Impact of trypsin and elastase in dynamics infection.

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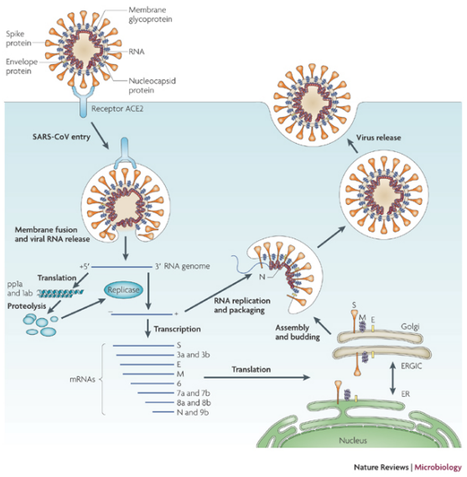
Motivation

- In vitro experiments are necessary to understand the processes driving viral infections and to develop antivirals and vaccines.
- However, experiments do not completely replicate the in vivo environment. To facilitate in vitro viral infections proteases are added.
- We use data from in vitro Severe Acute Respiratory Syndrome Coronavirus (SARS) infections in the presence of different proteases to parameterize a within-host mathematical model of SARS infection.

Severe Acute Respiratory Syndrome Coronavirus (SARS)

Severe acute respiratory syndrome (SARS) is caused by a SARS-associated coronavirus (SARS-CoV), a new virus member in a family of Coronaviridae. Unlike other human coronaviruses, SARS-CoV causes a fatal respiratory disease in humans.



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Proteases

Proteases are added to induce fusion of SARS-Co virus in Vero E6 cells.

Trypsin

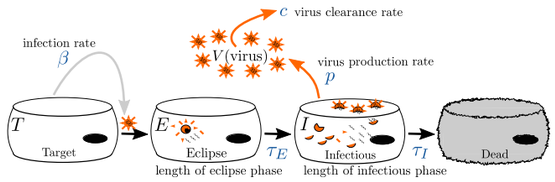
- Coronavirus is an enveloped virus with spikes in its surface, these spikes are composed of a trimer of the spike (S) protein.
- S protein binds to its receptor ACE2 and is cleaved. Trypsin induces cleavage of the S protein on virions.

Elastase

- It is one of the proteases produced by inflammatory cells in the lungs.
- Elastase enhances SARS-CoV infections in VeroE6 cells in terms of S protein cleavage.

Mathematical Model

We use a mathematical model to simulate the SARS virus life cycle.



Model Equations

- We use a gamma-distributed version of the basic infection model, where cells in the eclipse and infectious phases pass through multiple compartments.

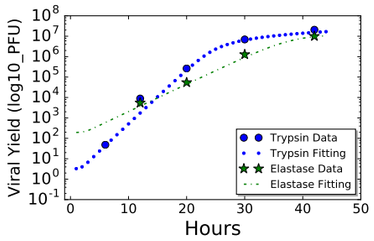
$$\begin{aligned}
\frac{dT}{dt} &= -\frac{\beta T V}{N} \\
\frac{dE_1}{dt} &= -\frac{\beta T V}{N} - \frac{n_E E_1}{\tau_E} \\
\frac{dE_j}{dt} &= \frac{n_E E_{j-1}}{\tau_E} - \frac{n_E E_j}{\tau_E} \quad \text{with} \quad j = 2, n_E \\
\frac{dI_1}{dt} &= \frac{n_E E_{nE}}{\tau_E} - \frac{n_I I_1}{\tau_I} \\
\frac{dI_k}{dt} &= \frac{n_i E_{k-1}}{\tau_I} - \frac{n_I I_k}{\tau_I} \quad \text{with} \quad k = 2, n_I \\
\frac{dV}{dt} &= p(I_1 + \sum I_k) - cV
\end{aligned}$$

where the parameters are:

- β infection rate
- p viral production rate
- c viral clearance
- τ_I infectious cell life span
- τ_E duration of the eclipse phase
- $t_{\text{inf}} = \sqrt{2/p\beta}$ time between release of virus and infection of next cell

Experimental data

- Data was taken from Matsuyama et al. Vero E6 cells were infected with SARS-CoV Frankfurt 1 strain.
- Viral growth kinetics after infection was examined in cultures in the presence or absence of trypsin (62.5 $\mu g/ml$) or elastase (125 $\mu g/ml$) .



We fit the model to the trypsin and elastase cases separately using least-squares minimization.

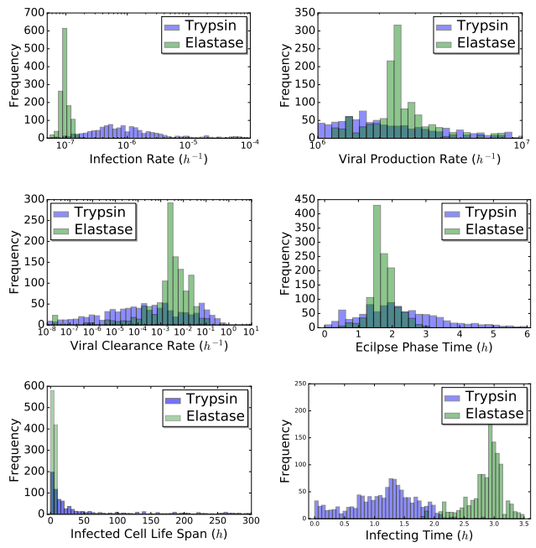
Bootstrapping

- We used bootstrapping to generate distributions for the different parameters.
- Bootstrapping is a general approach to statistical inference based on building a sampling distribution for a model by resampling from the data at hand.
- A separation in the distributions for a particular parameter in the trypsin and elastase cases indicates that the parameter has changed significantly.

T-test

- We use a t-test to determine whether there are statistically significant differences in the distributions of parameters.
- The t-test assesses whether the means of two groups are statistically different from each other. This is through analyzing the p-value, if the p-value is smaller than the threshold ($p < 0.05$) we reject the hypothesis of equal average values.

Parameter Distributions



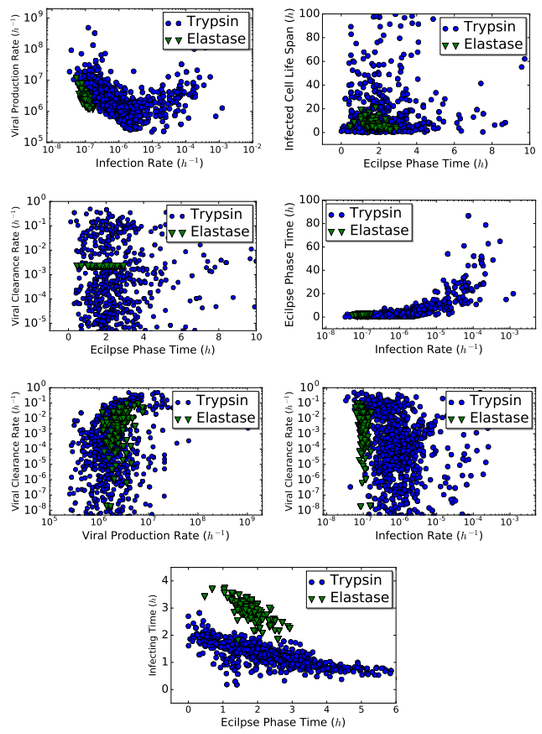
We measured how the average values differ for the different parameters performing the T-test. The p-values for the test are:

Parameter	p-value
β	< 0.05
c	< 0.05
p	0.302
τ_E	0.069
τ_I	< 0.05
t_{inf}	< 0.05

- There are no significant differences between p, and τ_E.
- β, τ_I, C and t_{inf} are different for the two proteases.

Parameter Correlation

To investigate changes in the parameters β, t_{inf} and τ_E we studied the parameter correlations. The simplest way to analyze possible relationships between variables is by using a scatter plot.



- We observe correlations for elastase between p and β, t_{inf} and τ_E.
- We observe correlations for trypsin between τ_E and β, t_{inf} and τ_E.
- All the correlations are negative, except the one for trypsin for the parametes τ_E and β.

Conclusions

- From the histograms and the t-test, we do not observe a significant differences in production rate p, and eclipse phase time τ_E, in the presence of trypsin and elastase.
- The infection rate β is lower for trypsin compared with elastase. Viral clearance c is higher for elastase compared to trypsin. Infected cell life span τ_I is lower for elastase and infecting time t_{inf} is higher for elastase.

Future Work

- Trypsin is also used to facilitate other viral in vitro infections, for example influenza. We will use data from different in vitro infections in the presence and absence of trypsin to parameterize a within-host mathematical model of viral infection, and compare the results between these different infections.
- We will construct a more detailed model of viral cell entry to hone in on the effect of trypsin.