

The cytokine responses in the pregnant mouse brain

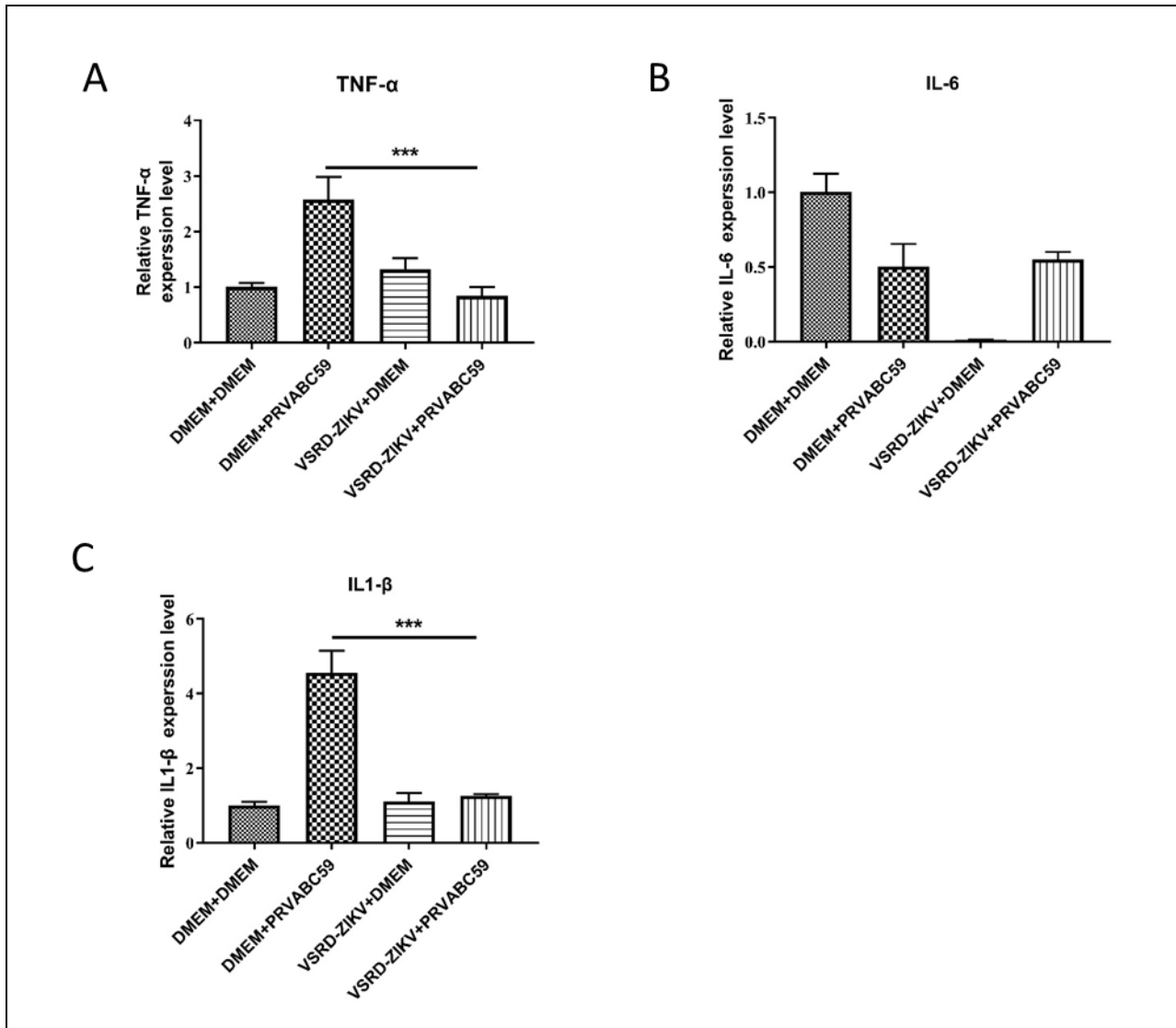
Five-week-old female AG129 mice were immunized with 1×10^6 FFU VSRD-ZIKV, or DMEM through the s.c. route. On day 14 post-immunization (p.i.) the 1×10^6 FFU VSRD-ZIKV group was immunized with 1×10^6 FFU VSRD-ZIKV again. The female AG129 mice received a prime-boost regimen of VSRD-ZIKV were mated on day 22 post-infection. The pregnant mice were then challenged with a lethal ZIKV infection (PRAVABC59 strain) and the placenta, fetal brain, and pregnant mouse brain were assessed 12 days post-infection for cytokine expression and histopathology (Figure, 4). Total RNA of cells was extracted by Trizol reagent (Invitrogen), and 1 μ g of RNA was used to synthesize cDNA using a first strand cDNA synthesis kit of SuperScript III System (ThermoFisher Scientific, Waltham, MA). Quantitative real-time RT-PCR was performed using a 7500 real-time PCR system (Applied Biosystems) and SYBR green PCR master mix (Toyobo, Osaka, Japan). Data were normalized to the level of β -actin expression in each sample. Primers used are listed in Table S1. The relative expression of TNF- α , CCL-5, IL-1 β , and IL-6 were normalized to the levels of endogenous control β -actin within each sample using the $2^{-\Delta\Delta CT}$ (where CT is threshold cycle) method.

Table S1. Primer pairs used for quantitative real-time RTPCR analyses.

Primer name	Sequence (5'-3')
β -actin-F	CACTGCCGCATCCTCTTCCTCCC
β -actin-R	CAATAGTGATGACCTGGCCGT
TNF-a-F	TGTCTCAGCCTCTTCTCATTCC
TNF-a-R	TTAGCCCACTTCTTCCCTCAC
CCL-5-F	TGCCACGTCAAGGAGTATTTC
CCL-5-R	AACCCACTTCTTCTGGGTTG
IL-6-F	CTGCTTCTGGTGATGGCTACTG
IL-6-R	GGCATCACCTTTGGCATCTT
IL-1 β -F	AACCTGCTGGTGTGTGACGTTC
IL-1 β -R	CAGCACGAGGCTTTTTTGTGT

Notes: F for forward primers, R for reverse primers.

Figure S1. The cytokine responses in the pregnant mouse brain.



Figure, S1. The cytokine responses in the pregnant mouse brain. Five-week-old female AG129 mice ($n = 9$) were immunized with 1×10^6 FFU VSRD-ZIKV, or DMEM through the s.c. route. On day 14 p.i. the 1×10^6 FFU VSRD-ZIKV group were immunized with 1×10^6 FFU VSRD-ZIKV again. On day 22 p.i. immunized AG129 female mice were mated with naïve male AG129 mice. At E6, mice were inoculated subcutaneously with 10^3 FFU of ZIKV PRVABC59 via intraperitoneal injection. All animals were sacrificed on E18 and the cytokine responses in the pregnant mouse brain were assessed by qRT-PCR: (A) TNF- α ; (B) IL-6; and (c) IL1- β . Data are expressed as means \pm SEM from three independent experiments. *** $P < 0.001$.