Virulence of the H1N1 2009 Pandemic Influenza Virus

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Siriraj Med J 2010;62:60-63

E-journal: http://www.sirirajmedj.com

Severity of the H1N1 2009 pandemic influenza

At the beginning of the pandemic, the outbreak in Mexico was reported to be rather severe with a large number of severe cases.[1] The case fatality ratio (CFR) was initially reported to be over 1% on average and as high as 6% in the elderly, which is considerably higher than that of seasonal influenza.[2] These figures were, however, calculated using confirmed cases as denominators, which caused an overestimation of CFR. Studies elsewhere conducted later in the course of the pandemic using various methods to estimate the total number of cases showed CFR of as low as 0.05 / 1,000.[3] It is still not clear whether the virus in the initial outbreak was more virulent than those spread to other regions or whether the discrepancy in the estimated CRF was purely due to differences in the methods for total cases calculation. Nevertheless, it is clear that the H1N1 2009 pandemic is a mild one. The disease burden calculated as DALY was found to be 35/100,000, which is not much higher than that of seasonal influenza.[4]

Despite being regarded as a mild pandemic, the H1N1 influenza can cause unusually severe diseases with can rapid progress to respiratory failure and death. Comparative studies in the same outbreak season showed that patients infected with the pandemic H1N1 virus were more likely to develop severe disease and die when compared to patients infected with seasonal influenza virus.[5,6] Therefore, the H1N1 pandemic influenza can be generally viewed as a mild virus and does not represent a Spanish flu-like highly pathogenic virus, it is not totally mild and has an elevated level of virulence as compared to seasonal influenza viruses.

Pathological studies showed that the H1N1 pandemic virus infects alveolar epithelial cells in fatal cases with ARDS (acute respiratory distress syndrome).[7] This is markedly different from pathological findings seen in seasonal influenza cases, where viral infection is mostly detected in airways. The infection of H1N1 pandemic virus in alveolar epithelial cells resembles the infection by H1N1 1918 Spanish flu and H5N1 highly pathogenic avian influenza virus.[8]

The enhanced virulence of the H1N1 pandemic virus and its ability to infect alveolar epithelial cells was also supported by animal models. Experimental infection in mice, ferrets, and macaques showed that the virus was more virulent and caused higher fatality as compared to seasonal influenza virus.[9-11] It was also shown that while seasonal influenza virus infected only the upper airways and bronchi, the pandemic virus could infect the airways and also alveolar epithelia.[9-11] These together indicate that the H1N1 pandemic influenza virus may be more virulent partly at least by infecting the lungs more efficiently.

Virological markers of virulence

There are several genetic determinants of virulence in influenza virus genome, many of which are strain specific. One of the most effective genetic determinants is the cleavage site of hemagglutinin precursor (HA) 0, which is digested by host protease to produce 2 HA proteins (HA1 and HA2). For low pathogenic influenza strains this cleavage site is recognized and cleaved by trypsin-like enzymes, which are available only in respiratory and digestive tracts. For some highly pathogenic influenza strains, there is an insertion of multiple positively charged amino acids in the cleavage site, which makes it cleavable by furin, which is ubiquitous in all cell types.[12] This cleavage site makes it possible for the virus to replicate outside respiratory and digestive tracts and cause disseminated infection.

Avian and human influenza viruses are known to be different in their receptor usage preference. While avian influenza viruses prefer α2,3-linked sialic acid, seasonal influenza viruses prefer α2,6-linked sialic acid. In human airways, the human-type receptor (α2,6-linked sialic acid) is expressed in the upper airway, whereas the avian-type receptor (α2,3-linked sialic acid) is found mainly in alveolar epithelial cells and terminal bronchioles.[14] This explains why the H5N1 highly pathogenic avian influenza virus causes severe pneumonia with little upper airway symptoms. The ability to target the lower airway is considered an important virulence factor.

Both 1918 pandemic influenza virus and highly pathogenic H5N1 virus were shown to induce inappropriately high levels of pro-inflammatory cytokine production in vitro[14-17] Kobasa, 2007 #30}. The ability to hyperinduce cytokines and chemokines is probably responsible for severe inflammation and tissue damage. However, we have recently shown that the cytokine hyperinduction property

was not always necessary for severe disease because some H5N1 isolates from severe cases failed to induce high levels of cytokine in vitro.[18]

Some other virulence markers were identified by comparing strains with different virulence in experimental animals, studying in vitro viral gene function, or by large scale viral sequence analyses. These virulence markers include NS1 mutation, PB1-F2 gene, and PDZ domain at the C-terminal of NS1.[19-24]

None of the above mentioned virulence markers is present in the H1N1 pandemic virus. The virus carries a usual trypsin-dependent HA cleavage site, lacks a PB1-F2 gene, and doesn't have a PDZ domain in NS1. However, it was shown to induce higher levels of pro-inflammatory cytokines as compared to seasonal influenza viruses.[25,26] It preferentially binds to α2,6-linked sialic acid, although a low level of binding to α2,3-linked sialic acid was also reported.[27]

Virulence determinants of H1N1 pandemic strains have been studied in animal models by comparing strains and variants. Some mutations have been found to be associated with higher virulence in animals.[28,29] Most of these mutations are located in HA and PB2 genes, which may enhance receptor binding and the viral polymerase activity, respectively.[30] An aspartate to glycine mutation at position 222 of HA (D222G) was shown by many reports to enhance the viral virulence in mice and confer a dual-specific receptor binding property. The mutation was also found to be associated with severe disease in humans.[28,29]

Interspecies transmission, evolution, and virulence

It is a common phenomenon that a virus shows increased virulence in a new host species after an inter-species transmission. Many highly virulence emerging viruses, like SARS coronavirus and Nipah virus, are zoonosis, which cause mild or no disease in their natural host. Because causing severe disease actually reduces the probability of transmission, having mild or asymptomatic infection benefits viruses and adaptive evolution would select for variants with less virulence. Selective pressure also works from the host side. The endemicity of a virus selects for host individuals with the ability to survive the infection. This provides a positive selection for individuals with genetic resistance to the infection. This viral-host co-adaptation usually results in a wide-spread transmission of low virulence viruses. This co-existence usually involves a delicate balance in viral replication and host defense. In a new host species, the balance cannot be established, and infection usually results in either of the two extremes: inefficient (abortive) infection or highly effective infection causing severe damages.

Influenza viruses are avian viruses in origin, and all human influenza viruses were once zoonosis. The 1918 pandemic influenza virus was highly virulent when it started the pandemic shortly after entering the human population.[31] The level of virulence was probably comparable to that of the H5N1 highly pathogenic avian influenza virus. As the 1918 pandemic expanded to Asia, the virulence gradually declined.[32] This suggested that the positive selective pressure for low virulence was strong and the viral adaptation to lower its virulence in the human population could occur relatively quickly in the course of the pandemic. The H2N2 and H3N2 pandemics were much less severe. This is likely because these two viruses were reassortants between the H1N1 human virus and some avian viruses,[33,34] whereas the 1918 H1N1 virus entered the human population as a totally new virus without any genetic component from a previous human virus. Having some genetic components from the H1N1 virus, which had circulated in the human population for decades, probably made the H2N2 and H3N2 viruses less virulent in humans. This is also the case for the current H1N1 pandemic virus. The virus is a reassortant with genetic components from multiple origins, including human seasonal influenza viruses.[9] It is also likely that the virus will become less virulent as the outbreak proceeds and will eventually have an optimal virulence for efficient transmission comparable to those of seasonal influenza viruses. This assumption is supported by the finding that strains of this pandemic virus showed heterogenous virulence in macaques.[35]

Possible mechanisms of the increased virulence

Because the H1N1 pandemic virus is new in the human population, most people do not have preexisting immunity against the virus, although some cross-reactivity has been reported especially in older age groups.[9] The lack of preexisting immunity may be, at least partly, be responsible for the severity. In seasonal influenza infection, most patients have preexisting cross-reactive antibodies to variable epitopes of HA or cellular responses targeting conserved internal epitopes. These immune responses provide partial suppression of viral replication and can prevent severe disease.

As mentioned above, the H1N1 pandemic virus lacks most of the known virulence markers. Although some mutations have been identified to be associated with higher virulence in mice, the underlying mechanism for the increased virulence is not clear. The D222G was found to be associated with severe disease in some patients.[28,36] The mutation probably facilitates infection in the lungs because it confers binding to α2,3-linked sialic acid, which is expressed on alveolar epithelial cells.[28] However, viruses from severe cases do not always carry this mutation, although variation at a quasi-species level cannot be ruled out.

It was recently reported that the pandemic H1N1 virus was less sensitive to surfactant protein D (SPD), which is a member of the collectin subgroup of the C-type lectin superfamily.[37,38] SPD plays important roles in innate defense against several bacteria and viruses, control of inflammatory responses, and surfactant function of the lung. SPD has been previously shown to bind influenza HA and inhibit infection by using its lectin function binding to N-linked glycan on HA. Certain N-linked glycosylation sites of HA (position 144 and 172 for H1N1 viruses; and 122, 133, and 144 for H3N2 viruses) were shown to be required for binding and inhibition by SPD.[39,40] It was also shown that these N-linked glycosylation sites accumulated over decades of circulation in the human population.[40] It is believed that the glycosylation helped the viruses to escape from antibody response by shielding their HA epitopes. After circulating in the human population for many decades, seasonal influenza viruses adapted themselves by gaining glycosylation sites and the ability to evade antibody response, and at the same time losing the ability to replicate in the lungs by gaining sensitivity to SPD. The H1N1 pandemic virus, being new in the human population, lacks these glycosylation sites, and thus is not sensitive to SPD. Resistance to SPD was shown to be associated with enhanced virulence in wild type but not in SPD-deficient mice. The resistance to SPD of the H1N1 pandemic virus is therefore likely to play an important role in its virulence and lung invasiveness.

It was also reported that the H1N1 pandemic virus was resistant to the γ inhibitor in the human sera.[41] The presence of influenza inhibitors in human and animal sera has been recognized since over five decades ago. These inhibitors bind to the viral HA and exhibit hemagglutination inhibition and/or viral neutralization activities. They were classified into α, β, and γ inhibitors according to their physical and chemical characteristics. While β inhibitors are lectins that bind glycan on HA molecules, α and β inhibitors are sialic acid-containing glycoproteins, which bind HA via their sialic acid, and are sensitive to sialidase. [42-44] These inhibitors were originally recognized as non-specific inhibitors interfering with antibody assays. It is however possible that they may play an important role in limiting the extent of infection and preventing systemic dissemination. Resistance to these inhibitors may therefore be an important factor in the virulence of the H1N1 pandemic virus.

Innate antiviral defense via soluble inhibitors may play an important role in the outcome of influenza infection, and their defect may contribute to severe disease. Better understanding of their mechanisms may provide another therapeutic approach. We have recently shown that lungs from H5N1 victims had low levels of SPD. [45] Down-regulation of SPD was likely due to apoptotic death of type II alveolar epithelial cells, which are the main production source of surfactant proteins.[46] Recently, there was a case report of severe H1N1 influenza in a child who was treated with surfactant replacement and recovered well after the treatment.[47]

CONCLUSION

Although the H1N1 influenza pandemic is generally considered as a mild pandemic and the virus is not as virulent as the 1918 H1N1 pandemic virus, cases with unusual severity have frequently been seen. The H1N1 pandemic virus is likely to be somewhat more virulent than seasonal viruses. The mechanism for its higher virulence is not yet clear. While the role of the lack of preexisting immunity cannot be ruled out, the virus showed less sensitivity to soluble innate antiviral factors in the blood and respiratory secretion. The resistance of these anti-viral factors may at least be in part responsible for the elevated virulence.

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