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Simulated pathogenesis of severe acute respiratory distress syndrome and leukopenia induced with influenza A/H5N1 virus infection and its treatment with immunoglobulins

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Abstract. Background: Severe acute respiratory distress syndrome (ARDS) is a major symptom of infection with influenza A/H5N1virus. Patients with influenza A/H5N1 suffer from leukopenia, which is regarded as a prognostic factor of cellular injury associated with ARDS. Nevertheless, the association of cellular injury with leukopenia remains unclear.
Methods and findings: A within-host mathematical model, a system of delay differential equations of virus dynamics and immune response, was applied to ascertain influenza pathogenesis. When leukocytes destroy infected cells, the ratio of leukocyte destruction becomes higher in the case of A/H5N1. Moreover, a comparison of therapies for leukopenia using the model reveals that immunoglobulin therapy is more effective than neuraminidase therapy.
Conclusion: Simulations show that the increased ratio of destruction of leukocytes induces leukopenia and cellular injury of A/H5N1. Furthermore, results of simulations demonstrate the possibility of immunoglobulin therapy for treating leukopenia associated with influenza A/H5N1.

1. INTRODUCTION

Emerging influenza, especially A/H5N1, is a threat to society. The influenza A/H5N1 pathogenesis must be elucidated, but the peculiar pathology of A/H5N1 remains unclear. Patients infected with influenza virus A/H5N1 become severely ill, exhibiting severe acute respiratory distress syndrome (ARDS) and leukopenia [**1**]. Patients with influenza A/H5N1 in Vietnam reportedly have fever, respiratory symptoms, and leukopenia [**2**]. Extraordinary cellular injury of the lung and leukopenia were also reported in a case in Thailand [**3**]. However, the association of cellular injury with leukopenia remains obscured.

In cases where etiological data related to infection with influenza A/H5N1 virus are limited, mathematical models can be useful to ascertain the pathological process within a host [**4**–**7**]. Various within-host models, from simple to complex, have been proposed for influenza [**8**–**13**]. To ascertain the pathological process of infectious disease, latency is important. Two methods are used to simulate latency; adding ordinary differential equations of the intermediate state to the model with

new parameters or simply using delay differential equation [**5, 16**]. Simple model is suitable to analyze the A/H5N1 pathology because identifying many parameters of complex models is difficult [**14, 15**]. For simple simulations of pathology with latency, models including delay differential equations have been exploited by Marchuk [**16**].

The following findings are our point of departure. Type II alveolar epithelial cells are the prime targets of influenza A/H5N1 [**17**]. The model mouse for ARDS, which is induced with influenza virus PR-8 A/H1N1, shows destruction of lung tissues after 7 days post infection (dpi) followed by invasion of neutrophils and macrophages into alveolar lavage [**18**]. Based on our clinical observations in cases of A/H5N1 where white blood cell (WBC) counts are a prognostic factor of cellular injury of the lung in patients, we infer that neutrophils and macrophages invade into alveolar lavage also in cases where A/H5N1 plays an important role in pathogeny [**1**]. The fraction of macrophages and neutrophils in WBC of infected mouse is approximately 0.95. That of lymphocytes is 0.05 [**19**]. However, in preceding studies, mainly of seasonal influenza, lymphocytes – T-cells, B-cells and plasma cells – are modeled as constituting the immune response system [**16**].

Furthermore, for seasonal influenza, neuraminidase (NA) inhibitor reduces the length of illness of patients by 1-2 days when they are treated 36-48 h from the onset of symptoms [**20**]. As described elsewhere in the literature, five Vietnamese patients infected with A/H5N1 were treated with NA inhibitor started after 5-12 dpi: one recovered, one was recovering, and three died [**2**]. For mice infected with A/H5N1, NA inhibitor is reportedly efficacious when therapy is initiated after 24 h or 36 h, but all mice die when treatment is delayed to 48 h [**21**]. Moreover, an NA inhibitor-resistant virus of A/H5N1 has been detected [**22**]. Alternative treatment should be prepared in cases where NA inhibitor is not efficacious.

In this study, profiles of WBC are modeled as a pathogenic factor of A/H5N1. Using the model, the possibility of therapy with NA inhibitor or immunoglobulins for influenza A/H5N1 is estimated.

2. METHODS

2.1. Within-host model of influenza

The lungs are modeled as the target organ of infection. For A/H5N1, in the human cases in Vietnam, A/H5N1 virus antigens were detected predominantly in lung tissues [**17**]. In the case of mice, influenza A/H5N1 virus titers in the lung were found to be much greater than those in the brain, spleen, liver, heart, blood, or kidney [**23,24**]. Furthermore, damage of lung epithelial cells is grave when seasonal influenza becomes severe [**25**]. Here, virus dynamics in epithelial cells of the lung and immune responses are modeled using the following delay differential equation systems; modifying Marchuk's model to simulate the pathology of A/H5N1[**8, 16**].

Virus dynamics in epithelial cells

$$\frac{dT}{dt} = -\alpha TV + g\left(T, I\right), \tag{1}$$

$$\frac{dI}{dt} = \alpha T\left(t-\tau\right)V\left(t-\tau\right) - \gamma_I I - \lambda IL, \tag{2}$$

$$\frac{dV}{dt} = \beta I - \gamma_V V - \mu FV. \tag{3}$$

Immune response system

$$\frac{dM}{dt} = \kappa M^* V - \gamma_M M, \tag{4}$$

$$\frac{dL}{dt} = \xi M\left(t-\tau_M\right)L\left(t-\tau_M\right) - \gamma_L\left(L-L^*\right) - \lambda\rho IL, \tag{5}$$

$$\frac{dF}{dt} = \eta L - \mu\sigma FV - \gamma_F F. \tag{6}$$

In those equations, T represents the fraction of the susceptible type II alveolar epithelial cells in the lung, i.e. target epithelial cells. I stands for the share of infected cells in the lung. V denotes the relative number of virus titer normalized by a reference value in the lung tissue. M denotes the relative number of antigen-presenting cells. L represents the relative number of activated WBCs in peripheral blood. Furthermore, F is the relative number of antibody titer. Parameter M^* represents naive antigen-presenting cells; L^* is the equilibrium value of precursor of WBC. They are assumed as constant values. Variable t denotes time; τ and τ_M are time lags. Other variables are γ_I, γ_V, γ_M, γ_L, and γ_F, which are the rate constants, and coefficients α, β, λ, μ, κ, ξ, η, ρ, and σ, which are the model parameters described in section 2.2. Finally, g is a function of cell regeneration in the lung.

The equations govern the following phenomena. Target epithelial cells (T) are infected by influenza viruses (V). They then turn into infected cells (I) with time lag τ. Infected cells die at a constant rate or are destroyed by WBC (L). Viruses are produced in the infected cells, die at a constant rate, or are neutralized by antibody (F). Furthermore, the lost target cells are recovered through regeneration of the lung. Regarding the immune system, naive antigen-presenting cells (M^*) in the peripheral blood are stimulated by the influenza virus and become antigen-presenting cells (M). WBCs, which are multiplied by antigen-presenting cells with time lag τ_M, decrease to an equilibrium value (L^*) at a constant rate. The WBCs are destroyed with ratio ρ when destroying infected cells. Antibodies are produced in WBC. They die at a constant rate or are consumed with ratio σ when neutralizing the virus.

During the recovery phase of infection, damaged lung regeneration occurs rapidly [**25**]. Regeneration is modeled as a linear function of T [**4, 11**] or as a quadratic function [**10**]. Here, all the regenerated cells are assumed to be susceptible, although the susceptibility of regenerated cells remains unclear. The function below is used in equation (1).

$$g\left(T, I\right) = \omega\left(1 - T - L\right) \cdot \chi T^n \tag{7}$$

Here, ω and χ are parameters; n is the growth index. The growth rate of cells is χT^n. In addition, $\omega(1 - T - I)$ is the rate of dead cells to be replaced when cured.

2.2. Parameters of the model

Rate constants and the parameters are fitted to simulations, as referred from acceptable ranges in preceding studies.

2.2.1 Rate constants

The fitted values and acceptable ranges of rate constants are following. The rate constants and time lags are $\gamma_I = 0.021$, $\gamma_V = 0.083$, $\gamma_M = 0.042$, $\gamma_F = 0.0018$ (1/h), $\tau = 3$, $\tau_M = 9.6$ (h). The acceptable ranges of destruction of infected cells because of cytopathicity γ_I is 0.021-0.083 (1/h) [**16**] and 0.055-0.086 (1/h) [**26**]. The rate constant of removal of antigen particles naturally by the immune system γ_V is 0.083-0.167 (1/h) [**16**], or the loss of infectivity is 0.105 (1/h) [**26**]. The rate constant of loss of stimulated antigen-presenting cells γ_M is 0.042-0.063 (1/h) [**16**]. The rate constant of natural death of antibodies γ_F is 0.0018 (1/h) [**16**]. Furthermore, the time lag of infection τ is 1.2-6 (h) [**26**]. For τ_M, the duration of lymphocyte cell division is 9.6-24 (h) [**16**] or 12 (h) [**8**]. No data are available in the literature for the loss rate of whole WBC γ_L. Instead of WBC as a whole, referring to the acceptable range of the rate constant of lymphocytes [**16**], loss of T-helper for CTL is 0.03-0.05 (1/h), loss of T-helper for B-cells 0.03-0.05 (1/h), natural death of CTL 0.01-0.02 (1/h), natural death of B-lymphocytes 0.002-0.004 (1/h), natural death of plasma cells 0.01-0.02 (1/h). The specified value is $\gamma_L = 0.007$ (1/h).

2.2.2 Parameters

Parameters in the model are normalized using reference values. The infection rate α specified in the model is 0.00035 (1/h). The virus product rate β is 120 (1/h). The ranges of α and β are described below. When the initial virus load is 3.2×10^6, 1×10^4, 4×10^2, 1×10^2 in units of 50 % egg infectious dose (EID), the corresponding infection rates α are 8.6×10^{-9}, 2.6×10^{-9}, 1.1×10^{-5}, 6.3×10^{-8} (1/(EID day)) [**4**]. Using 10^5 (EID) as the reference value, α is in the range of $1.1\times10^{-6} - 0.046$

(1/h). For parameter β, the corresponding values are 2.8×10^{-3}, 1.4, 1.5, 5.5×10 (EID/day) [**4**]. Normalizing by the 10^5 (EID) and the number of initial target cells 7×10^9, the range of β is 8.2-1.6 $\times 10^5$ (1/h).

The index of growth is set as $n = 1$. The acceptable range is 2/3-1, as described in an earlier report [**27**]. The recovery rate of susceptible cells $\omega\chi$ is specified as 0.0035 (1/h) to reproduce the experiment of mice infected with H1N1 influenza virus, which shows the peak of viral load at 4 dpi and the peak of lung damage at 11 dpi, with recovery at approximately 21 dpi [**25**].

The range of rate of antigenic stimulation to WBCs, κ, is $10^{11} - 4 \times 10^{16}$ (ml/(mol day)). The range of M^* is $5 \times 10^{-19} - 3 \times 10^{-18}$ (mol/ml) [**16**]. Then the product κM^* is in $2 \times 10^{-9} - 5 \times 10^{-3}$ (1/h). The specified value is κM^*=0.00015 (1/h).

The production rate of activated WBC: ξ is specified ξ =0.25 (1/h) based on the following. The number of cytotoxic T lymphocyte (CTL) creation by division 2-4 and rate of stimulation for CTL is $5 \times 10^{37} - 7 \times 10^{39}$((ml^2/(mol^2 day)), that of B-cell is $(1-100) \times 10^{37}$ ((ml^2/(mol^2 day)) and the range of naive antigen-presenting cells is $5 \times 10^{-19} - 3 \times 10^{-18}$ (mol/ml), specific precursor of CTLs is $(1-10) \times 10^{-19}$ (mol/ml) and specific precursor of B-lymphocytes is $5 \times 10^{-20} - 5 \times 10^{-19}$ (mol/ml) [**16**]. Using the reference value of antigen presenting 10^{-19}(mol/ml), lymphocytes 10^{-18} (mol/ml), the range of production rates is 0.06 − 116.6 (1/h). Here the equilibrium value of precursor is specified as $L^* = 0.1$.

The rate of synthesis of antibodies, η, is specified as η =0.4 (1/h) based on the following. The range of rate constant for synthesis of IgG molecules by one plasma cell is $8.5 \times 10^7 - 1.7 \times 10^8$ (molecule /(cell day)) [**16**]. Setting the reference value of antibodies 1013 (molecule/ml) and using the referred value of lymphocytes 10^{-18} (mol/ml), parameter η is between 0.21-0.43 (1/h).

The rate constant of antibodies neutralizing antigen: μ is specified as 6.1, which is adopted from a different normalized model based on Marchuk's model [**10**]. The ratio of destruction of antibodies σ when neutralizing viruses is specified as 10. The ratio of IgG molecules to neutralize viral particles is 1-10 [**16**].

The literature has no data available for the rate of destruction of infected cells by WBCs, λ, and the ratio of destruction of WBCs when destroying infected cells, ρ. In earlier studies, lymphocytes were shown to destroy infected cells for the seasonal case [**16**], but neutrophils and macrophages are regarded as playing an important role for A/H5N1 [**18**]. Furthermore, parameters λ and ρ differ for those cases. The fitted values of λ are 2.5 (1/h) for the seasonal case and 3.6 (1/h) for A/H5N1. Furthermore, ρ =0.01 is used for seasonal influenza. For A/H5N1, the fitted value is ρ =0.175 (1/h). The values of λ and ρ are fitted without information related to the acceptable range. For fitting, the parameters are specified to reproduce the cellular injury of ARDS in case of seasonal influenza or severe ARDS in case of A/H5N1. For ARDS, international standards [**28**] involve the acute onset P/F ratio

(Partial pressure of oxygen in arterial blood/ Fraction of oxygen in the space being measured) <200 mmHg. The P/F ratio <100 mmHg is used for severe ARDS, with the fact that the P/F ratio in case of A/H5N1-positive case in Vietnam is 61.4 ± 59.3 mmHg [**1**]. Usually, a P/F value higher than 450 mmHg is regarded as normal. Here, simply assuming cellular injury is proportional to P/F ratio, the values 200/450= 0.44 and 100/450=0.22 are adopted, respectively, for the criteria of ARDS and severe ARDS. In fitting, the fraction of susceptible cells must be less than the criterion.

Next the parameters are summarized. Two parameters differ: λ =2.5 (1/h), ρ =0.01 for severe seasonal influenza and λ =3.6 (1/h), ρ =0.175 for A/H5N1. The other parameters are common for both cases: $\gamma_I = 0.021$ (1/h), $\gamma_V = 0.083$ (1/h), $\gamma_M = 0.042$ (1/h), $\gamma_F = 0.0018$ (1/h), $\gamma_L = 0.007$ (1/h), $\tau = 3$ (h), $\tau_M = 9.6$ (h) and α =0.00035 (1/h), β =120 (1/h), μ =6.1 (1/h), $\kappa M^* = 0.00015$ (1/h), ξ =0.25 (1/h), η =0.4 (1/h), $\omega\chi = 0.0035$ (1/h), σ =10, and L^*=0.1. The parameters, adopted from different reports and interchangeable, might be unsuitable for quantitative research. However, the aim of this model is to ascertain the causes of leukopenia and its association with cellular injury of severe ARDS.

2.3. Initial conditions and numerical methods

As initial conditions, the influenza virus invades human organs at t=0, no virus was present in the organ earlier. The state of the system is $T = 1.0$, $I = 0.0$, $V = 0.0$, $M = 0.0$, $L = 0.1$, $F = 0.0$ before $t = 0$. At $t = 0$, $\delta V = 1.0$ is added to variable V. For $\delta V = 1.0$ corresponding to 10^5 (EID), substantial invasion of the virus into the target organ is assumed to simulate severe infection. The initial virus invasion is described as occurring between $10^2 - 3.2 \times 10^6$ (EID) by Handel et al. [**4**]. The duration of simulation is three weeks. The conventional fourth-order Runge-Kutta method is used for calculation. The time step width of simulation is 1 s because the delay differential equations of immune response become known as a stiff equation [**29**].

2.4. Therapy effect of NA inhibitor and immunoglobulins

The model is used to estimate the effectiveness of NA inhibitor therapy and immunoglobulin therapy for A/H5N1. Modification of the model to include the effects of both therapies is the following. NA inhibitor reduces the progeny virus yield in the infected cells, as described in an earlier report [**30**]. Equation (3) is modified to include the effect of NA inhibitor.

$$\frac{dV}{dt} = (1 - \varepsilon(t))\,\beta I - \gamma_V V - \mu F V \tag{8}$$

Here, ε=0, $t < T_m$, $\varepsilon = \varepsilon_0$, $t > T_m$, where T_m is the time at which the treatment starts. ε_0 represents the efficacy of NA inhibitor, which is specified as 0.98 [**30**].

However, immunoglobulin inhibits intracellular neutralization rather than yield reduction [**31**]. Equations (1) and (2) are modified to include the effects of immunoglobulin as shown below.

$$\frac{dT}{dt} = -\left(1-\varepsilon\left(t\right)\right)\alpha TV + g\left(T,I\right), \tag{9}$$

$$\frac{dI}{dt} = \left(1-\varepsilon\left(t\right)\right)\alpha T\left(t-\tau\right)V\left(t-\tau\right) - \gamma_I - \lambda IL. \tag{10}$$

Here, ε is the same function as (8).

The efficacy of immunoglobulin is unclear [**32**]. Therefore, the efficacy is inferred as 0.8 based on those of other inflammatory diseases [**33**].

3. RESULTS

3.1. Virus titer and infected epithelial cells in lung

The cases of influenza A/H5N1 and seasonal influenza are compared in Figs. 1 and 2. Relative changes of the portion of susceptible epithelial cells (T), the number of infected epithelial cells (I) and virus titer (V) in lung tissue are shown respectively in Figs. 1A, 1B, and 1C. In simulations, the lowest fraction of susceptible cells was approximately 0.32 for the seasonal case and approximately 0.1 for A/H5N1. For seasonal influenza, the peak of the virus number appeared at approximately 6 dpi. Using the normalization factor of 105 (EID), the amount of virus in the seasonal case was $10^4 - 10^6$ (EID) (Fig. 1C). It was in the order explained by Handel et al. [**4**]. For A/H5N1, the peak of the virus number was delayed and was higher.

3.2. Immune response to infection with influenza virus

The relative change of WBC counts (L) in the peripheral blood normalized to the equivalent value (L^*), antigen-presenting cells (M), and the relative antibody titer (F) are presented respectively in Figs. 2A, 2B, and 2C. The changes of WBC counts in peripheral blood revealed different profiles in the respective cases of seasonal and A/H5N1 influenza. For the seasonal case, the peak of the WBC count was at approximately 10 dpi (Fig. 2A); antibodies appeared after 15 dpi (Fig. 2C), showing the recovery of susceptible cells started after 9 dpi (Fig. 1A). However, for A/H5N1, WBC counts decreased. The appearance of antibody and the recovery of susceptible cells was delayed.

3.3. Effects of parameter λ and ρ on the severe ARDS

To clarify the respective roles of parameters λ and ρ, simulations are performed ρ ranging from 0.01 to 0.2 with fixed λ =3.6 and λ ranging from 2.25 to 3.9 with $\rho = 0.175$ fixed. Results of susceptible epithelial cells in lung (T) were as depicted in Fig. 3. The fraction of susceptible cells decreased from the level at

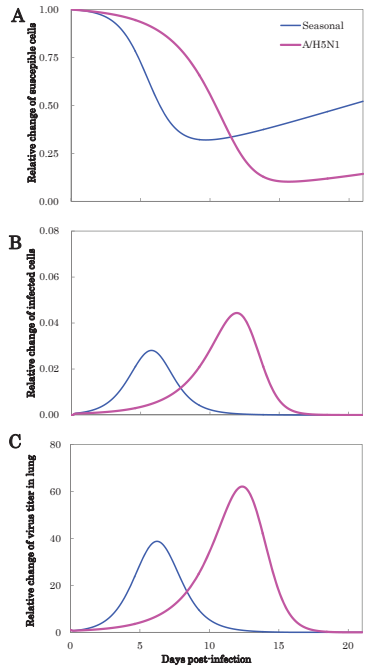

Figure 1. Virus dynamics in epithelial cells in lung infected with influenza virus of A/H5N1 and seasonal type: A, Relative change of susceptible epithelial cells (T); B, Relative change of infected epithelial cells (I); C, Relative virus titer (V) in lung tissue.

which ARDS did not occur to the level of severe ARDS when ρ increased with fixed λ (Fig. 3A). However, as Fig. 3B shows, irrespective of λ, the minimum of susceptible cells of all cases maintained $\rho = 0.175$, reaching the level of severe ARDS. Furthermore, for increasing λ, the minimum value of susceptible cells increased because more infected cells were destroyed by increasing λ. Moreover, the production of viruses was suppressed.

3.4. Effects of parameters λ and ρ on leukopenia

The results of WBC counts in peripheral blood (L) of the parameter runs are shown in Fig. 4. As portrayed in Fig. 4A, leukopenia did not occur for $\rho = 0.01$, but increasing ρ decreased WBCs and caused leukopenia. Furthermore, as shown in Fig. 4B, irrespective of the value of λ when maintaining $\rho = 0.175$, leukopenia occurred. Figures 3 and 4 clearly show that parameter ρ plays an important role in leukopenia and cellular injury with A/H5N1.

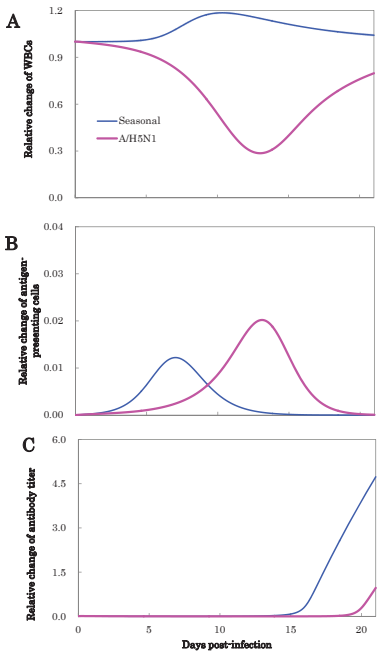

Figure 2. Immune responses in the peripheral blood to infection with influenza virus of A/H5N1 and seasonal type: A, Relative change of white blood cell counts (L); B, Relative number of antigen-presenting cells (M); C, Relative antibody titer (F).

3.5. Therapies with NA inhibitor and immunoglobulins

The model shows therapeutic effects of NA inhibitor and immunoglobulins and recovery from infection with A/H5N1, as presented in Fig. 5. The changes of the target epithelial cells (T) in lung, virus tissues (I), and WBC counts (L) are presented, respectively, in Figs. 5A, 5B, and 5C, showing that both therapies were efficacious on recovery, but with some differences in their effects. The virus titer in the treatment with NA inhibitor was reduced faster than that with immunoglobulins (Fig. 5B). However, the recoveries of susceptible cells and WBC counts by immunoglobulin treatment occurred more rapidly than those with NA inhibitor (Figs. 5A, 5C).

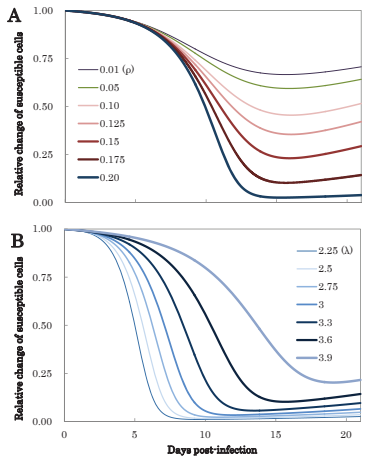

Figure 3. Relative change of susceptible epithelial cells in lung. Parameter λ is the rate of destruction of infected cells by WBCs. Parameter ρ is the ratio of destruction of WBCs when destroying infected cells. A: ρ =0.01-0.2 and λ =3.6. B: λ =2.25-3.9 and ρ =0.175. Case in which ρ =0.175 and λ =3.6 are the same as those for A/H5N1 in Figs. 1A.

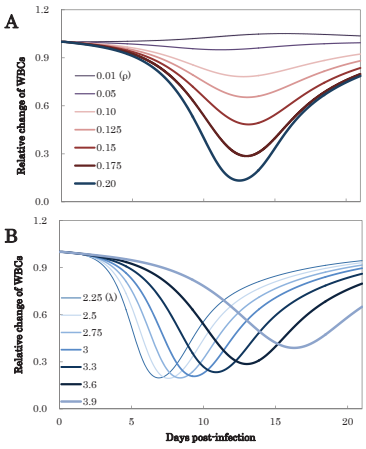

Figure 4. Relative change of leukocytes in peripheral blood. Parameter λ is the rate of destruction of infected cells by WBCs. Parameter ρ is the ratio of destruction of WBCs in turn when destroying infected cells. A: ρ =0.01-0.2 and λ =3.6. B: λ =2.25-3.9 and ρ =0.175. Case in which ρ =0.175 and λ =3.6 are the same as those for A/H5N1 in Figs. 2A.

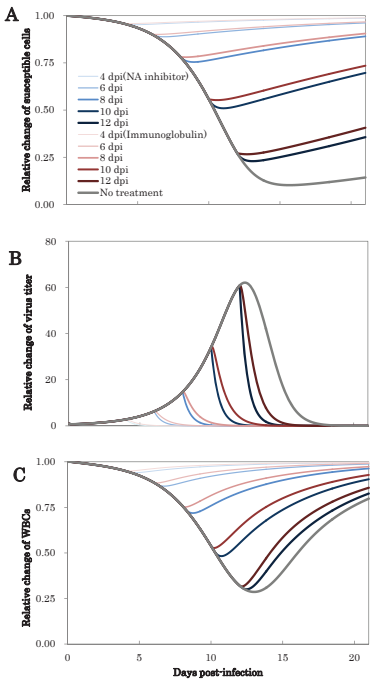

Figure 5. Treatment of neuraminidase inhibitor and immunoglobulins for A/H5N1: A, Relative change of susceptible epithelial cells (T) in lung; B, Relative change of virus tissues(V); C, Relative WBC counts (L). Treatment started after 4-12 dpi in Fig.5.

4. DISCUSSION

4.1. Profiles of symptoms after infection with influenza A/H5N1

Our simulations confirmed the course of viral titer and antibody production in patients infected with seasonal or A/H5N1 influenza, and reproduced symptoms for A/H5N1 delayed in comparison to those of seasonal influenza. The simulation showed a peak of viruses as 6 dpi, with existence until 12 dpi. For the actual course of seasonal infection, the virus peak comes on 3 dpi [**30, 34**]. A separate report describes that viruses peak at 5 dpi and exist until 10 dpi [**13**]. Furthermore, the peak of antibodies occurs at around 11 dpi [**35**]. In addition, an experiment with mice infected with H1N1 influenza virus shows the viral load peak at 4 dpi and lung damage at 11 dpi [**25**].

The real courses of A/H5N1 have been reported as follows. The latent period of A/H5N1 is usually 2-5 days [**32**]. The peak of A/H5N1 virus titer in the patients is apparent at 6 dpi and remains high at 8 dpi. Viral titer in the lung of a mouse

infected with A/H5N1 is high during 1-7 dpi, showing the peak at 6 dpi and a subsequent decrease to the level of detection on 8 dpi [**36**]. Moreover, antibody titer of A/H5N1 in plasma is not detected during 7 dpi, but is detected in 70% of patients in the second week, and in 80% in the third week, showing a peak at 2-10 weeks [**37**].

For the simulated A/H5N1, the virus titer peak was revealed by approximately 12 dpi in the present study. Antibodies appeared in the simulations at 19 dpi: after viruses had disappeared. The peak of viruses and the appearance of antibody in case of A/H5N1 infection were delayed compared to that of seasonal influenza in both real and simulated cases. In our simulations, the change of symptoms of A/H5N1 was attributable to two parameters: λ and ρ. When λ was increased, the delay of the cellular injury and leukopenia appeared because the increased λ showed reduction of both the number of infected cells and WBC destruction. Results show that reduction of the infected cells decreased production of virus and the spread of the infection. However, changing parameter ρ showed only a small effect on delay in this study. Simulations showed that the increase of destruction of infected cells by WBCs caused a delay of symptoms during A/H5N1 infection.

4.2. Cellular injury and leukopenia

Leukopenia, the characteristic feature of A/H5N1 infection, is associated with cellular injury of ARDS [**1**]. However, the cause of leukopenia has remained unclear. In the Vietnam cases, infected patients with A/H5N1-positive or A/H5N1-negative including infection with rhinovirus, adenovirus and/or bacteria showed severe ARDS. However, only the A/H5N1-positive patients showed leukopenia [**1**]. In the Thailand cases, WBC counts of fatal A/H5N1 patients were approximately half those of non-fatal A/H5N1 patients. All fatal A/H5N1 patients need treatment in ICU because of ARDS, but non-fatal patients infected with A/H5N1 did not [**3**], showing that ARDS and leukopenia are tightly associated in cases of A/H5N1. Regarding the cause of leukopenia, the infected model mouse for ARDS shows severe pneumonia with leukocyte infiltration into alveolar lavage [**18**]. In our simulations, leukopenia was shown for $\rho > 0.01$, but not for $\rho = 0.01$. Furthermore, leukopenia occurred irrespective of the value of λ, indicating that parameter ρ is the key to leukopenia. WBC counts are reduced in equation (5) if ρ becomes large. Furthermore, antibodies in equation (6) decreased, and viruses increased in equation (3). Viruses also increased because of the decrease of the destruction of infected cells by WBCs in equation (2). More viruses infect more susceptible cells into infected cells. The infected cells were destroyed or died in this study. Therefore, parameter ρ, if large, can allow leukopenia and cellular injury to occur simultaneously. For that reason, the degrees of leukopenia and ARDS are closely mutually associated. Our results show that the increased ratio of destruction of WBCs interacting with infected cells is the cause of leukopenia in cases of A/H5N1.

4.3. Therapeutic effect of NA inhibitor or immunoglobulins

Finally, we conducted simulations to compare NA inhibitor therapy and immunoglobulin therapy with cases of infection with A/H5N1. The simulated treatments started after 4-12 dpi because the median duration from onset to hospital admission was reported as 5.9 days in Vietnam [**2**], as 4 days in Thailand [**3**], and as 3 days in Taiwan [**38**]. Furthermore, treatment with NA inhibitor started after 5-12 dpi was reported [**2**]. Antiviral treatment using NA inhibitor is recommended for A/H5N1, but NA inhibitor resistance can emerge [**22**]. Some alternative treatment should be prepared. Monoclonal antibody is a candidate [**39**]. Another candidate is immunoglobulins [**40,41**]. Immunotherapy using convalescent plasma shows efficacy after NA inhibitor has lost efficacy [**42**]. The cells of mice are protected from A/H5N1 infection by treatment with immunoglobulins [**43**]. Furthermore, although few patients have been treated with immunotherapy and although treatments were uncontrolled including antivirals, the investigation of immunotherapy used against A/H5N1 should be encouraged [**32**]. Our simulations showed that NA inhibitor and immunoglobulin therapies have equivalent efficacy against A/H5N1. Comparing the results of two therapies in our simulation, the viruses in the treatment with NA inhibitor were reduced more rapidly than those with immunoglobulins because NA inhibitor suppressed the virus production first in the model. However, the recovery of susceptible cells by immunoglobulin treatment was faster than with NA inhibitor because immunoglobulins first change susceptible cells into infected cells. Furthermore, the recovery of WBCs by treatment with immunoglobulins was faster than that by treatment with NA inhibitor. The reason in the model is the following. With NA inhibitor treatment, the yield of viruses decreased first. Then infected cells decreased over time. The decreased destruction of WBCs followed. In addition, with the decreasing viruses, stimulation of antigen-presenting cells and the production of WBCs decreased. However, with immunoglobulin treatment, infected cells were reduced first; then the destruction of WBCs decreased. Consequently, the recovery by treatment with immunoglobulins might be faster than that by treatment with NA inhibitor. Results of our simulations suggest that immunoglobulins are more effective than NA inhibitor for leukopenia, especially in cases where treatment has been delayed.

4.4. Conclusions

Simulations in cases of infection with A/H5N1 virus show that increased destruction of WBCs interacting with infected cells caused leukopenia and cellular injury. Furthermore, simulation results showed the possibility of immunoglobulin therapy for leukopenia caused by influenza A/H5N1.

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