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Within-host influenza dynamics: A small-scale mathematical modeling approach

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ABSTRACT

The emergence of new influenza viruses like the pandemic H1N1 influenza A virus in 2009 (A(H1N1)pdm09) with unpredictable difficulties in vaccine coverage and established antiviral treatment protocols emphasizes the need of new murine models to prove the activity of novel antiviral compounds *in vivo*. The aim of the present study was to develop a small-scale mathematical model based on easily attainable experimental data to explain differences in influenza kinetics induced by different virus strains in mice. To develop a three-dimensional ordinary differential equation model of influenza dynamics, the following variables were included: (i) viral pathogenicity (P), (ii) antiviral immune defense (D), and (iii) inflammation due to pro-inflammatory response (I). Influenza virus-induced symptoms (clinical score S) in mice provided the basis for calculations of P and I. Both, mono- and biphasic course of mild to severe influenza induced by three clinical A(H1N1)pdm09 strains and one European swine H1N2 virus were comparatively and quantitatively studied by fitting the mathematical model to the experimental data. The model hypothesizes reasons for mild and severe influenza with mono- as well as biphasic course of disease.

According to modeling results, the second peak of the biphasic course of infection is caused by inflammation. The parameters (i) maximum primary pathogenicity, (ii) viral infection rate, and (iii) rate of activation of the immune system represent most important parameters that quantitatively characterize the different pattern of virus-specific influenza kinetics.

1. Introduction

In April 2009 a new pandemic H1N1 influenza A virus (A(H1N1)pdm09) emerged (Dawood et al., 2009; Ginsberg et al., 2009). It replaced seasonal H1N1 viruses and continued to circulate together with H3N2 and influenza B viruses causing millions of infections per year (WHO, 2013).

A(H1N1)pdm09 virus is M2 ion channel blocker-resistant and neuraminidase (NA) inhibitor (NAI)-sensitive (Dawood et al., 2009; Gubareva et al., 2009). However, since its emergence several mutations were identified that resulted in reduced NAI susceptibility or even resistance (Nguyen et al., 2012). Moreover, A(H1N1)pdm09 virus lacks the 150-cavity that is typical of group 1 NA (Li et al., 2010) and presents a structurally new target for the development of new inhibitory compounds (Kirchmair et al., 2011). In addition, the virus has a natural resistance against the potential nucleoprotein inhibitor nucleozin (Kao et al., 2010). Thus, there is an urgent need for the development of new anti-influenza compounds.

The efficacy of novel potential drug candidates for treatment of mild as well as lethal A(H1N1)pdm09 infections has to be demonstrated *in vivo*. Influenza can be modeled in a wide range of animals like mice, ferrets, pigs, nonhuman primates, rats and cotton rats (Barnard, 2009). Due to the moderate costs of the animals as well as their caging and the comparability of the disease to human illness, mice represent a good compromise for anti-influenza studies. During establishment of antiviral mouse models the dynamics of infection of used virus strains has to be characterized quantitatively. Influenza dynamics depends on viral pathogenicity that can be influenced for example by the efficiency of binding to host receptors, induction of apoptosis, or the replication potential of the virus

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(Tscherne and Garcia-Sastre, 2011). In addition, the virus-induced host immune response can worsen the disease course. In particular, the pro-inflammatory immune response that comprise cytokine as well as chemokine release of infected cells and the attraction of leucocytes can affect negatively the dynamics of infection and contribute to a severe course of influenza (Arankalle et al., 2010; de Castro et al., 2010).

Viral titers (Baccam et al., 2009) or measurements of at least one of the immunological components like interferons, macrophages, NK cells, B cell, T cells (Hancioglu et al., 2006; Handel et al., 2010; Miao et al., 2010; Lee et al., 2009; Pawelek et al., 2012; Saenz et al., 2010) were used as experimental data for modeling influenza kinetics in different hosts with the aim to identify factors explaining the course of illness (Canini and Carrat, 2011) and/or differences in pathogenicity of influenza strains (Wattrang et al., 2003; Smith et al., 2011). As reviewed by Smith and Perelson (2011), there are an increasing number of influenza kinetics models based on viral load data. However, these authors also express their doubts about using viral load as the only indicator of disease severity because immunopathology is discussed as an additional factor in severe infection (de Castro et al., 2010). For example, Kumar et al. (2004) have shown that a strong late immune response can increase the risk of persistent inflammation even after clearing the pathogen. Interestingly, Smith and Perelson (2011) suggest that assigning a symptom score throughout the infection could offer a new perspective into the characteristics of an infection. A symptom score is easily attainable and reflects how sick a host is.

Both small-scale mathematical models (e.g., Saenz et al., 2010; Baccam et al., 2009) and complex models with more than 10 equations and more than 50 parameters (e.g., Hao et al., 2013; Hancioglu et al., 2006; Lee et al., 2009) have been proposed. However, complex models require an increased number and quality of experimental data to calibrate the model parameters. Small-scale models have the advantage to be applicable for quantitative comparison of a (also large) set of experiments (e.g., with different virus strains and/or therapeutic strategies) with a limited experimental effort.

The aim of the present study was to use the symptom score as an easily attainable data source and to develop a small-scale mathematical model of influenza dynamics. Virus pathogenicity, antiviral immune defense, and pro-inflammatory response resulting from viral pathogenicity as well as inflammation were considered as main model variables in a three differential equation model. In the present work, the dynamics of influenza induced by three different A(H1N1)pdm09 strains and one swine H1N2 strain in mice was modeled by a single model with 8 parameters on experimental data given by the symptoms score.

2. 2 Materials and methods

2.1. Cells and viruses

Madin-Darby canine kidney (MDCK) cells (Friedrich-Loeffer Institute, Riems, Germany) were maintained in Eagle's minimum essential medium (EMEM) supplemented with 100 U/ml penicillin and 100 U/ml streptomycin, 10% fetal bovine serum, and 2 mM L-glutamine.

The A(H1N1)pdm09 influenza virus strains A/Jena/5258/09 ('Jena/5258'), A/Jena/5555/09 ('Jena/5555') (Kirchmair et al., 2011; Durrwald et al., 2010), and A/Jena/2688/10 ('Jena/2688') were isolated in MDCK cells from respiratory specimen that originated from patients with clinical symptoms of influenza infection. The European swine H1N2 influenza virus A/swine/Bakum/1832/00 ('Bakum/1832') was obtained from a nasal swab of a diseased pig (Bauer et al., 2012; Schrader and Suss, 2003).

Virus titers were determined by titration of 10-fold serial dilutions on confluent MDCK cells. The 50% tissue culture infectious dose ($TCID_{50}$) was calculated according to Reed and Muench (1938). For isolation, titration and propagation of viruses EMEM formulated with 100 U/ml penicillin and streptomycin, 2 μg/ml trypsin, and 0.1% sodium bicarbonate was used (test medium).

2.2. Animal experiments and data

Experiments were performed in female BALB/c mice (16–18 g; Charles River, Bad Sulzfeld, Germany). After isoflurane anesthesia, five mice were inoculated intranasally with 10^6 $TCID_{50}$/20 μl of each virus in EMEM. Three mice were mock-infected for control. Body weight and clinical score were used as study parameters and monitored for 21 days after virus challenge. Mice that lost more than 25% of their initial body weight were sacrificed. A laboratory clinical score was used to assess the severity of disease. It ranged from 0 to 7: 0 – no changes, 1 – scrubby coat in the neck, 2 – scrubby coat in the neck and on the back, 3 – scrubby coat on whole body, incipient hunchbacked posture, 4 – scrubby coat, hunchbacked posture, incipient inactivity, eyes half closed, 5 – scrubby coat, hunchbacked posture, inactivity, eyes closed, 6 – scrubby coat, hunchbacked posture, completely inactive, eyes closed, 7 – mouse deceased. The mean values and standard deviation (*Std*) for the clinical score (S) as well as the percentage of body weight changes in comparison to day 0 were calculated.

3. Theory and calculations

3.1. Model

The mathematical model representing the within-host influenza dynamics consists of a system of three differential equations in which the dependent variables represent the virus pathogenicity (P), antiviral immune defense (D) including both the innate immune response and the adaptive immune response, and inflammation due to pro-inflammatory response (I). The mathematical equations of our reduced model are:

$$\frac{dP}{dt} = \alpha * P * \left(1 - \frac{P}{k_p}\right) - \beta * D * \frac{P}{P + 0.01} \quad (1)$$

$$\frac{dD}{dt} = y * P - \theta * D \quad (2)$$

$$\frac{dI}{dt} = \varepsilon * f(D) - \rho * I \quad (3)$$

$$f(D) = 1 + \tan\ h\frac{(D - \delta)}{\omega} \quad (4)$$

$$S = P + I \quad (5)$$

The virus pathogenicity P represents the virulence of the virus strains. The dynamics of change of P is described by Eq. (1). It depends on viral infection and the immune response of the host. The first term is parameterized by the virus infection rate (parameterized by α) and the maximum primary pathogenicity (parameterized by k_P). The second term of Eq. (1) represents the efficiency of the early immune response to the virus (parameterized by β). For very small values of P this second term goes to zero due to the Michaelis–Menten function parameterized by a small and fixed Michaelis–Menten constant 0.01 (smaller values or other functions that go to zero if P becomes zero do not change the model behavior) to avoid that P becomes negative.

The antiviral defense is modeled by the variable D including both the innate immune response and the adaptive immune response. Although the defense system is very complex, in the small-scale model the change of the defense system is modeled in Eq. (2) by only