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Transgenic expression of full-length 2′,5′-oligoadenylate synthetase 1b confers to BALB/c mice resistance against West Nile virus-induced encephalitis

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ABSTRACT

Susceptibility of inbred strains to infection with West Nile virus (WNV) has been genetically associated with an arginine-to-a nonsense codon substitution at position 253 (R253X) in the predicted sequence of the murine 2′,5′-oligoadenylate synthetase 1B (OAS1B) protein. We introduced by transgenesis the *Oas1b* cDNA from MBT/Pas mice carrying the R253 codon ($Oas1b^{MBT}$) into BALB/c mice homozygous for the X253 allele ($Oas1b^{BALB/c}$). Overexpression of $Oas1b^{MBT}$ mRNA in the brain of transgenic mice prior and in the time course of infection provided protection against the neuroinvasive WNV strain IS-98-ST1. A 200-fold induction of $Oas1b^{MBT}$ mRNA in the brain of congenic BALB/c mice homozygous for a MBT/Pas segment encompassing the *Oas1b* gene was also efficient in reducing both viral growth and mortality, whereas a 200-fold induction of $Oas1b^{BALB/c}$ mRNA was unable to prevent virally-induced encephalitis, confirming the critical role of the R253X mutation on *Oas1b* activity in live mice.

Introduction

West Nile virus (WNV) is a positive-sense, single-stranded RNA flavivirus transmitted by mosquitoes that infects a wide range of vertebrate hosts and causes severe illness in humans, including encephalitis, meningitis, or flaccid paralysis. About 80% of WNV infections are asymptomatic, 20% result in self-limited West Nile fever, and <1% result in neurologic disease (Hayes and Gubler, 2006). Host genetic factors might be important to control the susceptibility and severity to WNV infection (Diamond et al., 2009). Our laboratories reported that classical inbred strains such as BALB/c, whose genome is 92% of *Mus m. domesticus* origin (Yang et al., 2007), were highly susceptible to intraperitoneal infection with the neuroinvasive and neurovirulent Israeli strain IS-98-ST1 of WNV while mouse strains derived from wild progenitors of the *M. m. musculus* (MBT/Pas) or *Mus spretus* (SEG/Pas) species were resistant. We mapped the resistance locus to a critical interval of approximately 1 Mb that contains a cluster of 10 members of the *Oas* gene family (*Oas1a* to *Oas1h*, *Oas2* and *Oas3*) encoding 2′,5′-oligoadenylate synthetases, and identified a premature stop codon within exon 4 of the *Oas1b* gene, hence encoding a truncated version of OAS1B, lacking ~30% of its C-terminal domain (Mashimo et al., 2002). There was a perfect correlation among the various inbred strains of mice between the presence of this mutation and the susceptibility phenotype, strongly supporting that the truncated *Oas1b* allele was responsible for the susceptibility of laboratory mice to infection with the IS-98-ST1 viral strain. Perelygin et al.(2002) simultaneously identified the premature stop codon in C3H/He mice susceptible to infection with WNV strain Eg101 and showed that viral growth was impaired in C3H/He fibroblasts expressing a full-length OAS1B protein. *In vitro* experiments further demonstrated that expression of full-length OAS1B protein but not the C-terminally truncated form inhibits WNV replication inside infected mouse cells (Kajaste-Rudnitski et al., 2006; Lucas et al., 2003). Together, there is mounting evidence that the OAS family may play a crucial role in antiviral host immunity to WNV mediated by type-I interferons (Kristiansen et al., 2011). Consistent with this finding, genetic variations in human and horse *OAS1* are risk factors for infection with neuropathogenic WNV (Lim et al., 2009; Rios et al., 2010). To establish that the alteration in the coding sequence of the *Oas1b* gene is causative of susceptibility to flaviviruses, Scherbik et al. (2007a) replaced exons 4 and 5 in the genome of 129/Sv mice with DNA that corresponds to the MBT/Pas allele and reported that the

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knock-in mice were resistant to lethal infection with neurotropic flavivirus via the intracranial route.

In addition to the nonsense mutation, several nucleotide sequence variants have been identified in the promoter region of the *Oas1b* gene (Mashimo et al., 2003). Although none of them was consistent with the pattern of resistance or susceptibility across the inbred strains tested, we suspected that one or more of the SNPs could alter activation of *Oas1b* transcription in response to viral infection. To gain a better understanding of the molecular basis of *Oas1b*-mediated WNV resistance *in vivo*, we took a complementary approach. A cloned cDNA corresponding to the *Oas1b* mRNA expressed in resistant MBT/Pas mice was introduced by transgenesis to induce constitutive expression of the full-length coding sequence in an otherwise susceptible genetic background (BALB/c). We report that up-regulation of a wild-type *Oas1b* cDNA confers to BALB/c mice resistance against lethal WNV-induced encephalitis.

Results and discussion

Full-length OAS1B is a key-factor for mouse resistance to WNV infection

Positional cloning identified *Oas1b* as a candidate gene accounting for the resistance of wild-derived inbred strains of mice to infection with WNV (Mashimo et al., 2002; Perelygin et al., 2002). To further assess the role of the *Oas1b* gene during lethal infection, we produced a C.MBT-*Oas1b* congenic line where the *Oas* gene cluster of the MBT/Pas resistant strain was introgressed into the BALB/c susceptible genetic background. C.MBT-*Oas1b* congenic mice survive an i.p. inoculation of 1000 FFU of the virulent IS-98-ST1 WNV strain while BALB/c mice die within 10 days post-infection (Fig. 1), indicating that the MBT/Pas segment carries one or more determinant(s) of resistance (Mashimo et al., 2002). Although this result demonstrates the importance of the *Oas* gene cluster, it does not point unambiguously to the *Oas1b* nonsense mutation.

To address this issue, a transgene consisting of the CAG promoter and *Oas1b* cDNA from MBT/Pas strain was constructed (Fig. 2) and micro-injected into (C57BL/6 J × SJL/J)F2 fertilized eggs. Eggs were implanted in pseudo pregnant females. Three resulting founder animals harboured the entire transgene (*Tg*), as shown by PCR analysis, and were crossed with BALB/c mice to generate transgenic lines in a BALB/c genetic background. After twelve generations of backcrossing, *Tg*/+ transgenic mice from each of the three lines were examined for their susceptibility to lethal infection with WNV. C.MBT-*Oas1b* congenic and BALB/c mice served as controls. Mice were inoculated i.p. with 1000 FFU of the virulent WNV IS-98-ST1 strain. All

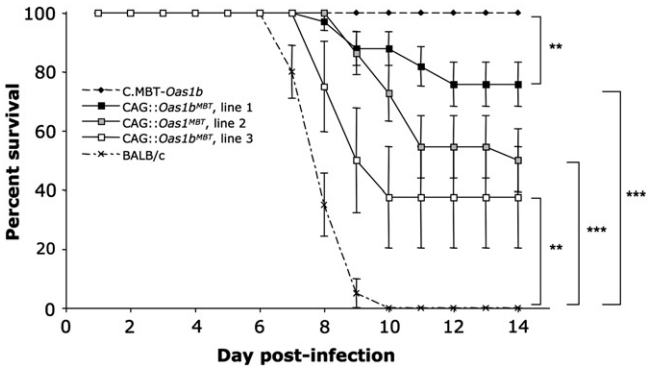

Fig. 1. Survival analysis of mice after West Nile virus infection. CAG::*Oas1b*^{MBT} transgenic mice from line 1 (N = 33), line 2 (N = 8) and line 3 (N = 22), C.MBT-*Oas1b* congenic mice (N = 20) and BALB/c control mice (N = 20) were inoculated with 1000 FFU of WNV IS-98-ST1 strain via the intraperitoneal route and monitored daily for mortality during 14 days. CAG::*Oas1b*^{MBT} transgenic mice from lines 1, 2 and 3 were significantly more resistant than BALB/c mice ($p<10^{-7}$, $p<10^{-8}$ and $p<0.005$, respectively).

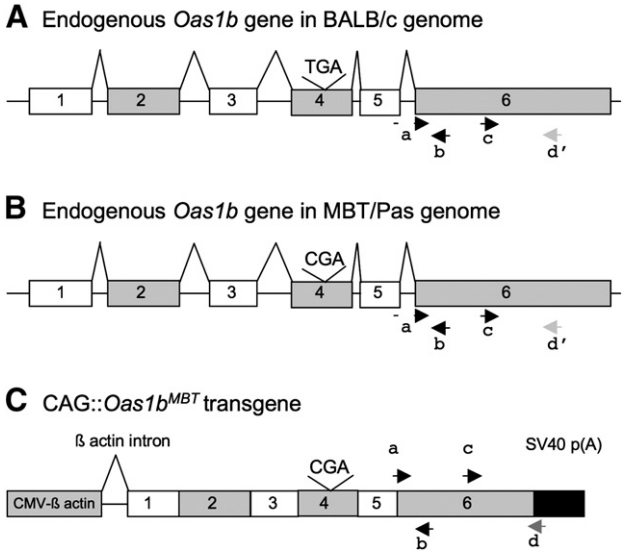

Fig. 2. Schematic structure of the endogenous *Oas1b* gene and CAG::*Oas1b*^{MBT} transgene. Structure of the endogenous *Oas1b* gene of BALB/c (A) and MBT/Pas (B) mice. Exons 1 to 6 of *Oas1b* are shown in boxes. Introns are shown by broken lines. The position of the CGA arginine codon in MBT/Pas mice that is mutated into TGA stop codon in BALB/c mice is also indicated. The positions of primers used for detecting (RT-PCR) and measuring (qRT-PCR) *Oas1b* mRNA are indicated. (C) The transgene contains the CMV/β-actin (CAG) promoter with β-actin intron and the complete MBT/Pas *Oas1b* cDNA and the SV40 polyadenylation sequence (SV40 p(A)).

BALB/c mice (N = 20) died within 10 days post-infection, whereas all C.MBT-*Oas1b* mice (N = 20) survived the infection, as expected. In CAG::*Oas1b*^{MBT} transgenic line 1, 75.7% (25/33) of the *Tg*/+ mice survived the infection (Fig. 1). There was no significant sex bias on survival. *Tg*/+ mice from transgenic lines 2 and 3 also survived the infection, though the percentage of survivors was lower (50 and 37% on average, respectively) (Fig. 1). These data indicate that the CAG::*Oas1b*^{MBT} transgene partially rescued the susceptible phenotype of BALB/c mice.

The CAG composite promoter has been shown to drive transgene expression in the whole body of adults and in embryos (Kawamoto et al., 2000; Kubo et al., 2002; Okabe et al., 1997). To assess CAG::*Oas1b*^{MBT} transgene expression *in vivo*, RT-PCR experiments were performed on various tissues of adult mice from each line. Organs were harvested and total RNAs were extracted from the heart, lung, liver, kidney, spleen and brain, RT-PCR assays were performed using primer pairs specific for the transgene (primers c–d in Fig. 2C) and the housekeeping aldolase gene, respectively. Fig. 3 shows that the transgene was expressed at detectable levels in the heart, lung, and brain in each line. However, transgene expression level was hardly detected in the liver in all lines. The CAG::*Oas1b*^{MBT} expression was not identical among the three lines suggesting that expression of the transgene was dependent on the site of integration, as commonly observed in transgenic mice (De Sepulveda et al., 1995). The more resistant transgenic line was used in subsequent investigations. To evaluate the expression of transgenic and endogenous *Oas1b* genes, we measured *Oas1b* mRNA levels in peripheral organ (spleen) and the central nervous system (brain) of BALB/c, C.MBT-*Oas1b* and CAG::*Oas1b*^{MBT} mice using primers that amplify the transcripts from the endogenous *Oas1b*^{MBT} and *Oas1b*$^{BALB/c}$ genes, and from the *Oas1b*^{MBT} transgene (primers a-b in Fig. 2). mRNAs containing a premature translation termination codon, such as *Oas1b*$^{BALB/c}$ mRNA, are generally degraded by nonsense-mediated decay (NMD) (Matsuda et al., 2008). Table 1 shows that, in the spleen, *Oas1b* mRNA level was approximately two fold higher in BALB/c than in C.MBT-*Oas1b* mice ($p<0.05$). This suggests that despite the premature translation termination codon in exon 4 of *Oas1b* in BALB/c mice, the transcript