Lessons Learned from Reconstructing the 1918 Influenza Pandemic

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The "Spanish influenza" pandemic of 1918 was the most devastating influenza epidemic reported in history and killed >30 million people worldwide. The factors contributing to the severe pathogenicity of this influenza virus are of great interest, because avian influenza viruses circulating today pose the threat of a new pandemic if they develop sustained human-to-human transmissibility. Recent characterization of the 1918 virus has illuminated which determinants may be the cause of virulence. Here, we wish to shed light on what has been learned to date about the 1918 virus with regard to pathogenicity and transmissibility, to supplement our understanding of the determinants of human virulence and transmission of pandemic influenza viruses. Monitoring the sequences of avian influenza viruses for genetic changes and diversity may help us to predict the risks that these viruses pose of causing a new pandemic.

Of all the pandemics caused by the influenza viruses in the 20th century, the 1918 pandemic, or "Spanish influenza," was by far the most devastating. It was associated with an exceptionally high mortality rate, killing an estimated 30–50 million people worldwide or possibly even more [1–4]. The unusually high mortality among the 15- to 34-year-old population significantly lowered the average life expectancy in the United States [5]. This degree of morbidity and mortality was not repeated in the later influenza pandemics of 1957 and 1968. It raises the question of what was so different about the 1918 pandemic influenza virus that caused so many deaths. It has been suggested that the high mortality rates were due to underlying conditions in humans at that time. Living conditions during the First World War among soldiers, a particularly susceptible group, were poor, as were those of some civilian populations [6]. Alternatively, or in combination with the conditions of that time, perhaps there was a particular unique characteristic of the virus that was responsible for high virulence. However, the influenza virus strain that caused the 1918 pandemic was not available for study, because the virus was not isolated in 1918, as a result of the lack of tissue-culture techniques. Nevertheless, Reid et al. [7, 8] were successful in determining the sequence of the hemagglutinin (HA) and neuraminidase (NA) genes and, more recently, of all 8 viral genes, using tissue samples from humans infected with the virus in 1918–1919 [9]. These samples did not contain infectious viruses but did contain small pieces of genetic material derived from the 1918 virus. However, although Taubenberger et al. [9] successfully genetically sequenced the 1918 virus, the result did not provide a clear understanding of its virulence, because the sequence of the 1918 virus did not reveal any known high virulence determinants.

The origin of the 3 known pandemic influenza viruses of the past century is of interest for understanding how human pandemic viruses are generated. Each pan-

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demic virus belonged to an antigenically distinct subtype: H1N1 (1918), H2N2 (1957), and H3N2 (1968). The absence of preexisting immunity against these viruses in humans during the pandemic years is thought to have played a major role in their rapid spread and in increased pathogenicity. Avian species are the reservoir for all the known antigenic subtypes of influenza viruses, with 16 known HA subtypes (H1–H16) and 9 NA subtypes (N1–N9). Strains circulating in pigs and horses, as well as in some other mammals, have also been described. Both the 1957 and 1968 human pandemic influenza viruses were a result of reassortment events involving 2 influenza viruses (of avian and human origin) exchanging genetic material. Reassortment is a common process in influenza virus evolution and is mediated by the fragmented nature of the viral genome, which allows the interchange of the 8 viral genes when a cell is infected with 2 different viral strains. The origin of the 1918 H1N1 virus is less certain. On the basis of sequence homology, it has been postulated that this virus was generated through homologous recombination of 2 HA genes, of swine and human origin [10]. This hypothesis is unlikely, because of the lack of experimental evidence of homologous recombination occurring in influenza viruses, and was challenged soon after publication [11]. More recently, the analysis of the complete sequences of the 1918 virus led to the hypothesis that the 1918 virus did not originate through a reassortment event between a human and an avian influenza virus but instead evolved from an avian virus that had adapted to infect humans [9]. However, this hypothesis is based on amino acid sequence homology and does not explain differences at the nucleotide level, unless one proposes that the avian influenza virus that led to the 1918 virus was in evolutionary isolation from other avian influenza viruses represented in the existing databases [9]. In fact, even the 1918 HA gene was found to be distinct from the H1 HA gene sequenced from a 1917 bird specimen preserved in the Smithsonian museum [12]. Therefore, the origin of the 1918 virus remains uncertain. In this respect, efforts at large-scale sequencing of human and animal influenza viruses might help us to understand more about the origin of the 1918 virus [13, 14]. Ideally, the identification of pre-1918 human samples containing genomic material from circulating influenza virus strains will definitely establish whether some of the genes were shared with those of the 1918 virus. It remains to be determined whether such a task is possible.

Understanding the determinants responsible for the origin of the 1918 influenza virus and the factors that contributed to its pathogenicity will help us to better recognize the risk of pandemics being initiated by circulating animal viruses. With the possible and immediate pandemic threat of a new avian H5N1 virus, which has successfully been transmitted between infected poultry and has also infected >200 humans to date, this understanding becomes highly important. Further, it will perhaps help us in the identification of novel targets for therapeutic and prophylactic intervention.

CHARACTERIZATION OF THE 1918 INFLUENZA VIRUS

The amino acid sequence of the polymerase proteins (PB1, PB2, and PA) from the 1918 influenza H1N1 virus was found to be very similar to the avian influenza sequences, with only a small number of differences [9]. The phylogenetic analysis conducted by Taubenberger et al. [9] described a total of 10 amino acid changes in the polymerase proteins, differentiating the 1918 human influenza virus from avian influenza virus. Similar sequence changes have been seen in the H5N1 avian influenza viruses circulating today. The transmission of an avian virus from bird to human and then from human to human will likely be the result of several genetic changes. Nevertheless, adaptation of avian influenza viruses to humans may also occur through a reassortment process with a human influenza virus strain, as happened in the case of the 1957 and 1968 human pandemics. The role of the PB1 gene appears to be particularly interesting, because it was found to have been transferred from an avian virus along with the HA gene in the 1957 and 1968 pandemic viruses. Taubenberger et al. [9] compared the PB1 protein from the 1918 human virus with the avian-derived PB1 segments found in the 1957 and 1968 pandemics. Only 1 change with respect to the avian consensus sequence—the N375S change—was shared among all 3 human pandemics. Most human influenza PB1 proteins have a serine at this site. This change is likely to be required for human adaptation of the avian PB1 gene. The role that an avian PB1 gene has, if any, in the origin of human pandemic viruses is not yet understood.

The underlying cause of the high virulence of the 1918 H1N1 influenza virus remained elusive until the virus was reconstructed through the synthesis of cDNAs corresponding to the sequence of the viral RNA genome and through the use of reverse-genetics techniques to rescue influenza viruses from plasmid DNA [15, 16]. The reconstructed 1918 virus demonstrated high lethality in mice, a characteristic not shared by any other primary isolate of human influenza virus [16]. These results highlight the intrinsic high virulence of this virus for mammalian species. Multiple genes were required for high virulence in mice, with the HA, NA, and polymerase genes contributing to increased pathogenicity [16–18].

HA gene. The HA surface glycoprotein of influenza virus is the receptor-binding protein, and it is also responsible for viral entry by mediating fusion of the viral envelope with the host endosomal membrane. This protein needs to be activated by a host protease that cleaves the HA into 2 subunits. A hallmark of highly pathogenic avian influenza viruses is the presence of a multibasic cleavage site recognized by ubiquitous proteases. The cleavage site of the HA of the 1918 influenza

virus did not belong in this category. Nevertheless, the 1918 HA gene, when introduced into a control influenza virus, conferred a virulent phenotype in mice [16–18]. The 1918 HA gene was also essential for the high virulence of the complete 1918 virus in both a mouse model and an embryonated egg model of pathogenicity [16]. High pathogenicity in mice correlated with higher levels of replication of the HA-containing viruses in mouse lungs. The mechanism by which the HA of the 1918 virus confers higher replication and higher virulence in mice is unclear. The crystal structure of the HA of the 1918 virus [19, 20] may guide future studies on the roles of different HA domains that might be responsible for increased virulence.

The HA of human influenza virus preferentially binds to host cell sialic acid with an α2,6 linkage in humans. By contrast, avian viruses preferentially bind with α2,3 linkage in the avian host [21, 22]. Therefore, changes in the HA receptor-binding specificity are thought to be important in the adaptation of avian HA genes to human hosts. The sequence of the 1918 HA gene from 5 different tissue samples has revealed 2 sequences differing only at aa 225 [23]. In a study examining the receptor-binding specificity of these 2 HA variants of the 1918 influenza virus, the 1918 HAs bearing a glutamic acid at aa 225 has specificity for sialic acids of the human type, whereas 1918 HAs bearing a glycine residue at this position have a dual receptor specificity and bind to both human (α2,6) and avian (α2,3) receptors. Moreover, a single amino acid change at aa 190 was found to change the receptor-binding specificity from human to avian cellular receptors [24]. Thus, it appears that receptor specificity of the 1918 HA is governed by only a few amino acid residues and can be switched easily through single point mutations.

NA gene. The influenza virus NA gene also encodes a surface viral glycoprotein, but, in contrast to HA, NA mediates viral release by cleaving off viral receptors. Whereas the 1918 HA gene conferred increased virulence, the addition of the 1918 NA gene enhanced the pathogenicity of the virus [17]. These results are not surprising, because the receptor-binding activity of HA and the receptor-destroying activity of NA are known to be finely tuned to complement each other. More interesting, however, is the observation that the 1918 NA gene allowed the trypsin-independent activation of HA in tissue culture [16]. Trypsin-independent activation of HA is a property of highly pathogenic avian influenza viruses, and, in this case, this is mediated by the presence of a multibasic cleavage site in HA [25–27]. The 1918 virus is also able to activate its HA in a trypsin-independent manner, but this property is mediated by its NA molecule and not by the cleavage site of HA. In this respect, the behavior of the 1918 NA is similar to that of the NA of a mouse-adapted, neurotropic strain of influenza virus, A/WSN/33 [28]. For WSN virus, this NA activity is one of the determinants of the virulence of this virus in mice [29]. It remains to be seen whether this unusual property of the 1918 NA is also involved in its high virulence.

Polymerase genes. The reconstruction of the 1918 virus also revealed a prominent role for the polymerase proteins in enhanced virulence [16]. The introduction of the 1918 polymerase genes into a virus containing the 5 remaining genes from 1918 decreased the 50% lethal dose of the virus in mice by ~2 log. The 1918 polymerase genes (PB1, PB2, and PA) allow the 1918 virus to replicate faster than other human H1N1 viruses in human respiratory epithelial cells and in the lungs of mice [16]. This enhanced replication is likely to contribute to the high virulence of the 1918 virus in mice.

NS1 gene. The NS1 gene of influenza virus encodes a nonstructural viral protein that contributes to viral replication and pathogenicity by inhibiting the host type I interferon (IFN)–mediated antiviral response [30]. However, the 1918 NS1 gene does not contribute to increased virulence of the 1918 virus in mice and, if anything, appears to decrease virulence in mice [31]. However, the NS1 protein displays host specificity, and the 1918 NS1 is an efficient inhibitor of the human IFN system [32]. The use of animal models other than mice should shed light on the potential contribution of the 1918 NS1 gene to virulence.

Host response. In studies conducted to understand the increased pathogenicity of the 1918 virus, recombinant influenza viruses containing the 1918 HA and NA glycoproteins demonstrated the induction of a profound host response accompanied by severe lung pathology and inflammation in mice through enhanced levels of alveolar macrophages, cytokines, and neutrophils [18, 33]. Historical histopathological analyses of lung tissue obtained from individuals who died in the 1918 pandemic showed severe pathology with acute pulmonary edema, as well as hemorrhage with acute bronchiolitis, aveolitis, and bronchopneumonia [34]. Although it is likely that severe inflammation and high levels of cytokines play a major role in the pathogenicity of the 1918 virus, depletion of neutrophils and alveolar macrophages in mice resulted in increased death rates, demonstrating that these cells also play a critical protective role [35]. Strategies based on down-regulation of the host inflammatory response as concomitant therapy with an antiviral should be carefully evaluated, because of the need to have a balanced host response that preserves its protective effects while diminishing its immunopathological consequences.

H5N1

It is of concern that several amino acid changes identified in the 1918 virus as potential contributors to human adaptation have been seen in the H5N1 virus circulating today. In 1997, the H5N1 virus was successfully transmitted from birds to humans, killing some of those infected. All 8 gene segments were acquired from Eurasian avian sources and retained character-

istics typical of avian influenza [36]. The mass culling of poultry successfully eliminated it from the poultry population, but it continued to circulate in geese in China [37]. In 2002, the virus underwent genotypic changes that resulted in increased pathogenicity in ducks and aquatic fowl, a rare event [38]. In 2003, the H5N1 virus underwent further genetic changes and was able to infect a family from Hong Kong in which 2 of the 3 presumably infected persons died [39]. As of March 2006, there have been more reports of H5N1 influenza among domestic poultry in many countries in Asia, Europe, and Africa, with the number of confirmed human cases exceeding 200, with >100 human deaths [40]. By now, this number may be an underestimate. However, no cases of human-to-human transmission have been confirmed, although there is a small number of cases occurring in families, which may be suggestive of human-to-human transmission. Moreover, it is also unclear how many cases of asymptomatic and mild H5N1 human infections have occurred; therefore, the lethality of this virus in humans is likely to be overestimated. Of note, most human isolates of H5N1 viruses have acquired a few changes responsible for increased virulence in mammals, and, as was the case with the 1918 virus, virulence appears to be associated with the HA, polymerase, and NS1 genes [41–43], as well as with a deregulated host response [44]. The ever-expanding geographic distribution of H5N1 increases the concern that the virus will eventually mutate and/or reassort and become capable of sustained transmission within the human population, causing a new influenza pandemic of unpredicted virulence. However, currently, no one can predict with certainty whether the H5N1 virus will become a human pandemic virus.

OTHER AVIAN INFLUENZA VIRUS SUBTYPES

There have been other avian influenza viruses—H9N2, H7N7, and H7N2, among others—that have also been successfully transmitted from birds to humans. These viruses have not caused as many human deaths as has the H5N1 virus, with 1 reported death due to an H7N7 isolate in a veterinarian [45]. Both H7N7 and H5N1 viruses are highly pathogenic for poultry. However, the success of a human pandemic virus does not require that it be highly pathogenic for poultry, and there is no indication that the 1918, 1957, and 1968 pandemics were due to a highly pathogenic avian virus precursor. Importantly, H2N2 viruses, which caused the 1957 pandemic and disappeared from humans in 1968, continue to circulate in birds. Every person born after 1968 lacks preexisting immunity against H2N2 viruses, and this creates a significant group of humans that are susceptible to a hypothetical H2N2 pandemic virus. Increased animal and human influenza virus surveillance is needed to more appropriately assess the risk that viral subtypes H2 and H4–H16 will be responsible for the generation of the next human pandemic virus. Monitoring the sequences of avian influenza strains for genetic changes may also help us to predict the possible adaptation of specific strains to new hosts and help us to prepare for a pandemic.

ANTIVIRALS AND VACCINES

Currently, the prophylactic and therapeutic options available for influenza are vaccination and NA and M2 inhibitor treatment. Both vaccination and viral inhibitors successfully prevented lethality, in mice, of highly virulent viruses containing 1918 genes, underscoring the benefit of these strategies even when a highly virulent virus is circulating [17, 46]. Ideally, a vaccine for a pandemic influenza virus should be available and mass produced by the time the first wave of the pandemic hits. Resources are currently being poured into the development of a vaccine effective against H5N1, and trials are under way. However, supply limitations and production capabilities are of concern. NA inhibitors and adamantanes are available for the treatment of influenza virus infections. Although adamantanes have been used to treat influenza, the 2004 H5N1 viruses are resistant to these drugs [47], which limits their use. The NA inhibitors oseltamivir and zanamivir, however, are effective against the H5N1 virus and may be useful as prophylaxis for those who are in contact with infected persons. Although resistance has been seen in a small number of patients, resistant viruses appeared to be less fit and less virulent than the wild-type virus [48–50]. Nevertheless, the generation of improved influenza virus vaccines and of novel antivirals will greatly increase our chances of being better equipped for the next pandemic influenza virus.

SUMMARY

Influenza A viruses are a major public health concern. Epidemic influenza causes significant illness, with thousands of deaths each year in the United States alone, despite available vaccines and antiviral therapy. Avian influenza viruses are a major concern as potential precursor viruses for new influenza pandemics. Although it is not clear whether H5N1 viruses will adapt to transmit from human to human, it is likely that a new pandemic influenza episode involving a virus with at least the HA gene derived from an avian influenza virus subtype will occur in the future. The virulence and fitness of that virus will depend on the genetic changes that take place. We understand so much more now of what potentially may cause severe pathogenicity of an influenza virus, but the matter of which amino acids are responsible for this increase remains elusive. The recent characterization of the 1918 virus has brought us closer to understanding these issues. However, there are several remaining questions about antigenicity, pathogenicity, and immunity of the 1918 virus. Research continues to move forward vigorously, because the time to a pandemic is not known. Moreover, heightened awareness and a greater public health infra-

structure will allow us to better prepare for such a pandemic. It is encouraging that studies have demonstrated that even a pandemic due to a highly virulent virus, such as the 1918 influenza virus, can be prevented with antivirals and vaccines that are in use today. Thus, we have an unparalleled opportunity to employ preventive measures against a new influenza virus pandemic, because we are at an enormous scientific and social advantage today compared with less than a century ago.

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