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The W-Shaped Mortality-Age Distribution of Novel H1N1 Influenza Virus Helps Reconstruct the Second Wave of Pandemic 1918 Spanish Flu

Lai KY[1]*, NG WYG[2] and Cheng FF[3]

[1]Chief-of-service, Department of Intensive Care Queen Elizabeth Hospital, B6, 30 Gascoigne Rd. Kowloon, Hong Kong SAR, China
[2]Associate Consultant, Department of Intensive Care Queen Elizabeth Hospital, Hong Kong SAR, China
[3]Consultant, Department of Medicine, Queen Elizabeth Hospital, Hong Kong SAR, China

Abstract

Interferon is essential in human defense against influenza virus. The non-structural gene segment (NS) of influenza virus has a critical role in counteracting human interferon-mediated antiviral responses. The second wave of 1918 H1N1 Spanish influenza pandemic was characterized by an enhanced mortality and a W-shaped mortality age distribution. In contrast to the U-shaped mortality-age distribution that targeted the very young and elderly during the first wave, young adult population were also affected during the second wave. The NS of the 1918 H1N1 Spanish influenza virus (1918PV) isolated during the second wave contributes to the virulence of 1918PV. This unique NS of 1918PV is able to inhibit human interferon production at both the pre-transcriptional and post-transcriptional level and induce cytokine dysregulation. The NS of 1918PV has entered the swine population in 1918 and re-emerged in the 2009 novel H1N1 influenza A pandemic virus (2009PV). Both seasonal and pandemic novel H1N1 influenza A viruses produced a W-shaped mortality age distribution. Information from the 2009 novel H1N1 Influenza A pandemic may help to reconstruct the mysterious surge in mortality during the second wave in the 1918 H1N1 Spanish influenza A pandemic. The W-shaped mortality-age distribution of 2009PV indicates the importance of a universal influenza vaccination policy for public protection. The high incidence of cytokine dysregulation and Streptococcus pneumoniae co-infection in hospitalized patients reflects the importance of pneumococcal vaccination and the development of immunomodulating agents that can control influenza-induced cytokine dysregulation.

Introduction

In 2009, 2009PV emerged in Mexico from swine population causing widespread human infection characterized by cytokine dysregulation and acute critical respiratory illness in young and relatively healthy individuals [1,2]. The high demand for extracorporeal membrane oxygenation (ECMO) therapy in these young critically ill patients made ECMO therapy an important consideration in future pandemic planning [3,4]. Novel H1N1 influenza pandemic has a W-shaped mortality-age distribution during the first wave [5,6]. The Centers for Disease Control and Prevention estimated that 77% of the novel H1N1 influenza related deaths in the United States were between 18 and 65 years of age during the first wave [7]. In 2010, the Advisory Committee on Immunization Practices (ACIP) first recommended annual influenza vaccination for all persons aged ≥6 months in the United States [8]. In spite of the universal vaccination policy for seasonal influenza [9], more than 60% of hospitalizations and death for laboratory-confirmed influenza during 2013–14 influenza seasons in the United States occurred among persons aged 18–64 years, and the majority is attributed to 2009PV. No significant antigenic changes in circulating 2009PV strains compared with vaccine strains have been detected since 2009. Bacterial co-infection especially with Streptococcus pneumoniae, Streptococcus pyogenes, or Staphylococcus aureus has been reported in critically ill patients [10,11]. This observed W-shaped mortality-age distribution of the 2009PV infection may help to reconstruct the event in the 1918 H1N1 Spanish influenza pandemic and demonstrate the need for the consolidation of current universal influenza vaccination policy in the United States for public safety. The high level of Streptococcus pneumoniae co-infection in these young individuals reflects the value of current pneumococcal vaccination program among individual aged 18 to 64 years who are at risk of pneumococcal disease. The high incidence of cytokine dysregulation in these hospitalized patients indicates further medical research should be directed towards the development of immunomodulating agents that can selectively suppress detrimental influenza-induced cytokine dysregulation without hindering protective anti-viral response of the host.

The Deadly Second Wave of 1918 H1N1 Spanish Influenza A Pandemic

Since the inception of historical records in the sixteenth century, cycles of pandemic and seasonal influenza had resulted in significant human morbidity and mortality [12]. In the last century, human pandemic influenza viruses namely 1918 H1N1 "Spanish" (1918PV), 1957 H2N2 "Asian" (H2N2PV), and 1968 H3N2 "Hong Kong" (H3N2PV) killed 40 million, 2 million, and 1 million people, respectively [13]. Pandemics can present in waves with peaks typically lasting several months associated with a greater number of cases and increased in mortality separated by troughs during which the number of cases is greatly diminished. During each wave the new influenza subtype undergo adaptation to human host or reassortment with other influenza viruses. The pandemic virus can sometimes re-emerge in a more pathological forms resulting in increased mortality in future waves [14]. In 1918, 1918PV emerged with the ability to spread among humans. The first wave of the 1918 H1N1 Spanish influenza pandemic had a mortality comparable to the usual seasonal influenza with a U-shaped mortality-age distribution that involved the very young and elderly. However, the second wave of the 1918 H1N1 Spanish influenza pandemic had a dramatic surge in mortality and a W-shaped mortality-age distribution that involved young adults with a distinct peak of death in individuals between 20 and 40 years of age [15] (Figure 1).

***Corresponding author**: LAI Kang Yiu, Chief-of-service, Department of Intensive Care Queen Elizabeth Hospital, B6, 30 Gascoigne Rd. Kowloon, Hong Kong, Tel: 852-35066363/35067309; Fax: 852-23881231; E-mail: laiky@ha.org.hk

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Type I alpha/beta interferons (IFN-α/β), encoded by a single interferon-beta (IFN-β) and 13 homologous interferon-alpha (IFN-α) genes in humans, represent an essential element of host defense against influenza virus infection. IFN-β plays an important role in the defense against influenza A virus that cannot be compensated for by IFN-α [16,17]. The non-structural protein 1 (NS1) encoded by the NS of influenza virus is critical in counteracting the interferon-mediated antiviral responses of the host [18]. Wilson Smith, Christopher Andrews, and Patrick Laidrow first isolated human influenza virus in 1933 [19]. However, it was not until 1997 that the genome of the 1918 H1N1 Spanish influenza virus was isolated from archived samples of the 1918 H1N1 Spanish influenza pandemic's second wave [20]. Analysis of individual gene segments showed that the NS contributed to the virulence of the 1918PV [21-23]. The virulence of 1918PV was related to the ability of its NS to disrupt the innate immune response, induce potent cytokine dysregulation, and block the transcription of certain lipid-based proinflammatory mediators that function as part of the host antiviral response [24,25]. NS1 protein encoded by the NS gene of 1918PV can inhibit human inducible pre-transcriptional IFN-β production [26] and post-transcriptional maturation and nuclear export of host interferon-related mRNAs (IFN-α/β) via optimal binding to human 30-kDa subunit of cleavage and polyadenylation specificity factor (CPSF30) [18,27]. The intense innate immune suppression led to an enhanced viral replication, increased viral load and cytokine dysregulation with early and excessive infiltration of macrophages and neutrophils in the lungs in 1918PV infection [28-31]. 1918PV infection was uniformly lethal in mice at low doses and produced severe lung pathology. In ferrets, 1918PV caused severe clinical disease and lung pathology with necrotizing bronchiolitis and alveolitis [32].

The H1N1 influenza virus circulated in humans from 1918 to 1957. During this period, there was substantial antigenic drift of H1N1 influenza virus from the original 1918PV strain. The H1N1 influenza virus with the same antigenic strain of the 1950s re-emerged in 1977 as seasonal H1N1 influenza virus with the usual characteristics of a low mortality and U-shaped mortality-age distribution. The NS of H1N1 viruses in 1940-1957 and 1977-1990 had lost the ability to suppress pre-transcriptional IFN-β production [26]. The seasonal H1N1 virus emerged after 1977 caused little disease in mice and ferret model [32].

The W-shaped Mortality-age Distribution of 2009 Novel H1N1 Influenza A Pandemic

Although the NS contributes to the virulence of 1918PV, whether it can induce the W-shaped mortality age distribution of the second wave of 1918 H1N1 Spanish influenza pandemic remains unresolved in the absence of human data. New information gained from the 2009 novel H1N1 pandemic may help to reconstruct the probable event in 1918 H1N1 Spanish influenza pandemic. 1918PV entered the swine population in 1918. The early classical swine H1N1 influenza virus caused severe clinical disease and lung pathology with necrotizing bronchiolitis and alveolitis in ferrets and mice similar to the 1918PV [32]. The NS of the 1918PV that had entered the swine population in 1918 re-emerged in the 2009PV [33]. 2009PV causes severe pathological lesions in the lungs while human seasonal H1N1 virus usually infected cells of the upper respiratory tract system. The assessment of human sera from different age groups suggests that infection with human H1N1 viruses antigenically closely related to viruses circulating in 1918 confers neutralizing antibody activity to 2009PV [34]. 2009PV produced an enhanced mortality with a W-shaped mortality-age distribution that involved the young population [5-7]. Although globally there were only an estimated 201,200 respiratory and 83,300 cardiovascular deaths associated with 2009PV pandemic during the first 12 months, 65% of these influenza-related respiratory and cardiovascular deaths were between 18 and 65 years of age [35].

Adaptation in pigs had led to several changes of the NS1 protein in the 2009PV as compared with the original 1918PV. The mutations include a K217E substitution that abolished binding to host Crk/CrkL signalling adapters, and a C-terminal truncation that deleted the PDZ binding motif. Both mutations have no major effect on replication, virulence, or transmissibility of the 2009PV [36,37]. However the truncated NS of 2009PV had retained the unique capacity to suppress human pre-transcriptional IFN-β production similar to the original 1918PV [26]. The NS1 protein of 2009PV re-emerged from the swine population has suboptimal binding to human CPSF30. Inefficient binding of the NS1 protein of 2009PV to human CPSF30 to inhibit IFN-α/β production at the post-transcriptional level may account for the suboptimal interferon antagonistic response and reduced mortality of 2009PV infection compared with the original 1918PV[27] The NS of influenza virus is able to inhibit innate and adaptive immunity [38-40]. The suppression of innate and adaptive immunity [41,42] has led to an enhanced viral replication and a delayed viral clearance and resulted in an increased viral load in 2009PV infection [43-46].

The 2009PV induced an early and sustained hyperactive pro-

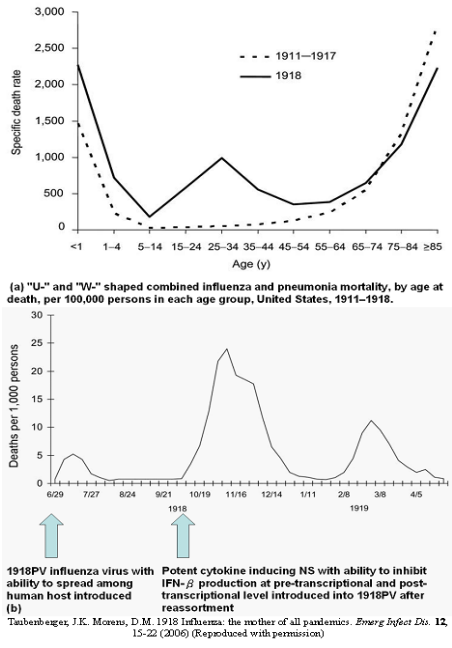

Figure 1: (a) Schematic diagram showing the W-shaped mortality-age distribution of combined influenza and pneumonia mortality of 1918 H1N1 Spanish influenza pandemic (solid line) in contrast with the U-shaped mortality-age distribution of seasonal influenza in the interpandemic years 1911-1917 (dashed line) in the United States. (b) Schematic diagram explaining the surge in mortality in the second wave of 1918 H1N1 Spanish influenza pandemic: The 1918PV with ability to spread among human host was introduced in 1918 in the first wave. The first wave of 1918 H1N1 Spanish influenza pandemic had a mortality comparable to the usual seasonal influenza with a U-shaped mortality-age distribution. Reassortment of an NS with ability to suppress human innate immune response and induce potent cytokine storm through the inhibition of inducible IFN- production at both the pre-transcriptional and post-transcriptional level into the 1918PV after the first wave had led to a dramatic surge in mortality and a W-shaped mortality-age distribution that involved young adults with a distinct peak of death between 20 and 40 years of age in the second wave of the 1918 H1N1 Spanish influenza pandemic as shown in diagram 1a.

inflammatory response and blocked the transcription of certain lipid-based proinflammatory mediators that function as part of the host antiviral response similar to 1918PV [47]. Adult patients with severe 2009PV pneumonia showed sustained hyper-activation of the innate pro-inflammatory cytokines (IL-6, CXCL8/IL-8, CCL2/MCP-1, and sTNFR-1), and markedly suppressed adaptive related cytokines (CXCL10/IP-10, CXCL9/MIG, and IL-17A). Patients who died five days after disease onset showed high viral load and undetectable IL-17 levels in serum. The suppression of adaptive immunity resulted in a delayed viral clearance, which in turn led to further sustained activation of the pro-inflammatory response. Elevated proinflammatory cytokines interleukin-6 (IL-6) predicted critical illness requiring ICU admission [48,49]. IL6 was a biomarker for severe disease in young adults below 18 years of age [50]. In children, high level of IL-6 was associated with mortality. Nonsurvivors were immunosuppressed with leukopenia and markedly reduced tumor necrosis factor-α (TNF-α) production capacity. A TNF-α response of less than 250 pg/mL was highly predictive of death and longer duration of ICU stay [51].

Bacterial co-infection was associated with adverse outcomes in hospitalized adult patient with 2009PV infection [52]. 41% of the deaths of 2009PV infection had bacterial co-infection and Streptoccoccus pneumoniae was the most common organism identified [53]. Most of the severe infection of 2009PV accompanied by pneumonia and increased mortality were between 15 and 44 years old [5,54]. Young patients in this age range and without any underlying disease had impaired immune responses for Streptococcus pneumoniae after 2009PV infection due to defective cytokine response with suppressed TNF-α production and alteration of adaptive immunity [55]. In contrast to previous seasonal influenza seasons, pediatric deaths related to 2009PV were less likely to have a bacterial co-infection with methicillin resistant Staphylococus aureus (MRSA). Many had bacterial co-infections with Streptococcus pneumoniae, as has been found in other studies [56,57].

Pathogenesis of the W-shaped Mortality Distribution of 1918 Spanish Influenza Pandemic and 2009 Novel H1N1 Pandemic: The Interplay of A Potent Innate Immune Suppressing NS, Heavy Viral Load, Cytokine Dysregulation and Bacterial Co-infection

Seasonal and pandemic influenza are an important cause of morbidity and mortality among the very young and elderly in both healthy population and in patients with chronic medical conditions and altered immune response [58]. H2N2 and H3N2 pandemics and seasonal influenza epidemics in the past century had a U-shaped mortality curves with most of the influenza-related deaths being the result of the exacerbation of an underlying condition or secondary to bacterial co-infections among the very young and the elderly [59]. The synergistic role of bacterial co-infection in enhancing the mortality of influenza infection has been known and documented for nearly a century [60]. Individual at the extremes of ages are more susceptible to bacterial co-infection after influenza infection due to bacterial colonization, immune dysfunction and co-morbidities [61,62]. The human upper respiratory tract of infants and children is the reservoir of a diverse community of potential pathogens such as Streptococcus pneumonia, Haemophilus influenza and Staphylococus aureus [63-65] which predispose these young individuals to invasive disease after an influenza infections [66-69]. Oral colonization by respiratory pathogens [70-73] and changes in pulmonary reserve, decreased mucociliary transport, decreased cough reflex, decreased elasticity of alveoli and poorer ventilation—all of which lead to diminished cough and airway patency—cause older adults to be more susceptible to bacterial pneumonia, especially after an influenza infection [74]. It has been estimated that persons who are 65 years and older account for more than 80% of all pneumonia- and influenza-related deaths. Most of the excess mortality caused by influenza and/or pneumonia is attributed to elderly with high-risk conditions [75]. Influenza increases clinical pneumococcal disease incidence in elderly patients. An additive effect was observed in reducing the need for hospital admission for influenza and pneumonia [76] and the prevention of all-cause mortality with influenza and pneumococcal vaccines given together in elderly people, including in those with underlying chronic disease [77]. Hence apart from underlying co-morbidities, bacterial co-infection has a major contribution to the enhanced mortality in the extremes of age and the U-shaped mortality age distribution during influenza seasons [78].

In the United States between 1911 to 1918, curves of influenza mortality by age at death are typically U-shaped, reflecting high mortality in the very young and elderly with low mortality at all ages in between [15]. The 1918 to 1919 pandemic and succeeding winter epidemic recurrences in 1919 and 1920 instead produced a W-shaped mortality curves, which featured a third mortality peak in healthy young adults between 20 to 40 years of age that accounted for approximately half of the total influenza deaths, including the majority of excess influenza deaths. Perhaps the most puzzling mystery of the 1918 pandemic is how to explain that extraordinary excess influenza mortality in persons between 20 to 40 years of age which was responsible for the W-shaped mortality age distribution during the pandemic. Although animal studies using the 1918PV regenerated from archived samples collected from patients who succumbed during the 1918 H1N1 Spanish influenza pandemic's second wave suggested the important role of cytokine dysregulation in the pathogenesis, historical data and autopsy series indicate that almost all deaths resulted from secondary bacterial bronchopneumonia and that frank acute respiratory distress (ARDS)-like syndromes in the absence of bacterial pneumonia, and thus, conceivably attributable to primary viral pneumonia and/or cytokine storms, have been uncommon causes of death. Many of these patients have acute aggressive bronchopneumonia featuring tracheobronchial epithelial necrosis [79]. The major bacteria identified in the pandemic were Streptococcus pneumoniae, Streptococcus pyogenes, and, less commonly, Staphylococcus aureus and Haemophilus influenza [80].

An intact interferon response plays an important role in determining the pathogenicity, tissue restriction and systemic dissemination of influenza infection. Mice that are unable to mount an interferon response due to disruption of the STAT1 gene are 100-fold more sensitive to lethal infection with influenza virus and develop fulminant systemic disease after influenza virus infection [81,82]. The NS1 protein encoded by the NS of influenza virus plays a critical role in inhibiting interferon-mediated antiviral responses. Influenza A virus lacking the NS1 protein encoding gene can only replicate in interferon-deficient systems [83,84]. Influenza A and B virus mutants containing a NS with "weak" anti-interferon activity are highly attenuated [85-88]. There is one single IFN-β and 13 homologous IFN-α genes in humans. IFN-β plays an important role in the defense against influenza A virus that cannot be compensated for by IFN-α. The unique NS of 1918PV is able to inhibit interferon production at both pre-transcriptional [26] and post-transcriptional level [18,27]. In contrast to other human influenza viruses in the last century, the NS1 protein of the 1918PV and 2009PV binds to retinoid-inducible gene 1 (RIG-I) to inhibit the downstream activation of interferon regulatory factor 3 (IRF3) and the subsequent production of IFN-β at the pre-transcriptional level [26,89-92]. In common with the other human influenza in the last century, the NS1 protein of 1918PV inhibit type I interferon (IFN-α/β)

production at the post-transcriptional level through optimal binding of the C-terminal effector domain to CPSF30. Subsequently, newly synthesized host cellular mRNAs including interferon and interferon-stimulated genes are unable to export from the nucleus after infection due to inhibition of 3' cleavage and polyadenylation. This results in the establishment of an influenza virus-specific translational system that selectively translates viral and not host mRNAs [18,27,93-96]. The intense innate suppression conferred by the NS allows 1918PV to replicate at a rate over 200 fold than that of seasonal H1N1 [31]. This allowed 1918PV to replicate swiftly at the "stealth phase" and attain high steady-state titers in the lungs within 48 hours after infection to induce direct cytopathic damages and cytokine dysregulation [97]. The NS mediated delay of interferon induction contributed to the virulence of 1918PV by influencing the spread to and replication efficiency of 1918PV in the lower respiratory tract [24]. 2009PV also demonstrated an enhanced replication in the lower respiratory tract and led to virus-associated diffuse alveolar damage in nonhuman primate model [34,98]. A comparison of the pathology of 1918PV 2009PV and seasonal H3N2 virus infection showed that 1918PV and 2009PV infect the mucosal epithelial cells of the airways, alveolar macrophages, and pneumocytes, whereas seasonal influenza H3N2 mainly infects mucosal epithelial cells of the larger airways [99]. The above evidences underlies the important role of an innate immune response suppressing NS in the production of severe pneumonia in the host through enhanced tropism of 1918PV to tissue of the lower respiratory tract and an increased viral load which is pivotal to cytokine dysregulation, pulmonary damage and mortality in influenza infection [100-102]. 1918PV showed an enhanced virulence in non-human primate [103,104]. The pathogenicity of the NS of 1918PV to human cell is species specific. The species specificity of NS was first discovered during testing of the individual gene segments of the 1918PV in mice. The NS gene that is virulent to human cells [21-23] is less virulent than the corresponding wild-type control virus in mice [105]. 2009PV which induces a potent cytokine dysregulation and produces an enhanced mortality and a mortality-age distribution that involved young adults in humans, only causes modest disease in ferrets [105],[106] and asymptomatic infection in pathogen free miniature pigs [34]. The asymptomatic infection of 2009PV in pathogen free miniature pig explains why there was no detectable outbreak of 2009PV infection in the swine population before the virus surfaced in humans.

Early cytokine dysregulation is a common feature in 1918PV [30,107] and 2009PV [48,49,108,109] infection. As a major transcription factor for antiviral and immune stimulatory activities, nuclear factor kappa-B (NF-κB) play an important role in the induction of interferon and other pro-inflammatory molecules such as IL-6 upon cellular responses against a virus infection [110,111]. Influenza A virus, being a small RNA virus with relatively small coding capacity and protected by the anti-interferon activity of its NS, has taken advantage of the host NFκB activation pathway [112] to enhance the synthesis of viral ribonucleic acid [113] and nuclear export of viral ribonucleoprotein (vRNP) complexes [114] and during the process leads to apoptosis of the host cell [115-117] and induces cytokine dysregulation [118,119]. The cytopathic damage and cytokine dysregulation result in an enhanced morbidity and mortality [120]. Either mechanism of tissue damage may predominate in individual patient [121]. NFκB activation is a prerequisite for influenza infection [122] and influenza-induced cytokine dysregulation [118,119,123,124]. The lack of hypercytokinemia in NF-κB-deficient mice during influenza infection confirms the central role of NF-κB in cytokine dysregulation [118]. Immune suppression conferred by the NS lead to an enhanced viral replication and an increased in viral load in 1918PV [28,29,31] and 2009PV [43-46] infection. The accumulation of endosomal haemagglutinin (HA) [125] and viral dsRNA [126] during an enhanced viral replication may in turn, activate NFκB to induce cytokine dysregulation [119,123,126] ROS acts as the second messenger in inducers of influenza-induced NF-κB activation [119,127-129]. HA activates NFκB through the production of reactive oxygen species via endoplasmic reticulum overload (ER-overload) [127,130-133]. Toll-like receptor 3 (TLR3) is expressed both intracellularly and on the cell surface of respiratory epithelial cells [134]. Influenza dsRNA inside the endosome activates NF-kB through upregulation of the TLR3 expression [135,136]. Stable influenza dsRNA released from dying influenza virus-infected cells [137] binds to TLR3 on the epithelial cell surface to activate NF-kB [138]. ROS enhances TLR3 induced NFκB activation in reaction to viral dsRNA in airway epithelial cells [139]. Apart from the activation of NF-κB via the TLR3 signal pathway, dsRNA is able to active NLRP3 inflammasome signal pathway. dsRNA can elicit an enhanced antigen-specific Th1-polarized immune response and dampened Th17 response [140], a feature of the cytokine dysregulation of 2009PV infection [49]. NF-κB-induced IL-6–STAT3 signaling pathway is an important pro-inflammatory response after influenza A infection [141]. Level of IL-6 correlated with the magnitude of influenza virus replication and cell damage in human tracheal epithelium [142] and is a prognostic biomarkers for progression to respiratory failure and mortality in influenza A infection [48-50,143]. Anti-oxidants are able to suppress NFκB-induced IL-6 production in influenza infection [144-146]. Inflammasome activation is important in the development of both innate [147] and adaptive immune responses [148,149] during influenza infection. An intact body commensal microbiota regulates immune defense against respiratory tract influenza A virus infection by priming basal levels of pro-IL-1β and pro-IL-18 at steady state.[150]. Influenza viruses activate inflammasome to induce interleukin-1β(IL-1β) and interleukin-18 (IL-18) production [151-153] through M2 protein expression [154] under the regulation of the N-terminal region of the NS1 protein that does not involved in RNA-binding activity [155-158]. On the other hand, the N-terminal RNA-binding domain of the NS1 protein restricts the production of the mature form of IL-1β and IL-18 through the inhibition of caspase-1-dependent post-translational processing of pro-IL-1β and pro-IL-18 to repress PKR-dependent apoptosis [159]. In spite of the suppression by the N-terminal RNA-binding domain of the NS1 protein, active IL-1β is secreted into bronchoalveolar lavage of mice infected with influenza virus in a dose-dependent manner through the caspase-1 dependent pathway [160-164]. 1918PV up-regulates inflammasome early after infection and produces early cytopathic damage of the respiratory epithelium and cytokine dysregulation [104]. Both IL6 and IL-1β are up-regulated in 2009PV infection. IL-6 was significantly correlated with specific clinical findings, such as severity of respiratory compromise and fever. No correlation was found between IL-1β expression and final outcome [165]. HA [166] and dsRNA [167-169] can also induce IL-1β and IL-18 through NLRP3 inflammasome activation. The M2 protein, HA and dsRNA accumulated during rapid viral replication may account for induction of IL-1β and IL-18 level during 1918PV because the 1918PV has been found to have a replication rate that is greater than 200-fold of seasonal H1N1 virus [31]. NLRP3 inflammasome activation in influenza infection is ROS dependent [[170-172]. Influenza induced M2-dependent IL-1β production can be inhibited by anti-oxidants [173].The hypersecretion of IL-18 induced by inflammasome activation may lead to the development of acute respiratory distress syndrome [174], multiple organ failure [175] and haemophagocytic syndrome [176,177]. Pathological evidence of haemophagocytic syndrome was a common finding in patient died of 1918PV infection [176]. 2009PV-induced haemophagocytic syndrome [179-182] is a major contribution to mortality in human 2009PV infection [183].

1918PV and 2009PV have an enhanced tropism to cells of the lower respiratory tract. Severe tracheobronchial/alveolar epithelial necrosis was a prominent feature in patients died of 1918PV infection. 1918PV, protected from the anti-interferon activities by its NS, produce a heavy viral load and induce cytokine dysregulation in humans. The accumulated HA and dsDNA resulting from an enhanced viral replication induce caspase 3-dependent apoptosis of the tracheobronchial tree through the NF-κB-IL-6 activation pathway. 1918PV up-regulates inflammasome early after infection and produces severe apoptosis of the respiratory epithelium as early as 12 hours after infection [104]. Hence, heavy viral load, IL6 hypersecretion, early inflammasome activation and enhanced tissue tropism to cells of the lower respiratory tract due to the presence of a potent innate immune suppressing NS may contribute to the severe mucosal damage of 1918PV infection. The NS-induced adaptive innate immune suppression predisposed these patients with severe tracheobronchial epithelial necrosis to bacterial co-infection, in particular Streptococcus pneumoniae. Upon introduction of 1918PV into humans in 1918, exceptionally high mortality was seen during the first 2 years that this subtype circulated, primarily due to bacterial pneumonia. Thereafter, the overall mortality rate declined, but excess mortality continued to be seen. H1N1 viruses reentered the population in 1976 as seasonal H1N1. The NS of seasonal H1N1 viruses had lost the ability to suppress pre-transcriptional IFN-β production [26] and induce necrotizing bronchiolitis and alveolitis [28] as the original 1918PV. Very little excess mortality has occurred during years where seasonal H1N1 viruses were the predominant circulating strain, and the relative contribution of bacterial superinfections to excess mortality has declined [184]. The NS of 1918PV entered the swine population in 1918 and emerged in 2009PV [29]. The NS of 2009PV has retained the ability suppress pre-transcriptional IFN-β production [26] and induce cytokine dysregulation as the original 1918PV [29,185]. Severe tracheitis, necrotizing bronchiolitis, alveolitis and haemophagocytic syndrome are common post-mortem finding in patient with fatal 2009PV infection [186,187]. 2009PV have altered the epidemiology of invasive pneumococcal disease and shifted the age distribution to healthy young adults 20–39 years of age, the age group that has a distinct peak of death in 1918 Spanish Influenza pandemic and contributed to the W-shaped mortality age distribution of the 1918PV. A high proportion of hospital admission during the first wave of 2009 novel H1N1 influenza pandemic due to invasive pneumococcal disease among 20–39 years of age required admission to intensive care unit. 2009PV has also led to invasive pneumococcal disease among persons 18–64 years of age with an increased prevalence of underlying conditions [188].With this new information from 1918PV and 2009PV, we may be able to reconstruct the probable events in the 1918 H1N1 Spanish influenza pandemic.

Reconstruction of the 1918 H1N1 Spanish Influenza Pandemic

1918PV which killed 40 million people worldwide was characterized by an enhanced mortality and a W-shaped mortality age distribution during the second wave in contrast to the U-shaped mortality-age distribution of pandemic H2N2 and H3N2 and epidemic seasonal influenza in the last decades. Most of these people who succumbed to 1918PV infection had pathological evidence of extensive mucosal damage of the tracheobronchial/alveolar tree and secondary bacterial co-infection. The unique NS of the 1918PV of the second wave is able to block interferon production at both pre-transcriptional and post-transcriptional level and induce cytokine dysregulation. We hypothesized that 1918PV with the ability to spread among human host was introduced in 1918 in the first wave. The first wave of 1918 H1N1 Spanish influenza pandemic had a mortality comparable to the usual seasonal influenza with a U-shaped mortality-age distribution. Reassortment of an NS with ability to induce potent cytokine dysregulation and suppress human innate immune response at both the pre-transcriptional and post-transcriptional level into the 1918PV after the first wave led to an enhanced viral replication and viral tropism to tissue of the lower respiratory tract. The enhanced viral replication and the subsequent heavy viral load led to an endosomal accumulation of HA and dsDNA which in turn, activate the NFκB-IL6 and inflammasome signaling pathway via the production of ROS through ER-overload. ROS also enhances TLR3 induced NFκB activation in reaction to viral dsRNA in endosome and in airway epithelial cells. The enhanced tropism to tissue of the lower respiratory tract, high viral load, hypersecretion of IL6 and early inflammasome activation induce extensive damage of the epithelium of the tracheobronchial tree and predisposed otherwise healthy human host to bacterial co-infection. Under the intense adaptive innate immune suppression of the NS, bacterial co-infection led to a dramatic surge in mortality in healthy young adults between 20 and 40 years of age. In couple with the usual U-shaped mortality distribution among the very young and elderly, the enhanced mortality among young adults between 20 and 40 years of age produced a W-shaped mortality-age distribution in the second wave of the 1918 H1N1 Spanish influenza pandemic. The enhanced mortality of bacterial co-infection among the young adults persisted in the first 2 years after the introduction of this unique NS into 1918PV. This resulted in a higher mortality during the third wave compared with the first wave (Figure 1b). The hypothesis outlines the contributing role of a potent innate immune suppressing NS of influenza viruses in producing cytokine dysregulation, bacterial co-infection and an enhanced mortality. It also explains why there is no major change in histopathology of fatal influenza pneumonias between pandemic and seasonal influenza as documented over the past 120 years [189] probably because the cytopathic damage, cytokine dysregulation and enhanced mortality are resulting from an increased viral load, a reflection of the severity of the underlying influenza virus infection that finally lead to the death of the patients.

Implication on the Importance of A Universal Influenza Vaccination Policy

There is a difference in seasonal influenza vaccination recommendation between the World Health Organization (WHO) and the ACIP of the United States. WHO recommends influenza vaccination for the very young and elderly and at risk groups such as patients with chronic medical conditions, pregnant women and healthcare workers [190]. ACIP recommended annual influenza vaccination for all persons aged ≥6 months in the United States since 2010 [8]. Pandemic influenza viruses containing an NS capable of suppressing inducible interferon production at either the pre-transcriptional (2009PV) [26,27] or post-transcriptional level (H2N2 and H3N2) [27] produced a mortality of less than 0.1% [15,31]. The 1918PV which suppressed inducible interferon production at both the pre-transcriptional [26,27] and post-transcriptional levels [18,27] led to a mortality of 2.5% [15] (Figure 2). If 2009PV were to acquire the amino acid structure essential for optimal binding to human CPSF30 during transmission in humans, the virulence of seasonal novel H1N1 influenza virus may escalate [27]. The W-shaped mortality-age distribution of seasonal novel H1N1 influenza virus and its potential for increased virulence after optimal adaptation to humans underscores the importance of the consolidation of the current universal vaccination policy for all persons 6 months and older in the United States [8,9]. The WHO should work towards a universal vaccination policy as

The Anti-interferon Activity of the NS1 protein of Human Influenza Viruses and the Mortality of Human Influenza Viruses during Pandemic

	Pre-transcriptional inducible IFN-β inhibition	Post-transcriptional interferon IFN-α/β inhibition (Optimal human CPSF30 binding)	Mortality
H2N2	× [26]	✓ [27]	<0.1% [15]
H3N2	× [26]	✓ [27]	<0.1% [15]
2009PV*	✓ [26,27]	× [27]	<0.1% [31]
1918PV*	✓ [18,26,27]	✓ [18,27]	~2.5% [15]

* 1918PV and 2009PV lead to a W-shaped mortality-age distribution that involved young adults in humans.

× = no ✓= yes.

Figure 2: Table showing the anti-interferon activity of the NS1 protein of human influenza viruses and the mortality of human influenza viruses during pandemic. 1918PV and 2009PV are able to inhibit pre-transcriptional IFN-production. H2N2, H3N2 and 1918PV are able to inhibit post-transcriptional maturation and nuclear export of host interferon-related mRNAs (IFN-α/β) via optimal binding to the human 30-kDa subunit of cleavage and polyadenylation specificity factor (CPSF30).

recommended by ACIP for public protection. The potential increase in virulence of H3N2 after the reassortment of the NS of 2009PV during transmission in humans should be monitored.

Implication on The Importance of Pneumococal Vaccination and Smoking Cessation Campaign

Pneumococcal vaccination program for persons at an increased risk of pneumococcal pneumonia and smoking cessation campaign should be consolidated to reduce the risk of Streptococcal pneumoniae co-infection in anticipation of an enhanced virulence of novel H1N1 influenza A virus after its NS1 protein can better adapt to human CPSF30 protein and in preparation of an H5N1/H7N9 avian influenza pandemic. During the influenza pandemics of 1918, 1957, and 1968, a bacterial etiology exists in as many as 50% to 95% of patients with fatal or life-threatening pneumonia. Streptococcus pneumoniae is the most common vaccine preventable organism in bacterial co-infection during these pandecmics [191-193]. Streptococcal pneumoniae co-infection is correlated with the severity of 2009PV infection [194] and was the most common organism identified in patients who died of 2009PV associated bacterial co-infection [53,195]. Streptococcus pneumoniae and influenza virus mutually potentiate the proliferation of each other through viral-bacterial synergistic interaction [196,197]. Influenza virus infection predisposes to Streptococcus pneumoniae co-infection by opening neuraminidase site for the attachment of Streptococcus pneumoniae [198,199] and impairing the host defenses against Streptococcus pneumoniae [200-203]. Highly pathogenic H5 and H7 avian influenza viruses possess polybasic amino acid motif at the haemagglutinin (HA)-cleavage site that allows cleavage activation via proteases produced during bacterial co-infection. These bacterial proteases could activate haemagglutinin (HA) directly or indirectly through the plasminogen/plasmin system to facilitate viral replication and enhance the pathogenicity of these plasmin-sensitive avian influenza virus strains [204-207] (Figure 3). This underscores the importance of pneumococcal vaccination for persons at increased risk of pneumococcal pneumonia during pandemic situation [208-210]. Current pneumococcal vaccination program should be expanded to include healthy young adults without underlying disease in the age range 20 to 40 because they have been shown to have impairments of the immune responses for Streptococcus pneumoniae after novel H1N1 infection due to defective cytokine response and alteration of adaptive immunity [55,188]. Pneumococcal vaccination to smoker should be emphasized because cigarette smoking activates platelet-activating factor receptor that allowed attachment of Streptococcus pneumoniae to lower airway cells [211-213]. Apart from increasing chance of invasive pneumococcal disease, tobacco smoke-induced oxidant stress and suppression of innate immunity are mechanistic factors leading to increases viral replication and increases severity of respiratory disease with influenza [214-217]. Smoking is the strongest independent risk factor for invasive pneumococcal disease among immunocompetent, nonelderly adults [218]. Smoking also increase influenza-associated mortality risks among elders [219].This is of particular importance in China where tobacco smoking is highly prevalent [220,221].

Implication on The Importance of The Development of Immunomodulatory Therapy to Control Influenza-induced Cytokine Dysregulation

Early cytokine dysregulation are associated with 1918PV [30], 2009PV [48,49], H5N1 [222,223] and H7N9 [224-226] infection. Annual influenza vaccination program and currently approved antiviral medications that are directed against the mutable targets of influenza viruses cannot directly prevent cytokine dysregulation. Since the adaptive cellular immunity and the associated cytokine responses are impaired/downregulated in 2009PV pneumonia, further immunosuppressive is unlikely beneficial and may even be harmful [49]. Early use of glucocorticoids was a risk factor for critical disease and death from 2009PV infection [227,228]. Steroid therapy for the treatment of cytokine dysregulation of human H7N9/H5N1 avian influenza infection adds to the complications of superinfection, hyperglycaemia and the development of drug resistant mutants without improving survival [229-231]. Hence, future research on immunomodulating therapy for severe influenza infection should consider medications that can selectively suppress the host pro-inflammatory response but maintain the host anti-viral activity intact. Like all viruses, the influenza virus largely relies on host cell factors and physiological processes to induce cytokine dysregulation and death of the host. Research focused on these non-mutable key steps in the pathogenesis of influenza-induced

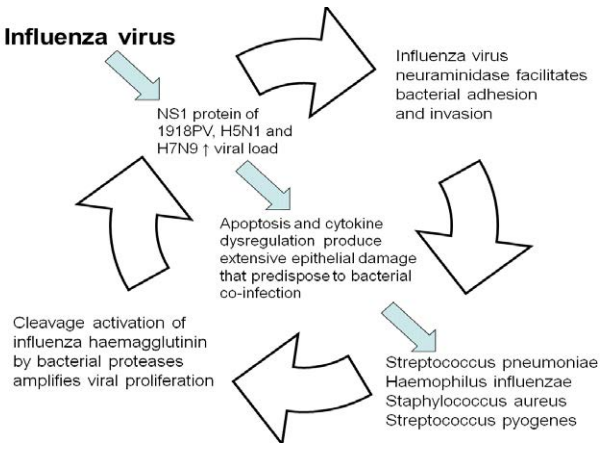

Figure 3: Schematic diagram outlining the mechanisms of viral-bacterial synergistic interaction in bacterial co-infection during influenza pneumonia: Influenza viruses open neuraminidase site for the attachment of bacteria and predispose to bacterial co-infection. Bacterial infection amplifies viral proliferation by producing extracellular proteases for cleavage activation of influenza haemagglutinin. Influenza viruses with a potent innate immune response suppressing NS (e.g. as 1918PV, H5N1 and H7N9) produce a heavy viral load and induce cytokine dysregulation. This results in extensive pulmonary epithelial damage and predisposes to secondary bacterial infection.

cytokine dysregulation inside the host may be novel targets for future therapeutic strategies against these rapidly mutating viruses. They may also have a complementary role to those anti-viral medications and vaccines under development that are directed against the mutable targets of influenza viruses. Immunomodulatory agents that has been proposed for influenza management may include N-acetylcysteine (NAC), macrolides, cyclooxygenase-2 inhibitors (COX-2 inhibitors), mesalazine, paracetamol, statins, peroxisome proliferator-activated receptor agonists, high dose intravenous gammaglobulin (IVIG), AMP-activated protein kinase agonists and high mobility group box 1 antagonists [232,233]. Many of these medications are approved by the Food and Drug Administration (FDA) for the treatment of other diseases. They are available and stockpileable for immediate use. They are currently produced as inexpensive generics, global supplies are huge, and they would be available to treat patients in any country with a basic health care system on the first pandemic day. These immunomodulatory agents may represent a new approach to reduce mortality caused by seasonal and pandemic influenza [234].

In human influenza infection, the anti-viral and proinflammatory cytokine response are activated through different pathways. The induction of anti-viral response is through pre-transcriptional IFN-β production via the activation of RIG-I/IRF-3 signaling pathway while the induction of proinflammatory cytokine response is through endosomal TLR3/HA-induced ROS-dependent NF-κB activation [125,126,135,235]. (Figure 4) NFκB activation, a prerequisite for influenza infection and influenza-induced cytokine dysregulation, is dependent on ROS. ROS also plays an important role in inflammasome activation in influenza virus infection. Hence by targeting the ROS signaling, anti-oxidant can selectively suppress the proinflammatory response without jeopardizing the anti-viral activities in influenza viral infection (Figure 4). Inhibitors of NFκB activation such as IκB kinase complex inhibitors [236], proteasome inhibitors [237,238] and anti-oxidants [239-242] reduce viral replication, attenuate cytokine dysregulation and improve survival of mice with lethal influenza infection. Among anti-oxidants with therapeutic potential to severe influenza infection [243], NAC is able to inhibit inflammasome [244,245] and NFkB [246]activation during influenza infection. NAC, at an oral dose of 1g/Kg daily, improved the survival of mice against lethal influenza infection [247] and was synergistic with ribavirin [248] or oseltamivir[249] in protecting mice from lethal influenza infection with an end point survival of 92% and 100% respectively (Figure 6). NAC, at 100 mg/Kg continuous intravenous infusion daily, suppressed the fever and C-reactive protein concentration with corresponding clinical improvement in a patient with 2009 novel H1N1 influenza virus (2009PV) pneumonia [250]. NAC inhibits replication of H5N1 and reduces H5N1-induced cytopathogenic effects, apoptosis and the pro-inflammatory cytokine production [246] at a serum concentration achievable with NAC infusion for the treatment of paracetamol overdose [251]. The inhibition of replication of influenza virus is strain dependent [252]. NAC enhances the development of influenza-specific CD8+ T cells, an important step in adaptive immunity for clearance of influenza virus [253,254]. Therefore, by targeting ROS-induced NF-κB activation, high dose NAC anti-oxidant therapy can inhibit viral replication, suppress cytokine dysregulation, and enhance the development of adaptive immunity during influenza infection. Given the poor oral availability of NAC in the range of 6% to 10% in humans, therapeutic dose of NAC for influenza infection can hardly be delivered by oral preparation [255]. NAC is a category B drug for pregnancy and is an affordable drug with a wide therapeutic window. NAC has an established safety profile even in high dose and prolonged use in humans[256-258]. With NAC's safety profile, it is ethically justifiable to validate its role in the management of cytokine dysregulation of severe influenza infection with large scale human randomized controlled trials. Selenium, a co-enzyme of glutathione peroxidase is essential in glutathione synthesis. It is able to suppress the activation of NFκB and has been shown to reduce influenza-induced pathology. It may have a complementary role to the anti-oxidant action of N-acetylcysteine [259-263]. The high selenium content of anti-influenza herbal mushrooms in traditional Chinese medicine may have contributed to their ability in protecting mice from lethal influenza infection [264].

The export of viral ribonucleoprotein (vRNP) complexes from the nucleus to the cytoplasm is a pivotal step necessary for the formation of progeny influenza virus particles [265].Caspase 3 activation is essential for nuclear vRNP export in influenza infection [115]. The influenza-induced NFκB activation up-regulates the pro-apoptotic factors tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Fas/FasL pathway and induces caspase 3-dependent apoptosis to facilitate the nuclear export of vRNP complexes to enhance influenza propagation [116]. Inhibition of NFκB activation by aspirin or COX-2 inhibitor blocks caspase activation, prevents the nuclear export of vRNP complexes. and improves the survival of mice from lethal influenza infection [266,267]. Salicylate and aspirin inhibit NFκB activation by preventing the phosphorylation of IκBα and its subsequent degradation by ubiquitin-proteasome pathway [268,269]. Their role in the management of influenza has declined after the association with Reye's syndrome with their use in children [270]. COX-2 inhibitors are being explored recently as an alternative immunomodulating agent for the treatment of severe influenza infection [271]. COX-2 inhibitors are able to suppress H5N1 virus replication in human macrophages [272]. H5N1-induced pro-inflammatory markers such as IL-6 was suppressible with celecoxib (COX-2 inhibitor), and mesalazine (5-amino salicylic acid) [273]. 5-amino salicylic acid is a

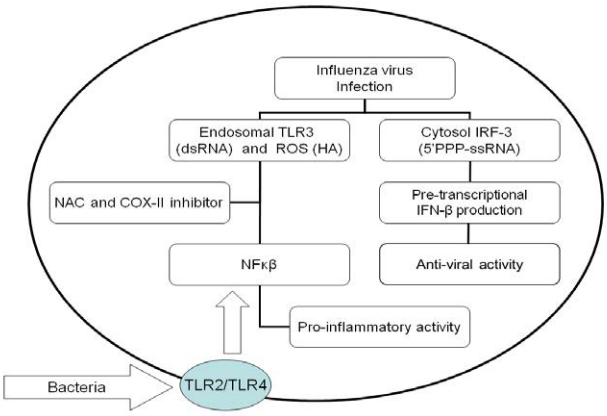

Figure 4: Schematic diagram showing the different activation pathway of pro-inflammatory and anti-viral activity in influenza infection: During the early phase of influenza infection, the induction of pro-inflammatory activity is through NFκB activation by toll-like receptor 3 (TLR3) stimulation via endosomal dsRNA and reactive oxygen species (ROS) production via haemagglutinin (HA)-induced endoplasmic reticulum overload (ER-overload) whereas the induction of early anti-viral activity is through the induction of pre-transcriptional interferon-β (IFN-β) production via activation of cytosol interferon regulatory factor 3 (IRF3) by binding of retinoid-inducible gene I (RIG-I) by 5'PPP-containing single stranded RNA (5'PPP-ssRNA). Therefore, anti-oxidants COX-2 inhibitor and paracetamol by targeting the NFκB pathway can block the human pro-inflammatory activity without compromising the human antiviral response. Since gram positive and gram negative bacteria can activate NFκB through surface membrane toll like receptor 2 and 4 (TLR2/TLR4) respectively [335-338] NAC is unable to block bacteria-induced NFκB activation.

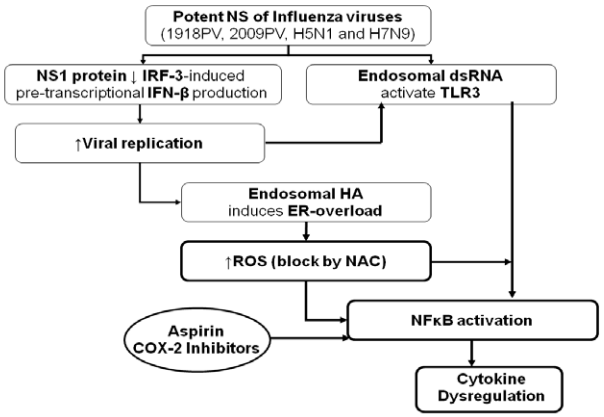

Figure 5: Schematic diagram showing the induction of cytokine dysregulation in severe influenza virus infection. Human infection with influenza viruses with a NS capable of suppressing pre-transcriptional IRF-3-induced IFN-β production (1918PV, 2009PV, H5N1 and H7N9) allows these influenza viruses to proliferate rapidly during the stealth phase of influenza infection. This enhanced viral replication results in an accumulation of endosomal haemagglutinin (HA) and double-stranded RNA (dsRNA). The endosomal HA activates NF-κB via the production of ROS through endoplasmic reticulum overload (ER-overload). ROS enhances TLR3 induced NFκB activation in reaction to an accumulated endosomal viral dsRNA. Profound activation of NFκB by accumulated endosomal HA and dsRNA results in cytokine dysregulation. Since NFκB activation is a prerequisite for influenza-induced cytokine dysregulation, blockade of NF-κB activation by NAC, paracetamol and COX-2 inhibitors leads to suppression of cytokine dysregulation in influenza infection.

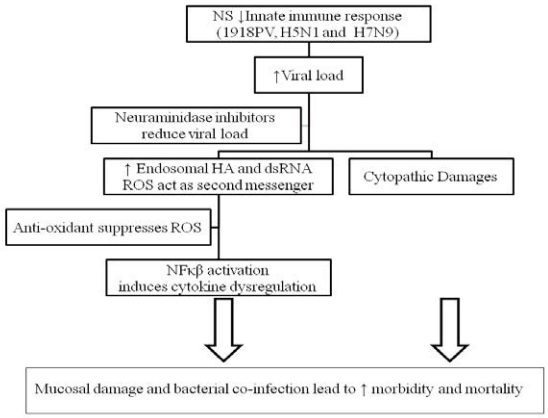

Figure 6: Influenza viruses (1918PV, H5N1 and H7N9) with potent innate immune response suppressing NS induce a heavy viral load which in turn leads to direct cytopathic damage and an accumulation of endosomal haemagglutinin (HA) and double-stranded RNA (dsRNA). ROS then acts as the second messenger for both dsRNA and HA induced NFκB activation to induce cytokine dysregulation. The cytopathic damage and cytokine dysregulation result in an enhanced morbidity and mortality. Either mechanism of tissue damage may predominate in an individual patient. Neuraminidase inhibitors reduce viral load. Anti-oxidant at a dose that can suppress endosomal ROS reduces influenza-induced cytokine dysregulation. This may explain the synergistic action of neuraminidase inhibitor and high-dose NAC in reducing mortality in mice with lethal influenza infection.

derivative of salicylic acid with anti-oxidant property for the treatment of inflammatory bowel disease [274]. Paracetamol has been shown to significantly decrease the infiltration of inflammatory cells into the airway spaces, reduced pulmonary immunopathology associated with acute infection and improved the overall lung function of mice, without adversely affecting the induction of virus-specific adaptive responses [275]. The role of paracetamol and COX-2 inhibitors in the management of influenza virus induced cytokine dysregulation should be confirmed by prospective randomized studies in humans (Figure 5).

Apart from control of cytokine dysregulation, reduction in viral load is pivotal to survival in influenza virus infection. Although blockage of NF-kB activation can abolish cytokine dysregulation, it is unable to prevent apoptosis of the host cells because influenza virus also induce extracellular Ca2+ influx, leading to mitochondrial dysfunction to induce host cell apoptosis [276,277]. Influenza virus-induced accelerated extracellular Ca2+ influx are critical for influenza replication [278]. Early neuraminidase inhibitor therapy reduces viral load and improves survival in 2009PV and H5N1 infection in humans [279,280]. Patients receiving treatment within 48 hours after symptom onset of H5N1 infection have the best survival benefit with a mortality of 20%. The mortality of H5N1 was 76% in the absence of anti-viral therapy [281]. Neuraminidase inhibitors are complementary to NAC and COX-2 inhibitors in improving the survival of mice in lethal influenza infection probably by controlling the viral load and influenza-induced apoptosis. A triple therapy with a combination of zanamivir, celecoxib (COX-2 inhibitor), and mesalazine reduce viral load and inflammatory marker and improve survival of mice with lethal H5N1 avian influenza infection. Zanamivir alone reduced viral load but not inflammation and mortality. The survival benefits of adding celecoxib and mesalazine to zanamivir could be caused by their synergistic effects in reducing cytokine dysregulation [282].

Future Perspectives

Although no significant antigenic changes in circulating 2009PV strains compared with vaccine strains have been detected since 2009, seasonal novel H1N1 influenza in 2013–14 influenza seasons produced an enhance mortality and W-shaped mortality age distribution [10,11,283,284]. Besides monitoring the antigenic drift in hemagglutinin and neuraminidase gene segment, the surveillance of NS mutation is also necessary to determine whether the NS1 protein adapatation of novel H1N1 influenza A virus to human CPSF30 may have contributed to the adverse clinical outcome of 2009PV infection in 2014. The virulence of H3N2 may increase if this potent innate immune suppressing NS of 2009PV is reassorted to seasonal H3N2 during transmission in humans.

A potent innate suppressing NS capable of inhibiting human pre-transcriptional IFN-β production has emerged in highly pathogenic H5N1 and low pathogenic H7N9 avian influenza viruses [26,285]. This had resulted in cytokine dysregulation [225,286-288] and an enhanced mortality [289,290] in human infections by these viruses. The H5N1 responsible for the human outbreak in 1997 (A/HK/97/H5N1) produced a mortality of 33% [291]. A/HK/97/H5N1 contained an NS capable of suppressing constitutive IFN-β release [292] and inducible IFN-β production at the pre-transcriptional level [26]. The NS1 protein of A/HK/97/H5N1, which contains an L103 and I106 structure, binds human CPSF30 to a significant, though not optimum extent [293]. Over 98% of the NS1 proteins of H5N1 isolated from humans after 2003 contain the F103 and M106 amino acid structures that bind optimally to human CPSF30 [294]. The mutation results in a 20-fold enhancement in virus replication in tissue culture and 250-fold enhancement of virulence in mice [295,296]. Human infection with H5N1 after 2003 has a mortality of 55% [297]. This unique potent innate immune suppressing NS of influenza A/HK/97/H5N1 is also present in H9N2 [298,299] and H6N1 [300,301] avian influenza viruses endemic in China. Constitutive interferon-beta (IFN-β) and inducible type I interferon (IFN-α/β) are complementary in protection against

influenza infection. Low level of constitutively produced IFN-β is important to maintain immune cells in an activated state ready for a timely response for the production of inducible interferon to influenza infection [302-304].The suppression of both constitutive and inducible interferon at multiple levels may account for the enhanced mortality in human H5N1 infection [26,292,294] (Figure 7). The intense innate immune suppression conferred by this NS enhances tropism of H5N1 for human tissues and allows H5N1 to invade and replicate in human tissues without the need for the avian sialic acid alpha-2,6-galactose receptor [305-308]. Introduction of the NS of H5N1 into the highly pathogenic H7N1 avian influenza virus enabled H7N1 to replicate efficiently in different human cell lines without prior adaptation. The enhanced viral replication and tissue tropism were attributed to a stronger suppression of IFN-β production and better binding efficiency of the NS1 protein to human CPSF30. This observation shows that the NS of H5N1 is able to increase the virulence and enhance the adaptation of avian influenza viruses to human hosts [309,310].This potent NS of H5N1 that had resided in H9N2 influenza viruses had reassorted into low pathogenic H7N9 avian influenza virus [311-314], leading to a mortality of 33% during the first human outbreak of H7N9 in China in 2013 [315]. The NS1 protein of H7N9 exert potent inhibition of RIG-I-dependent upregulation of the IFN-β promotor in human cells and allow H7N9 to replicate efficiently in human alveolar tissue. Although NS1 protein of H7N9 can inhibit IFN-β at the pre-transcriptional level, it has suboptimal binding to CPSF30 to allow it to block interferon production at the post-transcriptional level, a situation similar to A/HK/97/H5N1 in 1997 [316]. The presence of this human adapted potent innate immune suppressing NS in low pathogenic avian influenza viruses endemic in China may give rise to the emergence of highly lethal reassortant avian influenza viruses with pandemic potential through the poultry market in China [317]. Outbreak of human avian influenza virus infection in China and South East Asia usually occurs during the months around the Chinese New Year when poultry movement and sales grow exponentially [318-321].

There is an evidence for a protective effect of influenza immunization against bacterial infections, and vice versa of pneumococcal vaccination against influenza-associated pneumonia and lethality [322]. While antibiotics and vaccines will certainly reduce the rate of individual mortality, the factor contributing most to the relatively lower anticipated lethality of a pandemic with a 1918-like influenza virus in contemporary population is to reduce bacterial co-infection, in particular Streptococcus pneumonia [323]. This can be achieved through vaccination policy and smoking cessation campaign. Pandemic preparedness plans against novel influenza viruses such as H5N1/H7N9 avian influenza viruses should consolidate existing pneumococcal vaccination program, in particular among persons aged 18–64 years who are at risk of pneumococcal disease [324]. Pneumococcal vaccination program should expand to include healthy young adults in between 20 to 40 years of age in anticipation of an W-shaped mortality distribution of 2009PV and H5N1 avian influenza viruses infection [54,188,325]. Smoking cessation campaign should be incorporated into national pandemic preparedness plans in countries where tobacco smoking is highly prevalent. The world should be working towards a tobacco free initiative on both national and global levels as promoted by the World Health Organization [326].

Cytokine dysregulation is a common feature in 1918PV, 2009PV, H5N1, and H7N9 infection and contributes to influenza-related mortality. In the event of a human H7N9/H5N1 avian influenza pandemic that carries a mortality of over 30%, more than 85% of the world's population will not have meaningful access to pandemic vaccines or antiviral agents [327]. From the experience of the novel H1N1 pandemic, pandemic vaccines are unlikely to be available for effective prevention during the first wave of a pandemic [328,329], and non-pharmaceutical interventions such as quarantine and containment failed to contain the pandemic in the initial mitigation phase even in developed countries [330]. In combating such unprecedented global public-health crisis, additional rapidly activated intervention measures that may control influenza-induced cytokine dysregulation will be required if high mortality rate are to be avoided [331]. Research is urgently needed to determine whether alternative treatments that are inexpensive, can be stockpiled and would be available on the first pandemic day might be useful in managing the cytokine dysregulation of human H7N9/H5N1 avian influenza infection [332]. Since interferon-induced transmembrane protein-3 dysfunction that predisposed an individual to cytokine dysregulation and an enhanced mortality in severe influenza infection such as H7N9/H5N1 avian influenza virus is more common in Han Chinese [226,333,334], medications that can effectively suppress cytokine dysregulation without impairing the human anti-viral response is urgently needed for strategic preparation of a H7N9/H5N1 pandemic in China. By targeting the NFκB and inflammasome activation pathway, high dose NAC anti-oxidant therapy, paracetamol and COX-2 inhibitors may have a complementary role to anti-viral agents in the management of influenza-induced cytokine dysregulation. If the efficacy of these agents against human H7N9/H5N1 avian influenza virus infection is confirmatory, the availability and affordability of these agents make them ideal medications in pandemic situation and for use in countries with limited resources. It may also signify a major breakthrough in the future management of all human influenza A induced cytokine dysregulation as these medications are directed against non-mutable determinants of the host common to the pathogenesis of all influenza A viruses.

Authors' contributions

KY Lai and GWY NG contributed to the conception and drafting of the manuscript. KY Lai, GWY Ng contributed to the conception

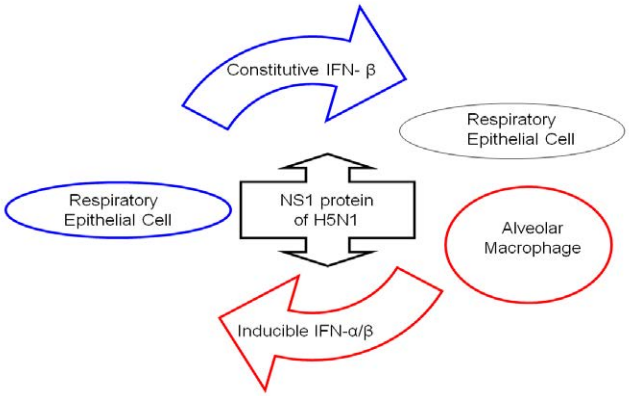

Figure 7: Schematic diagram showing the important complementary role of constitutive IFN-β and inducible type I interferon (IFN-α/β) during influenza infection: The respiratory epithelial cell has a basal secretion of constitutive IFN-β to maintain the alveolar macrophage in a primed state for timely production of IFN-α/β in response to influenza infection. The release of constitutive IFN-β by influenza infected respiratory epithelial cell allows the activation of interferon-related genes in the respiratory epithelial cell during the early phase of influenza infection. The inducible IFN-α/β released from infected alveolar macrophages initiates antiviral signaling, triggers apoptosis and limits influenza replication in the respiratory epithelial cells in the early phase of influenza infection. The NS1 protein of H5N1 isolated in humans after 2003 can inhibit release of constitutive IFN-β and production of inducible IFN-β at the pre-transcriptional level and IFN-α/β at the post-transcriptional level.

and writing of the manuscript. All authors have read and approved the manuscript. This paper is dedicated to Johnny Sze Wah LAU, Mei Yin HO and Fuk Hong LAI.

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