General Medicine: Open Access

Review Article | **Open Access**

Mechanisms of Increased Severity of Influenza-Related Pneumonia

Masafumi Seki*

Department of Infection Control and Prevention, Graduate School of Osaka University, Suita, Osaka, Japan

Abstract

Influenza-related pneumonia is an important complication of influenza, and it has been suggested that excessive inflammatory reactions, including "cytokine storm", may contribute to the mechanisms underlying severe pneumonia. Human data and mice co-infected with influenza virus and *Streptococcus pneumoniae* show increased severity of illness according to the elevation of cytokines/chemokines, and mice with genetic knock-out of immune molecules such as Toll-like receptor 3 and IRAK-M also show hyper-immune responses and reduced survival following influenza virus infection. Such findings suggest that innate immune responses and excessive neutrophil activation might be related to severe inflammatory changes in the lungs, and immunomudulatory therapy may thus be effective against severe influenza-related pneumonia.

Keywords: Influenza; *Streptococcus pneumonia*; Cytokine storm; Innate immunity

Introduction

A pandemic of a new strain of influenza became a reality in 2009 [1]. In addition, avian H7N9 type influenza appeared in China, 2013 [2]. Although even regular seasonal influenza is a very serious disease, particularly for elderly patients and those with underlying disorders, few deaths result from influenza itself. Rather, complications centered on pneumonia are usually the cause of death [3-5].

Pneumonia associated with influenza is divided into primary viral pneumonia due to the virus itself and secondary pneumonia or co-infection with bacteria. Involvement of an excessive immune response (cytokine storm) in the host is seen, especially in primary viral pneumonia [6,7] (Table 1).

In secondary bacterial pneumonia, involvement of cytokine storm has been suggested in addition to the synergy of viruses and bacteria [8-12]. We found that in humans, bacterial pneumonia becomes much more severe when co-infection with influenza virus is present; this may be due to immune mechanisms via cytokines or other substances [5,13,14].

In primary viral pneumonia, alveolar edema as the results of hyperpermeability of alveolar walls due to cell damages were suggested by cytokine theory [7].

In this article, centered on our own patient data, we selected 32 of 203 papers, and reviewed the mechanisms associated with increased severity of influenza-related secondary bacterial pneumonia and primary influenza pneumonia.

Pathology of Secondary Bacterial Pneumonia in Humans and Animal Models

An infection experiment using mice showed that pneumococcus infections following influenza virus vaccination became much more severe than various single infections [8].

In humans, we have reported that the severity of pneumonia with co-infection of influenza virus and bacteria was significantly greater than in those infected with bacteria alone [5]. Plasma levels of high mobility group box chromosomal protein-1 (HMGB-1), as well as of other inflammatory molecules, such as interleukin (IL)-6, regulated on activation normal T-cell expressed and secreted (RANTES), and soluble intercellular adhesion molecule-1 (sICAM-1), were determined in patients with bacterial pneumonia co-infected with influenza virus,

revealing that HMGB-1 levels were significantly elevated in these patients compared to patients experiencing mild bacterial pneumonia alone [14]. Among cases of co-infection, we found a significant correlation between the concentration of HMGB-1 and white blood cell counts. These data suggest that infection with influenza virus exacerbates bacterial pneumonia, and a synergic interaction between influenza virus and bacteria is mediated by cytokines/chemokines.

In addition, Mauad et al. analyzed autopsy findings from 21 Brazilian patients with confirmed influenza virus (H1N1pdm 2009) infection [1]. They found that eight of the 21 patients showed bacterial co-infection, and 20 of 21 patients showed diffuse alveolar damage on histological examination. Marked expression of Toll-like receptor (TLR)-3 and interferon (IFN)-gamma and large numbers of CD8-positive T cells and granzyme B-positive cells were apparent within the lung tissue. Such findings suggest the importance of bacterial co-infection in influenza virus-related pneumonia and an association between ongoing aberrant pulmonary immune response and increased severity of disease.

The association between influenza and bacterial co-infection has

Mechanisms	Related Molecules	References
Cytokine	IL-6, RANTES, sICAM-1, HMGB-1	[7-9,14,23,25]
Neutrophils	Elastase, lysozyme, CXCR-2	[7-9,11,12]
Innate Immunity	TLR-3, IFN-gamma, CD8+ T Cells, Granzyme B	[1,7,18,19]
Apoptosis	Epithelial cells, Caspase-3, -8	[7,10]
Endothelial cells	S1P(1)	[23,24]
MAP kinase	p-ERK, p38, c-jun	[8]
Platelets	Platelets activate factor (PAF)	[10]
Nucleoporin protein	RANBP2	[26]

Table 1: Possible mechanisms of increased severity of influenza-related pneumonia.

*Corresponding author: Masafumi Seki, Division of Infection Control and Prevention, Osaka University Hospital, 2-15 Yamadaoka, Suita, Osaka 565-0871, Japan, Tel: +81-6-6879-5093; Fax: +81-6-6879-5094; E-mail: seki@hp-infect.med.osaka-u.ac.jp

been related to increased morbidity and mortality in earlier pandemics [15]. The Centers for Disease Control reported that 29% of fatal cases in the United States presented with at least one bacterial co-infection (10 cases with *S. pneumoniae*, six with *S. pyogenes*, and seven with *Staphylococcus aureus)* [16]. The pathways and intermediate signaling molecules have been suggested to be similar in both *S. pneumoniae* and influenza virus infections, creating an opportunity for either interference with or augmentation of immune responses during dual or sequential infection [15].

In an animal study of co-infected mice, similar to the data reported by McCullers using co-infected mouse models [15-17], we observed pathological changes accompanied by pulmonary alveolar hemorrhage in addition to a significantly decreased survival rate [18]. Influenza virus or *S. pneumoniae* infection alone induced moderate pneumonia, whereas co-infected mice showed severe bronchopneumonia with massive hemorrhage, resulting in death of these mice two days after inoculation with *S. pneumoniae*. These pathological changes correlated with strong expression of immune-related molecules, such as cytokines, chemokines, TLRs, and the related mitogen-activated protein kinase signaling molecules (phosphorylated extracellular signal-regulated kinase-1 and -2, p38 and c-Jun N-terminal kinase).

Similarly increased severity was seen when mice with chronic bronchitis were infected first with *Pseudomonas aeruginosa*, and then co-infected with influenza virus. At the same time, excessive activation of neutrophils following a transient decline in neutrophil function was also seen [9]. Excessive functioning or failure of immune mechanisms is thought to be induced by co-infection with influenza virus.

It is predicted that not only factors related to the viruses or bacteria, but also immune-related factors of the host are strongly involved in severe pneumonia. When detection of important factors related to increased severity was undertaken at the protein level in addition to the gene level, activation of platelet-activating factor (PAF) and related PAF-AF enzyme was first observed in an analysis using DNA microarrays [10].

Meanwhile, in protein tip (Figure 1) and two-dimensional
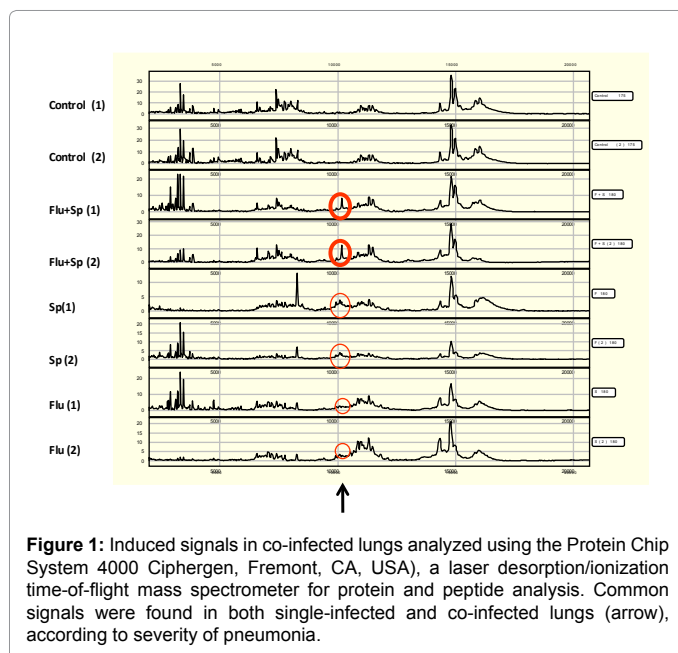
Figure 1: Induced signals in co-infected lungs analyzed using the Protein Chip System 4000 Ciphergen, Fremont, CA, USA), a laser desorption/ionization time-of-flight mass spectrometer for protein and peptide analysis. Common signals were found in both single-infected and co-infected lungs (arrow), according to severity of pneumonia.

electrophoresis analyses [11], the possibility was shown that the following play important roles as aggressive factors: activated α-antitrypsin, which inhibits neutrophil function; enzymes and proteases, such as neutrophil elastase, myeloperoxidase and lysozymes; and cytokines and chemokines such as macrophage inflammatory protein (MIP)-2, which corresponds to IL-8 in humans [11]. All molecules and enzymes detected in this paper were known as neutrophil related, and suggested the possible of therapeutic application of neutrophil modulation.

Possible Mechanisms of Increased Severity of Primary Influenza Pneumonia

The effect of cytokine storms and other host immune responses is thought to be even larger in primary influenza pneumonia.

In influenza virus infection experiments using TLR-3-knockout mice, Le Goffic et al. showed that inhibition of excessive inflammatory responses may contribute to improved survival rates [17,19]. More specifically, immune responses, which are essentially supposed to benefit the body, may function as a "double-edged sword" that has the opposite effect of causing tissue damage from excessive inflammatory reactions [20]. In addition to collagen disease and allergic diseases, this possibility is suggested even in infectious diseases such as sepsis [21].

From an infection experiment using gene-deficient mice for TLR-related signaling factors, we discovered again that excessive host immune responses, typified by cytokine storms, do in fact play a key role in increasing the severity of influenza infections [7].

Thus, when mice lacking the IRAK-M gene (*IRAK-M-/-* mice), a negative regulator of Myd88, which plays a major role in TLR intracellular signaling, were infected with fairly large amounts of influenza virus, the natural immune response was enhanced significantly compared with normal wild-type (WT) mice. As a result, increased expression of cytokines and chemokines was seen. These responses were thought to be excessive and pathologically more severe inflammation and stronger tissue damage than in WT mice were seen. These data suggested that the polymorphism of genetic background in immune responses might influence significantly in the pathogenesis of severe influenza pneumonia.

In addition, these phenomenons were blocked by administration of anti-CXCR-2, known as modulator of neutrophils, and also suggested the possible of adjunctive therapeutic use for neutrophil modulators.

One mechanism for the increased severity of pneumonia in these gene-deficient mice is thought to be that excessive apoptosis is induced from an early stage in lung cells [7,22]. Apoptosis is originally a normal body response that occurs to get rid of viruses-infected cells, but an excessive reaction seems to occur in association with massive viral infection, particularly in gene-deficient mice, and over-elimination of airway epithelial cells and other cells infected with the virus may have the effect of exacerbating tissue damage.

Furthermore, lung permeability, as measured by albumin concentration of bronchoalveolar lavage fluid, was very greatly enhanced in gene-deficient mice, corresponding to a pathological state, and endovascular cells were also strongly damaged, suggesting that severe pulmonary edema had been induced [7].

Wang et al. analyzed the impact of the cytokine storm on the pathogenesis of vascular hyperpermeability in severe influenza, and found that influenza A virus infection resulted in significant increases in TNF-alpha, IL-6, IL-1beta, and viral hemagglutinin-processing protease trypsin levels, and viral replication with vascular

hyperpermeability in the lung and brain in the first six days of infection [22]. Trypsin upregulation was suppressed by transcriptional inhibition of cytokines in vivo and by anti-cytokine antibodies in endothelial cells, and endothelial hyperpermeability were inhibited by a protease-activated receptor-2 antagonist and a trypsin inhibitor. They concluded that the influenza virus-cytokine-protease cycle is one of the key mechanisms underlying vascular hyperpermeability, and endothelial cells were one of the most important components in severe influenza.

Teijaro et al. reported that S1P(1) receptor, which is expressed on endothelial cells and lymphocytes within lung tissue and acts to suppresses cytokines and innate immune cell recruitment, identified endothelial cells as central regulators of cytokine storm [23]. Their data reveal immune cell infiltration and cytokine production as distinct events that are both orchestrated by endothelial cells, and the modulation of endothelium with a specific agonist suggested that diseases in which amplification of cytokine storm was a significant pathological component could be chemically tractable.

In human encephalitis/encephalopathy, Kawashima et al., reported national survey data of 207 cases of pediatric encephalopathy due to H1N1 pdm 2009 during one season in Japan [24]. They found that 10 cases were accompanied by high cytokine levels, and three of these children died. Wang and Li reviewed many influenza-associated encephalopathy (IAE) cases and reported that the pathogenesis of IAE may involve viral invasion of the central nervous system (CNS), proinflammatory cytokines, metabolic disorders, or genetic susceptibility [25]. An autosomal-dominant viral acute necrotizing encephalopathy (ANE) was recently found to possess missense mutations in the Ran-binding 2 (RANBP2) gene [26]. Another recurrent ANE case following influenza A infection was also reported in a genetically predisposed family showing RANBP2 mutation.

Encephalitis (inflammation) and encephalopathy (edema) are generally seen as typical CNS complications of influenza, primarily in children, and the above findings suggest the possibility that a similar pathological state is produced at the same time in the lungs, although significant differences may also exist between the CNS, which is fundamentally pathogen-free and isolated from the outside world, and the respiratory organs, which are constantly exposed to the outside world and its irritants.

For Practical use as Adjunctive Therapies and Immunomodulatory Agents

In any event, activation of mainly neutrophils might play a major role, as in the case of secondary bacterial pneumonia and primary influenza pneumonia. In fact, a treatment experiment using a protease inhibitor (gabexate mesilate) revealed that the clinical condition could be controlled within a certain range, although this was with a single influenza virus infection. Among immunomodulatory agents for severe influenza, not only corticosteroids and immunoglobulins, but also statins and macrolides are well known and have been reviewed [27-30].

Hui et al. reviewed adjunctive therapies and immunomodulatory agents in the management of severe influenza, and discussed as the divided three groups: 1) therapies with evidence of improved patient outcome: including convalescent plasma and intravenous immunoglobulin (IVIG) preparations, 2) therapies of uncertain benefit: including , N-acetylcysteine (NAC) , polymyxin B-immobilized fiber column hemoperfusion, therapeutic plasma exchange, statins, macrolides, peroxisome proliferator-activated receptors agonists, and combination of celecoxib and mesalazine, 3) therapies with evidence of worsened patient outcome, such as systemic corticosteroids [26].

In the future, additional investigation of the application of these agents to treatment for severe influenza pneumonia patients is needed [12], in addition to the current approach including antibiotics, anti-influenza drugs (e.g., osertamivir) and vaccinations.

Conclusions

In this review, we have examined the mechanisms underlying the increased severity of influenza-related pneumonia, particularly in terms of co-infection with bacteria. Human and animal studies have suggested that excessive inflammatory reactions might contribute to the mechanisms of severe pneumonia. Activation of neutrophils might offer a theraupeutic target.

Furthermore, reverse genetic technologies have also advanced for viruses in recent years, and the role of viral genetics in Spanish flu and other severe influenza infections is being elucidated [31,32]. Using genetically modified strains of viruses and bacteria in addition to examinations of hosts, we plan to continue elucidating the characteristics of secondary bacterial pneumonia related to the influenza virus, as well as the mechanisms involved in the increased severity of viral pneumonia itself.

References

1. Mauad T, Hajjar LA, Callegari GD, da Silva LF, Schout D, et al. (2010) Lung pathology in fatal novel human influenza A (H1N1) infection. Am J Respir Crit Care Med 181: 72-79.

2. Gao R, Cao B, Hu Y, Feng Z, Wang D, et al. (2013) Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med 368: 1888-1897.

3. Mandell LA, Wunderink R, Anzueto A, Bartlett JG, Campbell GD, et al. (2007) Infectious Diseases Society of America; American Thoracic Society. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 44: S27-72.

4. Committee for the Japanese Respiratory Society guidelines for the management of respiratory infections (2006) Guidelines for the management of community acquired pneumonia in adults, revised edition. Respirology 11 Suppl 3: S79-133.

5. Seki M, Kosai K, Yanagihara K, Higashiyama Y, Kurihara S, et al. (2007) Disease severity in patients with simultaneous influenza and bacterial pneumonia. Intern Med 46: 953-958.

6. Seki M, Suyama N, Hashiguchi K, Hara A, Kosai K, et al. (2008) A patient with fulminant influenza-related bacterial pneumonia due to Streptococcus pneumoniae followed by Mycobacterium tuberculosis infection. Intern Med 47: 2043-2047.

7. Seki M, Kohno S, Newstead MW, Zeng X, Bhan U, et al. (2010) Critical role of IL-1 receptor-associated kinase-M in regulating chemokine-dependent deleterious inflammation in murine influenza pneumonia. J Immunol 184: 1410-1418.

8. Seki M, Yanagihara K, Higashiyama Y, Fukuda Y, Kaneko Y, et al. (2004) Immunokinetics in severe pneumonia due to influenza virus and bacteria coinfection in mice. Eur Respir J 24: 143-149.

9. Seki M, HigashiyamaY, Tomono K, Yanagihara K, Ohno H, et al. (2004) Acute infection with influenza virus enhances susceptibility to fatal pneumonia following Streptococcus pneumoniae infection in mice with chronic pulmonary colonization with Pseudomonas aeruginosa. Clin Exp Immunol 137: 35-40.

10. Seki M, Kosai K, Hara A, Imamura Y, Nakamura S, et al. (2009) Expression and DNA microarray analysis of a platelet activating factor-related molecule in severe pneumonia in mice due to influenza virus and bacterial co-infection. Jpn J Infect Dis 62: 6-10.

11. Kosai K, Seki M, Yanagihara K, Nakamura S, Kurihara S, et al. (2008) Two-dimensional gel electrophoresis analysis in simultaneous influenza pneumonia and bacterial infection in mice. Clin Exp Immunol 152: 364-371.

12. Kosai K, Seki M, Yanagihara K, Nakamura S, Kurihara S, et al. (2008) Gabexate mesilate suppresses influenza pneumonia in mice through inhibition of cytokines. J Int Med Res 36: 322-328.

13. Seki M, Hashiguchi K, Kosai K, Higashiyama H, Yanagihara K, et al. (2006) A patient with fulminant primary influenza pneumonia which developed into secondary bacterial pneumonia. Acta Medica Nagasakiensia 51: 121-124.

14. Kosai K, Seki M, Yanagihara K, Nakamura S, Kurihara S, et al. (2007) Elevated levels of high mobility group box chromosomal protein-1 (HMGB-1) in sera from patients with severe bacterial pneumonia coinfected with influenza virus. Scand J Infect Dis 40: 338-342.

15. McCullers JA (2006) Insights into the interaction between influenza virus and pneumococcus. Clin Microbiol Rev 19: 571-582.

16. Centers for Disease Control and Prevention (CDC) (2009) Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) - United States, May-August 2009. MMWR Morb Mortal Wkly Rep 58: 1071-1074.

17. Le Goffic R, Pothlichet J, Vitour D, Fujita T, Meurs E, et al. (2007) Influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. J Immunol 178: 3368-3372.

18. Iverson AR, Boyd KL, McAuley JL, Plano LR, Hart ME, et al. (2011) Influenza virus primes mice for pneumonia from Staphylococcus aureus. J Infect Dis 203: 880-888.

19. Le Goffic R, Balloy V, Lagranderie M, Alexopoulou L, Escriou N, et al. (2006) Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. PLoS Pathog 2: e53.

20. Si-Tahar M, Touqui L, Chignard M (2009) Innate immunity and inflammation--two facets of the same anti-infectious reaction. Clin Exp Immunol 156: 194-198.

21. Cavassani KA, Ishii M, Wen H, Schaller MA, Lincoln PM, et al. (2008) TLR3 is an endogenous sensor of tissue necrosis during acute inflammatory events. J Exp Med 205: 2609-2621.

22. Wang S, Le TQ, Kurihara N, Chida J, Cisse Y, et al. (2010) Influenza virus-cytokine-protease cycle in the pathogenesis of vascular hyperpermeability in severe influenza. J Infect Dis 202: 991-1001.

23. Teijaro JR, Walsh KB, Cahalan S, Fremgen DM, Roberts E, et al. (2011) Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. Cell 146: 980-991.

24. Kosai K, Seki M, Tanaka A, Morinaga Y, Imamura Y, et al. (2011) Increase of apoptosis in a murine model for severe pneumococcal pneumonia during influenza A virus infection. Jpn J Infect Dis 64: 451-457.

25. Kawashima H, Morichi S, Okumura A, Nakagawa S, Morishima T (2012) collaborating study group on influenza-associated encephalopathy in Japan., National survey of pandemic influenza A (H1N1) 2009-associated encephalopathy in Japanese children. J Med Virol 84: 1151-1156.

26. Hui DS, Lee N, Chan PK (2013) Adjunctive therapies and immunomodulatory agents in the management of severe influenza. Antiviral Res 98: 410-416.

27. Wang GF, Li W, Li K (2010) Acute encephalopathy and encephalitis caused by influenza virus infection. Curr Opin Neurol 23: 305-311.

28. Neilson DE, Adams MD, Orr CM, Schelling DK, Eiben RM, et al. (2009) Infection-triggered familial or recurrent cases of acute necrotizing encephalopathy caused by mutations in a component of the nuclear pore, RANBP2. Am J Hum Genet 84: 44-51.

29. Yamaya M, Shinya K, Hatachi Y, Kubo H, Asada M, et al. (2010) Clarithromycin inhibits type a seasonal influenza virus infection in human airway epithelial cells. J Pharmacol Exp Ther 333: 81-90.

30. Ishii H, Komiya K, Yamagata E, Yatera K, Chojin Y, et al. (2012) Clarithromycin has limited effects in non-elderly, non-severe patients with seasonal influenza virus A infection. J Infect 64: 343-345.

31. Fedson DS (2009) Confronting the next influenza pandemic with anti-inflammatory and immunomodulatory agents: why they are needed and how they might work. Influenza Other Respi Viruses 3: 129-142.

32. Kawaoka Y (2007) Orthomyxovirus. Field's Virology Fifth Edition. 1691-1699.