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Partial dysfunction of STAT1 profoundly reduces host resistance to flaviviral infection

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ABSTRACT

The genetic basis for a dramatically increased virus susceptibility phenotype of MHC-II knockout mice acquired during routine maintenance of the mouse strain was determined. Segregation of the susceptibility allele from the defective MHC-II locus combined with sequence capture and sequencing showed that a Y37L substitution in STAT1 accounted for high flavivirus susceptibility of a newly derived mouse strain, designated *Tuara*. Interestingly, the mutation in STAT1 gene gave only partial inactivation of the type I interferon antiviral pathway. Accordingly, merely a relatively small impairment of interferon α/β signalling is sufficient to overcome the ability of the host to control the infection.

1. Text

In an investigation that addressed the contribution of antibody and T cells to the recovery from infection with the flavivirus, *Japanese encephalitis virus* (JEV), we reported that a strain of MHC-II k/o mice lacking the 4 classical MHC-II genes (*MHCII-Aα/β*$^{-/-}$ mice) (Madsen et al., 1999) were significantly more susceptible than congenic wt C57Bl/6 (B/6) mice to low-dose JEV infection; *MHCII-Aα/β*$^{-/-}$ mice uniformly succumbed to the infection with a mean survival time (MST) of 12 days (Larena et al., 2011). Surprisingly, a second strain of MHC-II k/o mice, in which only the Aα locus of the MHC-II complex was inactivated (*MHCII-Aα*$^{-/-}$ mice) (Kontgen et al., 1993), presented with a more severe phenotype than *MHCII-Aα/β*$^{-/-}$ mice in response to JEV infection (MST=8.7 ± 3 days; Fig. 1A). The difference in flavivirus susceptibility between *MHCII-Aα*$^{-/-}$ and *MHCII-Aα/β*$^{-/-}$ mice was even more pronounced when a low-virulence strain of *West Nile virus* (WNV_{KUN}) was used (Fig. 1B): while all B/6 wt and *MHCII-Aα/β*$^{-/-}$ mice survived the challenge, *MHCII-Aα*$^{-/-}$ mice showed 100% mortality (MST =9 ± 2.0 days). *MHCII-Aα*$^{-/-}$ mice infected with JEV or WNV_{KUN} presented with severe signs of encephalitis and high viral load in brain and spinal cord (data not shown). The increased susceptibility phenotype was consistently observed when *MHCII-Aα*$^{-/-}$ mice were challenged with a live-attenuated flaviviral vaccine, ChimeriVax-JE (Fig. 1 C,D).

To investigate whether the high sensitivity of *MHCII-Aα*$^{-/-}$ mice to the flaviviral infections was at least in part the result of an unknown defect other than the deficiency in the adaptive immune response mediated by the mutation in the MHC-II *Aα* gene, bone marrow-derived macrophages from *MHCII-Aα*$^{-/-}$ and B/6 wt mice were infected with JEV, WNV_{KUN} or the prototype flavivirus, *yellow fever virus* (YFV) and virus yield in culture supernatants measured by plaque assay (Fig. 1E). The three flaviviruses produced 50- to 100-fold higher titers in cultured cells from *MHCII-Aα*$^{-/-}$ than B/6 wt mice, suggesting the possibility of a spontaneous mutation that is independent of the MHC-II defect.

Collectively, these data show that *MHCII-Aα*$^{-/-}$ mice display an extraordinary sensitivity to flaviviral challenge, and that this susceptibility phenotype cannot be explained solely by an MHC-II defect. Moreover, the findings support the results from a second study published during the course of this investigation showing that the strain of *MHCII-Aα*$^{-/-}$ mice used is also more susceptible to alphavirus (Semliki Forest virus) and poxvirus (ectromelia virus) infections than *MHCII-Aα/β*$^{-/-}$ mice (Alsharifi et al., 2013). Accordingly, it was likely that the *MHCII-Aα*$^{-/-}$ mice had acquired an unidentified virus susceptibility allele as the result of a spontaneous mutation.

1.1. The unknown susceptibility allele is a recessive trait with full penetrance and is localized on chromosome 1

Genetic mapping enables localization of a spontaneous mutation to a particular chromosomal region by co-segregation of the mutation with genetic markers interspersed throughout the genome (reviewed in

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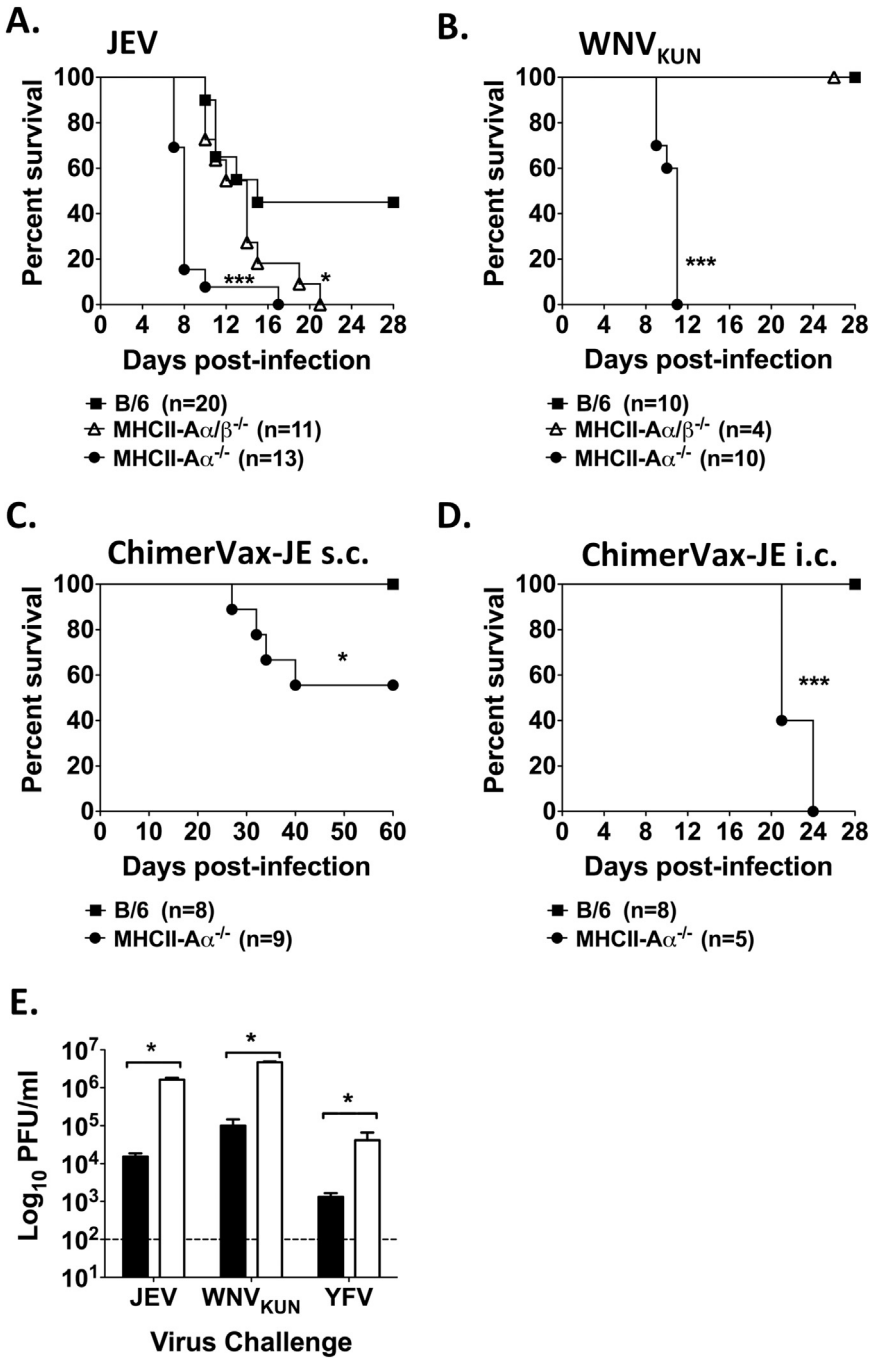

Fig. 1. Susceptibility of MHC-II k/o and B/6 wt mice and ex vivo macrophage cultures to flavivirus infections. Groups of 8-week-old mice were infected into the footpad with 10^3 PFU of JEV (A) or WNV_{KUN} (B) as described (Purtha et al., 2012). Morbidity and mortality were recorded daily, and surviving mice were monitored for 28 days. Groups of 6-week-old mice were inoculated subcutaneously (s.c.) with 10^5 PFU of ChimeriVax-JE (C) as described (Purtha et al., 2012), and morbidity and mortality recorded daily for a 60-day-observation period. Groups of 4-week-old mice were inoculated intracranially (i.c.) with 10^3 PFU of ChimeriVax-JE (D), and morbidity and mortality recorded daily for a 28-day-observation period. The data shown were constructed from two independent experiments. Asterisks denote statistical significance in mortality between B/6 and various knockout mice (*, $P < 0.05$; ***, $P < 0.001$). (E) Bone marrow cells were isolated from lower extremity bones of B/6 wt and *MHCII-Aα*$^{-/-}$ mice, and cultured for 7 days towards macrophage differentiation. Mature macrophages were then infected with JEV, WNV_{KUN}, or YFV at MOI of 1. Bars represent mean viral load with SEM (n =3) in culture supernatants at 24 pi, determined by plaque titration in Vero cells as described (Lobigs et al., 2009). Asterisks denote statistical significance (*, $P < 0.05$), ***, $P < 0.001$).

(Nelms and Goodnow, 2001)). To identify the unknown virus susceptibility allele, *MHCII-Aα*$^{-/-}$ mice were first crossed with a mapping strain (CBA mice) to produce F1 (B6xCBA) progeny. CBA mice were known to be resistant to WNV_{KUN} infection. The virus susceptibility of a group of F1 (n=6) in comparison to CBA control mice (n =5) was tested by WNV_{KUN} challenge: all F1 and CBA mice survived the challenge, while 100% of a group of control *MHCII-Aα*$^{-/-}$ mice (n=5) succumbed to the infection (data not shown). This suggests that the unknown susceptibility allele is a fully penetrating recessive trait. Next, F1 mice were backcrossed to *MHCII-Aα*$^{-/-}$ mice to generate N2 progeny mice (*MHCII-Aα*$^{-/-}$xF1). Challenge of N2 (n=24) mice with WNV_{KUN} gave the expected outcome of resistant (46%) and susceptible (54%) mice, and showed that the virus susceptibility allele segregated independently from the MHC-II locus in a simple Mendelian pattern of inheritance (Table 1).

Meiotic recombination in the F1 and subsequent N2 generations acts as a genetic shuffle that can be used to distinguish regions of the genome that are closely linked to the susceptibility allele from regions