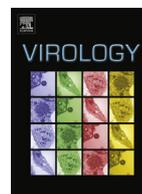




ELSEVIER

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Brief Communication

Varicella zoster virus DNA does not accumulate in infected human neurons



Nicholas L. Baird^a, Jacqueline L. Bowlin^a, Xiaoli Yu^a, Stipan Jonjić^{b,c}, Jürgen Haas^{d,e}, Randall J. Cohrs^{a,f}, Don Gilden^{a,f,*}

^a Department of Neurology, University of Colorado School of Medicine, Aurora, CO, USA

^b Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

^c Center for Proteomics, Department of Histology and Embryology, University of Rijeka, Rijeka, Croatia

^d Max von Pettenkofer Institut, Ludwig-Maximilians Universität München, München, Germany

^e Division of Pathway Medicine, University of Edinburgh, Edinburgh, United Kingdom

^f Department of Microbiology, University of Colorado School of Medicine, Aurora, CO, USA

ARTICLE INFO

Article history:

Received 24 March 2014

Returned to author for revisions

31 March 2014

Accepted 7 April 2014

Available online 5 May 2014

Keywords:

VZV DNA

Human neurons

Tissue culture

ABSTRACT

Varicella zoster virus (VZV) is an exclusively human neurotropic alphaherpesvirus. It is unclear why human neurons infected *in vitro* with VZV at low multiplicity of infection do not exhibit a cytopathic effect (CPE) even though all VZV genes are transcribed, VZV proteins from all kinetic classes are translated and minimal infectious virus is produced. Here, we show that the lack of VZV-induced CPE correlates with the low abundance of viral DNA.

© 2014 Elsevier Inc. All rights reserved.

Introduction

Varicella zoster virus (VZV), an exclusively human alphaherpesvirus, becomes latent in ganglionic neurons along the entire neuraxis after primary infection. Decades later, virus can reactivate to produce zoster and additional serious neurological and ocular complications (Gilden et al., 2011; Nagel and Gilden, 2013). VZV can infect neurons *in vitro* from multiple sources, including those derived from human embryonic stem cells [hESC; (Markus et al., 2011; Sloutskin et al., 2013)], human neural stem cells [hNSC; (Pugazhenthhi et al., 2011)] and human induced pluripotent stem cells [iPSC; (Baird et al., 2014; Grose et al., 2013; Yu et al., 2013)]. Although human neurons die after VZV infection at high multiplicity of infection (MOI; $> 1 \times 10^{-3}$) (Markus et al., 2011; Sloutskin et al., 2013), no CPE develops within 2 weeks after infection at low MOI ($< 1 \times 10^{-3}$) (Baird et al., 2014; Grose et al., 2013; Yu et al., 2013). While the absence of a CPE in neurons 5 days after infection at low MOI has been attributed to non-productive infection based on the absence of detectable VZV proteins (Sloutskin et al., 2013), we have shown that VZV transcripts and

proteins as well as infectious virus are produced in neurons 14 days after low MOI infection, and ultrastructural analysis revealed numerous empty capsids in the nuclei of VZV-infected neurons (Grose et al., 2013; Yu et al., 2013). Furthermore, Next Generation RNA sequencing (NextGen RNAseq) showed that every annotated VZV open reading frame (ORF) is transcribed in human neurons 14 days post-infection (dpi), and that the abundance of VZV transcripts does not differ significantly from that found in VZV-infected human fibroblasts with extensive CPE (Baird et al., 2014). Thus, we examined VZV DNA abundance in VZV-infected human neurons at multiple times over a 2-week period.

Results

Virus production is both delayed and reduced in human neurons compared to fibroblasts

No virus was produced in VZV-infected neurons at 1, 3 and 7 dpi; at 14 dpi, 31 PFU/culture were detected (Fig. 1). The few PFU detected in infected human neurons 14 dpi were not due to residual inoculum since no infectious plaques were seen at earlier times. In contrast, VZV-infected fibroblasts began producing infectious virus 1 dpi, which peaked at 2.1×10^4 PFU/culture 7 dpi then decreased slightly 10 dpi concomitant with cell death.

* Correspondence to: Department of Neurology and Microbiology, University of Colorado Denver School of Medicine, 12700 E. 19th Avenue, Mail Stop B182, Aurora, CO 80045, USA. Tel.: +1 303 724 4326; fax: +1 303 724 4329.

E-mail address: don.gilden@ucdenver.edu (D. Gilden).

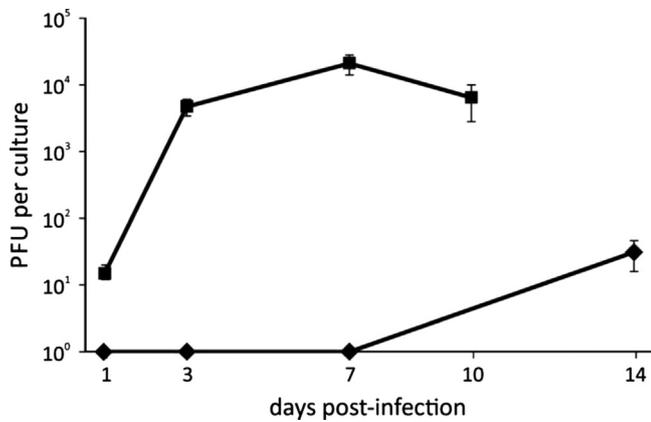


Fig. 1. VZV production is both delayed and reduced in human neurons. Cells of duplicate cultures of human neurons (diamonds) and fibroblasts (squares) infected with VZV at a low MOI were harvested at four time points with trypsin-EDTA, washed with PBS, and analyzed in plaque assays by co-cultivation with uninfected fibroblasts. The inoculum was removed 3 h after co-cultivation and cell cultures were fixed, stained and plaques counted 7 days later.

VZV-infected human neurons do not accumulate viral DNA

To determine whether reduced and delayed VZV production was due to limited numbers of VZV genomes available for packaging, virus DNA abundance was monitored in infected human neurons. The abundance of VZV DNA in neurons infected with a low MOI remained constant over the 2-week study period (Fig. 2A). Importantly, viral genomes detected at 1 dpi were likely from virus DNA in the inoculum because a vial of Zostavax contains 1.4×10^{10} genomes ($\pm 0.1 \times 10^{10}$; $n=2$) and 200 PFU (± 100 PFU; $n=4$) (Fig. 2B), resulting in 7×10^7 VZV genomes per PFU. At 1 dpi, the virus-to-cell DNA ratio was equivalent in infected fibroblasts and neurons. By 10 dpi, the virus-to-cell DNA ratio had increased 6.7-fold in fibroblasts.

Discussion

Overall, VZV DNA did not accumulate in productively infected human neurons that lacked a CPE, but did accumulate in fibroblasts with extensive CPE. The lack of VZV-induced CPE in human neurons is likely due to defective viral DNA replication or rapid degradation of VZV DNA. An absolute block in viral DNA replication in neurons is unlikely since late viral transcripts and their proteins, for which viral DNA replication is required, were detected (Azarkh et al., 2011; Baird et al., 2014; Grose et al., 2013; Yu et al., 2013). VZV DNA replication was ultimately sufficient to produce 31 PFU/culture, a level that would not have been detected above the $\sim 10^9$ input genomes. Importantly, human fibroblasts infected with the same inoculum replicated VZV genomes above input virus DNA. A similar inhibition of HSV-1 growth and lack of CPE in neurons obtained from explanted fetal human brain, spinal cord or ganglia has been reported, although in these *ex vivo* cultures, HSV-1 replicates and a CPE develops in non-neuronal cells (Kennedy et al., 1983).

Materials and methods

Cells and virus

Human iPSCs (iCell neurons; Cellular Dynamics International, Madison, WI) and human fetal lung fibroblasts were cultured and infected with cell-free VZV (Zostavax; Merck, Whitehouse Station,

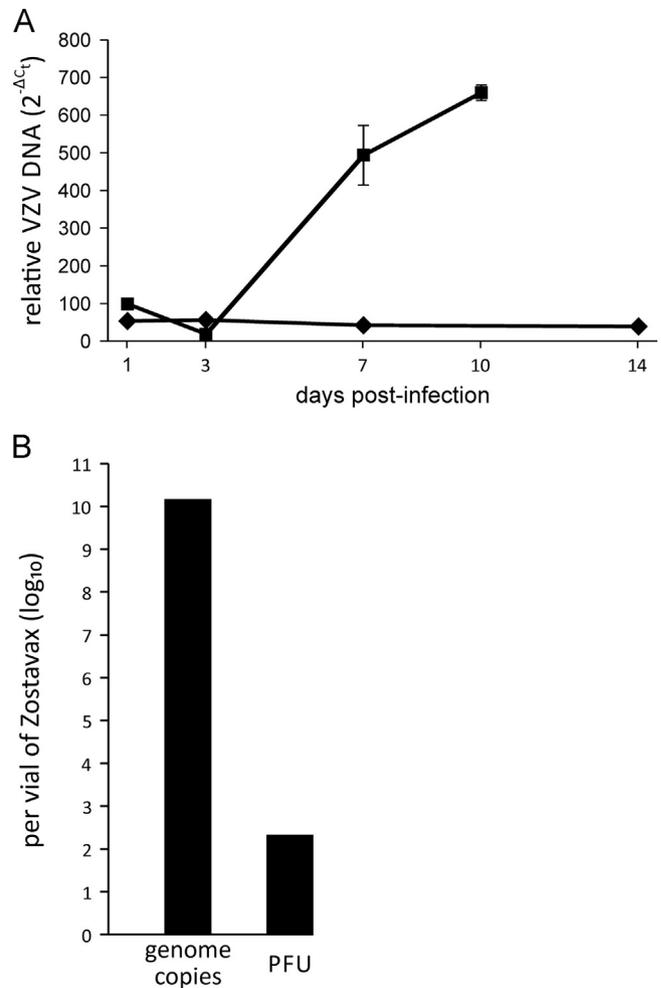


Fig. 2. VZV DNA does not accumulate in VZV-infected neurons. (A) Duplicate cultures of human neurons (diamonds) and human fetal lung fibroblasts (squares) were infected with cell-free VZV at low MOI as in Fig. 1 and total DNA was extracted at four time points post-infection. Viral and cellular DNA was detected by qPCR using primers for VZV ORF 29 and GAPDH, respectively. Relative quantities of VZV DNA, normalized to cell DNA, were determined as detailed in the text. Error bars represent standard deviation. (B) Viral genome copy number and PFU were determined. DNA extractions of two separate vials of Zostavax were analyzed by qPCR for VZV genomes; plaque assay was performed from 4 separate vials of Zostavax.

NJ) at an MOI $< 1 \times 10^{-3}$ as described (Grose et al., 2013; Yu et al., 2013).

Plaque assay

To quantify infectious virus production, both VZV-infected human neurons and fibroblasts were harvested with trypsin-EDTA at multiple dpi, washed with PBS, diluted and co-cultivated with uninfected fibroblasts; after 3 h at 37 °C, cells were washed, medium was replenished and plaques were visualized by crystal violet staining and counted 7 days later.

qPCR

VZV-infected human neurons and fibroblasts were harvested on multiple dpi and at each time point, DNA was extracted (GenElute; Sigma, St. Louis, MO) and quantified by TaqMan-based quantitative PCR (qPCR) using 10 ng of total DNA and primer/probe sets specific for both cell (GAPDH) and virus (VZV ORF 29) DNA as described (Yu et al., 2013). The amount of VZV

DNA compared to cell DNA was determined using the formula $2^{-\Delta C_t}$, where $\Delta C_t = C_t(\text{VZV ORF29}) - C_t(\text{GAPdH})$.

DNA was isolated from Zostavax (GenElute; Sigma) and quantified by TaqMan-based qPCR. Standards to determine copy number were prepared from purified VZV bacterial artificial chromosome (BAC) DNA (Tischer et al., 2007).

Acknowledgments

This work was supported by Public Health Service Grants AG006127 (D.G.), AG032958 (D.G. and R.J.C.) and NS082228 (R.J.C.) from the National Institutes of Health. Dr. Baird was supported by Training Grant NS007321 to Dr. Gildea from the National Institutes of Health. Dr. Jonjić was supported by the Instrument for Pre-Accession Assistance (IPA), component III C, Science and Innovation Investment Fund (SIIF), phase; grant number IPA2007/HR/16IPO/001-040517. We thank Marina Hoffman for editorial review and Lori DePriest for manuscript preparation.

References

- Azarkh, Y., Dolken, L., Nagel, M., Gildea, D., Cohrs, R.J., 2011. Synthesis and decay of varicella zoster virus transcripts. *J. Neurovirol.* 17, 281–287.
- Baird, N.L., Bowlin, J.L., Cohrs, R.J., Gildea, D., Jones, K.L., 2014. Comparison of VZV RNA sequences in human neurons and fibroblasts. *J. Virol.* 88, 5877–5880.
- Gildea, D., Mahalingam, R., Nagel, M.A., Pugazhenti, S., Cohrs, R.J., 2011. Review: the neurobiology of varicella zoster virus infection. *Neuropathol. Appl. Neurobiol.* 37, 441–463.
- Grose, C., Yu, X., Cohrs, R.J., Carpenter, J.E., Bowlin, J.L., Gildea, D., 2013. Aberrant virion assembly and limited glycoprotein c production in Varicella-Zoster virus-infected neurons. *J. Virol.* 87, 9643–9648.
- Kennedy, P.G., Clements, G.B., Brown, S.M., 1983. Differential susceptibility of human neural cell types in culture to infection with herpes simplex virus. *Brain* 106 (Pt. 1), 101–119.
- Markus, A., Grigoryan, S., Sloutskin, A., Yee, M.B., Zhu, H., Yang, I.H., Thakor, N.V., Sarid, R., Kinchington, P.R., Goldstein, R.S., 2011. Varicella-zoster virus (VZV) infection of neurons derived from human embryonic stem cells: direct demonstration of axonal infection, transport of VZV, and productive neuronal infection. *J. Virol.* 85, 6220–6233.
- Nagel, M.A., Gildea, D., 2013. Complications of varicella zoster virus reactivation. *Curr. Treat. Options Neurol.* 15, 439–453.
- Pugazhenti, S., Nair, S., Velmurugan, K., Liang, Q., Mahalingam, R., Cohrs, R.J., Nagel, M.A., Gildea, D., 2011. Varicella-zoster virus infection of differentiated human neural stem cells. *J. Virol.* 85, 6678–6686.
- Sloutskin, A., Kinchington, P.R., Goldstein, R.S., 2013. Productive vs non-productive infection by cell-free varicella zoster virus of human neurons derived from embryonic stem cells is dependent upon infectious viral dose. *Virology* 443, 285–293.
- Tischer, B.K., Kaufer, B.B., Sommer, M., Wussow, F., Arvin, A.M., Osterrieder, N., 2007. A self-excisable infectious bacterial artificial chromosome clone of varicella-zoster virus allows analysis of the essential tegument protein encoded by ORF9. *J. Virol.* 81, 13200–13208.
- Yu, X., Seitz, S., Pouton, T., Bowlin, J.L., Cohrs, R.J., Jonjić, S., Haas, J., Wellish, M., Gildea, D., 2013. Varicella zoster virus infection of highly pure terminally differentiated human neurons. *J. Neurovirol.* 19, 75–81.