Mechanistic viral dynamic modeling of in vitro cytomegalovirus infection based on viral DNA and viral titer

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Background

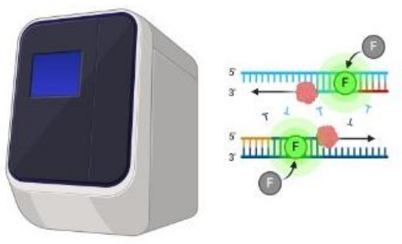
Cytomegalovirus and viral dynamic modeling

- Cytomegalovirus (CMV) is a herpesvirus which causes high mortality in immunocompromised patients. The first-line drugs (i.e., ganciclovir, valganciclovir) have narrow therapeutic window.
- Viral dynamic model can be linked with population PK model and inform dose optimization, which needs viral load data as input.

Viral load quantification

qPCR

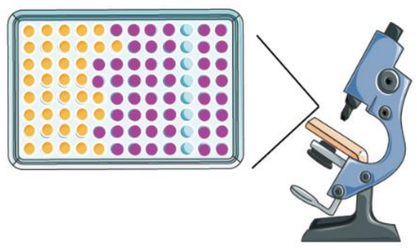
Quantifying total viral genome copies



- Faster
- Cheaper
- More accessible
- Cannot distinguish infectious & non-infectious virus

TCID50

Quantifying infectious viral titer



- More functional data about infectivity
- Time-consuming
- Labor-intensive

Research gap

- Published *in vivo* CMV viral dynamic models relied solely on viral DNA
- No in vitro CMV model has been published

Aims

- Develop a viral dynamic model of *in vitro* CMV infection
- Characterize the relationship between viral DNA and viral titer

Methods

Experiments

Viral load quantification

1.5×10^6 cells → MOI = 0.1, 2 hrs → Wash-out and replenish → Sampling, 25 days → Quantification (qPCR + TCID50)

Cell viability

1.5×10^4 cells → 1. w/o virus, 2. MOI = 0.1 → Sampling, 17 days → Quantification (WST-1 assay)

Model development

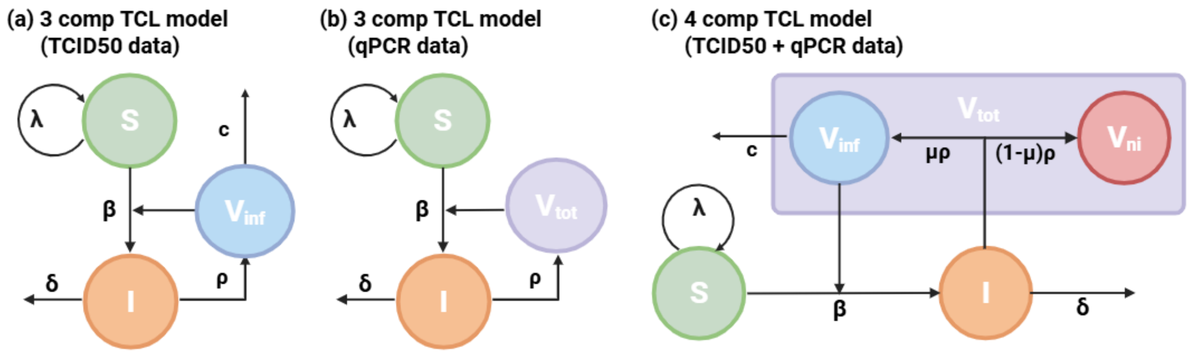


Figure 1. Schematic diagrams of different structural models. S, susceptible cells; I, infected cells; V_{inf}, infectious virus; V_{tot}, total viral genome; V_{ni}, non-infectious virus

- A 3-compartment target-cell limited (TCL) model was used to fit viral load data from PCR or TCID50 readout, respectively.
- Viral dynamic parameter estimates derived from two quantification methods were compared.
- A joint model was developed and fitted using data from both methods to characterize the relationship between total viral DNA and infectious viral titer.

Results

Parameter comparison across models

Table 1. Final estimates (RSE%) of different models

Parameter	3 comp (TCID50)	3 comp (qPCR)	4 comp (TCID50 + qPCR)
β (PFU^{-1} or $copy^{-1}$/day)	$1.42*10^{-8}$ (80%)	$1.04*10^{-10}$ (7%)	$8.4*10^{-8}$ (93%)
λ (day^{-1})	0.225 (45%)	0.266 (12%)	0.246 FIX
δ (day^{-1})	0.803 (5%)	0.741 (2%)	0.772 FIX
ρ (PFU or copy/cell/day)	117 (103%)	$0.97*10^{4}$ (12%)	$2.63*10^{4}$ (28%)
μ	-	-	$0.394*10^{-3}$ (99%)
c (day^{-1})	0.402 (21%)	-	0.402 FIX
EC_{50} (μg/mL)	0.028 (73%)	0.626 (34%)	0.519 (26%)
Hill coefficient	0.5 (12%)	1.11 (7%)	1.36 (6%)

Abbreviations: β, viral infection rate; λ, cell growth rate; δ, death rate of infected cells; ρ, virus production rate; μ, fraction of infectious virus; c, viral clearance; EC_{50}, drug concentration yielding half maximal effect; RSE, relative standard error.

Three-compartment model: qPCR vs. TCID50

- Cell-related parameters – including susceptible cell growth rate (λ) and death rate of infected cells (δ) – remained consistent regardless of the quantification method.
- qPCR-based model yielded lower infection rate constants (β) and higher viral production rates (ρ) compared to TCID50-based model. This discrepancy may result from non-infectious viral particles detected by qPCR, which inflate viral DNA counts without contributing to infectivity.
- The estimated EC_{50} derived from TCID50 data was lower than the estimation from qPCR data.

Four-compartment model: Joint fitting with qPCR and TCID50

- The jointly estimated infection rate (β) aligned more closely with the value obtained from TCID50 data alone.
- In contrast, the viral production rate (ρ) and EC_{50} estimates were more consistent with those derived from qPCR-based fits.

Model evaluation

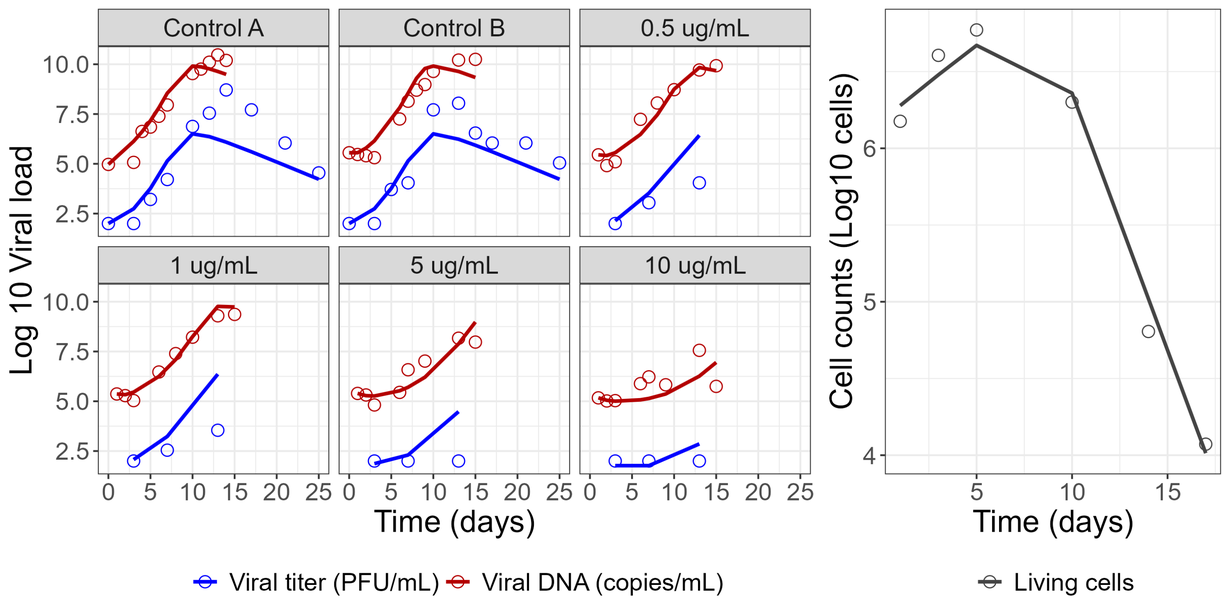


Figure 2. DV vs. IPRED plots of the 4-compartment joint model

- The individual plots demonstrated good agreement between prediction and observation in all data types.
- In the control group, qPCR detected peak viral DNA (~10 log_{10} copies/mL) at day ~13, followed by a plateau. $TCID_{50}$ showed a lower peak (~8 log_{10} PFU/mL) with subsequent decline.
- In the treatment group, progressive reduction in viral load can be seen in both methods with increasing drug concentration.
- The readout from qPCR was generally higher than TCID50 in all concentration groups.
- Living cell counts (log_{10} scale) started to drop significantly from day 10, aligning with the viral load peak.

Conclusions

- This is the first known viral kinetic model of in vitro CMV infection that characterizes the dynamics of CMV as well as the relationship between viral DNA and viral titer.
- Viral kinetic parameters as well as EC50 are sensitive to the data quantification method. It is important to adjust the model structure to fit the type of data used in model development.

References

1. Duke, Elizabeth R et al. (2021)
2. Iyaniwura, Sarafa A et al. (2024)
3. Yamaguchi et al. (2024)
4. Iwami et al. (2012)

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