Mechanisms of Increased Severity of Influenza-Related Pneumonia

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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 immunomodulatory therapy may thus be effective against severe influenza-related pneumonia.

Keywords: Influenza; Streptococcus pneumoniae; 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
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, chemokines such as macrophage inflammatory protein (MIP)-2, which correlates 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 phenomena 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

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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.
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., oseltamivir) 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 therapeutic 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


