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Influenza A Virus Infection Kinetics: Quantitative Data and Models

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Abstract

Influenza A virus is an important respiratory pathogen that poses a considerable threat to public health each year during seasonal epidemics and even more so when a pandemic strain emerges. Understanding the mechanisms involved in controlling an influenza infection within a host is important and could result in new and effective treatment strategies. Kinetic models of influenza viral growth and decay can summarize data and evaluate the biological parameters governing interactions between the virus and the host. Here we discuss recent viral kinetic models for influenza. We show how these models have been used to provide insight into influenza pathogenesis and treatment, and we highlight the challenges of viral kinetic analysis, including accurate model formulation, estimation of important parameters, and the collection of detailed data sets that measure multiple variables simultaneously.

1 Introduction

Influenza A virus infection is characterized by dissemination of the virus in the airways and by rapid viral replication followed by complex interactions with the immune system [98, 107]. The mechanisms driving the virulence of pathogenic influenza strains and their interaction with the immune system are poorly understood [98, 107]. Identification of virus characteristics and host components crucial to virus control are important aspects that have been addressed using both theory and experiments.

Kinetic models (Box 1) describing viral infections are valuable tools that can be used to analyze experimental results and explain biological phenomena [74]. The use of such quantitative models can improve the state of knowledge about influenza by making predictions about the dynamic differences in strains [85, 89] and the importance of immune responses [37, 38, 60, 65, 82]. They can also be utilized to test hypotheses about antiviral mechanisms [2, 6, 39, 47], i.e., whether antivirals prevent virus replication or infection of cells. The successfulness of these models, however, is dependent on the availability of experimental data that can be compared with model predictions. For these purposes it would be ideal if the influenza data is frequently measured, is obtained using sensitive assays, has simultaneous measurements of both virus and immune components and is representative of a natural infection.

Currently, viral titers are the most frequently used type of data in modeling influenza dynamics. Typically, virus is measured in plaque forming units (PFU) or 50% tissue culture infectious dose (TCID₅₀), which represent infectious virus only, whereas total virus is reflected by measuring viral RNA levels. Such data has been obtained from experimental

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infections in the laboratory using cell culture and animals models. Although viral titers alone are not a complete representation of influenza pathogenesis, they are easily attainable and are fairly consistent over experimental systems. A recent focus on the host response to infection has resulted in an increase in model complexity and the use of immunological measurements in addition to viral titers [60, 65]. These data, although not always frequently measured, are most often obtained from laboratory experiments using mice since sampling in larger hosts is challenging.

Here, we review the current state of modeling influenza viral kinetics, the data currently available to parameterize these models, and discuss the future of using mathematical models in coordination with quantitative data. We focus on the model formulations, the techniques involved in analyzing such models, and the fundamental questions pertaining to influenza infection dynamics.

2 Modeling Influenza Kinetics

In humans, influenza A virus usually causes an acute and self-limiting infection. As a short-lived infection with an incubation period of ~2 days, an infectious period of 4-7 days and, in the vast majority of cases, confinement to the respiratory tract [98], studying influenza infections with mathematical models has been difficult because the dynamics are rapid and complex. It is unclear what mechanisms are responsible for controlling viral growth resulting in the viral titer reaching a peak (3-4 days postinfection) and then declining leading to eventual infection resolution (usually within 10 days).

Remarkably, modeling studies of *in vivo* infections have successfully shown that it is possible to exclude innate and adaptive immune system responses and still effectively describe the viral titer dynamics [2, 39, 89]. Similarly, models have successfully described *in vitro* viral titer dynamics while excluding the effect of innate immune responses [6, 68, 85]. In these models, depletion of susceptible target cells (e.g., epithelial cells) can result in the decline of virus. There is evidence *in vitro* suggesting that, with a multiplicity of infection of 0.025, up to 80% of cells become infected within 24 hours [68] with few viable cells remaining after 3-4 days [31, 68]. However, *in vivo*, complete destruction of the entire respiratory is not evident [82]. This supports the idea that immune regulation may play a large role in controlling viral growth [38, 65, 82]. Nevertheless, models involving only target cell limitation agree well with much of the available viral titer data [2, 6, 39, 68, 89]. Thus, it is currently unclear if more model complexity is necessary to fully explain the course of viral load changes during an experimental influenza infection.

The mathematical approaches to modeling influenza infections within a host most often use systems of ordinary differential equations [2, 6, 11, 37, 38, 39, 46, 60, 65, 68, 82, 85, 89, 101] but agent based, cellular automaton, and partial differential equation models have also been implemented [92, 5, 47]. Significant effort has been invested in the development and parameterization of these models. Models constructed with mild complexity and the ability to accurately estimate parameters have proven useful, particularly when analytical solutions that facilitate interpretation of the time scales and rates of viral growth and decay can be derived [90]. For this reason, simple models that can be readily analyzed are used, although more complex models that may have more than 10 equations and more than 50 parameters have been developed to provide a comprehensive description [11, 37, 60, 65]. For these complex models, analysis has been limited to simulation [11, 37, 60] and only in one case has parameter estimation been done [65].

Regardless of complexity, influenza model formulations thus far have attempted to increase the knowledge of the viral life cycle [87], the differences in pathogenicity of influenza strains [89], and the mechanisms and efficacy of antiviral treatment [2, 6, 39, 47]. They have

highlighted aspects of influenza biology, such as viral growth rates and loss of infectivity, and have resulted in estimates of the death rate of infected cells, the half-life of infectious viral particles, and the basic reproductive number, R_0 , characterizing the spread of virus during early infection [2, 6, 39, 65, 68, 82, 89].

3 Virus Control By Target Cell Limitation

In a classic viral dynamic model, first derived to study HIV (summarized in [73, 74, 91]), cells that are susceptible to infection (i.e., target cells (T)) become infected upon interaction with infectious viral particles, V , according to the law of mass action at rate βTV , where β is the infection rate constant. Target cells have a constant supply rate, s , and natural death rate, d . Virus is produced at rate p per infected cell, I , and is cleared at rate c per virion, which corresponds to a half-life $t_{1/2} = \ln(2)/c$. An infected cell has an average lifespan of $1/\delta$, where δ is the death rate of infected cells, and thus produces an average of $N = p/\delta$ virions during its lifetime. Fig. 1(a) shows a schematic diagram of these dynamics and the associated equations are given in Box 2.

For influenza virus, the classic model has been modified to provide a more accurate representation of the infection dynamics. First, since influenza dynamics are rapid (lasting approximately 7-10 days), including regeneration and natural death of these target cells, which have longer time scales, has little effect on the dynamics [2, 38] and are typically excluded ($s = 0$, $d = 0$).

Second, the eclipse phase, i.e., the time it takes for viral production to begin after initially becoming infected, is missing from the classic model, although it was added in later HIV models [45, 67]. The eclipse phase can be incorporated into models by including a second class of infected cells (I_1) that are in the eclipse phase and cannot yet produce virus. In one class of models, cells in the eclipse phase (I_1) transition to a productive state (I_2) at rate k . This model, shown schematically in Fig. 1(b) and given by the equations in Box 2, simplifies matters and assumes that no cell death occurs before the initiation of virus production. In other classes of influenza models, the time spent in the eclipse phase is described by either a fixed or distributed delay [6, 46, 68], as has been done in HIV modeling [45, 67].

In general, including the eclipse phase does not change the model behavior and may not be statistically justifiable since the number of parameters is increased, but parameter estimates made by fitting the model to data enter more biologically realistic ranges [2, 6] and growth kinetics are more accurate [90] when taking into account these dynamics.

Third, the virus lost from entry into cells is typically excluded in viral kinetic models since this is usually a negligible amount of virus compared with the amount lost by other processes such as phagocytosis and mucociliary clearance. However, in cell culture, this is not the case [6, 68]. Furthermore, particularly in influenza infection, it is typical to measure only infectious virus. Therefore, loss of viral infectivity can be modeled as clearance of infectious virus. Parameters estimated using viral titers from infection of MDCK cells *in vitro* suggested that the loss of viral infectivity ($c = 0.1 \text{ h}^{-1}$) is much smaller than the loss of virus from cell entry ($\beta T_0 = 5.0 \text{ h}^{-1}$) [6]. Thus, in this system where only infectious virus was modeled, an extra term ($-\gamma TV$) was added to the virus equation in Box 2, such that $dV/dt = pI - cV - \gamma TV$, since the viral entry dynamics could not be neglected [6]. Here, γ is the adsorption rate of virus, which we expect to be greater than the infection rate (β) since it may take more than one infectious viral particle to infect a single cell. Even though model fits were better, γ was less than β when estimated by data fitting [6]. The issue of why γ was less than β has yet to be fully resolved, but one possible explanation proposed by Beauchemin et al. [6] was that the number of infectious virions counted by the plaque assay,

V , was an underestimate of the true number, V_{real} . They showed that changing variables from V to $V_{real} = \zeta V$, with $\zeta < 1$, and rewriting the model equations could resolve the paradox. Other solutions, although non-mechanistic, are to enforce $\beta T_0 < c$ and exclude the γTV term [89], fix the value of γ/β [68] or constrain it to be ≥ 1 .

Characterizing influenza infection kinetics using data fitting and models to estimate parameters is a useful technique, but obtaining an accurate model in which all parameters are identifiable is challenging. The target cell limited model (Box 2) has 4 equations and 7 parameters (β , p , c , δ , k , the initial viral inoculum, V_0 , and the initial target cell density, T_0) for which nonlinear least squares or the maximum likelihood analog are the most frequently used methods to establish parameter values. However, not all parameters are identifiable [82, 89, 93] and it has been necessary to fix some parameters (e.g., the eclipse phase parameter, k) or restrict the ratios of parameters (e.g., $\beta T_0 < c$) to ensure they take on biologically relevant values. Additionally, it has been shown that the initial target cell density, T_0 , and the rate of virus production, p , appear in the model solution only as a product, pT_0 , and, hence, only one of these parameters can be estimated [65, 93]. Most often, T_0 is fixed and p is estimated [2, 6, 39, 82, 89].

Another difficulty in estimating the parameters characterizing an influenza infection is the variety of experimental systems and virus strains used in various studies. For example, the data used to parameterize models has come from humans infected with influenza virus strains A/Hong Kong/123/77 (H1N1) [2, 70] or A/Texas/91 (H1N1) [39, 42], Welsh ponies infected with influenza A/equine/Kildare/89 (H3N8) virus [78, 82], BALB/cJ mice infected with influenza A/Puerto Rico/8/34 (H1N1) virus [38, 49, 89] or a variant expressing the 1918 PB1-F2 protein [89], C57BL/6 mice infected with influenza A/Hong Kong/X31 (H3N2) virus [65], and Madin-Darby canine-kidney (MDCK) cells infected *in vitro* with influenza A/Albany/1/98 (H3N2) virus [6], influenza A/Puerto Rico/8/34 (H1N1) [85], influenza A/Wisconsin/67/2005 (H3N2) [85], or equine influenza virus strain A/equine/Newmarket/1/93 (H3N8) [68]. The existing data suggest that parameter estimates drawn from certain systems are strain and cell type specific and cannot be generalized to all influenza infections (Table 1).

The estimates of the infected cell lifespan are an example of this, where estimated values have varied depending on the type of data used and the strain of influenza. Cells in the productively infected state were estimated to have a lifespan of 5 hours in an experimental human infection [2], 13 hours in cell culture [6], and 16 hours for one influenza strain and 27 hours for another in mice [89]. Furthermore, in addition to possibly inducing cell death at different rates, influenza viruses (e.g., H1N1 versus H3N2) may differ in their rates of infection. The rate of virus production may also be different depending on the virus strain but also dependent on the cell type (e.g., MDCK cells versus A549 cells).

To aid in the identification of model parameters, analysis of the model equations has been useful (Box 2). Approximate solutions of Eq. (2) in Box 2 have elucidated the contributions of parameters to the initial viral growth rate and the decay rate of viral titers in the recovery phase [90]. Early in the infection, many of the parameters influence the dynamics, but viral decay is described by the infected cell death rate (δ) when it is much smaller than the rates of viral clearance (c) and eclipse phase transition (k) [90], as it is thought to be *in vivo*. *In vitro*, however, the immune response is absent and, hence, c may be much less than δ . This implies that, in such circumstances, viral decay is determined by the rate of viral clearance. From this, a value for δ (or c , *in vitro*) can be easily found by fitting a line to the decay of log viral titers in the recovery phase (typically beginning on 3 days post-inoculation), which reduces the number of parameter that need to be fitted. *In vivo*, quantitative measurements of the number of infected cells or the number of dead cells as a result of virus infection are

difficult to obtain, and thus direct measurements of δ have not been obtained. However, some data does exist [49, 82, 85], and a few models have incorporated this type of data [38, 82, 85].

Furthermore, studying influenza dynamics *in vitro* has strengthened the understanding of viral production and clearance rates in the absence of an immune response. For example, the loss of infectious virus due to an infectivity loss was examined by incubating virus in cell culture media in the absence of cells and then evaluating the amount of infectious virus, measured by plaque assay, at various times. The amount of infectious virus decayed with time yielding an estimated viral infectivity half-life of 6.6 hours for influenza A/Albany/1/98 (H3N2) virus [6]. The clearance rate of infectious virus within a host can be expected to have an even shorter half-life, on average, since virus can be lost by cell entry and by immune-mediated mechanisms. For instance, in a murine model, the infectious virus half-life of influenza A/Puerto Rico/8/34 (H1N1) was 0.6 hours [89], which is substantially shorter than 6.6 hours.

Understanding the differences in pathogenicity of virus strains is an important aspect of influenza biology. Modeling influenza kinetics using the target cell limited model (Box 2) played an integral role in teasing apart the differences and potential mechanisms of a virulent influenza strain compared to a less pathogenic one [89]. In this case, an analysis was done of two influenza viruses that differed only in the PB1-F2 protein that they express. In one virus, the PB1-F2 was from the 1918 pandemic strain A/Brevig Mission/1/1918 (H1N1), while the other was the natural PB1-F2 of influenza A/Puerto Rico/8/34 (H1N1). The PB1-F2 protein is thought to increase inflammation and contribute to viral virulence [17, 62, 63, 109], and the variant carrying the 1918 PB1-F2 was expected to be more virulent [17, 63]. Fitting the model showed a significant difference in the kinetics of the two infections with the most notable influences of PB1-F2 on the viral replication rate (p) and the infected cell death rate (δ) [89].

4 Virus Control By Host Immune Responses

The limitation in viral growth that defines the viral titer peak may be controlled by innate immunity rather than by loss of target cells. Furthermore, adaptive immunity contributes to the eradication of virus [21], as virus can persist for long periods in immunocompromised hosts [26]. Using both *in vitro* and *in vivo* experimental systems can provide information about the efficiency of a host's immune response at controlling influenza infection. However, only comparing viral titer measurements from these systems gives no indication about adaptive immune responses versus innate immune responses. Therefore, quantitative measurements of immunological components are necessary to evaluate individual contributions of the host response.

One difficulty of this approach, however, is the limited availability of such kinetic data in coordination with viral titers. Early models focused on developing a comprehensive view of immune response dynamics but did not incorporate sufficient data to validate the models [11, 37]. More recent modeling efforts have examined these host responses by using data from experimental infections in horses [78, 82] and in mice [7, 38, 60, 65].

4.1 Innate Immune Control

During primary influenza infection, the release of proinflammatory cytokines by antigen presenting cells and the infected lung epithelium initiates the innate immune response [53]. In particular, the type I interferons, interferon alpha and beta (IFN- α and IFN- β), are thought to play a major role in limiting viral replication by interacting with susceptible cells and rendering them "resistant" to infection [30, 49, 83]. This effectively prevents virus from

establishing a productive infection within the cell and is thought to allow time for the adaptive immune system to respond and clear the virus [77]. These antiviral effects have been explored with Eq. (2) in Box 2 [2, 13, 38] and in a more complicated extension of this model [82].

Interferon levels begin to rise shortly after virus becomes detectable and correlate well with viral replication [49]. The time lag that occurs between the initiation of an influenza infection and the appearance of IFN has been modeled with the equation $dF/dt = \eta I(t-\tau) - \alpha F$, where η is the IFN production rate, α is the IFN decay rate, and τ is a time delay (Box 3). Because IFN can reduce viral replication in an infected cell [72], the rate of viral production in the presence of IFN has been modeled as $p = \hat{p}/(1 + \varepsilon_1 F)$. The rate that cells in the eclipse phase begin virus production (k) may also be altered and was accounted for by redefining this parameter, $k = \hat{k}/(1 + \varepsilon_2 F)$. The efficiency of these interferon effects is reflected by the parameters ε_1 and ε_2 [2].

Estimating these parameters is difficult since obtaining well characterized IFN kinetics in the lungs is challenging, especially in human infections. Thus, incorporating the dynamics of IFN into Eq. (2) in Box 2, which increases the number of parameters, could not be statistically justified in one model given the lack of supporting data [2]. However, including these dynamics could explain a potential biphasic increase or plateau of titers during the viral decay phase [2].

Because IFN- α is produced not only by infected cells but also by cells such as plasmacytoid dendritic cells [16], it was assumed in one model (Box 3) that the rate of IFN production was proportional to the amount of free virus rather than to infected cells [38]. Interestingly, comparing this model to IFN measurements from influenza infected mice [49], it was necessary to enforce a decrease in interferon concentration on day 5 by setting the production rate to zero (i.e., $\omega = 0$) since this cytokine disappeared even when viral titers remained at high levels [38].

Another way to model the effects of IFN is to explicitly consider cells that enter an antiviral state, in which they are refractory to infection (Box 3) [13, 37, 82]. Simultaneously fitting the model to viral titers, IFN concentrations and death of infected cells in horses experimentally infected with the equine influenza [78] suggested that the contribution of the innate immune response in viral control outweighed the effects of target cell limitation in this system [82].

4.2 Adaptive Immune Control

Adaptive immune responses to influenza, involving B cells and T cells, are thought to dictate the decrease and eventual clearance of virus [21, 100], particularly in individuals previously exposed to influenza [15]. In addition to their antiviral effects, type I interferons stimulate effector cells, such as natural killer (NK) cells and cytotoxic T lymphocytes (CTLs), that destroy influenza infected epithelial cells [21, 83]. Virus specific B cells and plasma cells, on the other hand, produce antibodies that can neutralize the virus and reduce its infectivity (anti-HA antibodies) or reduce viral levels (anti-NA antibodies) [52], possibly by reducing virus release from infected cells.

Including components of the adaptive response such as these to influenza models increases their complexity and requires sufficient data to support such a model. However, even with well characterized data, a large number of model parameters may not be identifiable. In this case, simulating the equations with biologically plausible parameter values may be the only option [11, 37, 60].

Less complicated models can still be useful for teasing apart the effects that adaptive immunity has on viral kinetics (Box 4). In several instances, modeling efforts have been focused on the CTL response [7, 13, 38, 101], which in one study in mice was shown to begin increasing around day 5 after inoculation, to peak around 10 days and to return to a baseline level around 20 days postinfection [65]. Each of the models suggested that the CTL response plays an important role in complete viral clearance.

While CTLs have effects on infected cells, which in turn affects virus concentrations, antibodies have direct effects on free virus. The antibody response is important to consider since efficient removal of virus without damaging the respiratory tract, as with CTL killing of cells, is ideal. In mice, virus-specific antibodies begin increasing around 5 days after inoculation which is followed by a peak in IgM level around 8-10 days and in IgG levels around 25 days and eventual decay [65]. The dynamics of antibody (A), as measured in mice infected with A/Puerto Rico/8/34 (H1N1) [49], were modeled in combination with the effects of the innate response (i.e., IFN) (Box 4). While the model fit the data well, replacing the antibody response with the CTL response resulted in fits that were equally as good [38]. Therefore, additional data would be necessary to distinguish the contributions of these two responses to viral clearance.

Detailed models of the immune response to influenza virus infection highlight three important components: the effects of interferon (i.e., the removal of target cells available for infection), the effects of cellular immunity (i.e., the killing of infected cells), and the effects of antibodies (i.e., the effective virus concentration is reduced) [37, 60, 65]. Incorporation of the dynamics of antigen presenting cells, such as dendritic cells, has also been done [37, 60]. Only one study, however, used quantitative data from a large number of mice, in which virus, CD8 T cells, and antibody (both IgG and IgM) were all measured, and combined with a detailed model. By doing so, model parameters could be estimated and the effects of the CTL response on reducing infected cell half-life and of antibody on increasing infectious virus loss were quantitatively assessed [65].

5 Influenza Kinetics in Response to Antivirals

Influenza antiviral agents are used therapeutically and prophylactically to prevent individuals who are exposed from acquiring the virus, to reduce the severity of infection, and to limit virus transmission to others who are susceptible. Two classes of antiviral drugs are currently approved for treatment of influenza [25]: the adamantanes (amantadine and rimantadine) and the neuraminidase inhibitors (NIs, oseltamivir and zanamivir) (reviewed in references [19, 35, 51]). Viral resistance is prevalent with use of both classes of drugs, although resistance to the adamantanes is more common particularly when used therapeutically rather than prophylactically [40].

Adamantanes reduce the ability of the virus to infect cells by blocking the activity of the M2 protein ion channel which has been modeled by reducing β by $(1-\varepsilon(t))$, where $\varepsilon(t)$ represents the effectiveness of the drug and may not be constant due to the pharmacokinetic and pharmacodynamic effects of the drug (Box 5). Fitting Eq. (2) in Box 2 to viral titers obtained in the presence of amantadine resulted in the estimate that this drug may at best only be 74% efficacious *in vitro* with the low efficacy most likely due to rapid development of drug resistance [6].

To analyze the effects of giving the antiviral drug oseltamivir, which blocks the release of virus from infected cells, the viral production rate (p) is altered (Box 5). With p multiplied by $(1-\varepsilon_{NI})$, where ε_{NI} is the efficacy of the drug (e.g., $\varepsilon_{NI} = 1$ implies 100% effectiveness), the model was fitted to viral titers in patients post-treatment (the antiviral was administered early (26 or 32 hours postinoculation) or late (50 hours postinoculation) [42]). With this

data, the drug was estimated to stop 97-99% of viral production in humans infected with influenza A/Hong Kong/123/77 (H1N1) virus [2].

The emergence of drug-resistant virus can alter infection dynamics. To model this scenario (Box 5), the virus population can be split into two classes: virions that are sensitive to the drug (V_s) and virions that are resistant to the drug (V_r). Mutations leading to resistant virus typically have an associated fitness cost such that either virus production (p) is lowered or the infection rate (β) is lowered [39]. Examining the properties of resistant strains, such as the differences in viral titers and in plaque size and growth velocity, has also been done using modeling approaches [47]. To do this, Holder and Beauchemin [47] developed a partial differential equation model to examine plaque growth of an NI-resistant strain versus a wild-type strain [47], and the approximate solutions of the target cell limited model [90] were used to examine the viral titers of the resistant and sensitive strains in cell culture [47].

To reduce the effects that drug-resistant mutations have on viral dynamics during treatment, combination therapy can be used. The scenario was examined with a model, which assumed the effects of each drug were additive, and indicated that patients could benefit from treatment with both an amantadine and a neuraminidase inhibitor [60].

In practice, antiviral drugs need to be given within the first 48 hours of infection for maximum results [1]. Indeed, the viral growth phase in influenza infected patients was estimated to last between 30 and 63 hours [90], which was found to be similar in mice [89]. At the point when viral growth significantly slows and a peak is reached, any drug induced changes in the production of virus is predicted to have minimal effects [2].

6 Concluding Remarks

In the last 5 years, new kinetic models for influenza A virus infection driven by quantitative data from experimental infections have been developed. The number of these models has increased significantly in the last 2 years, which has led to an array of models being in the literature with varying complexity [2, 5, 6, 11, 37, 38, 39, 46, 47, 60, 65, 68, 82, 85, 89, 92, 101]. Although controversy still exists about the necessity of including immune responses to describe influenza viral dynamics, kinetic analyses have provided a way to investigate the properties of viral growth and decay and give insights into the control mechanisms involved in combating influenza infection. One general difficulty in developing, validating, and refining within-host influenza models is the lack of available data detailing immune kinetics and epithelial cell pathology, particularly for a natural infection.

An abundance of influenza viral load data exists (e.g., [12, 14, 23, 29, 44, 59, 64, 86, 94, 95, 99, 104, 110]), some of which has been used in modeling studies [2, 6, 38, 39, 46, 47, 65, 68, 82, 85, 89], but analyzing only this type of data may result in exclusion of important dynamics. For example, a recent study comparing influenza infections in aged and adult mice [99] and another comparing influenza strains [62] suggest that lung immunopathology, rather than viral load, is a better indicator of disease severity.

One type of data that is easily attainable and offers a new perspective into the characteristics of an infection is the assigning of a symptom score throughout the infection, which provides insight into how sick a host is. Data of this sort, which has been recorded in mice [99] and in humans (see [12] and references therein), indicates the extent to which cytokines (e.g., TNF- α) are involved in pathogenesis since lung immunopathology is strongly correlated with increased cytokine production [24, 41].

Furthermore, increased viral replication, increased numbers of alveolar macrophages (AMs) and neutrophils, and elevated cytokines and chemokines have all been associated with

influenza lethality (reviewed in reference [58]). While some cytokine data does exist (e.g., [3, 18, 20, 27, 34, 41, 43, 56, 55, 78, 88, 99, 103, 106, 108]), these molecules are typically excluded from models because their effects on virus concentrations are indirect. Fewer data sets contain measurements of cells involved in the innate immune response, like macrophages and neutrophils [56, 76, 96, 97, 99, 102, 106], and the effects of these cells have not been incorporated into influenza models. However, these cells may have a crucial role in viral control and clearance, particularly since higher viral titers are observed when AMs and, to a lesser extent, neutrophils are absent [28, 29, 56, 96, 97, 102].

Alveolar macrophages may have another role in influenza infections that could be investigated using a mathematical model. These cells can be infected by influenza virus [8, 71, 76, 79, 84, 61, 105], but it is unclear if the infection is productive (i.e., if infectious virus is produced within and released by AMs) and how much the infection of these cells contribute to the overall dynamics of the infection. That is, it may be of importance to understand whether the virus infection in AMs acts as a sink or a source for free virus.

An additional source of virus, such as from infected AMs, could explain the plateau, or potential second peak, in viral titers observed in some experimental infections during the recovery phase of infection (usually between days 5-7) [9, 10, 22, 36, 41, 50, 69, 70, 80]. One explanation of this phenomena came from a model which suggested that the decreasing interferon response could allow for an increase in viral production [2]. However, other circumstances that allow the virus to undergo a surge in viral titer, such as the virus migrating into new areas of the respiratory tract resulting in an increase in the number of susceptible target cells, could be evaluated with a mathematical model. Besides AMs, other cells in the lung may act as influenza target cells. Furthermore, heterogeneity among lung epithelial cells may make some cells better targets than others. The effects of multiple types of target cells could be investigated with models, as has been done in the field of HIV modeling [75].

The development of influenza kinetic models and the merging of theoretical analyses with experiments has increased significantly over the past several years. So far, these models have used quantitative descriptions of infection kinetics to aid the interpretation of experimental data and improve our understanding of viral control and treatment. The modeling techniques described here focused on viral growth, viral decay, target cell depletion and, to some extent, the immunological involvement. However, several aspects have not yet been addressed. While the dynamics of influenza A virus have been investigated in various studies, the dynamics of influenza B virus [4], which is also an important public health threat, have not been analyzed using kinetic models. The models developed thus far also generally do not incorporate multiple compartments, such as the lung, trachea and nasopharynx [59], that may have interesting dynamical differences. Integrating the extracellular events (e.g., virus production and infected cell death) with the intracellular kinetics (e.g., RNA replication and virus assembly) [87] or the transmission between hosts (e.g., [32, 66]) may give insight into the various aspects of influenza pathogenicity including why some strains cause more inflammation or are more easily transmitted. As the amount of information available continues to increase with new and different data, such as imaging and microarray analysis of gene expression [33, 54, 57, 81], old models should become more refined and new models formulated thus increasing our understanding of influenza infections and how to better treat infected hosts.

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Box 1**Definitions**

Viral Kinetics: The rate of change of virus as a function of the time postinfection.

Kinetic Model: A mathematical model, typically a set ordinary differential equations, that describes the viral kinetics.

R_0 : The basic reproductive ratio. Defined as the number of infected cells that are produced by a single infected cell at the initiation of infection.

Box 2**Modeling Target Cell Limitation**

A classic model of viral dynamics, which includes target cells (T), infected cells (I), virus (V) (parameters defined in the text), is described by the equations:

$$\frac{dT}{dt} = sT - dT - \beta TV \quad \frac{dI}{dt} = \beta TV - \delta I \quad \frac{dV}{dt} = pI - cV \quad (1)$$

Assuming that target cells (T) are limited and become depleted during an acute influenza infection ($s=d=0$) and including the eclipse phase dynamics (splitting infected cells into two classes, I_1 and I_2), the viral kinetics are described by the following equations (parameters defined in the text) [2]. These equations have been used to estimate parameters by fitting the equations to viral titers from experimental influenza infections [2, 6, 39, 89].

$$\begin{aligned} \frac{dT}{dt} &= -\beta TV \\ \frac{dI_1}{dt} &= \beta TV - kI_1 \\ \frac{dI_2}{dt} &= kI_1 - \delta I_2 \\ \frac{dV}{dt} &= pI - cV \end{aligned} \quad (2)$$

Numerical solution of Eq. (2) using parameter values and viral titer data (boxes) from a human infected with influenza [2].

Initially, T is relatively constant as virus enters an exponential growth phase that is described by $V(t) = \alpha_1 e^{\lambda t}$, where λ is a complex combination of parameters [90]. As the viral growth slows and peaks, target cells are rapidly depleted causing viral decay and eventual infection resolution. Around the peak, $V(t) = \alpha_2 e^{-\delta t} + \alpha_3 e^{-ct} + \alpha_4 e^{-kt}$, followed by a phase of decay according to the law $e^{-\delta t}$ [90]. The rates of virus rise and fall (λ and

δ , respectively) can also be calculated by fitting lines to the phases of viral growth and decay [47, 89, 90].

Comparison of the fit (circles) to viral titer data (boxes) from a human infected with influenza [2] and the approximate solution to Eq. (2)) [90]. The viral growth phase (red) lasts for t_1 days and the viral decay phase (blue) begins at t_2 days [90]. (Reprinted with permission from Ref. [90]. Copyright 2010 Springer Science+ Business Media.)

Log-linear fits [90] to viral titer data (boxes) from a human infected with influenza [2]. Titers during days 1-3 were considered part of the initial viral growth phase, and titers during days 4-7 as part of the viral decay phase [90]. (Reprinted with permission from Ref. [90]. Copyright 2010 Springer Science+Business Media.)

Box 3**Modeling the Effects of Interferon**

The effect that interferon (F) has during a viral infection can be described by modifying Eq. (2) in Box 2 to account for the reduction in the rates of virus production (p) and of cells entering a productively infected state (k) in the presence of interferon [2]. This model was fit to viral titer data from humans experimentally infected with influenza [70] and was able to reproduce the bimodal titers observed in several patients [2]:

$$\begin{aligned} \frac{dF}{dt} &= \eta I(t - \tau) - \alpha F \\ p &= \frac{\hat{p}}{(1 + \varepsilon_1 F)}, \quad k = \frac{\hat{k}}{(1 + \varepsilon_2 F)} \end{aligned} \quad (3)$$

Numerical solution (virusblack, IFN-blue) of Eq. (2)-(3) using parameter values and viral titer data (boxes) from a human infected with influenza [2].

Other authors assumed the rate of IFN production was proportional to the amount of virus present [13, 38]:

$$\frac{dF}{dt} = \omega V - \alpha F \quad (4)$$

The interferon response has also been modeled by explicitly including the dynamics of cells that become resistant to infection in the presence of interferon [13, 37, 82]. Saenz et al. [82] used 6 classes of cells (target cells (T), two classes of latently infected cells (E_1 , E_2), prerefractory cells (W), refractory cells (R), and productively infected cells (I)), virus (V) and interferon (F). Interferon was assumed to be secreted by productively infected cells at a constant rate q and by cells in the eclipse phase at a constant rate nq . Production

of interferon was assumed to render target cells resistant to infection at a rate ϕF but only after they spend an average time $1/a$ in the prerefractory state, where they can be infected at the reduced rate $m\beta V$ [82]:

$$\begin{aligned} \frac{dT}{dt} &= -\beta TV - \phi TF & \frac{dI}{dt} &= k_1 E_1 + k_2 E_2 - \delta I & \frac{dV}{dt} &= pI - cV \\ \frac{dE_1}{dt} &= \beta TV - k_1 E_1 & \frac{dW}{dt} &= \phi TF - m\beta VW - aW & \frac{dF}{dt} &= nqE_2 + qI - \alpha F \\ \frac{dE_2}{dt} &= m\beta VW - k_2 E_2 & \frac{dR}{dt} &= aW \end{aligned} \quad (5)$$

Simultaneously fitting Eq. 5 to viral loads (RNA copies/ml nasal secretion (NS)) and interferon concentrations in the nasal washes of a Welsh pony experimentally infected with influenza [82]. Reprinted from Saenz et al. [82]. Used with permission from the *American Society of Microbiology*.

Box 4**Modeling the Effects of Adaptive Immunity**

Cytotoxic T lymphocytes (CTLs, Z) respond during an influenza infection and kill infected cells (I). One model, which was utilized to compare infection dynamics in normal and T cell deficient mice, excluded viral dynamics (i.e., no V equation) but assumed that cells became infected at rate βTI . The CTLs (Z) were assumed to decay at rate b and be recruited proportional to infected cells (I) at rate v . When CTLs encounter an infected cell, the infected cell is killed at rate r [7]:

$$\frac{dT}{dt} = s - dT - \beta TI \quad \frac{dI}{dt} = \beta TI - \delta I - rIZ \quad \frac{dZ}{dt} = vI - bZ \quad (6)$$

A similar model also included the dynamics of infected cells that are in complexes with CTLs, J , and assumed that the expansion rate of CTLs, was given by a function $v(Z, I, J)$ [101]:

$$\begin{aligned} \frac{dT}{dt} &= sT(1 - T - I - J) - \beta TV & \frac{dI}{dt} &= rIZ - \psi J & \frac{dZ}{dt} &= v(Z, I, J) - rIZ \\ \frac{dJ}{dt} &= \beta TV - \delta I - rIZ & \frac{dV}{dt} &= pI - cV \end{aligned} \quad (7)$$

In the presence of virus-specific antibody (A), the virus can be neutralized and effectively removed from circulation. In this model, both the innate (i.e., IFN (F)) and the adaptive (i.e., antibody) immune responses, and the dynamics of cell death (D) were integrated. Antibody production was assumed to occur at rate f proportional to the amount of virus present and decay at rate h . Antibody-mediated clearance of virus was assumed to occur at rate κ and follow the law of mass action, κVA [38]:

$$\begin{aligned} \frac{dT}{dt} &= \chi D - \beta TV & \frac{dI_1}{dt} &= \beta TV - kI_1 & \frac{dF}{dt} &= \omega V - \alpha F \\ \frac{dD}{dt} &= \delta I_2 - \chi D & \frac{dI_2}{dt} &= kI_1 - \delta I_2 & \frac{dA}{dt} &= fV - hA \end{aligned} \quad (8)$$

$$\frac{dV}{dt} = \frac{\hat{p}}{1 + \varepsilon_1 F} I_2 - cV - \gamma TV - \kappa VA$$

In more complicated models of influenza kinetics, several components of the adaptive immune response have been considered [11, 37, 60, 65]. In one of these models [65], sufficient data was available for estimation of parameters:

Fits of the model in [65] to data (viral titers (not shown), CD8 T cells, IgG and IgM (not shown)) collected from experimentally infected mice [65]. Reprinted from Miao et al. [65]. Used with permission from the *American Society of Microbiology*.

Box 5**Modeling Antiviral Treatment**

The two antiviral drugs approved for treatment of influenza virus infections are adamantanes and neuraminidase inhibitors.

Neuraminidase Inhibitors (NIs)

When NIs are given, they are assumed to reduce the rate of viral production, p , from infected cells with efficacy ε_{NI} , with $\varepsilon_{NI} = 1$ corresponding to 100% effectiveness [2, 39]:

$$p \Rightarrow (1 - \varepsilon_{NI})p \quad (9)$$

Adamantanes

When adamantane antiviral drugs are given, they are assumed to reduce the rate of cell infection, β , with efficacy ε_A [6]:

$$\beta \Rightarrow (1 - \varepsilon_A)\beta \quad (10)$$

The drug efficacy may be dependent on the pharmacodynamic effect of the drug. This can be described by $\varepsilon(t)$, where ε_{max} is the maximal drug effect, $D(t)$ is drug concentration at time t , IC_{50} is the half-maximal inhibitory drug concentration, and n is the Hill coefficient characterizing the steepness of the response [48]:

$$\varepsilon(t) = \frac{\varepsilon_{max} D(t)^n}{D(t)^n + IC_{50}^n} \quad (11)$$

Drug-resistant strains can emerge during treatment of influenza infections and may influence viral dynamics. To model this, viruses can be classified as either sensitive (V_s) or resistant (V_r) to the drug, where a fitness cost (σ) is associated with the mutations (μ) that lead to resistance [39]:

$$\begin{aligned} \frac{dT}{dt} &= -\beta T (V_s + V_r) & \frac{dZ}{dt} &= rZ \\ \frac{dI_s}{dt} &= \beta T V_s - \delta I_s & \frac{dV_s}{dt} &= (1 - \varepsilon_{NI})(1 - \mu) p I_s - c V_s - r Z V_s \\ \frac{dI_r}{dt} &= \beta T V_r - \delta I_r & \frac{dV_r}{dt} &= (1 - \varepsilon_{NI}) \mu p I_s + (1 - \sigma) p I_r - c V_r - r Z V_r \end{aligned} \quad (12)$$

Fits of Eq. (12) [39] to viral titer data from humans infected with influenza that were either given no treatment (blue) or antiviral treatment early (29 hours, red) or late (50 hours, green) [42]. Total virus ($V_s + V_r$) - solid lines, Resistant virus (V_r) - dashed lines. Reprinted from Handel et al. [39].

Figure 1.

Schematic diagram of the viral dynamics models. (a) Classic model of viral dynamics. Target cells (T) are supplied at constant rate s and die at rate d per day. These cells become infected at rate βV per day. Free virions are produced from infected cells (I) at a rate p and

are removed at a rate c . Infected cells are lost at a rate δ . (b) Acute virus infection model modified from the classic model. Target cell regeneration and death are not included. Infected cells were split into two classes, I_1 and I_2 , where virus production initially undergoes an eclipse phase (k).

Table 1

Estimates of parameter values for influenza infection.

Experimental System	Parameters						
	V_0	β	p	c	δ	$k(\tau)$	T_0
Human	TCID ₅₀ /ml	(TCID ₅₀ /ml) ⁻¹ day ⁻¹	TCID ₅₀ cell ⁻¹ day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	cells
A/Hong Kong/123/77 (H1N1)	[2]	7.5×10^{-2}	4.6×10^{-2}	5.2	5.2	4.0	4.0×10^8 *
A/Texas/91 (H1N1)	[39]	1.0×10^{-5}	1.1×10^{-5}	3.0	1.3	-	4.0×10^8 *
Horse	RNA/ml	(RNA/ml) ⁻¹ day ⁻¹	RNA/ml cell ⁻¹ day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	cells
A/equine/Kildare/89 (H3N8)	[82]	1.4×10^{-4}	1.4×10^{-5}	5.2*	2.0*	2.0*	3.5×10^{11} *
Mouse	TCID ₅₀ /ml	(TCID ₅₀ /ml) ⁻¹ day ⁻¹	TCID ₅₀ cell ⁻¹ day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	cells
A/Puerto Rico/8/34 (H1N1)	[38]	4.0×10^4	1.9×10^{-7}	10.0*	2.0*	4.0*	7.0×10^9 *
A/Puerto Rico/8/34 (H1N1)	[89]	2.0×10^0	2.8×10^{-6}	25.1	28.4	0.9	1.0×10^7 *
PR8-PB1-F2(1918)	[89]	2.6×10^{-1}	9.1×10^{-7}	72.8	9.2	1.5	1.0×10^7 *
Cell Culture	EID ₅₀ /ml	(EID ₅₀ /ml) ⁻¹ day ⁻¹	EID ₅₀ cell ⁻¹ day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	cells
A/Hong Kong/X31 (H3N2)	[65]	1473	2.4×10^{-6}	100*	4.2	0.6	5.8×10^5
Cell Culture	1/ml	ml/day	day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹ (hours)	cells/ml
A/Albany/1/98 (H3N2)	[6]	0^{*f}	5.5×10^{-5}	1.9×10^0	2.5*	1.9	6.7×10^6 *
A/Puerto Rico/8/34 (H1N1) NIBSC	[85]	N/A	3.1×10^{-4}	1.7×10^1	0.3	13.4	$2 - 3 \times 10^5$ *
A/Puerto Rico/8/34 (H1N1) RKI	[85]	N/A	2.4×10^{-6}	5.7×10^3	13.2	0.7	$2 - 3 \times 10^5$ *
A/Wisconsin/67/2005 (H3N2)	[85]	N/A	9.1×10^{-6}	3.8×10^2	2.6	0.7	$2 - 3 \times 10^5$ *
A/equine/Newmarket/1/93 (H3N8)	[68]	2.6×10^7 *	3.4×10^{-2}	1.2×10^4	0.2	6.2	1.2×10^6 *

For each virus strain and experimental system, the best fit estimate of the initial viral titer (V_0), infection rate constant (β), death rate of productively infected cells (δ), viral release rate per infected cell (p), viral clearance rate (c), the initial number of target cells (T_0), and the transition rate for infected cells to produce virus (k or τ for fixed delays) are given.

* Parameters that were fixed.

[†] Cells preinfected with virus were used to inoculate the cell culture. In this case, $I_0=6.7$ cells/ml.