Cell Research (2017) 27:158-160.

www.nature.com/cr

LETTER TO THE EDITOR

Transfer of convalescent serum to pregnant mice prevents Zika virus infection and microcephaly in offspring

Cell Research (2017) **27**:158-160. doi:10.1038/cr.2016.144; published online 6 December 2016

Dear Editor,

Zika virus (ZIKV) is spreading rapidly around the world in over 60 countries. There is a mounting concern over the association of ZIKV infection and devastating cases of fetal and newborn microcephaly cases [1]. The connection between ZIKV infection and microcephaly was first proposed based on the presence of ZIKV in microcephalic fetal brain tissues [1]. The causal link between ZIKV infection and microcephaly was recently confirmed in animal models and human cerebral organoids [2-4]. The infection is likely to cause deregulation of genes related to neural progenitor cell (NPC) development, cell death and immune response, and subsequently microcephaly [2-4].

As a ZIKV vaccine is likely years away, there is an urgent need to explore immediately available therapeutics for effective treatment against ZIKV. Over the past decades, human convalescent plasma derived from patients recovered from diseases has been used in emergency response to emerging viral diseases, such as severe acute respiratory syndrome, H5N1 influenza, Ebola virus disease and Middle East respiratory syndrome [5]. Notably, convalescent plasma is safe for pregnant women and children. Here we describe the effects of treatment with human convalescent serum in an established animal model of microcephaly [2].

Firstly, we inspected the *in vitro* neutralizing activity of the convalescent serum from a donor who recovered from ZIKV infection 2 months before [6] by a standard plaque reduction neutralization test (PRNT) using BHK21 cells, and PRNT50 against ZIKV was calculated to 1: 161 by non-linear regression analysis (Figure 1A). Injection of approximately 650 PFU ZIKV into the cerebroventricular space of embryonic day 13.5 (E13.5) brains has been shown to result in microcephaly at E18.5 [2]. We adopted the same method to investigate the potential protective effect of convalescent serum during pregnancy. 100 μl convalescent serum was injected into the peritoneal cavity of pregnant ICR mice once daily on day 1 and 2 after the brains of embryos were infected with ZIKV. Fetal brains were inspected at E18.5 by immunocytochemistry staining. Similar to what was shown previously, a large number of cells in the cortex were infected in the brains injected with ZIKV [2], and the number of infected cells in the fetal brains from serum-treated pregnant mice decreased substantially (Figure 1B). Accordingly, the number of cells positive for activated form of caspase-3 also reduced dramatically (Figure 1C). These findings indicate that convalescent serum can inhibit ZIKV infection and suppress cell death in infected brains, which have been shown previously to contribute to the smaller brain sizes [2, 4, 7]. To exclude the possibility that the protective effects might be caused by immunological responses elicited by challenges with heterogeneic elements in the human serum, we repeated the experiments with serum from a healthy human and no protective effect was detected (Supplementary information, Figure S1).

We next investigated whether convalescent serum can prevent microcephaly induced by ZIKV infection. Compared with their mock-infected littermates, a mild reduction in brain sizes was observed in ZIKV-infected fetuses 5 days after infection (data not shown), and the administration of convalescent serum to the pregnant mother reversed such reduction (Figure 1D). In addition, the thinning of the cortical plate (CP) and ventricular zone (VZ)/subventricular zone (SVZ) which was observed in the infected fetal brains was also effectively rescued by the serum treatment (Figure 1D and 1E). This was confirmed by staining with NeuN (mature neurons) and Tbr1 (immature neurons) (Figure 1F). Because the thinning of VZ/SVZ in the infected brains was shown to be caused by dysregulation of NPC cell cycle and proliferation [2, 4], we inspected whether convalescent serum could rescue the reduction of NPCs caused by ZIKV infection. As shown in Figure 1G-1I, there were significantly fewer cells positive for phosphorylated H3, a marker for cells in M phase of the cell cycle, Sox2, a marker for NPC, and Tbr2, a marker for intermediate/basal progenitor cell (IPC/BPC) in the VZ/SVZ of ZIKV-infected brains, indicating a decreased number of NPCs and IPCs/BPCs. This

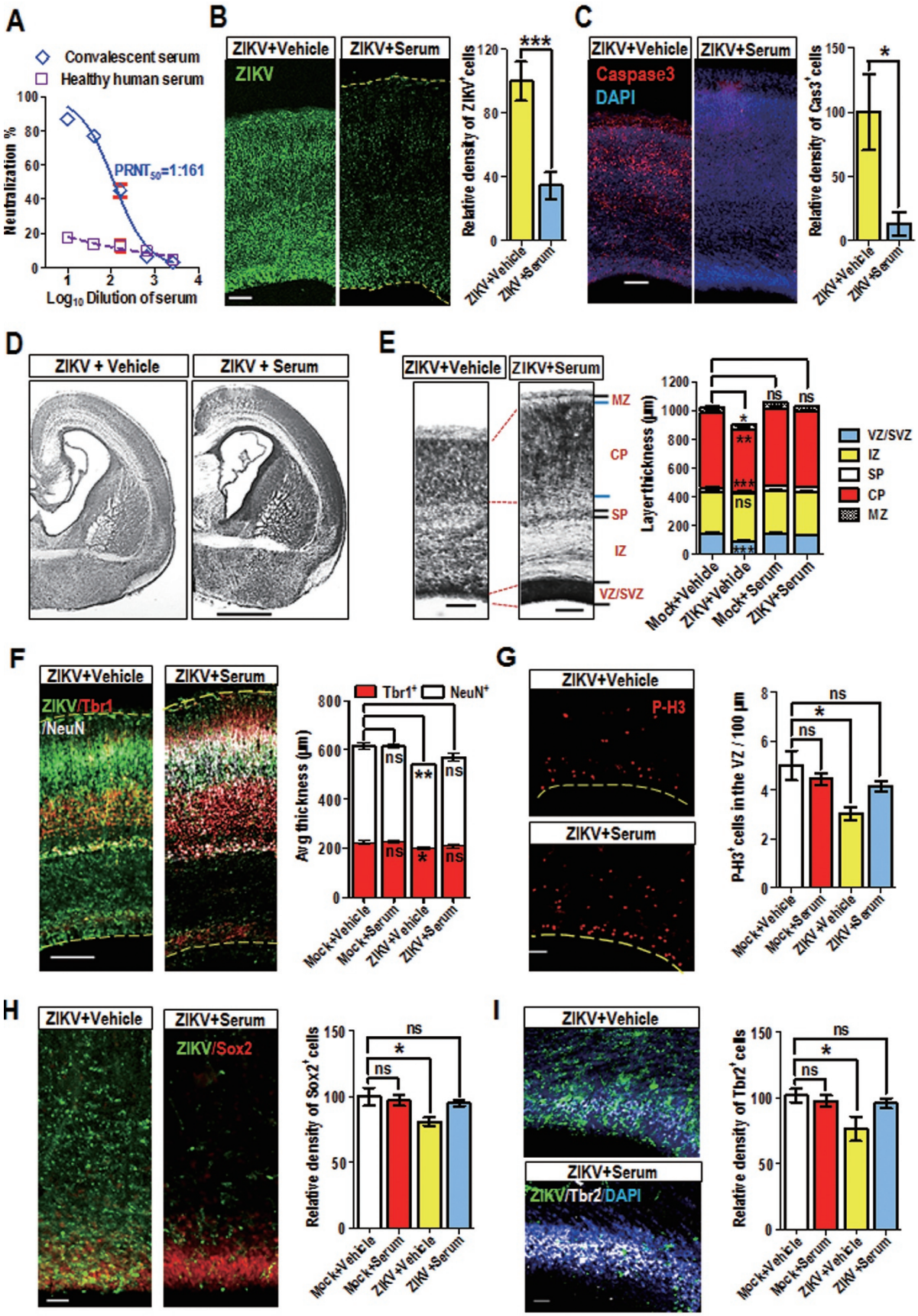
A
Convalescent serum
Healthy human serum
Neutralization %
PRNT50=1:161
Log10 Dilution of serum
B
ZIKV+Vehicle
ZIKV+Serum
ZIKV
Relative density of ZIKV+ cells
***
C
ZIKV+Vehicle
ZIKV+Serum
Caspase3
DAPI
Relative density of Cas3+ cells
*
D
ZIKV + Vehicle
ZIKV + Serum
E
ZIKV+Vehicle
ZIKV+Serum
MZ
CP
SP
IZ
VZ/SVZ
Layer thickness (μm)
Mock+Vehicle
ZIKV+Vehicle
Mock+Serum
ZIKV+Serum
F
ZIKV+Vehicle
ZIKV+Serum
ZIKV/Tbr1
/NeuN
Tbr1+ NeuN+
Avg thickness (μm)
G
ZIKV+Vehicle
ZIKV+Serum
P-H3
P-H3+ cells in the VZ / 100 μm
H
ZIKV+Vehicle
ZIKV+Serum
ZIKV/Sox2
Relative density of Sox2+ cells
I
ZIKV+Vehicle
ZIKV+Serum
ZIKV/Tbr2/DAPI
Relative density of Tbr2+ cells

Figure 1 Convalescent serum protects embryos from ZIKV brain infection and microcephaly. **(A)** *In vitro* neutralizing activity of human convalescent serum from a ZIKV-infected patient compared with serum from a healthy human**.** ZIKV were mixed with four-fold serial dilutions of serum, and standard plaque reduction assay was performed on BHK-21 cells. *n* = 4, human convalescent serum; *n* = 2, healthy human serum. **(B**-**I)** Fetal brains were injected with ZIKV or medium at E13.5 and inspected at E18.5 with or without treatment with human convalescent serum. **(B)** Left panel: images of coronal sections stained with ZIKV antiserum (green). Right panel: quantification of relative levels of $ZIKV^+$ cells. ZIKV+Vehicle: *n* = 22, ZIKV+Serum: *n* = 16. **(C)** Left panel: images of cortices stained with the activated form of caspase3 (red) and DAPI (blue). Right panel: quantification of relative levels of caspase3-positive cells. ZIKV+Vehicle: *n* = 6, ZIKV+Serum: *n* = 7. **(D**, **E)** Similar position of coronal sections of Vehicle- or Serum-treated ZIKV-infected brains with Nissl staining. Right panel in **E**: quantification of layer thickness. *n* = 39 for each. MZ: marginal zone, CP: cortical plate, SP: subplate, IZ: intermediate zone, SVZ: subventricular zone, VZ: ventricular zone. **(F)** Images of cortices stained for NeuN (white) and Tbr1 (red). Right panel: quantification of thickness stained with individual markers. Mock+Vehicle: *n* = 10 ($NeuN^+$), 10 ($Tbr1^+$); Mock+Serum: *n* = 13 ($NeuN^+$), 14 ($Tbr1^+$); ZIKV+Vehicle: *n* = 16 ($NeuN^+$), 6 ($Tbr1^+$); ZIKV+Serum: *n* = 12 ($NeuN^+$), 12 ($Tbr1^+$). **(G)** Images of cortices stained with phospho-Histone H3 ($P\text{-}H3^+$, red, left panel). Right panel: quantification of the $P\text{-}H3^+$ cells in the VZ. Mock+Vehicle: *n* = 5, Mock+Serum: *n* = 15, ZIKV+Vehicle: *n* = 5, ZIKV+Serum: *n* = 10. **(H)** Coronal sections of cortices stained with ZIKV (green) and Sox2 (red). Right panel: quantification of the relative density of $Sox2^+$ cells in the VZ/SVZ. Mock+Vehicle: *n* = 11, Mock+Seurm: *n* = 12, ZIKV+Vehicle: *n* = 8, ZIKV+Serum: *n* = 13. **(I)** Coronal sections of cortices stained with ZIKV (green) and Tbr2 (white). Right panel: quantification of the relative density of $Tbr2^+$ cells in the VZ/SVZ. All data are means ± SEM, Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. n: slice numbers from 4 Mock+Vehicle and Mock+Serum brains **(B**-**I)**, 9 ZIKV+Vehicle and ZIKV+Serum brains **(B**-**E)** or 6 ZIKV+Vehicle and ZIKV+Serum brains **(F**-**I)**. Scale bars for **B** and **C**: 100 μm; **D**: 1mm; **E**: 100 μm; **F**: 200 μm; **G**-**I**: 50 μm.

reduction was rescued by convalescent serum treatment, in agreement with the rescue of the thinning of VZ/SVZ.

Until an effective vaccine becomes available, utilizing existing counter-measurements represents an immediately attainable approach in treating ZIKV-infected pregnant women. Recent studies have shown that several antibodies are able to neutralize ZIKV and inhibit viremia in mouse model of ZIKV infection [8, 9]. However, how to protect pregnant women, especially those with potential fetal brain ZIKV infection, is significantly more challenging. Our results demonstrate that passive transfer of convalescent serum containing high-titer neutralizing antibodies to pregnant mice can not only suppress ZIKV replication but also inhibit cell death and reduction of NPCs in infected fetal brains, thus preventing microcephaly. Our results indicate that antibodies in the convalescent serum can pass through both the placental barrier of pregnant mice and the blood-brain barrier of fetuses. Our study reveals the potential of convalescent serum for the prevention and treatment of ZIKV infection in pregnant women, which is particularly important in countries and regions where abortion is prohibited by law or religion. A panel of human monoclonal antibodies with potent neutralizing activities have now been characterized [10, 11], and our results highlight their potential application in pregnant women in the endemic regions.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31430037, 31271156, 81522025 and 81621005), the National Basic Research Program ("973" program; 2014CB942801, 2013DFA31990 and 2012YQ03026006) and the National Key Research Program of China (2016YFD0500304).

Shuo Wang[1, 2, *], Shuai Hong[1, 2, *], Yong-Qiang Deng[3, *], Qing Ye[3], Ling-Zhai Zhao[4], Fu-Chun Zhang[4], Cheng-Feng Qin[3], Zhiheng Xu[1, 5]

[1]State Key Laboratory of Molecular Developmental Biology, CAS Center for Excellence in Brain Science and Intelligence Technology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China; [2]University of Chinese Academy of Sciences, Beijing 100101, China; [3]Department of Virology, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China; [4]Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China; [5]Parkinson's Disease Center, Beijing Institute for Brain Disorders, Beijing 100101, China

*These three authors contributed equally to this work.
Correspondence: Zhiheng Xu[a], Cheng-Feng Qin[b]
[a]E-mail: zhxu@genetics.ac.cn
[b]E-mail: qincf@bmi.ac.cn

References

1 Lessler J, Chaisson LH, Kucirka LM, *et al*. *Science* 2016; **353**:aaf8160.
2 Li C, Xu D, Ye Q, *et al*. *Cell stem cell* 2016; **19**:120-126.
3 Qian X, Nguyen HN, Song MM, *et al*. *Cell* 2016; **165**:1238-1254.
4 Wu KY, Zuo GL, Li XF, *et al*. *Cell Res* 2016; **26**:645-654.
5 Winkler AM, Koepsell SA. *Curr Opin Hematol* 2015; **22**:521-526.
6 Zhang FC, Li XF, Deng YQ, *et al*. *Lancet Infect Dis* 2016; **16**:641-642.
7 Xu D, Zhang F, Wang Y, *et al*. *Cell Rep* 2014; **6**:104-116.
8 Zhao H, Fernandez E, Dowd KA, *et al*. *Cell* 2016; **166**:1016-1027.
9 Stettler K, Beltramello M, Espinosa DA, *et al*. *Science* 2016; **353**:823-826.
10 Barba-Spaeth G, Dejnirattisai W, Rouvinski A, *et al*. *Nature* 2016; **536**:48-53.
11 Dai L, Song J, Lu X, *et al*. *Cell Host Microbe* 2016; **19**:696-704.

(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)