



Chikungunya viral RNA and viral titration in tissue samples in cynomolgus macaques as model

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CONTEXT

The Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that induces in humans a disease characterized by fever, rash, and pain in muscles and joints. The aim of this study is to propose a new model for CHIKV infection in adult, immunocompetent cynomolgus macaques. CHIKV infection in these animals recapitulated the viral, clinical, and pathological features observed in human disease [1].

MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit: 03961-1-002 (ceramic beads 2.8mm) for smooth tissues and 03961-1-001 (metals beads 2.8mm) for joint and muscle.
- Samples: Splenic, hepatic, muscular, and joint tissues from cynomolgus macaques inoculated with CHIKV (30 mg /~100mg).
- Buffer: 1 ml lysis buffer (Macherey Nagel) for Viral RNA extraction or 1 ml DMEM supplemented with 10% FCS for Viral titration

PROTOCOL

- Precellys®24: 5000 rpm, 2x20 sec or 2x10 sec
- Tissue viral RNA extraction: Nucleospin 96 RNA kit (Macherey Nagel), Relative quantitative RT-PCR simultaneously with CHIKV and GAPDH primers and probes.
- Determination of viral titers (TCID₅₀/g): on mammalian BHK-21 cell lines based on their TCID₅₀ using 4–5 replicates.

[1] K.labadie et al, J Clin Invest. 2010;120(3):894–906. doi:10.1172/JCI40104. <http://www.jci.org>



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RESULTS

The figure 1 shows the infectious virus titers in spleen, liver, muscle, and joint collected from CHIKV-infected macaques, 6 and 44 dpi.

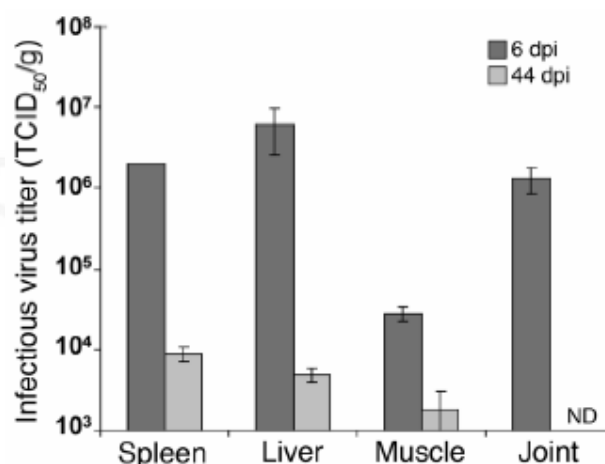


Figure 1. Tissues were collected at 6 dpi from macaques inoculated i.v. with 10⁷ PFU CHIKV, or at 44 dpi from macaques inoculated i.v. with 10⁶ PFU CHIKV, and the amount of infectious virus present in tissues was quantified by TCID₅₀. Data are mean ± SEM of 2 independent virus titrations. The detection threshold was 700 TCID₅₀/g.

Our results provide insights into the pathogenesis of CHIKV. We have developed a relevant macaque model of CHIKV infection, in which we demonstrated long-term CHIKV persistence in various tissues and identified macrophages as cellular reservoirs during the late stages of CHIKV infection in vivo.

CONCLUSION

The Precellys®24 is an ideal tool to evaluate both viral RNA and viral titers from various tissues of cynomolgus macaques inoculated with Chikungunya virus.

Precellys®24 is very easy to use, it is as simple as using a centrifuge. Comparing to the former method (mortar), we appreciate the cleanness and the cross-contamination free provided by consumables of Precellys®24 lysing kit. The reproducibility given for various tissues of this study is also a strong advantage of this smart homogenizing equipment.

For more details, please contact precellys@bertin.fr



Problem



Solution

