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Current Opinion in Systems Biology

Progress and trends in mathematical modelling of influenza A virus infections

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Abstract

Mathematical modelling of influenza A virus infection has seen increased use over the last several years. Models applied to both *in vitro* and *in vivo* data have provided important new understanding of the kinetics of the virus, the role of different components of the immune response, the importance of non-infectious influenza A virus particles, the issue of drug treatment and resistance, and the interaction mechanisms during bacterial co-infections. We review these contributions by mathematical models, with a focus on studies performed in the last several years. For continued progress, we emphasize robust data and parameter estimation approaches.

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Current Opinion in Systems Biology 2018, **12**:30–36

This review comes from a themed issue on **Infectious diseases and host pathogen interact (2018)**

Edited by **Denise Kirschner** and **Ramit Mehr**

For a complete overview see the Issue and the Editorial

Available online 25 August 2018

https://doi.org/10.1016/j.coisb.2018.08.009

Keywords

Influenza virus, Mathematical modelling, Antiviral resistance, Defective interfering particles.

Introduction

Mathematical models (MM) have seen an increased use in all areas of biology. They have been successfully applied to the study of influenza A virus (IAV), at the population level and at the level of an infected host (*in vivo*) or cell culture (*in vitro*). This review focuses on MM efforts at the *in vivo* and *in vitro* scales, where we specifically focus on mechanistic, dynamical MM simulations, applied to describing an *in vivo* or *in vitro* IAV infection system. Those MMs are most commonly described by sets of ordinary differential equations. We review and summarize how these MMs have been applied to different scenarios and questions concerning IAV, and summarize MM findings of the last few years. For reviews on earlier influenza MM efforts, see e.g., Ref. [1,2].

Improved methodology for *in vitro* infection analyses

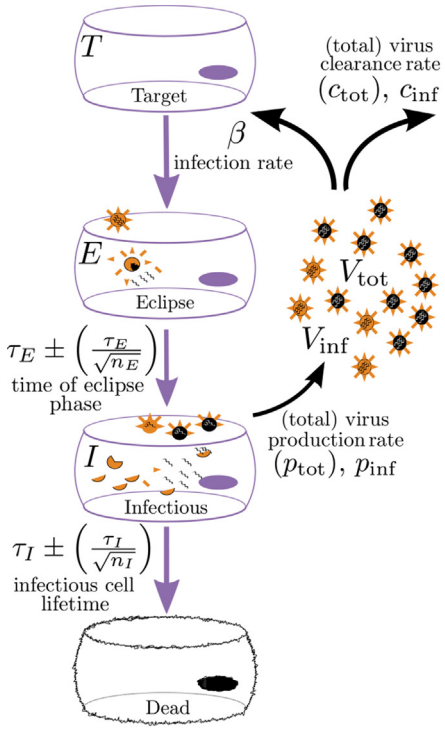
In vitro MMs offer an opportunity to study properties of the virus and cell-virus interactions in a relatively controlled system that lacks confounding effects from host factors. This has enabled the development of experiments best suited to inform MMs; namely the single-cycle assay, the multiple-cycle assay, and the mock-yield or viral decay assay [3,4]. The single-cycle experiment consists in infections initiated with enough virus to infect most cells almost simultaneously (typically $\sim$3 PFU/cell), providing information about the average kinetic of a single cell as it progresses from infection to virus progeny production to death. The multiple-cycle assay is inoculated with a heavily diluted inoculum (typically $\sim$1 infectious virus per 10^5 cells), providing information about the kinetics of virus spread and infectivity through multiple cycles of cells infecting other cells. The mock-yield or viral decay assay quantifies virus concentration over time, under the same conditions as the infection assays, but in the absence of cells. Supported by this rich dataset, MMs for IAV infections *in vitro* have largely crystallized into the form presented in Figure 1.

Using such MMs, it was established that the duration of the eclipse and infectious phases for cells infected with IAV *in vitro* is normal-like [5]. The rate of loss of IAV infectivity was determined to be $c_{\text{inf}} \sim 0.1\ \text{h}^{-1}$, whereas that due to IAV RNA degradation is $c_{\text{tot}} \sim 0.01\ \text{h}^{-1}$ or less [6–8]. This now relatively standardized combination of MM and 3 experimental assays has enabled identification of likely mechanisms for the loss of IAV fitness due to certain antiviral resistance mutations [6,8], and identified key differences in the virus replication characteristics of human-vs avian-adapted IAV strains [7].

Progress towards better MMs of *in vivo* immunity to influenza virus

In 2013, Dobrovolny et al. [9] systematically compared *in vivo* IAV infection MMs against a substantial

Figure 1



$$\frac{dT}{dt} = -\beta T V_{inf}$$

$$\frac{dE_1}{dt} = \beta T V_{inf} - \frac{n_E}{\tau_E} E_1$$

$$\frac{dE_{i=2,\ldots,n_E}}{dt} = \frac{n_E}{\tau_E} E_{i-1} - \frac{n_E}{\tau_E} E_i$$

$$\frac{dI_1}{dt} = \frac{n_E}{\tau_E} E_{n_E} - \frac{n_I}{\tau_I} I_1$$

$$\frac{dI_{j=2,\ldots,n_I}}{dt} = \frac{n_I}{\tau_I} I_{j-1} - \frac{n_I}{\tau_I} I_j$$

$$\frac{dV_{inf}}{dt} = p_{inf} \sum_{j=1}^{n_I} I_j - c_{inf} V_{inf}$$

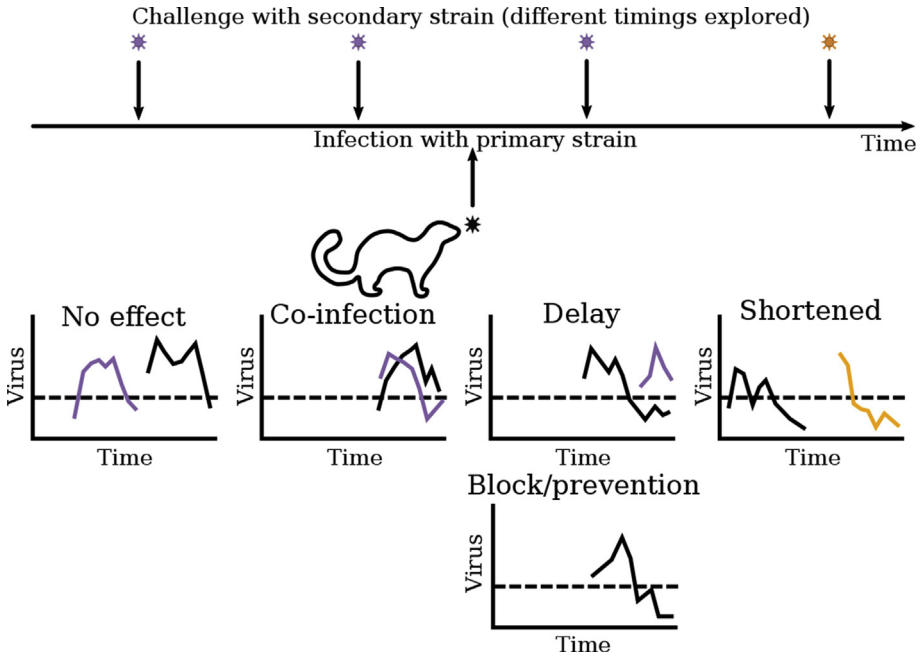
$$\frac{dV_{tot}}{dt} = p_{tot} \sum_{j=1}^{n_I} I_j - c_{tot} V_{tot}$$

MM describing IAV infections *in vitro*. Upon infection by virus, V_{inf}, an uninfected, susceptible target cell T enters the eclipse phase $E_{i=1,2,\ldots,n_E}$. During the eclipse phase, intracellular virus replication gets underway, but the cell is not yet releasing virus progeny. After traversing all E_i stages, the cell enters the productively infectious phase $I_{j=1,2,\ldots,n_I}$. While in that phase, it is assumed to produce virus at a constant rate, where p_{inf} and p_{tot} are the rates of production of infectious and total virus per cell, respectively. Only a subset of produced virions are infectious such that $p_{tot}/p_{inf} \gg 1$. Infectious virus (V_{inf}) are typically measured through infectious dose assays ($TCID_{50}$) or plaque or focus forming assays (PFU or FFU), while total virus is typically quantified via quantitative RT-PCR. The rate at which these virus populations decay or degrade (c_{inf}or c_{tot}) corresponds to the rate at which they cease to be countable by these methods. The time spent by cells in the eclipse and productively infected phases is commonly modelled with an Erlang distribution, with a mean ± standard deviation duration of $\tau_E \pm (\tau_E/\sqrt{n_E})$ or $\tau_I \pm (\tau_I/\sqrt{n_I})$. Through adjusting the number of compartments in each phase (n_E and n_I), the Erlang distribution can represent phase durations that follow either exponential, log-normal, normal or even Dirac delta-like distributions.

collection of data available from animal and human studies. While all MMs tested could reproduce some of the dynamics, none could fully capture all patterns observed. More recently, Boianelli et al. [10] reviewed MMs up to around 2015, highlighting the fact that most data are not collected with MMs in mind, often only reporting viral load kinetics, and more rarely, some sparse measurements of a few immune response components. Even when data is collected with MMs in mind, obtaining rich enough datasets to allow discrimination between alternative hypotheses regarding immune response interactions is difficult.

One solution to this issue appears to be the collection of infection data under serially varying conditions. For example, Laurie et al. [11] performed a series of infection experiments in ferrets with two distinct IAV strains, a primary and a secondary, wherein the timing of infection with the secondary strain was varied serially to occur a times prior to or post infection with the primary strain, as depicted in Figure 2. Analyzing this viral kinetic data with MMs, McCaw and colleagues were able to rule out some potential innate response mechanisms [12], and to explain how different mechanisms of CD8 T-cell memory led to shortened secondary infections observed in the data [13,14]. They also showed that data from ferrets simultaneously co-infected with 2 strains of IAV allow MMs to discriminate between mechanisms responsible for fitness differences between strains [15,16], and showcased a robust approach to investigate the role of CD8 T-cells [17]. Another example work exploiting serially perturbed conditions is that of Li et al. [18] which performed infections at serially increasing inoculum doses. Dose-dependent virus kinetics, especially when measured alongside components of the immune response, is invaluable in discriminating between alternative mechanisms proposed for innate and adaptive immune responses, as was recently demonstrated for IAV infections [19]. Given the clear advantage such perturbation-based datasets offer, we expect that experiments combining multiple such serial perturbations, e.g. serial or co-infection

Figure 2



Serially varying infection times with two strains. In Ref. [11], ferrets were infected with a primary strain (influenza A virus; black) and secondary strain (purple or orange), where the timing of infection with the secondary strain was varied. The secondary strain was either of the same type as the primary strain (influenza A virus; orange) or of a different type (influenza B virus; purple). A variety of virus shedding patterns were observed, including seemingly no effect or a co-infection with no significance interference, or interference in the form of a delayed, a shortened or even a blocked secondary infection.

experiments at different doses, with measurement of viral load and several immune response components, would be invaluable in enabling MMs to further elucidate the underlying virus and immune response dynamics.

Other topics of influenza virus infection modelling

Defective interfering and semi-infectious particles

Total IAV particles are consistently found to significantly outnumber infectious particles [6–8,16,20]. MMs typically assume that particles whose activity does not register in infectivity assays play no role in the course of infection. However, it has become increasingly evident that "non-infectious" particles likely possess biological activities, potentially playing a role in infection, transmission and pathogenicity [21,22].

Defective interfering particles (DIPs) are one subset of non-infectious, biologically active particles, as illustrated in Figure 3. MMs began to quantitatively account for IAV DIPs after their accumulation hampered cell-based vaccine production [23], and their presence became apparent in high MOI infection experiments with IAV stocks produced by reverse genetics that could not be passaged at low MOI [7,8,24].

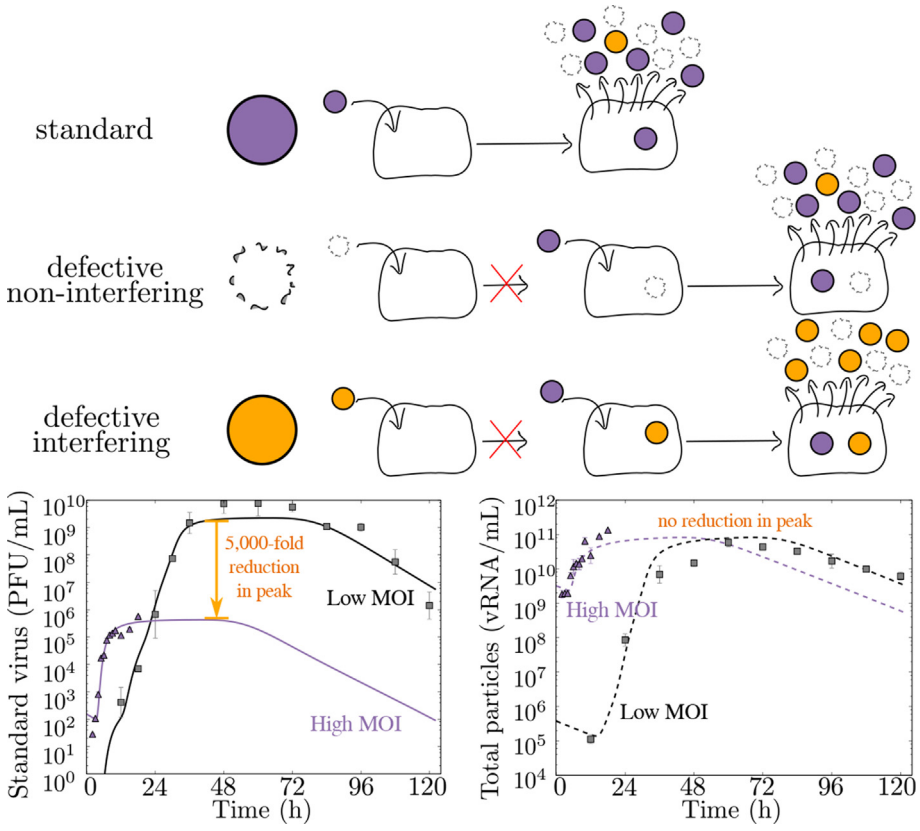
Using a MM, Frensing et al. [23] found that the accumulation of DIPs could only be controlled if the stock was completely DIP-free or if de novo generation of DIPs was exactly zero. Liao et al. used a MM to establish that an *in vitro* assay provides accurate counts of DIPs only if certain criteria regarding both the biology of co-infection and the experimental procedure were met [24]. Laske et al. used a detailed MM of intracellular IAV replication to examine one possible mechanism of interference: shorter DI RNAs replicating faster than full-length RNAs [25]. These and future DIP MMs will be critical in discriminating between mechanisms of interference [26], ensuring quality control of virus preparations [27,28], evaluating their potential as antiviral agents [29,30], and the effect of their presence on vaccine efficacy [27,31].

Similar to DIPs, semi-infectious particles (SIPs) also require co-infection to propagate, but are not thought to interfere with IAV replication [22]. To date, only one MM incorporates both SIPs and DIPs, and it suggests SIPs promote reassortment while DIPs suppress reassortment [32].

Co-infections

It has long been noted that IAV infection can lead to an increased probability of severe secondary bacterial infection, most notably pneumococcal infections [33]. A number of MM studies have investigated the potential mechanisms for this interaction between IAV and pneumococcal infections [34,35], the implications for

Figure 3



Defective interfering influenza particles suppress infectious virus titer. (Top) When standard infectious virus (purple) infects a cell, the progeny comprises standard virus as well as defective interfering (orange) and non-interfering (white) particles. When a defective non-interfering particle infects a cell, no progeny is produced, and further infection by replication competent virus is not impacted. In contrast, when a defective interfering particle infects a cell on its own, no progeny is produced, unless the cell becomes co-infected with standard virus. In such a case, the co-infected cell will produce mostly DIPs instead of standard virus. The co-infection kinetics of standard virus + DIPs in a population of cells is affected by factors such as the timing of infection with DIPs relative to standard virus, and the ratio of DIPs to standard virus progeny produced by co-infected cells. (Bottom) Compared to low MOI infections (~10^{-5} PFU/cell), the presence of DIPs in the virus stock causes the collapse of standard infectious virus titer in high MOI infections (~3 PFU/cell) where many co-infection events occur, while the total particle concentration remains the same [Image is adapted from Figures 1 and 2 in Ref. [24] (©2016, the Authors of [24]), used under the terms of the Creative Commons Attribution License https://creativecommons.org/licenses/by/4.0/, which permits unrestricted use, provided the original author and source are credited.].

co-infection risk [36,37], and its effects on drug treatment [38,39]. MMs have also studied simultaneous infection with IAV and a variety of other viruses [40]. The general challenge for these kinds of studies is that 2-pathogen MMs are generally more complex than MMs for a single pathogen, and thus require more data to yield robust MM analyses.

Anti-influenza interventions

MMs have been applied to IAV infections to inform therapeutic interventions with existing antiviral drugs [39,41–43], to study antiviral resistance emergence [44–48], to propose new interventions [49], and to explore the impact of vaccines [50]. To use MMs for these purposes, it is important to understand the main underlying mechanisms of interaction between pathogen, immune response and intervention. While some progress has been made in that regard, the increased usefulness of MMs as robust predictive tools for specific interventions will rely on further refinements of our overall understanding regarding the details of the infection [42].

Cross-scale models

The realization that the dynamics of IAV spans multiple temporal and spatial scales is well-appreciated [51]. MMs bridging the within- and between-host scales have explored the role of within-host and environmental factors for avian influenza [52,53], investigated the role of tissue tropism on virus fitness [54], and assessed the impact of individual heterogeneity on population-level outcomes [55,56]. Working at a lower scale, Heldt et al. [57] extended their intracellular IAV MMs to bridge the scale of intracellular virus replication with

that of intercellular *in vitro/vivo* IAV spread. The challenge for all such MMs is that their increased complexity requires even more data, self-consistent over all scales; such data is rather rare.

Conclusion

Over the last several years, MMs for influenza have become more refined, robust and applied to a variety of questions. To continue progress, several conditions need to be met: 1) Further increases in data quantity and quality. This not only applies to the richness of data coming from different sources (e.g., different *in vitro* assays or *in vivo* infection conditions). The data also need to be robust and reliable. As an example, Paradis et al. [8] encountered reproducibility issues *in vitro* when analyzing the impact of the I223V neuraminidase (NA) mutation in a 2009 IAV pandemic strain background. They showed that parameters for the wild-type (WT) strain varied more between experiments than the WT varied from its mutant within each experiment [8]. The issue of robust data is likely even more important for *in vivo* experiments, where it is widely known that e.g., the 'same' mouse strain purchased from different sources might lead to different results. The issue of reliable and reproducible data and findings is of course not limited to IAV and MMs, and has recently received wide attention [58]. 2) Improved MM parameter estimation (PE) techniques. With wider awareness of the issue of parameter identifiability and correlations, simplistic "best-fit" PE are giving way to more accurate and robust Bayesian PE methods, e.g., partially observed Markov processes for stochastic models [59], Markov chain Monte Carlo (MCMC) methods [7,8,60]. Increasingly, authors provide, or reviewers rightfully demand, diagnostic plots showing the degree of correlation between parameter pairs and proofs of PE convergence. 3) Further strengthening of collaborations between modellers and experimentalists will help drive the previous two conditions. While it is common in areas such as epidemiology to involve modellers and statisticians from the beginning of a project, this is not as common yet for *in vitro* or *in vivo* studies, where an experiment is often done without the initial goal of fitting MMs to the data. But the number of tightly integrated, successful collaborations between modellers and experimentalists is increasing [20,34,37,61], promising future progress toward understanding the many complicated mechanisms of IAV infections.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors wish to acknowledge Benjamin P. Holder for providing the original artwork which was adapted for the purposes of, and which now appears in, Figure 1.
In working on this review, C.A.A.B. was supported by the Interdisciplinary Theoretical and Mathematical Sciences (iTHEMS) programme at RIKEN, the Natural Sciences and Engineering Research Council of Canada [grant number 355837-2013], and the Ministry of Research and Innovation of the Government of Ontario, Canada [grant number ER13-09-040]. Work by L.E.L. was performed under the auspices of the U.S. Department of Energy [contract number DE-AC52-06NA25396] and supported by the National Institutes of Health [grant numbers R01-OD011095, R01-AI078881]. A.H. was partially supported by the National Institutes of Health/National Institute of Allergy and Infectious Diseases [grant number U19AI117891].

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