Scientific Insights into the 1918 Influenza Pandemic and Their Implications for the Future[1]

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This paper briefly summarizes scientific insights gained from studying the 1918 influenza virus and the implications of these findings for future pandemic planning. With the recent centenary of the 1918–1919 pandemic in 2018–2019, a number of more comprehensive reviews have been published on this subject, which can provide the reader with additional information (Taubenberger, Kash, and Morens 2019; Morens and Taubenberger 2018a, 2018b, 2019; Taubenberger and Morens 2019).

Influenza A Virus Biology and Ecology

As a background to examining the impact of the 1918 influenza virus (Taubenberger, Kash, and Morens 2019; Morens and Taubenberger 2019), it is first necessary to provide a brief background to influenza virus biology and ecology. Influenza A viruses (IAV) are enveloped negative-strand RNA viruses with segmented genomes containing eight gene segments. IAV are the major cause of annual, epidemic influenza but also pose a significant risk of zoonotic infection (that is, human infection with an animal virus), host switch events, and the formation of pandemics. IAV are enveloped with a host cell–derived lipid membrane. The eight gene segments encode at least 11 open reading frames (Shaw and Palese 2013; Taubenberger and Kash 2010).

IAV encode two major surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). HA functions both as the viral receptor-binding protein and fusion protein. HA binds to the tips of host cell glycoproteins, specifically those with a terminal sialic acid bound to underlying sugars in various configurations. IAV adapted to birds have an HA receptor binding specificity for sialic acids bound via α2,3 glycosidic linkages, while HA from IAV adapted to humans have higher

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specificity for sialic acid bound via α2,6 linkages. After HA binds its cellular receptors, the virus is internalized, leading to a conformational change in the HA protein (termed *fusion*) allowing release of the viral RNA segments bound to the viral nucleoprotein and associated with the viral RNA polymerase complex into the cytoplasm to initiate viral replication within the cell.

NA is a glycoprotein with sialidase (neuraminidase) enzymatic activity, which is required to cleave host cell sialic acids, allowing newly produced (budding) viruses to be released. The complementary functions between sialic acid binding by HA and sialic acid cleavage by NA requires balancing coadaptation. In addition to their functions in the viral life cycle, both HA and NA are the major antigenic targets of the humoral immune response to IAV, and NA is the target of the antiviral neuraminidase inhibitor drugs oseltamivir and zanamivir.

Typical of RNA viruses, IAV are evolutionarily dynamic viruses with high mutation rates. Genetic mutations that change amino acids in the antigenic portions of the surface glycoproteins HA and NA may produce selective advantages for viral strains by allowing them to evade preexisting immunity. This is especially important in IAV adapted to humans, which are subjected to strong population immunologic pressures. Such selective mutation in the mapped antigenic domains of HA and NA has been termed *antigenic drift*. Similarly, mutations in NA can result in resistance to antiviral neuraminidase inhibitors.

Because the IAV genome consists of eight discrete RNA segments, co-infection of one host cell with two different IAVs can result in progeny viruses containing gene segments from both initially infecting viruses (Taubenberger and Kash 2010). When this process of gene segment mixing (reassortment) involves the gene segments encoding the HA and/or NA genes, it has been termed *antigenic shift*. There are theoretically 256 possible combinations of the eight gene segments from reassortment between two parental IAV. Reassortment has been shown to be both common and important in IAV evolution (Dugan et al. 2008; Holmes et al. 2005) and host switch events (Morens, Taubenberger, and Fauci 2009).

IAVs are subdivided by antigenic characterization of the HA and NA surface glycoproteins. Sixteen HA and nine NA subtypes have been identified in avian hosts, with two other HA and NA subtypes identified in bats. Theoretically, therefore, 144 possible HA-NA subtype combinations are possible from the IAV that circulate in birds and host switch to humans and other mammals (Krauss et al. 2004; Munster et al. 2007). These HA and NA subtype combinations are abbreviated using H1–H16 and N1–N9 nomenclature; for example, H1N1 was the

subtype of the 1918 pandemic virus, while H3N2 was the subtype of the 1968 pandemic virus.

Genetically and antigenically diverse IAVs are widely distributed in wild avian species around the world. They are maintained predominantly by asymptomatic infections, most frequently documented in aquatic birds of the orders Anseriformes (ducks, geese, swans, etc.) and Charadriiformes (gulls, terns, etc.). Over 100 species of wild birds have been identified as harboring IAV (Munster et al. 2007). IAV in wild aquatic birds tend to be predominantly transmitted via a fecal-oral route and to infect epithelial cells in the lower intestinal tract where they cause little to no apparent disease.

IAV maintained in wild birds have been associated with stable host switch events to novel hosts, including domestic gallinaceous poultry (e.g., chickens and turkeys), horses, swine, dogs, and humans, leading to the emergence of viral lineages transmissible in the new host. Adaptation to domestic poultry species is the most frequent (Wright, Neumann, and Kawaoka 2013). Stable host switching likely involves the acquisition of several mutations depending on the virus and the species that serve to separate an individual, clonally derived IAV strain from the large wild bird IAV gene pool. Because adaptation to a new host likely limits the ability of these viruses to return to the wild bird IAV gene pool (Swayne 2007), these emergent viruses must evolve as distinct eight-segment genome constellations within the new host (Dugan et al. 2008; Taubenberger and Morens 2009).

Overview of Influenza Pandemics

Influenza viruses are among the most frequent causes of human respiratory infections and the most significant because they cause high morbidity and mortality. Influenza outbreaks have occurred since at least the Middle Ages, and likely since ancient times (Morens and Taubenberger 2011). In the elderly, infants, and people with chronic diseases, influenza is associated with especially high mortality. In the United States, influenza results in approximately 200,000 hospitalizations and up to 61,000 deaths in a single season. In addition to annual winter outbreaks, pandemic IAVs occasionally emerge, as they have every 8 to 41 years for at least several centuries. IAV pandemics are global outbreaks due to viruses with novel antigenic subtypes. Up to 50 percent of the population can be infected in a single pandemic year and can be associated with a dramatic increase in number of deaths. In the last 500 years, since 1510, there have been approximately 14 IAV pandemics; in the past 120 years, there were pandemics in 1889, 1918, 1957, 1968, 1977, and 2009 (Taubenberger and Morens 2009). In

1918, the worst pandemic in recorded history caused approximately 675,000 total deaths in the United States and killed up to 50–100 million people globally (Johnson and Mueller 2002). The 1957 H2N2 and 1968 H3N2 IAV pandemics caused approximately 70,000 and 34,000 excess deaths in the United States, respectively (Noble 1982). The 2009 H1N1 IAV pandemic resulted in approximately 12,500 excess deaths in the United States (CDC 2019).

The Historical Impact of the 1918 Influenza Pandemic

A little more than a century ago, the world experienced a catastrophic and unprecedented natural disaster—the influenza pandemic of 1918–1919 (Taubenberger, Kash, and Morens 2019; Jordan 1927; Taubenberger and Morens 2006). The 1918 pandemic spread and caused infections in almost all inhabited places on Earth, resulting in symptomatic disease in approximately one-third of the world's population over the course of a year. The majority of people presenting with clinical illness in the 1918 pandemic had typical, self-limiting influenza, but a disproportionate number developed lower respiratory involvement and died of the consequences of viral and bacterial co-infection and pneumonia (Morens, Taubenberger, and Fauci 2008). Case-fatality ratios in the United States were approximately 1 percent (Viboud et al. 2013), but case fatality was much higher in many developing countries (Chandra, Kuljanin, and Wray 2012) and in many crowded environments, from inner cities (Mamelund 2006) to military training camps set up during World War I (Chertow et al. 2015). The global mortality estimates range from 50 million to as high as 100 million in the first pandemic year (Johnson and Mueller 2002).

The impact of the pandemic virus—now known to be an H1N1 influenza A virus—was not, however, limited to 1918–1919. The 1918 influenza A virus became a "founder" virus, initiating a century-long pandemic era by evolving into progeny pandemic viruses through a number of separate genetic mutational and reassortment events (Morens, Taubenberger, and Fauci 2009). Since 1918, all subsequent influenza A pandemics and seasonal epidemics have been caused by descendants of the 1918 virus, including the antigenically drifted seasonal descendants of the 1918 H1N1 virus, and the reassorted pandemic viruses that appeared in 1957 (H2N2), 1968 (H3N2), and 2009 (H1N1pdm). Each of these descendant pandemic viruses contained gene segments descended from the founder 1918 virus. Some of these gene segments drifted substantially over time, and 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 gene segments that had become incorporated into newly evolving swine influenza viruses (Shope 1931; Garten et al. 2009).

Consequently, the 1918 virus was not only responsible for the millions of global deaths during the pandemic itself, but also for the millions of subsequent influenza deaths occurring during the past century of this ongoing pandemic era. In one recent year alone (2014–2015), 710,000 Americans were hospitalized for influenza and 56,000 died (Budd et al. 2018). In other years, the toll has been even higher (Thompson et al. 2003). CDC data report that the 2017–2018 influenza season resulted in the death of 61,000 Americans. Over the last century, the 1918 influenza virus has forever affected our evolving conception of pandemic influenza (Taubenberger, Hultin, and Morens 2007); public health preparedness and future pandemic planning must always consider the theoretical appearance of a future pandemic of a similar magnitude to that of 1918. The ongoing public health impact of the 1918 influenza virus, in the form of continually evolving descendant human IAV over the last 100 years, cannot be downplayed; humans have experienced previous influenza pandemics for at least 500 years, and likely before that. Significant influenza epidemics and pandemics have occurred since at least the Middle Ages, if not since ancient times (Taubenberger and Morens 2009; Morens and Taubenberger 2011; Morens et al. 2010). In the 510 years since 1510, there have been 14 influenza pandemics at irregular intervals. In the past 150 years, there were undoubted pandemics in 1889, 1918, 1957, 1968, 1977, and 2009. Thus, during this 510-year interval, recognized pandemics have appeared every 36 years on average.

Recovery and Reconstruction of the 1918 Influenza Virus

The H1N1 IAV that caused the 1918 pandemic was not identified as the etiologic agent at the time (Taubenberger, Hultin, and Morens 2007), but influenza A viruses were eventually isolated from swine in 1930 (Shope 1931) and from humans in 1933 (Smith, Andrewes, and Laidlaw 1933). Serological data from the 1930s first suggested that the 1930s "classical" swine virus and the 1918 pandemic virus were closely related antigenically, a finding later supported by viral genetic sequence analysis and by antigenic and pathogenesis studies (Taubenberger, Kash, and Morens 2019). The origin and pathogenicity of the 1918 virus, however, remained unelucidated for most of the 20th century (Jordan 1927). But in the 1990s, tiny degraded viral RNA fragments recovered from preserved lung tissues of victims of the 1918–1919 pandemic (Taubenberger, Kash, and Morens 2019; Taubenberger,

Hultin, and Morens 2007; Taubenberger et al. 1997), from a handful of the virus's many millions of victims, along with advances in viral reverse genetics, permitted the "resurrection" of infectious 1918 influenza A virus allowing for experimental studies of viral pathogenesis in high containment laboratory facilities (Qi et al. 2012; Tumpey et al. 2005). The effort to sequence the genome of the 1918 virus began in 1995 using formalin-fixed, paraffin-embedded 1918 influenza autopsy tissues in the collection of the Armed Forces Institute of Pathology (Taubenberger et al. 1997). Additional viral RNA-positive cases were subsequently identified (Xiao et al. 2013; Sheng et al. 2011; Reid et al. 2003; Reid et al. 1999), allowing the complete genome to be sequenced and reconstructed over a nine-year period. Genetic analyses of viral RNA showed high levels of viral sequence identity between cases separated by thousands of miles, and between May 1918 and February 1919. These sequence data also suggested that the 1918 pandemic virus likely derived from a wild waterfowl influenza A virus that had somehow directly or indirectly switched hosts to become a human-adapted virus. Ongoing studies with the 1918 virus have addressed questions of host adaptation, factors associated with transmission in mammals, pathogenesis, and the host inflammatory response (Taubenberger, Kash, and Morens 2019).

Disease, or Pathogenicity, Caused by the 1918 Influenza Virus

The 1918 viral genome has been studied for molecular features potentially associated with human adaptation. Results of these observations suggest that adaptive mutations in avian waterfowl IAV 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 might be multiple paths by which mutations and genetic epistasis can lead to novel host adaptation (Taubenberger, Kash, and Morens 2019; Taubenberger and Kash 2010).

Influenza pathogenicity must consider the interrelated factors of viral virulence, host inflammatory response, and secondary bacterial co-infections (Kash and Taubenberger 2015). Progression to severe disease is a multifactorial process involving viral, host, and bacterial factors. Viral virulence factors that contribute to pathogenicity of the 1918 virus have also been investigated in experimental animal infections using IAV containing one or more gene segments from the 1918 virus. Viral constructs expressing the 1918 pandemic H1 HA gene segment alone on the backbone of the remaining seven gene segments of seasonal H1N1 or H3N2 human IAV showed greater pathology in

the murine respiratory tract than the seasonal virus infections, that were characterized by a prominent infiltration of neutrophils and macrophages into lung air spaces, and virus replication in alveolar epithelial cells, analogous to that induced by the fully reconstructed 1918 virus (Qi et al. 2012; Kobasa et al. 2004; Qi et al. 2011; Qi et al. 2009).

In subsequent studies, a chimeric 1918 virus in which the 1918 H1 HA gene had been replaced by a modern wild-waterfowl-derived H1 HA gene segment was equally pathogenic in experimental animals as the 1918 virus itself (Qi et al. 2012). The severe pathogenicity associated with a virulence factor in the 1918 pandemic H1 HA seems to be shared with other wild avian H1 HAs (Qi et al. 2014; Watanabe et al. 2014). Avian influenza A viruses expressing H1 subtype HAs genetically similar to those that existed in 1918 still circulate in nature today. Therefore, an avian H1 virus is presumably capable of reemerging as a genetic component of a future pathogenic pandemic virus. Other avian IAV HA subtypes in addition to H1 were also shown to be inherent virulence factors in mammalian influenza pathogenesis. Modern avian IAV expressing H1, H6, H7, H10, and H15 subtype HAs also induced enhanced pathogenicity in experimental animals (Qi et al. 2014). The pathology caused by infection with these H1, H6, H7, H10, and H15 viruses is characterized by a marked neutrophil infiltration into lung air spaces, a principle feature of the pathological findings in infections with the 1918 virus.

Experiments have also highlighted the importance of the host inflammatory response in disease caused by the 1918 virus. 1918 virus infection induced recruitment and activation of neutrophils associated with robust activation of host inflammatory and cell death responses (Kobasa et al. 2007; Kash et al. 2006) in animal models consistent with the typical features of 1918 autopsy studies (Taubenberger, Kash, and Morens 2019; Walters et al. 2016). For example, transcriptomic analysis of a 1918 pandemic autopsy sample (Xiao et al. 2013) showed a marked concordance with the host gene expression patterns of experimentally infected mice (Kash et al. 2006) and cynomolgus macaques (Kobasa et al. 2007). An important observation with the potential for treating current and future severe influenza disease in humans, mice infected with a lethal dose of the 1918 virus subsequently treated with an anti-inflammatory drug that blocks damage caused by reactive oxygen species, induced less severe lung pathology, greater activation of tissue repair responses, and showed enhanced survival (Kash et al. 2014).

Even though the 1918 influenza pandemic virus caused tens of millions of deaths worldwide, the vast majority of those who were

infected had typical self-limited illness followed by full recovery. Influenza infections can however induce more severe disease with bronchitis, diffuse alveolar damage (DAD) in the lung, and primary viral and secondary bacterial pneumonias (Taubenberger and Morens 2008; Kuiken and Taubenberger 2008).

The features of primary influenza viral pneumonia, including DAD, pulmonary edema, and alveolar hemorrhage, were observed in autopsy studies of the 1918 pandemic and in autopsy studies of subsequent pandemics. Fatal 1918 pandemic cases were also characterized by widespread pulmonary vascular thrombus formation and prominent infiltration of lung tissue by neutrophils (Sheng et al. 2011; Walters et al. 2016; Taubenberger and Morens 2008; LeCount 1919a, 1919b; Winternitz, Wason, and McNamara 1920). Although fatal primary influenza viral pneumonias have been documented, the vast majority of severe influenza-associated pneumonias in 1918–1918 were associated with secondary bacterial co-infections (Morens, Taubenberger, and Fauci 2008). Increased incidence of secondary bacterial pneumonias in persons with influenza may be thus considered an intrinsic property of viral pathogenicity, and this is likely to be the case for other pathogenic influenza viruses. Co-infection-induced pulmonary thrombosis in 1918 likely exacerbated vascular leak and alveolar edema, limiting compensatory ventilation responses and contributing to severe hypoxia and death.

Implications of 1918 Influenza Studies and Future Outlook

Influenza has been a significant public health concern for centuries, and now, 100 years after the 1918 pandemic, it remains a huge public health issue, including the appearance of unpredictable pandemics and the predictable annual seasonal epidemic recurrences of varying severity. The historical record strongly suggests that there will be future influenza pandemics, but even with a century of advancement in our understanding of influenza viruses and how they cause disease, it is still not possible to predict when and where the next pandemic will appear, what viral subtypes they will be, or how pathogenic they will be. Greater influenza virus surveillance, especially at the animal–human interface, is critical, but we currently do not possess the knowledge to identify pre-pandemic zoonotic animal IAV before their emergence in a pandemic.

Do all avian IAV or IAV adapted to other mammals have the potential to acquire host adaptive mutations that lead to human pandemic emergence (Morens, Subbarao, and Taubenberger 2012; Morens and

Taubenberger 2010), or is such evolution prevented by structural or functional evolutionary constraints associated with adaptation to the prior host? Among the 16 IAV HAs and nine NAs known to circulate in wild waterfowl, just three of the 144 possible subtype combinations—H1N1, H2N2, and H3N2—have been observed in any human adapted or pandemic influenza A virus since 1918. An analogous situation can be observed with waterfowl influenza A viruses that have adapted to other mammalian hosts including horses (H3N8 and H7N7) and dogs (H3N2 and H3N8). Are there as yet unelucidated restraints on the ability of wild waterfowl or mammalian IAV to become human-adapted pandemic viruses? Looking backward to the century before 1918, epidemiological and archaeserological evidence is consistent with the speculative possibility that pandemics between the 1830s and 1889 may, like those of the past century, have only expressed H1, H2, or H3 HA subtypes (Taubenberger, Kash, and Morens 2019).

Human volunteer influenza A virus challenge studies have accelerated evaluation of immune correlates of protection and phase II clinical evaluations of novel therapeutics and vaccines (Walters et al. 2019; Park et al. 2018; Memoli et al. 2016; Memoli et al. 2015). Recent studies have shown that serum antibodies against the HA head and stalk along with antibodies against the NA are all correlates of protection, and yet anti-NA antibodies are the only independent predictor of a reduction of all assessed influenza clinical outcome measures (Park et al. 2018). Currently, it is difficult to predict whether an individual infected with IAV at the outset will have a mild or severe infection. Developing prognostic biomarkers of influenza infection would be of great clinical utility. In a recent study, gene expression studies of peripheral blood leukocytes identified populations of genes early in infection that correlated with active viral shedding, predicted length of shedding, and disease severity (Walters et al. 2019).

Even with our current armamentarium of antiviral drugs, antibiotics, influenza and bacterial vaccines, and intensive care treatment, we still continually observe high morbidity and mortality from influenza, both in its seasonal and pandemic forms. We need additional antiviral drugs, as antiviral resistance can emerge quickly in many IAV that circulate in humans. We also need more effective vaccines against bacteria associated with pneumonia. Most critically, however, we need effective broadly protective or "universal" influenza vaccines to prevent or at least mitigate the impact of future pandemics and to prevent deaths from seasonal influenza in the periods between pandemics (Morens and Taubenberger 2019; Krammer, Garcia-Sastre, and Palese 2017; Erbelding et al. 2018). New generation vaccines that induce long-lasting, broad immune responses against all influenza viruses, and

especially against viruses with the most pathogenic HAs found in nature, would significantly enhance public health preparedness, and would be the greatest advance in reducing the morbidity and mortality burden associated with influenza infection. For a century now, the 1918 pandemic has been a critical yardstick for measuring the crucial public health importance of influenza. Scientists continue to use insights initially garnered from tiny, degraded RNA fragments from the 1918 pandemic virus preserved in century-old autopsy tissues. Hopefully these insights from the 1918 pandemic are providing a path to better prepare for future influenza pandemics. The 1918 pandemic influenza virus continues to inform clinical and public health preparedness decisions. The threats posed by influenza remain daunting but efforts to prevent or mitigate current and future outbreaks remain an urgent, global public health necessity.

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