Multigenic control of resistance to Sendai virus infection in mice

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ABSTRACT

Experimental infection of mice with Sendai virus (SeV) is frequently used as a model of viral pathogenesis of human respiratory disease. To understand the differences in host response to SeV among mice strains, we carried out genetic mapping studies in DBA/2 (D2) (susceptible) and C57BL/6 (B6) (resistant) mice. F_1, F_2, and N_2 backcrossed mice were generated and examined for their disease resistance and susceptibility. For the determination of virulence, percentage body weight loss and survival time were used as phenotypes. We, then, carried out a genome wide scan on 108 backcrossed mice for linkage with percentage body weight loss as phenotype. A major quantitative trait locus (QTL) showing significant linkage was mapped to the distal portion of Chr 4 (*SeV1*). In addition, two other QTLs showing suggestive statistical linkage were also detected on Chr 8 and 14. We, further, performed genome scan for interactions with least squares analysis of variance of all pairs of informative makers in backcrossed progenies. We identified a highly significant epistatic interaction between *D3Mit182* and *D14Mit10*, then denoted as *SeV2* and *SeV3*, respectively, and the latter was the same locus showing a suggestive level on Chr 14 in QTL analysis. Considered genotypes of these three loci, we could account for more than 90% of genetic effect on the differential response to SeV infection between B6 and D2 mice. These findings revealed a novel gene interactions controlling SeV resistance in mice and will enable the identification of resistance genes encoded within these loci.

Keywords: Sendai virus; resistance; DBA/2; C57BL/6; quantitative trait locus

1. Introduction

Sendai virus (SeV), the murine counterpart of human parainfluenza virus type 1 (HPIV1), is a natural respiratory pathogen of mice, which is highly related both structurally and serologically to HPIV1 that causes severe respiratory disease in children with the increasing risk of asthma. The virus belongs to the *Paramyxoviridae* family which remains endemic and it is the leading cause of pneumonia among rodent's colonies in various laboratories world wide; hence the concepts of specific pathogen-free (SPF) laboratory animals (Chanock et al., 2001).

Recently, it has been reported that the virus was able to replicate in the upper and lower respiratory tract of chimpanzees and African green monkeys and therefore may cause zoonotic disease in humans (Skiadopoulos et al., 2002). Furthermore, the virus has been used extensively in research studies that define most of the basic biochemical and molecular biological properties of *Paramyxoviruses* (Fascia and Desmecht, 2007), and experimental infection of mice with SeV is frequently used to study the viral pathogenesis of human respiratory diseases (Kim et al., 2008).

In mice, generally resistant strains are up to 20,000-fold more resistant to the lethal effects of SeV than susceptible strains. In addition, mice with resistant phenotypes abrogate the infection by confining infected regions primarily to the air ways, whereas those with susceptible phenotypes allow the infection to spread from the air way into the lung parenchyma (Parker et al., 1978; Brownstein and Winkler, 1986; Itoh et al., 1991; Fascia et al., 2005). The two inbred mice, DBA/2 (D2) and C57BL/6 (B6), differ markedly in the susceptibility to SeV infection. For instance, infected B6 mice have 10-300 times lower viral titers than D2 mice and the virus replicates predominantly in air epithelial cells which causes the inflammation of airways. In addition, B6 mice mount vigorous interferon (IFN) response

and recruit large numbers of lymphoid cells into the infected airway where viral replication terminates. In contrast, D2 mice fail to mount augmented IFN and NK cell response, resulting in few lymphoid cells being recruited into the infected airways, thereby leading to the spreading of the lesions into the lung parenchyma and massive replication of the virus in alveolar lining cells (Itoh et al., 1991; Baig and Fish, 2008)

Earlier reports based on genetic differences generated an array of hypotheses about associated phenotypic characteristic (sex and coat colour) or underlying gene polymorphisms such as IFN, mucocilliary transport or *Sas-1*, toll-like receptors (TLR), and H-2 haplotype, none of which fully confirmed the genetic basis of the varying spectrum of resistance and susceptibility to SeV (Brownstein and Winkler, 1986; Breider et al., 1987; Hou et al., 1992; Mo et al., 1995; Koenraad et al., 2003). Since resistance and susceptibility to infection is complex genetic trait, and as a step towards the elucidation of the contributing genetic host factors for SeV infection in mice, we exploited the contrasting response of inbred D2 and B6 to SeV infection. We carried out the genetic mapping studies in these two mouse strains with the goal of dissecting these genetic variants, which might provide valuable insights into the molecular basis of host variations to SeV infection.

Using the two informative mouse strains and quantitative trait locus (QTL) mapping approach, we identified a major significant QTL on the distal portion of Chr 4 and two contributing suggestive QTL loci on Chr 8 and 14. Further, we identified a highly significant epistatic interaction between *D14Mit10* and *D3Mit182*. Finally, with these three loci, we could account for more than 90% of genetic effect on the differential response to SeV infection between B6 and D2 mice.

2. Materials and methods

2.1 Mice

SPF B6 and D2 mice were purchased from Japan SLC (Shizuoka, Japan). (D2 x B6)F_1 (DBF_1), [(D2 × B6)F_1 × (D2 × B6)F_1]F_2 (DBF_2), and D2 × (D2 × B6)F_1 (DBN_2) mice were generated to conduct the genetic and phenotypic assessment experiments. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Hokkaido University.

2.2 Infection

The MN strain of SeV was kindly provided by Prof. Hiroshi Iwai, Rakuno Gakuen University (Yamaguchi et al., 1988). Seed virus stock in modified Eagle medium (Sigma, MO, USA) with 1% bovine serum albumin was inoculated into 10-day-old embryonated chicken eggs and incubated for 72 h at 35ºC. After allantoic fluid recovered from inoculated eggs was centrifuged at 2,500 xg for 20 min, the supernatant was collected and stored at -80 ºC until used for infection to mice. Virus titer in the allantic flued was determined by hemadsorption assay using a monkey kidney cell line, LLC-MK2, and chicken red blood cells. The values of virus titer were indicated as median tissue culture infectious dose ($TCID_{50}$).

Eight-week-old mice were used for all infection experiments. Mice were infected with various doses of $TCID_{50}$ of SeV in Medium 199 (Sigma, MO, USA). A volume of 25 µl of the viral innoculum was slowly instilled intranasally, following anesthetization with intra-peritoneal injection of pentobartital sodium (Somnopentyl, Schering-Plough Animal Health, NJ, USA). Generally, for the determination of virulence, infected mice were weighed daily

and monitored by visual inspection twice per day, with the main visual disease signs being lethargic, ruffled fur, hunching, and dyspnea. Mice were sacrificed by cervical dislocation, if the weight loss exceeded 40% of the weight from day 0 or if the animals were obviously moribund.

2.3 Genotyping analysis

To identify and map QTL, a total of 125 informative microsatellite markers were used for the genotyping analysis. Initially we genotyped a set of 100 markers, that differentiated between the alleles of B6 and D2 mice. The genetic map positions (cM) and physical map positions (Mb) of marker loci were obtained from the Mouse Genome Informatics of the Jackson Laboratory (http://www.informatics.jax.org/), which provides an average genome scan of approximately 10-20 cM selected to provide coverage of all 19 autosomes and the X chromosome (Table 1). Additional 25 markers were used at chromosomal regions of interest to estimate the QTL positions more precisely. After genomic DNA preparation and PCR amplification, the amplified samples were electrophoresed with 9% polyacrylamide gels and stained with ethidium bromide. The stained gels were then visualized and photographed under ultraviolet lamp.

2.4 QTL and statistical analysis

For the genome scans, only two phenotypic categories, 40% body weight loss (value = 0) and less than 40% body weight loss (value = 1) were used as survival phenotype of mice. Analyses of linkage of survival phenotypes to chromosomal loci were performed using the MapManager QTXb20, a software program that uses a maximum likelihood algorithm with

"interval mapping" and "simultaneous search", and permits better localization of loci and exclusion mapping (Manly et al., 2001). Recombination frequencies (%) were converted into genetic distance (cM) using the Kosambi map function. This program provides linkage data as Likelihood ratio statistic (LRS) score, which is usually converted to the more frequently used LOD score by dividing the LRS by 4.601. The interval mapping function of MapManager QTX is more accurate, when the phenotype data are normally distributed (Manly KF, personal communication). Therefore, the values for free, dominant, and additive models of inheritance were calculated in terms of LRS by carrying out permutation test. For main effects, threshold for suggestive and significant linkage followed the established guidelines (Lander and Kruglyak, 1995), which was based on 5,000 permutation test. The thresholds in the backcrossed progenies under the assumption of a free model were determined by the QTL soft program to be LRS 6.8 and 12.5 for finding suggestive and significant linkages, respectively. Two-way interactions (epistasis) were estimated with a QTL scan and statistical significance for these gene to gene interaction tests were based on $P < 0.05$ using 5,000 permutations of the observed data. Significant interactions found in the QTL were confirmed with standard ANOVA, including cross terms for two-way interactions for all maker pairs. All computations were performed with the Stat view program statistical package (SAS Institute, Cary, NC). $P < 0.05$ was considered to be significant.

3. Results

3.1 Determination of the genetic spectrum and mode of resistance and susceptibility to SeV

Inbred strains of mice with different susceptibilities to SeV infection have been identified previously (Parker et al., 1978). B6 and D2 mice differ markedly in response to SeV infection. Percentage body weight changes and survival times were chosen as parameters for the evaluation of SeV resistance and susceptibility phenotype for an infection period of 21 days.

Initially we tested the spectrum of sensitivity of D2 and B6 mice, by challenging them at different viral titer levels of $TCID_{50}$ of SeV inoculums. In a two independent experiments, mice (males and females, n = 4 to 5) were inoculated intra-nasally with SeV inoculums, with dose ranges of 1×10^2, 1×10^3, 1×10^4, and 1×10^5 $TCID_{50}$. All of mice showed obvious signs of infection between 3-5 days, which they appear to be lethargic, ruffled fur, hunching, dyspnea, and loss of weight (Percy et al., 1994). D2 showed a progressive loss of percentage body weight and mortality in all dose ranges, while B6 mice showed slight loss of body weight which returned to normal and only 10% mortality even at very virulent dose of 1×10^5 $TCID_{50}$ (data not shown). In addition, resistance and susceptibility were not linked to sex. These results reconfirmed the earlier reports on differences in the spectrum of susceptibility to SeV between these two strains (Brownstein and Winkler, 1986; Itoh et al., 1991).

In order to investigate the mode of resistance to SeV, we infected parental strains in replicate experiments (males and females, n = 17) with 1×10^3 $TCID_{50}$, of which dose clearly discriminated between susceptible D2 and resistant B6 mice and served as a choice dose for our genetic mapping studies. In addition, as a control for each analysis, groups of D2 were infected at the same time as the DBF_1, DBF_2, and backcrossed progenies. D2 mice showed a

progressive loss of weight and mean survival time was 10.0 ± 1.0 days for females and 9.0 ± 1.5 days for males, while B6 mice (male and females) from day 0, showed approximately 20% decline in body weight between day 6-7, and by day 8 they started regaining weight which returned to normal with a 100% rate of survival (Fig.1a, b). Thus, these results indicate that B6 has a conferred genetic molecular mechanism of abrogating the infection.

We, therefore, investigated the genetic inheritance of resistance and susceptibility of DBF_1 mice. In two independent experiments, we tested 16 DBF_1 mice (males and females) using 1×10^3 $TCID_{50}$ (Fig.1c, d). Interestingly, DBF_1 mice were more resistant than both parental strains and they showed approximately 15% decline in percentage body weight loss between day 6-7, and survival rate was 100%. In addition, we also found that resistance was not sex-dependent, but remained dominant.

3.2 Phenotypic characterization of SeV resistance and susceptibility

To estimate the effect of genetic trait of SeV susceptibility, segregation analysis was performed. The null hypothesis was that a single dominant gene controls resistance. If so, then according to Mendelian rule, 100% of the F_1 hybrid of resistant B6 strain crossed to sensitive D2 strain should display a resistant phenotype and 50% of the backcrossed mice of the F_1 hybrid crossed to sensitive D2 should be resistant. Chi-square analysis of the DBF_1 response supported the null hypothesis ($\chi^2 = 0.22$, $P < 0.05$). Further, 61/108 (56.5%) of the backcrossed DBN_2 mice were resistant, which agrees with predicted 50% according to the Mendelian rule. Chi-square analysis of DBF_2 mice, however, rejected the null hypothesis ($\chi^2 = 23.34$: $P > 0.05$), suggesting that sensitivity to SeV is a muligenic trait.

3.3 Linkage analysis for the identification of genetic loci of SeV resistance and susceptibility

We investigated the resistance and susceptibility phenotype of the progenies of DBN_2 mice. We used backcrossed progenies for genetic analysis, because the phenotypes of DBF_1, mainly the loss of body weight showed a dominant genetic trait. This allows for optimal discrimination between the phenotypes recovered in the backcrosses.

A total of 108 (54 males and 54 females) backcrossed mice were infected with 1×10^3 $TCID_{50}$ SeV and phenotypes of body weight change were determined as QT. Alternatively, the susceptible or resistant phenotype was determined as 40% body weight loss or less than 40% body weight loss, respectively, because these phenotypes were consistent with susceptible (non-survival) or resistant (survival) phenotype in the pilot study (data not shown).

We carried out simple interval mapping and the values for the free, dominant, and additive models of inheritance were calculated in terms of LRS. When percentage body weight loss was used as QT, none of significant or suggestive QTL was detected. Therefore, we used 40% body weight loss (value = 0) or less than 40% body weight loss (value = 1) as QT, and our linkage analysis, then, revealed a major significant QTL, which mapped to the distal region of Chr 4, residing within 8.3 cM (23 Mb) between *D4Mit146* and *D4Mit204* (LRS 14.5), denoted as *SeV1* locus (Fig. 2a). Additional QTLs showing suggestive linkages were also detected on Chr 8 (between *D4Mit4* and *D8Mit100*, LRS 8.4) and 14 (between centromere and *D14Mit10*, LRS 7.4), $P < 0.005$ (Fig. 2b, c).

3.4 Identification of epistatic interaction involved in SeV resistance and susceptibility

Epistatic interactions result from the combined effect of two or more genes on a phenotype, which could not have been predicted as the sum of their separate effects (Frankel and Schork, 1996). Recent evolutionary trends have shown that in crosses involving a strain

of model organism susceptible to certain disease conditions with resistant strains, the genes of the susceptible strain show differential effects with the different background strain genomes (Rapp et al., 1994). Based on the hypothesis of our genetic background and evolutionary evidence of gene to gene interactions, we investigated the possibilities of epistatic interactions by performing ANOVA of all informative markers in 108 DBN_2 progenies using Stat view program statistical package (SAS Institute, Cary, NC). All informative pairs of markers were tested one by one, with a *P* value of <0.05 as the threshold for finding gene to gene interactions. The analysis also includes both the previously identified significant and suggestive QTLs, and at the set *P* value threshold we identified a highly significant epistatic interaction between *D3Mit182* and *D14Mit10*, *P* <0.0001 (Fig. 3). Thus, *D14Mit10* is one of the previously identified suggestive QTLs (Fig. 2d) and it is interacting epistastically with a locus on Chr 3 (*D3Mit182*), therefore denoted as *SeV3* and *SeV2* loci, respectively (Fig. 3, Table 2).

Finally, we interrogated our likely candidate region of the QTL on Chr 4, *SeV1*, which showed a peak level on *D4Mit308*, and carried out a mean survival rate analysis of mice with respect to genotype at this locus. A single genotyping analysis at this locus revealed a mean survival rate of 66% for heterozygous (*B6*/*D2* allele) and 34% for homozygous (*D2* allele) (Fig. 4a). Next, addition of the effect of the suggestive interacting locus, *SeV3* (*D14Mit10*), revealed a novel increase in survival rate, with the *B6*/*D2* allele mice with respect to both *D4Mit308* (*SeV1*) and *D14Mit10* (*SeV3*) loci having 74% mean survival rate, while mice carrying *D2* allele at both loci had 14% mean survival rate (Fig. 4b). We, therefore, went ahead to include the locus effect that showed epistatic interaction by fixing *D3Mit182* (*SeV2*) to be heterozygous, and carried out an estimation of mean survival rate with genotypes at the above two loci. Cumulatively 93.3% of heterozygous mice were resistant to SeV infection, whereas homozygous mice showed a value of 0% survival rate (Fig. 4c), indicating the

combined effects of the significant QTL and the loci showing highly significant epistatic interaction in the control of resistance to SeV infection. Thus, these data revealed that there could be several genetic factors that are localized within the mouse genome regulating the differential sensitivity of these mice to SeV infection.

4. Discussion

One of the major challenges of modern biology is to achieve a better understanding of the molecular genetic basis for complex trait variation. Complex genetic traits are derived from the interplay between genetic variants and environmental exposures (Zondervan and Cardon, 2004), where a one-to-one relationship between genotype and phenotype does not exist (Darvansi, 1998). SeV infection in mice, like many diseases and biological phenotypes, is thought to arise as a consequence of the interplay of two or more genes.

Earlier reports have shown that there is differential sensitivity to SeV among various inbred mouse strains. SeV-infected B6 mice elicit a strong IFN and $CD4^+/CD8^+$ T cell response in the respiratory tract and it is believed that they have a coordinated interaction for the effective clearance of SeV in these mice; however, whether they act directly to eliminate the virus-specific response is not clear. Adoptive transfer studies suggest that $CD4^+$ T cells may contribute to the B6 anti-SeV response by providing help to cytotoxic T cells in the form of IL-2 (Kast et al., 1986; Hou et al., 1992; Cole et al., 1994). Lopez et al. (2006) have shown that type I IFNs facilitate virus clearance and enhance the migration and maturation of dendritic cells after SeV infection *in vivo* soon after infection. However, mice cleared the virus from their lungs and efficiently generated cytotoxic T cells independently of type I IFN signaling. Furthermore, mice that are unresponsive to type I IFN developed long term anti-SeV immunity, including $CD8^+$ cells and antibodies. Recently Kim et al. (2008) have shown that wild type B6 mice no longer have detectable viral titer and a trace level of SeV-specific RNA expression after infection and that macrophages derived from these mice express IL-13 mRNA. Further, the analysis of macrophage-deficient mice (*Csf3-/-*) indicates that both IL-13 and Muc5ac production after SeV infection depends on the presence of macrophage. Furthermore, it is believed that both host and viral factors play a role in the resistance and

susceptibility to SeV virus, and the activation of innate immune system depends on the recognition of the molecules that are specific for the pathogen. For instance, for the *Paramyxoviridae*, the anti-host defense mechanism is due mostly to C and V proteins. SeV C protein is a multifunctional protein that plays important roles in regulating viral genome replication and transcription, antagonizing host IFN system, suppressing virus-induced apoptosis, and facilitating virus assembly and budding. Similarly, the V protein is shown to participate in the establishment of antiviral state that is required for viral pathogenesis (Kato et al., 1997; Garcin et al., 2000; Strahle et al., 2003; Kato et al., 2007).

Host response to SeV infection involved complex interactions of a number of factors, including cellular infiltration and their induction of chemokines (Kast et al., 1986; Mo et al., 1995; Strahle et al., 2007). However, the definition of this host response to SeV infection will require an understanding of the host genetic susceptibility to the virus. In this study we performed QTL analysis to examine differences in SeV sensitivity between inbred D2 and B6 mice. The identification of clear phenotypes of resistance and susceptibility to SeV is a valuable tool for genetic analysis in mice. We carried out a QTL analysis on body weight loss in backcrosses from D2 and B6 mice after infection with SeV. These two mouse strains represent polar extremes in severity of SeV-triggered disease (Fig. 1a), which in addition to the rate of body weight loss, is manifested by several phenotypes previously identified. These phenotypes include mean survival time, viral loads as well as histopathology of the lungs (Parker et al., 1978; Itoh et al., 1991; Percy et al., 1994; Fascia et al., 2005). However, the abrogation of early onset of SeV-induced loss of weight in backcrossed mice is the most attractive phenotype for analysis, because of its ease of accurate determination. We have experience that we have failed to detect significant QTLs due to the choice of complex phenotypes as QT. Complex phenotypes generally tend to include a lot of errors to be determined, leading to the fail of QTL analysis. As a matter of fact, we have succeeded in this

report to detect significant QTLs using a simple QT, loss of body weight, leading to the elucidation of genetic contribution to the strain difference in resistance to SeV. However, it remains obviously to determine other QTLs by performing QTL analysis using other phenotypes as QT. One interesting phenomenon we found was the mode of inheritance for the sensitivity to SeV infection. The phenotypes of weight loss of the DBF_1 progenies of D2 cross to B6, as well as in DBF_2 mice demonstrate that resistance is inherited as a dominant trait. The results from DBF_2 mice after infection suggest that multiple genes influence susceptibility to SeV. The three-fold difference in body weight loss found between D2 and DBF_1 mice suggest that resistance and susceptibility is a QT amenable to further genetic analysis and mapping (Lander and Kruglyak, 1995). Using information on microsatellite polymorphisms evident between these strains, we have identified chromosomal region that contains the gene(s) that are responsible for strain differences to SeV for the first time.

Our analysis revealed a QTL on the distal portion of Chr 4 (*SeV1*), which was significantly linked to post infection body weight change with an LRS of ≥14.5, in addition suggestive linkages were found for QTL on Chr 8 and 14. Furthermore, we report a highly significant epistatic interaction between *D3Mit182* (*SeV2*) and *D14Mit10* (*SeV3*, suggestive locus in QTL analysis) at a threshold value of $P < 0.0001$. Taken together the QTLs and inter-allelic interaction had explained more than 90% of the genetic effect on disease severity between D2 and B6 mice.

In terms of candidate genes as in many QTL study, chromosomal regions significantly influencing the outcome of a disease process are rather large and contain several potential "candidate genes". Although it might be a pure coincidence that the analysis in this study allows us to identify exact genes involved in resistance to SeV, there could be many genes of interest that regulate the function of macrophages and other immune cells as well as cytokines as presented in Table 2. We assessed the most likely genes that could account for three

responsible regions, the significant peak as well as the epistatic interaction. For the *SeV1* region we search between 109 and 132 Mb and identified more than 900 transcripts (RIKEN OmicBrowse, http://omicspace.riken.jp/db/genome.html). Genes were ranked in terms of likelihood based on the amount of evidence extracted during database searches. The search elucidated very strong candidates including the colony stimulating factor 3 receptor (*Csf3r*) and toll-like receptor 12 (*Tlr12*). *Csf3r* functions in neutrophil trafficking and interferon receptor activity. Further, it has been recently reported that neutrophils play a critical role in SeV-induced asthma phenotype (Akk et al., 2008). Mammalian TLRs have been shown to initiate immune responses to infection by recognizing microbial nucleic acids, thereby linking innate and acquired immunity. Recently it has been reported that two intracellular RNA sensor molecules RIG-I and MDA5, that recognize RNA viruses, tend to interact with TLRs towards mounting immune response to these viruses (Kato et al., 2006; Strahle et al., 2007; Yount et al., 2008). *SeV2* locus includes interleukin 2 (*Il2*), interleukin 21 (*Il21*), fibroblast growth factor 2 (*Fgf2*), and interleukin 12a (*Il12a*). For the *SeV3* locus, we found that thymic deletion 3 (*Rthyd3*), interleukin 3 receptor alpha chain (*Il3ra*), and interleukin 17 receptor B and D (*Il17rb* and *Il17rd*) are likely candidates. The list of candidates may be even greater than discussed here; however, an interesting aspect of our study is the localization of QTLs as well as epistatic interaction influencing the differential response to SeV infection in mice. QTLs detected can be fine-mapped in future studies and tested as to whether they represent single or more linked QTLs. Regions harboring QTLs from one selection line can be made congenic on the opposite line, and phenotype testing of congenic lines can be used to increase the mapping precision and better assessment of the physiological basis of gene function. We are currently attempting to explore this possibility in congenic lines.

Taken together, these observations raise the intriguing possibility that loci controlling basic aspects of resistance and susceptibility to SeV are localized to homologous regions in

the genome of other species; hence by exploiting the region of human chromosome showing homologous synteny (SeV1 for human Chr 1, SeV2 for human Chr 3, and SeV3 for mosaic human Chr), prime candidates for resistance and susceptibility to parainfluenza viral infections in humans could be identified, which could further enhance our understanding of the signaling pathway as well as host response to these closely related viruses.

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Figure Legends

Fig. 1 The time course and body weight changes as relative values following SeV infection. Sensitivity to SeV infection between parental strains and DBF_1 mice was determined. Relative body weights are presented as percent of initial body weight before viral inoculation for individual mouse. Initial body weights for each group were as follows; male D2, 20.4 ± 0.6 g; female D2, 21.0 ± 0.4 g; male B6, 20.0 ± 0.1 g; female B6, 20.0 ± 0.9 g; male DBF1, 23.3g ± 0.5; female DBF1, 22.1g ± 0.7 g.

Fig. 2 QTLs affecting SeV resistance and susceptibility in backcrossed progenies. Each vertical axis represents the genetic map for the mouse chromosome and the markers at which genotypes were determined. The threshold in the backcrossed progenies under the assumption of a free model were LRS 6.8 and 12.5 for suggestive (Su, dotted line) and significant (Si, solid line) linkages, respectively (P <0.05). (A) *SeV1* was defined by a peak LRS value of 14.5 on Chr 4 observed at a confidence interval of 8.3 cM (24 Mb) extending from *D4Mit146* to *D4Mit204*, while Chr 8 (B) and 14 (C) showed suggestive loci. Suggestive QTL on Chr 14 was denoted as *SeV3*, because it showed a significant epistatic interaction with another locus (see Fig. 3).

Fig. 3 Epistatic interaction affecting SeV resistance and susceptibility in backcrossed progenies. (A), Plot of pair-wise interaction for the least square analysis of variance of all informative markers in 108 backcrossed progenies showed a highly significant epistatic interaction between *D3Mit182* and *D14Mit10*, P <0.0001 and percentage of trait variance accounted for. Both loci were denoted as *SeV2* and *SeV3,* respectively. Error bars represent standard error. (B), Kaplan-Meier plot of SeV-infected backcrossed mice with respect to their

genotypes of *Sev2* and *SeV3* loci, which show epistatic interaction. In both (A) and (B), the survival rate was determined as the rate of mice showing less than 40% body weight loss after SeV infection in the light of "humane endpoint" concept.

Fig. 4 Kaplan-Meier plot of SeV-infected backcrossed mice with respect to their genotypes of *Sev1*, *Sev2*, and *SeV3* loci. (A), Kaplan-Meier plot of mice possessing heterozygous or homozygous with respect to *SeV1*. (B), Kaplan-Meier plot of mice with respect to *SeV1* and *SeV3*. (C), Kaplan-Meier plot of mice with respect to *SeV1, SeV2,* and *SeV3*. In this analysis, *SeV2* (*D3Mit182*) is fixed to heterozygous to show epistatic interraction. In (A), (B), and (C), the survival rate was determined as the rate of mice showing less than 40% body weight loss after SeV infection in the light of "humane endpoint" concept.

END

Fig.1.

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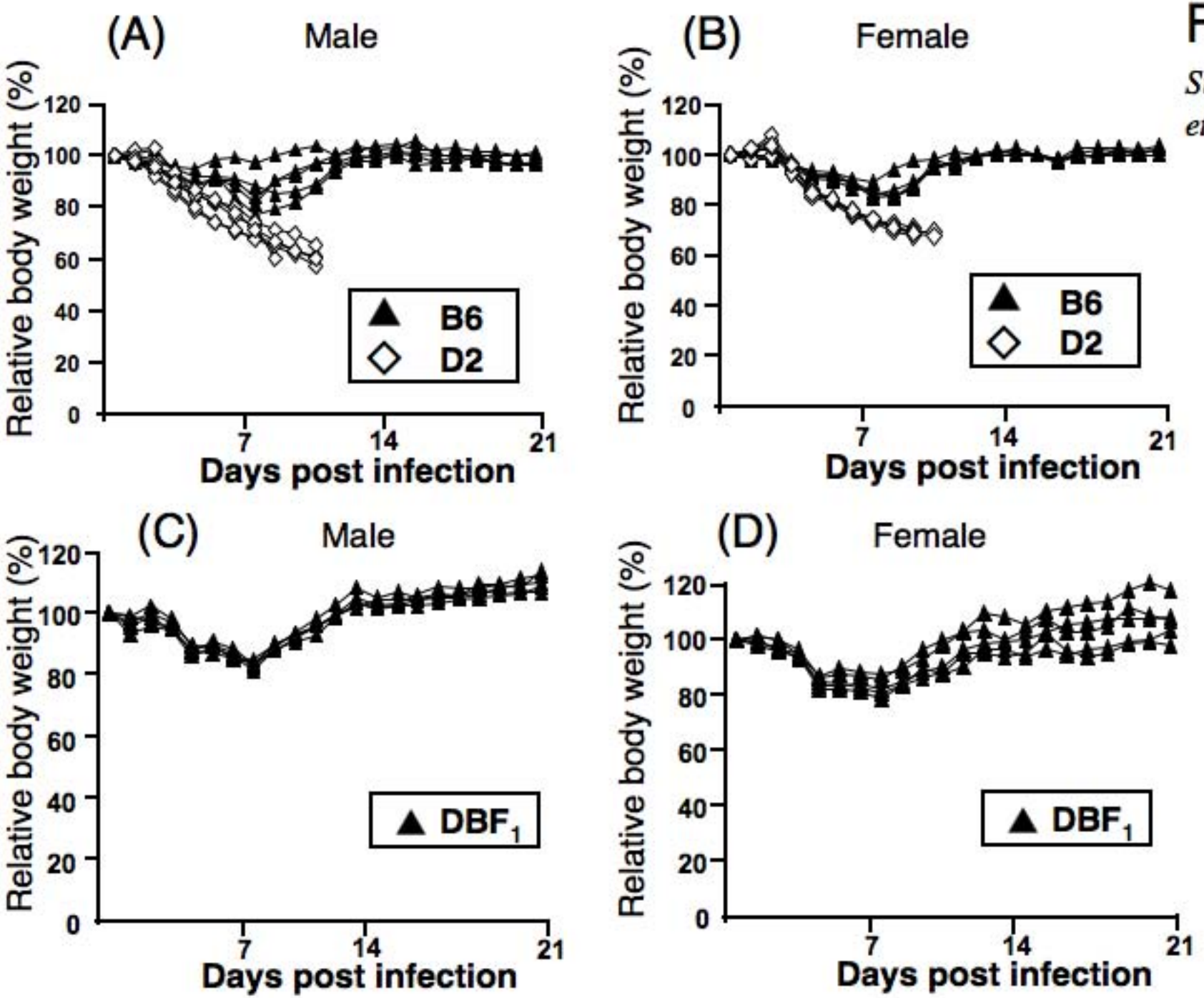


Fig. 2 (A) (B) (C)

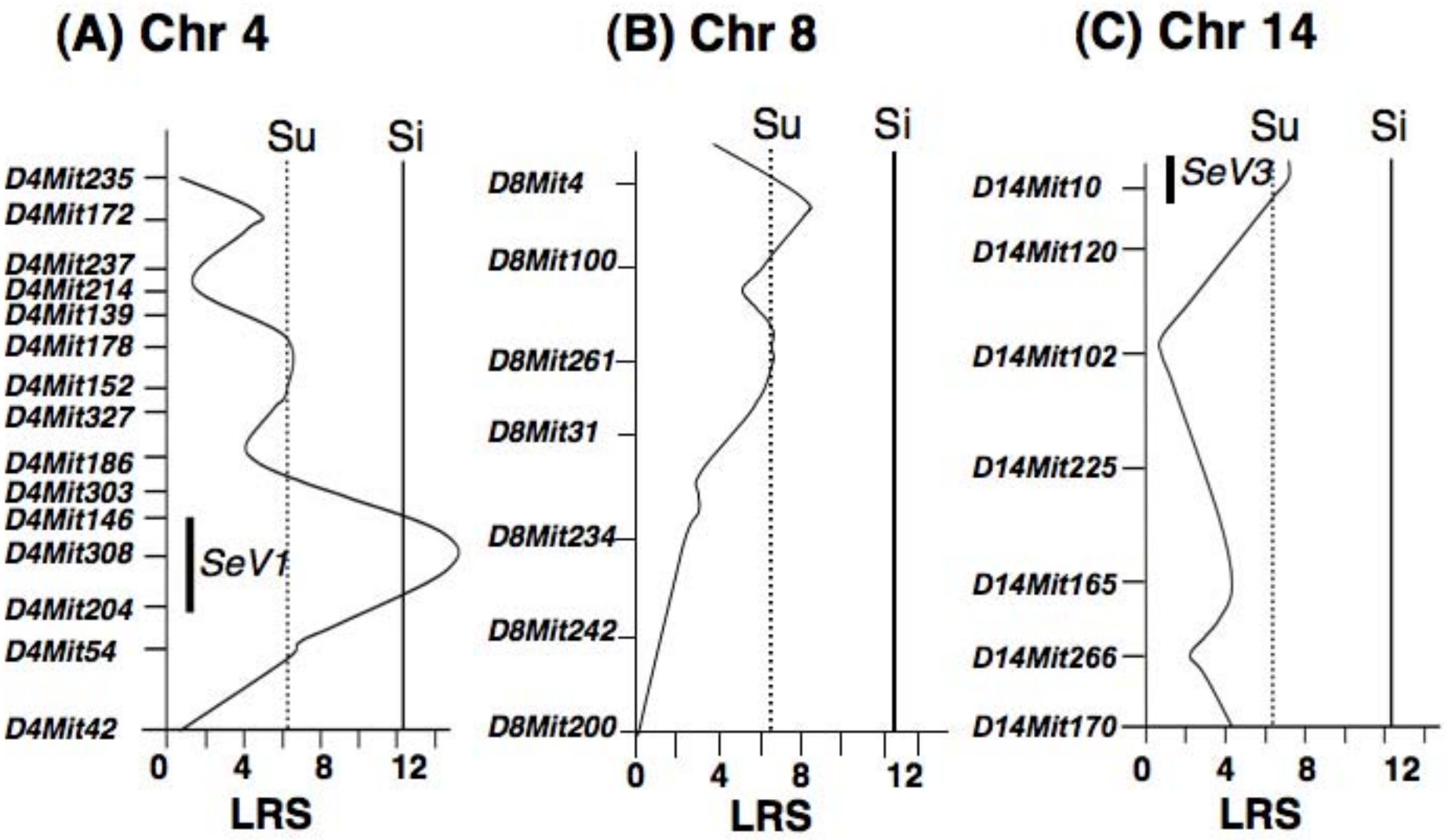
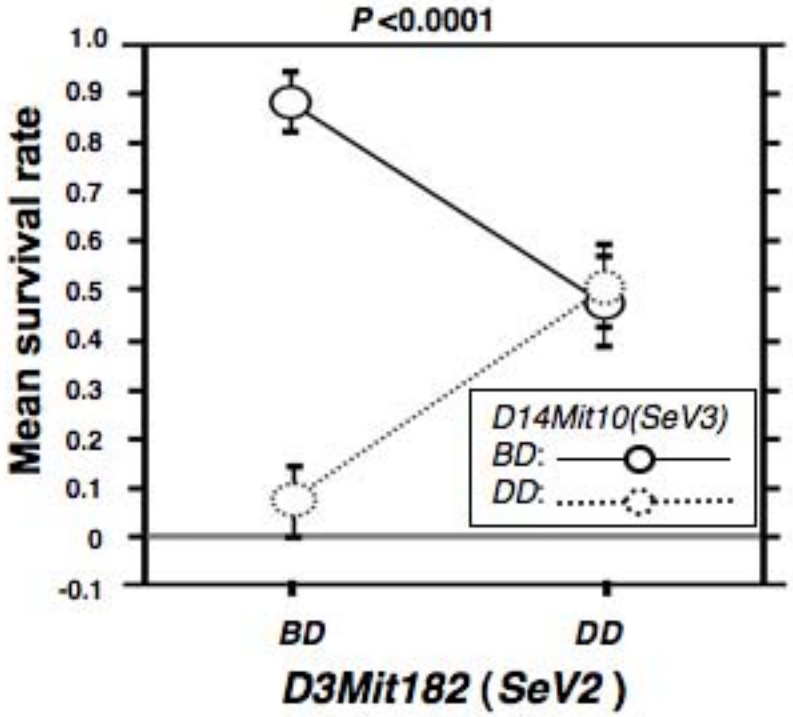

(A) Chr 4
Su
Si
D4Mit235
D4Mit172
D4Mit237
D4Mit214
D4Mit139
D4Mit178
D4Mit152
D4Mit327
D4Mit186
D4Mit303
D4Mit146
D4Mit308
D4Mit204
D4Mit54
D4Mit42
SeV1
0
4
8
12
LRS
(B) Chr 8
Su
Si
D8Mit4
D8Mit100
D8Mit261
D8Mit31
D8Mit234
D8Mit242
D8Mit200
0
4
8
12
LRS
(C) Chr 14
Su
Si
D14Mit10
D14Mit120
D14Mit102
D14Mit225
D14Mit165
D14Mit266
D14Mit170
SeV3
0
4
8
12
LRS

Fig. 3

(A)


P<0.0001
Mean survival rate
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
D14Mit10(SeV3)
BD:
DD:
BD
DD
D3Mit182 (SeV2)

(B)

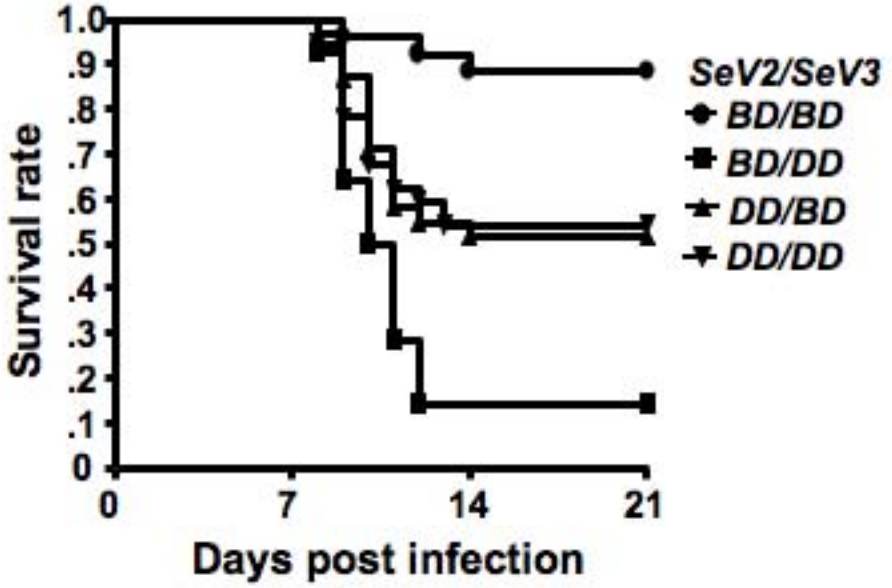

Survival rate
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
0
7
14
21
Days post infection
SeV2/SeV3
BD/BD
BD/DD
DD/BD
DD/DD

Fig. 4 (A)

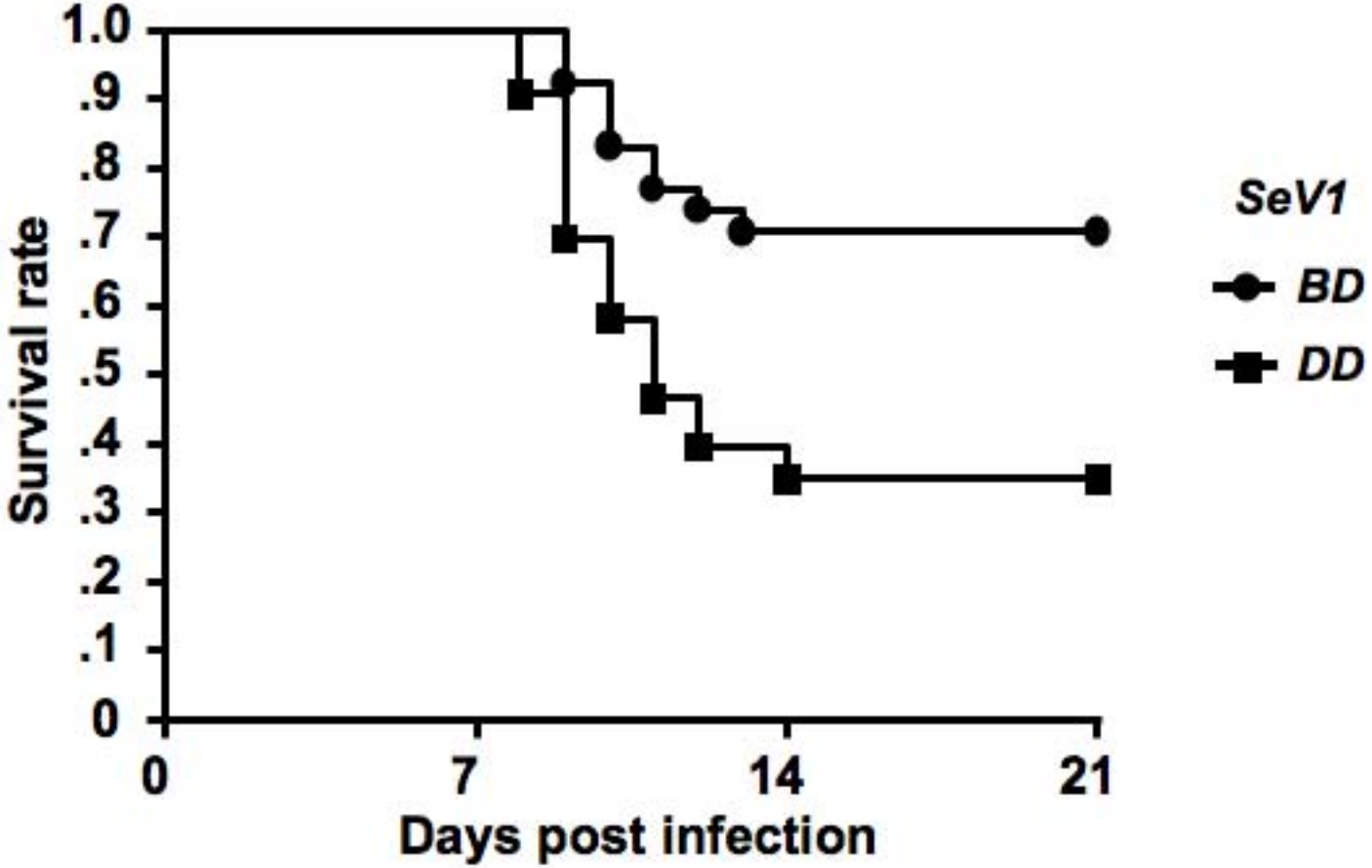

1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
Survival rate
0
7
14
21
Days post infection
SeV1
BD
DD

Fig. 4 (B)

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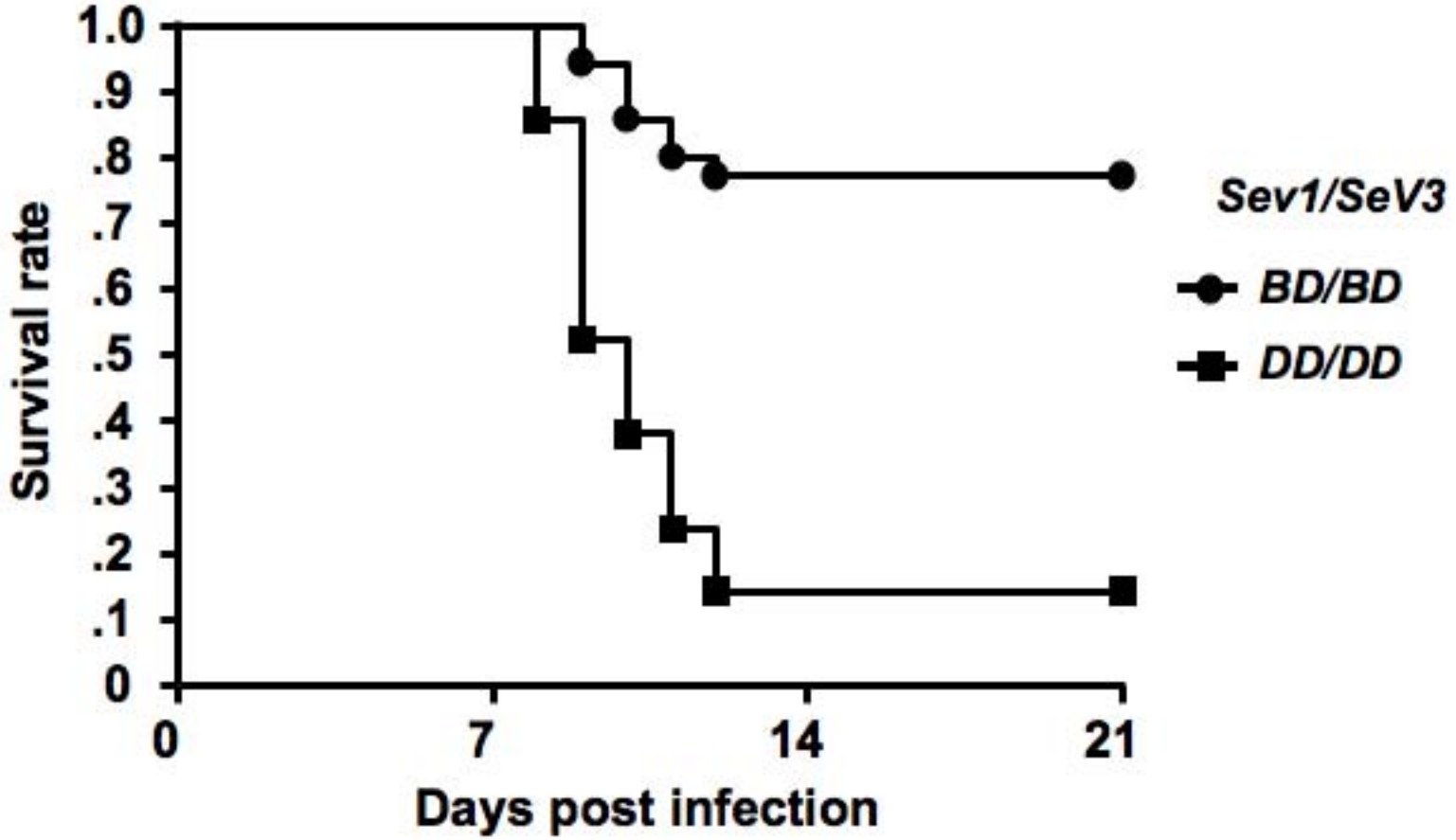

Fig. 4 (C)

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