PEER-REVIEWED ORIGINAL ARTICLE

# Simulation of leukopenia developed with influenza A/H5N1 and its recovery with treatment of an antibody to influenza A/H5N1 virus

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## Abstract

**For highly pathogenic avian influenza A/H5N1, white blood cell count rapidly decreases after onset and then engender leukopenia. To elucidate the pathogenesis of leukopenia associated with highly pathogenic avian influenza A/H5N1 infection, simulations were conducted using a mathematical model of mice. Those simulation results suggest the possibility that therapy with an antibody to influenza A/H5N1 virus showed better improvement from leukopenia than neuraminidase inhibitor therapy.**

**Keywords**: Highly pathogenic avian influenza A/H5N1, Leukocytes, H5-specific antibody therapy, mathematical model

## Introduction

Innate immune response plays an important role in influenza A/H5N1[1]. In cases of seasonal influenza, counts of leukocytes, i.e. macrophages, neutrophils and lymphocytes in peripheral blood, increase slightly. By contrast, these leukocytes decrease rapidly in cases of influenza A/H5N1. Kawachi *et al*[2]. reported that patients infected with influenza A/H5N1 show leukopenia, but patients infected with rhinovirus, adenovirus, or bacteria in Vietnam do not. Numerous apoptotic leukocytes have been observed in lung tissues of a patient who died of avian influenza A/H5N1[3]. Furthermore, their possibly major role in destroying alveolar epithelial cells of lung has been pointed out, but apoptosis of infiltrating leukocytes into the lungs alone might not be sufficient to cause leukopenia. Because lung lesions might engender acute respiratory distress syndrome (ARDS), both leukopenia and severe ARDS are characteristic symptoms of avian influenza A/H5N1[2].

In addition, the rapid decrease of leucocytes in peripheral blood has been demonstrated in experiments of mice infected with A/H5N1[4]. Moreover, leukopenia associated with the pathogenesis of highly pathogenic avian influenza A/H5N1 (HPAI A/H5N1) has been reported from experiments conducted with mice[5,6].

One method to investigate the influenza A pathogenesis is simulation using a mathematical model. Several mathematical models are proposed to simulate the innate and adaptive immune responses to influenza A infection[7-11]. Especially Handel model is developed to understand quantitatively the whthin-host dynamics of seasonal influenza infection, and revealed both an innate and an adaptive immune response play an important role to explain adequately the experimental data[9]. On the other hand, the mathematical model without immune response is used to estimate the effect of therapy using NA-inhibitor based on the data in vitro[12]. We modified Handel model to apply the case of HPAI A/H5N1, and estimate the effect of therapy to HPAI A/H5N1 using the modified model involving immune response.

For simulation, one apparent difficulty is ascertaining the parameters to apply to the pathogenesis[13,14]. Frequently, no other avenue is available to identify parameters than using data from different experiments or reports of the patients. However, consistency of the parameters should be kept in mind.

In this study, simulations were conducted with a mathematical model to elucidate the leukopenia associated with the HPAI A/H5N1 infection. Model parameters for leukopenia were adapted from experimental data of mice. The model was applied to estimate therapies with neuraminidase (NA) inhibitor or an antibody to HPAI A/H5N1. It should be noted that although the model is not mouse specific, the parameters of model are determined by the experimental data of mice.

## Methods

### Mathematical models

We modified the model reported by Handel *et al* [9]. developed for quantitative investigation of the pathogenesis of seasonal influenza A in mice. The Handel model was developed to simulate seasonal influenza, and the parameters were set using the experiment of Iwasaki and Nozima (IN experiment) [15]. The proposed Handel model for influenza A including immune response is explained below.

$$\dot{U} = \lambda D - bUV \quad (1)$$
$$\dot{E} = bUV - gE \quad (2)$$
$$\dot{I} = gE - dI \quad (3)$$
$$\dot{D} = dI - \lambda D \quad (4)$$
$$\dot{V} = pI/(1+\kappa F) - cV - \gamma bUV - kVX \quad (5)$$
$$\dot{F} = wV - \delta F \quad (6)$$
$$\dot{X} = fV + rX \quad (7)$$

In those equations, $U$ represents uninfected or susceptible cells to influenza. $E$ stands for latently infected cells, $I$ signifies productively infected cells, $D$ denotes dead cells, $V$ represents free virus, $F$ represents innate immune response, and $X$ stands for the active immune response. The units above values follows the Handel model; $U$, $E$, $I$, $D$ are count by cell number and $V$ by PFU. And $F$, $X$ are shown in the same mode of Handel *et al* [9]. Parameters $\lambda$, $b$, $p$, $\kappa$, $\gamma$, $k$, $w$, $\delta$, $f$, and $r$ are all constants and mentioned later.

The model shows that susceptible cells $U$ can become infected cells $E$ by virus $V$. After some time, productively infected cells $I$ start to produce virions. Virus-producing infected cells die at some rate and new susceptible cells replace dead cells $D$. Free virus is clarified by non-specific mechanisms, absorbed in cells, or killed by adaptive immune response. The innate immune response $F$ is triggered upon infection and increases proportionally to free virus. Although innate immune response is triggered rapidly, adaptive immune response is assumed to take longer to high levels. For the Handel model, the humoral component of adaptive immune response (antibodies) is modeled assuming antibodies X are activated proportional to free virus load and followed by antigen-independent clonal expansion. Only the expansion phase of adaptive immune response is modeled because the peak and contraction of adaptive immune response occurred days after the virus cleared as described below. Regarding the pathogenesis of influenza A, virus titers had declined to an undetectable level by 10 days post-infection (dpi), but the antibody peak occurred at around 11 dpi in the experiment of seasonal influenza[16]. The peak viral load comes at 4 dpi, but lung damage caused by immune cell infiltration appeared to peak at 11 dpi[17].

To fit the data of the IN experiment, the Handel model used the interpolation formula of the data for innate immune response instead of eq. (6).

$$\log_{10} F(t) = 0.5388t - 0.08429 \text{ for } t \le 5,$$
$$\log_{10} F(t) = -0.7435t + 6.328 \text{ for } t > 5 \qquad (8)$$

Here, $t$ represents the days after infection (dpi). Parameters were fitted with or without time delay $\tau$ day in the form of $F(t-\tau)$. Although the case with delay improved the fitting, either is acceptable.

For seasonal influenza, adaptive immune response plays major role. However, for HPAI A/H5N1, innate immune response will become important[1]. For this reason, the Handel model is modified as presented below.

$$\dot{I} = gE - dI - \alpha IL \qquad (9)$$
$$\dot{D} = dI + \alpha IL - \lambda D \qquad (10)$$
$$\dot{M} = zV - \varepsilon M \qquad (11)$$
$$\dot{L} = \xi M - \beta (L - L^*) - \alpha\sigma IL \qquad (12)$$
$$\dot{X} = eM + qX \qquad (13)$$

In those equations, $M$ stands for the stimulated antigen-presenting macrophage, $L$ signifies circulating leukocytes, and $L^*$ denotes a steady state. The units of $M$ and $L$ are adjusted to the innate immune response of the Handel model. Parameters $\alpha$, $z$, $\varepsilon$, $\xi$, $\beta$, $\sigma$, $e$, and $q$ are constants mentioned later. The modified equations consist of (1), (2), (9), (10), (5), and (11)–(13). In the modified model, leukocytes kill infected cells in (9). In turn, leukocytes interacting with infected cells are destroyed in (12). Details are discussed later.

## Model parameters

The parameters of modified model are set to follow the Handel model as stated below. For the parameters of modified model to IN experiments in (1), (2), (5), (9), and (10), the same values as those used in the Handel model are used for (1)–(5) for $g$, $d$, $c$, $b$, $p$, $\gamma$, $\kappa$, $k$, and $\lambda$. Furthermore, $\alpha = 0$ is assumed, because the destruction of infected cells by innate immune response is negligible in cases of seasonal influenza. Then eq. (12) is separated from the system. In eq. (5), $M$ is used instead of $F$, assuming that $F$ is proportional to $M$. The other parameters of immune response are fitted using least-squares method into the result of the susceptible cells $U$, virus titer $V$ and antibody concentration $X$ of Handel model without delay $\tau = 0$ in eq. (8). The values of parameters of modified model used for seasonal case are listed in Table 1.

Next, the parameters in the equations of virus and innate immune response used for HPAI A/H5N1 case were refitted into the experimental data of virus titer and leukocytes in the experiment of Xu *et al*[6]. The virus used in the experiment designated as Chicken/HB/108 (a strain of HPAI A/H5N1), was isolated from chicken. It is highly lethal to mice. The values of refitted parameters are in Table 2. For fitting, the experimental data were digitized from figures. The other parameters were the same as those for seasonal case. For leukocytes, the data of the leukocyte counts of mice vary in response to genetic and environmental factors, e.g. the range is $5.87\times10^3$–$11.62\times10^3$ (1/μl)[18] or $11.1\times10^3$–$19.6\times10^3$ (1/μl)[19]. The circulating blood volume of mice is estimated 1.0 – 2.4ml. The leukocyte count in a steady state $L^*$ is set as $7.57\times10^6$, as it was in Xu's experiment. The parameter of immune response $\kappa = 0$ was set in (5), considering the effect of immune response by which infected cells are killed by leukocytes in (9). The rate of death of leukocytes interacting with infected cells $\sigma$ was set as 0.1 with reference to the case of lymphocytes[10]. Life-span of virus 1/c or rate constant of non-specific virus removal c is unclear. The estimated value of c is $c=10^9$, or $c=2^{7,10}$, $c=3^{11}$. Here, c=2 is used for refitting. The recovery rate of leukocytes $\beta$=0.005 fitted in the case of HPAI A/H5N1 is lower than that of seasonal cases $\beta$=0.05 – 0.5 described in[10] and discussed later.

## Models for medical treatment

NA inhibitor reduces the progeny virus yield in the infected cells, as described in an earlier report[12]. The equation of virus (5) is changed to include the treatment effects.

$$\dot{V} = (1-\varepsilon(t))\, pI/(1+\kappa F) - cV - \gamma bUV - kVX \qquad (14)$$

Here, $\varepsilon(t)=0$, $t<T_m$, $\varepsilon(t)=\varepsilon_0$, $t>T_m$, where $T_m$ is the time at which the treatment starts, the efficacy of NA inhibitor is specified as $\varepsilon_0$=0.98[12].

However, H5-specific antibody, inhibits intracellular neutralization rather than yield reduction[20]. Mice cells are protected from HPAI A/H5N1 infection by treatment with H5-specific antibody[21]. Equations (1) and (2) are changed to include the H5-specific antibody treatment effects as presented below.

$$\dot{U} = \lambda D - b(1-\varepsilon(t))\, UV \qquad (15)$$
$$\dot{E} = b(1-\varepsilon(t))\, UV - gE \qquad (16)$$

Here, $\varepsilon(t)$ is the same function as that of eq.(14). The H5-specific antibody efficacy is set as 0.90 – 0.98 based on the experience of other inflammatory diseases studied by one of the authors (SK)[22] and with reference to earlier reports[20,21].

## Results

### Seasonal influenza

For seasonal influenza, simulated results of the modified model were compared to that of the Handel model. The initial

**Table 1. Model parameters for seasonal influenza**

| symbol | meaning | values | comments |
|---|---|---|---|
| *1/g* | duration of latent eclipse phase | 6 h (g=4 per day) | fixed by Handel et. al. |
| *1/d* | lifespan of infected, virus-producing cell | 12 h (d=2 per day) | fixed by Handel et. al. |
| *1/c* | lifespan of free virus (1/rate constant of non-specific virus removal) | 2.4 h (c=10 per day) | fixed by Handel et. al. |
| *b* | infection rate | $2.1\times10^{-7}$ | fit to IN's experiment by Handel et. al. |
| *p* | virus production rate | 0.05 | fit to IN's experiment by Handel et. al. |
| *λ* | rate of regeneration of epithelial cells | $1.9\times10^{-8}$ | fit to IN's experiment by Handel et. al. |
| *γ* | conversion between infection virons and EID/PFU units | 0.00075 | fit to IN's experiment by Handel et. al. |
| *κ* | strength of innate immune response | 0.018 | fit to IN's experiment by Handel et. al. |
| *k* | kill rate of adaptive immune response | 1.8 | fit to IN's experiment by Handel et. al. |
| *f* | recruitment rate of adaptive immune response (Handel model) | $2.7\times10^{-6}$ | fit to IN's experiment by Handel et. al. |
| *r* | expansion rate of adaptive immune response (Handel model) | 0.3 | fit to IN's experiment by Handel et. al. |
| *z* | conversion rate of antigen-presention macrophage | 0.0001 | fit to results of Handel model |
| *ε* | reciprocal of life span of antigen-presenting macrophage | 0.0744 | fit to results of Handel model |
| *e* | recruitment rate of adaptive response (modified model) | 0.0574 | fit to results of Handel model |
| *q* | expansion rate of adaptive immune response (modified model) | $1.3\times10^{-8}$ | fit to results of Handel model |

IN: Iwasaki and Nojima

**Table 2. Model parameters for HPAI A/H5N1**

| symbol | meaning | values | comments |
|---|---|---|---|
| *α* | rate of destruction infected cell by leukocytes | $2.45\times10^{-9}$ | fit to data of Xu's experiment |
| *ξ* | migration rate of leukocytes | $4.17\times10^{-7}$ | fit to data of Xu's experiment |
| *β* | rate of recovery of leukocytes to steady state | 0.0054 | fit to data of Xu's experiment |
| *1/c* | lifespan of free virus (1/rate constant of non-specific virus removal) | 12h (c=2 per day) | fixed, see text |
| *σ* | destruction rate of leukocytes to infected cells | 0.1 | fixed, see text |
| *p* | virus production rate | 0.015 | fit to data of Xu's experiment |
| *κ* | strength of innate immune response | 0 | fixed, see text |
| *γ* | conversion between infection virons and EID/PFU units | $1.91\times10^{-5}$ | fit to data of Xu's experiment |
| *k* | kill rate of adaptive immune response | $3.31\times10^{-10}$ | fit to data of Xu's experiment |

value of uninfected or susceptible cells in simulation for the IN experiment is $U_0$=7.0×10$^9$, $V_0$=3.8×10$^4$, as used by Handel *et al*[9]. Calculation was performed using the conventional fourth-order Runge–Kutta method. The uninfected or susceptible cells $U$ are presented in Figure 1A. Latently infected cells $E$ and productively infected cells $I$ are presented in Figure 1B. Virus titer $V$ is shown in Figure 1C. The cells in Figures 1A and 1B are correspond originally to the epithelial cells in lungs of IN experiment, and also virus titer in Figure 1C swabbed in lungs of IN experiment. As presented in Figure 1C, the virus titer of the modified model decayed faster than that of the Handel model after 5 dpi. In Figure 1D, the innate immune response $F$ of Handel model and $M$ in the modified model are shown using interferon concentration in the same way as that used by Handel *et al*[9]. At 5 dpi, the interpolation equations for the variable $F$, i.e. the interferon production from innate immunity of the Handel model were changed. Furthermore, as presented in Figure 1E, the adaptive immune responses $X$, the neutralizing antibody production of Handel model and the modified model are shown in the same way of Handel *et al*[9]. Because the adaptive response plays an active role in cases of seasonal influenza, variable $M$ is regarded as the equivalent concentration necessary to produce the titer of neutralizing antibody $X$ to fit the result of the Handel model.

## HPAI A/H5N1

Simulation of HPAI A/H5N1 was conducted using the modified model. Some parameters related mainly to the virus and innate immune response are refitted into Xu's experiment for mice infected with HPAI A/H5N1 as described above[6]. The calculated pathogenesis of HPAI A/H5N1 is presented in Figure 2. Calculation starts after 1 dpi. The initial values were $U_0$=7.0×10$^9$ and $V_0$=4.17×10$^5$. Leukocytes were set as $L_0$=$L^*$=7.57×10$^6$. The values of $V_0$ and $L_0$ are digitized as the figures of Xu's experiment[6].

As presented in Figure 2A, the number of susceptible cells in epithelial cells decreased rapidly. Latently infected and productively infected cells increased and subsequently decayed. Calculated results of virus titer and counts of leukocytes in the peripheral blood were compared to those obtained from Xu's experiment. For virus titer in Figure 2B, results from simulations in the expansion phase from 1 dpi to 6 dpi well fit the data reported from Xu's experiment. In the contraction phase, the virus titer of the experiment decayed more rapidly than that of simulation. The simulated leukocyte counts in peripheral blood showed coincidence to the experimental data from 1 dpi to 6 dpi in Figure 2C. The decay of simulation after 6 dpi became slower than that of experiment.

## Treatments for HPAI A/H5N1

Because leukopenia might be a key role of disease activity in patients with HPAI A/H5N1 infection, the effectiveness of treatments for HPAI A/H5N1 with NA inhibitor or H5-specific antibody was simulated using the modified model. Figure 3 and Figure 4 present a comparison of both therapies to virus titers and leukocytes. Administration of NA inhibitor and H5-specific antibody started on 2–4 dpi. As depicted in Figure 3, both NA inhibitor and H5-specific antibody were effective in the simulation using the modified model. NA inhibitor suppressed the virus titer promptly. H5-specific antibody also achieved suppression with hours of delay in the simulation. By contrast, although both treatments provided symptomatic improvement in leukopenia (Figure 4), H5-specific antibody therapy improved the symptoms to a greater degree than NA inhibitor therapy in the simulation in cases of treatment starting on 3 dpi (Figure 4B).

## Discussion

In our simulation of seasonal influenza, the results of the modified model approximate those reported from the Handel model well in the expansion phase. However, in the contraction phase after 5 dpi, when the interpolation equation of innate response is changed at 5 dpi in eq. (8) of Handel model, differences are apparent mainly in the innate immune response. The change of eq. (8) of innate immune response in the Handel model may suggest the mechanism of innate immune response in contraction phase may be different from that of expansion phase. For HPAI A/H5N1, at a glance, the reported simultaneous development of leukopenia and lung lesion in cases of HPAI A/H5N1 infection is strange, because cellular injury in the lung is induced by leukocytes. However, the simulation using the modified model reproduced the simultaneous development of leukopenia and lung lesion in cases of HPAI A/H5N1 infection. The simulated result reproduced the data of Xu' experiment well in the expansion phase, but the difference between the simulated result and the data of Xu's experiment appears in the contraction phase in case of HPAI A/H5N1.

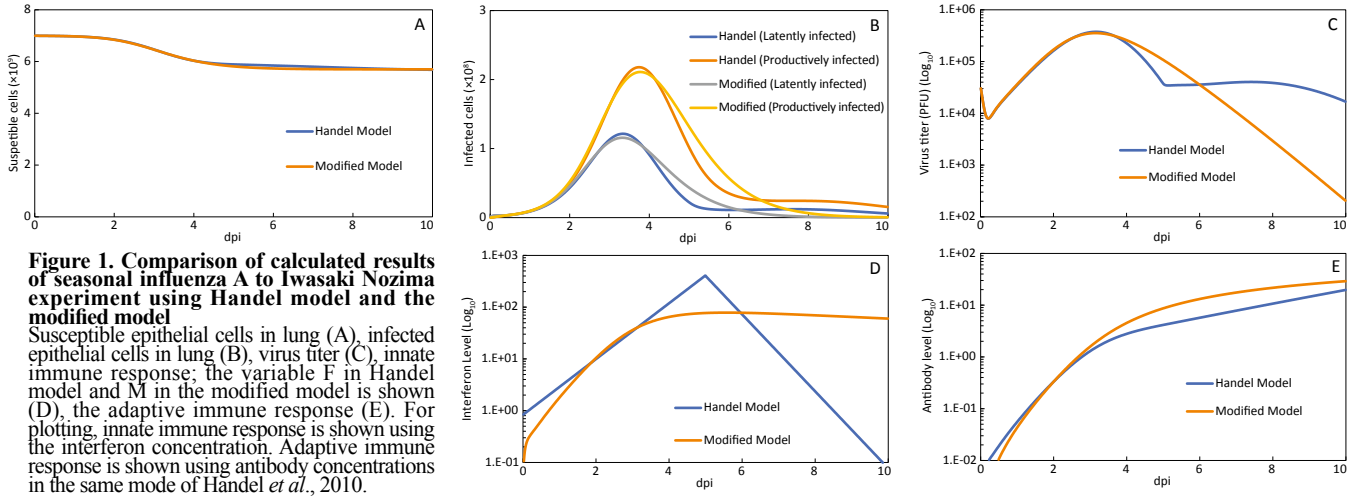


**Figure 1. Comparison of calculated results of seasonal influenza A to Iwasaki Nozima experiment using Handel model and the modified model**
Susceptible epithelial cells in lung (A), infected epithelial cells in lung (B), virus titer (C), innate immune response; the variable F in Handel model and M in the modified model is shown (D), the adaptive immune response (E). For plotting, innate immune response is shown using the interferon concentration. Adaptive immune response is shown using antibody concentrations in the same mode of Handel *et al*., 2010.

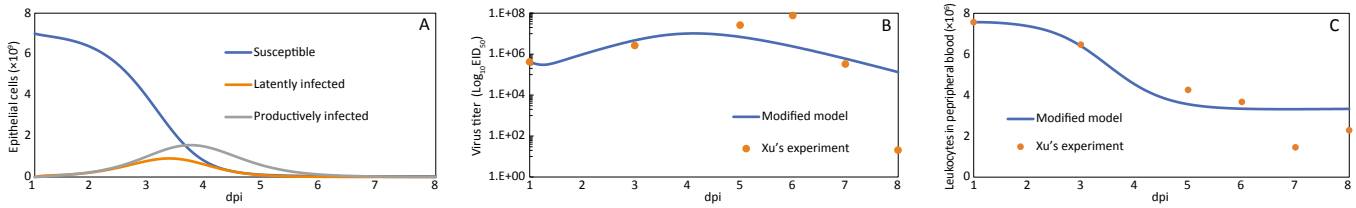


**Figure 2. Comparison of calculated results of the modified model and the experimental data of highly pathogenic A/H5N1**
Susceptible, latently or productively infected epithelial cells (A), virus titer (B), leukocytes (C). Dots show experimental data reported by Xu *et al*., 2006.

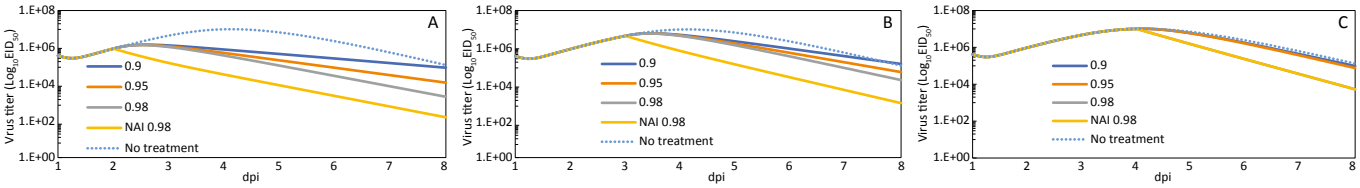


**Figure 3. Virus titer during treatment for highly pathogenic influenza A/H5N1 using NA inhibitor and H5-specific antibody. The NA-inhibitor efficacy is 0.98. That of H5-specific antibody is 0.90–0.98**
Administration starts after 2 dpi (A), 3 dpi (B), 4 dpi (C).

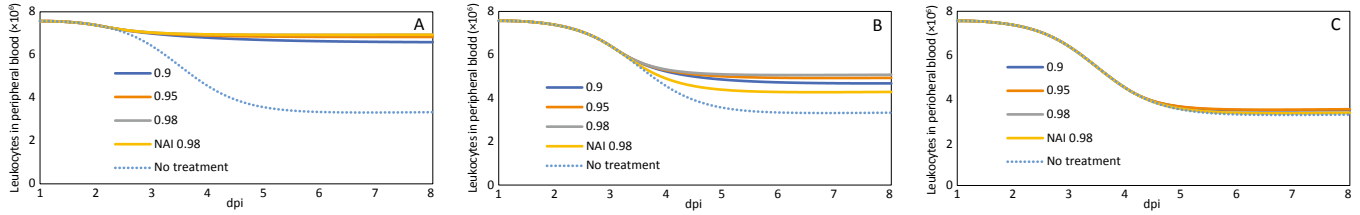


**Figure 4. Leukocyte counts during treatment for highly pathogenic influenza A/H5N1 using NA inhibitor and H5-specific antibody. The NA-inhibitor efficacy is 0.98. That of H5-specific antibody 0.90–0.98**
Administration starts after 2 dpi (A), 3 dpi (B), 4 dpi (C).

In the contraction phase, the difference between the simulated result and the experimental data appeared both in case of HPAI A/H5N1 and seasonal influenza. For IN experiment, Interpolation of data, i.e. eq. (8) is used for innate immune response instead of the equations of rate law in Handel model. The two interpolation equations in eq. (8) will show the behavior of innate immune response in contraction phase differs quantitatively from that in expansion phase. If so, it might be difficult to describe the model of innate immune response by a single equation like Handel model or the modified model. To estimate the effectiveness of therapies for HPAI A/H5N1, simulated result suggests the expansion phase is more important than the contraction phase.

The simulation for the case of HPAI A/H5N1 using the data reported from Xu's experiment[6] demonstrated that susceptible cells in the lung decreased markedly after viral infection. In fact, the lung is the target organ of infection with A/H5N1 in mice. An experiment examining mice infected with HPAI A/H5N1 demonstrated that most mice showed prominent signs of respiratory distress: approximately 80% of mice (13 of 16) died on 6–8 dpi[6].

For Xu's experiment, the virus peak appears on 6 dpi. It is below the detectable level on 8 dpi[6]. The production of virus titer in the expansion phase was reproduced in the simulation, but no rapid decrease in contraction phase was reproduced. However, in another experiment, the virus titer in case of lethal HK483 maintains a high level during the experiment (7 dpi), although virus titer in cases of nonlethal HK486 (A/H5N1) begins to decrease after 5 dpi[5].

Innate immune response plays an important role in case of HPAI A/H5N1[1]. For killing infected cells by immune response, some models for seasonal influenza include the term the killing infected cells by adaptive immune response, i.e. CTL (cytotoxic T lymphocyte)[7,8,10,11], but no above model has the term of the killing infected cells by innate immune response. For innate immune response, Handel *et al*[9]. pointed out the possiblity of killing infected cells by leukocytes. However, the results of simulation are not presented and the destruction of leukocytes, mainly neutrophils, interacting with infected cells is not considered[9]. Antiviral mechanisms of neutrophils to host defense are considered as below[23]. Neutrophils can phagocytose the virus and produce various antimicrobiral agents that inactivate the virus. However, for producing antimicrobiral agents, neutrophils can become excessively activated, triggering overt immune activation and leading to host tissue damage. The study of mice infected with H1N1 by Sugamata *et al*. demonstrated the important role of neutrophil-derived myeloperoxidase (MPO), a kind of antimicrobial, in exacerbating acute lung damage[24]. And further, the important role of neutrophils on killing infected cells has been reported in the experiments of mice infected with H3N2[25] and H1N1[26]. In addition, it is shown that in vitro model system, although few or no neutrophils adhered to uninfected cells, neutrophils adherence to infected epithelia increased as the number of infected cells increase[27]. And, greatly enhanced infiltration of macrophages and neutrophils into the lung airway has been shown in experiments examining mice infected with A/H5N1[6].

Next, the destruction of neutrophils interacting with infected cells is characteristic for HPAI A/H5N1 as follows. The numbers of apoptotic cells in bronchial epithelial cells and subepithelial layer of mice infected with HK/483 (a strain of HPAI A/H5N1) are high[5]. In the experiment of mice infected with strains of HPAI A/H5N1, leukopenia was reported in cases of lethal HK483[5] and Chicken/HB/108[6]. It is noteworthy that the decrease of leukocytes of the sort does not occur in cases of nonlethal A/H5N1. Comparing data obtained for lethal H483 and nonlethal H486, although virus titers in the lung remained at approximately the same level up to 5 dpi, leukocytes in the case of H483 decreased greatly after 2 dpi, but leukocytes in the case of H486 decreased slightly at 2 dpi and began to recover after 3 dpi[5]. Furthermore, for mice infected with high or low doses of HK483 and HK486, only mice infected with HK483 at either dose showed profound reduction of total leukocytes in circulation[4]. The above braces the modification of the equation for innate immune response to HPAI A/H5N1. The innate immune response of the modified model in case of HPAI A/H5N1 is sketched in Figure 5.

For the therapy of HPAI A/H5N1, NA-inhibitor therapy and immunoglobulin therapy are simulated. NA inhibitor drugs are often the first choice for treatment. However, because of resistance during treatment, anti-influenza antibody is a candidate for alternative therapy. For the treatment of NA inhibitor, therapy is reportedly efficacious when the therapy is initiated after 24 h or 36 h, but all mice die when treatment is delayed to 48 h[28]. However, for the treatment with H5-specific antibody, experiments have demonstrated that the mortality and morbidity of mice infected with HPAI A/ H5N1 were reduced to a marked degree[29]. H5-specific human monoclonal antibodies reportedly show therapeutic benefits in mice. After administering H5N1-specific antibodies derived from chicken eggs to mice, the peak of virus titer in lung was much less than

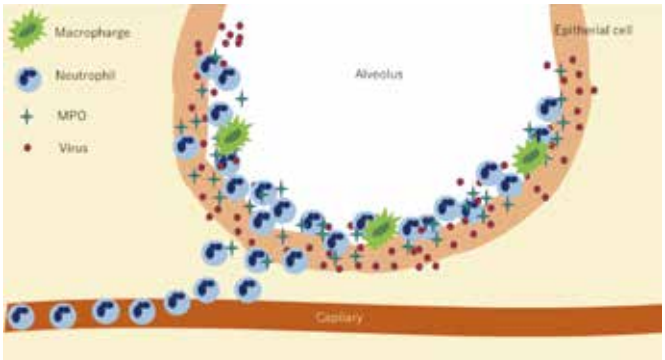


**Figure 5. Sketch of innate immune response in case of HPAI A/H5N1**
MPO: neutrophil-derived myeloperoxidase

in the case of normal antibodies[30]. Our simulations demonstrate that both therapies were effective, but some differences were apparent. For leukopenia symptoms, H5-specific antibody therapy improves symptoms to a greater degree than NA inhibitor therapy in the simulation using the modified model in case of 3 dpi. In experiments conducted with mice, H5-specific antibody protects cells from HPAI A/H5N1 infection[21]. Consequently, the destruction of leukocytes has been expected to be reduced. However, little recovery occurs after treatment in our simulation, mainly because the fitted recovery rate of leukocytes into Xu's experiment $\beta$=0.01 is low compared to seasonal values $\beta$=0.05–0.5[10]. The recovery is greater in the simulation for $\beta$=0.5. The cause of the low recovery rate might be that widespread apoptosis of leukocytes in lymphoid organs by cytokine dysregulation occurs locally while lymphocytes are circulating through the infected lung[3], although it is not the case in mice. Matters related to the low recovery rate of leukocytes present further difficulties.

**Conclusion**

Leukopenia associated with HPAI A/H5N1 infection was simulated using a mathematical model. The model follows the experimental data of mice infected with HPAI A/H5N1. The characteristic symptom of HPAI H5N1 is reproduced in the simulation. Furthermore, the effectiveness of therapies with NA inhibitor and H5-specific antibody was estimated through a simulation. Our model simulation results suggest that both therapies are effective and the possibility that H5-specific antibody therapy shows symptomatic improvement from leukopenia that is superior to that of neuraminidase inhibitor therapy.

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