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The Nonlinear Relations that Predict Influenza Viral Dynamics, $CD8^+$ T cell-Mediated Clearance, Lung Pathology, and Disease Severity

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Abstract

Influenza A viruses cause a significant amount of morbidity and mortality. Understanding how the infection is controlled by host immune responses and how different factors influence severity are critical to combat the infection. During infection, viral loads increase exponentially, peak, then decline until resolution. The viral decline is often biphasic, which we previously determined is a consequence of density-dependent infected cell clearance. The second, rapid clearance phase corresponds with the infiltration of $CD8^+$ T cells, but how the rate changes with infected cell density and T cell density is unclear. Further, the kinetics of virus, infected cells, and $CD8^+$ T cells all contribute to disease severity but do not seem to be directly correlated. Thus, we investigated the relations between viral loads, infected cells, $CD8^+$ T cells, lung pathology, and disease severity/symptoms by infecting mice with influenza A/PR8, simultaneously measuring virus and $CD8^+$ T cells, and developing and calibrating a kinetic model. The model predicted that infection resolution is sensitive to $CD8^+$ T cell expansion, that there is a critical T cell magnitude below which the infection is significantly prolonged, and that the efficiency of T cell-mediated clearance is dependent on infected cell density. To further examine the latter finding and validate the model's predicted dynamics, we quantified infected cell kinetics using lung histomorphometry. These data showed that the area of lung infected matches the predicted cumulative infected cell dynamics, and that the area of resolved infection parallels the relative $CD8^+$ T cell magnitude. Our analysis further revealed a nonlinear relationship between disease severity (i.e., weight loss) and the percent of the lung damaged. Establishing the predictive capabilities of the model and the critical connections that map the kinetics of virus, infected cells, $CD8^+$ T cells, lung pathology, and disease severity during influenza virus infection aids our ability to forecast the course of infection, disease progression, and potential complications, thereby providing insight for clinical decisions.

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Author Summary

Influenza A viruses infect millions of people each year. An understanding of how virus growth and host responses impact disease progression is critical to identify disease-specific markers that help predict hospitalization, complications, and therapeutic efficacy. To establish these relations, we developed and validated a mathematical model that accurately forecasts the kinetics of virus, infected cells, and $CD8^+$ T cells. We discovered that the rate of infected cell removal by $CD8^+$ T cells increases as infected cells decline, that there is a critical number of $CD8^+$ T cells below which recovery is prolonged, and that recovery time depends on the number of $CD8^+$ T cells rather than their efficiency. Further, examining lung pathology showed that the area of the lung infected and the area of the lung resolved parallel the model's predicted cumulative infected cell and $CD8^+$ T cell dynamics, respectively. Our analysis also revealed a nonlinear relation between the lung pathology and disease severity. These connections illustrated the predictive capabilities of our model and established how the spread and clearance of influenza virus within the lung contributes to disease progression. This information aids our ability to predict the infection course and potential complications, and make robust clinical decisions.

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Introduction

Over 15 million respiratory infections and 200,000 hospitalizations result from influenza A viruses (IAVs) each year [1–4]. The incidence and severity of IAV infections increases when new strains emerge and/or when there is a lack of prior immunity. A robust immune response is crucial for resolving viral infections, but immune-mediated pathology can exacerbate disease [5–9]. High viral loads also play a role in disease progression [10], but these do not always correlate with the strength of the host response or with disease severity [11–14]. An understanding of how viral loads, host immune responses, and disease progression are related is critical to identify disease-specific markers that may help predict hospitalization or other complications.

During IAV infection, viral loads increase rapidly for the first 1-2 days of infection before reaching a peak (e.g., as in [13, 15–19]). In naïve hosts, viral loads then begin to decline, first slowly (< 1 $\log_{10}$ TCID_{50}/d; 3–7 d) then rapidly ($4-5$ $\log_{10}$ TCID_{50}/d; 7–9 d) [15]. We previously quantified this biphasic viral decline with a mathematical model, which indicated that the rate of infected cell clearance increases as the density of infected cells decreases [15]. The timing of the second, rapid viral decay phase coincides with the expansion of CD8^+ T cells, which are the primary cell responsible for clearing infected cells and resolving the infection [20–23]. It remains unclear whether CD8^+ T cell efficacy is dictated by their own density [24, 25], infected cell density [26–28], or both [29]. While quantifying CD8^+ T cell efficacy is difficult *in vitro* and *in vivo*, the question is ripe for *in silico* investigation. Indeed, several modeling studies have described CD8^+ T cell-mediated infected cell clearance for various viral infections, including IAV, HIV, and LCMV (e.g., as in [17, 24, 26, 29–35]). However, for IAV infections, in particular, nonlinearities in the efficiency of the CD8^+ T cell response and the consequences on viral loads have yet to be investigated in detail.

A better understanding of infected cell clearance may also yield insight into the damage induced to the lung during IAV infection. In general, widespread alveolar disease is observed in patients who succumb to the infection [36]. Further, in hospitalized patients who died as a result from infection with the 2009 H1N1 influenza virus had large numbers of CD8^+ T cells present in their lung tissue [7]. Large pulmonary populations of CD8^+ T cells may result in 'bystander damage' to uninfected epithelial cells [37] in addition to targeting IAV-infected cells. The accumulation of damage to the epithelium during IAV infection, either from virally-induced cell lysis or immune-mediated effects, is relatively understudied. This is in part due to the difficulty in measuring the dynamics of infected cells and in establishing how damage correlates to different

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host responses. Recent technological advances, including the use of reporter viruses [38–41] and lung histomorphometry [12, 42–44], have provided an opportunity to acquire these types of measurements. However, even with these techniques, quantitative data over the course of infection is not currently available. Having data such as these should help reconcile potential nonlinearities in infected cell clearance and provide insight into the accumulated lung damage, which we hypothesize is a marker of disease severity.

In general, measures of disease severity do not seem to be directly correlated to viral loads or to specific immunological components. In humans infected with IAV, viral loads typically increase prior to the onset of systemic symptoms, which peak around 2–3 d post-infection (pi) [18, 45, 46]. Respiratory symptoms often last longer and can remain following viral clearance [45]. In the murine model, weight loss is used as an indicator of disease progression and severity, where greater weight loss corresponds to more severe disease [47–49]. Animal weights typically drop slowly in the first several days during an IAV infection and more rapidly as the infection begins to resolve [19, 50]. This is unlike viral load dynamics in these animals, which increase rapidly during the first 0–3 d pi then remain relatively constant prior to resolution [15]. Because weight loss often occurs following resolution of the infection, immune-mediated pathology is thought to play a role [5, 49, 51–53]. Host and pathogen factors, such as age, viral proteins, and inoculum size, can also influence disease progression [13, 14, 54, 55]. While the causes of IAV-associated disease and mortality remain elusive, this gap in knowledge impairs our ability to effectively predict, understand, and treat the disease.

To gain deeper insight into the dynamics of viral resolution and investigate the connection between viral loads and disease severity, we simultaneously measured viral loads and $CD8^+$ T cells daily from the lungs of BALB/cJ mice infected with influenza A/Puerto Rico/8/34 (H1N1) (PR8). We then developed a model that describes their kinetics to explore the mechanisms and dynamics of $CD8^+$ T cell influx and their efficiency in removing virus-infected cells. The model verified our previous results that infected cells are cleared in a density-dependent manner. We further determined that infection duration is dependent on the magnitude of $CD8^+$ T cells rather than their efficacy. Exploring these findings through quantitative whole lung histomorphometry corroborated the predicted infected cell dynamics and the direct relation between infected cell clearance and $CD8^+$ T cell expansion. In addition, these data revealed a nonlinear connection between disease severity, as measured by weight loss, and the amount of the lung impacted by the infection. The data, model, and analyses provide a robust quantification of the density-dependent nature of $CD8^+$ T cell-mediated clearance, and the critical connections between these cells and

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the dynamics of viral loads, infected cells, lung pathology, and disease severity.

Results

Virus and CD8$^+$ T cell kinetics

In animals infected with 75 $TCID_{50}$ PR8, virus rapidly infects cells or is cleared within 4 h pi (Fig 1). Virus then increases exponentially and peaks after ~2 d pi. Following the peak, virus enters a biphasic decline. In the first phase (2–6 d pi), virus decays slowly and at a relatively constant rate (0.2 $\log_{10}$ $TCID_{50}$/d) [15]. In the second phase (7–8 d pi), virus is cleared rapidly with a loss of $4-5$ $\log_{10}$ $TCID_{50}$ in 1–2 d (average of -3.8 $\log_{10}$ $TCID_{50}$/d) [15]. CD8$^+$ T cells remain at their baseline level from 0–2 d pi before they infiltrate the lung tissue and increase slightly at 2–3 d pi. The population briefly contracts (3–5 d pi) before expanding rapidly (6–8 d pi). This expansion corresponds to the second viral decay phase. Sixty percent of mice clear the infection by 8 d pi, and the other forty percent by 9 d pi. CD8$^+$ T cells decline following viral clearance (8–10 d pi), but do not return to their baseline level. These cells then increase again slightly from 10–12 d pi.

Viral kinetic model with CD8$^+$ T cell-mediated clearance

We previously described the viral load kinetics and biphasic decline using the density-dependent (DD) model in Eq (1)–(4), which assumes that the rate of infected cell clearance increases as the density of infected cells decreases (i.e., $\delta_d(I_2) = \delta_d/(K_\delta + I_2)$) [15]. Because the second viral decay phase is thought to be due to the clearance of infected cells by CD8$^+$ T cells, we developed a model to describe the dynamics of these cells and their efficiency in resolving the infection (Eq (5)–(10); Fig 1A). The model includes equations for effector (E, denoted $CD8_E$) and memory (E_M, denoted $CD8_M$) CD8$^+$ T cells, and two mechanisms of infected cell clearance. During the first viral decay phase (2–6 d pi), the rate of infected cell clearance by non-specific mechanisms is relatively constant (δ). During the rapid, second viral decay phase (7–8 d pi), $CD8_E$-mediated infected cell clearance occurs at a rate that increases as the density of infected cells decreases ($\delta_E(I_2, E) = \delta_E E/(K_{\delta_E} + I_2)$). Excluding this density dependence resulted in a significant and premature decline in viral loads, which disagreed with the experimental data. Although memory CD8$^+$ T cells are not the focus here, it was necessary to include the $CD8_M$ population because CD8$^+$ T cells are at a significantly higher level at 10–12 d pi than at 0 d

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Table 1: **CD8$^+$ T cell model parameters.**

Parameter	Description	Units	Value	95% CI
β	Virus infectivity	TCID_{50}^{-1} d^{-1}	6.2×10^{-5}	$[5.3 \times 10^{-6},\ 1.0 \times 10^{-4}]$
k	Eclipse phase transition	d^{-1}	4.0	[4.0, 6.0]
p	Virus production	TCID_{50} cell^{-1} d^{-1}	1.0	$[5.8 \times 10^{-1},\ 1.1 \times 10^{2}]$
c	Virus clearance	d^{-1}	9.4	$[5.6,\ 9.5 \times 10^{2}]$
δ	Infected cell clearance	d^{-1}	2.4×10^{-1}	$[1.0 \times 10^{-1},\ 6.6 \times 10^{-1}]$
δ_E	Infected cell clearance by CD8_E	cells CD8_E^{-1} d^{-1}	1.9	$[3.3 \times 10^{-1},\ 2.0]$
K_{δ_E}	Half-saturation constant	cells	4.3×10^{2}	$[1.0 \times 10^{2},\ 2.9 \times 10^{5}$
ξ	CD8_E infiltration	CD8_E^{2} cell^{-1} d^{-1}	2.6×10^{4}	$[1.3 \times 10^{2},\ 8.7 \times 10^{4}]$
K_E	Half-saturation constant	CD8_E	8.1×10^{5}	$[1.0 \times 10^{3},\ 1.0 \times 10^{6}]$
η	CD8_E expansion	cell^{-1} d^{-1}	2.5×10^{-7}	$[1.6 \times 10^{-8},\ 6.7 \times 10^{-7}]$
τ_E	Delay in CD8_E expansion	d	3.6	[2.1, 5.9]
d_E	CD8_E clearance	d^{-1}	1.0	$[5.1 \times 10^{-2},\ 2.0]$
ζ	CD8_M generation	CD8_M CD8_E^{-1} d^{-1}	2.2×10^{-1}	$[1.0 \times 10^{-2},\ 9.4 \times 10^{-1}]$
τ_M	Delay in CD8_M generation	d	3.5	[3.0, 4.0]
$\hat{E}_0$	Baseline CD8	CD8	4.2×10^{5}	$[3.3 \times 10^{5},\ 5.3 \times 10^{5}]$
$T(0)$	Initial uninfected cells	cells	1×10^{7}	-
$I_1(0)$	Initial infected cells	cells	75	-
$I_2(0)$	Initial infected cells	cells	0	-
$V(0)$	Initial virus	TCID_{50}	0	-
$E(0)$	Initial CD8_E	CD8_E	0	-
$E_M(0)$	Initial CD8_M	CD8_M	0	-

Parameters and 95% confidence intervals obtained from fitting the CD8$^+$ T cell model (Eq (5)–(10)) to viral titers and CD8$^+$ T cells from mice infected with 75 TCID_{50} PR8. CD8_E and CD8_M denote effector (E) and memory (E_M) CD8$^+$ T cells, respectively. The total number of CD8$^+$ T cells is $\hat{E} = E + E_M + \hat{E}_0$ and is denoted by CD8.

pi (Fig 1B). The model includes terms for CD8_E infiltration ($\xi I_2/(K_E + E)$), which accounts for the initial increase at 2–3 d pi, and for CD8_E expansion ($\eta E I_2(t - \tau_E)$), which accounts for the larger increase between 5–8 d pi. To capture the contraction of CD8$^+$ T cells between these times (3–5 d pi), it was necessary to assume that CD8_E infiltration is reduced by their own population (i.e., $\xi(E) = \xi/(K_E + E)$). In both terms, the increase is proportional to the number of infected cells. Fitting the model simultaneously to viral loads and CD8$^+$ T cells from the lungs of infected animals illustrated the accuracy of the model (Fig 1B). The resulting parameter values, ensembles, histograms, and 95% confidence intervals (CIs) are given in Table 1 and Figs 2 and S1–S2.

Plotting the model ensembles indicated that the behavior of the virus-specific parameters (i.e., the rates of virus infectivity (β), virus production (p), virus clearance (c), and eclipse phase transition (k)) in the CD8$^+$ T cell model were similar to the results obtained fitting the DD model to the viral load data [15] (Fig 2A). In addition, they revealed a correlation between the

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two infected cell clearance parameters (δ and δ_E; Fig 2B), which represent the efficacy of the non-specific and CD8$^+$ T cell immune responses, respectively. Performing a sensitivity analysis showed that the viral load dynamics do not change substantially when these parameters are increased or decreased. However, decreasing the rate of non-specific infected cell clearance (i.e., lower δ) resulted in a significant increase in the number of CD8$_\mathrm{E}$ due to the small increase in the number of infected cells (Fig S3). Even with a larger CD8$_\mathrm{E}$ population, recovery was delayed by only ~ 0.1 d. Given the correlation between δ and δ_E (Fig 2B), a more efficient CD8$_\mathrm{E}$ response (i.e., higher δ_E) may be able to overcome this short delay in resolution. The lack of sensitivity to changes in the infected cell clearance parameters is in contrast to the DD model, where the viral dynamics were most sensitive to perturbations in δ_d (Fig S5) [15], which encompasses multiple processes. With CD8$^+$ T cells explicitly included in the model, the infection duration was most sensitive to changes in the rate of CD8$_\mathrm{E}$ expansion (η) (Figs S3 and S5; discussed in more detail below).

Examining the parameter ensembles and sensitivity analysis also yielded insight into how other model parameters affect the CD8$^+$ T cell response. The rates of CD8$_\mathrm{E}$ expansion (η) and clearance (d_E) were slightly correlated, indicating a balance between these two processes (Fig 2C). This correlation and the sensitivity of η produce model dynamics that were also sensitive to changes in the CD8$_\mathrm{E}$ clearance rate (d_E) (Fig S4). As expected, the rates of CD8$_\mathrm{M}$ generation (ζ) and CD8$_\mathrm{E}$ clearance (d_E) were correlated (Fig 2C). It has been estimated that approximately 5–10% of effector CD8$^+$ T cells survive to become a long-lasting memory population [56]. Despite the inability to distinguish between CD8$_\mathrm{E}$ and CD8$_\mathrm{M}$ in the data, the model predicts that 17% of CD8$_\mathrm{E}$ transitioned to the memory class by 15 d pi. Additional insight into the regulation of the CD8$^+$ T cell response, results from the model fitting, and a comparison of the DD model and the CD8$^+$ T cell model are in the Supporting Information.

Density-dependent infected cell clearance

To gain further insight into the nonlinear dynamics of CD8$^+$ T cell-mediated infected cell clearance, we plotted the clearance rate ($\delta_E(I_2, E) = \delta_E E/(K_{\delta_E} + I_2)$) as a function of infected cells (I_2) and CD8$_\mathrm{E}$ (E) (Fig 3). This confirmed that there is minimal contribution from CD8$_\mathrm{E}$-mediated clearance to viral load kinetics or infected cell kinetics prior to 7 d pi (Fig 3A–C, markers a–b). At the initiation of the second decay phase (7 d pi), the clearance rate is $\delta_E(I_2, E) = 3.5/\mathrm{d}$ (Fig 3A–B, marker c). As the infected cell density declines towards the half-saturation constant

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(K_{δ_E} = 4.3 $\times 10^2$ cells), the clearance rate increases rapidly to a maximum of 4830/d (Fig 3A–C, markers d–g). The model predicts that there are 6×10^5 infected cells remaining at 7 d pi, which can be eliminated by $\mathrm{CD8_E}$ in 6.7 hours.

To explore how recovery time is altered by varying $\mathrm{CD8_E}$ levels, we examined the resulting dynamics from increasing or decreasing the rate of $\mathrm{CD8_E}$ expansion (η). When η was increased by 50%, the $\mathrm{CD8_E}$ population increased by a substantial 670% (Fig S3). However, this was insufficient to significantly shorten the infection (8.4 d versus 7.8 d). The infection duration can be reduced if $\mathrm{CD8_E}$ expansion began earlier (i.e., smaller τ_E; Fig S4). Although recovery is not significantly expedited by a larger $\mathrm{CD8_E}$ population, the infection is dramatically prolonged if these cells are depleted (Figs 3D–E and S3). This *in silico* analysis revealed a bifurcation in recovery time, such that the infection is either resolved within ~15 d pi or may last up to ~45 d if $\mathrm{CD8_E}$ are below a critical magnitude required to resolve the infection (Fig 3D–E). The critical number of total $\mathrm{CD8^+}$ T cells needed for successful viral clearance was $\hat{E}^{\mathrm{crit}}_{\mathrm{max}} = 7.4 \times 10^5$ CD8, which was 39.2% of the maximum number of $\mathrm{CD8^+}$ T cells obtained from the best-fit solution (i.e., $\hat{E}_{\mathrm{max}} = 1.9 \times 10^6$ CD8). This corresponds to 17% of $\mathrm{CD8_E}$ (i.e., $E^{\mathrm{crit}}_{\mathrm{max}} = 2.3 \times 10^5$ $\mathrm{CD8_E}$; Fig 3D–E). The model predicts that decreasing the total number of $\mathrm{CD8^+}$ T cells by as little as 0.1% from this critical level (i.e., 39.2% to 39.1%) lengthens the infection from 15 d pi to 25 d pi (Fig 3D).

Lung histomorphometry validates infected cell dynamics

To investigate the dynamics of infected cells, we quantified these cells and the infection progression and resolution within the lung using serial whole lung histomorphometry (Fig 4A). Antigen-positive areas of the lung ("active" lesions) were first detectable at 2 d pi (Fig 4A–B), which coincides with the peak in viral loads. The infected area continued to increase until 6 d pi, whereas viral loads remained high until 7 d pi (Fig 1B). At this time, resolution of the infection began and ~28.7% of the infected area was cleared between 6–7 d pi (Fig S6A). Few to no infected cells were present by 8 d pi (Fig 4A). Correspondingly, virus was undetectable in most animals by 8 d pi (Fig 1B). Because the percent active lesion is a reflection of the influenza-positive cells, we examined whether the $\mathrm{CD8^+}$ T cell model accurately predicted these dynamics. In the model, the accumulated infection is defined by the cumulative area under the curve (CAUC) of the productively infected cells (I_2). Plotting the percent active lesion against the CAUC of I_2 showed that the model accurately predicts the cumulative infected cell dynamics and, thus, the

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infection progression within the lung (Fig 4B).

Antigen-negative, previously-infected or damaged areas of the lung ("inactive" lesions) are evident beginning at 5 d pi (Fig 4A,C). This resolution of the infection continued from 5–8 d pi, causing a 14.6%/d increase in the area of inactive lesions (Fig S6B). Following this, healing of the lung is apparent as the inactive lesioned areas decline (-14.5%/d from 9–10 d pi; Figs 4C and S6B). These dynamics parallel the $CD8^+$ T cell dynamics (Fig 1B). Fitting a line to the $CD8^+$ T cell data from 5–8 d pi indicated that the influx rate is 4.7×10^5 cells/d (Fig S6C). Thus, every 100,000 $CD8^+$ T cells clear ~ 3.1% of the lung. During $CD8^+$ T cell contraction phase (8–10 d pi; Fig 1B), a similar linear regression analysis suggested that these cells decline at a rate of ~ 3.3×10^5 CD8/d (Fig S6C). Similar to the relation discussed above, the dynamics of the damaged areas of the lung corresponded precisely to the dynamics of the percent maximum $CD8_E$ (i.e., E/E_{max}) in the model (Fig 4C).

Weight loss predicts area of lung affected

To monitor disease progression, weight loss was measured daily throughout the course of infection (Fig 4D). During the first 5 d pi, animals gradually lost ~4% of their initial weight. This was followed by a sharper drop (8%) at 6 d pi. Animal weights increased slightly at 7 d pi (~6%) before reaching peak weight loss (10–14%) at 8 d pi. Following virus resolution, the animals' weights began to restore as the inactive lesions resolved (9–10 d pi; Fig 4D). We hypothesized that these weight loss dynamics may reflect the area of the lung impacted by the infection. Indeed, plotting weight loss together with the percent total (active and inactive) lesioned area of the lung revealed the similarity in their dynamics (Fig 4D-E). To further quantify their relationship, we plotted the percent weight loss against the percent total lesioned area and observed a nonlinear relation. Thus, we fit the saturating function $L = l_{max}W^n/(W^n + K_w^n)$ to these data, where L is the percent total lesioned area, W is percent weight loss, l_{max} is the maximum rate of the interaction, K_w is the half-saturation constant, and n is the Hill coefficient. This function provided a close fit to the data (R^2 = 0.9; Fig 4E) with best-fit parameters l_{max}=39.7% total lesioned area, K_w=2.6% weight loss, and n = 5.2.

Discussion

Influenza A virus infections pose a significant threat to human health, and it is crucial to understand how the virus spreads within the respiratory tract, how specific immune responses

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contribute to infection control, and how these relate to disease progression. Although it has been difficult to directly relate these features, we circumvented the challenge by pairing comprehensive, experimental data with robust mathematical models and analyses. Our iterative model-driven experiment approach [57, 58] revealed important dynamic relations between virus, infected cells, $CD8^+$ T cells, lung damage, and weight loss (Fig 5). Identifying these nonlinear connections allows for more accurate interpretations of viral infection data and significant improvement in our ability to predict disease severity, the likelihood of complications, and therapeutic efficacy.

Our histomorphometric data provided the first quantification of the spread of influenza virus infection within the lung. These data allowed us to validate our model's predicted infected cell dynamics and, thus, confirm that their density impacts the rate at which they are cleared by effector $CD8^+$ T cells ($CD8_E$) (Fig 4). We first detected this density-dependence in a model that excluded specific immune responses (i.e., Eq (1)–(4)) [15]. That model was a mathematically elegant way to capture the nonlinearity in the viral load decline, but it could not distinguish between different mechanisms of viral clearance. Here, we verified that the second viral clearance phase reflects $CD8^+$ T cell-mediated clearance, determined that their efficiency increases as more infected cells are removed, and identified the critical level needed for a timely recovery (Fig 3).

Several factors may contribute to the density-dependent change in the rate of $CD8_E$-mediated clearance. One possibility is that the slowed rate of clearance at high infected cell densities is due to a "handling time" effect, which represents the time required for an immune cell to remove an infected cell (e.g., as in [15, 24, 29, 59–62]). When $CD8_E$ interact with infected cells, a complex is formed for ~20–40 min [32, 63–66]. Because $CD8_E$ cannot interact with other infected cells during this time, the global rate of infected cell clearance would be lowest when infected cells outnumber $CD8_E$. In addition, contact by a single $CD8_E$ may be insufficient to remove an infected cell [28]. Infected cell clearance is more frequently observed after interactions with multiple $CD8_E$, with an average of 3.9 contact events either serially or simultaneously [28]. Thus, the high density of infected cells early in the infection reduces the probability that a single virus-infected cell would be targeted a sufficient number times to induce cell death. However, as $CD8_E$ accumulate and the density of infected cells decreases (Fig 3A), the probability of simultaneous interactions will increase. This should reduce the handling time required to remove of an infected cell and, thus, result in a higher efficiency. While this is a likely explanation, it is possible that spatial limitations also contribute, such that a high infected cell density may hinder $CD8^+$ T cells from reaching infected cells on the interior of the infected tissue. Crowding of immune cells at the periphery of infected lesions has been observed in other infections (e.g.,

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in tuberculosis granulomas [67, 68]) and has been suggested in agent-based models of influenza virus infection [69].

The new knowledge about how the infection spreads throughout the lung also uncovered a novel connection between the percent weight loss of an infected animal (i.e., disease severity) and the percent total area of the lung impacted by the virus infection (Fig 4D–E). This discovery is significant because it suggests that disease severity is linked to the amount of lung involvement, which we showed is directly connected to infected cell and $CD8_E$ kinetics (Fig 4B–C) and indirectly connected to viral load kinetics (Fig 5). Other studies utilizing histomorphometry data, although not quantitatively, provide support for the relationship between weight loss and lung involvement during IAV infection [12]. For example, animals treated with antivirals in various conditions (single or combination therapy and in immunocompetent or immunosuppressed hosts) demonstrated reduced weight loss that corresponded to reduced infected areas of lung [12, 42]. Examining the histomorphometric kinetics and the connection between weight loss and lung pathology in various infection settings should improve our predictive capabilities. This may be particularly helpful in understanding the exacerbated morbidity and mortality in elderly individuals. In the animal model, elderly mice typically have lower viral loads compared to adult mice yet experience more weight loss, symptoms, and/or mortality [13, 14].

The nonlinear relations derived from the histomorphometric analysis further provide a way to interpret and utilize weight loss data, which is the most readily available type of data and commonly used as a measure of disease severity in infection models (Figs 4–5). In our data and others', there is a spike in weight loss, symptom score, and/or inflammation at ~6 d pi [50, 54, 70, 71] (Fig 4D). Our data suggests that this is due to $CD8_E$ infiltration and activity while the infection continues to spread (Fig 4). Utilizing this observation and the function describing the correlation between the percent lung lesion and weight loss ($L(W)$; Fig 4E), the kinetics of the active and inactive lesioned areas can be directly estimated from weight loss data (Fig 5). Taking this one step further, the dynamics of the CAUC of the infected cells and the relative $CD8_E$ can be obtained. Finally, the $CD8^+$ T cell model (Eq (5)–(10)) can be employed to estimate the viral load and $CD8^+$ T cell dynamics (Fig 5). These connections can be reversed to estimate disease severity (i.e., weight loss) from viral load data.

Weight loss increases following infection resolution (8–9 d pi), which is likely attributed to $CD8^+$ T cell-mediated pathology (Fig 4C). This is supported by other studies that suggest a large number of $CD8^+$ T cells poses a risk of acute lung tissue injury [5–9]. According to our model predictions, excessive $CD8^+$ T cell numbers may augment disease progression yet do not

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improve recovery time (Fig S3). Instead, an earlier onset of $CD8_E$ proliferation (i.e., smaller τ_E) would be required to significantly shorten the infection (Fig S4). This aligns with evidence that hosts with adaptive immune responses primed by vaccine or prior infection recover more rapidly [72, 73]. While higher $CD8^+$ T cell numbers have little impact on viral kinetics, the model agrees with clinical and experimental studies from a wide range of host species that impaired $CD8^+$ T cell responses can prolong an IAV infection [17, 22, 74–77]. That is, virus can persist for up to several weeks if $CD8^+$ T cell-mediated clearance is unsuccessful (Fig 3; [74, 78, 79]). The bifurcation in recovery time revealed by the model suggests that this may occur when the total number of $CD8^+$ T cells are <39.2% of their maximum (Fig 3D–E)). This number is expected to vary depending on parameters like the rate of virus replication and the infected cell lifespan, which has been noted in another modeling study that detailed similar bifurcation behavior [80]. Although some previously published models also predict delayed resolution with depleted $CD8^+$ T cell responses [17, 33, 34, 81], this bifurcation has not been observed and their estimated delays in recovery do not amount to the long-lasting infections in immunodeficient patients [74, 78, 79].

In addition to illuminating the connections between virus spread, virus clearance, the associated pathology, and the severity of disease, the histomorphometric data validated the model's infected cell dynamics (Fig 4B). The dynamics of susceptible and infected cells throughout the infection and, thus, the accuracy of the target cell limited approximation used within influenza viral kinetic models have been questioned for several years [58, 82–87]. The data here corroborate the use of this approximation, which does not define what limits the availability of target cells. The data also agree with the model that there are few infected cells during the time when viral loads are growing most rapidly (0–2 d pi; Figs 1B and 4B). We previously used this information to derive approximations for the model and gain deeper insight into how each parameter influences the kinetics [88], which has been utilized by numerous studies [15, 17, 89, 90]. Further, the data support the model's hypothesis that that there is minimal clearance of infected cells prior to $CD8_E$ infiltration (Fig 3A–B). The knowledge of the model's accuracy and of the spatial growth throughout the lung should aid investigation into the mechanisms that limit virus growth during the early stages of the infection.

Employing targeted model-driven experimental designs to examine and validate the predictions of models like the one presented here is pivotal to elucidating the mechanisms of infection spread and clearance [57, 58]. Determining the factors that influence disease severity/weight loss is the first step to understand the disproportionate mortality in at-risk populations (e.g., elderly) and to improve therapeutic design. This is particularly important because current antivirals alleviate

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symptoms but do not effectively lower viral loads [91–96]. Validated models like the one here have enormous predictive capabilities and should prove useful in forecasting infection dynamics for a variety of scenarios. These tools and analyses provide a more meaningful interpretation of infection data and a deeper understanding of the progression and resolution of the disease, which will undoubtedly aid our ability to make robust clinical decisions.

Materials and methods

Use of experimental animals

All experimental procedures were approved by the Animal Care and Use Committee at St. Jude Children's Research Hospital under relevant institutional and American Veterinary Medical Association guidelines and were performed in a biosafety level 2 facility that is accredited by AALAAS.

Mice

Adult (6 week old) female BALB/cJ mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed in groups of 5 mice in high-temperature 31.2cm × 23.5cm × 15.2cm polycarbonate cages with isolator lids. Rooms used for housing mice were maintained on a 12:12-hour light:dark cycle at 22 ± 2°C with 50% humidity in the biosafety level 2 facility at St. Jude Children's Research Hospital (Memphis, TN). Prior to inclusion in the experiments, mice were allowed at least 7 d to acclimate to the animal facility such that they were 7 weeks old at the time of infection. Laboratory Autoclavable Rodent Diet (PMI Nutrition International, St. Louis, MO) and autoclaved water were available ad libitum. All experiments were performed under an approved protocol and in accordance with the guidelines set forth by the Animal Care and Use Committee at St. Jude Children's Research Hospital.

Infectious agents

All experiments were done using the mouse adapted influenza A/Puerto Rico/8/34 (H1N1) (PR8).

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Infection experiments

The viral infectious dose ($TCID_{50}$) was determined by interpolation using the method of Reed and Muench [97] using serial dilutions of virus on Madin-Darby canine kidney (MDCK) cells. Mice were intranasally inoculated with 75 $TCID_{50}$ PR8 diluted in 100µl of sterile PBS. Mice were weighed at the onset of infection and each subsequent day to monitor illness and mortality. Mice were euthanized if they became moribund or lost 30% of their starting body weight. For viral load and $CD8^+$ T cell quantification, experiments were repeated three times to ensure reproducibility and two complete experiments (10 animals per time point) were used for these studies. Histomorphometry was performed on five animals per time point.

Lung harvesting for viral and cellular dynamics

Mice were euthanized by CO_2 asphyxiation. Lungs were aseptically harvested, washed three times in PBS, and placed in 500µl sterile PBS. Whole lungs were digested with collagenase (1mg/ml, Sigma C0130), and physically homogenized by syringe plunger against a 40µm cell strainer. Cell suspensions were centrifuged at 4°C, 500xg for 7 min. The supernatants were used to determine the viral titers ($TCID_{50}$) by serial dilutions on MDCK monolayers. Following red blood cell lysis, cells were washed in MACS buffer (PBS, 0.1M EDTA, 0.01M HEPES, 5mM EDTA and 5% heat-inactivated FBS). Cells were then counted with trypan blue exclusion using a Cell Countess System (Invitrogen, Grand Island, NY) and prepared for flow cytometric analysis as indicated below.

Lung titers

For each animal, viral titers were obtained using serial dilutions on MDCK monolayers and normalized to the total volume of lung homogenate supernatant. The resulting viral loads are shown in Fig 1B and were previously published and utilized for calibration of the density-dependent model (Eq (1)–(4); see below) [15].

Flow cytometric analysis

Flow cytometry (LSRII Fortessa; Becton Dickinson, San Jose, CA) was performed on the cell pellets after incubation with 200µl of a 1:2 dilution of Fc block (human-γ globulin) on ice for 30 min, followed by surface marker staining with anti-mouse antibodies: CD11c (eFluor450, eBioscience), CD11b (Alexa700, BD Biosciences), Ly6G (PerCp-Cy5.5, Biolegend), Ly6C (APC,

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eBioscience), F4/80 (PE, eBioscience), CD3e (PE-Cy7, BD Biosciences or BV785, Biolegend), CD4 (PE-Cy5, BD Biosciences), CD8α (BV605, BD Biosciences), DX5 (APC-Cy7, Biolegend or APC-e780, Affymetrix, Inc) and MHC-II (FITC, eBioscience). The data were analyzed using FlowJo 10.4.2 (Tree Star, Ashland, OR) where viable cells were gated from a forward scatter/side scatter plot and singlet inclusion. Following neutrophil (Ly6G^{hi}) and subsequent macrophage (CD11c^{hi}F4/80^{hi}) exclusion, CD8$^+$ T cells were gated as CD3e$^+$CD8α^+CD4$^-$DX5$^-$. The absolute numbers of CD8$^+$ T cells was calculated based on viable events analyzed by flow cytometry as related to the total number of viable cells per sample. The gating strategy is shown in Fig S7.

Lung histomorphometry and immunohistochemistry

The lungs from IAV infected mice were fixed via intratracheal infusion and then immersion in 10% buffered formalin solution. Tissues were paraffin embedded, sectioned, and stained for influenza virus using a primary goat polyclonal antibody (US Biological, Swampscott, MA) against influenza A, USSR (H1N1) at 1:1000 and a secondary biotinylated donkey anti-goat antibody (sc-2042; Santa Cruz Biotechnology, Santa Cruz, CA) at 1:200 on tissue sections subjected to antigen retrieval for 30 minutes at 98°C. The extent of virus spread was quantified by capturing digital images of whole-lung sections stained for viral antigen using an Aperio ScanScope XT Slide Scanner (Aperio Technologies, Vista, CA) then manually outlining defined fields. Alveolar areas containing virus antigen-positive pneumocytes were highlighted in red (defined as "active" infection), whereas lesioned areas containing minimal or no virus antigen-positive debris were highlighted in green (defined as "inactive" infection). Lesions containing a mix of virus antigen-positive and antigen-negative pneumocytes were highlighted in orange (defined as "mixed" infection). The percentage of each defined lung field was calculated using the Aperio ImageScope software. Representative images and quantitative analyses of viral spread and lung pathology during IAV infection are shown in Fig 4A–C.

Mathematical models

Density-dependent model

We previously developed a density-dependent (DD) viral kinetic model that describes the biphasic decline of viral loads [15]. This model tracks 4 populations: susceptible epithelial ("target") cells (T), two classes of infected cells (I_1 and I_2), and virus (V) [15].

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$$\frac{dT}{dt} = -\beta TV \tag{1}$$
$$\frac{dI_1}{dt} = \beta TV - kI_1 \tag{2}$$
$$\frac{dI_2}{dt} = kI_1 - \delta_d(I_2)I_2 \tag{3}$$
$$\frac{dV}{dt} = pI_2 - cV \tag{4}$$

In this model, target cells become infected with virus at rate βV per day. Once infected, target cells enter an eclipse phase (I_1) before transitioning at rate k per day to a productively-infected state (I_2). These infected cells produce virus at rate p TCID_{50} per infected cell per day. Virus is cleared at rate c per day and virus-producing infected cells (I_2) are cleared according to the function $\delta_d(I_2) = \delta_d/(K_\delta + I_2)$, where δ_d/K_δ is the maximum per day rate of infected cell clearance and K_δ is the half-saturation constant. This model provides a close fit to the viral load data in Fig 1B and replicates the biphasic viral load decline [15].

CD8^+ T cell model

To examine the contribution of CD8^+ T cells to the biphasic viral load decay, we expanded the DD model (Eq (1)–(4)) to include two mechanisms of infected cell clearance (non-specific clearance (δ) and CD8^+ T cell-mediated clearance ($\delta_E(I_2, E)$)) and two CD8^+ T cell populations: effector (E, denoted CD8_E) and memory (E_M, denoted CD8_M) CD8^+ T cells. The model is given by Eq (5)–(10).

$$\frac{dT}{dt} = -\beta TV \tag{5}$$
$$\frac{dI_1}{dt} = \beta TV - kI_1 \tag{6}$$
$$\frac{dI_2}{dt} = kI_1 - \delta I_2 - \delta_E(I_2, E)I_2 \tag{7}$$
$$\frac{dV}{dt} = pI_2 - cV \tag{8}$$
$$\frac{dE}{dt} = \xi(E)I_2 + \eta E I_2(t - \tau_E) - d_E E \tag{9}$$
$$\frac{dE_M}{dt} = \zeta E(t - \tau_M) \tag{10}$$

In this model, virus-producing infected cells (I_2) are cleared by non-specific mechanisms

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(e.g. apoptosis and innate immune responses) at a constant rate δ per day and by $\mathrm{CD8_E}$ at rate $\delta_E(I_2, E) = \delta_E E/(K_{\delta_E} + I_2)$ per day, where the rate of infected cell clearance is δ_E/K_{δ_E} per $\mathrm{CD8_E}$ per day and K_{δ_E} is the half-saturation constant. The $\mathrm{CD8_E}$-mediated clearance rate ($\delta_E(I_2, E)$) is dependent on the density of infected cells and is similar to the infected cell clearance term in the DD model (see Eq (3)) [15]. Similar density-dependent forms have also been used in models that describe the $\mathrm{CD8^+}$ T cell response to other virus infections [31, 59]. Models that exclude this density-dependence were examined, but these models resulted in a poor fit to the data. The model assumes that $\mathrm{CD8_E}$ infiltrate the lung proportional to infected cells at rate $\xi(E) = \xi/(K_E + E)$ $\mathrm{CD8_E}$ per cell per day, which is down-regulated by the $\mathrm{CD8_E}$ already present in the lung. The associated half-saturation constant is K_E. Similar terms for $\mathrm{CD8_E}$ regulation have been used in modeling HIV infections [31, 98] and in models that examine $\mathrm{CD8^+}$ T cell proliferation mechanisms [99]. In our model, $\mathrm{CD8_E}$ expansion occurs at rate η per infected cell per day with time delay τ_E. The delay may signify activation by antigen presenting cells, differentiation, and/or proliferation in lymphoid organs outside of the lung. However, the model does not define the site of proliferation (i.e., lung, lymph node, or spleen [20, 100–104]). The lung $\mathrm{CD8_E}$ population declines due to cell death and/or migration at rate d_E per day. These cells transition to $\mathrm{CD8_M}$ (E_M) at rate ζ $\mathrm{CD8_M}$ per $\mathrm{CD8_E}$ per day after τ_M days. The model schematic and fit to the viral load and $\mathrm{CD8^+}$ T cell data are in Fig 1.

Parameter estimation

Given a parameter set θ, the cost $C(\theta)$ was minimized across parameter ranges using an Adaptive Simulated Annealing (ASA) global optimization algorithm [15] to compare experimental and predicted values of $\log_{10}\mathrm{TCID_{50}}$/lung virus ($V$) and of $\log_{10}$ total $\mathrm{CD8^+}$ T cells/lung ($\hat{E} = E + E_M + \hat{E}_0$, where $\hat{E}_0$ is the initial number of $\mathrm{CD8^+}$ T cells at 0 d pi). The cost function is defined by

$$C(\theta) = \sum_{i,j}(V(\theta, t_i) - v_{i,j})^2 + s_E\left[\sum_{i,j}(\hat{E}(\theta, t_i) - e_{i,j})^2 + \sum_i \sqrt{\gamma_i}\left(\frac{\hat{E}(\theta, t_{i+1}) - \hat{E}(\theta, t_{i-1})}{t_{i+1} - t_{i-1}} - \frac{1}{\gamma_i}\sum_j \frac{e_{i+1,j} - e_{i-1,j}}{t_{i+1} - t_{i-1}}\right)^2\right],$$

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where $(t_i, v_{i,j})$ is the viral load data, $(t_i, e_{i,j})$ is the CD8$^+$ T cell data, and $V(\theta, t_i)$ and $\hat{E}(\theta, t_i)$ are the corresponding model predictions. Here, $s_E = (v_{\max} - v_{\min})/(e_{\max} - e_{\min})$ is a scaling factor, and $\gamma_i = J_{i+1}J_{i-1}$ where J_i is the number of observations at time t_i. Errors of the $\log_{10}$ data were assumed to be normally distributed. To explore and visualize the regions of parameters consistent with the model, we fit Eq (5)–(10) to 2000 bootstrap replicates of the data. If the fit was within $\chi^2 = 0.05$ of the best-fit, then the bootstrap was considered successful [15, 89, 105]. For each best-fit estimate, we provide 95% confidence intervals (CI) obtained from the bootstrap replicates (Table 1). All calculations were performed in Python using the *simanneal* package [106] followed by a L-BFGS-B [107, 108] deterministic minimization through SciPy's *minimize* function. SciPy integrate.ode using *lsoda* and PyDDE [109] were used as the ODE and DDE solvers.

Estimated parameters included the rates of virus infection (β), virus production (p), virus clearance (c), eclipse phase transition (k), non-specific infected cell clearance (δ), CD8$_\mathrm{E}$-mediated infected cell clearance (δ_E), half-saturation constants (K_{δ_E} and K_E), CD8$_\mathrm{E}$ infiltration (ξ), CD8$_\mathrm{E}$ expansion (η), delay in CD8$_\mathrm{E}$ expansion (τ_E), CD8$_\mathrm{E}$ clearance (d_E), CD8$_\mathrm{M}$ generation (ζ), delay in CD8$_\mathrm{M}$ generation (τ_M), and the baseline number of CD8$^+$ T cells ($\hat{E}_0$). Bounds were placed on the parameters to constrain them to physically realistic values. Because biological estimates are not available for all parameters, ranges were set reasonably large based on preliminary results and previous estimates [15]. The rate of infection (β) was allowed to vary between 10^{-6}–10^{-1} TCID$_{50}^{-1}$ d^{-1}, and the rate of virus production (p) between 10^{-1}–10^{3} TCID$_{50}$ cell^{-1} d^{-1}. Bounds for the virus clearance rate (c) were 1 d^{-1} ($t_{1/2}$ = 16.7 h) and 10^3 d^{-1} ($t_{1/2}$ = 1 min). To insure biological feasibility, the lower and upper bounds for the eclipse phase transition rate (k) were $4 - 6$ d^{-1} as done previously [15].

The rate of non-specific infected cell clearance (δ) was given limits of $0.05 - 1$ d^{-1}. The CD8$_\mathrm{E}$-mediated infected cell clearance rate (δ_E) varied between $0.01 - 2$ cells CD8$_\mathrm{E}^{-1}$ d^{-1}, and the associated half-saturation constant (K_{δ_E}) was bounded between $10^2 - 10^6$ cells. The upper bound of δ_E was chosen to maintain the convergence of δ to nonzero values. Bounds for the rate of CD8$_\mathrm{E}$ infiltration (ξ) were $10^2 - 10^6$ CD8$_\mathrm{E}^2$ cell^{-1} d^{-1}, and bounds for the half-saturation constant (K_E) were $10^3 - 10^6$ CD8$_\mathrm{E}$. The CD8$_\mathrm{E}$ expansion rate (η) varied between $10^{-8} - 10^{-6}$ cell^{-1} d^{-1}, and the delay in CD8$_\mathrm{E}$ expansion (τ_E) between 2–6 d. The rate of CD8$_\mathrm{E}$ clearance (d_E) had limits of $0.05 - 2$ d^{-1}. The rate of CD8$_\mathrm{M}$ generation (ζ) varied between $0.01 - 1$ CD8$_\mathrm{M}$ CD8$_\mathrm{E}^{-1}$ d^{-1}, and the delay in CD8$_\mathrm{M}$ generation (τ_M) varied between 3–4 d. Larger bounds were examined for this parameter, however, the parameter is non-identifiable and a small range was required for

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convergence. Bounds for the baseline number of $CD8^+$ T cells ($\hat{E}_0$) were set to the upper and lower values of the data at 0 d pi ($3.0 \times 10^5 - 5.3 \times 10^5$ CD8).

The initial number of target cells ($T(0)$) was set to 10^7 cells [15, 89, 105]. The initial number of infected cells $I_1(0)$ was set to 75 cells to reflect an initial dose of 75 $TCID_{50}$ [15]. We previously found that estimating $I_1(0)$, fixing $V(0) = 75$ $TCID_{50}$, or estimating $V(0)$ did not improve the fit and could not be statistically justified [15]. The initial number of productively infected cells ($I_2(0)$), the initial free virus ($V(0)$), and the initial number of $CD8_E$ ($E(0)$) and $CD8_M$ ($E_M(0)$) were set to 0.

Linear regression

The function *polyfit* in MATLAB was used to perform linear regression on the percent active lesioned area, the percent inactive lesioned area, and the $CD8^+$ T cells during the expansion phase (5–8 d pi) and the contraction phase (9–10 d pi). Linear fits are shown in Figure S6.

Area under the curve

The function *cumtrapz* in MATLAB was used to estimate the cumulative area under the curve (CAUC) for the infected cells (I_2) for the best-fit model solution.

Author contributions

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Supporting Information

The Supporting Information contains additional parameter ensembles, sensitivity of the $CD8^+$ T cell model, comparison of the DD model and the $CD8^+$ T cell model, linear regression analysis of the whole lung histomorphometry, and the flow gating strategy.

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Figures

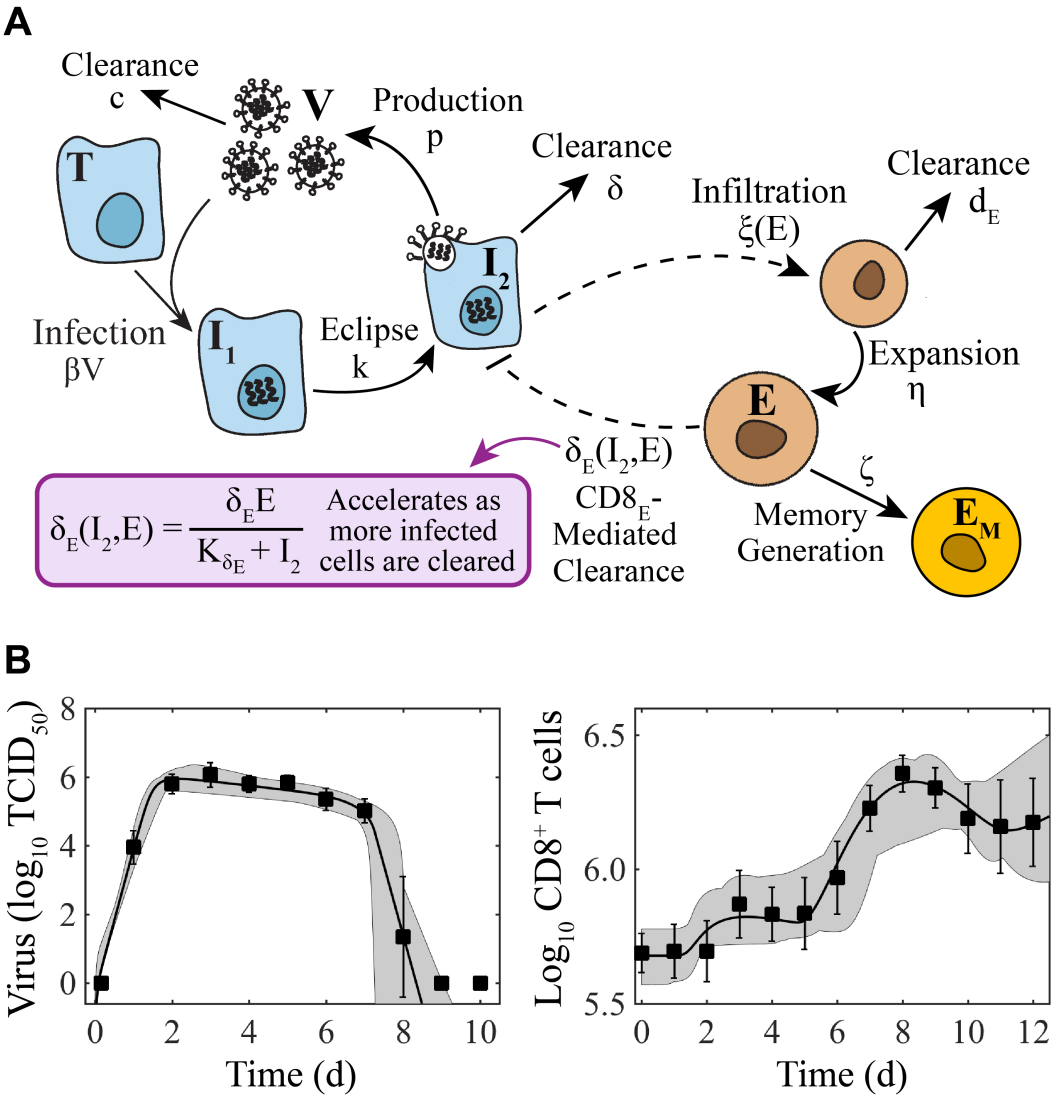

Fig 1: **Schematic and fit of the CD8$^+$ T cell viral kinetic model.** (A) Schematic of the CD8$^+$ T cell model in Eq (5)–(10). In the model, target cells (T) are infected at rate βV. Infected cells (I_1) undergo an eclipse phase and transition to become productively-infected cells (I_2) at rate k. Virus (V) is produced by infected cells at rate p and is cleared at rate c. Infected cells are cleared at rate δ by non-specific mechanisms and at rate $\delta_E(I_2, E)$ by effector CD8$^+$ T cells (E; denoted CD8$_\mathrm{E}$). The dashed lines represent interactions between infected cells and CD8$_\mathrm{E}$. CD8$_\mathrm{E}$ infiltration ($\xi(E) = \xi/(K_E + E)$) is proportional to infected cells and is limited by CD8$_\mathrm{E}$ with half-saturation constant K_E. CD8$_\mathrm{E}$ expansion (η) occurs proportional to infected cells τ_E days ago. Memory CD8$^+$ T cell (E_M; denoted CD8$_\mathrm{M}$) generation occurs at rate ζ and proportional to CD8$_\mathrm{E}$ τ_M days ago. (B) Fit of the CD8$^+$ T cell model (Eq (5)–(10)) to virus and CD8$^+$ T cells from the lungs of mice infected with 75 TCID$_{50}$ PR8 (10 mice per time point). The total number of CD8$^+$ T cells is $\hat{E} = E + E_M + \hat{E}_0$. The solid black line is the optimal solution and the gray shading is the minimum and maximum of the model solution using parameter sets within the 95% CIs. Parameters are given in Table 1. Data are shown as mean ± standard deviation.

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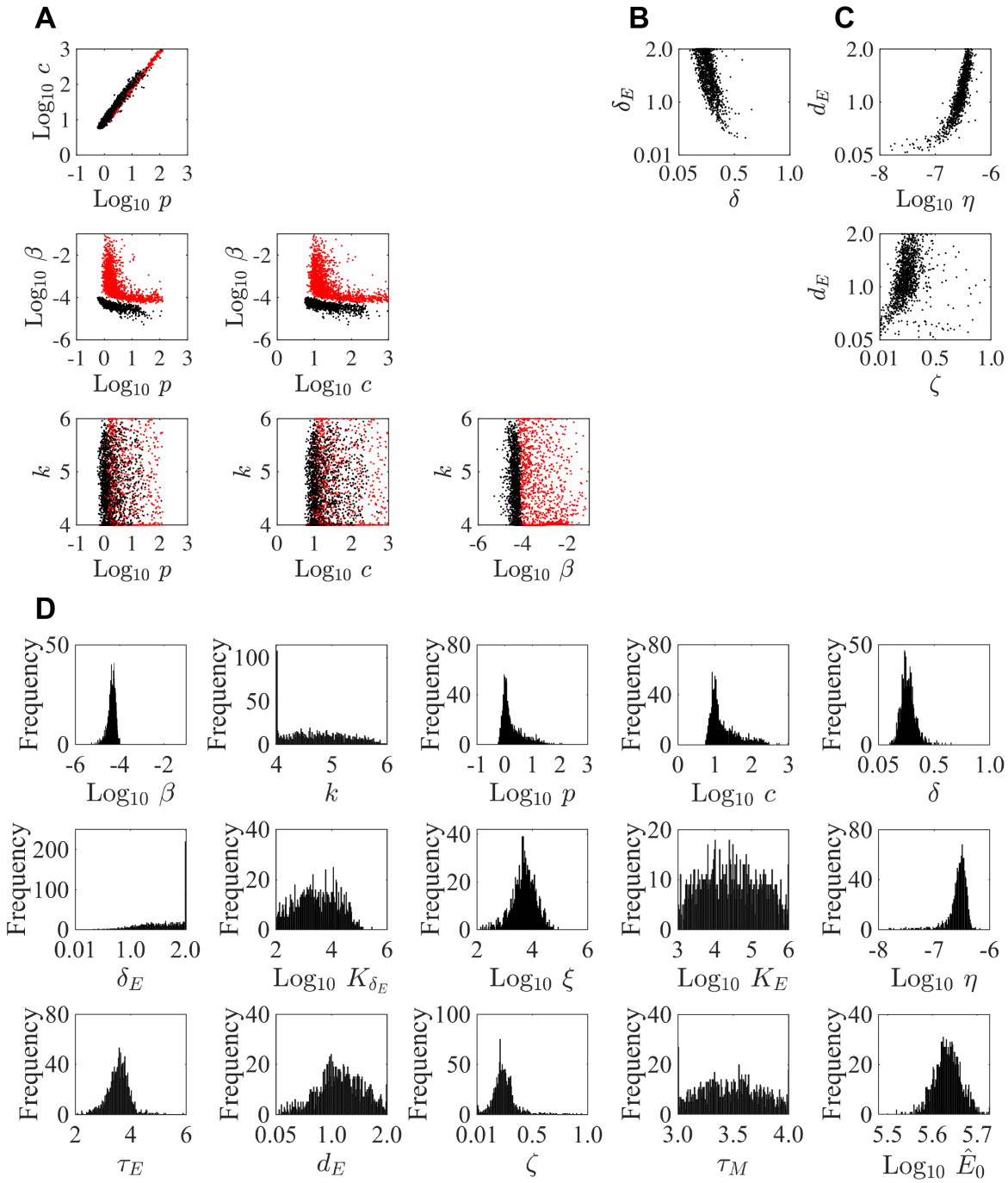

Fig 2: **Parameter ensembles and histograms.** Parameter ensembles (Panels A-C) and histograms (Panel D) resulting from fitting the density-dependent (DD) model (Eq (1)–(4)) [15] or the $CD8^+$ T cell viral kinetic model (Eq (5)–(10)) to viral titers and $CD8^+$ T cell counts from mice infected with 75 $TCID_{50}$ PR8. (A) Comparison of parameters that were consistent between the DD model (red) and the $CD8^+$ T cell model (black). Correlations are evident between parameters relating to the rates of virus infectivity (β), virus production (p), and virus clearance (c). However, the strength of the correlation was significantly reduced in the $CD8^+$ T cell model. The eclipse phase parameter (k) is not well-defined in either model. (B) The rates of infected cell clearance by non-specific mechanisms (δ) and by $CD8_E$ (δ_E) are slightly negatively correlated. (C) Additional correlations were present between the rates of $CD8_E$ clearance (d_E), expansion (η), and memory generation (ζ). The axes limits reflect imposed bounds. Additional ensemble plots are in Figs S1–S2.

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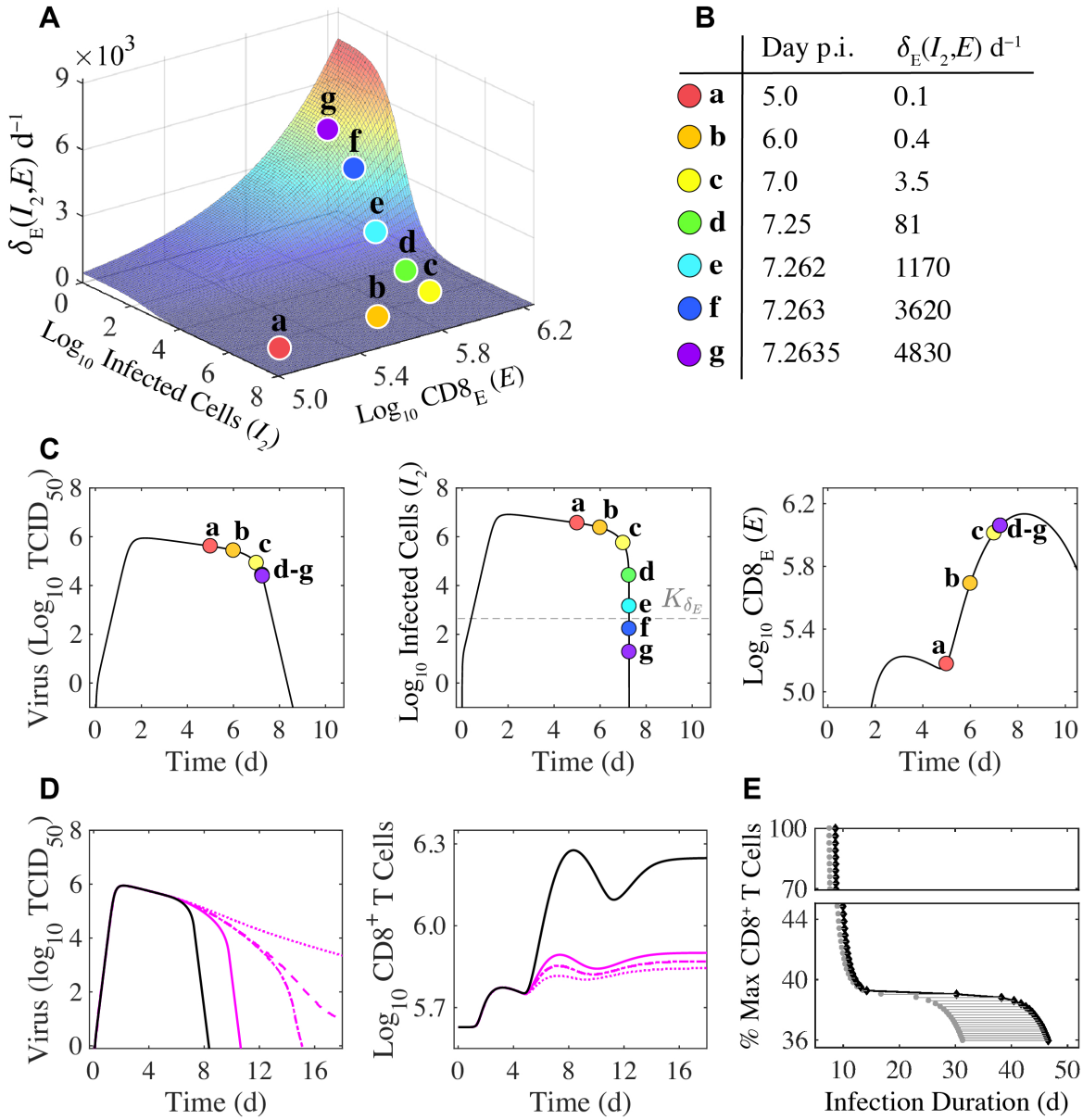

	Day p.i.	$\delta_E(I_2,E)$ d^{-1}
a	5.0	0.1
b	6.0	0.4
c	7.0	3.5
d	7.25	81
e	7.262	1170
f	7.263	3620
g	7.2635	4830

Fig 3: **Density-dependent infected cell clearance by CD8$^+$ T cells and their impact on recovery time**. (A) The rate of CD8$_E$-mediated infected cell clearance ($\delta_E(I_2, E) = \delta_E E/(K_{\delta_E} + I_2)$) plotted as a function of infected cells (I_2) and effector CD8$^+$ T cells (E; CD8$_E$). The colored markers (denoted a–g) indicate the infected cell clearance rate that corresponds to different time points during the infection for the best-fit solution. (B) Values of $\delta_E(I_2, E)$ for the indicated time points associated with the markers a–g. (C) Corresponding locations of the various $\delta_E(I_2, E)$ values (markers a–g) on the best-fit solution of the CD8$^+$ T cell model for virus (V), infected cells (I_2), and CD8$_E$ (E). (D) Solutions of the CD8$^+$ T cell model (Eq (5)–(10)) for virus (V) and total CD8$^+$ T cells ($\hat{E}$) using the best-fit parameters (black line) and when varying the CD8$_E$ expansion rate (η; magenta lines) to illustrate how different total CD8$^+$ T cell magnitudes alter infection duration. The magenta lines are solutions from when the percent $\hat{E}_{max}$ relative to $\hat{E}_{max}$ from the best-fit solution was 42% (solid line), 39.2% (dash-dotted line), 39.1% (dashed line), or 37% (dotted line). (E) The time at which infected cells reach the half-saturation constant ($I_2 = K_{\delta_E}$; gray circles) and the infection duration (time where $\log_{10} V = 0$; black diamonds) are shown for the various CD8$^+$ T cell magnitudes. The gray line between these points is the time required to eliminate K_{δ_E} infected cells and achieve complete resolution of the infection ($\log_{10} V = 0$).

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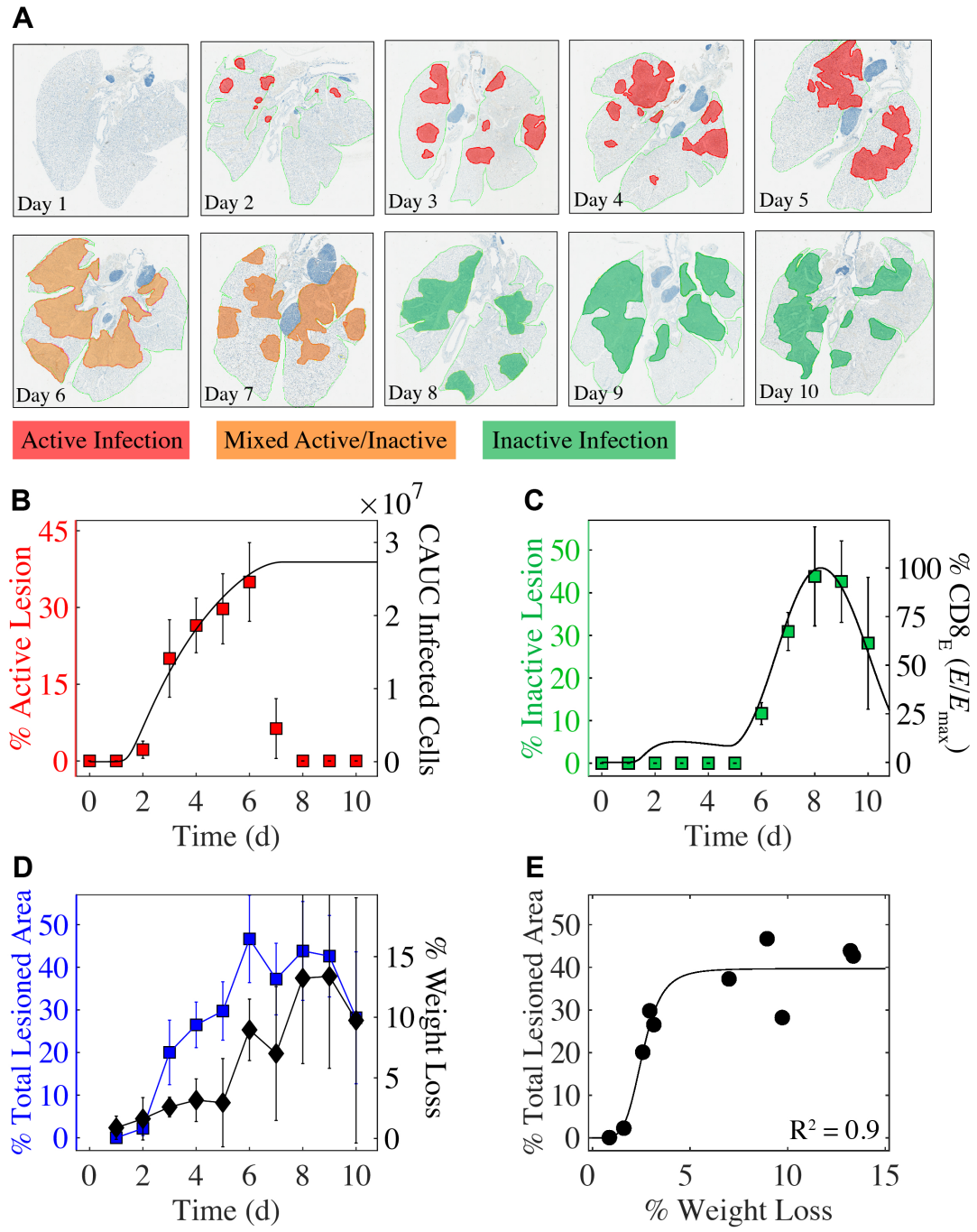

Fig 4: **Histomorphometry of the lung during IAV infection and its relation to weight loss and model dynamics.** (A) Whole lung sections with histomorphometry showing the areas of influenza NP-positive "active" lesions (red), "inactive" lesions with minimal antigen-positive debris (green), or mixed active and inactive regions (orange) throughout the infection. (B) Percent active lesion (red squares) plotted together with the cumulative area under the curve (CAUC) of the predicted infected cell dynamics (I_2) obtained from fitting the CD8$^+$ T cell model. (C) Percent inactive lesion (green squares) plotted together with the percent maximum CD8$_E$ (E/E_{max}) obtained from fitting the CD8$^+$ T cell model. (D) The combined percent active and inactive lesion (blue squares) plotted together with the percent weight loss (black diamonds) to illustrate their similar dynamics. (E) Fit of a saturating function ($L(W) = l_{max}W^n/(K_w^n + W^n)$) to the percent total lesioned area (L) and weight loss (W). The best-fit parameters were l_{max} = 39.7% total lesioned area, K_w = 2.6% weight loss, and n = 5.2.

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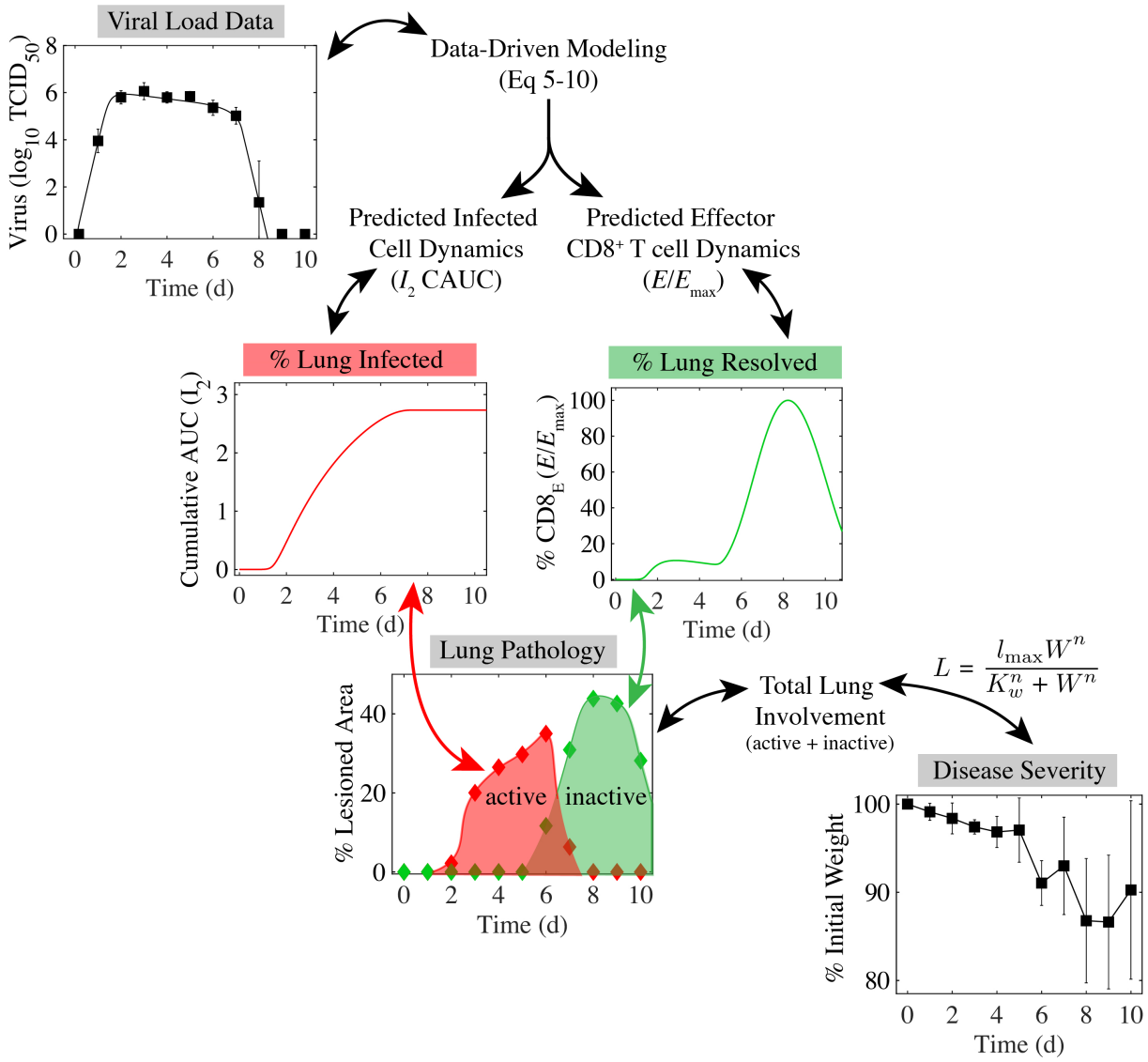

Fig 5: **Connection between the kinetics of virus, infected cells, CD8$^+$ T cells, lung pathology, and disease severity.** Viral loads and weight loss are the two most easily measured pieces of data. Our analysis relates these through mathematical models. That is, given viral loads, the CD8$^+$ T cell model (Eq (5)–(10)) can be used to predict the kinetics of infected cells and CD8$^+$ T cells. The cumulative area under the curve (CAUC) of the predicted infected cell dynamics (I_2) yields an estimate of the percent lung infected (active lesion) while the predicted relative $\mathrm{CD8_E}$ dynamics (E) yield an estimate of the percent lung resolved (inactive lesion). The total amount of lung involved (% lung infected and % lung resolved) can then be used to estimate weight loss through the function $L(W)$. These connections can be reversed and weight loss used to predict viral load kinetics.