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COMMENTARY

Why Does Pandemic Influenza Virus Kill?

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The most terrifying aspect of pandemic influenza A infection is its ability to cause respiratory failure and death in young adults. There is a major debate in the literature regarding whether such severe outcomes result from more robust viral infection in the lung and associated cellular injury or from lung injury caused by a pathogenic immune response to some extent uncoupled from viral replication (eg, cytokine storm). The article by Gao et al[1] in this issue makes useful contributions in this regard. The authors made use of an important resource of lung tissue obtained from 48 subjects who died of infection with the 2009 H1N1 pandemic virus (2009 pH1N1). Although the pandemic did not result in such massive death and morbidity as was caused by the 1918 H1N1 pandemic, it was clearly associated with a disturbing incidence of pneumonia and respiratory death in young adults.[2] In the group reported by Gao et al,[1] the mean age was 37.33 years. As in other influenza A pandemics and seasonal epidemics,[3] a significant proportion of the deaths in 2009 were associated with bacterial superinfection (28%). There was a relatively high incidence (68%) of underlying medical conditions in this cohort of patients, including factors known to predispose to more severe influenza infection, such as asthma, obesity, pregnancy, or immunosuppression.

An important finding in these fatal cases was the demonstration of protracted viral replication in lung tissue. Notably, the viral load was positively correlated with duration of illness and with elevated levels of some key proinflammatory cytokines in the lung (ie, MIP-1β, MCP-1, IP-10, and RANTES). Other proinflammatory cytokines were also elevated, although these were not correlated with viral load. Of interest, however, was the significantly greater elevation of IL-6 and TNF-α in patients who had bacterial superinfection. Other researchers have shown an association of severe outcomes with 2009 pH1N1 in humans with high serum levels of IL-6,[4] although these studies did not assess the association of viral load or bacterial superinfection with cytokine induction. The association between viral replication and expression of proinflammatory cytokines was further substantiated by pathological analysis showing that viral antigen and cytokine expression occurred in the same areas of the lung.

An additional important finding of the Gao et al[1] study is a marked elevation of Fas ligand mRNA and a decrease in Fas antigen mRNA in patient lungs, compared with controls. Thus, there was an imbalance between the levels of Fas ligand and Fas antigen. The Fas ligand levels also positively correlated with viral load. Pathological studies confirmed the presence of extensive apoptosis in areas of viral infection. Overall, the findings of Gao et al[1] support the concept of linkage among sustained viral infection, immune response, and cellular damage.

Intrinsic Differences in Pathogenicity between Pandemic and Seasonal Influenza A Strains

Seasonal influenza A epidemics are also associated with mortality, which can be substantial if the current vaccine does not closely match the prevalent viral strain. Seasonal epidemics cause mortality predominantly in the elderly and very young. So what makes pandemic influenza different, and why does it cause more mortality in young adults? One key factor separating pandemic and seasonal influenza A is the greater intrinsic pathogenicity of pandemic strains. The 1918 H1N1 is particularly notable in this regard; it is highly lethal in a variety of animal models, because of increased replication and profound inflammatory pathology in the lung.[5] Similarly, 2009 pH1N1 causes increased weight loss and mortality in ferrets, compared with recent seasonal H1N1 strains.[6] The ferret model is frequently used in influenza studies because it appears to mirror human infection more

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closely than do mouse models. It should be noted that the increased pathogenicity of 2009 pH1N1 is less profound than that of the 1918 virus, consistent with the far greater death toll caused by the 1918 virus.

Reverse genetics is a powerful virological technique now extensively used in influenza studies. This technique allows construction of novel viruses from plasmids containing intact or mutated versions of the eight genome segments of influenza A viruses. With this method, it has been possible to specifically identify the role of the viral hemagglutinin (HA) or other viral genes in influenza pathogenesis. To some extent, the immune pathology and increased viral replication of the 1918 virus can be dissociated using a reverse genetics approach.[5] The inflammatory pathology and mortality relate largely to properties of the 1918 HA molecule, whereas the increased viral replication involves the distinct properties of the polymerase complex. Similarly, HA of other pandemic viruses [eg, 1957 (H2), 1968 (H3), or 2009 (H1)] directly contributes to the greater illness and pathology of these viruses in mice, compared with seasonal influenza.[7]

Glycosylation of HA appears to be an important determinant of pathogenicity. Viruses containing just the HA of the pandemic strains combined with gene segments of seasonal H1N1 are resistant to inhibition by surfactant protein D, because of minimal glycosylation of the head region of HA.[7,8] Surfactant protein D plays an important role in initial containment of influenza.[9] Other innate immune inhibitors are also inactive against pandemic H1N1.[8] Thus, the ability of pandemic influenza to bypass components of the innate response could contribute to pathogenesis. Other properties of 2009 pH1N1 also contribute to greater pathogenicity, compared with seasonal H1N1 (eg, the ability to suppress activation of retinoid-inducible gene 1 protein and consequently interferon responses).[10]

Differences in Host Response to Pandemic Influenza A Strains

Differences in host responsiveness to influenza virus likely also account for differences in disease severity. Newly weaned ferrets were shown to have less disease severity than adult animals infected with pandemic H1N1 of 2009.[11] In this case, viral replication did not differ between the young and adult animals, but the young exhibited reduced disease severity and pulmonary pathology, because of more robust interferon and IL-10 responses. This might account for the observation of relatively less severe clinical symptoms among pediatric patients during the 2009 pandemic.[11] In contrast, pregnant women were particularly vulnerable during the 2009 H1N1 pandemic. A pregnant mouse model demonstrated increased severity of infection with 2009 pH1N1 and linked the increased severity to pathogenic immune responses and not to increased viral replication.[12] Pregnant women were also shown to have reduced interferon responses during the 2009 pH1N1 pandemic.[13] These two findings are not necessarily incompatible, because failure of the initial type 1 interferon response could result in more damaging secondary innate responses.[14] A useful paradigm in evaluating the innate response to influenza A virus is that there are initial defense factors that may be able to contain or down-regulate infection at the outset. These would include interferons produced by airway epithelial cells and soluble inhibitors such as surfactant proteins or anti-microbial peptides. If the virus succeeds in bypassing these constraints, then a more costly inflammatory response must occur to contain the virus.

Differences in Prior H1N1 Exposure among Elderly and Young Adults

Another key factor in determining disease severity in different age cohorts is the history of prior exposure to related viral strains. For instance, elderly people were less affected during the 1918 and 2009 pandemics, probably because of retention of cross-reactive neutralizing antibodies from remote H1N1 exposure.[15] It does not appear that 2009 pH1N1 was intrinsically less pathogenic in the elderly. Elderly macaques had both increased viral load and more severe innate immune pathology in response to this virus.[16] Based on mouse models,[17] a compelling hypothesis has been advanced that increased illness in young adults infected with 2009 pH1N1 relates to lack of adequate neutralizing antibody protection, despite a robust cell-mediated response. According to this hypothesis, young adults exposed only to the highly glycosylated HA of seasonal H1N1 strains from 1977 to 2008 had poor antibody protection against the less glycosylated 2009 pH1N1. In contrast, the cell-mediated response to influenza is determined by conserved internal viral proteins, such as polymerase and nucleoproteins. Thus, the same subjects had robust cell-mediated response to the pandemic strain, perhaps contributing to lung injury. Older subjects who retained antibody responses to H1N1 strains from 1918 through 1957 (when H1N1 strains were replaced by H2N2) were able to effectively neutralize the 2009 strain.

Conclusions

Clearly, the innate and adaptive immune responses to influenza A virus are highly complex. It is extraordinary that an apparently genetically limited virus has so many effects on the human population and on human immune responses. One obvious factor in the success of influenza A viruses is the existence of animal reservoirs, which allow for exchange of whole genome segments between human and animal strains. In addition, the virus appears to make maximum use of its limited genomic repertoire. Recently, it has become clear that alternative reading frames allow the virus to encode additional proteins that modulate the host response,[18] such that there are now 13 defined gene products for influenza. In addition, the viral proteins interact with an extraordinary number of host proteins, leading to pleomorphic effects on the host

response.[19] One need only look at the viral NS1 protein, which suppresses innate antiviral responses on many levels, including inhibition of type 1 interferon production, inhibition of action of interferon-related genes (eg, protein kinase R and oligoadenylate synthetase), and inhibition of PI-3 kinase and RIG-1 activation (among many other activities).[20]

It is obvious from this cursory review of the massive literature on influenza A virus infection that many factors are involved in determining pandemic influenza severity, and that animal model systems support a role both for increased replication potential of these viruses and for increased ability to trigger potentially pathogenic immune responses. The findings of Gao et al[1] cannot address the issue of which of these two aspects is more responsible for pandemic influenza A—associated mortality. Their study does, however, provide valuable human data and overall lends support to the contention that proinflammatory cytokine generation and lung injury are closely linked to sustained viral replication in the lungs of fatal cases. From their findings, it would seem that efforts to improve vaccine delivery and effectiveness and antiviral therapies should continue to take precedence over inhibition of pathogenic immune responses. Increasing knowledge of the contributions of specific viral genes to pathogenesis should allow for novel antiviral strategies.

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