

# Modelling within-host macrophage dynamics in influenza virus infection

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## Abstract

Human respiratory disease associated with influenza virus infection is of significant public health concern. Macrophages, as part of the front line of host innate cellular defence, have been shown to play an important role in controlling viral replication. However, fatal outcomes of infection, as evidenced in patients infected with highly pathogenic viral strains, are often associated with prompt activation and excessive accumulation of macrophages. Activated macrophages can produce a large amount of pro-inflammatory cytokines, which leads to severe symptoms and at times death. However, the mechanism for rapid activation and excessive accumulation of macrophages during infection remains unclear. It has been suggested that the phenomena may arise from complex interactions between macrophages and influenza virus. In this work, we develop a novel mathematical model to study the relationship between the level of macrophage activation and the level of viral shedding in influenza virus infection. Our model combines a dynamic model of viral infection, a dynamic model of macrophages and the essential interactions between the virus and macrophages. Our model predicts that the level of macrophage activation can be negatively correlated with the level of viral shedding when viral infectivity is sufficiently high. We further identify that temporary depletion of resting macrophages in response to viral infection is a major driver in our model for the negative relationship between macrophage activation and viral shedding, providing new insight into the mechanisms that regulate macrophage activation. Our model serves as a framework to study the complex dynamics of virus-macrophage interactions and provides a

mechanistic explanation for existing experimental observations, contributing to an enhanced understanding of the role of macrophages in influenza viral infection.

11 *Keywords:* Mathematical modelling, Influenza virus, Macrophage

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## 12 **1. Introduction**

13 Influenza is a contagious respiratory disease caused by influenza viruses.  
14 Infection with influenza A virus (IAV) in particular remains as a major public  
15 health concern, resulting in heavy morbidity worldwide every year [1].  
16 Epithelial cells, which line the upper respiratory tract (URT) of the host, are  
17 the primary target cells for influenza virus infection [2, 3], and virus-induced  
18 cell damage is often thought to be the main cause for clinical symptoms and  
19 a determinant of virulence [4, 5, 6, 7]. During an infection, host immunity  
20 plays an important role for viral resolution and host recovery. The innate  
21 (or nonspecific) immune system is the first and primary defence mechanism  
22 that is triggered upon detection of an IAV infection. Macrophages, as part  
23 of the innate immune cellular response, are activated at the early stages of  
24 infection [8, 9, 10]. They perform two important antiviral functions. One  
25 is the uptake of viruses mediated by the interaction of pattern-recognition  
26 receptors (PRRs), such as the Toll-like receptors (TLRs), in macrophages  
27 with pathogen-associated molecular patterns (PAMPs) on the virus, and the  
28 phagocytosis of apoptotic virus-infected cells [11, 12, 13, 14]. The other is the  
29 secretion of cytokines and chemokines, such as tumor necrosis factor-alpha  
30 (TNF- $\alpha$ ), interleukins-6 (IL-6) and interferons (IFNs), by which macrophages  
31 can modulate inflammatory responses and help trigger an adaptive immune  
32 response, attracting effector cells to the site of infection. [15, 16, 17].

33 Macrophages are highly heterogeneous in the host and can alter their  
34 phenotypes and functions rapidly in response to local stimuli [18, 19, 20]. In  
35 response to a viral infection, resting macrophages are activated and give rise  
36 to two major types of macrophages, denoted  $M_1$  and  $M_2$  in terms of function-  
37 ality [21].  $M_1$  macrophages have a stronger capability to engulf free virions,  
38 present antigens to other immune cells and produce pro-inflammatory cy-  
39 tokines which contribute to both host inflammatory responses and pathogen  
40 clearance [18]. In contrast,  $M_2$  macrophages primarily secrete anti-inflammatory  
41 cytokines to mitigate inflammation and maintain host homeostasis [22]. While  
42 viral infection-induced cell death and tissue damage are thought to be the

43 primary contributors to host morbidity, further evidence has shown that  
44 over-expression of pro-inflammatory cytokines and chemokines mediated by  
45 activated macrophages may also be a cause for lung pathology [23, 24, 25,  
46 26, 24, 27]. Unregulated pulmonary infiltration of macrophages is often the  
47 hallmark of severe influenza virus infection [28, 29, 30], as reviewed in [31].  
48 For instance, mice infected with highly pathogenic (HP) influenza virus ex-  
49 perience rapid infiltration of macrophages and excessive accumulation of  
50 macrophages during infection, showing fatal infection results with a high  
51 level of viral shedding. These outcomes were not observed in mice infected  
52 with low virulent strains [32]. The observations suggest a positive correlation  
53 between the level of macrophage activation and the level of viral shedding.  
54 However, since the interactions between different types of macrophages and  
55 between macrophages and influenza virus involve both positive and negative  
56 feedback mechanisms (see review [33]), it is not clear how this relationship  
57 can arise from a dynamical system of virus-macrophage interactions and un-  
58 der what condition(s) such a relationship may no longer be valid. In this  
59 paper, we study these interactions using a mathematical model.

60 Mathematical models have been used to explore macrophage dynamics  
61 in different pathological environments, e.g., in bacterial infection [34, 35]  
62 (particularly in tuberculosis infection (TB) [36]) and for tumors [37]. Some  
63 models in the literature have been used to specifically investigate the inter-  
64 actions between  $M_1$  and  $M_2$  macrophages [38, 39]. Models have also been  
65 used to study within-host influenza virus dynamics, many of which have been  
66 designed to explore how the viral load kinetics is modulated by different im-  
67 munological factors (see reviews [40, 41, 42]). However, in the literature,  
68 there are no influenza virus infection models which explicitly include both  
69  $M_1$  and  $M_2$  macrophage dynamics as part of the innate immune responses.

70 In this paper, we develop a novel mathematical model, which combines a  
71 dynamic model of influenza virus infection, a dynamic model of macrophages  
72 and the essential interactions between virus and macrophages. We use the  
73 model to explore the dynamics of macrophages in response to influenza viral  
74 infection and investigate how the level of macrophage activation is influenced  
75 by the viral infectivity (which is a critical parameter determining the level  
76 of viral shedding). Our aim is to explore in detail possible explanation(s) for  
77 the mechanism determining the aforementioned relationship between viral  
78 shedding and macrophage activation. Finally we discuss our findings and  
79 the biological implications of our model results.

## 80 2. Methods

81 We first introduce a dynamic model of macrophages, which incorporates  
82 three distinct populations and the conversion processes between them, in the  
83 absence of viral infection. We then incorporate the macrophage model into an  
84 influenza viral infection model which captures the minimal essential processes  
85 to describe IAV kinetics, including viral multiplication via the infection of  
86 epithelial cells and viral resolution by antibodies.

### 87 2.1. A dynamical model of macrophages

88 The model of macrophage dynamics contains three macrophage popula-  
89 tions:  $M_1$  (so called “classically activated” macrophages),  $M_2$  (so called “al-  
90 ternatively activated” macrophages) [21] and resting macrophages  $M$  which  
91 have low efficiency to present antigen and produce cytokines. Resting macrophages  
92 can convert into either  $M_1$  or  $M_2$  macrophages in response to different stimuli  
93 [21, 36]. A diagram representing the model is shown in Fig. 1.

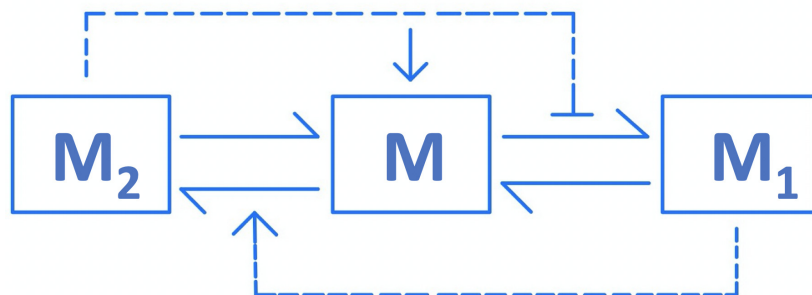


Figure 1: **Macrophage dynamics in the absence of viral infection.** The dashed arrow line indicates that the presence of  $M_1$  promotes the conversion process of  $M$  to  $M_2$ . The dashed-bar line denotes suppression of the process of  $M$  to  $M_1$  due to  $M_2$ . The solid full-arrow line denotes recruitment of  $M$ , and solid half-arrow lines denote the conversion processes among macrophages.

94 In the absence of infection,  $M$  macrophages can be converted into  $M_1$   
95 in response to apoptotic cells as well as tissue debris, and  $M_1$  macrophages  
96 stimulate the secretion of pro-inflammatory cytokines, e.g.  $\text{TNF-}\alpha$  and  $\text{IFN-}\gamma$   
97  $\gamma$  [43], which subsequently reinforce the activation process in an autocrine  
98 or paracrine manner [44].  $M$  macrophages can also be converted into  $M_2$   
99 macrophages stimulating the secretion of anti-inflammatory cytokines, e.g.

100 interleukin-10 (IL-10), to mitigate the host inflammatory response and main-  
101 tain homeostasis [19]. The conversion processes are modelled by a set of  
102 ordinary differential equations (ODEs):

$$\begin{aligned} \frac{dM}{dt} = & g \left( 1 - \frac{M + M_1 + M_2}{M_0} \right) M - \frac{k_1}{1 + s_1 \frac{M_2}{M_0}} M - k_2 \left( 1 + s_2 \frac{M_1}{M_0} \right) M \\ & + k_{-2} M_2 + k_{-1} M_1, \end{aligned} \quad (1)$$

$$\frac{dM_1}{dt} = \frac{k_1}{1 + s_1 \frac{M_2}{M_0}} M - k_{-1} M_1 - \delta M_1, \quad (2)$$

$$\frac{dM_2}{dt} = k_2 \left( 1 + s_2 \frac{M_1}{M_0} \right) M - k_{-2} M_2 - \delta M_2. \quad (3)$$

103 Eq. 1 describes the rate of change of resting macrophages  $M$ . It is gov-  
104 erned by five processes (corresponding to the five terms on the righthand side  
105 of Eq. 1).  $M$  are produced at a rate  $g(1 - (M + M_1 + M_2)/M_0)M$  mimicking  
106 a logistic growth model. To phenomenologically capture the established reg-  
107 ulatory effects of  $M_1$  and  $M_2$  [23] as reviewed in [21], the rate of conversion  
108 from  $M$  to  $M_1$  is modelled by a decreasing function of  $M_2$  with a maximum  
109 of  $k_1$  (see the second term on the righthand side of Eq. 1) and the rate of  
110 conversion from  $M$  to  $M_2$  is modelled by an increasing function of  $M_1$  with  
111 a minimum of  $k_2$  (see the third term on the righthand side of Eq. 1). The  
112 parameters  $s_1$  and  $s_2$  modulate the dependence of the conversion rates on the  
113 number of activated macrophages.  $M_1$  and  $M_2$  macrophages return to the  
114 resting state  $M$  at rate  $k_{-1}$  and  $k_{-2}$  when stimuli are diminished, respectively  
115 [45].

116 Eq. 2 and Eq. 3 model the dynamics of  $M_1$  and  $M_2$ , respectively. In  
117 addition to the terms describing the conversion between  $M$  and  $M_1$  (i.e. the  
118 first and second terms on the righthand side of Eq. 2) and between  $M$  and  $M_2$   
119 (i.e. the first and second terms on the righthand side of Eq. 3), macrophages  
120  $M_1$  and  $M_2$  decay naturally at rate  $\delta$ .

## 121 2.2. A model coupling macrophage dynamics and viral infection dynamics

122 Upon detection of virus, resting macrophages  $M$  are promptly activated  
123 and converted into  $M_1$  via Toll-like receptor (TLR)-dependent signalling  
124 pathways, and strong inflammatory responses are initiated [14]. A wide  
125 range of inflammatory cytokines and chemokines, such as tumor necrosis

126 factor-alpha (TNF- $\alpha$ ), interleukins-6 (IL-6) and interferons (IFNs), are se-  
 127 creted by proinflammatory macrophages  $M_1$  and lead to the recruitment of  
 128 more immune cells to the site of infection [46]. Activated macrophages have  
 129 been shown to have a stronger capacity for phagocytosis of apoptotic cells  
 130 and antigen presentation compared to those in an inactivated state [47].

131 Macrophages are activated in response to viral infection and are crucial  
 132 in providing negative feedback to viral reproduction. For example, activated  
 133  $M_1$  macrophages uptake free virions and prime various adaptive immune re-  
 134 sponses [16, 21]. Here we propose a model to capture the essential regulatory  
 135 processes between macrophages and influenza virus. A diagram representing  
 136 the model is shown in Fig. 2, and the processes are modelled by a system of  
 137 ODEs:

$$\frac{dT}{dt} = -\beta TV, \quad (4)$$

$$\frac{dI}{dt} = \beta TV - \delta_I I, \quad (5)$$

$$\frac{dV}{dt} = pI - cV - \kappa M_1 V - \kappa_a AV, \quad (6)$$

$$\begin{aligned} \frac{dM}{dt} = & g \left( 1 - \frac{M + M_1 + M_2}{M_0} \right) M - \frac{k_1}{1 + s_1 \frac{M_2}{M_0}} M - k_2 \left( 1 + s_2 \frac{M_1}{M_0} \right) M \\ & + k_{-2} M_2 + k_{-1} M_1 - q_1 IM - q_2 VM, \end{aligned} \quad (7)$$

$$\frac{dM_1}{dt} = \frac{k_1}{1 + s_1 \frac{M_2}{M_0}} M - k_{-1} M_1 - \delta M_1 + q_1 IM + q_2 VM, \quad (8)$$

$$\frac{dM_2}{dt} = k_2 \left( 1 + s_2 \frac{M_1}{M_0} \right) M - k_{-2} M_2 - \delta M_2, \quad (9)$$

$$\frac{dA}{dt} = \mu M_1 + \rho \left( 1 - \frac{A}{A^*} \right) A. \quad (10)$$

138 Eqs. 4–6, proposed based on the classic target cell-infected cell-virus  
 139 (TIV) model, describe the essential dynamics of virus turnover through the  
 140 infection of target cells and the resolution of infection by immune responses.  
 141 In detail, target cells ( $T$ ; i.e. epithelial cells in influenza infection) are in-  
 142 fected with virus ( $V$ ) and become infected cells ( $I$ ) at a rate  $\beta V$ . Infected  
 143 cells produce and release viral progenies (at a rate  $p$ ) which invade target cells  
 144 leading to further infection. Free virus ( $V$ ) decays due to three processes:

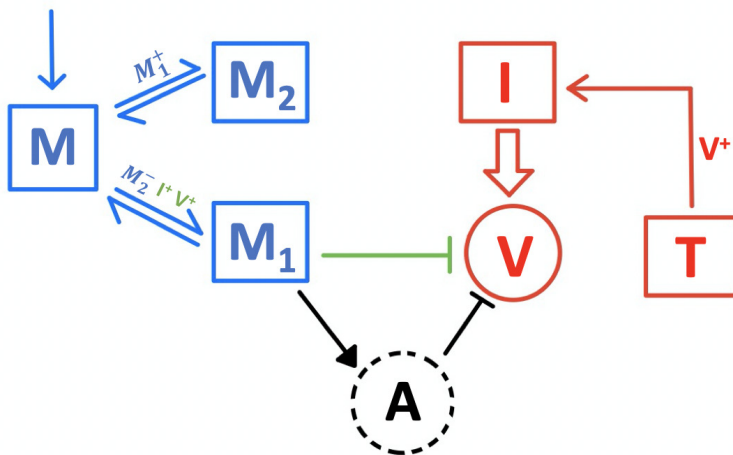


Figure 2: **Macrophage dynamics in the presence of viral infection.** Resting macrophages ( $M$ ) replenish their population in the system (blue arrow towards  $M$ ) and are activated into either pro-inflammatory macrophages  $M_1$ , or anti-inflammatory macrophages  $M_2$  (blue arrows from  $M$  to either  $M_1$  or  $M_2$ ). The activated macrophages return back to  $M$  (as indicated by the blue arrows from  $M_1/M_2$  to  $M$ ).  $M_1^+$  indicates that  $M_1$  increases the rate of conversion from  $M$  to  $M_1$ . In a similar way,  $M_2^-$ ,  $V^+$  and  $I^+$  indicate that the rate of conversion from  $M$  to  $M_1$  is suppressed by  $M_2$  but enhanced by virus  $V$  and infected cells  $I$ . Virus ( $V$ ) infects epithelial cells ( $T$ ), which become infected cells ( $I$ ) (indicated by the red solid arrow with  $V^+$ ), and infected cells ( $I$ ) produce virus (large arrow).  $M_1$  internalise free virions (green solid bar line), and stimulate adaptive immunity (black solid full-triangle line) in which antibodies ( $A$ ) are produced, by which virions are neutralised (black solid bar line).

145 natural decay at a rate  $c$ , internalisation by activated macrophages  $M_1$  at a  
 146 rate  $\kappa M_1$  and neutralisation by antibodies at a rate  $\kappa_a A$ . Infected cells ( $I$ )  
 147 die naturally at a rate  $\delta_I$ .

148 Eqs. 7–9 are adapted from the macrophage model (Eqs. 1–3) with some  
 149 additional terms capturing the effect of infected cells and virus on the con-  
 150 versions from  $M$  to  $M_1$  and  $M_2$ . For example, the term  $q_1 I M$  models the  
 151 conversion of resting macrophages  $M$  to  $M_1$  due to the presence of various  
 152 infected cell-producing cytokines [48, 49]. The term  $q_2 V M$  models virus-  
 153 induced macrophage activation via TLR-dependent pathways [16].

154 Eq. 10 models the activation and expansion of adaptive immune re-

155 sponses, in particular the production of antibodies, which are responsible  
156 for clearing virus at the late stages of infection, providing long-term pro-  
157 tection. In detail, the production of antibodies ( $A$ ) is phenomenologically  
158 modelled by a logistic growth model (i.e. the second term on the righthand  
159 side of Eq. 10) with a growth rate  $\rho$  and a carrying capacity  $A^*$ , coupled with  
160 a “trigger” term due to antigen presentation,  $\mu M$ , which assumes that the  
161 strength of triggering the adaptive immune response is proportional to the  
162 level of activated macrophage  $M_1$ .

### 163 *2.3. Model parameters*

164 The values of model parameters are given in Table 1. The parameter  
165 values and initial conditions for influenza viral dynamics (such as  $p$ ,  $\delta_I$ ,  $T(0)$ ,  
166  $I(0)$  and  $V(0)$ ) are chosen from the study in [50], in which the authors fitted  
167 the TIV model to a set of data from humans infected with A/H1N1 virus.  
168 The parameter  $\beta$  is estimated and chosen from the literature such that the  
169 viral load shows at least a three-fold increase in infection [51]. To the best of  
170 our knowledge, the values for the model parameters which govern either the  
171 macrophage dynamics (such as  $M_0$ ,  $k_1$ ,  $k_{-1}$ ,  $k_2$  and  $k_{-2}$ ) or the influenza virus-  
172 macrophage interactions (such as  $q_1$  and  $q_2$ ), are not available. Therefore,  
173 we choose those parameter values from [52] in which macrophage dynamics  
174 are investigated in a tumour environment. We assume macrophages ( $M_1$  and  
175  $M_2$ ) have comparable impact on each other and set  $s_1 = s_2 = 1$ . Also, the  
176 assumed carrying capacity for antibodies ( $A^*$ ) during infection is chosen such  
177 that virus can be efficiently cleared.

### 178 *2.4. Numerical simulation methods*

179 The ordinary differential equations are solved using the ode solver *ode15s*  
180 in MATLAB R2019b with a relative tolerance of  $1 \times 10^{-5}$  and an absolute  
181 tolerance of  $1 \times 10^{-10}$ . The initial values for the target cell  $T$ , the infected cell  
182  $I$  and the virus  $V$  are given in Table 1. The initial values for the macrophage  
183 populations  $M$ ,  $M_1$  and  $M_2$  are given by the virus-free steady state and are  
184 obtained by numerically integrating Eqs. 1–3 (using *ode15s* in MATLAB) for  
185 a sufficiently long time interval (see Fig. S1), and choosing the values of  $M$ ,  
186  $M_1$  and  $M_2$  at the final time point. MATLAB code to produce all the figures  
187 in this study can be found at <https://github.com/keli5734/Matlab-Code>.



### 188 3. Results

#### 189 3.1. Dynamics of macrophages and viral shedding

190 The simulated time series of viral load ( $V$ ) and two populations of acti-  
191 vated macrophages ( $M_1$  and  $M_2$ ) are shown in Fig. 3A (model solutions for  
192 other variables are given in Fig. S2 in the *Supplementary Material 1*). The  
193 viral load curve shows a three-phase shape—an exponential growth followed  
194 by a slow decay (or a plateau) and finally a rapid decay to viral resolution—  
195 typical of observed infection data and simulations of *in vivo* influenza infec-  
196 tion [50, 51, 53, 54, 55]. In response to viral infection, activated macrophages  
197  $M_1$  undergo a rapid increase followed by a decrease (Fig. 3A red solid line),  
198 while  $M_2$  macrophages experience a decline followed by a replenishment (Fig.  
199 3A red dashed line). The different behaviours of  $M_1$  and  $M_2$  are due to com-  
200 petition for the limited resource (i.e. resting macrophage  $M$ ). There is a  
201 dramatic increase in the conversion from  $M$  to  $M_1$  induced by the initial ex-  
202ponential viral growth that rapidly consumes  $M$  (see Fig. S2) which in turn  
203reduces the conversion from  $M$  to  $M_2$ .  $M_1$  and  $M_2$  gradually return to their  
204homeostatic state upon the resolution of infection (after approximately day  
20512 in Fig. 3A).

206 To better understand how the viral load is influenced by macrophages, we  
207 present the time series of the four terms on the righthand side of Eq. 6 (Fig.  
208 3B). These four time-series represent the four major processes determining  
209 the rate change of viral load ( $dV/dt$ ) in the model and include viral produc-  
210 tion ( $pI$ ), natural death ( $cV$ ), internalisation by  $M_1$  macrophage ( $\kappa M_1 V$ )  
211 and neutralisation by antibodies ( $\kappa_a AV$ ). The macrophage-mediated innate  
212 immune response (dash-dotted line) plays a dominant role in controlling viral  
213 replication before antibody takes over on approximately day 9 in the model.  
214 A qualitatively similar model behaviour was observed in [51] where the innate  
215 immune response was assumed to be mediated by interferon.

#### 216 3.2. Relationship between the level of macrophage activation and the level of 217 viral shedding

218 Having examined the time-series behaviour of viral shedding and  $M_1$   
219 macrophages, we now examine the relationship between the level of macrophage  
220 activation and the level of viral shedding. The level of macrophage activation  
221 is assumed to be the cumulative number of  $M_1$  macrophages because of their  
222 key role in producing massive pro-inflammatory cytokines in influenza virus

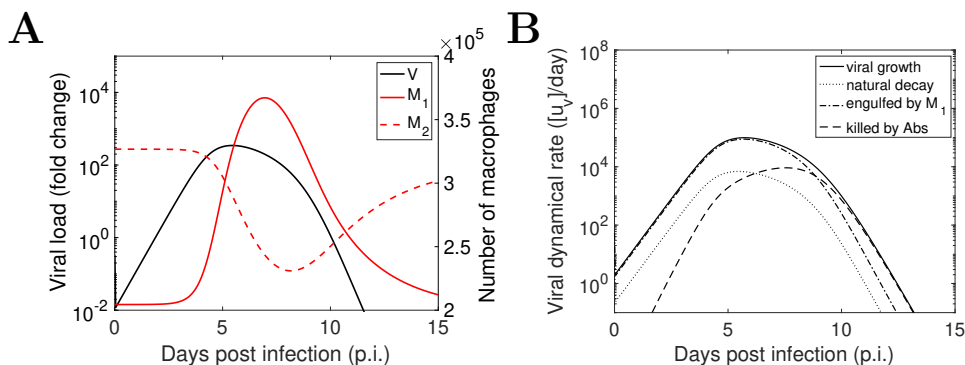


Figure 3: **Model simulation results of viral shedding kinetics and macrophage dynamics in infection using the parameters values in Table 1.** (A) Viral shedding dynamics (Eq. 6; black solid line), pro-inflammatory macrophages  $M_1$  (Eq. 8; red solid line) and anti-inflammatory macrophages  $M_2$  (Eq. 9; red dashed line). (B) The rate of change of the components on the right-hand side of  $dV/dt$  (Eq. 6), which are the rates of viral growth  $pI$  (solid line), viral natural decay  $cV$  (dot line), virus engulfed by  $M_1$  macrophages  $\kappa M_1 V$  (dash-dotted line), and virus neutralized by antibodies  $\kappa_a AV$  (dashed line).

223 infection [56], and we quantify the cumulative number by the area under the  
 224  $M_1$  time-series curve ( $AUC_{M_1}$ )

$$AUC_{M_1} = \int_0^{\tau} M_1(t) dt,$$

225 where  $\tau$  is a cut-off day for computation. The level of viral shedding is  
 226 assumed in the model to be the cumulative viral load, which has been con-  
 227 sidered as a surrogate for viral infectiousness of influenza infection [57] and  
 228 an important marker for viral pathogenicity [58, 59]. It is quantified by the  
 229 area under the viral load time-series curve ( $AUC_V$ )

$$AUC_V = \int_0^{\tau} V(t) dt.$$

230 In this study, we set  $\tau = 15$  which is an appropriate value to cover both  
 231 the duration of viral infection and the duration of macrophage activation as  
 232 shown in [32].

233 Fig. 4 shows the relationship between  $AUC_{M_1}$  and the  $AUC_V$  as viral in-  
234fectivity (model parameter  $\beta$ ) varies. We chose to vary the viral infectivity  
235because it is a key parameter determining the ability of virus to cause infec-  
236tion. We see that for intermediate values of  $\beta$ , the  $AUC_{M_1}$  and the  $AUC_V$  are  
237positively correlated (e.g. in Region II; the definition of the regions are pro-  
238vided in the caption of Fig. 4), consistent with experimental observation [32].  
239However, we also identify in the model a region where the two quantities are  
240negatively correlated for relatively high  $\beta$  (i.e. Region III), which suggests  
241that a highly pathogenic virus strain may cause a compromised activation of  
242 $M_1$  macrophages while maintaining a high level of viral shedding. In addi-  
243tion, for very small  $\beta$  (i.e. in Region I), viral infection cannot be established  
244because the basic viral reproduction number (provided in the caption of Fig.  
2454) is less than the infection threshold 1.

246 It is unclear from Fig. 4 why the negative relationship between the  $AUC_{M_1}$   
247and the  $AUC_V$  in region III arises, so we examine the time series for the viral  
248load and  $M_1$  macrophages. Fig. 5A and 5B show the time series of viral load  
249and different types macrophages for  $\beta = 6.08 \times 10^{-5}$  which is the critical  
250value separating regions II and III. Fig. 5C and 5D show similar time series  
251for a  $\beta$  value inside the region III (i.e.  $\beta = 10.08 \times 10^{-5}$ ). Although the viral  
252load curve for the larger  $\beta$  exhibits a shorter duration of infection compared  
253to that for the smaller  $\beta$  (Fig. 5C v.s. Fig. 5A), it also exhibits a higher peak  
254value such that a higher  $AUC_V$  is possible. In contrast, the  $M_1$  macrophage  
255curve for the larger  $\beta$  exhibits both a lower peak value and a shorter duration  
256of activation—quickly reaching a peak and declining—compared to that for  
257the smaller  $\beta$  (Fig. 5D v.s. Fig. 5B), which explains the decrease in  $AUC_{M_1}$ .  
258 In the next section, we will explore the mechanism(s) leading to the reduction  
259in  $AUC_{M_1}$ .

### 260 3.3. Temporary depletion of $M$ is a mechanism driving the decrease of $AUC_{M_1}$ 261 in region III

262 Since the production of  $M_1$  macrophages is fundamentally driven by the  
263conversion of resting macrophages  $M$  in the model (shown in Fig. 1), we  
264hypothesise that the decrease in  $AUC_{M_1}$  in region III might be attributed to  
265a more severe (albeit temporary) depletion of  $M$ , which is partly supported  
266by Fig. 5B and 5D where the level of  $M$  is driven lower and earlier for a  
267larger  $\beta$ .

268 To investigate our hypothesis, we increase the regrowth rate of  $M$  (i.e.  
269the model parameter  $g$ ), which should mitigate the extent of  $M$  depletion

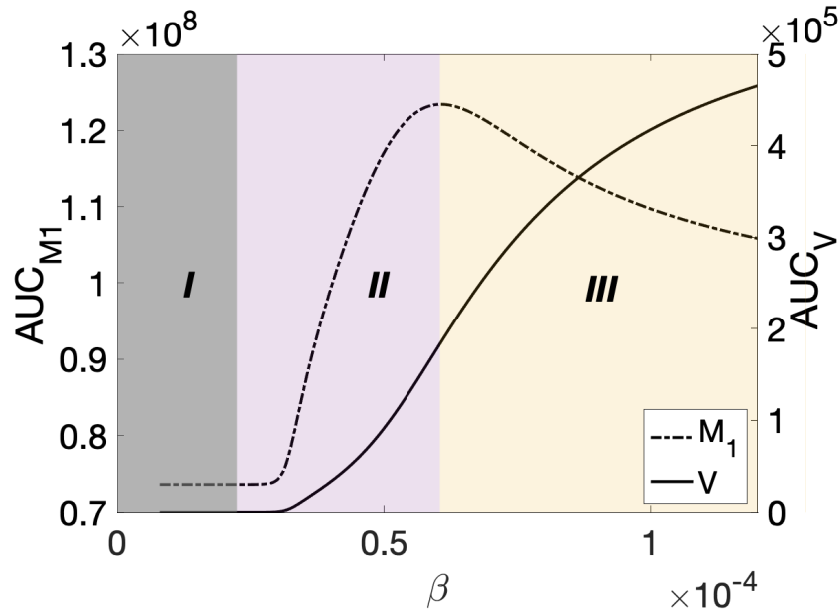


Figure 4: **Simulation results of the change of  $AUC_{M_1}$  (dash-dotted line) and  $AUC_V$  (solid line) to the modulation of viral infectivity  $\beta \in [8 \times 10^{-6}, 1.2 \times 10^{-4}]$ .** We classify the results into three regions. Regions I and II are separated by the viral basic reproduction number which is given by  $R_{V,0} = p\beta T(0)/(\delta_I(c + \kappa M_1(0) + \kappa_a A(0)))$ . In region I,  $R_{V,0} < 1$ . At the boundary between regions I and II,  $R_{V,0} = 1$ . In regions II and III,  $R_{V,0} > 1$ . The boundary between regions II and III is determined by a change in the correlation between  $AUC_{M_1}$  and  $AUC_V$ . In region II these two areas are positively correlated whereas in region III they are negatively correlated.

270 by increasing both the rate of  $M$  replenishment and the initial number of  $M$   
 271 macrophages (i.e. the homeostatic state, see Fig. 6A). As seen in Fig. 6B, as  $g$   
 272 increases, region III shrinks while both region I and region II expand. Fig. 6C  
 273 provides a more detailed view of how the regions shift for two selected values  
 274 of  $g$  (in particular the expansion of region II at the expense of a shrinking  
 275 region III). These results show that mitigating  $M$  depletion (by increasing  
 276 the regrowth rate  $g$  of  $M$  macrophages in the model) can turn a decreasing  
 277  $AUC_{M_1}$  to increasing for a range of  $\beta$ , confirming our hypothesis that a  
 278 temporary depletion of  $M$  is a mechanism driving the decrease of  $AUC_{M_1}$   
 279 in region III.

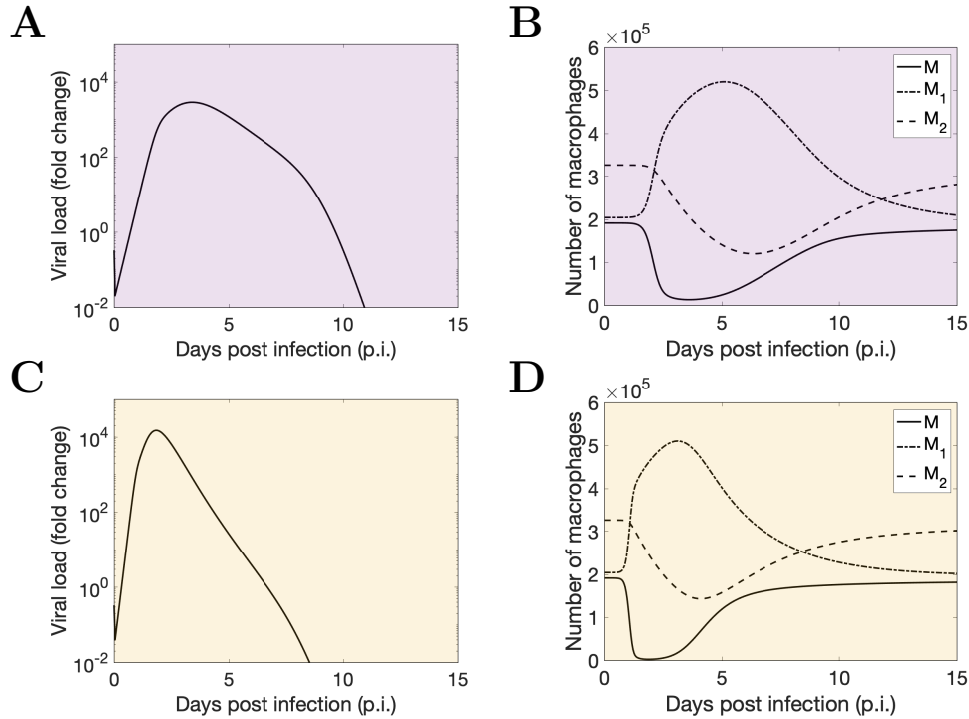


Figure 5: **Model simulation results of macrophage dynamics and viral shedding kinetics with different viral infectivity  $\beta$  in different regions.** First row (A and B): viral shedding kinetics, and the dynamics of  $M$  macrophages (solid line),  $M_1$  macrophages (dash-dotted line) and  $M_2$  macrophages (dashed line) in region II ( $\beta = 6.08 \times 10^{-5}$ ). Second row (C and D): viral shedding kinetics and macrophage dynamics in region III ( $\beta = 10.08 \times 10^{-5}$ ).

280 *3.4. Dependence of the  $AUC_V$ - $AUC_{M_1}$  relationship on other model parameters*  
 281

282 So far we have varied the viral infectivity  $\beta$ , as a means to examine the re-  
 283 lationship between the level of viral shedding and the level of  $M_1$  macrophage  
 284 activation. We now examine whether our results and conclusions are robust  
 285 to a change in other virus-related parameters. For example, we vary the vi-  
 286 ral production rate  $p$  and produce a series of figures similar to Figs. 4–6 (see  
 287 Figs. S3–S5 in *Supplementary Material 1*). We find that the results are qual-  
 288 itatively the same as those for varying  $\beta$ , in particular the existence of region

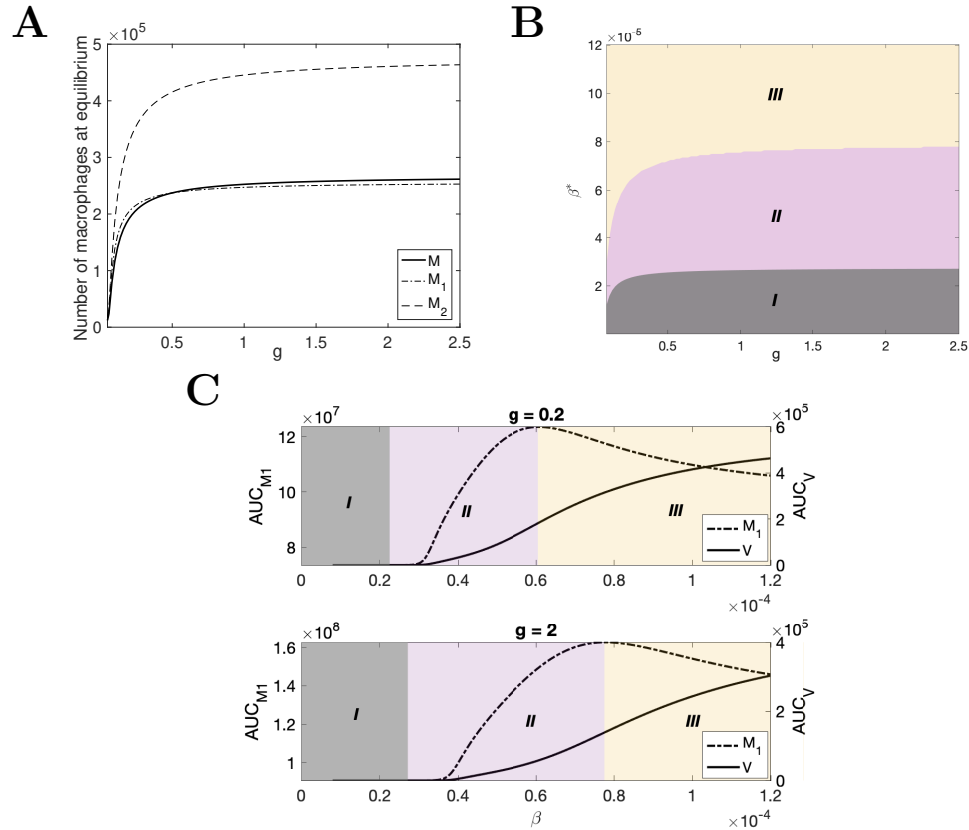


Figure 6: **Model simulation results of macrophages,  $AUC_{M1}$  (dashed line) and  $AUC_V$  (solid line) as functions of  $M$  regrowth rate  $g$ .** (A) The dependence of initial values of macrophage populations on the  $M$  regrowth rate  $g$ .  $g$  is varied from 0.02 to 2.5. Note that the numbers of macrophages become saturated for relatively large  $g$ . We show mathematically that there always exists a maximum capacity for  $M$  as  $g \rightarrow \infty$  (see *Supplementary Materials 2* for detail). (B) shows how regions I, II and III change as  $g$  increases. (C) The dependence of the  $AUC_{M1}$  and the  $AUC_V$  on  $\beta$  for two selected values of  $g$  (i.e.  $g = 0.2$  and  $g = 2$ ).  $\beta \in [8 \times 10^{-6}, 1.2 \times 10^{-4}]$ .

289 III (see Fig. S3) and the observed reduction in region III when mitigating  
 290 depletion of  $M$  by increasing the regrowth rate  $g$  (see Fig. S5). Varying  $\kappa$   
 291 (the rate of viral engulfment by  $M_1$ ) yields similar results (see Figs. S6–S8;

292 note that the effect of decreasing  $\kappa$  is similar to that of increasing  $\beta$  or  $p$   
 293 because of the antagonistic processes described by the model).

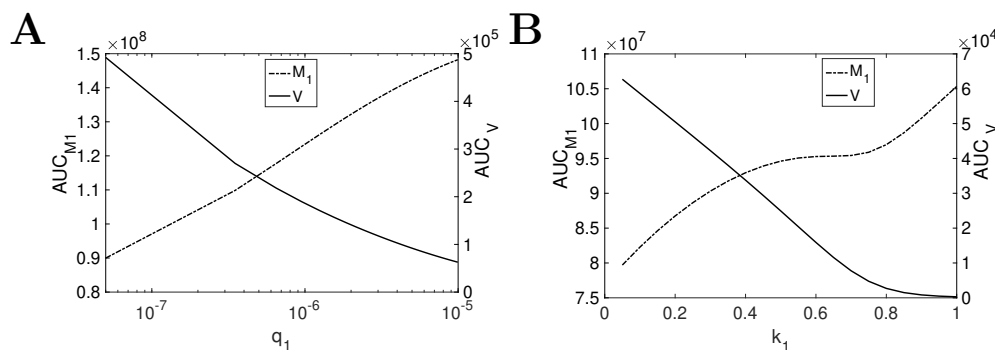


Figure 7: **Model simulation results of  $AUC_{M1}$  (dashed line) and  $AUC_V$  (solid line) as functions of  $q_1$  and  $k_1$ , respectively.** (A) The dependence of the  $AUC_{M1}$  and the  $AUC_V$  on the virus-induced macrophage activation rate  $q_1$ .  $q_1$  is varied from  $3 \times 10^{-8}$  to  $1 \times 10^{-5}$ . (B) The change of the  $AUC_{M1}$  and the  $AUC_V$  to modulation of the proinflammatory macrophage activation rate  $k_1 \in [0.02, 1]$ . The  $AUC_{M1}$  is indicated by dashed line, and the  $AUC_V$  is shown in solid line.

294 Since experimental studies [26, 27, 29, 30] have suggested highly pathogenic  
 295 influenza virus strains can infect macrophages and modulate the rate of cy-  
 296 tokine production, resulting in rapid macrophage infiltration and strong in-  
 297 flammatory response, we further examine the effect of the rate of  $M_1$  acti-  
 298 vation, either virus-induced ( $q_1$ ) or virus-independent ( $k_1$ ), on the  $AUC_V$ -  
 299  $AUC_{M1}$  relationship. Fig. 7A shows that increasing  $q_1$  leads to an increase  
 300 in  $AUC_{M1}$  but a decrease in  $AUC_V$ . A similar result is observed for increas-  
 301 ing  $k_1$  (Fig. 7B). The negative relationship between  $AUC_V$  and  $AUC_{M1}$  in  
 302 response to a change in the rate of  $M_1$  activation in the model may imply  
 303 that a viral mutation conveying a change in the rate of  $M_1$  activation will  
 304 result in either compromised viral shedding or compromised macrophage re-  
 305 sponse and therefore cannot solely explain the aforementioned observation  
 306 that highly pathogenic influenza virus strains can induce both a higher cell  
 307 infiltration and a higher level of viral shedding than less pathogenic strains.

## 308 4. Discussion

309 In this work, we have studied the relationship between viral shedding  
310 and macrophage activation during influenza virus infection through numeri-  
311 cal analysis of a mathematical model which integrates viral infection dynam-  
312 ics, macrophage dynamics and the essential interactions between virus and  
313 macrophages. We find based on the model that viruses with a higher ability  
314 to cause infection (e.g. a higher viral infectivity  $\beta$  or a higher production  
315 rate  $p$  in the model) will always lead to a higher level of viral shedding (i.e.  
316 a higher  $AUC_V$ ) but not necessarily a higher level of macrophage activation  
317 (i.e. a higher  $AUC_{M1}$ ). For an intermediate range of viral infectivity, the  
318 level of viral shedding and the level of macrophage activation are positively  
319 correlated (shown in Fig. 4; region II), which has been observed in avian IAV  
320 infection [32, 60, 61, 62]. But when the viral infectivity becomes sufficiently  
321 high, the level of macrophage activation declines, leading to an unexpected  
322 negative correlation with the level of viral shedding (shown in Fig. 4; region  
323 III), which is then shown to be caused by a temporary depletion of resting  
324 macrophages  $M$ . Our findings not only suggest that a higher viral shedding  
325 may not be accompanied by a higher macrophage response but also high-  
326 light the importance of the pool size of resting macrophages in modulating  
327 the pro-inflammatory response.

328 To the best of our knowledge, our model is the first work to incorporate  
329 the dynamics of heterogeneous macrophage populations into a model of in-  
330 fluenza viral dynamics. Although no macrophage data in the literature can  
331 be used to directly test our model results, there is some indirect evidence from  
332 cytokine data to support our findings. For example, activated macrophages  
333  $M_1$  can secrete a large amount of cytokines such as interleukin 6 (IL-6) and  
334 tumor necrosis factor (TNF) [21, 63], and evidence from experimental stud-  
335 ies [12, 64, 65] has shown that the level of those pro-inflammatory cytokines  
336 normally rises in the early days of influenza infection and then gradually  
337 decreases afterwards—a kinetic behaviour consistent with the kinetics of  $M_1$   
338 macrophages predicted by our model. Furthermore, alternatively activated  
339 macrophages  $M_2$  are responsible for producing anti-inflammatory cytokines,  
340 such as IL-4 and IL-10, and the time series data of IL-4 from [66] (in which  
341 mice are experimentally inoculated influenza A/PR/8/34 H1N1 virus) show  
342 qualitatively similar kinetics to that of the  $M_2$  macrophages produced by  
343 our model (i.e. an initial decrease followed by a recovery back to baseline, as  
344 shown in Fig. 3A).



345 Understanding the cause of symptoms due to IAV infection is an impor-  
346 tant but challenging task. Because the mechanism causing various symptoms  
347 remains unclear, we do not have the capacity to accurately model symptom  
348 dynamics. However, we can take a heuristic approach to predict the kinetics  
349 of symptoms using our model. For example, given that macrophage-mediated  
350 inflammatory response contributes to the formation of illness [24, 64, 65],  
351 then if we assume a positive correlation between symptom dynamics and the  
352 number of  $M_1$  macrophages, our model predicts a delayed presence, peak  
353 and resolution of symptoms compared to viral shedding dynamics (Fig. 3A).  
354 This prediction is qualitatively consistent with a previous finding that vi-  
355 ral shedding preceded the occurrence of symptoms by approximately one  
356 day and finished earlier than the symptom resolution [67]. Although there  
357 are assumptions to be validated by further experiments, such as the posi-  
358 tive correlation between the formation of symptoms and the dynamics of  $M_1$   
359 macrophages, our model provides promising directions to probe the mecha-  
360 nism of symptom formation and establish the relationship between symptoms  
361 and immune response dynamics.

362 Further, macrophages have been shown to have pathological effects in  
363 mice infected with highly pathogenic influenza virus [68, 69]. Our model  
364 results provide new insight into the possible mechanisms for regulation of  
365 macrophage activation, suggesting that the pathological effects can be min-  
366 imized by influencing the replenishment rate and reducing the available  
367 number of resting macrophages (e.g., region III Fig. 4). This may de-  
368 crease macrophage accumulation and restrict the strength of inflammatory  
369 responses. For example, a study in [70] has shown that lethality of IAV in-  
370 fection to mice could be ameliorated when interferon-I (IFN-I) signalling is  
371 blocked. The similar knockout can be applied to macrophages and modulate  
372 the activation process of macrophages.

373 Our model can be extended to study other biological processes which  
374 are highly dependent upon macrophage dynamics in influenza infection. For  
375 instance, macrophages have been shown to have an important role in effec-  
376 tive activation of adaptive immunity [12, 68], and quantifying the impact of  
377 macrophages upon adaptive immune responses in influenza infection will be  
378 a promising direction for further study. Also, biological activities of cell sur-  
379 face mucin (cs-mucin) glycoproteins, MUC1 particularly, have been shown  
380 to have an important role in reducing the severity of influenza infection, as  
381 reviewed in [71]. MUC1 provides two-fold protection to the host—a physical  
382 barrier to prevent virus from infecting healthy cells and more importantly

383 a regulator of host inflammatory responses via inhibition of signalling path-  
384 ways on macrophages [72]. Our model has the capacity to model the two  
385 protective roles of MUC1. For instance, we could model the physical effect  
386 of MUC1 against influenza infection by reducing the viral infectivity  $\beta$  or  
387 model the inhibitory effect of MUC1 on inflammatory response by reducing  
388 the activation rate of  $M_1$  macrophages. With the data available from [72],  
389 we may be able to quantitatively study the effect of MUC1 on reduction of  
390 infection severity and inflammatory responses in influenza infection. Another  
391 potential application of our model is to predict the effect of novel antiviral  
392 treatments, such as Pam2Cys, a novel immunomodulator shown to be able  
393 to enhance protection against influenza in mice by stimulating innate im-  
394 munity and recruiting macrophages to the site of infection [73, 74]. These  
395 applications are beyond the scope of this paper and are left for future work.

#### 396 **Author contributions**

397 **Ke Li:** Conceptualization, Methodology, Software, Formal analysis, Writing-  
398 Original Draft. **James M. McCaw** Methodology, Formal analysis, Writing-  
399 Review and Editing, Supervision. **Pengxing Cao:** Methodology, Formal  
400 analysis, Writing- Review and Editing, Supervision

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#### 407 **Declarations of interest**

408 None.

Par.	Description	Value	Unit	Reference
$p$	Viral production rate	$7.1 \times 10^{-2}$	$[u_V][u_T]^{-1}d^{-1}$	[50]
$c$	Viral natural death rate	20	$d^{-1}$	[51]
$s_1$	Effectiveness of $M_2$ attenuates $M \rightarrow M_1$	1	-	-
$s_2$	Effectiveness of $M_1$ promotes $M \rightarrow M_2$	1	-	-
$\delta_I$	Natural death rate of infected cells	3.6	$d^{-1}$	[50]
$\delta$	Decay rate of $M_1$ and $M_2$	0.02	$d^{-1}$	[52]
$\kappa$	Rate of virus internalisation by $M_1$	$7.7 \times 10^{-4}$	$[u_{M_1}]^{-1}d^{-1}$	[34]
$\kappa_a$	Neutralisation rate of virus by antibody	0.2	$[u_A]^{-1}d^{-1}$	[51]
$\mu$	Rate of macrophage-induced activation of adaptive immunity	$10^{-6}$	$d^{-1}$	-
$\rho$	logistic growth rate of antibody response	1	$d^{-1}$	[53]
$\beta$	Viral infectivity	$3.8 \times 10^{-5}$	$[u_V]^{-1}d^{-1}$	-
$g$	Regrowth rate of $M$	0.2	$d^{-1}$	-
$M_0$	Carrying capacity of macrophage regrowth	$10^6$	$[u_M]$	[34, 37]
$k_1$	Conversion rate of $M \rightarrow M_1$	0.5	$d^{-1}$	[52]
$k_{-1}$	Conversion rate of $M_1 \rightarrow M$	0.33	$d^{-1}$	[52]
$k_2$	Conversion rate of $M \rightarrow M_2$	0.5	$d^{-1}$	[52]
$k_{-2}$	Conversion rate of $M_2 \rightarrow M$	0.33	$d^{-1}$	[52]
$q_1$	Rate of infected cell-induced conversion from $M$ to $M_1$	$1 \times 10^{-6}$	$[u_I]^{-1}d^{-1}$	[34]
$q_2$	Rate of virus-induced conversion from $M$ to $M_1$	$1 \times 10^{-6}$	$[u_V]^{-1}d^{-1}$	[34]
$A^*$	Assumed carrying capacity of antibody upon an infection	$10^5$	$[u_A]$	-
$T(0)$	Initial value of uninfected epithelial cells in the upper respiratory tract	$4 \times 10^8$	$[u_T]$	[50]
$I(0)$	Initial value of infected cells	0	$[u_I]$	[50]
$V(0)$	Initial value of viral load	$3.3 \times 10^{-1}$	$[u_V]$	[50]

Table 1: **Parameter values used for numerical simulation.**  $[\cdot]$  denotes the unit for each variable, e.g., the unit of  $T$  is denoted as  $[u_T]$ .  $d^{-1}$  denotes per day.

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