Spatially explicit model of the lymphocyte diaspora in influenza-infected lung reveals constraints on chemokine directed migration

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Abstract

During the primary immune response, clearance of influenza in the lung requires the homing of activated CD8 T cells from regional lymph nodes to infected foci comprising a fraction of the total lung. T cell navigation to this region is undirected but migration is made possible by local cytokine and chemokine production from infected epithelial cells. We examined the efficiency of local chemokines to induce migration of activated CD8 T cells using an agent-based model (CyCells).

Avian H5N1, seasonal H1N1, and 2009 pandemic influenza strains were used to induce chemokine production *in vitro*. Of the chemokines tested, secretion of CXCL10 (IP-10) and CCL5 (RANTES) were stimulated by infection, with IP-10 dominating the migration due to higher concentrations. A differential equation model was fit to empirical chemokine production rates and coupled with published T cell parameters to calibrate the spatial model to test inter-strain variation on T cell recruitment in the lungs. We observe that T cell sensitivity to chemokine is high enough to maximize their efficiency. The modeled immune response is unable to clear the pandemic strain due to its high rate of viral production.

The spatial nature of the model reveals unique challenges to T cell recruitment not visible in standard differential equation models. Infected cells can become isolated in expanding plaques, impeding infection control. In the model, T cells executed an efficient search with sensitivity to either chemokine. A key limitation imposed on T cells was illustrated by their failure to clear the pandemic H1N1 virus after day 6, when T cells became inefficient in large infected foci as revealed in cinematics. This spatial model is consistent with efficient T cell recruitment to infected lung. Without an apparent means to induce increased efficiency, vaccination remains the key to shortening the interval between initial infection and

T cell activation.

Author Summary

Clearance of influenza from the lung depends strongly on the efficiency with which T cells travel from the lymph node to the site of infection. However, T cells in the lung must navigate the large branching bronchial network. This maze-like structure is a challenge for T cell search, and the vast majority of T cells exit the lung without encountering infection. Infected cells produce cytokines and chemokines, which signal T cells to exit the vasculature and enter tissue. We developed an agent-based model to quantify how these molecular signals affect search. Our experimental data show that two chemokines, IP-10 and RANTES, are important for T cell search. We incorporated the data in our spatially explicit model, which allowed us to identify challenges to the search process not obvious in conventional differential equation models. We ran three sets of simulations, using parameters from seasonal influenza, avian influenza, and 2009 pandemic influenza. The simulated immune response contains the first two infections but is unable to contain the highly virulent pandemic strain due to the rapidly expanding plaque size. Direct interventions to improve this T cell search process are unlikely to be effective, and therefore vaccination is required to contain more virulent strains.

Introduction

The adaptive immune response induced during acute influenza A infection is a complex web of defense mechanisms that control viral replication of all but the most virulent strains. Understanding the behavior and interactions of each cellular component may lead to improved vaccines and strategies to control immunopathology. The innate immune response is critical for early control of viral replication, followed by antibody-mediated viral neutralization [1–3]. Complete resolution of influenza pneumonia depends on antigen-specific CD8+ T effector cells [4, 5]. The murine model has been critical in the reductive analysis of each phase and component, permitting temporally explicit descriptions of the induction phase in the regional lymph node [6–8] where recruitment of clonal precursor CD8 T cells is highly efficient [9]. On approximately day 5 after infection activated CD8 T cells are released from the secondary lymphoid tissue [10] and distributed throughout the body in a process known as the lymphocyte diaspora [11].

The process of recruitment of activated CD8 effector cells to infected sites in the lung is not completely understood. Initial localization and extravasation may depend on a number of inflammatory signals but viral antigen does not appear to induce recruitment into tissue [12]. Effector cells must localize to sites of viral replication [4], but it is not clear whether cells passing through uninfected lung tissue leave through the pulmonary vein or exit the capillary bed and leave the lung through lymphatic channels. Chemotactic proteins play a major role in recruitment in both acute infections and chronic inflammatory diseases [13,14] where recruited T cells mediate either protection or immunopathology. Infected epithelial cells secrete chemokines [15] and contact with CD8 T cells also triggers secretion [16]. Activated CD8 T effector cells upregulate chemotactic protein receptors, particularly CXCR3 and CCR5 [17,18], responding to their primary ligands CXCL10 (IP-10) and CCL5 (RANTES) respectively.

The consequences of chemotactic ligand-receptor interactions, however, are complex [18, 19] and variable in the models studied. For example, in the lymphochoriomeningitis virus model the CXCR3 receptor mediated T cell recruitment to infected brain and subsequent immunopathology [20, 21], while the CCR5 mediated the opposite effect [22]. In contrast, in the West Nile virus model [23] and the dengue model [24], deficiency of CXCL10 reduced T cell recruitment to the brain resulting in higher viral burden and increased mortality. In the herpesvirus model CXCL10 was critical in T cell recruitment and disease control in the HSV-2-infected brain [25, 26]. In the parainfluenza virus model CXCR3 receptor is critical in CD4+ T cell migration to the lung [27]. In the influenza A model initial studies with chemokine receptor knock-out mice obtained mixed results with respect to changing the course of disease [28, 29], concluding that redundancy in chemokine signals may confound interpretation. The CXCR3 receptor mediated T cell localization and spared the increased mortality of CCR5 deficiency but viral clearance was not altered [30]. The CXCR3 receptor also mediates the balance between effector versus memory cell differentiation among recruited CD8 T cells in the lung [31].

Although leukocytes exhibit directional behavior to chemokines [32, 33], T cells have not be shown to climb chemokine gradients. Following arrival at the infected site, CD8 T cells secrete cytokines when mononuclear cells are present but in their absence kill infected epithelial cells only by contact cytolysis [34]. Finally the exit of T cells from tissue is tightly regulated to keep effector cells from leaving or dying prematurely [35, 36] but effective life-span is unknown.

A number of mathematical models have constructed the entire adaptive response and key components [37–42], filling gaps with reasonable assumptions of cellular behavior in the absence of experimental

support. Focused math models can examine each event in detail and narrow the range of possible solutions. We sought to examine the CD8 T cell diaspora and recruitment to infected lung in detail. Models using ordinary differential equations are able to predict events with temporal fidelity, but lack the ability to examine the spatial detail. Activated T cells searching for and homing into infected tissue do so in a spatially complex environment. We therefore utilized the strategy of agent-based modeling (ABM) to represent the physical environment of the searching T cell. Thus T cells travel in vascular and lymphatic channels during part of their search, and respond to localized inflammatory signals to achieve compartmentalized cell-cell interactions.

We ask how small foci of infected tissue, scattered through a very large space of uninfected tissue, can attract and retain limited numbers of activated CD8 T cells. Our model does not consider important features of tissue immunity such as T cell proliferation, resident T cells, tertiary lymphoid structures, and regulatory interactions with other cell phenotypes [43]. The model does not aim to predict control of viral replication but only compare simulated outcome among three influenza strains. To achieve this focused goal on the efficacy of the chemotactic signals alone, we use chemotactic protein data from in vitro cultures of bronchial epithelial cells infected with three strains of influenza virus with different replication rates [44] previously described in our laboratory. Using known parameters from the extensive literature on the murine model, we offer several insights on the control of viral replication in lung tissue derived from spatially explicit simulations.

Models

Delay Differential Equation Model

We estimate chemokine production rates by adapting the delay differential equation model described in [44] by adding one new equation to model chemokine production. Initial population sizes and parameter

values are taken from the previous study.

$$\begin{aligned}
\dot{T} &= -\beta TV \\
\dot{I}_1 &= \beta TV - \beta T[t-\tau_1]V[t-\tau_1] \\
\dot{I}_2 &= \beta T[t-\tau_1]V[t-\tau_1] - \delta I_2 \\
\dot{V} &= \frac{p}{1+eF} I_2 - \beta TV \\
\dot{F} &= I_1[t-\tau_2] \\
\dot{C} &= rI[t-\tau_3] - dC
\end{aligned} \tag{1}$$

Table 1 summarizes population and parameter values and descriptions. Strain-specific values for r were found by fitting the equations to experimental data (see Results and Table 3).

Computational Modeling

Computational modeling used CyCells [45], a modeling platform for two- or three-dimensional agent-based simulations of the immune response. A simplified model of T cell activation and recirculation (Fig. 1) was implemented in CyCells (Fig. 2), and simulations measured efficiency of infection clearance under different environmental conditions. The lung was represented as a two-dimensional sheet of healthy epithelial cells. Vasculature was represented as a binary tree with fourteen branches originating at a single lymph node. Activated T cells descend through the vascular tree until cytokine signal is detected on the local endothelium, at which point they exit the vasculature and follow the chemotactic gradient to the site of infection. T cells that do not encounter cytokine recirculate to the lymph node. At the site of infection when a T cell encounters an infected epithelial cell it induces apoptosis.

The simulation begins when a single cell in the center of the tissue is infected. After the eclipse phase (incubation), the infected cell begins secreting virus and chemokine. Virus diffuses locally, infecting nearby cells, and continuing the cycle. Chemokine diffuses from secreting cells, creating a ball of stimulation around the infected region. After a five day delay to simulate lymph node stimulation and T cell proliferation, activated T cells exit the lymph node and circulate through the vasculature to the tissue. Because T cells cannot choose their path through the branching network, we assume they arrive in tissue at random locations.

Model Definition

In the model, epithelial cells are stationary and can be in one of five different states: *healthy*, *virus-incubating*, *virus-expressing*, *apoptotic*, and *dead*. *Healthy* cells remain unchanged unless infected by virus. Once infected, the cell transitions from incubating to *expressing*. *Expressing* cells secrete virus and chemokine for approximately 17 hours and then die. *Expressing* cells become *apoptotic* if they encounter activated T cells. Apoptoic cells continue to secrete virus until they die one hour after their transition. *Dead* cells remain inert and do not regenerate over the course of an infection.

T cells have three states. *Circulating* T cells begin to emerge from the lymph node at five days post infection. *Emigrating* T cells arrive at a random location on the lung's surface, wander randomly in the tissue for 10 minutes, and transition to *circulating* in the absence of chemokine. *Circulating* cells spend six minutes recirculating to the lymph node, transition to *emigrating* and are reintroduced to a new random location in the lung. When a *emigrating* T cell encounters chemokine, it converts to *chemotaxing* and begins following the chemotactic gradient to the source of infection. *Chemotaxing* T cells move continuously up the gradient, inducing apoptosis if they encounter *expressing* epithelial cells. *Chemotaxing* cells decay exponentially with an average lifespan of two hours.

The model contains two kinds of particles: virus and chemokine. Both are produced at constant rates by *expressing* epithelial cells. Virus diffuses through the lung tissue, infecting healthy cells probabilistically according to the local virus concentration. Chemokine diffuses across the tissue but has no direct effect beyond activating T cells. Both particle types decay exponentially. IgM is modeled by increasing the viral decay rate by a factor of three after the third day.

Parameters that are consistent between every model are shown in Table 2. Strain-specific values are shown in Table 3.

Materials

Chemokine secretion: Epithelial cell culture and supernatant collection was performed as described [44]. Briefly, undifferentiated human tracheal epithelial cells (University of Miami) were cultured for 4 weeks to achieve fully differentiated confluent monolayers on collagen-coated transwell inserts, or commercial differentiated human bronchial epithelial cells (EpiAirway Tissue, MatTek Corp., Ashland, MA) used immediately upon receipt, were infected at an MOI of 0.01 with either seasonal H1N1 virus A/New

Caledonia/20/99 (sH1N1), the 2009 H1N1 pandemic strain A/California/04/09 (pH1N1), or avian H5N1 virus A/Hong Kong/483/97 (aH5N1) derived from a fatal human infection. Apical fluid for viral secretion, and basal media for chemokine secretion collected before treatment of the monolayer with protease, was collected from previously undisturbed triplicate or quadruplicate wells at 0, 6, 10, 12, 16, 20, 24, 30, 36, 42, 48, and 72 hours after infection, and stored at -80C until assay. Quantitative viral culture was performed by standard plaque assay. Quantitative chemokine levels were performed in 30 l aliquots for a panel of chemokines (IL-8, MCP-1, MIP-1α, MIP-1β, RANTES, IP-10, eotaxin) and cytokines (interferon-gamma, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-6, IL-10, IL-12p40, IL-15, IL-17, TNFα) (Luminex Assay, Luminex Corp.) and reported as ng/mL basal media sampled from a total volume of 4 mL. Only IP-10, RANTES, and TNFα showed increases in production. TNF was ignored as its effects are outside the scope of this paper.

Results

Chemokine production

To provide estimates of chemokine concentrations and secretion rates present in lung tissue, chemokine levels were measured at 4-6h intervals during the first 48 h of infection in wells containing approximately one million human bronchial epithelial cells (Fig. 3). The dynamic viral loads at these intervals in these cultures infected with seasonal H1N1 virus, pandemic H1N1 virus, and avian H5N1 virus have been reported previously [44]. IP-10 concentration increases were observed by 8h post-infection (p.i.), and RANTES by 16h p.i.. To estimate per-cell production rates, we extended the ordinary differential equation (ODE) model of Ref. [44] to represent chemokine production from infected cells (Eq. 1). Model fits (Table 3) were computed for three strains (Fig. 3) using Matlab's `nlinfit` function (Levenberg-Marquardt algorithm). The resulting chemokine production values were used in the CyCells ABM. Best-fit expression rates were similar for all strains except for significantly higher RANTES production in aH5N1. There is no positive correlation between viral production and induced chemokine production across the three strains.

T cell sensitivity to chemokine

The model simulates a chemokine gradient surrounding an infected focus (Fig. 5), based on the calculated per-infected cell secretion rate (Table 3) and known chemical parameters for a 10 kDa protein (Table 2). T cell sensitivity depends on receptor density [46] and this was assumed to be constant.

Because this parameter is unknown, we simulated T cell sensitivity levels ranging over 10 orders of magnitude and found a threshold (Fig. 4) at a concentration of 100 ng/ml (10 ng/ml in aH5N1), beyond which there is no detectable effect on model behavior (single runs were used due to computational limitations, model variance is discussed in S2.1.). We then set the sensitivity to 10 ng/mL for all future runs (1 nM concentration assuming a chemokine molecular weight of 10 kDa) [47].

Spatial effects

Spatial effects of viral and chemokine diffusion play an important role in both the spreading and clearing of infections. Free virus particles diffuse from virus secreting cells and infect healthy cells. Chemokine produced by infected cells attracts T cells to the infected cells. Although virus is produced at a higher rate than chemokine, its larger size diffuses much more slowly, while chemokine decays more quickly. These countervaling effects result in similar spatial profiles for the two particle types (Fig. 5). Until day 4 the plaque is dominated by active (incubating and secreting) cells, whereas dead cells are rare. Over time, cells in the plaque's interior die, and active cells form a decreasing proportion of the plaque. T cells arrive at day 5 and begin killing the virus-secreting cells. By day 6 many expressing cells have been eliminated and the plaque is dominated by dead cells. Refer to the videos in Figures S3-S5 to see a representative simulation of seasonal influenza.

In aH5N1, the plaque is dense, allowing T cells to find secreting cells easily, and infection is eliminated. However, secreting cells were not eliminated in either H1N1 simulations (Fig. S1). Secreting cells accounted for at most 10% of the active cell population and less than 1% of the total plaque at 6 days p.i.. T cells still accumulate, but they arrive more slowly than the plaque is growing, leading to lower average T cell killing rate. Further, the regions of concentrated chemokine lag behind the cell and virus spatial layout. It takes time for newly secreting cells to produce chemokine while pockets of high chemokine density are slow to decay. Thus, T cells can fail to detect cellular changes in the plaque. Taken together, the delayed response and low proportion of virus secreting cells prevent T cells from completely clearing

the infection.

T cells are unable to kill cells that have not yet presented antigen. At day 5.5, the ratio of secreting cells to the total plaque size is high (Fig. 5-6). By day 7, this ratio is approximately 1:100 for both the pH1N1 and sH1N1 strains. The high replication rate of pH1N1 enhances this effect (Fig. 6C) and the T cells can control (but not eliminate) the sH1N1 infection. The video in Figure S6 illustrates this event.

A cell infected with pH1N1 produces new virus at the rate of 5.08e-3 PFU/s [44]. That is, in each secreting cell a new viral particle is produced approximately every 200 seconds. Assuming that secretion continues for one hour after apoptosis is initiated, the best a T cell could do is limit production to 18 new viral particles. Thus, T cells alone cannot contain the pH1N1 infection. In contrast, a sH1N1 virus-secreting cell produces a new virus particle every 2,632 seconds, allowing T cells to limit a single infected cell to 1.4 viral particles in the one-hour window. Avian H5N1 virus-secreting cells produce only 0.2 viral particles in the interval after induced apoptosis.

Discussion

Modeling Methodology

Limitations in the model are primarily due to a number of simplifications to and deletions of elements in the innate immune response, allowing us to build a tractable model where data was available. Antigen presentation T cell clonal expansion in secondary lymphoid organs is represented solely by the constant rate of emigration of activated T cells from regional lymph nodes. Virus-specific strain replication rates are represented as constant rates, and virus clearance is also constant. The contribution of IgM antibody clearing free virus is represented at a constant rate, and the class switch to higher affinity antibody mediated by CD4+ T cells is not represented. All of these rates may in fact be time-variable as indicated by superior data-fitting models [48]. Immigration and contributions of virus-nonspecific immune cells such as macrophages and/or dendritic cells are not represented in our model. Finally, the proliferation of activated T cells in lung tissue is not represented, but is thought to be crucial in the control of lung infection [37]. Thus, our model is not intended to predict clearance of virus from the lung. Rather our goal was to examine the features of the response that permitted T cells to sense and contact infected target cells.

The use of an ABM has certain advantages over a spatially homogeneous ODE model. An ODE

model assumes that any virus particle is capable of infecting any healthy cell. Figure 5 shows that this is clearly not the case. In fact, most free virus exists on top of infected cells that are no longer candidates for viral binding and fusion. ODE models account for this discrepancy by lowering rates of infection by a constant amount, but this assumes that the proportion of unsuitable virions will always be the same. This is limiting as can be seen in Figure 5 where the early infection has a higher proportion of virus overlapping healthy cells.

Our ABM renders the model in OpenGL (Fig. 5). Seeing the model in action reveals spatial dynamics that are absent in ODEs and difficult or impossible to observe in *in vitro* and *in vivo* systems, for example, the spatial dynamics discussed earlier. First, because T cells find infected cells by climbing a gradient, we see T cell clustering at local maxima of chemokine concentration, a possible explanation for why T cells do not increase in effectiveness as their numbers increase. Second, T cell clustering persists after all virus-secreting cells have been eliminated. The local chemokine maxima takes time to diffuse and decay so that T cells can climb the gradient to a new maximum. Finally, we can see that infected cells are more disperse as infection size grows. Because T cells are clustered they cannot cover the increasing plaque effectively. These spatial observations provide explanations for the pH1N1 resurgence that would be obscured without the visualization tools provided by the ABM. The behavior of searching T cells described in this paper can enhance future global immune response models.

Chemokine Directed T Cell Search

The actual quantitative chemokine environment in the infected lung is difficult to estimate for a math model. The hypercytokinemia documented in virulent influenza infections [49] likely does not reflect lung tissue concentrations. Dynamic chemokine concentrations secreted by bronchial epithelial cells in vitro depend on infection intensity and cell maturation state [15, 44, 50, 51] but may better approximate actual levels. Interestingly, the attenuation of the type I interferon response by H5N1 strains is not associated with attenuation of chemokine secretion [52]. The model did not incorporate the potential contributions from other chemokines such as CXCL8/IL-8 detected in bronchial cell cultures [53, 54], nor did the model account for chemokines secreted by immigrant macrophages [55] and amplification of epithelial cell secretion by CD8 T cells [16].

A key determinant in the efficiency of chemokine-directed T cell migration towards virus-secreting epithelial cells is the communication distance, defined by the threshold of sufficient chemokine signal

required to induce directed motion of the cell up the chemical gradient [56]. The diameter of this gradient generated by a single cell is a function of production rate, decay rate, protein diffusion and the sensitivity threshold. For the threshold of 10 ng/mL and maximal levels of concentration in tissue of 10,000 pg/mL, we calculated the effective communication distance to be approximately 150 microns in our model by simulating a single chemokine producing cell and observing the radius of the resulting chemokine gradient. This calculation is similar to the distance calculated for generic cytokines secreted by a suspended solitary cell [57]. Spatially explicit modeling can provide support for not only communication distance but also the role of immigrant CD8 T cell proliferation, contribution of resident memory T cells and B cells, and effector cell lifespan.

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    KEY: Dawson2000

    ANNOTATION: –¿ description of the general effect on lung "inflammation" of CCR5 dependent T cell response during influenza infection - CCR5 KO triggers higher mortality rate in influenza infected mice but does not significantly decrease the clearance of the virus (

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    KEY: Fadel2008

    ANNOTATION: These data could be interesting if we want to model both CCL5 and CXCR3 (receptor for CXCL10 or IP-10) because CXCR3 has regulating properties on CCR5 (receptor for CCL5 or RANTES) dependent and/or independent response.

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    KEY: Desmetz2006

    ANNOTATION: –¿ the important factor seems to be the fluctuations of CCR5 (receptor) expression on T cells and less the fluctuations of CCL5 (chemokine) concentration on T cell chemotactic activity. - CCL5 concentration dependent T cell migration (in vitro) –¿ CCL5 T cell sensitivity ?

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KEY: Gao2003

ANNOTATION: CCL5 (RANTES) and CXCL10 (IP-10) concentrations inducing T cell chemotaxis (adhesion)

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Figure Legends

Figure 1. Model of T cell search. Activated T cells originate in the lymph node and enter the bloodstream after which they randomly navigate through 14 vascular bifurcations of the bronchial network. Upon reaching a capillary, T cells exit into tissue if cytokine signal is present. In the absence of signal, the T cell recirculates either through the lymph network or through the pulmonary vein back to the top of the network.

Figure 2. Visual representation of the model. Healthy epithelial cells infected by virus begin secreting virus after the incubation delay. Activated T cells traverse the bronchial vascular network and may be recruited by inflammatory cytokine. Chemotaxing T cells climb the chemokine gradient and induce apoptosis in infected cells. Solid arrows represent a cell state transition from one behavior to another. Dashed arrows display the mechanism used to induce a transition. Dotted arrows indicate the production of new virus.

Figure 3. Empirical viral and cytokine titers for three strains of influenza: Avian H5N1, Seasonal sH1N1, and Pandemic pH1N1. Viral titer (blue circles) is in PFU/mL, and IP-10 (red triangle) and RANTES (green square) are shown in ng/mL. sH1N1 IP-10 registered three values, not included in model fitting, above the measurement threshold of 8,500 pg/mL (empty red triangles). Dashed lines show model fits to IP-10 and RANTES data. Human bronchial epithelial cells were infected at an MOI of 0.01 with one of the three strains of influenza. Apical fluid for viral secretion and basal media for chemokine secretion was collected at the given time intervals post infection. Viral culture was performed by a standard plaque assay and chemokine levels were measured using 30 l aliots for a panel of 17 chemokines and cytokines (not shown).

Figure 4. Varying T cell sensitivity to chemokine. H5N1 model results use RANTES only, and sH1N1 and pH1N1 use both IP-10 and RANTES. Total number of incubating, secreting and apoptotic cells are plotted for each infection. The sensitivity value specifies the minimum level of chemokine concentration required for T cells to detect it.

Figure 5. Simulated sH1N1 infection. Screenshots from day 4, day 5.5, and day 7. The top row shows the spreading focus of infection through the color coding of individual cells: healthy cells in uninfected tissue (gray), virus-incubating cells (yellow), virus-secreting cells (orange), apoptotic cells (red), dead cells (brown), and T cells arriving at day 5 (green). Free virus and chemokine particles are represented by compartmentalized concentrations of mols/mL and ng/mL. The individual cell images for days 5.5 and 7 have been enhanced by hand for improved T cell visibility. Individual groups of T cells appear larger than they are and only cover an area of one or two epithelial cells in the model. Original images available upon request.

Figure 6. Simulated infections of aH5N1, sH1N1, and pH1N1. Plotted values: total plaque size (blue), number of virus incubating cells (yellow), number of virus secreting cells (green), and T cells (red). T cells clear secreting and incubating cells in aH5N1, fail to clear incubating cells in sH1N1, and fail to clear either type of infected cell in pH1N1. The number of expressing cells (green) after day 5 differs markedly among the three strains indicating that the T cells have differing success at controlling the infection.

Tables

Population	Description	Initial Value
T	Healthy target eptithelial cells	1,000,000
I_1	Virus-incubating cells	0
I_2	Virus-secreting cells	0
V	Free virus particles	10,000
F	Interferon quantity	0
C	Chemokine quantity (ng/mL)	0
Parameter	**Description**	**Value**
β	Viral infection rates	
	Avian	5.3e-7 $(\text{PFU}\cdot\text{h})^{-1}$
	Seasonal	6.1e-7 $(\text{PFU}\cdot\text{h})^{-1}$
	Pandemic	2.7e-6 $(\text{PFU}\cdot\text{h})^{-1}$
p	Viral production rates	
	Avian	0.20 (PFU/h)
	Seasonal	1.4 (PFU/h)
	Pandemic	18.3 (PFU/h)
e	Interferon strengths	
	Avian	1.0e-8
	Seasonal	1.6e-6
	Pandemic	3.4e-3
τ_2	Interferon production delays	
	Avian	21.5 (h)
	Seasonal	23.6 (h)
	Pandemic	21.0 (h)
δ	Virus-secreting cell decay rate	0.6 (h^{-1})
d	Chemokine decay rate	1.386 (h^{-1})
τ_1	Viral incubation time	10 (h)
τ_3	Chemokine production delays	
	IP-10	8 (h)
	RANTES	16 (h)

Table 1. All parameters and populations are taken from [44] except for C, d, and τ_3. The value chosen for d corresponds to a 30 minute half-life and values for τ_3 were take from the observed chemokine data (Fig. 3). Interferon (F) is an abstracted unitless quantity and thus e is a unitless multiplier.

Paramter	Value	Source
Viral Diffusion in Airway	$.0318\mu m^2/s$	[58]
Viral Decay in Airway	$1/day$	[42]
Chemokine Diffusion Rate	$.318\mu m^2/s$	[58]
Chemokine Decay Rate	$3.8508 \cdot 10^{-4}$/s	Selected[1]
Infection Sensitivity Rate	$2hour/virion$	Selected[2]
Incubation Time	$10hours$	[44]
Expression Time	$16.7hours$	[44][3]
Apoptosis Time	$1hour$	[59]
T cell Production Rate	$777/hour$	[37]
Blood Circulation Time	$6min$	[60]
Search Time In Chemokine-Free Tissue	$10min$	[60]
T Cell Speed (Search)	$30\mu m/s$	[6]
T Cell Speed (Chemotaxis)	$3\mu m/s$	[6]
T Cell Sensitivity to Chemokine	$10ng/mL$	[47]
T Cell Expected Kill Time	$10min$	Selected[4]
Epithelial Cell Radius	$25\mu m$	Selected[5]
T Cell Age (at FOI*)	$2hours$	Selected[6]
T Cell Age (in Blood)	$3days$	Selected[6]
Onset of ATC‡ Lymph Node Exit	$5days$	[61]
IgM Strength	Viral decay of $3/day$	Selected[7]

Table 2. 1) Corresponds to a 30 minute half-life. 2) Epithelial cells are infected at a probabilistic rate such that the expected time for infection in the presence of a single virion is 2 hours. This scales linearly with the number of virions in the cell's vicinity. 3) Chosen as a plausible median time (1,000 minutes) between 6 hours and 24 hours. 4) T cells induce apoptosis in nearby virus-secreting epithelial cells at a probabilistic rate such that the expected time to induce apoptosis is 10 minutes. This rate does not scale with T cell numbers. 5) The mean surface area of the epithelial cell available for virus contact and entry includes cilia and the radius is estimated to be 25 microns. 6) Parameters used after discussions with David Woodland, Trudeau Institute. 7) IgM presence is abstracted by increasing viral decay by a factor of three. *Focus of Infection. ‡Activated T Cell.

Strain	IP-10 Production ($pg/s \cdot cell$)	RANTES Production ($pg/s \cdot cell$)	Viral Production ($PFU/s \cdot cell$)
Avian H5N1	2.0e-4 8.4e-5 — 4.2e-4	1.3e-5 7.9e-6 — 1.9e-5	5.4e-5 4.4e-5 — 3.7e-4
Seasonal H1N1	1.8e-4 1.2e-4 — 3.0e-4	8.9e-7 4.8e-7 — 1.6e-6	3.8e-4 2.8e-4 — 1.5e-3
Pandemic H1N1	8.7e-5 1.7e-5 — 7.1e-4	4.3e-6 5.0e-7 — 3.5e-5	5.1e-3 2.8e-3 — 5.3e-3

Table 3. Strain-specific model parameters. Small text values show 95% confidence intervals resulting from 1,000 bootstrapping runs for each parameter [62]. Viral production values are taken from [44].