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Hyperactive immune cells (T cells) may be responsible for acute lung injury in influenza virus infections: A need for early immune-modulators for severe cases

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SUMMARY

It has been believed that acute lung injury in influenza virus infections is caused by a virus-induced cytopathy; viruses that have multiplied in the upper respiratory tract spread to lung tissues along the lower respiratory tract. However, some experimental and clinical studies have suggested that the pathogenesis of acute lung injury in influenza virus infections is associated with excessive host response including a cell-mediated immune reaction. During the pandemic H1N1 2009 influenza A virus infections in Korea, we experienced a dramatic effect of immune-modulators (corticosteroids) on the patients with severe pneumonia who had significant respiratory distress at presentation and those who showed rapidly progressive pneumonia during oseltamivir treatment. We also found that the pneumonia patients treated with corticosteroids showed the lowest lymphocyte differential and that the severity of pneumonia was associated with the lymphocyte count at presentation. From our findings and previous experimental and clinical studies, we postulated that hyperactive immune cells (T cells) may be involved in the acute lung injury of influenza virus infections, using a hypothesis of 'protein homeostasis system'; the inducers of the cell-mediated immune response are initially produced at the primary immune sites by the innate immune system. These substances reach the lung cells, the main target organ, via the systemic circulation, and possibly the cells of other organs, including myocytes or central nerve system cells, leading to extrapulmonary symptoms (e.g., myalgia and rhabdomyolysis, and encephalopathy). To control these substances that may be possibly toxic to host cells, the adaptive immune reaction may be operated by immune cells, mainly lymphocytes. Hyperimmune reaction of immune cells produces higher levels of cytokines which may be associated with acute lung injury, and may be controlled by early use of immune-modulators. Early initiation and proper dosage of immune-modulators with antiviral agents for severe pneumonia patients may reduce morbidity and prevent progressive fatal pneumonia.

Introduction

Influenza virus infections have been a major global concern since the pandemic 1918 'Spanish flu' affected humans. The pandemic H1N1 2009 influenza A virus (H1N1 2009 virus) was first seen in Mexico in February 2009, and its spread was pandemic during 2009, reaching Korea [1,2]. Although the mortality of H1N1 2009 influenza does not exceed that of seasonal influenzas, it has been reported worldwide that some previously healthy patients with H1N1 2009 influenza suffer severe pneumonia, progressive acute respiratory distress syndrome (ARDS) and even death [2,3].

Despite extensive clinical and experimental studies, the pathogenesis of influenza infections, including their mechanism of lung injury, species-specificity, and different clinical manifestations among individuals, is not fully understood [1,4–6]. Studies on influenza viruses including human H5N1 virus revealed that a majority of patients had leukopenia and lymphopenia [3,7–9] and that a higher viral load in the pharynx and a lower peripheral T-lymphocyte count were associated with a poor outcome [7]. It has also been reported that mice infected with H5N1 virus show marked lymphopenia with lymphocyte depletion in lymphoid tissues [10]. Patients with the potentially fatal pneumonia caused by coronavirus-associated severe acute respiratory syndrome (SARS), measles, or *Mycoplasma pneumoniae* infections have constantly shown lymphopenia, especially in severe cases [11–13].

Some experimental and clinical studies have also suggested that the pathogenesis of lung injury in influenza infections is associated with excessive host response including the cell-mediated immune reaction [7,14–17]. It is postulated that infected hosts with progressive pneumonia may have an inadequate innate immune response to the initial viral insult. An abnormally activated innate immune system may produce higher levels of inflammatory cytokines and chemokines, which induce more inflammatory immune

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cells and their activation [7,16,17]. Severely affected hosts progress to ARDS, multiple organ dysfunction syndrome, and death.

Therefore, it can be inferred that circulating immune cells, including lymphocytes, may be associated with lung injury in the early stages of these infections. During the pandemic H1N1 2009 virus infections in Korea, we experienced a dramatic effect of immune-modulators (corticosteroids and intravenous immunoglobulin) on the patients with severe pneumonia who had severe respiratory distress at presentation and those who showed rapidly progressive pneumonia during oseltamivir treatment (Kil et al., in submission) [18]. We also found that the pneumonia patients treated with corticosteroids showed the lowest lymphocyte differential and the severity of pneumonia was associated with lymphocyte count at presentation (Rhim et al., in submission) [18].

In this study we review the characteristics of influenza virus infections and propose a hypothesis regarding the pathogenesis of lung injury in influenza virus infections.

Influenza viruses

Influenza A viruses are grouped in Orthomyxoviruses, and have eight segmented, negative sense single-stranded RNA genomes with 10 genes. Influenza viruses are classified by their two structural glycoproteins, namely hemagglutinin (H) and neuraminidase (N), and up till now 16 H and 9 N subtypes have been discovered, respectively. Influenza A viruses affect mainly birds and mammals and show a strict species-specificity although some subtypes evoke infection across the species. Human are affected by only three subtypes of H (H1, H2 and H3) and two subtypes of N (N1 and N2), while aquatic birds regarded as a natural reservoirs carry all subtypes without apparent disease [19]. The viruses can easily exchange the RNA genome between the species (antigenic shift) and tend to mutate in a single RNA genome (antigenic drift). Rapid changes of genomic materials including the appearance of a new pandemic subtype have been problematic for prevention of influenza.

In an avian subtype infection, interestingly, affected chickens show a nearly 100% of mortality with severe clinical manifestations, whereas ducks show a nearly 0% of mortality with few symptoms although immune system of two avian subspecies may be similar [4,19]. Furthermore, chickens are not susceptible to all kinds of avian subtypes [19,20]. The reason for this extreme discrepancy is unknown, but this phenomenon suggests a clue in human influenza virus infections; the majority of infected patients recover uneventfully, but some patients have severe pneumonia and progress to ARDS and death.

How do tiny changes of viral glycoproteins on the envelope lead to different virulence of viruses and different clinical phenotypes such as asymptomatic condition to ARDS and death? If we are convinced that all pathologic processes in influenza virus infections originate from viruses themselves, the viruses should multiply in enough numbers to cover all extent of lesions and reach various pathologic regions. Recent studies have revealed that highly pathogenic avian subtype (H5N1) and less virulent subtypes have different affinities to the host respiratory cell receptors which are composed of sialic acid with linked galactose (α2,3-linkage or α2,6-linkage). Two subtypes have different point mutations in genes of neuraminidases which are involved in cleavage and release of progeny from the infected cells, and in genes of polymerases, including PB2, which are involved in virus replication, and in other genes of viral proteins [1,17,21]. However, these genetic variances do not fully corroborate our clinical findings [21]. Therefore, we need to emphasize the role of the host immune reaction against virus infection and a new concept of pathogenesis of acute lung injury in influenza infections is necessary (see hypothesis).

Host response to viral insults

After adherence to upper respiratory tract cells via receptors, viruses replicate and may spread to regional lymph nodes and to lower respiratory tract cells inducing pneumonia and ARDS. In initial viral replication sites, the cells of the innate immune system such as circulating monocyte/macrophages, granulocytes including neutrophils and natural killer cells may be involved in the control of virus spread. In virus infected cells, type I interferons (IFNs α/β) are produced and which in turn activate production of other antiviral proteins, including Mx1, which may protect virus replication and spread to other cells [21,22]. It is a common concept that innate immune system and adaptive immune system of the host form a continuum, not separated or complementary [23]. The mediators (proteins) from the innate immune reaction may affect adaptive immune reaction; Toll-like receptors and intracellular sensors, including RAG-1, in infected cells and macrophages, that recognize viral RNA and/or other substances from viruses induce antiviral proteins and other proteins including pro-inflammatory cytokines [22,24]. These proteins may affect cells of the adaptive immune system.

In the regional lymphoid organ (lymph nodes) near initially infected cells, adaptive immune response is provoked; antigen presentation cells and corresponding T cells induce viral specific CD4+ T cells and CD8+ T cells with clonal expansion. The CD4+ T cells help to produce virus specific antibodies from B cells and cytotoxic CD8+ T cells attack the virus infected cells which express viral antigens. Specific B cells produce specific antibodies for viruses and perform a neutralization of the exposed virus particles from killed cells and protection from re-infection with same subtype of viruses.

However, it is likely that the innate immune response plays a crucial role in influenza virus elimination from the host [5,17]. It has been reported that nude mice which have no T cells [15,25] and 'Rag2' knockout mice which lack an acquired immune system [26] showed a similar clinical course compared to wild type animals when infected with influenza viruses. In cotton rat models, virus specific T cells and antibodies appear after virus clearance in the airways and lung inflammatory response peaks after virus is cleared [5,27]. In this pandemic, we experienced a patient with nephrotic syndrome who had received long-term cyclosporine A (5 mg/kg) which suppresses the CD4+ T cells, but his clinical outcome was uneventful, without pneumonia, despite the maintenance of his medication regimen. Recently, it has been reported that human immunodeficiency virus (HIV) positive children with 2009 H1N1 virus infection had mild clinical courses with lower lymphocyte count compared to HIV negative children, although none of the positive children had severe immune-suppression [28].

Lymphopenia in influenza virus infections

Leukopenia with lymphopenia, as well as leukocytosis with lymphopenia at the early stage, may be a characteristic finding in severe cases of influenza virus infections [10,29]. Since leukocyte count and differentials are non-specific findings in various infectious diseases and they are influenced by the age of patients and the stage of illness, physicians may tend to overlook this parameter. We found that the patients with pneumonia had a higher leukocyte count with lower lymphocyte differential compared to the patients without pneumonia, and the more severely affected patients had the lowest lymphocyte differential in the early stages of infection (within 2 days of fever onset). Previous human studies of H5N1 virus infection revealed that a lower lymphocyte count was associated with a poor outcome [7–9]. Mice infected with the influenza viruses show lymphopenia and H5N1 viruses induce

a marked lymphopenia with systemic lymphoid tissue depletion [10]. The leukopenia with lymphopenia is also observed in other systemic infections which can induce severe pneumonia, ARDS and multi-organ failure syndrome, such as measles, SARS due to coronavirus, and *M. pneumoniae* infections [11–13]. It has also been reported that development of ARDS in SARS due to coronaviruses is associated with the degree of lymphopenia [30,31]. The reason for lymphopenia in influenza virus infections is unknown but some studies suggested that apoptosis of immune cells by Fas-FasL signaling after viral infection [29], or by viral components or products during viral infection is responsible [10]. Initial viral insults on host cells should be controlled by circulating immune cells, and subsequent tissue injury also controlled by immune cells which are only effectors of the host. Therefore the association between the severity of lymphopenia and the clinical severity in the hosts gives a clue into the pathogenesis of lung injury in these infections (see below).

Pathologic studies

All earlier and recent experimental studies of influenza virus infections have revealed that immune cells, especially numerous small lymphocyte infiltrates, appear in the peribronchial and perivascular areas around the alveoli within 1–3 days of viral inoculation, despite different study designs. In mice models, the route of inoculation, including intranasal, intraperitoneal, or peroral inoculation, did not affect the pathological lung findings [32]. In one intragastric inoculation study, a large-dose inoculation facilitated the infiltration of immune cells and changes in the alveoli within several hours, but there were few viruses in the lungs [32]. Interestingly, earlier human autopsy studies performed in late 1950s during 'Asian flu' indicated that half of dead patients had no viruses in their lung lesions, although the majority of them had bacteria [33,34]. Suzuki et al. reported that mice in which the T cells were suppressed by anti-lymphocyte serum or anti-lymphocyte immunoglobulin showed an apparent reduction in lung consolidation and a 50% decrease in mortality, whereas the control mice all died with progressive consolidation. The viral titers in the lungs and the hemagglutinin antibody titers did not differ in the two groups [14]. Wyde et al. observed that athymic nude (T cell deficient) mice exhibited increased survival times and had less cell infiltration with no destruction of the lungs, compared with immune-competent control mice which suffered severe lung destruction [15]. The thymuses of the control mice were markedly reduced in size at the time of death, suggesting the depletion of cortical lymphocytes [10,15]. Eichelberger reported that viral clearance in the airways of cotton rats appeared before the establishment of adaptive immunity (antibodies and antigen-specific T cells) in the host and that the expression of genes which may be associated with the innate immune response was upregulated until day 10 after infection [5,27]. Recently, it has been reported that H1N1 2009 virus is less contagious than seasonal influenza viruses in mice and ferrets, and systemic viremia in other vital organs such as brain, liver and spleen has not been observed [6].

Given the results of the experimental studies described above, short incubation period (1–3 days), early appearance of pneumonia within 2 days after fever onset (85% of patients in our series, unpublished observation), the random sites of pneumonic consolidation, the very rapid progression of pneumonia in some patients, and the dramatic resolution of severe lung consolidation induced by early administrated immune-modulators (corticosteroids) within 24 h, one suggests that lung injury in influenza virus infections is associated with the host response to viral insult rather than with virus-induced cytopathies.

Hypothesis

How then do immune cells (mainly T cells) cause lung injury? All multicellular living organisms on earth have evolved through genes and all living activities including embryonic development, physiologic and pathologic phenomena in a living organism may be controlled by proteins that derive from genetic information in a molecular level. It is believed that the number of proteins in a living organism surpasses the number of genes in the organism, and proteins have variable sizes and functions. Since the majority of external insults (natural toxins and microbes) to the host in nature are proteins, the host may evolve to control these pathogenic proteins. The host may have a 'protein homeostasis system' which controls the balance of proteins and removes pathogenic proteins in vivo. For example, in cell levels, certain small proteins attach to receptors on cell membrane and induce new proteins through activation of signal transduction and intra-nuclear transcription factors. Newly produced proteins, if released outside of the cell, can also attach to their own receptors on cells and produce other proteins via similar pathways. Therefore, there should be a mechanism that controls this endless protein production. We postulated that the adaptive (specific) immune system of the host may be one of the 'protein homeostasis systems' in vivo. It is well known that very small proteins (peptides) cannot induce antibodies from B cells. T cells recognize a variety of peptides via T cell receptors (TCR) which are constructed by various gene recombinations. We postulated that B cells control pathogenic proteins except small proteins through antibodies, and T cells control small proteins through cytokine production or through their effect on cell-bound pathogenic proteins.

For influenza virus infections, it is postulated that the inducers (including small pathogenic proteins) of the cell-mediated immune response are initially produced at the primary immune sites of the upper respiratory tract during the incubation period. These substances (components related to influenza virus and/or other inflammatory mediators from the host during innate immune reaction), which may have an affinity for host tissues (mainly lung tissues), are produced during the innate immune reaction. These substances reach the lower respiratory tract cells, the main target organ, mainly via the systemic circulation, and possibly the cells of other organs, including myocytes or central nerve system cells to create extrapulmonary manifestations (e.g., fever, myalgia and rhabdomyolysis, and encephalopathy). To control these substances that are possibly toxic to host cells and induce chemokines for immune cells, the adaptive immune reaction may be initiated by immune cells, mainly T lymphocytes. Because the specific immune cells involved in viral elimination appear 3–5 days after the appearance of the clinical symptoms, this reaction may involve non-specific T cells initially, at least in part, until the specific T cells that can control pathologic proteins efficiently are produced. During this process, various inflammatory cytokines and counter-inflammatory cytokines are produced by the immune cells, and a cytokine imbalance may be associated with the progression of lung injury during these infections. The concept of the 'cytokine storm' is well documented in a variety of fields, including in infectious and rheumatological diseases [35,36].

Why do virus specific B cells and T cells appear 3–4 days after disease onset at the earliest? If viruses are inoculated directly into lymphoid organs (lymph nodes) in which primed antigen presentation cells and naïve lymphocytes exist, do virus specific antibodies and specific T cells appear within 1–2 days? If they do not, it could be assumed that adaptive immune cells are the 'cleaner' for debris which the innate immune cells made during the initial immune reaction, since the innate immune system has a crucial role in influenza virus infections as previously described. It is

assumed that a massive polyclonal activation of immune cells (B cells and T cells) observed in some infection-related disorders plays a role in controlling the acute disturbance of the homeostasis for the internal environment (protein balance) of the host [37].

One logical step from this hypothesis is that it may be possible to explain the pathogenesis of nearly all human diseases, including genetic disorders, immune-mediated (rheumatic) disorders and even malignancies that all arise from a breakdown of the 'protein homeostasis system' of the host. A variety of clinical and pathologic characteristics in infection-related diseases such as Kawasaki disease and acute poststreptococcal glomerulonephritis may depend on the affinity of pathogenic proteins to target cells and corresponding immune cells (T cell clones) with different cytokines for control of different pathogenic proteins [38]. Excessive and aberrant immune reaction (production of cytokines) may be associated with disease progression and development of autoimmune diseases. Briefly, a person who has a genetic defect in controlling the pathogenic proteins produced during any external or internal insults may not avoid a disaster.

Corticosteroids and influenza virus infections

During the 2009 H1N1 pandemic (1 September 2009–31 January 2010) in Deajeon, Korea, we experienced approximately 3000 outpatients and 217 inpatients with 80 pneumonia patients in our hospital. All inpatients received oseltamivir, 94.5% of patients within 48 h after fever onset. All children of age (0–15 years of age) except infants were affected by the viruses with a relatively even distribution according to age. The pneumonia patients showed lymphopenia, and severity of pneumonia was associated with lymphocyte count (Rhim et al., in submission). In addition, 17 severe pneumonia patients who were treated with corticosteroids showed a dramatic improvement of clinical manifestations and radiographic findings, regardless of severity of consolidation, after corticosteroid treatment (Kil et al., in submission). Twelve patients received intravenous methylprednisolone (10 mg/kg/day at presentation, 5 mg/kg/day at next day and then tapered off within a week) and five patients received oral prednisolone (1 mg/kg for 3 days, tapered off within a week). We previously experienced a similar phenomenon in *M. pneumoniae* pneumonia (MP) patents during two epidemics. Although MP is regarded as a bacterial infection, epidemiological and some clinical characteristics of MP, including appearance of lymphopenia in severe cases and radiographic findings, are similar to those of systemic viral infections [39,40]. We also reported previously that additional prednisolone treatment for antibiotic non-responsive patients was very effective in improving of clinical and radiographic findings [13]. Although there are no controlled clinical studies of corticosteroid treatment in influenza patients, we expected that the early administration of immune-modulators (corticosteroids) would be beneficial for prevention of the disease progression, based on our postulation that host cell-mediated reaction may be involved in acute lung injury in both infections. In addition, when we performed a comparative study with pediatricians in a neighbouring hospital where the doctors did not use corticosteroids at all, even for ARDS patients, the severe pneumonia patients who were treated with corticosteroids (17 cases) showed shortened total fever duration and hospitalization, rapid resolution of pneumonic infiltrations, and possibly no progression to ARDS, compared to the patients without corticosteroid treatment (15 cases) (Kil et al., in submission).

Although the beneficial effects of corticosteroid on various infectious diseases including viral infections are known [41], there are few controlled clinical and experimental studies of corticosteroid treatment for influenza virus infections. Ottolini et al. reported that mice treated with triamcinolone via intranasal lavage had dramatically fewer lung lesions than the control mice or mice treated with only antiviral drugs, and the beneficial effects of corticosteroids on lung lesions was dose-dependent. Corticosteroid therapy alone did not lead to prolonged viral replication in this animal model [42]. But some experimental studies reported no beneficial effect of corticosteroids on influenza virus infected animals [43]. In clinical studies of human avian H5N1 infections, corticosteroid treatment was reported to be ineffective in improving the mortality rate of ~50% [7–9]. However, many confounding factors must be considered when the results of these studies are analyzed. The small uncontrolled sample sizes, the broad range of subject ages, the different numbers of patients with high-risk factors, the use of antiviral drugs (or not), and more importantly, the different timing and doses of corticosteroids administered make it difficult to interpret the effects of corticosteroids [7–9]. Our patients treated corticosteroids and patients without corticosteroid treatment in the neighbouring hospital were young and previously healthy without underlying diseases. Since our patients were treated as early as possible with same dosage of corticosteroid, there might be few confounding factors for evaluation of corticosteroid effect. Recently Quispe-Laime et al. reported that prolonged medium-dose of corticosteroid administration for adult ARDS patients treated in the intensive care unit was effective in improvement of lung injury score and multiple organ dysfunction score [44]. To et al. reported that the fatal cases of H1N1 virus infection showed a delayed clearance of viral load and a higher level of cytokines compared with the non-fatal cases and the lung pathologic findings were similar to those of highly pathogenic H5N1 cases. They suggested the possible role of immune-modulator on severe pneumonia leading to ARDS in influenza viral infections [45]. It is suggested that ARDS itself, including that caused by influenza viruses, may be a predisposing factor to bacterial infections, leading to multi-organ failure syndrome and death [46,47]. Although the immune reaction of host in bacterial sepsis may be more intense than viral insult, a meta-analysis study reported that prolonged corticosteroid use for ARDS patients was associated with improved mortality and morbidity outcomes [46].

Fatal patients with secondary bacterial sepsis in influenza infections did not respond to early and adequate doses of antibiotics despite isolated bacterial pathogens being sensitive to antibiotics [33]. Since the immune/repair system in mammals has a limitation to the extent of controllable microbes (lethal doses in experimental animals), i.e., a critical point for the life and death against any toxic insults including infections, patients who progress to this point require prolonged time to recover or they would die despite all interventions including high-dose antibiotics and corticosteroids. It is known that ARDS could come from various conditions including blunt chest trauma, inhalation burn and autoimmune disorders [36,48]. Lung tissue injury by influenza viral insult (by direct or immune mediated), either compounded by bacterial infections or not, may lead to greater immune reaction of the host cells (by more pathogenic proteins and corresponding immune cells) and greater production of cytokines and further lung injuries. The findings of total lymphocyte depletion of whole lymphoid tissues in fatal cases in experimental animals and in autopsy findings of the patients may be explained by this hypothesis.

Our patients who received corticosteroids presented with rapid development of marked dyspnea and some patients showed cyanosis at presentation or during hospitalization. This type of pneumonia has been very rarely observed in the last decade in our department, with an incidence of <5 cases/year. These patients had relatively small areas of pulmonary infiltration on chest radiographs considering the severity of their respiratory distress and they had the lowest lymphocyte differential. Among them, one patient showed rapid progression of small patch the infiltrates on the left upper lobe to total left lung consolidation within 12 h after

admission, but he showed a dramatic near-complete resolution of massive consolidation within 24 h after corticosteroid treatment (methylprednisolone, 10 mg/kg) as well as in other patients with less severe pulmonary consolidations. Therefore, it can be inferred that the initial pneumonia in some of these patients would have progressed to ARDS if prompt corticosteroid treatment had not been initiated. Our findings suggest that early control of the patients who have any possibility of progressing to ARDS would be crucial in order to prevent further lung tissue destruction.

Corticosteroids have multi-potent modes of action as an immune-modulator and anti-inflammatory drug for almost all immune-mediated diseases. The whole mode of action of corticosteroid is unknown; numerous genes, including pro-inflammatory cytokines, are suppressed but some genes are activated by corticosteroids [49]. Corticosteroid is a potent immune-modulator against immune cells, including immature immune cells (B cell progenitors and thymocytes), activated immune cells and eosinophils and its effects are dose-dependent [50].

As an alternative immune-modulator, indications of high-dose intravenous immunoglobulin (IVIG) have been extended for immune-mediated diseases including Kawasaki disease [51–53]. We also experienced a beneficial effect of IVIG on pulmonary lesions during the 2009 H1N1 viral pneumonia and MP, although the cases were small [39]. Mechanism of the immune-modulation and anti-inflammatory effects of IVIG on immune diseases is unknown and its effects are also dose-dependent [37,52]. We previously observed that high-dose IVIG induces systemic protein modulation in vivo and proposed a theory unifying the various IVIG effects on immune-mediated diseases in the 'protein homeostasis system' [37].

Antiviral therapy has been reported to be effective in the acute stage of influenza infections in humans and experimental animals [54,55]. In 217 inpatients of our series, we also found that the majority of patients (97%) defervesced within 48 h after medication, and a majority of pneumonia patients showed improvement of their pneumonic infiltrations at discharge (Rhim et al., in submission). However, it is still unclear whether antiviral drugs protect against rapidly progressive pneumonia or extrapulmonary diseases, because there have been no controlled clinical studies [55]. Since the initial viral load of an inoculation is associated with the clinical phenotype (tracheitis model and fatal pneumonia model) [4,5,15] and the time of appearance of lung lesions in experimental animals [32], early antiviral treatment is beneficial in reducing the initial immune response. Our results of corticosteroid treatment on influenza virus infections were obtained from a small series of patients. Further prospective controlled studies with a large number of patients are required to confirm the role of corticosteroids in the treatment of influenza virus infections.

In conclusion, we found that pneumonic infiltrations during the 2009 H1N1 virus infections appeared early after the onset of the illness, and the severity of the pulmonary lesions was associated with the lymphocyte count at presentation. Some patients had rapidly progressive pneumonia, and early corticosteroid treatment halted their decline and rapidly improved their clinical and chest radiographic findings. These results suggest that immune cells, including lymphocytes, are involved in the mechanism of lung injury, and also suggest a role for immune-modulators in severe and progressive cases. We also proposed a new theory of pathogenesis of acute lung injury in influenza virus infections using a 'protein homeostasis system'. The early administration of antiviral agents and the proper use of immune-modulators may reduce morbidity and prevent the progression to fatal pneumonia.

Conflict of interest statement

We declare that no authors have any conflict of interest.

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