Pathogenesis, biophysical stability and phenotypic variance of SAT2 foot-and-mouth disease virus

Supplementary Material

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Data Description

A real-time cell analysis system to monitor cell growth has been applied to quantify virus-induced cytopathic effects (CPE) in cell culture [1,2]. The objective of this study was to investigate the differences in cell lysis of two genetically related SAT2 foot-and-mouth disease (FMD) viruses (FMDV), ZIM/5/83 and ZIM/7/83. An electrical impedance assay was used to characterise the effect of the FMDV on cultured cells. The data showed that a decrease in impedance of ZIM/5/83-infected BHK-21 cells took place late in the course of infection. However, cell lysis and decrease in impedance of ZIM/7/83-infected BHK-21 cells were significantly quicker.

Real-time analysis of FMDV-mediated cytopathogenicity

The cytopathic effect (CPE) generated by ZIM/5/83 and ZIM/7/83 viruses on BHK-21 cells differed when viewed by light microscopy. SAT2/ZIM/5/83 never achieved complete CPE after 40 h p.i (Fig. 1A). Infection with ZIM/7/83 caused complete lysis of more than 95% of the cells within 8-16 h p.i. (Fig. 1B). To investigate the infection properties of the two viruses in more detail, a real-time cell analysis system (xCELLigence system; Roche Applied Science) to monitor cell growth has been applied to quantify virus-induced cytopathic effects (CPE) in cell culture.

The real-time cell analysis system measures impedance of individual monolayers over time in a 96-well microelectronic plate format where any decrease in impedance is equated to a proportionate increase in monolayer permeability (Atienza *et al*., 2005, 2006; Kirstein *et al*., 2006; Li *et al*., 2006). The measurement is reported as a Cell Index (CI) as a function of time (hours post-infection or CIT). The point of 50% decline in the CI value (CIT_{50}) was regressed as a function of

the infectious dose in log_{10} $TCID_{50}$. To perform the assay, BHK-21 cells in 100 µl of growth medium were seeded into the wells ($3{,}2 \times 10^4$ cells/well) of a 96-well E-Plate (Roche Applied Science) and monitored at hourly intervals following incubation for 24 h at 37°C with 5% CO2 influx to obtain background readings. For the assessment of virus-mediated cytopathogenicity the BHK-21 cell monolayers in the top row of the E-plate were infected with FMDV at an MOI of 1 PFU/cell, followed by a 1:1 serial dilution down each column. As a control, wells were mock-infected by adding 100 µl of growth medium only. The E-Plate 96 was then immediately placed back into the real-time cell analysis workstation in the incubator, and the CI values were measured every 30 minutes for up to 12 h, then hourly until 36 hpi. The average, normalised CI half-life (CIT_{50}) of four infected wells was plotted against the virus titre (Fig.1C).

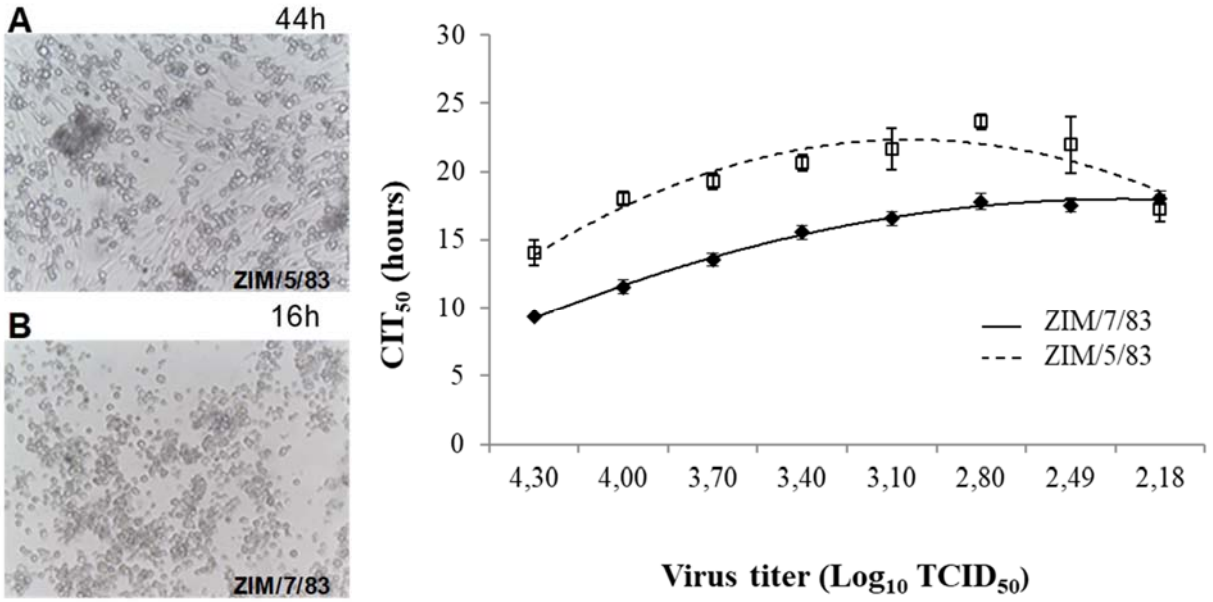

Figure 1: (A & B) Light microscopic observation of the cytopathic effect (CPE) produced by the two related SAT2 viruses on BHK-21 cells 16 h p.i. with ZIM/7/83 (B) and 44 h p.i. with ZIM/5/83 (A). (C) Real-time monitoring of SAT2 viruses ZIM/5/83 and ZIM/7/83-induced CPE on BHK-21 cells. Normalized cell index (CI) plotted as a function of time (hpi) (CIT_{50}) for E-wells inoculated with $TCID_{50}$ of 100-20,000 particles per well in a two-fold dilution of viruses (ZIM/5/83 or ZIM/7/83) and cell-only controls. The curve is an average of four independent replicate wells. The point of 50% decline in the CI value was regressed as a function of the infectious dose in log_{10} $TCID_{50}$.

Real-time monitoring of the growth of ZIM/5/83 and ZIM/7/83 in BHK-21 cultured cells revealed that at 37ºC the virus-induced cytolysis, the cause of CPE, was detected as early as 8.5 and 6 hpi, respectively, at 2 x 10^4 PFU. A rapid decline of the cell index (CI) followed for ZIM/5/83 and ZIM/7/83 infected cells, which decreased by 50% within 14 and 9 hpi, respectively, at a titre of 4.3 log_{10}. Each order of magnitude (serial two-fold dilution) decrease in the infectious dose of the ZIM/7/83 inoculum resulted in a delay of approximately 1.7 h in the CIT_{50} (time required for the CI to decrease 50%) after virus infection (Fig. 1C). Similarly, the CIT_{50} of ZIM/5/83 reduced by approximately 1.3 h with every two-fold dilution of its infectious dose.

Acknowledgments

Research findings documented here were in part supported by funding from MSD Animal Health (previously Intervet SPAH). The authors would like to express their sincere gratitude to Tina Kresfelder, Team Manager at Roche, South Africa, for training provided and analysis of the data.

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