BL/6j and C57BL/10j, suggesting that antibody responses to these antigens are positively linked to H-2^b. TC0480 is an ortholog of CT208 from *C. trachomatis* serovar D, a 3-deoxy-D-manno-octulosonic-acid transferase or KdtA; TC0912 is an ortholog of CT622 of serovar D or CTA0675 of *C. trachomatis* serovar A. CTA0675 is highly polymorphic among ocular isolates [48] while CT622 has recently been shown to be a secretion protein [24]; TC0229 is an ortholog of CT841, an ATP-dependent metalloprotease or FtsH; TCA04 is an ortholog of PgP3, a plasmid-encoded secretion protein; TC0289 is an ortholog of CT020, a signal peptidase I; TC0892 is an ortholog of CT603, an anti-oxidant AhpCTSA family protein. In addition to H-2^b linkage, TC0480 was also recognized by antibodies from the two H-2^d mouse strains Balb/c and DBA/2 while TC0912 by C3H, CBA & A/J (all express the H-2^k allele at multiple H-2 loci). Thus, antibody responses to TC0480 are likely positively linked to both H-2^b and H-2^d while antibody responses to TC0912 are positively linked to both H-2^b and H-2^k.

Antigens TC0035, TC0387, TC0052, TC0781, TC0373, TC0117 & TC0396 were all significantly recognized by Balb/c and DBA/2 mice (H-2^d) and thus, antibody responses to these antigens are likely positively linked to H-2^d. TC0035 is an ortholog of CT664, a phosphopeptide binding protein likely involved in type III secretion system; TC0387 is an ortholog of CT111, 10kDa molecular chaperone or GroES; TC0052 is an ortholog of CT681, the major outer membrane protein or MOMP; TC0781 is an ortholog of CT494, a signal peptide peptidase (serine protease with a catalytic S-K dyad); TC0373 is an ortholog of CT098, a 30S ribosomal protein S1; TC0117 is an ortholog of CT741, a preprotein translocase subunit YajC; TC0396 is an ortholog of CT119, an inclusion membrane protein A or IncA. It is worth noting that TC0035 and TC0387 were also significantly recognized by C3H, CBA & A/J mice. Thus, their antibody responses are likely positively linked to both H-2^d and H-2^k haplotypes, which is consistent with the fact that H-2^a in A/J consists of multiple k (K, A β , A α , E β , E α) and d (S, D & L) loci. Interestingly, TC0117 was

significantly co-recognized by A/J but neither C3H nor CBA, suggesting that antibody response to TC0117 is likely positively associated with S^d, D^d or L^d but not other H-2 gene loci.

Finally, TC0512, TC0177, TC0589, TC0794 and TC0596 were preferentially recognized by C3H, CBA & A/J mice and thus, antibody responses to these antigens are likely positively restricted by H-2^k. TC0512 is an ortholog of CT241 that is an Outer membrane protein or Omp; TC0177 is an ortholog of CT795, a hypothetical protein (HP) that is secreted into host cell cytosol; TC0589 is an ortholog of CT315, a DNA-directed RNA polymerase subunit beta; TC0794 is an ortholog of CT507, a DNA-directed RNA polymerase subunit alpha and TC0596 is an ortholog of CT322, a translation elongation factor Tu. Somehow, all these antigens were also more or less (although not significantly based on our 60% recognition frequency requirement) recognized by Balb/c and DBA/2 mice, suggesting that H-2^d haplotype is also linked to antibody responses to these antigens. It is worth pointing out that most H-2^k-linked antigens are also positively associated with H-2^d.

In addition to the above positive linkage of H-2 haplotypes to antibody responses to *C. muridarum* antigens, negative associations were also noted. When a recognition frequency of an antigen by a strain of mice at 20% or less was determined as insignificant antibody responses to the antigen by the mouse strain, multiple negative linkages could be identified. TC0289 & TC0892 were not recognized by any strains of mice tested except C57BL/6 & C57BL/10. Thus, antibody responses to these two antigens are only positively linked to H-2^b but negatively restricted by both H-2^d and H-2^k mice. We designated TC0389 and TC0892 as H-2^b-unique antigens. However, H-2^b is negatively linked to antibody responses to many other antigens including TC0035, TC0387, TC0781, TC0373, TC0066, TC0396, TC0177, TC0589, TC0794 & TC0596 (since no significant antibody responses to any of these antigens by either C57BL/6 or C57BL/10 mice were detected). Interestingly, TC0373, TC0066 & TC0396 are negatively associated with H-2^k. Thus, these 3 antigens are H-2^d-unique antigens. Overall, H-2^b is more frequently negatively associated with antibody responses to chlamydial antigens than H-2^d and H-2^k.

4. Discussion

The objective of the current study is to use the mouse *C. muridarum* infection model to evaluate the effects of host factors on antibody responses to *C. muridarum* infection by comparing 7 different inbred strains of mice. We selected these 7 strains because they share common H-2 haplotypes or H-2 loci between two or more strains, thus allowing us to distinguish antibody responses impacted by strain-specific background or H-2 genes. After identifying 5 commonly recognized antigens by all 7 strains, we were able to map 19 antigens to which antibody responses are likely linked to H-2 genes. For TC0117, the linkage was further localized to S, D or L loci due to its unique recognition patterns by different strains of mice. These results have demonstrated that chlamydial antigen-specific immune responses during chlamydial infection can be significantly impacted by host immunogenetics.

The five immunodominant antigens (TC0660, TC0727, TC0828, TC0726 & TC0268) were recognized by >90% of the 35 mice regardless of their strain background and H-2 haplotypes. Their orthologs in *C. trachomatis* (CT381, CT443 or OmcB, CT541, & CT442) were also immunodominant during *C. trachomatis* infection in humans [30, 31]. Thus, the information acquired from the murine model can be relevant to *C. trachomatis* infection in humans.

We have identified 19 antigens to which antibody responses were positively or negatively linked to mouse H-2 haplotypes or loci. The questions are whether antibody responses to the orthologs of these antigens during *C. trachomatis* infection are also impacted by human HLA and what are the consequences of these HLA-restricted responses in terms of host susceptibility to reinfection and development of upper genital tract pathology. *C. trachomatis*-associated reactive arthritis has been linked to HLA-A27 [4, 5] and women carrying certain alleles of DQ were found to be more susceptible to upper genital infection and infertility [6, 7]. However, it is not known whether these genetic linkages are mediated by host immune responses to specific chlamydial antigens. Thus, it will be worth testing whether human antibody responses to these antigens have any relationships with human immunogenetics and susceptibility to reinfection and pathologies. Some of these proteins may be developed into biomarkers for diagnosis. If immune responses to these antigens are indeed heavily influenced by immunogenetic variation in humans, it is unlikely to consider them as vaccine antigens.

The next question is why different strains of mice make antibodies to different chlamydial antigens when they are similarly infected with chlamydial organisms. Chlamydial organisms are known to be able to vary their protein expression at individual organism levels [28]. Furthermore, chlamydial organisms are able to vary their protein distribution patterns in response to different environmental conditions [49]. Although it is difficult to directly detect chlamydial protein expression and distribution during natural infection, host antibody responses to chlamydial antigens has been used to monitor the expression of immunogenic chlamydial proteins in animals and humans [29-31]. All of 19 H-2-restricted antigens were immunogenic in Balb/c mice when the mice were immunized with the corresponding fusion proteins (data not shown). However, Balb/c mice failed to produce antibodies to some of the antigens in the context of chlamydial infection. This analysis suggests that lack of antibody production to some antigens during chlamydial infection may not be necessarily due to lack of host ability to recognize the antigens but due to lack of antigen expression by the chlamydial organisms or failure of the chlamydial antigens to access to host immune systems. Of course, a positive antibody response can always indicate both antigen expression by chlamydial organisms and immunogenicity of the expressed antigens. It will be interesting to test whether chlamydial antigen expression and distribution can contribute to the observed H-2 associated antibody responses to chlamydial antigens.

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Fig. 1. Reactivity of 35 mouse antisera with 257 *C. muridarum* GST fusion proteins

A total of 257 *C. muridarum* open reading frames (ORFs, expressed as GST fusion proteins) as listed along the Y-axis were each reacted with 35 individual mouse antisera from 7 inbred strains of mice as shown horizontally on top of the figure. Each antiserum was used at a dilution of 1:1000. The reactivity was listed in order of the ORFs encoded in the *C. muridarum* genome (starting with TC0003) followed by ORFs encoded in the plasmid (ending with TCA07, panel A) or in order of recognition frequency by mouse antisera (panel B). The reactivity patterns of 109 *C. muridarum* ORFs recognized by at least one of the 35 mice were emphasized in panel C and 5 ORFs recognized by at least 60% of mice from each strain were shown in panel D. The brightness of color represents the reactivity intensity between a given antiserum and ORF. Each color represents reactivity from one strain of mice.

Fig. 2. Preferential recognition of *C. muridarum* antigens by antisera from 7 different inbred strains of mice

When the antigen specificities of the antibody responses were compared between the 7 strains of mice, a total of 41 antigens were each recognized by 60% or more mice from any given strain (panel A), of which, 19 were preferentially recognized by strains of mice that share the same H-2 haplotype but not mice with other H-2 haplotypes (B). The H-2-associated antigens were further marked in panel C with plus for positive association and minus for negative linkage. TC0289 & TC0892 are only positively linked to H-2^b while TC0373, TC0066 & TC0396 linked to H-2^d were highlighted by bold face plus signs. Antibody response to TC0117 is highlighted with star because it is likely linked to S^d, D^d & L^d due to its recognition by H-2^d & H-2^a but not H-2^k mice. The color scale is the same as described in figure legend.

Table 1

H-2 haplotype, *C. muridarum* organism shedding and anti-*C. muridarum* antibody responses in 7 different strains of mice

Mouse Strain	H-2	Allele at H-2 locus								Organism shedding (Log10 IFU)		Anti- <i>C. muridarum</i> Antibody titers (log10)
		K	A β	A α	E β	E α	S	D	L	Day 7	Day 14	
C57BL/6J	b	b	b	b	b	-	b	b	b	5.55 $\pm$ 0.21	4.86 $\pm$ 0.29	5.98 $\pm$ 0.29
C57BL/10J										5.11 $\pm$ 0.12	4.72 $\pm$ 0.32	5.9 $\pm$ 0.19
BALB/cJ	d	d	d	d	d	d	d	d	d	5.18 $\pm$ 0.33	3.60 $\pm$ 0.61	5.48 $\pm$ 0.45
DBA/2J										5.06 $\pm$ 0.08	3.99 $\pm$ 0.29	5.2 $\pm$ 0.31
C3H/HeN	k	k	k	k	k	k	k	k	-	4.26 $\pm$ 0.44	3.71 $\pm$ 0.65	5.34 $\pm$ 0.35
CBA/J		k	k	k	k	k	d	d	d	4.91 $\pm$ 0.08	4.33 $\pm$ 0.14	5.06 $\pm$ 0.13
A/J	a									4.47 $\pm$ 0.16	3.33 $\pm$ 0.36	5.68 $\pm$ 0.41

Footnote: The alleles of H-2 loci of the 7 strains of mice are listed in the left columns. A total of 35 female mice with 5 in each strain were infected with *C. muridarum* 5 days after injection with Depopovera. After infection, vaginal swab was taken from each mouse on a weekly basis. Live organisms from each swab were quantitated using a cell culture immunofluorescence assay. The results were calculated into log IFUs (inclusion forming units) and expressed as mean and standard deviation for each time point (only day 7 and day 14 data were shown). Mice were sacrificed on day 54 post infection for measuring antibodies in the sera. Anti-*C. muridarum* antibody titer from each mouse was defined as the highest dilution of the antiserum that still gave positive immunofluorescence staining and expressed as log10. All mice produced significant levels of antibodies and there were no statistically significant differences between different strains.