REVIEW

Pathogenesis of pandemic H1N1 2009 influenza virus infection and the implication on management

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Abstract The pandemic H1N1 2009 influenza virus has caused the first influenza pandemic of the 21st century, leading to disproportionate fatalities in the low-risk population despite the generally mild nature of the illness. Advances in science and technology have allowed very detailed study on the pathogenesis of this novel virus, and many have already been published in less than a year after the start of the pandemic. Information generated from cell lines, animal models, and clinical data analysis has provided us with greater understanding of the behavior of this virus and the associated host response. The new knowledge will allow us to formulate scientifically sound and evidence-based management plans.

Keywords influenza A virus, H1N1 subtype; pandemic; pathogenesis; virulence factors; immunity; management, antiviral

1 Introduction

The emergence of human cases of H5N1 influenza virus infection in 1997 [1] and severe acute respiratory syndrome (SARS) coronavirus infection in 2003 [2] has alerted the world of the devastating consequences of novel human respiratory viruses. In anticipation of a pandemic influenza, which may cause even greater morbidity and mortality worldwide, antiviral stockpiling and clinical trials for H5N1 vaccines were started in many developed countries [3]. As expected, the current pandemic H1N1 2009 influenza virus, which was first detected in March 2009, has spread worldwide [4,5]. Although most patients infected with this novel strain present with mild upper respiratory tract symptoms [6], the higher fatality rate in children and young adults clearly differentiate this virus from seasonal influenza virus [7,8]. Moreover, obese patients [9] and pregnant or postpartum women [10–13] are at increased risk of complications requiring intensive care or even death. These distinct clinical observations pose important scientific questions on our understanding of the differences in pathogenesis between this pandemic virus and the seasonal influenza virus. The marked advances in science and technology since our last pandemic in 1968 allow an unprecedented opportunity to do very detailed study on the pathogenesis of this infection. Results from these studies, which have important potential implications on the treatment of 2009 H1N1 virus, are reviewed and discussed in this paper.

2 Virus tropism and receptor binding

In contrast with the seasonal influenza virus, the 2009 H1N1 virus had greater predilection for the lower respiratory tract, as demonstrated by studies in ferrets and mice [14–16]. In fact, some patients may have the virus detected exclusively from the lower respiratory tract specimens [17]. This may be partially explained by the broader receptor binding properties. Binding of influenza virus to host cell surface glycoproteins or glycolipids is mediated by viral hemagglutinin (HA), and amino acid substitution in HA could affect the binding. In the 1918 H1N1 virus, both Asp190Glu and Asp225Gly (H3 numbering) of the HA altered the binding specificity to avian type receptor [18–20]. For H2 and H3 subtypes, avian to human adaptation can be achieved by substitutions at positions 226 and 228 [21]. By constructing theoretical HA-glycan structural complexes, it was found that Lys145, which is unique in the 2009 H1N1 virus, together with Lys133 and Lys222, may form a positively charged lysine fence at the base of the binding site and favor the binding to both α2-3 and α2-6 glycans [22]. In addition, Asp190, a residue critical in the binding to α2-6
glycans in the 1918 H1N1 virus, was postulated to be
stabilized by a unique set of residues Ser186, Thr187, and
Ala189. In human seasonal influenza virus, Asp190
substitution reduced the binding to α2-3 glycans, but in
the 2009 H1N1 virus, this interaction was rescued by the
lysine fence. This prediction was confirmed by carbohy-
drate microarray analysis, which showed that the 2009
H1N1 virus could also bind to the α2-3 sialic acid residue
[23], similar to H5N1 virus [24]. However, direct glycan
binding assay from another research group showed that the
HA of 2009 H1N1 virus could bind to α2-6 sialic acid
residue but minimally to α2-3 sialic acid residue [15]. This
apparent contradiction may be related to the selection of
different sialyl probes and the differences in binding
assays. The ability to bind α2-3 glycan is important since
this may be associated with the binding to lower
respiratory tract in human [25].

In vitro studies showed that the 2009 H1N1 virus could
replicate in human cell lines originating from extrapul-
monary tissues, including gastrointestinal, genitourinary,
neuromuscular, immune cells, and conjunctiva [26,27].
Viral load was greatest in colorectal adenocarcinoma
(Caco-2) and rhabdomyosarcoma (RD) cell lines, consist-
tent with the clinical observations of gastrointestinal
symptoms and rhabdomyolysis [28–31]. However, the
level of viral replication in cell lines was similar to
seasonal H1N1 virus except in the human conjunctiva,
where 2009 H1N1 virus had better replication [26]. The
broad viral tropism in cell lines is in accordance with the
detectable virus in extrapulmonary specimens, including
blood, urine, and stool from patients infected with 2009
H1N1 virus [32,33].

Since receptor binding is critical in the pathogenesis of
influenza virus infection, mutations at the binding site of
the HA may alter the virulence. An Asp222Gly (H1
numbering) substitution in HA of 2009 H1N1 virus was
found to be more common among fatal human cases [34].
Strains carrying Asp274Glu substitution in HA have also
been found in the United States, Spain, and Italy, but the
clinical data from these cases were not available [35].
Analysis using Informational Spectrum Method bioinfor-
matics platform has found that position 274 may be
associated with change in binding specificity. However, the
binding property of this mutant virus has not been
determined, and further studies are required to verify the
clinical significance of this substitution.

3 Viral virulence factors

Severe disease can be attributed to specific virulence
factors of the virus that may lead to excessive inflamma-
tion, as in the case of H5N1 and 1918 H1N1 viruses.
Known virulence factors in H5N1 virus include Lys627 of
PB2, which increases the viral replication; an Asn66Ser
substitution in PB1-F2, a protein that induces apoptosis,
enhances inflammation in mice, and predisposes to
secondary bacterial infection; NS1, which antagonizes the
antiviral effect of interferon; and a multibasic HA cleavage
sequence, which allows cleavage by ubiquitous proteases
[36]. For the 1918 H1N1 virus, a full-length PB1-F2 has
been postulated to be an important virulence factor [37].
However, these virulence factors are absent from the 2009
H1N1 virus: a glutamic acid is found at residue 627 of
PB2; PB1-F2 is truncated due to the presence of stop
codon at position 12; a deletion of a PDZ ligand domain is
found in NS1; and a single basic amino acid is found at the
HA cleavage site [36,38]. Even the introduction of known
virulence-determining mutations into PB2 of the 2009
H1N1 virus did not enhance virulence [39]. However, the
sequence analysis of the 2009 H1N1 virus PB2 genes has
identified Ser590 and Arg591, which is rare in seasonal
influenza [40]. In that study, it was demonstrated that the
paired substitution was associated with an increase in
polymerase activity and enhanced viral replication.

4 Viral load

The level of viral load at the pathophysiological focus of
infection represents the dynamic interaction between the
virus and host immunity. This level is the result of viral
replication, and its destruction by the host immune
response may be an important determinant of disease
severity. A higher viral load in respiratory tract samples
was associated with fatal disease from H5N1 [41] and with
disease severity in infection due to respiratory syncytial
virus and human metapneumovirus [42]. However, when
compared with seasonal influenza virus infection, the
nasopharyngeal viral load was slightly lower in patients
with 2009 H1N1 virus infection [32]. For severe cases of
2009 H1N1 virus infection, the initial viral load was found
to be similar to that of patients with mild disease, but there
was a slower decline in viral load [33]. Therefore, the
temporal rate of clearance of virus, rather than the initial
viral load, is an important determinant of severity. This is
in accordance with a study conducted in mice, which
showed that effective host innate immune response only
occurred after 48 h of infection, which is the time when
viral load started to decrease [43].

5 Pre-existing immunity

The reassortment between the triple-reassortant swine
influenza virus (which contains genes originated from
H1N1 avian virus, H3N2 seasonal human virus, and H1N1
classical swine virus) and the Eurasian “avian-like” swine
influenza virus (originated from H1N1 avian virus)
resulted in the 2009 H1N1 virus that is antigenically
distinct from recent seasonal influenza H1N1 viruses
[36,38,44]. Serological surveys have been conducted
worldwide to determine the level of pre-existing immunity in the population. In the United States, 34% of patients born before 1950, but only 4% born before 1980, had a microneutralization titer ≥160 [45]. In that study, it was determined that a microneutralization titer of 80–160 corresponded to a hemagglutination inhibition titer (HAI) of 40, which was associated with >50% reduction in the risk of infection [46]. In England, the level of cross-reactive antibody increased significantly with age, with 31.3% in those over 80 years old having a HAI of ≥32 [47]. Similar findings were reported from Finland, where 55.6% of people born before 1919 had HAI ≥40, but only present in 21.2% born between 1920 and 1930 and <1% in those born after 1940 [48]. The level of pre-existing cross-reactive antibody is much lower in studies conducted in Asia. In a serological survey performed in Guangxi, China, only 1.7% of serum samples had a HAI ≥40, and none were over 60 years old [49]. Vaccine studies showed that only 4% of participants aged 61 years or above had a HAI of ≥40 before vaccination [50,51]. In Japan, pre-existing neutralizing antibody was only present in those born before 1920 [14]. Most of these prevalence studies showed that the level of cross-reactive antibody tends to be higher in the older population. The higher rate of cross-reactive antibody in the older population was proposed to be due to cross reactivity generated from exposure to the 1918 H1N1 virus or the immediate descendants. Monoclonal antibodies generated against the HA sites Sa of the 1918 H1N1 virus had neutralizing activity against the 2009 H1N1 virus and reduced the replication of 2009 H1N1 virus in the lungs of mice [52]. Furthermore, antibodies generated by the 1976 swine origin H1N1 virus vaccine also protected mice infected with the 2009 H1N1 virus [53]. The lack of exposure to these viruses may partially explain why younger adults have higher clinical attack rate and more serious disease for the 2009 H1N1 influenza than seasonal influenza [54–56].

Despite the fact that severe disease disproportionately affected young adults, still, most infected patients in this age group had mild disease. Antibody levels cannot fully explain the situation, because protective immunity is dependent not only on the level of antibody but also on the T cell immunity. It has been found that 69% of CD8+ T cell epitopes and 41% of CD4+ T cell epitopes are conserved in the 2009 H1N1 virus when compared with seasonal influenza virus [57,58]. Furthermore, memory T cell response, as measured by interferon-γ secretion after stimulation by T cell epitopes, was present for both conserved and nonconserved epitopes. Similarly, a study using blood samples from donors with no history of 2009 H1N1 virus infection showed that the 2009 H1N1 virus peptides could induce specific T cell response [59]. These results are consistent with a previous study in human volunteers that demonstrated the importance of T cell immunity in the absence of cross reactive antibodies [60]. These studies have highlighted the role of T cell immunity, especially in patients without protective antibody. If the cell-mediated immune response, such as cytotoxic T lymphocyte, can be recruited early enough, the viral load can be controlled at a lower level at the early stage of the disease, and the viral load may drop faster, which would result in less cell death and cytokine activation. In addition to T cells, natural killer (NK) cells are also involved in the defense against influenza [61]. The major NK activating receptors involved in NK cell cytotoxicity, NKp46, was able to recognize the HA of 2009 H1N1 virus with subsequent direct killing [62]. This is in contrast with H5N1 virus, in which NKp46 could not elicit direct killing of the virus, and this may be one of the reasons why H5N1 virus is the most pathogenic influenza virus for human.

6 Cytokine/Chemokine response

Hypercytokinemia has been associated with the disease severity of several respiratory viruses, including SARS coronavirus [63] and H5N1 virus [41]. In vitro studies in human macrophages infected with 2009 H1N1 virus showed that the induced proinflammatory cytokine levels were similar to cells infected with seasonal influenza virus but much lower than those of H5N1 virus [64]. Weaker induction of interferons, CXCL10, and TNF-α was found in dendritic cells infected with 2009 H1N1 virus or seasonal influenza virus than those infected with mouse adapted H1N1 and H3N2 viruses [65]. Another study showed comparable cytokine response in bronchial epithelial cells and alveolar type I-like pneumocytes infected by 2009 H1N1 virus or seasonal H1N1 virus [26]. In a macaque model of 2009 H1N1 virus infection, there were persistently elevated levels of monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP-1α), IL-6, and IL-18 in the inflammatory lungs [14]. IFN-γ was overexpressed in the lung of mouse model coinfected with influenza virus and Staphylococcus aureus and in the lung tissue of fatal 2009 H1N1 human cases [66,67]. For patients with 2009 H1N1 virus infection, levels of proinflammatory cytokines or chemokines were higher in patients with most severe disease who died or developed acute respiratory distress syndrome than in those with disease of moderate severity or mild symptoms. Granulocyte colony-stimulating factor (G-CSF), MCP-1, TNF-α, IL-1α, IL-6, IL-10, and IL-15 levels were higher in patients with most severe disease than those with moderate or mild severity during the initial three days after symptom onset, with IL-6, IL-10, and IL-15 levels persistently elevated through the course of illness [33]. On the other hand, IL-17 levels were found to be lower in the more severe groups for samples collected within three days of symptom onset but did not differ significantly if samples were collected later than four days after symptom onset. In another study comparing single serum samples collected at a median of five days after
symptom onset, highest levels of IL-6, IL-15, and IL-12p70 were found in the most severe cases, and IL-17 level was noted to be similar between the critical cases and noncritical hospitalized cases but higher than in outpatients or controls [68]. High levels of proinflammatory cytokines may be mediated by a sustained elevation of Toll-like receptor 3 (TLR-3), as seen in human postmortem lung tissues [67]. Taken together, immunodysregulation is a key factor in patients with severe disease, but the degree of cytokine activation may be much less than the more pathogenic avian H5N1 virus.

7 Host factors

The severity of 2009 H1N1 virus infection can range from asymptomatic infection to fatal disease. Host genetic makeup has been proposed to be one of the determinants. Since different strains of inbred mice exhibited variable susceptibility to infection by seasonal influenza virus [69], understanding the host determinants of influenza viral replication can facilitate the development of anti-influenza treatment directed at host factors instead of viral targets. Using yeast two-hybrid analysis and genome wide expression profiling, 616 human factors have been proposed to be involved in viral-host interaction for seasonal influenza virus, which can be broadly divided into those that are involved in viral replication and those involved in the regulation of IFNβ [70]. Another study has identified interferon-inducible transmembrane proteins to be critical for the antiviral action of interferon type I and II in seasonal influenza viruses [71]. Using genome-wide RNA interference screen, many host factors were shown to be associated with the replication of 2009 H1N1 virus [72,73]. The significance of certain host factors has been confirmed by either knockdown mutants that could not produce the specific host factor or by the inhibition of the factor using small molecule inhibitors. These proteins included SON DNA binding protein (trafficking of influenza virons during early infection), CDC-like kinase 1 (splicing of viral M2 messenger RNA), and p27 (required during late stage of viral replication) [72]. In another study, the inhibition of gene expression using siRNA confirmed the importance of 12 genes in viral replication of 2009 H1N1 virus, which included those involved in viral entry (CD81, MID1P1, ARCN1, ATP6V0C, MAP2K3, FGFR4, GSK3B, and CSE1L), postviral entry steps including transcriptional regulation (CAMK2B) and HA cleavage (PRSS35), and nuclear trafficking (CSE1L, PRSS35, and CAMK2B). The mechanism of inhibition on viral cycle is unknown for SUMO2 and GABBR1 [73].

An Australian study demonstrated the association between immunoglobulin G2 (IgG2) deficiency and severe disease [74]. In that study, patients with severe H1N1 infection, as defined by admission to intensive care unit (ICU) for respiratory and/or vasopressor support, had significantly lower levels of IgG2 than patients with disease of moderate severity. Most of these patients also had low levels of IgG2 in their convalescent sera. Since the half-life of IgG2 is three weeks [75], the authors suggested that these patients were likely to have underlying IgG2 deficiency rather than a low IgG2 level that was precipitated by the 2009 H1N1 virus infection, and patients with underlying IgG2 deficiency may not be able to mount early innate immune response to influenza, thus being predisposed to severe disease.

8 Pathology

Most fatal cases of 2009 H1N1 virus infection suffered from respiratory failure [76], and lung pathologies represented the most prominent findings in autopsy studies. The main pathological features in the lung included diffuse alveolar damage (DAD), necrotizing bronchiolitis, and DAD with intense alveolar hemorrhage [33,67,77], which were similar to those reported for fatal H5N1 cases and previous pandemics [1,78]. Pulmonary artery microthrombi were present in 15/55 (27.2%) of cases from the two series. This phenomenon has been described in patients with seasonal influenza, and one postulation was that the thrombosis may have been triggered by the transient appearance of anticardiolipin antibody [79]. In addition to the lower respiratory tract, tracheitis was also found.

Evidence of secondary bacterial infection was seen in up to 55% of postmortem cases [80]. Bacterial pathogens included Streptococcus pneumoniae, Staphylococcus aureus, Streptococcus pyogenes, Haemophilus influenzae, and Streplococcus mitis [81]. The coinfection by community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) has led to a fatal disease in a 42 year-old immunocompetent man [82]. In this patient, there were areas with typical changes of bacterial pneumonia, namely, patchy consolidation with polymorphs inside alveolar cells. In other areas, there were features typical of viral pneumonitis, which were alveolar spaces with necrotic debris and detached pneumocytes or atypical pneumocytes with mildly enlarged nuclei and granular to vacuolated cytoplasm. Influenza virus infection may predispose to bacterial infection by destruction of the physical barrier that exposes potential binding sites for the bacteria, impaired mucociliary clearance, immunodysregulation, upregulation of receptors, and expression of bacterial genes that encode toxin [83]. Conversely, S. aureus specific protease, which cleaves influenza HA, may increase viral replication [84].

Extrapulmonary pathology was prominent in some patients [33]. Lymphoid aggregate and myofibril necrosis of the myocardium, typical features of viral myocarditis, were found. In one case series, reversible cardiac dysfunction occurred in 4.9% of 2009 H1N1 virus
infection [85], and these cases may have myocarditis. Hemophagocytosis, a frequent histopathological finding in patients with severe infection by intracellular pathogens, was evident in lymph nodes of some cases [86]. Splenic vein thrombosis was found in a patient who has suffered from multiorgan failure. Hepatic necrosis was found in the autopsy of a pregnant woman [67].

9 Implications on treatment

Viral load studies informed us that the speed of viral clearance, but not the initial viral load, was important in predicting severity. Antiviral drugs have been considered to be a cornerstone in the treatment of influenza [87]. The current strain of pandemic influenza is universally resistant to amantadine or rimantadine, due to Ser31Asn mutation in the M2 gene [88]. Most remain sensitive to neuraminidase inhibitors, including oseltamivir, zanamivir, and peramivir. In retrospective studies, delayed treatment with oseltamivir was associated with ICU admission or death [12,89]. This may be explained by a faster viral load reduction in patients who have received oseltamivir within two days of symptom onset [90]. However, the reduction in viral load was slow, with more than 50% of patients still having detectable virus in nasopharyngeal aspirate after five days of treatment regardless of the timing of starting oseltamivir. Furthermore, oseltamivir-resistant strains have emerged worldwide [91–97]. As of 3 February 2010, there have been 225 oseltamivir-resistant strains reported to the World Health Organization [97]. All of these strains contained the His275Tyr mutation. Most of these were unrelated, but clusters have been found in the community and in the hospital [97,98]. Oseltamivir-resistant strains may exist as quasi-species, which may hinder the detection of the resistant strains [93]. Oseltamivir resistance may also emerge in patients taking oseltamivir as postexposure prophylaxis [99]. Treatment with intravenous zanamivir has been successful in a 10-year-old girl with acute lymphoblastic leukemia and another 22 year-old woman with Hodgkin’s disease and infected with an oseltamivir-resistant strain [100,101], but the virus was still detectable 5 and 13 days after intravenous zanamivir, respectively. Furthermore, one of the two cases was started on high doses of steroid concomitantly. Thus, the cause of the improvement is still uncertain. Although zanamivir resistance has not been detected in the current pandemic strain, it has been reported in seasonal influenza A virus [102]. Intravenous peramivir is also available, but the IC50 for oseltamivir-resistant strains is high and unlikely to be useful in oseltamivir-resistant cases [103]. In addition to neuraminidase inhibitors, other classes of antiviral agents against influenza are developing, including adamantane derivatives, inosine monophosphate (IMP) dehydrogenase inhibitors, RNA polymerase inhibitors, and siRNAs [104]. In addition to drugs that target the virus directly, drugs that alter host factors are also developed. In vitro studies have shown that DAS181, a sialidase fusion protein which removes cell-surface sialic acid residues from respiratory epithelia, has potent antiviral effect on 2009 H1N1 virus, including oseltamivir-resistant strains [105]. It has been shown that standardized extracts from Echinacea purpurea has activity against the 2009 H1N1 virus, including oseltamivir-resistant strains, but the mechanism of action is unclear [106]. More effective antiviral drugs will be urgently needed to decrease the viral burden and hence may improve the prognosis in patients with severe disease.

As the virus can be found in extrapulmonary tissues, optimal antiviral agents should be able to achieve high concentrations in these tissues. Oral oseltamivir has good absorption, but it cannot be used in patients who cannot tolerate oral feeding or in those who have poor gastrointestinal absorption. Although zanamivir has good antiviral activity, even against oseltamivir-resistant strains, nebulized, or inhaled route would achieve very low systemic concentration. Therefore, the only currently available options are the parenteral forms of zanamivir or peramivir. Unfortunately, resistance in seasonal H1N1 virus was reported even before these parenteral agents were marketed [101]. It is important to remember that early treatment of mild cases by antiviral drugs would decrease viral load more rapidly with earlier resolution of symptoms by one more day, but whether early treatment of those with risk factors for severe disease will prevent progression and death is uncertain. Since the viral load has a plateau by the time of presentation for most severe cases, the efficacy of these parenteral antiviral drugs remains to be determined in formal clinical trials of patients with severe disease.

Apart from antiviral treatment, antibacterial coverage is also necessary because of the high prevalence of bacterial coinfection. Empirical antibiotics should be able to cover potential bacterial pathogens, especially S. aureus, including CA-MRSA, S. pneumoniae, and S. pyogenes. Diagnostic tests for bacterial copathogens should be employed to guide antibiotics therapy [87].

As persistent cytokine activation is evident in patients with severe disease, strategies to damp down the deleterious inflammatory response are required. Pooled immunoglobulin in the form of convalescent plasma or hyperimmune globulin may serve both antiviral and immunomodulatory function. In a meta-analysis of the use of convalescent plasma in the 1918 influenza pandemics, there was lower mortality rate in the convalescent plasma group [107]. Convalescent plasma was also shown to be effective in a patient with severe H5N1 infection [108]. However, the effectiveness in 2009 H1N1 virus infection has not been reported. Pooled immunoglobulin was also successful in two patients with severe disease and IgG2 deficiency [74]. A study with a mouse model showed that monoclonal antibody was more effective than oseltamivir in the treatment of H5N1 and
seasonal H1N1 virus infection [109]. Hyperimmune globulin may be a useful strategy. In a mathematical model, it has been shown that passive immunotherapy is a feasible option even if there is a low percentage of donors [110]. Corticosteroid has been a matter of debate. A study involving patients in the ICU has reported success using corticosteroid [111]. It has been suggested that a low dose of steroid is helpful in severe cases to compensate for relative adrenal insufficiency [112]. The addition of celecoxib and mesalazine to antiviral drug has improved survival in mice [113]. Other potential immunomodulators include fribates and statins [114]. However, these agents have not been tested in the setting of pandemic influenza and await further studies to establish the efficacy.

With the advance in medical science and technology, knowledge regarding the 2009 H1N1 virus has expanded at an unprecedented pace. Further characterization of viral and host determinants of severe disease would be important. The understanding of the pathogenesis will form the basis for formulating scientifically sound strategies in clinical management.

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