INFLUENZA

The 1918 influenza pandemic: 100 years of questions answered and unanswered

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The 2018–2019 period marks the centennial of the "Spanish" influenza pandemic, which caused at least 50 million deaths worldwide. The unprecedented nature of the pandemic's sudden appearance and high fatality rate serve as a stark reminder of the threat influenza poses. Unusual features of the 1918–1919 pandemic, including age-specific mortality and the high frequency of severe pneumonias, are still not fully understood. Sequencing and reconstruction of the 1918 virus has allowed scientists to answer many questions about its origin and pathogenicity, although many questions remain. This Review summarizes key findings and still-to-be answered questions about this deadliest of human events.

A CENTURY OF PANDEMIC INFLUENZA

The year 2018 marks two somber, temporally linked centennials—one marking the end of World War I, which claimed 18 million lives, and the other marking the most unprecedented natural disaster in recorded history, the so-called Spanish influenza pandemic of 1918–1919, which claimed tens of millions more lives (*1*, *2*). The pandemic (a geographically widespread or global infection in people) appeared in all but the remotest places on Earth, causing symptomatic disease in at least one-third of the world's population. Whereas most sick persons had self-limiting influenza, indistinguishable from influenza as seen today, an unexpectedly high number went on to die of pneumonia (*3*). U.S. case fatality ratios were approximately 0.5 to 1% (*4*), but case fatality was much higher in many developing countries (*5*, *6*) and in many crowded environments, from urban slums (*7*) to Western military training camps (*8*). The global mortality will never be known with certainty, but estimates range from 50 million to as high as 100 million in the first pandemic year (*9*).

The 1918 pandemic was caused by an influenza A virus (*10*). The RNA virus family Orthomyxoviridae contains seven genera (*11*), including four genera of influenza viruses and three genera of non-influenza viruses. Delta influenza viruses (influenza D viruses) infect pigs and cattle; the non-influenza Orthomyxoviridae (*Isavirus*, *Quaranjavirus*, and *Thogotovirus*) rarely cause human disease. Human influenza infections caused by alpha influenza viruses (influenza A viruses) are the only members of this viral family known to cause pandemics and to be associated with high mortality. The beta influenza viruses (influenza B viruses) often spread widely but cause moderate or low mortality, typically in younger persons. The gamma influenza viruses (influenza C viruses) cause mild local outbreaks of illness. Here, we will discuss only the influenza A viruses, including the virus that caused the 1918 influenza pandemic.

The natural global reservoir of influenza A viruses is birds, mainly waterfowl and shorebirds. Influenza A viruses of humans, other mammals, and poultry result from uncommon waterfowl host-switching events associated with critical viral genetic changes that are poorly understood. In addition, mammal-to-mammal transmission of influenza A viruses (e.g., humans to pigs, or horses to dogs) is also well docu-

mented. In the past century, all pandemic and endemic influenza A viruses have been descended from the 1918 pandemic influenza virus (*12*), which was itself likely descended from a wild waterfowl virus at some point in or shortly before 1918.

Influenza A viruses are enveloped negative-strand RNA viruses with eight segmented genes, each encoding one or more proteins, any or all of which may undergo reassortment when a cell is co-infected with two or more influenza A viruses. This reassortment mechanism was responsible for the post-1918 pandemics of 1957, 1968, and 2009 (Fig. 1) (*2*). Pandemic and endemic influenza A viruses circulate and evolve continually in humans, resulting in the eventual development of widespread population immunity, which can only be escaped by further viral evolution. Such escapes are associated with mutations that create new epitopes or glycosylation sites (referred to as antigenic "drift"), with genetic reassortment of entirely new hemagglutinin (HA) or other gene segments (referred to as antigenic "shift"), or with importation of preexisting variant human HA genes (referred to as "intrasubtypic reassortment"). Host cellular immunity is mostly directed against internal influenza A virus proteins. In contrast, the three external influenza virus proteins—HA, neuraminidase (NA), and matrix 2 (M2)—predominantly encounter the humoral immune system, elicit protective immunity which stimulates virus immune escape (Fig. 1). At least 16 genetically unique HA and 9 NA influenza virus subtypes exist in wild waterfowl, providing the basis for the simplest nomenclature: H1 to H16 and N1 to N9. This allows any influenza A virus to be described by its two most important external proteins, e.g., H1N1 in the case of the 1918 pandemic influenza virus. Although most of the 144 possible HA and NA subtype combinations are found in wild waterfowl, during the past century of virological observation, only H1N1, H2N2, and H3N2 influenza A viruses have circulated in humans.

The impact of the pandemic H1N1 virus was not limited to 1918–1919. The 1918 influenza A virus (Fig. 1) was a new "founder virus" that initiated the current era of circulating influenza A viruses by evolving into progeny pandemic viruses through genetic reassortment. All influenza A pandemics and seasonal epidemics since that time, and almost all cases of human influenza A worldwide, have been caused by descendants of the 1918 influenza virus (Fig. 2) (*12*). These include not only the antigenically "drifted" descendants of the 1918 H1N1 virus itself but also the genetically reassorted pandemic viruses that appeared in 1957 (H2N2), 1968 (H3N2), and 2009 (H1N1pdm). The exceptions include human infections caused by

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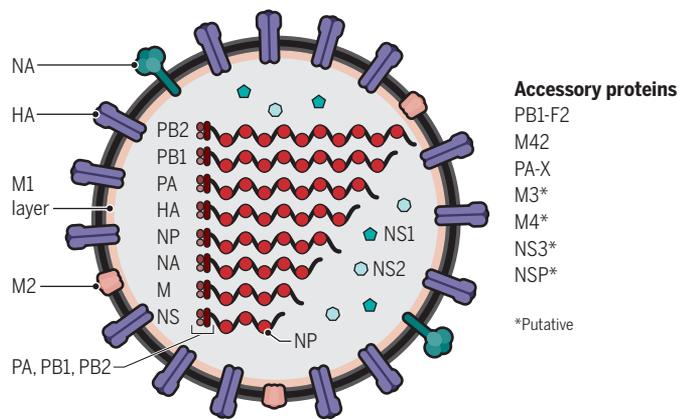
Fig. 1. Structure of influenza A viruses. Influenza A viruses are composed of 8 gene segments and 11 or more proteins. The surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) are the major antigenic targets of the host immune response to influenza A virus infection. NA is the target of the antiviral drugs oseltamivir and zanamivir. Matrix 1 (M1) is a structural protein, and matrix 2 (M2) is an ion channel protein. Nucleoprotein (NP) encapsidates the viral RNA. Each gene segment is associated with a trimeric RNA-dependent RNA polymerase complex consisting of the PB1, PB2, and PA proteins. The nonstructural 1 (NS1) protein has pleiotropic functions, including binding to double-stranded RNA, enhancement of viral mRNA translation, inhibition of host mRNA processing, and blocking the type I interferon response of the host. NS2 [also referred to as nuclear export protein (NEP)] is found in virions and facilitates nuclear export of viral ribonucleoprotein complexes. Another small viral protein, PB1-F2, is variably encoded within the PB1 gene by an alternative reading frame; it targets the mitochondrial inner membrane and may play a role in apoptosis during influenza A viral infection. Influenza pandemics over the past century have emerged in several different ways. They can emerge directly due to influenza A viruses that inhabit wild waterfowl switching to a human host, as likely occurred in the 1918 pandemic. They can result from acquisition of gene segments through reassortment of different HA subtypes with or without reassortment-associated acquisition of other gene segments (so-called antigenic shift). They can also result from complex reassortment and host adaptation events, such as occurred in the 2009 pandemic, involving reassortment between human, swine, and avian influenza A viruses. Major HA changes in seasonal endemic viruses, arising from intrasubtypic reassortment, may also cause pandemics, as happened in 1946 (*147*). Since 1918, there have been pandemics caused by 1918 descended viruses: in 1957 (H2N2), in 1968 (H3N2), the reemergence of H1N1 in 1977, and the emergence of a new H1N1 virus in 2009 (*12*). Between pandemics, annual seasonal influenza A viruses are generated by continuous viral genetic mutations (antigenic drift) and by intra-subtypic reassortments (*148–150*). The figure is adapted from (*10*).

animal-derived (zoonotic) influenza A viruses, e.g., those from poultry-adapted influenza A viruses such as H5N1 and H7N9. Interestingly, the H1 HA of the 2009 pandemic virus (H1N1pdm) is genetically and antigenically related to the 1918 pandemic virus through its evolution in pigs (*13*). This provides not only a fascinating but also a disturbing example of a direct connection between the 1918 founder virus and the currently circulating H1N1pdm influenza virus strain (*12*).

Each of these descendant pandemic viruses contained gene segments that evolved from the 1918 virus. Some of these gene segments drifted over time, whereas others were eventually "updated" through genetic reassortment by influenza A virus genes derived from waterfowl or, in the case of the 2009 pandemic virus, by different 1918 virus-derived genes that had become incorporated into swine influenza viruses (*14*). The 2009 H1N1 pandemic virus (H1N1pdm) contained HA, nucleocapsid (NP), and nonstructural (NS) gene segments derived from the classical swine H1N1 (1918 origin) lineage.

The 2009 H1N1 pandemic virus also contained NA and matrix (M) gene segments from a European avian-like H1N1 swine virus lineage that had circulated in pigs for several decades enzootically (that is, ongoing infections in animals) (*15*). Its three remaining gene segments (PB2, PB1, and PA) were derived from a North American swine H1N2, "triple" reassortant lineage, the PB2 and PA genes having been derived from a waterfowl influenza A virus and the PB1 gene from a human seasonal H3N2 virus (*16*). The evolution of the 1918 founder virus over the past century has thus been complex and multidirectional. Like the original 1918 pandemic virus, each of the three descendant pandemic viruses that appeared subsequently soon began to drift antigenically, causing major global mortality in virtually every year of the past century (Fig. 3). In one recent year alone (2014–2015), 710,000 Americans were hospitalized for influenza and 56,000 died (*17*). In other years, the toll has been far higher (*18*): Preliminary data suggest that the 2017–2018 influenza season may have killed 80,000 Americans (*19*).

The founding 1918 pandemic virus is truly the "mother" of all subsequent influenza pandemics (*2*), and we are still in its "pandemic era" today. Its descendants are still evolving, still killing tens of thousands of people every year, with no end in sight (*12*). However, the 1918 pandemic has also spurred advances in virology, bacteriology, clinical infectious diseases, public health, and vaccinology (*20*). Here, we address what has been learned about the 1918 pandemic from the perspective of science and public health, how that knowledge is helping to prevent and control influenza, and what remaining questions are guiding future influenza research.

FINDING THE HOLY GRAIL: SEQUENCING AND RECONSTRUCTING THE 1918 INFLUENZA VIRUS

The 1918 pandemic occurred in an era when viruses, as we know them today, were largely theoretical conceptualizations. Back in 1918, the extraordinarily high pandemic mortality, especially in young adults, frustrated physicians and scientists, who were unable to identify an etiological agent and thus were unable to diagnose and successfully treat the disease it caused. As virology matured as a scientific discipline, influenza A viruses were eventually isolated from pigs (1930) (*21*) and from humans (1933) (*22*). Serological data from the 1930s first suggested that the 1930s "classical" swine virus and the 1918 pandemic virus were closely related antigenically (*23*). This was later verified by viral genetic sequence analysis and by antigenic and pathogenesis studies (*13, 24–26*). The subsequent pandemics, beginning with the 1957 H2N2 pandemic, revealed that new pandemic viruses could arise from previous pandemic viruses through genetic reassortment (Fig. 1) (*12, 27, 28*). But where the 1918 virus had come from and what the basis of its severity had been remained among the most discussed medical mysteries throughout most of the 20th century (*1*). Solving that mystery was often referred to as a scientific "Holy Grail," and few believed that it would ever be found. In 1976, historian Alfred Crosby (1931–2018) wrote that "It has been the dream of scientists working on influenza for over a half century to somehow obtain specimens of the virus of Spanish influenza, but only something as unlikely as a time capsule could provide them" (*29*).

The development of polymerase chain reaction (PCR) technology in the 1980s was an important first step in finding that time capsule. Reverse transcription PCR (RT-PCR) made it possible at last to recover and sequence highly degraded fragments of viral RNA retained in preserved tissues from victims of the 1918–1919
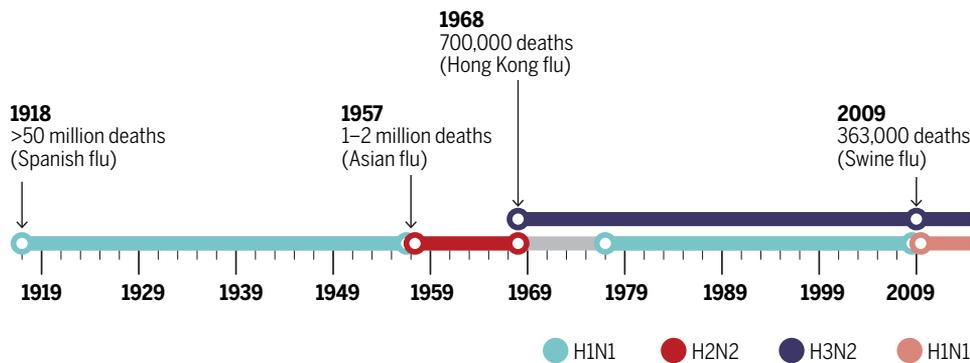
Fig. 2. Influenza pandemics of the past 100 years. The 1918 "Spanish flu" pandemic was caused by a founder H1N1 influenza A virus. The three subsequent pandemics of 1957, 1968, and 2009 (black arrows) were caused by descendants of the 1918 virus, which acquired one or more genes through reassortment (*12*). Colored horizontal lines reflect the years of annual epidemics of seasonal influenza that occurred after each pandemic. In 1977, pre-1957 human H1N1 viruses reemerged presumably through accidental release of an older human strain from a laboratory (*151*). This resulted in a 20-year gap in H1N1 circulation as indicated by the discontinuous turquoise line. Consequently, human H3N2 viruses co-circulated with human 1918 lineage seasonal H1N1 viruses from 1977 to 2009, when this lineage was replaced by the new swine-origin H1N1 2009 pandemic virus. Since 2009, H3N2 viruses have co-circulated with 2009 pandemic lineage H1N1 viruses in humans.

pandemic (*24*, *30–36*). Using PCR and the new approach of viral "reverse genetics," plasmids containing DNA clones of tiny viral RNA gene sequences permitted eventual reconstruction of the complete sequences of all eight genes of the 1918 influenza virus (*37*), allowing its "resurrection" through reverse genetics as an infectious virus that could be studied experimentally. Remarkably, miniscule degraded viral RNA fragments recovered from just a few of the pandemic's many millions of victims are now yielding, a century after their deaths, new insights into influenza virus biology and pathogenesis and are providing important lines of investigation into the prevention, treatment, and control of future pandemics. The details of the effort to find and sequence the 1918 virus genome (*20*), and to reconstruct and study it, are briefly summarized here.

The effort to sequence the pandemic viral genome was initiated in 1995 using formalin-fixed, paraffin-embedded autopsy tissues in the collection of the National Tissue Repository of the now-defunct Armed Forces Institute of Pathology. The tissue archives of the Armed Forces Institute of Pathology had preserved materials from more than 100 autopsies of 1918 influenza victims, more than 70 of which had formalin-fixed, paraffin-embedded tissues samples. Review of the medical records and histological examinations of these tissues identified a dozen that appeared most likely to be influenza RNA positive. Of these, one case was found to be positive and complementary DNA (cDNA) sequence fragments of four of the eight viral gene segments from this case were published in 1997 (*30*). This study confirmed an influenza A virus H1N1 subtype and demonstrated the lack of an HA cleavage site mutation. This indicated that the virus was unlikely to have originated from a highly pathogenic poultry-adapted influenza virus and was consistent with descent from a waterfowl influenza A virus. Another round of screening of Armed Forces Institute of Pathology cases in 1997 revealed a second positive formalin-fixed, paraffin-embedded tissue sample. Simultaneously, the laboratory received extremely important frozen lung samples from a 1918 victim, contributed by pathologist Johan Hultin (*24*).

In 1951, Hultin had conducted an expedition to Brevig Mission, Alaska, to recover lung tissue samples from 1918 pandemic victims

for viral isolation. Unfortunately, no virus had been isolated. In 1997, nearly a half century later, the establishment of PCR and reverse genetics techniques, not imagined in 1951, led Hultin to propose returning to Alaska for another exhumation to secure additional specimens, this time for molecular genetic analysis. After obtaining permission from the Brevig Mission Council to reopen a mass grave, a victim was identified whose body remained well preserved, presumably because her subcutaneous fatty tissue insulated and preserved the internal organs during periods of thawing within the permafrost.
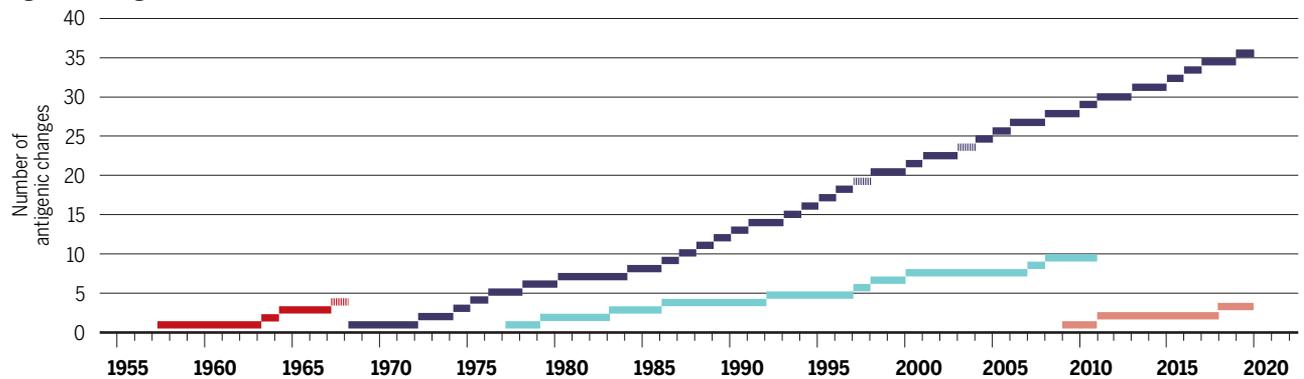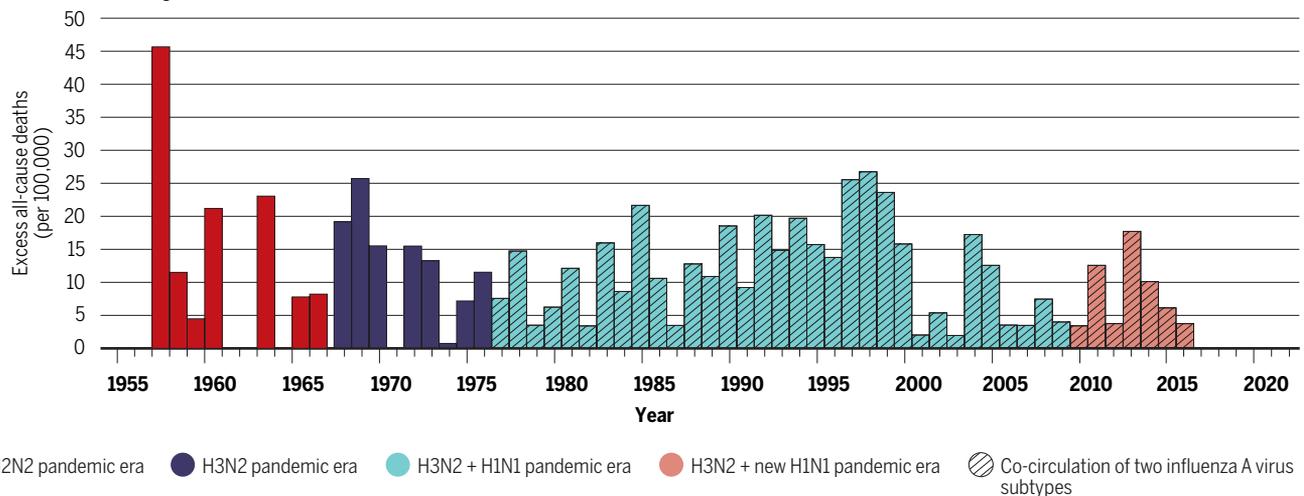
Samples of frozen lung were biopsied in situ and placed directly in fixatives, including ethanol, formalin, and guanidine. Influenza virus RNA fragments were detected in this case as well. The H1 domains of the HA genes were sequenced from all three cases (*24*). They differed from each other by only a single nucleotide over 1200 bases, indicating a true clonal pandemic virus. The remaining seven gene segments were sequenced from RNA isolated from lung tissue from the Alaskan case. Determining the complete coding sequences of all eight genes of the 1918 virus would take nine more years (*36*).

The search for additional 1918 influenza RNA-positive cases was expanded by screening autopsy tissue blocks from the collection of the Royal London Hospital, in collaboration with the laboratory of virologist John Oxford. Several additional 1918 cases were found. Sequencing of the H1 HA gene again revealed extremely high sequence identity (*34*). Next, 68 of the 70 Armed Forces Institute of Pathology cases with autopsy material were reexamined by histopathological analysis, immunohistochemistry for viral antigen, and RT-PCR for viral RNA (*38*). The lung tissues of 37 of these cases were positive for influenza viral antigens or viral RNA, including four tissue samples from the period of May–August 1918, before the pandemic had extended globally.

In 2013, next-generation sequencing approaches were used to obtain the complete genomic sequence of an additional 1918 virus from a formalin-fixed, paraffin-embedded autopsy tissue sample. The sequence was determined at approximately 3000× per base coverage, and the library also had good representation of host mRNA and bacterial RNA, providing the first "transcriptome" of a fatal 1918 case of influenza pneumonia (*39*). It was thus certain that the 1918 pandemic had been caused by a single recently introduced virus that was essentially genetically identical in its near-simultaneous appearances around the globe. This virus shared many features with, and was likely derived in the very recent past from, a wild waterfowl influenza A virus that had somehow directly or indirectly switched hosts to become a human-adapted influenza A virus.

Reverse genetics technology for influenza viruses, developed in 1999 (*40*, *41*), also allowed production of infectious influenza A virions containing one or more 1918 virus genes for in vitro and in vivo study. A multicenter collaborative project involving

A Antigenic changes

B Influenza mortality rates

Legend: ● H2N2 pandemic era ● H3N2 pandemic era ● H3N2 + H1N1 pandemic era ● H3N2 + new H1N1 pandemic era ⊘ Co-circulation of two influenza A virus subtypes

Fig. 3. Evolution and annual mortality of four pandemic influenza virus strains. The four pandemic strains shown are descended from the 1918 pandemic virus and arose by antigenic shift between 1955 and 2016. (**A**) Antigenic changes in post-pandemic viruses are indicated. The colored bars represent the prevalence of the 1957 H2N2 pandemic virus (red), the 1968 H3N2 pandemic virus (dark blue), the unexpected return of a 1950s-era descendant of the 1918 pandemic virus, presumably released accidentally from a laboratory (turquoise), and the 2009 H1N1 pandemic virus (orange). Changes in antigenic drift of sufficient magnitude to require reformulation of the annual vaccine are indicated on the *y* axis. Notably, the 1968 H3N2 virus has been drifting at a greater rate (an average of 0.7 genetic changes per year) than the other three pandemic viruses (an average of 0.27 genetic changes per year for the three combined). Antigenic changes in post-pandemic influenza viruses are associated with antigenic drift, which introduces new epitopes or new glycosylation sites, and by intrasubtypic reassortment of an antigenically different HA of the same subtype, represented by vertical white lines. (**B**) Annual excess U.S. mortality rates attributed to influenza from 1955 to 2016. Pandemic eras since 1957 are represented by different colors, as shown by the key below the graph. Data are missing for some early years. Data were obtained from the U.S. Centers for Disease Control and Prevention and reflect excess all-cause mortality, the most common calculation method available for this time period (which may overestimate mortality). Figures are modified and updated from (*152*).

the Armed Forces Institute of Pathology laboratory and colleagues from the U.S. Centers for Disease Control and Prevention, the U.S. Department of Agriculture, the Mount Sinai School of Medicine, the Scripps Research Institute, and the University of Washington provided initial characterization of 1918 viral pathogenesis (*32, 37, 42–51*). These initial 1918 virus studies evaluated key questions of host adaptation, pathogenesis, and the role of the host inflammatory response in disease control and pathogenesis, as well as factors associated with transmission among mammals.

Reconstruction of the 1918 virus has ushered in a new era of influenza research and has greatly expanded our understanding of influenza as a pandemic and endemic disease (*52*). What have we learned that will help us prevent or control future deadly influenza pandemics and what knowledge gaps remain?

WHERE DID THE 1918 PANDEMIC H1N1 VIRUS COME FROM?
Wild waterfowl and shorebirds (mainly from the orders Anseriformes and Charadriiformes) are the major global reservoir of influenza A viruses, which cause predominantly asymptomatic gastrointestinal tract infections in more than 100 of these species (*53, 54*). Mixed influenza A virus infections, consisting of infection with two or more influenza viruses and subsequent gene segment reassortments between these viruses, are common in these reservoir hosts (*54*). Waterfowl influenza A viruses form transient genome constellations without a strong selective pressure to be maintained as linked genomes, leading to continual emergences, disappearances, and new emergences of different viral genotypes through reassortment. Relative genetic stability of an influenza A virus occurs only when a wild waterfowl virus switches hosts to adapt to a new gallinaceous poultry or mammalian host population and is then transmitted within that population.

Pinpointing the origin of the 1918 pandemic virus, including discovering exactly how, where, and when it emerged to initiate sustained human-to-human pandemic transmission, will likely never be possible. Because human-adapted influenza A viruses are only moderately contagious and moderately fatal, it is the nature of influenza pandemics that many weeks, and more likely many months, must pass between the emergence of a pandemic and its detection. During this time, there would be relatively few deaths, given (relatively) low influenza case fatality, and those deaths that occurred would be difficult or impossible to recognize beneath the background of deaths from seasonal influenza and from other prevalent respiratory agents. It remains important to seek genomes of additional influenza viruses from the months and years before May 1918, when the earliest virally confirmed fatal 1918 case occurred (38). The hope is that new viral sequences identified from before 1918 will help to answer fundamental questions about the origin of the 1918 pandemic virus and population immunity before the pandemic, but the viral evolutionary and host adaptational sequences of events that bridge wild waterfowl gene constellations and pandemic viral genomes occur inside a "black box" that currently remains largely invisible to science. We have information about the genome of the 1918 virus at a very early stage of its emergence, but we do not yet know anything about evolutionary steps that may have occurred before it became adapted to human hosts.

An instructive example of our inability to predict pandemic emergence, or to precisely characterize pandemic viral genetic evolutionary pathways, is that of the 2009 swine influenza pandemic caused by the H1N1pdm virus (14). This virus appeared in an era of unprecedented human and animal influenza viral surveillance and the near real-time deposition of thousands of influenza virus genome sequences into public databases (55, 56). The first recognized human cases caused by the 2009 pandemic H1N1pdm virus occurred in Mexico (14, 57). However, multiple co-circulating genotypes of related swine influenza A viruses, resulting from multiple complex reassortments of various swine influenza A virus lineages (including those similar to the 1918 pandemic H1N1 virus from which the ancestral 2009 swine virus lineage had been derived in 1918), were identified not only in swine populations in central Mexico (57) but also in Asia (16). Clearly, most, if not all, of the major pre-emergence genetic events of 2009 had happened at some time, and in some unknown place, that escaped detection.

Even if the future pandemic virus had been identified in swine populations in the months and years before 2009, it would likely not have been recognized as a virus with pandemic potential. This is because viral phenotypic properties associated with human adaptation and transmissibility cannot yet be predicted from genetic sequences. The implications are sobering: Identifying pre-pandemic viruses by increased viral surveillance in mammals and birds may be difficult or impossible. There is reason to believe that every influenza virus pandemic and panzootic/epizootic event (the animal counterparts to pandemics and epidemics in humans) may be fundamentally different from every other. Although these pandemic (or panzootic/epizootic) viruses-to-be already exist in nature, or evolve and adapt in humans or other mammals, there is a growing realization that each seems to achieve its host switch success through different cooperative polygenic adaptive mutations that, in unique combination, are able to support pandemic or panzootic spread (10, 52, 58–62).

Can epidemiology tell us anything about the origin of the 1918 virus? Several locations of pandemic origin have been proposed, including Étaples (France) (63), China (64), or Camp Funston/Haskell (Kansas) (65), but it is hard to decide whether any of these proposed origins are likely. Among other problems, most of these supposed originating outbreaks occurred at times of the year that are favorable to influenza spread, yet they spontaneously and completely disappeared long before pandemic influenza was detected.

If, however, we take the occurrence of extreme excess respiratory disease mortality as an epidemiological marker, the 1918 pandemic seems to have appeared globally in just a few months (July–September 1918, perhaps slightly earlier in China and India), in an "everywhere at once" pattern rather than a "place to place" pattern (66). This suggests that the virus had already been seeded around the globe, but that fatal infections remained below the threshold of excess mortality detection, thereby epidemiologically obscuring its place of origin. Such indolent pre-pandemic transmission, likely beginning in or before 1918, eventually reached pandemic explosivity (and therefore recognition) only after exceeding critical excess mortality thresholds in multiple large urban populations around the globe (67).

Among the first of these, in July–August 1918, were pandemic emergences identified by excess mortality—although presumably blunted by summer temperature and humidity, which are unfavorable to influenza virus spread—in China and India, and in parts of northern Europe including England, northern Germany, and Scandinavia (1, 68). Despite enormous wartime traffic across the English Channel, it is curious that whereas many English cities, including London, had a marked summer 1918 "wave" of influenza mortality, Paris (only about 200 miles south of London and linked at the time by major interurban population movement) did not (69).

Also poorly understood are occurrences in the 1917–1918 winter and spring months of scattered outbreaks of respiratory disease, consistent with influenza, in many parts of Europe and in several U.S. cities and military training camps. Epidemiological observations, however, are seemingly inconsistent with most of these outbreaks being early appearances of the 1918 pandemic influenza virus (1, 4, 8). Most of them featured local explosivity but lacked wide geographical extension (despite favorable winter-spring conditions), low to negligible mortality, and a clinical and epidemiological pattern that differed from the pandemic "waves" seen later in 1918 (1, 70). A question arises whether the 1918 pandemic virus could have been highly prevalent in the northern hemisphere in the winter of 1917–1918 without becoming pandemic or even spreading widely, but readily doing so in the summer of 1918, a season that is unfavorable to influenza spread (because the higher temperature and humidity, coupled with less indoor crowding, retards virus persistence and spread) (61).

Further evidence is provided by detailed epidemiological and clinical data from the U.S. Army. Examination of 1917–1918 occurrences of acute respiratory diseases in 39 U.S. Army training camps, including their rates of incidence, case complications, mortality, and case fatality, revealed three peaks of acute respiratory disease incidence preceding and during the 1918 pandemic (8). Influenza outbreaks peaking in December 1917 and again in April 1918 were of low incidence (~5% of soldiers were clinically ill) and were associated with case fatality ratios fivefold lower than during the true fall pandemic (~1% versus ~5% case fatality ratios). Moreover, the clinical picture was different between the winter-spring and fall cases. Far fewer of the winter 1917–spring 1918 cases were complicated by pneumonia, and those that were, were less likely than in the fall of 1918 to be complicated by bronchopneumonia (10 to 20% versus 50 to 75%) (71), which became a hallmark of the 1918 pandemic (3). Such data

suggest that the December 1917 and March–April 1918 influenza-like respiratory illness peaks were largely due to one or more non-pandemic respiratory viruses, rather than representing early pandemic waves (*4*, *72*). Such outbreaks may have been caused instead by seasonal influenza viruses, which often circulate throughout the winter and early spring.

To explain the apparent paradox of explosively transmissible but largely uncomplicated and nonfatal spring 1918 outbreaks of influenza-like illness, some have postulated that the emerging pandemic virus was originally, in the spring of 1918, of low pathogenicity but became more pathogenic as it circulated in humans (*73*). The earliest pre-pandemic cases identified in May–August 1918, however, had pandemic HA sequences identical to those seen during the pandemic peaks in late 1918 and in 1919 (*38*). These early H1 HAs shared pathogenic properties with H1 HAs found in wild waterfowl influenza A viruses, making hypotheses of evolving viral pathogenesis problematic.

Further evidence is provided by study of clinical, pathological, bacteriological, and virological findings from 68 fatal influenza/pneumonia patients from U.S. military training camps. Nine of the 68 fatal cases occurred before the September–November 1918 pandemic wave (*38*), with dates of death ranging between 11 May 1918 and 8 August 1918, a time when there was no evidence of elevated influenza mortality in the United States. The pre-pandemic and pandemic peak cases were indistinguishable clinically and pathologically, as were their viral sequences. Partial HA sequences from four of these cases have been determined, covering the receptor binding domain on the head of the HA molecule. These sequences match HA sequences from fall wave cases and together only vary at one amino acid residue in the receptor binding region (at site 222, numbered from the start codon of the HA open reading frame). Viral antigen distribution in the respiratory bronchoalveolar system, however, was not apparently different between pre-pandemic and pandemic peak cases, or between infections with viruses bearing the different receptor binding polymorphisms, including those with more "human-like" and those with more "avian-like" receptor binding preferences.

In an important set of experimental studies designed to characterize 1918 viral pathogenicity, 7:1 chimeric 1918 influenza viruses were created in which each of the eight 1918 virus gene segments was replaced, one at a time, by the corresponding gene segment of a modern wild waterfowl H1N1 virus (*74*). In murine studies, seven of the 1918 7:1 chimeric viruses replicated and caused disease that was equivalent to the extreme pathogenicity of the fully reconstructed 1918 virus, including the 1918 pandemic chimeric virus with the modern waterfowl H1 HA. Only the chimeric 1918 virus containing the waterfowl influenza PB2 gene segment was attenuated, but this attenuation was corrected by a single amino acid change (E627K) in PB2. This E627K mutation is one of the mutational changes at the C-terminal end of the influenza A virus PB2 polymerase protein frequently observed in nonpandemic avian-descended influenza A viruses that infect humans and mammals (*10*, *75*), apparently representing an easily acquired adaptational change in these new hosts. Clearly, the 1918 pandemic virus, whatever its origin, was similar pathophysiologically to a wild waterfowl influenza A virus. These data, coupled with 1918 HA sequence data from early (pre-pandemic) 1918 cases (*38*), strongly support the hypothesis that the earliest circulating 1918 viruses were virtually identical to, and inherently as pathogenic as, the viruses sequenced from the main pandemic wave in September–November 1918.

The origin of the 1918 virus has also been investigated by studying the viral genome and its coding sequences compared to those of other influenza A viruses (*10*, *76*). Gene segments from the 1918 pandemic virus have a nucleotide composition and a high guanosine-cytosine content like those of influenza A viruses that (then and now) circulate in wild waterfowl, and unlike influenza A virus strains adapted to humans (*15*, *77*, *78*). This indicates that, with or without adaptation in an intermediate host, the 1918 virus was likely derived from a waterfowl influenza A virus. Phylogenetic analyses have also been used to model 1918 virus origin but have yielded different dates for the estimated origin of the pandemic virus (*36*, *76*, *79*, *80*). Until pre-1918 influenza A virus sequences become available, reconciling the dates of the origin of the 1918 virus phylogenetically will remain difficult. It seems highly unlikely on epidemiological and biological grounds that a virus expressing the pandemic H1 HA, with its inherent virulence, could have widely circulated in humans much before 1918.

A unique epidemiological feature of the 1918 influenza virus, related to its origin, was infection of both humans and swine. Influenza was first recognized as a clinical entity in swine in the United States in autumn 1918 (*81*), concurrent with the spread of the pandemic in humans (*25*, *82*), having apparently been transmitted from humans to pigs. This host switch split the virus off into two independent viral lineages, one human and the other porcine. After 1918, the epizootic disease became widespread among herds of swine in the U.S. midwest. Epizootic viruses appeared annually thereafter, leading to Shope's 1930 isolation of the first influenza virus, A/swine/Iowa/30 (*21*), 3 years before the first human isolation of a descendant of the parent 1918 virus, A/WS/33 (*22*). The two 1918 viral H1N1 lineages, one human and the other porcine, evolved and antigenically drifted at different rates until 2009. In the 2009 pandemic, the human-adapted H1N1 descendant was replaced by a different H1N1 virus that was also a 1918 viral descendant, ironically one that had been circulating enzootically in pigs. The original 1918 classical swine lineage still circulates enzootically today (*83*).

WHAT HAVE WE LEARNED ABOUT INFLUENZA VIRUS PATHOGENICITY?

Intrinsic, evolutionary, and adaptational determinants of influenza virus pathogenicity

The phenotypic properties of the influenza virus include infectivity, transmissibility, pathogenicity, cell tropism, sensitivity to environmental factors (such as temperature, humidity, and pH), and a host of other variables. When an influenza A virus switches hosts, as the 1918 pandemic virus did, it must be able to survive in a new infectious ecosystem. It must be able to infect target epithelial cells, replicate, and be transmitted efficiently between members of the new host species. It does not have to be highly pathogenic, given that killing or incapacitating the host would not enhance viral spread. Whereas the 1918 pandemic virus was inherently pathogenic, its subsequent history (e.g., between 1918 and 1946 and after its 1977 reappearance until 2008) was one of apparent viral attenuation over decades of post-pandemic circulation (*12*, *84*, *85*). Regardless of accumulating humoral and cellular immunity in the human population, human seasonal influenza A/H1N1 viruses of the last few decades do not share the pathogenic properties of the 1918 pandemic virus in various animal models (*26*, *86*, *87*).

Viral adaptation to new hosts

Although there is no certainty that it had to adapt markedly from its waterfowl influenza A virus precursor to become pandemic, the 1918 viral genome has been studied for molecular features potentially associated with human adaptation (*10*). These include amino acid changes that have been associated with mammalian adaptation in nature and in experimental systems, such as alterations in the HA receptor binding domain and in the viral polymerase complex. Unfortunately, when other waterfowl-to-mammalian influenza A virus host switch events are compared, there is little evidence for shared adaptational mechanisms (*15*). For example, some of the amino acid changes that distinguish the 1918 pandemic virus from waterfowl influenza A virus are not observed in unrelated viral lineages derived from influenza A virus host switches, such as a European waterfowl-like swine H1N1 virus that emerged in the late 1970s or wild waterfowl-derived equine influenza A virus (*88*). Similarly, the 2009 H1N1 pandemic virus demonstrated the independent and polygenic nature of individual influenza A virus host switching (*89–91*). Individual host-switching events are apparently achieved by unique sets of cooperating mutations that allow adaptations for host cell entry, replication, and transmissibility within recipient host populations. There may be many different states and pathways by which wild waterfowl influenza A viruses adapt to humans and other hosts. Of course, the critical viral evolutionary events happen obscurely in nature, preventing us from observing them.

Infectivity

Among the most important properties associated with influenza A virus host switching is the ability of a virus to infect cells of the new host. Viral attachment to respiratory epithelial cells is mediated by binding of HA to cellular receptor glycans with terminal sialic acids linked in different configurations to underlying sugars (e.g., α2-3– and α2-6–linked sialic acids). The HA receptor binding domain region was sequenced in 16 fatal 1918 autopsy cases (*38*), revealing several naturally occurring viral mutations in persons who died between May 1918 (before pandemic recognition) and February 1919 (after the pandemic had peaked in most of the world). All such H1 viruses had the E187D mutation in the HA receptor binding domain when compared to the HA of conserved waterfowl influenza A virus. In addition, some cases also had either a G222D or G222N mutation in the HA receptor binding domain and one case had a Q189R mutation. Structural analyses and assays of in vitro binding to glycan arrays indicate that the 1918 pandemic influenza virus HA carrying a G222 mutation had a blended α2-3 > α2-6 sialic acid binding specificity, whereas HA with a D222 mutation had a predominantly α2-6 sialic acid binding specificity. The 1918 pandemic influenza virus HA with a Q189R mutation has not yet been evaluated using a glycan array.

It might be suspected that these mutational differences in the HA receptor binding domain would lead to differences in viral binding, infectivity, and pathogenic potential. However, there were no differences in distribution of viral antigens along the respiratory tract of the 1918 influenza cases with different HA receptor binding domain mutations and no apparent differences in the clinical course or pathological findings (Fig. 4, A to D) (*38*). Moreover, when the 1918 pandemic influenza variant viruses were evaluated for both viral entry and for replication in primary human bronchial epithelial airway cells in vitro, all variants efficiently bound to and infected airway cells in a comparable manner (*92*).

These observations, supported by mouse and ferret studies (*48, 74, 93*), indicate that cellular tropism of 1918 influenza viruses bearing the various HA receptor binding domain mutations noted above—whether associated with an α2-6 sialic acid binding preference or a mixed α2-3/α2-6 sialic acid binding preference—has no discernible effect on viral infectivity. Whether these HA receptor binding domain mutations preexisted or developed during individual infections is not known (*94*), nor is it known whether these mutations affect human-to-human transmissibility. In a ferret study, 1918 virus variants with blended α2-3/α2-6 sialic acid binding specificity were less transmissible (one of three contact animals) than 1918 viruses with an α2-6 sialic acid binding specificity (three of three contact animals), although the relevance of these data for human infections is unknown (*48*).

Mutations in the polymerase protein PB2 have long been associated with influenza A virus host switching, especially the E627K mutation in the C-terminal region of the PB2 protein (*95*). The E627K mutation was identified in the 1918 pandemic virus and in some naturally occurring avian influenza A virus infections of humans and was also identified as a virulence factor in experimental mammalian host studies (*74, 96*). Other independent mammalian-adapted influenza A virus lineages have mutations at residue 701 in PB2. These include the waterfowl-origin European swine H1N1 viruses (*15*) and some highly pathogenic poultry-adapted H5N1 viruses isolated from humans (*97, 98*), although other PB2 mutations may be important for adaptation to humans as well (*36*). Residues 701 and 702 in PB2 have also been implicated in nuclear localization of the virus (*99, 100*).

With the emergence of the H1N1pdm pandemic virus in 2009, it became clear that such PB2 changes were not necessary for human adaptation. The 2009 pandemic virus PB2, which was not derived from either the 1918 or European avian-like swine H1N1 viral lineages, did not have any of the key changes at residues 627, 701, or 702 in PB2 (*14*). In fact, in experimental systems, engineering of these mutations into viruses expressing the 2009 pandemic virus polymerase complex had no effect on virus replication or pathogenicity (*89, 90*). After the 2009 pandemic, mutations in two adjacent C-terminal PB2 residues (590 and 591) were proposed as an alternate "strategy" for viral adaptation to humans (*91*).

Together, these observations suggest that adaptive mutations in waterfowl influenza A virus host-switching events, including those associated with emergence of a pandemic virus like the 1918 virus, might be unique to specific viruses and their new hosts, and that there may be multiple pathways by which intergene cooperation can lead to host adaptation. Furthermore, mutations that do occur in host-switched viruses may be results of, rather than prerequisites for, adaptation. This also suggests that surveillance for pre-pandemic viruses may be problematic, because many different constellations of mutations might predispose to pandemic host-switching events, and we currently have no way to recognize them.

Pathogenicity

Viral factors contributing to pathogenicity of the 1918 pandemic influenza virus have also been sought in experimental animal infections using chimeric influenza viruses containing one or more genes from the 1918 virus. Remarkably, a chimeric 1918 virus in which the 1918 H1 HA gene had been replaced by a modern wild waterfowl-derived H1 HA gene was just as pathogenic in experimental animals as the 1918 virus itself, producing a pattern of disease and histopathology indistinguishable from that caused by the 1918 virus (*74*). Viruses
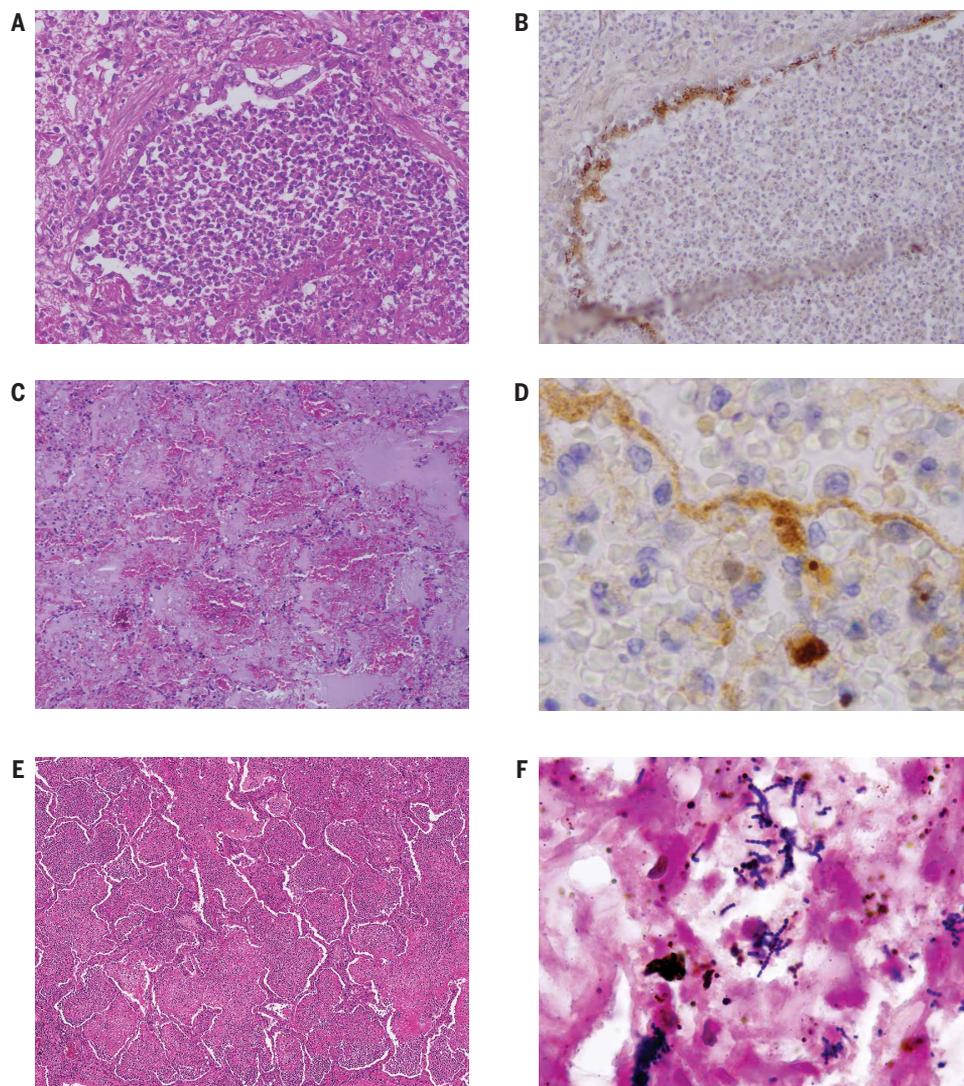
Fig. 4. Pathological features of the 1918 pandemic influenza virus. (**A**) Photomicrograph of a hematoxylin and eosin (H&E)–stained section of lung from autopsy material from a 1918 influenza case with acute pneumonia. The image shows a necrotizing bronchiolitis with massive infiltration of neutrophils through the wall and into the lumen of a bronchiole. Original magnification, ×200. (**B**) Immunohistochemical staining of influenza viral antigens in a bronchiole from lung autopsy material from a 1918 influenza case. Viral antigens stain human respiratory epithelial cells reddish brown on a hematoxylin-stained background. Original magnification, ×200. (**C**) H&E-stained section of lung obtained from a 1918 influenza case at autopsy. The image shows diffuse alveolar damage with acute pulmonary edema and hemorrhage filling the lung air spaces. The alveolar air spaces contain edema fluid, strands of fibrin, red blood cells, and inflammatory cells. Original magnification, ×100. (**D**) Immunohistochemical staining of a section of lung obtained from a 1918 influenza case showing influenza viral antigens in alveolar epithelial cells and lung alveolar macrophages. Viral antigens stain alveolar cells reddish brown on a hematoxylin-stained background. Original magnification, ×1000. (**E**) Section of lung obtained from a 1918 influenza case with acute pneumonia. The image shows a bacterial bronchopneumonia characterized by a necrotizing bronchitis and bronchiolitis and massive infiltration of neutrophils into the lung air spaces of surrounding alveoli. Original magnification, ×100. (**F**) Gram staining of a lung tissue section obtained at autopsy from a 1918 influenza case with pneumonia. Gram-positive cocci morphologically compatible with *S. pyogenes* are purple. Original magnification, ×1000. Figure panels represent histological findings for autopsy lung samples from 1918 fatal influenza cases.

expressing a wild waterfowl H1 HA that can replicate in mammals therefore are inherently pathogenic for mammals and likely humans.

Viral chimeras expressing the 1918 pandemic H1 HA alone on the backbone of seasonal H1N1 or H3N2 human influenza viruses (*86*, *93*, *101*) also led to enhanced pathology in the murine respi-

ratory tract. This was characterized by a prominent infiltration of alveolar neutrophils and macrophages into lung air spaces, and virus replication in alveolar epithelial cells, similar to that induced by the fully reconstructed 1918 virus (*74*). This suggested that the 1918 HA specifically, rather than the paired 1918 HA and NA glycoproteins, was acting as a major virulence factor. The other genetic components of the 1918 virus also likely contributed to the overall pathogenicity of the pandemic virus (*58*). The influenza A virus polymerase genes have also been shown to contain virulence factors in animal models. Chimeric influenza A virus genomes encoding the four ribonucleoprotein gene segments of the 1918 virus (PB2, PB1, PA, and NP) in the context of the remaining genes from seasonal influenza viruses showed enhanced pathogenicity in mice and ferrets (*90*, *102*).

The extreme pathogenicity associated with the 1918 pandemic H1N1 virus seems to be shared with other, presumably all, waterfowl H1 influenza A viruses (*103*, *104*). Apparently, a major determinant of 1918 pandemic mortality was incorporation into the viral genome of an inherently pathogenic H1 HA gene that preexisted in nature. Avian influenza A viruses expressing the H1 HA subtype that presumably is very similar to those that existed in 1918 still circulate in nature today. Thus, an avian H1 virus is presumably capable of reemerging as a genetic component of future pandemic viruses, possibly within influenza A viruses that are fundamentally different genotypically in the other viral gene segments when compared to the 1918 H1N1 virus.

This unexpected finding begs the question of whether the 16 HAs found in wild waterfowl in nature sit on a spectrum of inherent pathogenicity and whether extremely pathogenic HAs other than H1 may exist in nature. Studies have demonstrated that, as was true for the 1918 H1N1 virus (*37*, *74*, *105*, *106*), modern wild waterfowl chimeric influenza A viruses expressing H1, H6, H7, H10, and H15 HA subtypes also show increased

pathogenicity in mice and cytopathicity in human bronchial airway epithelial cells. Moreover, the experimental pathology of these H1, H6, H7, H10, and H15 viruses was characterized by marked pulmonary neutrophil infiltration into lung air spaces, a prominent feature of pathology findings in animals infected with the 1918

virus. In addition, there was higher expression of genes associated with recruitment and activation of these neutrophils and other inflammatory cells, including genes encoding proinflammatory cytokines and chemokines. Other pathology included alveolitis induced by neutrophil infiltration, tissue damage due to reactive oxygen species, and lung cell death, all consistent with the hallmarks of 1918 autopsy findings (*103*, *105*, *107*).

Experimental mouse infections with influenza A viruses containing 1918 HA and other pathogenic HAs showed a pathological picture that was similar to that for highly virulent human influenza A virus infections, including those caused by the 1918 virus. This pathology included increased cytopathicity and robust activation of host inflammatory and cell death responses (*46*, *87*, *108*). Experimental nonhuman primate infections additionally revealed that within 1918 virus-infected upper airway cells, increased expression of proinflammatory chemokines and cytokines was accompanied by suppression of type I interferon responses and other antiviral responses (*87*). The NS1 protein of influenza A viruses plays an essential role in the inhibition of antiviral responses by host cells. In vitro studies have shown that the NS1 protein of the 1918 virus (which has >98% identity with NS1 proteins from a number of wild bird influenza A virus sequences) appears to be a particularly effective regulator of these host immune responses (*32*, *42*, *74*, *109–111*). Such aberrant inflammatory responses, including elevated expression of cytokines, chemokines, expression of acute phase response reactants, and suppression of host antiviral responses, lead to robust viral replication and potent activation of immune cells, cell death responses, oxidative damage, and increased disease severity (*105*). Transcriptomic analysis of a single 1918 pandemic autopsy sample (*39*) showed a marked concordance with the transcriptomic patterns of experimentally infected mice (*46*) and cynomolgus macaques (*87*). Of potential importance for understanding and treating severe influenza disease, mice infected with a lethal dose of the 1918 influenza virus and then treated with the drug EUK-207 (a catalytic catalase/superoxide dismutase mimetic) had reduced alveolar epithelial cell damage due to reactive oxygen species, less severe lung pathology, greater activation of tissue repair responses, and prolonged survival (*105*).

These pathogenesis findings indicate that pandemic and panzootic/epizootic viruses, such as the 1918 virus, that potentially emerged from waterfowl influenza A viruses can bring with them preexisting genetic determinants of pathogenicity for human and other mammalian hosts, expressed as extreme cytopathicity coupled with destructive host immune responses. This raises the possibility that influenza A viruses with HAs other than those we have already encountered in pandemic viruses in the past century (H1, H2, and H3) may potentially emerge to cause disease severity comparable to that of the 1918 pandemic influenza virus.

The potential for emergence of future pandemic viruses containing such pathogenic HAs is unknown. However, for the purposes of pandemic planning, we must now consider the emergence of pandemics with extreme pathogenicity and high fatality, a concern underscored by the apparent variability in pandemic influenza severity recorded over a period of more than 500 years (*61*). As we ponder the possibility of future influenza pandemics caused by viruses with other pathogenic HAs, we must also remember that the pathogenicity of the 1918 pandemic virus seems to have been inherent to an H1 HA subtype that remains in nature today. Thus, if current global population immunity to H1 viruses wanes, another H1 pandemic could arise with the same deadly consequences as those experienced

in 1918. All such pathogenic HAs existing in nature therefore represent a fundamental threat and an important target for pre-pandemic vaccine development.

WHAT MADE THE 1918 PANDEMIC VIRUS SO DEADLY?

Influenza viruses, including the 1918 pandemic virus, usually cause acute self-limited respiratory infections in humans. Symptoms typically include fever, coryza (inflammation of the nasal mucosa), cough, headache, fatigue, and malaise, often persisting for 7 to 10 days, followed by complete recovery. In most cases, lower respiratory involvement is not clinically prominent, if it occurs at all. Although the 1918 influenza virus caused tens of millions of deaths worldwide, the vast majority of those who were infected had a typical self-limited illness indistinguishable from clinical influenza seen today, and they promptly recovered. However, on occasion, influenza infections of any sort can be complicated by hemorrhagic bronchitis, diffuse alveolar damage in the lung air sacs, and pneumonia (*112*, *113*). Progression to such severe disease is likely a multifactorial process involving viral, host, and bacterial factors (*10*).

Histopathological features of fatal primary influenza viral pneumonia, including diffuse alveolar damage with pulmonary edema and alveolar hemorrhage, were observed in autopsy studies of the 1918 pandemic and subsequent pandemics (*112–115*). However, the 1918 pandemic was also characterized by widespread pulmonary vascular thrombus formation and prominent infiltration of lung tissue by neutrophils (*38*, *107*, *112*, *116–118*). Areas of the lung showing these features in 1918 autopsies are usually coincident with areas showing histological features of tissue repair and other areas showing features of bacterial bronchopneumonia (*38*, *112*, *113*). This supports an asynchronous pathology associated with primary damage due to the virus, subsequent tissue repair by the host, and secondary bacterial pneumonia, as is observed in experimental animal models of influenza and bacterial co-infection (*107*, *119*, *120*). Although fulminant fatal primary influenza viral pneumonias have been documented, most severe influenza-associated pneumonias in 1918 were associated with secondary bacterial infections (Fig. 4, E and F) (*3*, *121*), a pathological feature that had been established before 1918 and remains common today (*1*, *115*, *121*). In 1918, however, there was a higher rate of severe or fatal secondary bacterial pneumonias in persons with influenza than was expected based on pre-1918 data, including those of the 1889 pandemic. Data from U.S. military training camps in 1918 indicated that the odds of influenza cases being complicated by bronchopneumonia in September–October 1918 were approximately 25-fold higher than they had been in the December 1917–April 1918 pre-pandemic peaks of influenza-like illnesses (*8*). This supported the conclusion (*122*) that high pandemic case fatality during the fall 1918 pandemic resulted primarily from an increased frequency, not an increased severity, of secondary bacterial pneumonias, especially in young adults.

Another unusual epidemiological determinant of 1918 pandemic severity was the unprecedented age-specific mortality pattern, in which young adults were at extraordinarily high risk of dying, a feature not observed in influenza outbreaks before or since (*1*, *2*, *4*, *67*). Classically, influenza age-specific mortality is roughly "U-shaped," with high mortality in the very young and the very old, and with low mortality in healthy persons in between these ages. The 1918 mortality curve, however, was roughly "W-shaped," with an additional mortality peak in persons 20 to 40 years old (Fig. 5) (*67*), and slightly

increased mortality in the elderly (although still less than predicted). The less than predicted mortality in the elderly conceivably could be attributed to 19th century exposure either to then-prevalent influenza A viruses containing H1 or N1 surface proteins or to conserved "minor" epitopes shared with the 1918 virus (2, 4). Although the 1918 virus appears to have been pathogenic for persons of all ages, the disproportionate increase in frequency of secondary bacterial pneumonias in healthy young adults might be an additional manifestation of viral virulence associated with differential host immune responses (8, 39, 58, 105, 107).

The bacteria most frequently associated with secondary infections after influenza in 1918–1919 were the pneumopathogens *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus* (3). Almost all of the tens of millions of deaths worldwide during the 1918 pandemic were associated with secondary bacterial infections, primarily with Gram-positive bacteria (3). Had it not been for secondary bacterial pneumonias caused by these and other pneumopathogens, the 1918 pandemic likely would have been associated with a far lower mortality (8). Increased susceptibility to secondary bacterial pneumonias in persons with influenza should be considered an intrinsic property of influenza virus pathogenicity, and this is likely to be the case for other pathogenic influenza viruses.

Whether such viral-bacterial co-pathogenesis is a 1918 virus–specific property or an unfortunate by-product of other aspects of HA-associated pathogenicity such as cytopathicity or host immune activation remains an open question. In a mouse co-infection model of the 1918 virus and *S. pneumoniae* (107), secondary bacterial infection was associated with increased early bacterial replication,

increased activation of neutrophils, and aggregation of platelets leading to abundant small blood vessel thrombi. Altered host immune responses to infection as compared to infection with the 1918 virus or *S. pneumoniae* alone greatly enhanced lung pathology and shortened survival. Co-infected mice showed histopathological changes affecting >50% of the lung parenchyma. Acute pneumonia featured alveolar airspaces packed with infiltrating neutrophils and macrophages, extensive acute suppurative pleuritis, widespread necrotizing bronchiolitis, and abundant fibrin thrombi in lung veins, venules, and capillaries (107).
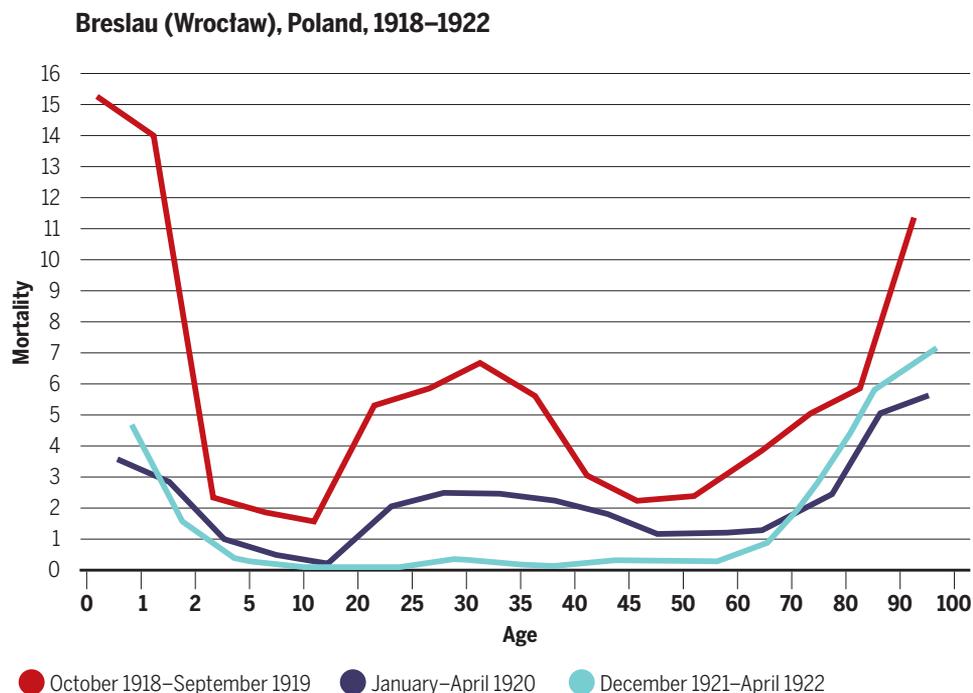
In co-infected mice, neutrophil infiltrates also expressed greater amounts of myeloperoxidase and neutrophil elastase, with increased extracellular deposition of neutrophil elastase along the endothelium of blood vessels and on alveolar and bronchiolar epithelium. Mice co-infected with the 1918 virus and *S. pneumoniae* also showed prominent staining for tissue factor throughout the lungs, especially in areas of acute pneumonia, bronchiolitis, and pleuritis. A unique gross pathological feature of the mice infected with the 1918 virus and *S. pneumoniae* was the presence of extensive fibrinous pleuritis, with fusion of the lungs to the chest wall and diaphragm, as was observed in the 1918 pandemic (118).

These experimental findings are consistent with observations from 1918 autopsy cases positive for both the influenza virus and *S. pneumoniae*, including marked expression of tissue factor in monocytes, macrophages, neutrophils, and epithelial cells and abundant thrombi in small blood vessels (107, 116). As was the case in the experimental studies, fibrinous thrombi were also commonly observed at autopsy in small veins, venules, and capillaries.

Co-infection–induced pulmonary thrombosis apparently exacerbated vascular leak and alveolar edema, limiting compensatory ventilation responses and contributing to severe hypoxia and death, and probably to the many 1918 influenza deaths associated with terminal "heliotrope cyanosis" (123).

A final aspect of influenza viral pathogenesis that has been little studied is the possible association of individual genetic host susceptibilities to severe influenza disease, suggested by epidemiological data on poultry-associated influenza A virus infection of humans (124–127). Identifying and characterizing such genetic susceptibilities might allow identification of persons at heightened risk for severe influenza disease so that they could receive influenza and pneumococcal vaccinations, post-exposure antiviral prophylaxis, and close medical follow-up during periods of influenza prevalence.

Because influenza is an important cause of global mortality, our research emphasis must remain focused on preventing deaths that result both from pandemics and from endemic viral circulation. Characterizing the natural history and pathogenesis of severe 1918 pandemic

Breslau (Wrocław), Poland, 1918–1922

● October 1918–September 1919 ● January–April 1920 ● December 1921–April 1922

Fig. 5. Age-specific influenza mortality (1918–1922). The 1918 Spanish influenza pandemic appeared in Breslau (now Wrocław), Poland, in October 1918, causing high mortality. The "W-shaped" age-specific mortality pattern indicated in the graph was seen worldwide. Influenza age-specific mortality is usually U-shaped with higher mortality in infants and the elderly. A third peak of mortality in young adults (peaking at about age 27) was uniquely associated with the 1918 pandemic. Pandemic recurrences 15 and 26 months later were, however, associated with lower overall mortality and the virtual disappearance by 1922 of mortality in young adults. Modified from (84).

disease, including mechanisms underlying severe bacterial co-infection, is a key research goal. But despite progress in understanding the pathological correlates of severe disease, we still lack knowledge about how to effectively prevent them. Co-pathogenic bacterial bronchopneumonias can develop and progress so rapidly, in persons with otherwise uncomplicated influenza, that avoidable deaths constitute a big part of total influenza mortality. High on the list of tools we lack are prognostic biomarkers of impending bacterial pneumonias (*128*), given that very early antibiotic treatment in an intensive care setting is often the only means of preventing fatal outcomes.

WHAT REMAINS TO BE DONE?

Influenza was a major public health problem for centuries before 1918, and remains so today, in terms of both sporadic pandemics and annual seasonal epidemic recurrences of variable severity. There will undoubtedly be influenza pandemics in the future, but despite an enormous amount of study, it is not possible to predict when and where they will appear, what viral subtypes they will be caused by, or what pathogenic properties they will display (*59*, *129*, *130*). Enhanced influenza virus surveillance, especially at the animal-human interface, is clearly important, but we lack any real ability to identify pre-pandemic viruses before they become pandemic.

We therefore need to better understand the potential for human adaptation of the various types of influenza A viruses to which humans are exposed and their possible mechanisms of host switching. These include viruses of wild waterfowl, and viruses of hosts to which waterfowl viruses may switch and adapt, including mammals and poultry. The 2009 H1N1 pandemic was "game changing" in being the first observed instance of a pandemic virus emerging from another mammal to which it had adapted, albeit a virus that had descended from the 1918 human virus. The multiple emergences of outbreaks in animals (epizootics) or zoonotic infections of humans with poultry-adapted influenza A viruses, such as H5N1 and H7N9, make it clear that much remains to be clarified about the implications of influenza A virus mutational changes within the host and switching to different hosts, including better genotypic-to-phenotypic correlations. We must also ask whether the 1918 virus was inherently human-adapted before it emerged or whether it underwent critical adaptive changes that facilitated emergence. If the latter, what were these changes and in what host did they occur? How can we identify these changes by experimental study of the very few mutations that distinguish pandemic viruses from their waterfowl influenza A virus precursors? We have few answers to any of these questions. Wild waterfowl are the natural reservoir of influenza A virus genetic and antigenic diversity; thus, it seems reasonable to suspect that adaptation and emergence of new pandemic influenza A viruses must result from the acquisition of key mutations on a path to pandemicity. The ability to distinguish human-like mutations from avian-like signatures, including mutations affecting HA receptor binding specificity and polymerase mutations associated with efficient viral replication in human cells, might facilitate better predictions of the emergence of potential pandemic strains.

However, this may not be the case after all. Rather than being markers of inevitable progression to human adaptation and pandemic emergence, such mutations may simply be markers that any waterfowl- or poultry-adapted influenza A viruses are likely to develop when they replicate zoonotically in humans (whether or not they can adapt to human population infectivity and transmissibility).

So, is host adaptation a result of viral problem solving in which the virus gets the "right answer," or are there many right answers for a lucky virus to potentially discover? Or is it the case that for most would-be pandemic viruses, there are no possible right answers, pandemic success being an accidental outcome of an extraordinarily rare virus with a favorable set of cooperative mutations arising in nature by unique happenstance?

In attempting to address these questions, poultry-adapted influenza A viruses such as A/H5N1, A/H7N9, and others are of particular concern, because they have been associated with high case fatality (*131*). H5N1 viruses have been circulating for more than two decades and have caused at least 860 human cases with 454 deaths since 2003 (*132*). H7N9, which has caused epizootic/zoonotic outbreaks throughout China since 2013, has resulted in another 1625 human infections and 623 deaths (*132*). What risks do such viruses pose for pandemic emergence?

Although poultry-adapted influenza A viruses seem to have difficulty productively infecting the great majority of humans, they can replicate in, and cause severe disease in, at least a small percentage of humans. Some H7N9 viruses have acquired mutations that enhance H7 HA receptor binding specificity for α2,6-linked sialic acids (*133*), as well as mutations in the viral polymerase PB2 associated with enhanced replication and virulence in mammals (*134*), features shared with the 1918 pandemic virus. The current H5N1 and H7N9 viruses, however, primarily infect humans only in single, dead-end events, so far without ongoing human-to-human transmission and without evidence of adaptation to humans.

What variables have so far prevented wild H5N1 and H7N9 waterfowl viruses from becoming efficiently and pandemically transmissible in human populations, as the 1918 virus did? Do all influenza viruses, including poultry-adapted viruses, have the potential to acquire host adaptive mutations that lead to pandemicity (*60*), or is such evolution prevented by structural or functional evolutionary constraints associated with adaptation to the previous host? Does influenza A virus poultry adaptation place the virus in a particular evolutionary dead end with respect to subsequent human adaptation? Or can poultry-adapted influenza A viruses acquire mutations like those of the known pandemic viruses? If not, can they adapt to humans through different mechanisms? Again, we have no answers to these important questions.

Although many thousands of people are continually exposed to a huge array of avian influenza A viruses, few pandemics have emerged over the past millennium (*61*); as the global human population has greatly increased, pandemic frequency has not (*61*, *67*). This suggests that despite a low species barrier for individual zoonotic infection by wild and poultry-adapted influenza A viruses, barriers against productive viral adaptation and onward transmission in humans must be high. Among the 16 influenza HAs and 9 NAs known to exist in wild waterfowl influenza A viruses, only 3 of the 144 subtype combinations—H1N1, H2N2, and H3N2—have been incorporated into any human-adapted or pandemic influenza A virus since 1918. An analogous situation is apparent with waterfowl influenza A viruses that are host-adapted to other mammals such as horses (H7N7 and H3N8) and dogs (H3N8 and H3N2) (*135*). Such HA and NA subtype restriction is puzzling because, for example, H1 and H2 are uncommonly found in wild waterfowl, whereas more common waterfowl subtypes, such as H4 and H6, have never been observed in human-adapted viruses. Are there real but unappreciated restraints on the ability of wild waterfowl viruses to become pandemic viruses?

In the late 1950s and 1960s, Francis and others (*136*) hypothesized that only a very few naturally occurring influenza A virus subtypes had the ability to cause pandemics. These few virus subtypes reappeared in regular cycles determined by the lifespans of human birth cohorts exposed to them and rendered immune to them (*137*). This hypothesis has not yet been refuted by the past century of influenza experience. Extending observations back in time to the century before 1918, epidemiological and archaeserological evidence is consistent with the possibility that pandemics between the 1830s and 1889 may, like those of the past century, have only expressed HA subtypes H1, H2, or H3. Moreover, anecdotal observations going back to the mid-1700s are consistent with such HA recycling. If pandemics are explained by recycling of only a few influenza HA and NA subtypes, our efforts to develop preventive vaccines and our viral surveillance strategies need to be targeted accordingly. We currently do not know whether pandemic influenza threats of the future will be few in number and restricted in severity, or multitudinous and deadly.

Despite our modern arsenal of antibiotics, of viral and bacterial vaccines, of antiviral drugs, advanced intensive care treatment, and nonpharmaceutical interventions (*138*), we are still doing a poor job of preventing influenza deaths. The most important lesson from the devastation of the 1918 pandemic may be the need to produce better antiviral drugs and prophylactic and therapeutic monoclonal antibody therapies (*139*, *140*). We need effective vaccines against multiple bacterial pneumopathogens, especially *S. aureus* (*141*) and *S. pyogenes* (*142*), and effective broadly protective "universal" influenza vaccines to prevent, or at least mitigate the impact of, future pandemics and to prevent deaths from seasonal influenza in the periods in between pandemics. Vaccines that could confer long-term broad immune responses against all influenza viruses, and especially against viruses with the most pathogenic HAs found in nature, would greatly enhance public health preparedness (*143*–*146*).

The 1918 pandemic has long served as a benchmark for the critical public health importance of influenza. The fields of microbiology, immunology, vaccinology, and epidemiology continue to use information gleaned from very degraded viral RNA fragments from the 1918 pandemic influenza virus. It is hoped that by better understanding the now century-old 1918 pandemic, we can better manage the future. Information obtained from experimentation with the 1918 virus and analysis of its genome continue to inform clinical and public health decisions that form the basis for saving lives and improving health. The past century of unlocking the secrets of the 1918 virus has been extremely rewarding, but even greater rewards surely lie ahead. The challenges are daunting, but overcoming them remains an urgent necessity.

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Taubenberger *et al.*, *Sci. Transl. Med.* **11**, eaau5485 (2019)   24 July 2019

12 of 15

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Taubenberger *et al.*, *Sci. Transl. Med.* **11**, eaau5485 (2019)   24 July 2019

15 of 15

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