CLINICAL IMPLICATIONS OF BASIC RESEARCH

The Genetic Archaeology of Influenza

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Over the past century, three influenza pandemics occurred because of the emergence of novel influenzaviruses to which little or no immunity existed. In 1918 and 1919, the "Spanish" influenza pandemic killed more than 20 million people, with many of the deaths due to an unusually severe, hemorrhagic pneumonia. Now, Kobasa and colleagues[1] have used modern molecular methods to show that the hemagglutinin antigen from this strain (HA^{sp}) is a key determinant of virulence.

Using reverse genetics, Kobasa et al.[1] synthesized the *HA*^{sp} and neuraminidase (*NA*^{sp}) genes on the basis of the genetic sequences of the 1918–1919 influenza[2] strain and constructed influenzaviruses using one or both of these genes (Fig. 1). The resulting viruses that expressed the HA^{sp} protein were significantly more virulent than the wild-type strains in a mouse model, regardless of the neuraminidase antigenic subtype. These viruses were also more pathogenic, not simply because they were associated with increased levels of in vivo replication but also because they stimulated massive increases in the responses of inflammatory cytokines in the lungs of infected mice. The mice infected with HA^{sp}-containing virus had increased recruitment of leukocytes to the sites of lung infection and had severe hemorrhage resembling the hemorrhagic pneumonia associated with human infections during the 1918–1919 pandemic. Kobasa et al. went on to show that people born after 1920 have little or no serum-neutralizing activity against viruses expressing HA^{sp}.

This new study has important clinical and epidemiologic implications. Assuming that the mouse model at least partially reflects the important factors in the virulence of influenza in humans, further dissection of the HA^{sp} molecule is warranted to help identify the critical structural motifs that confer enhanced virulence. This can be accomplished by performing site-directed mutational analyses of the *HA*^{sp} gene and investigating the effects of these mutations on infection in the mouse model. The identification of these motifs may provide a new epidemiologic tool for surveillance of circulating animal and human influenzaviruses that could be used to predict the emergence of a new, highly virulent pandemic strain. In addition, these detailed molecular studies could facilitate the identification of antigenic epitopes to include in vaccines in order to protect people against related pandemic strains.

The study by Kobasa et al. also suggests potential strategies for the treatment of patients infected with highly virulent strains of influenzavirus. In the mouse model, infection with influenza strains that expressed HA^{sp} was associated with massive induction of inflammation, including increased chemokine and cytokine responses, and an increased influx of neutrophils into the lungs (Fig. 1B and 1C). These events were associated with more severe pathological features and higher mortality, suggesting that overactive host immune responses stimulated by the HA^{sp} molecule may trigger severe disease.

The epidemiologic features of fatal cases of influenza during the 1918–1919 pandemic support this hypothesis.[3] Death rates were highest among young-to-middle-aged adults, with lower rates among both adolescents and the elderly. In contrast, the rates of death associated with infections caused by interpandemic influenza strains increase with age, peaking in the oldest age groups because of immunologic senescence and underlying frailty in the elderly. Because of their preserved immune function and previous exposures to unrelated influenza strains, the young-to-middle-aged adults in the 1918–1919 pandemic may have been more prone to severe disease, owing to overexuberant inflammation triggered by pandemic strains. Therefore, immunomodulatory therapies designed to minimize potent inflammatory responses may be appropriate as adjunctive treatments for people infected with influenza strains expressing hemagglutinin antigen molecules with activities similar to those of HA^{sp}. The mouse model may offer a useful means of exploring the potential benefits of other antiinflammatory adjunctive treatments.

Finally, the results of Kobasa et al. show that influenza-specific memory immunity can persist and

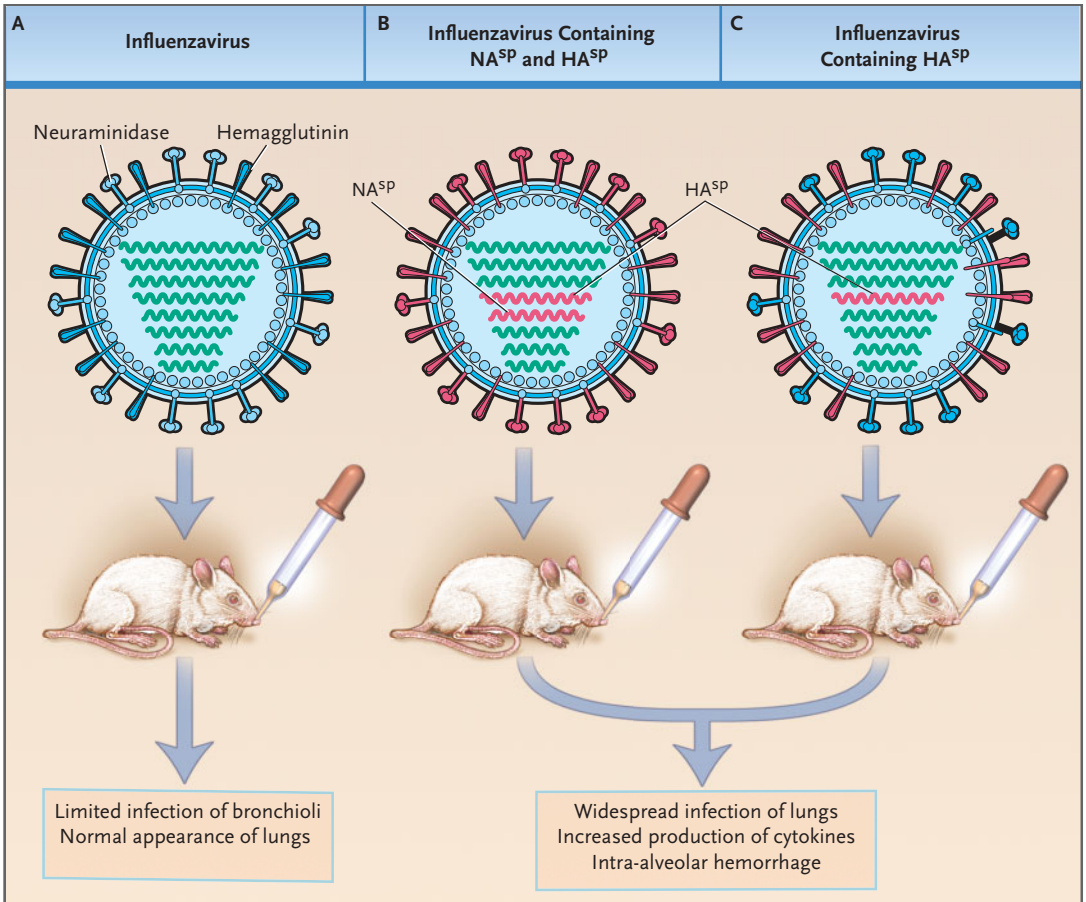

Figure 1. Hemagglutinin as a Driver of Influenza Virulence.

Different strains of influenzavirus have different pathologic effects. For example, infection by the so-called Spanish influenzavirus caused more than 20 million deaths in 1918 and 1919, many of which were due to hemorrhagic pneumonia. To identify the critical components of this virus, mouse-adapted influenza A viruses (Panel A) were modified by Kobasa et al.[1] so that these viruses expressed the form of hemagglutinin encoded by the gene of the 1918 Spanish influenza strain (*HA^sp*), alone (Panel C) or in combination (Panel B) with the form of neuraminidase encoded by the gene of the 1918 Spanish influenza strain (*NA^sp*). They concluded that the HA^sp protein is critical to the enhanced cytokine production, inflammation, and hemorrhagic pneumonia that characterized this virulent influenza.

maintain its strain specificity for up to 80 years after infection. As expected, people born after 1920 had no neutralizing serum immunity against the 1918–1919 pandemic strain, presumably owing to antigenic drift of the HA^sp antigen. Interestingly, neutralizing activity against a more recently circulating interpandemic virus failed to develop in persons who had been exposed to the 1918–1919 pandemic strain. Such persons may be at increased risk for serious complications of infection with currently circulating strains. Alternatively, their failure to mount protective antibody responses against currently circulating strains may reflect a more effective cross-protective T-cell immunity. It will be important to determine which of these scenarios is correct, given the need to identify persons at high risk and to develop new vaccination strategies.

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1. Kobasa D, Takada A, Shinya K, et al. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. Nature 2004;431:703-07.
2. Taubenberger JK, Reid AH, Krafft AE, Bijwaard KE, Fanning TG. Initial genetic characterization of the 1918 "Spanish" influenza virus. Science 1997;275:1793-6.
3. Dauer CC, Serfling RE. Mortality from influenza. Am Rev Respir Dis 1961;83:15-28.