Published in final edited form as:

Virus Res. 2011 December ; 162(1-2): 2–7. doi:10.1016/j.virusres.2011.09.003.

Insights on influenza pathogenesis from the grave

Jeffery K. Taubenberger and John C. Kash

Viral Pathogenesis and Evolution Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Abstract

The 1918–1919 ‘Spanish’ influenza virus caused the worst pandemic in recorded history and resulted in approximately 50 million deaths worldwide. Efforts to understand what happened and to use these insights to prevent a future similar pandemic have been ongoing since 1918. In 2005 the genome of the 1918 influenza virus was completely determined by sequencing fragments of viral RNA preserved in autopsy tissues of 1918 victims, and using reverse genetics, infectious viruses bearing some or all the 1918 virus gene segments were reconstructed. These studies have yielded much information about the origin and pathogenicity of the 1918 virus, but many questions still remain.

Introduction

Influenza A viruses (IAV) pose a significant and continual threat to public health in the form of annual influenza epidemics and occasional and unpredictable pandemics, but also pose an agricultural and economic threat by the continual emergence of strains infecting domestic birds and mammals. The biology and ecology of IAV, with rapid evolution driven by various selection pressures, the production of novel genotypes through reassortment following mixed infections, and the inter-related ability of IAV to stably adapt to new avian and mammalian species, makes both the control of and predictions about influenza virus outbreaks particularly challenging (Palese and Shaw, 2007; Taubenberger and Kash, 2010; Webster et al., 1992; Wright et al., 2007).

In the United States approximately 36,000 deaths occur annually following influenza infection (Thompson et al., 2003), and the morbidity and mortality impact is even more severe in some epidemic years and in the first years of circulation of pandemic influenza virus strains (Morens et al., 2009). Planning for a future influenza pandemic, including concern about the continuing zoonotic infections of highly pathogenic avian IAV of H5N1 subtype (Peiris et al., 2007), has been in many ways informed by the worst influenza virus outbreak in recorded history, the so-called ‘Spanish’ influenza pandemic of 1918–1919 (Jordan, 1927; Taubenberger and Morens, 2006).

Influenza pandemics have emerged sporadically for at least 500 years, and it is likely that pandemics have occurred for 1000 years or more (Morens and Taubenberger, 2011). In the

*Corresponding author.s contact information: Jeffery K. Taubenberger, M.D., Ph.D., Viral Pathogenesis and Evolution Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 33 North Drive, Room 3E19A.2, MSC 3203, Bethesda, MD 20892-3203 USA, Tel. 1-301-443-5960; Fax. 1-301-480-1696; taubenbergerj@niaid.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

past century, four pandemics have occurred: 1918–1919 ‘Spanish’ H1N1 influenza; 1957–1958 ‘Asian’ H2N2 influenza; 1968–1969 ‘Hong Kong’ H3N2 influenza; and 2009–2010 ‘Swine-origin’ H1N1 influenza (Morens et al., 2009). What has become clear from the analysis of just these four pandemics, but supported by a larger historical review of remote pandemics before 1918, is that pandemic viruses are quite variable in their emergence and impact measured both by their initial pandemic and subsequent impacts as recurrent annual influenza.

The 1918–1919 pandemic is now thought to have resulted in the deaths of 675,000 people in the United States, and approximately 50 million people globally (Johnson and Mueller, 2002), a mortality impact that is hard to comprehend. What is perhaps equally important to consider however, is that with a case fatality rate of approximately 2.5% (compared to rates of <0.1% in other pandemics) (Taubenberger and Morens, 2006), the vast majority of infected individuals in 1918 (>97%) had a typical, self-limited course of influenza, without any antivirals, antibiotics, or vaccines. Other epidemiological features of the 1918–1919 pandemic were also unique, including its appearance in up to three ‘waves’ within the first year, and a “W-shaped” (tri-modal) age-specific mortality curve featuring a still unexplained peak in young adults.

The effort to identify the causative agent of pandemic influenza started prior to the 1918 pandemic (Taubenberger et al., 2007), and was intensified during and in the aftermath of the devastating outbreak. Highly pathogenic avian influenza virus was actually isolated as a filterable agent in 1901–1903 (Centanni, 1902; Lode and Gruber, 1901; Maggiora and Valenti, 1903; Morens and Taubenberger, 2010), but these early ‘fowl plague’ viruses were not recognized as influenza A viruses until the 1950.s (Schäfer, 1955).

Shope succeeded in isolating the first mammalian-adapted influenza A virus from a pig in Iowa in 1930 (Shope, 1931), followed quickly by the isolation of the first human influenza virus by Smith, Andrewes, and Laidlaw (Smith et al., 1933), and tremendous advances in our understanding of the biology, ecology, and treatment of influenza viruses occurred in the following decades. In the mid-1990s a project was initiated in the laboratory of co-author JKT to amplify and sequence small cDNA fragments of the 1918–1919 pandemic influenza viral RNA preserved in formalin-fixed, paraffin-embedded autopsy lung tissues of fatal cases archived in the National Tissue Repository of the Armed Forces Institute of Pathology in Washington, DC [reviewed in (Taubenberger et al., 1997)]. In 1997, in collaboration with Dr. Johan Hultin, viral RNA fragments were also obtained from frozen, unfixed lung tissue of a 1918 influenza victim buried in the permafrost in northern Alaska. While RNA quality was not better than in the fixed tissue samples, the amount of frozen lung tissue obtained greatly facilitated the completion of the genome of the 1918 pandemic virus (Reid et al., 1999). In 2005, after a nine-year effort, the 1918 viral genome was completed (Taubenberger et al., 2005), and in a collaborative, multi-center effort, the 1918 virus was reconstructed by plasmid-based reverse genetics and its pathogenicity was first assessed in mice (Tumpey et al., 2005). This brief review will discuss a variety of insights into the biology and pathophysiology of the sequenced and reconstructed 1918 influenza virus.

Insights from the 1918 genome on the origin and emergence of pandemic influenza

Obtaining the genomic sequence of the 1918 influenza virus has provided important information about its origin, but with the limited samples available from 1918 and before, a number of unanswered questions remain. In 2009, the first pandemic of the 21st Century, and the first pandemic in 41 years was initially detected in Mexico and the Southwest United States. The 2009 pandemic H1N1 virus was a reassortant of two well-known swine

influenza viral lineages (Garten et al., 2009), each circulating for over a decade prior to the emergence of the pandemic virus (Dunham et al., 2009; Webby et al., 2004). However, despite the unprecedented current scale of human and animal influenza surveillance globally, and the near real-time deposition in public databases of thousands of influenza A viral genomes, the timing and place of origin, the host species in which the reassortment event took place, and characterization of the genome changes that were required for adaptation of the virus to humans (and how long this process took) remain unanswered (Lam et al., 2011; Smith et al., 2009b). The 1957 and 1968 pandemic viruses are also known to be reassortant viruses in which a virus of then circulating human viruses acquired three and two avian influenza-like gene segments, respectively by reassortment [reviewed in (Taubenberger and Kash, 2010)]. Yet, similar to the 2009 pandemic virus, the place, timing, and host species of these reassortment events are not known. Also unanswered for all these pandemics is how long these nascent zoonotic viruses circulated (and in what host) before the pandemic was recognized in each case.

Given the above uncertainties about the origin of pandemics occurring in the virology era, it is even more challenging to understand the origin of the 1918 influenza, with our lack of definitive information about which influenza viruses circulated in humans and animals before the 1918 pandemic. As no human pre-1918 IAV sequences are currently available, the origin of the pandemic virus, including timing of its emergence in humans, whether an intermediate host was involved, and whether the virus was a reassortant virus, remain unresolved (Smith et al., 2009a; Taubenberger and Morens, 2006).

The 1918 influenza pandemic had another unique feature, the likely simultaneous (or nearly simultaneous) infection of humans and swine. It has been suggested that the 1918 virus adapted in swine in the decade before 1918 (dos Reis et al., 2009; Kanegae et al., 1994; Smith et al., 2009a) using phylogenetic models. Interestingly, however, no convincing data exists in the medical or veterinary literature for outbreaks consistent with swine influenza prior to the main peak of the 1918 outbreak (Dorset et al., 1922; Morens and Taubenberger, 2010; Taubenberger et al., 2001). Contemporary investigators were convinced that the influenza virus had not circulated as an epizootic disease in swine before 1918 and that the infection spread from humans to pigs because the swine epizootics occurred after the pandemic had started in people (Shope, 1936). Regression analyses based on human and classical swine H1N1 virus sequences have placed the 1918 precursor virus in the 1915–1918 timeframe (Taubenberger et al., 2001). The identification of pre-1918 human and swine influenza A virus-positive samples will likely be needed to definitively answer questions about the timing of the origin and evolution of human and classical swine H1N1 viruses in relation to the 1918 pandemic.

The evolutionary relationships between the 1918 influenza virus and subsequent classical swine viruses were highlighted with the unexpected emergence of the 2009 H1N1 pandemic. Because the 2009 pandemic H1N1 virus contains the HA gene derived from the classical swine H1N1 lineage, it is antigenically very similar not only to classical swine H1N1 viruses, but also to the 1918 virus (Easterbrook et al., 2011; Kash et al., 2010), the likely ancestor of both human and classical swine H1N1 lineages. Consequently, seroepidemiologic studies demonstrated cross-protective immunity in the population, primarily in people >60 years (Hancock et al., 2009). In a recent set of experiments, it was shown that mice vaccinated with the monovalent 2009 pandemic H1N1 vaccine were completely protected in a lethal challenge model with the 1918 influenza virus. These data suggest that either prior infection or vaccination with the 2009 pandemic H1N1 virus may protect the population from a possible future 1918 or 1918-like pandemic (Easterbrook et al., 2011; Kash et al., 2010).

Analyses of the 1918 genome by comparison of the amino acid sequences of its encoded proteins (Taubenberger et al., 2005) and by genome nucleotide composition have also suggested that the 1918 pandemic virus was avian influenza virus-like. Avian IAV have a higher GC nucleotide composition than influenza viruses adapted to humans (Greenbaum et al., 2008; Rabadan et al., 2006). All the gene segments from the 1918 virus have a genomic GC content similar to avian IAV, supporting an avian derivation for the pandemic virus. Similar changes in nucleotide composition with a decline in GC content were also shown for swine-adapted IAV (Dunham et al., 2009), suggesting a common but still poorly understood selection pressure as viruses adapted to birds adapt to mammalian hosts.

Insights from the 1918 genome on host adaptation

The genome of the 1918 pandemic virus has also been used to model hypotheses regarding host adaptation. These questions are more broadly applicable than just trying to understand how the 1918 pandemic virus emerged; it has been hoped that these lessons would also be useful to understanding basic mechanisms to help prevent or mitigate future pandemic threats. The mutations underlying the ability of avian IAV to switch hosts and infect humans and other mammals, and what mutations might allow these viruses to become transmissible in these new hosts are poorly understood. Biological properties of IAV, including human infectivity, transmissibility, and pathogenicity, all appear to be complex and polygenic (Parrish et al., 2008; Taubenberger and Kash, 2010). Further complicating these questions are the inter-related natures of host adaptation, transmissibility, and virulence/pathogenicity, as these are likely independent, polygenic, and possibly competing viral properties.

As discussed, the coding sequences of the 1918 pandemic virus are very avian IAV-like, and a relatively small number of amino acid changes in each protein distinguish human IAV strains from avian IAV, and it has been hypothesized that a subset of these changes might be critically important in human adaptation (Finkelstein et al., 2007; Reid et al., 1999; Reid et al., 2004a; Reid et al., 2002; Reid et al., 2000; Reid et al., 2004b; Taubenberger et al., 2005). A factor complicating these analyses is that 5 of the 8 gene segments introduced with the 1918 pandemic virus (segments 1 [PB2], 3 [PA], 5 [NP], 7 [M], and 8 [NS]) were retained in the reassortment events leading to the 1957 H2N2 and 1968 H3N2 pandemics and thus have circulated in humans monophyletically in both pandemic and seasonal human H1N1, H2N2, and H3N2 IAVs from 1918 to 2011 (represented currently by seasonal H3N2 viruses). The novel reassortant 2009 swine-origin H1N1 pandemic virus also retains 3 segments derived from the classical swine H1N1 lineage (and thus ultimately also derived from the 1918 pandemic virus – segment 4 [HA], 5 [NP], and 8 [NS]). Thus, all the pandemic viruses since 1918 contain at least some gene segments ultimately derived from the 1918 virus, and consequently, it has been suggested that the past 93 years are part of a larger single “pandemic era” with the 1918 pandemic strain as a founder virus (Morens et al., 2009).

When independent avian-to-mammalian host switch events are examined, however, the data strongly suggest a lack of parallel evolution (Dunham et al., 2009; Taubenberger and Kash, 2010). Thus, the amino acid changes that define the 1918 virus were in general not observed in unrelated mammalian adapted IAV lineages, like the European avian-like swine H1N1 viruses that emerged in late 1970s. Similarly, the emergence of the 2009 H1N1 pandemic further demonstrated the lack of parallel evolution and demonstrated the independent and polygenic nature of individual IAV host switch events (Herfst et al., 2010; Jagger et al., 2010; Mehle and Doudna, 2009). Amino acid changes in the 1918 genome that have been associated with mammalian adaptation in experimental systems include changes in the hemagglutinin (HA) receptor-binding domain, and changes in the viral polymerase complex (reviewed in (Taubenberger and Kash, 2010)), summarized briefly below.

Viral attachment is mediated by the HA surface glycoprotein binding to cellular receptor glycans terminating in sialic acids (SA) linked in different configurations to underlying sugars. The amino acids making up the HA receptor-binding domain are conserved in avian IAV, which have specificity for $\alpha 2-3$ SA, but key changes have been observed in IAVs adapted to human and other mammals including sites 138, 190, 194, 225, 226, and 228 (H3 numbering), which have been shown to enhance specificity for $\alpha 2-6$ SA. In H1 subtype HAs, changes at residues 190 and 225 have been shown to be very important for enhancing specificity for $\alpha 2-6$ SA, while changes at residues 226 and 228 have been shown to enhance $\alpha 2-6$ SA specificity in H2 and H3 subtype HAs (Wright et al., 2007). The receptor-binding domain of the 1918 HA gene was sequenced from five 1918 autopsy cases and these sequences differed at HA residue 225 (wherein 2 strains retained the avian glycine, and 3 cases had a mutation at this codon to aspartic acid, a residue commonly seen at this site in post-1918 seasonal H1N1 virus strains (Reid et al., 2003). Structural analyses and *in vitro* binding assays have shown that the 1918 HA with glycine at 225 has a mixed $\alpha 2-3/\alpha 2-6$ SA binding specificity, whereas the 1918 HA with aspartic acid at 225 has an $\alpha 2-6$ SA binding specificity. Even so, there were no apparent differences in the clinical courses, or pathological findings between these cases (Reid et al., 2003).

In recent work, additional 1918 virus HA receptor-binding domain sequences have been determined (Sheng et al., 2011), including several cases from before the pandemic peak. These new sequences suggest a possible chronological trend from more avian-like to more human-like receptor binding with later cases more likely to bear an aspartic acid at residue 225. Interestingly, when viral cellular tropism was examined in these 1918 autopsy samples by immunohistochemistry, there was no apparent difference. Isogenic IAVs expressing the 1918 HA with different receptor-binding specificities were all pathogenic in mice (Qi et al., 2009) and ferrets (Tumpey et al., 2007), but a 1918 virus construct with only an $\alpha 2-3$ binding specificity was not transmissible in ferrets. In summary, HA receptor-binding changes are likely associated with host-switch events, but 1918 viruses that retain a mixed $\alpha 2-3/\alpha 2-6$ SA binding specificity were able to cause lethal infections in people, which has been supported in animal models.

Mutations in the genes encoding the IAV polymerase complex have also been associated with host switch events in IAV. The RNA-dependent RNA-polymerase gene segment PB1 was replaced by reassortment with an avian IAV-derived PB1 segment in both the 1957 and 1968 pandemics (Wright et al., 2007), and the PB1 gene in the 1918 virus was shown to be very avian IAV-like (Taubenberger et al., 2005). The PA and NP genes have also been associated with host restriction, and several mutations in each gene have been proposed for human adaptation (Finkelstein et al., 2007; Reid et al., 2004a; Taubenberger et al., 2005), but further experiments to assess the significance of these proposed changes is needed.

The polymerase protein PB2 has also long been identified as an important host switch determinant, especially a mutation at amino acid 627 (Subbarao et al., 1993), and as a virulence factor in mammalian host models. Residue 627 is usually a glutamine in avian IAV, while the 1918 virus and subsequent human viruses encode a lysine. Residues 701–702 have been implicated in nuclear localization (Gabriel et al., 2008; Tarendeau et al., 2007). Independent emergence of changes at residue 701 has also been observed - in avian-origin European swine H1N1 viruses (Dunham et al., 2009), and in some highly pathogenic avian H5N1 viruses.

With the emergence of the H1N1 pandemic virus in 2009, it became clear that these above changes in PB2 were not necessary for human adaptation, as the independent origin of the pandemic PB2 (not derived from either the 1918 viral or European avian-like swine H1N1 lineages) did not possess these key changes at 627, 701, or 702 (Garten et al., 2009). In fact,

in experimental systems when these mutations were engineered into viruses expressing the 2009 pandemic virus polymerase complex, no enhanced replication or pathogenicity was observed (Herfst et al., 2010; Jagger et al., 2010). Recently mutations in two adjacent residues (590–591) were proposed as an alternate strategy for human adaptation (Mehle and Doudna, 2008). Thus, the 2009 pandemic has demonstrated clearly that host adaptation mutations in a past pandemic virus like 1918 might not be predictive of a future pandemic the emerges independently.

Insights from the 1918 genome on virulence and pathogenicity

As described above, the 1918 influenza virus caused the most severe pandemic on record, with a case fatality rate of approximately 2.5%. Data collected in the aftermath of the 1918 pandemic and recently reviewed show that the vast majority of fatal cases were associated with secondary or co-incident bacterial pneumonias (Morens et al., 2008). Various common pneumopathogenic Gram-positive bacteria, predominantly streptococci and staphylococci, were most frequently cultured from the lungs at autopsy. Even in the antibiotic era, bacterial pneumonias were seen in the majority of fatal infections in the 1957, 1968, and 2009 pandemics (Gill et al., 2010; Mulder and Hers, 1972). This suggests that bacterial co-infections play a significant role in morbidity and mortality in pandemic influenza infection in general, and further that the high incidence of bacterial co-infections in fatal cases can be thought of as a marker of the inherent pathogenicity of primary influenza viral pneumonias. Proposed mechanisms for enhanced disease in influenza viral/bacterial co-infection include increased colonization of the upper respiratory tract and bacterial-viral synergistic co-pathogenesis, influenza viral denuding of the respiratory epithelium that could increase the rate of colonization by bacteria in the nasopharynx, exposure of bacterial attachment sites through the activity of influenza viral neuraminidase (McCullers, 2006), and loss of airway epithelial cell repopulation and repair (Kash et al., 2011). Autopsy studies have shown that the range of histopathology associated with fatal 1918 influenza viral infections is asynchronous, and a mixture of changes associated with primary viral infection, secondary bacterial pneumonias, and repair and remodeling (Kuiken and Taubenberger, 2008; Taubenberger and Morens, 2008). Importantly, the 1918 virus apparently did not induce a unique set of pathologic changes as compared with other pandemic influenza viruses, including the 2009 H1N1 virus (Gill et al., 2010).

Mapping viral virulence and host factors in influenza virus pathogenesis studies is complicated by the animal models generally employed. Mice are commonly used, but clearly are not ideal models of human disease. Ferret models may better match human infection and transmission, but the lack of reagents and their outbred nature, make characterization of host factors involved in pathogenesis difficult. Nonhuman primates experiments are difficult to perform, and no clear data are available on whether these species are better models of human influenza infection than mice or ferrets.

Whether or not it can be related directly to human pathogenicity, the 1918 influenza virus is pathogenic without prior adaptation in mouse, ferret, and macaque models. However, these studies have allowed for molecular virologic mapping of associated virulence motifs, and an examination of changes in host response, particularly when compared to less pathogenic IAV, like seasonal H1N1 viruses. Comparison of the fully reconstructed 1918 virus with constructs containing various combinations of gene segments from the 1918 virus and gene segments from a non-pathogenic human seasonal H1N1 strain (A/Texas/36/1991) (Kash et al., 2004; Pappas et al., 2008; Tumpey et al., 2005; Tumpey et al., 2004) showed that the hemagglutinin gene (HA) in particular, but also the neuraminidase (NA), and the polymerase PB1 each encoded murine virulence factors. None of the chimeric viruses retained the pathogenicity of the fully reconstructed 1918 virus, strongly suggesting that

pathogenicity is polygenic (Tumpey et al., 2005). Similar results were observed with other chimeric viruses in which the 1918 HA (with or without the 1918 NA) was expressed in seasonal influenza virus backgrounds using A/Kawasaki/173/2001 (H1N1), A/Memphis/8/88 (H3N2) (Kobasa et al., 2004), or A/New York/312/2001 (H1N1) (Qi et al., 2009).

All of these studies have shown that the 1918 HA gene seems to encode a virulence factor in mice. While the HA serves as a primary virulence factor in poultry in highly pathogenic avian influenza viruses of H5 or H7 subtypes because of their expanded polybasic cleavage site, the 1918 virus does not possess a polybasic cleavage site. Isogenic IAV constructs expressing the 1918 HA with different receptor-binding specificities were all shown to be pathogenic in mice (Qi et al., 2009) and ferrets (Tumpey et al., 2007), but the 1918 HA viral construct with a receptor-binding configuration specific to $\alpha 2-3$ SA did not transmit between ferrets. In recent work, isogenic New York/312/2001 (H1N1) viruses expressing as 7:1 viruses the HA genes of the four extant pandemic viruses (1918 H1, 1957 H2, 1968 H3 and 2009 H1) were all shown to be pathogenic in a mouse model (Qi et al., 2011). The viruses expressing the pandemic HAs were all associated with significant histopathology in the lower respiratory tract, including acute inflammation, viral tropism for alveolar epithelium and alveolar macrophages, and showed low *in vitro* binding activity for surfactant protein D (SP-D). In contrast, the HAs from seasonal influenza strains (H1 and H3) induced only mild disease with little lung pathology in infected mice and exhibited strong *in vitro* binding to SP-D. These data demonstrate that the 1918 virus is not unique in having virulence factors encoded by its HA gene. How structural changes in the 1918 HA and in the HA of other pandemic IAV relate to virulence is still poorly understood.

The gene segments encoding the proteins of the viral polymerase complex have also been shown to contain virulence factors in mouse and ferret models. Replacing the PB1 gene segment of seasonal H1N1 viruses with the 1918 PB1 enhanced viral replication *in vitro* and in mice and ferrets (Pappas et al., 2008; Watanabe et al., 2009), and in a complementary experiment, replacing the PB1 in the 1918 virus with the PB1 from seasonal influenza strain attenuated the 1918 virus in mice (Pappas et al., 2008). This segment also encodes a second protein, PB1-F2 (Chen et al., 2001), which is highly variable in avian IAV, and is variably encoded by IAV adapted to humans and swine. The PB1-F2 of the 1918 virus has also been shown to be a virulence factor, specifically related to a mutation at amino acid 66, also observed in some highly pathogenic H5N1 viruses (Conenello et al., 2007), but also possibly by enhancing secondary bacterial pneumonias (McAuley et al., 2007). Viruses encoding the four gene segments of the 1918 polymerase complex (PB2, PB1, PA, and NP) in the context of the remaining genes from seasonal H1N1 viruses (A/Kawasaki/173/2001 or A/New York/312/2001) showed enhanced pathogenicity in mice and ferret models (Jagger et al., 2010; Watanabe et al., 2009). Interestingly, isogenic chimeric viruses expressing the 1918 polymerase complex but with the single mutation of PB2 residue 627 lysine changed to the consensus avian IAV glutamic acid were highly attenuated (Jagger et al., 2010).

Experimental studies in mouse and non-human primate models with the fully reconstructed 1918 virus have revealed host molecular mechanisms that may partially explain the pathogenicity of the virus in humans. Comparison of a seasonal human H1N1 virus (A/Texas/36/1991) with the reconstructed 1918 influenza virus in a murine model showed that infection with the 1918 virus resulted in severe disease, with high viral lung titers, with death at 4–5 days post-infection, characterized by severe pulmonary lesions and severe necrotizing bronchitis with accompanying severe alveolitis and edema (Kash et al., 2006). Expression microarray analysis of the lung response to 1918 virus infection demonstrated a profound activation of immune cell-related genes and inflammatory and cellular death receptor response genes. These results suggest that a component of the severe lung pathology during 1918 infection was immunopathogenic and resulted from increased

immune cell killing. Similarly, cynomolgus macaques (*Macaca fascicularis*) infected with the 1918 virus similarly resulted in a uniformly lethal disease with signs of severe viral pneumonia (Kobasa et al., 2007). Analysis of the host response to infection in bronchial tissue revealed that 1918 viral infection was associated with a suppression of type I interferon response, as compared to seasonal H1N1 virus infection, and an increased expression of pro-inflammatory chemokines. These studies showed that the 1918 influenza virus was very efficient at suppressing cellular defense and antiviral responses, while resulting in significant activation of inflammatory mediators and immune cell populations.

Conclusions

The fifteen years of effort to find 1918 case material containing RNA fragments of the pandemic virus, determining the genomic sequence, and studying the origin and pathogenicity of the virus has yielded many insights, but much work is still needed to explain the epidemiological and pathophysiological consequences of this severe pandemic (Taubenberger et al., 2007). While understanding what happened in 1918 has great historical and biological interest, most relevant for the future will be to understand more fully how IAV host adaptation occurs and to identify both virulence factors but also novel therapeutic strategies based on these insights to help prevent or mitigate a future pandemic influenza virus.

Acknowledgments

This work was supported by the intramural funds of the NIH and the NIAID.

References

- Centanni E. Die Vogelpest. Beitrag zu dem durch Kerzen filtrierbaren Virus [Fowl plague. Report on the candle filterable virus]. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten 1 Abteilung: medizinische-hygienische Bakteriologie und tierische Parasitenkunde. 1902; 31:145–152.
- Chen W, Calvo PA, Malide D, Gibbs J, Schubert U, Bacik I, Basta S, O'Neill R, Schickli J, Palese P, Henklein P, Bennis J, Yewdell JW. A novel influenza A virus mitochondrial protein that induces cell death. Nat Med. 2001; 7(12):1306–1312. [PubMed: 11726970]
- Conenello GM, Zamarin D, Perrone LA, Tumpey T, Palese P. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. PLoS Pathog. 2007; 3(10):1414–1421. [PubMed: 17922571]
- Dorset M, McBryde CN, Niles WB. Remarks on hog flu. Journal of the American Veterinary Medical Association. 1922; 62:162–171.
- dos Reis M, Hay AJ, Goldstein RA. Using non-homogeneous models of nucleotide substitution to identify host shift events: application to the origin of the 1918 'Spanish' influenza pandemic virus. Journal of Molecular Evolution. 2009; 69(4):333–345. [PubMed: 19787384]
- Dunham EJ, Dugan VG, Kaser EK, Perkins SE, Brown IH, Holmes EC, Taubenberger JK. Different evolutionary trajectories of European avian-like and classical swine H1N1 influenza A viruses. J Virol. 2009; 83(11):5485–5494. [PubMed: 19297491]
- Easterbrook JD, Kash JC, Sheng ZM, Qi L, Gao J, Kilbourne ED, Eichelberger MC, Taubenberger JK. Immunization with 1976 swine H1N1- or 2009 pandemic H1N1-inactivated vaccines protects mice from a lethal 1918 influenza infection. Influenza and Other Respiratory Viruses. 2011; 5(3):198–205. [PubMed: 21477139]
- Finkelstein DB, Mukatira S, Mehta PK, Obenauer JC, Su X, Webster RG, Naeve CW. Persistent host markers in pandemic and H5N1 influenza viruses. J Virol. 2007; 81(19):10292–10299. [PubMed: 17652405]

- Gabriel G, Herwig A, Klenk HD. Interaction of polymerase subunit PB2 and NP with importin alpha 1 is a determinant of host range of influenza A virus. *PLoS Pathog.* 2008; 4(2):e11. [PubMed: 18248089]
- Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, Sessions WM, Xu X, Skepner E, Deyde V, Okomo-Adhiambo M, Gubareva L, Barnes J, Smith CB, Emery SL, Hillman MJ, Rivailler P, Smagala J, de Graaf M, Burke DF, Fouchier RA, Pappas C, Alpuche-Aranda CM, Lopez-Gatell H, Olivera H, Lopez I, Myers CA, Faix D, Blair PJ, Yu C, Keene KM, Dotson PD Jr, Boxrud D, Sambol AR, Abid SH, St George K, Bannerman T, Moore AL, Stringer DJ, Blevins P, Demmler-Harrison GJ, Ginsberg M, Kriner P, Waterman S, Smole S, Guevara HF, Belongia EA, Clark PA, Beatrice ST, Donis R, Katz J, Finelli L, Bridges CB, Shaw M, Jernigan DB, Uyeki TM, Smith DJ, Klimov AI, Cox NJ. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science.* 2009; 325(5937):197–201. [PubMed: 19465683]
- Gill JR, Sheng ZM, Ely SF, Guinee DG, Beasley MB, Suh J, Deshpande C, Mollura DJ, Morens DM, Bray M, Travis WD, Taubenberger JK. Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. *Arch Pathol Lab Med.* 2010; 134(2):235–243. [PubMed: 20121613]
- Greenbaum BD, Levine AJ, Bhanot G, Rabadan R. Patterns of evolution and host gene mimicry in influenza and other RNA viruses. *PLoS Pathog.* 2008; 4(6):e1000079. [PubMed: 18535658]
- Hancock K, Veguilla V, Lu X, Zhong W, Butler EN, Sun H, Liu F, Dong L, DeVos JR, Gargiullo PM, Brammer TL, Cox NJ, Tumpey TM, Katz JM. Cross- reactive antibody responses to the 2009 pandemic H1N1 influenza virus. *The New England Journal of Medicine.* 2009; 361(20):1945–1952. [PubMed: 19745214]
- Herfst S, Chutinimitkul S, Ye J, de Wit E, Munster VJ, Schrauwen EJ, Bestebroer TM, Jonges M, Meijer A, Koopmans M, Rimmelzwaan GF, Osterhaus AD, Perez DR, Fouchier RA. Introduction of virulence markers in PB2 of pandemic swine-origin influenza virus does not result in enhanced virulence or transmission. *J Virol.* 2010; 84(8):3752–3758. [PubMed: 20130063]
- Jagger BW, Memoli MJ, Sheng ZM, Qi L, Hrabal RJ, Allen GL, Dugan VG, Wang R, Digard P, Kash JC, Taubenberger JK. The PB2-E627K mutation attenuates viruses containing the 2009 H1N1 influenza pandemic polymerase. *mBio.* 2010; 1(1):e00067–e00010. [PubMed: 20689744]
- Johnson NP, Mueller J. Updating the accounts: global mortality of the 1918–1920 "Spanish" influenza pandemic. *Bulletin of the History of Medicine.* 2002; 76(1):105–115. [PubMed: 11875246]
- Jordan, EO. *Epidemic Influenza: A Survey.* Chicago: American Medical Association; 1927.
- Kanegae Y, Sugita S, Shortridge KF, Yoshioka Y, Nerome K. Origin and evolutionary pathways of the H1 hemagglutinin gene of avian, swine and human influenza viruses: cocirculation of two distinct lineages of swine virus. *Arch Virol.* 1994; 134(1–2):17–28. [PubMed: 8279953]
- Kash JC, Basler CF, Garcia-Sastre A, Carter V, Billharz R, Swayne DE, Przygodzki RM, Taubenberger JK, Katze MG, Tumpey TM. Global host immune response: pathogenesis and transcriptional profiling of type A influenza viruses expressing the hemagglutinin and neuraminidase genes from the 1918 pandemic virus. *J Virol.* 2004; 78(17):9499–9511. [PubMed: 15308742]
- Kash JC, Qi L, Dugan VG, Jagger BW, Hrabal RJ, Memoli MJ, Morens DM, Taubenberger JK. Prior infection with classical swine H1N1 influenza viruses is associated with protective immunity to the 2009 pandemic H1N1 virus. *Influenza and Other Respiratory Viruses.* 2010; 4(3):121–127. [PubMed: 20409208]
- Kash JC, Tumpey TM, Proll SC, Carter V, Perwitasari O, Thomas MJ, Basler CF, Palese P, Taubenberger JK, Garcia-Sastre A, Swayne DE, Katze MG. Genomic analysis of increased host immune and cell death responses induced by 1918 influenza virus. *Nature.* 2006; 443(7111):578–581. [PubMed: 17006449]
- Kash JC, Walters KA, Davis AS, Sandouk A, Jagger BW, Chertow DS, Qi L, Kuestner RE, Ozinsky A, Taubenberger JK. Lethal synergism of 2009 pandemic H1N1 influenza virus and *Streptococcus pneumoniae* co-infection is associated with loss of murine lung repair responses. *mBio.* 2011 In press.
- Kobasa D, Jones SM, Shinya K, Kash JC, Copps J, Ebihara H, Hatta Y, Kim JH, Halfmann P, Hatta M, Feldmann F, Alimonti JB, Fernando L, Li Y, Katze MG, Feldmann H, Kawaoka Y. Aberrant

- innate immune response in lethal infection of macaques with the 1918 influenza virus. *Nature*. 2007; 445(7125):319–323. [PubMed: 17230189]
- Kobasa D, Takada A, Shinya K, Hatta M, Halfmann P, Theriault S, Suzuki H, Nishimura H, Mitamura K, Sugaya N, Usui T, Murata T, Maeda Y, Watanabe S, Suresh M, Suzuki T, Suzuki Y, Feldmann H, Kawaoka Y. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. *Nature*. 2004; 431(7009):703–707. [PubMed: 15470432]
- Kuiken T, Taubenberger JK. The pathology of human influenza revisited. *Vaccine*. 2008; 26(Suppl 4):D59–D66. [PubMed: 19230162]
- Lam TT, Zhu H, Wang J, Smith DK, Holmes EC, Webster RG, Webby R, Peiris JM, Guan Y. Reassortment Events Among Swine Influenza A Viruses in China: Implications for the Origin of the 2009 Influenza Pandemic. *Journal of Virology*. 2011 E-published July 29, 2011.
- Lode A, Gruber F. Bakteriologische Studien über die Aetiologie einer epidemischen Erkrankung der Hühner in Tirol 1901 [Bacteriological studies on the aetiology of epidemic illness of chickens in Tirol 1901]. *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten 1 Abteilung: medizinische-hygienische Bakteriologie und tierische Parasitenkunde*. 1901; 30:593–604.
- Maggiora A, Valenti G. Ueber eine Seuche von exsudativem Typhus bei Hühnern [Mittheilung [Regarding an epidemic of exudative typhus in chickens Part I]. *Zeitschrift für Hygiene und Infektionskrankheiten; medizinische Mikrobiologie, Immunologie und Virologie*. 1903; 42:185–243.
- McAuley JL, Hornung F, Boyd KL, Smith AM, McKeon R, Bennink J, Yewdell JW, McCullers JA. Expression of the 1918 influenza A virus PB1-F2 enhances the pathogenesis of viral and secondary bacterial pneumonia. *Cell Host & Microbe*. 2007; 2(4):240–249. [PubMed: 18005742]
- McCullers JA. Insights into the interaction between influenza virus and pneumococcus. *Clinical Microbiology Reviews*. 2006; 19(3):571–582. [PubMed: 16847087]
- Mehle A, Doudna JA. An inhibitory activity in human cells restricts the function of an avian-like influenza virus polymerase. *Cell Host & Microbe*. 2008; 4(2):111–122. [PubMed: 18692771]
- Mehle A, Doudna JA. Adaptive strategies of the influenza virus polymerase for replication in humans. *Proc Natl Acad Sci U S A*. 2009; 106(50):21312–21316. [PubMed: 19995968]
- Morens DM, Taubenberger JK. Historical thoughts on influenza viral ecosystems, or behold a pale horse, dead dogs, failing fowl, and sick swine. *Influenza and Other Respiratory Viruses*. 2010; 4(6):327–337. [PubMed: 20958926]
- Morens DM, Taubenberger JK. Pandemic influenza: certain uncertainties. *Reviews in Medical Virology*. 2011; 21(5):262–284.
- Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis*. 2008; 198(7):962–970. [PubMed: 18710327]
- Morens DM, Taubenberger JK, Fauci AS. The Persistent Legacy of the 1918 Influenza Virus. *N Engl J Med*. 2009; 361(3):225–229. [PubMed: 19564629]
- Mulder, J.; Hers, JFP. *Influenza*. Groningen: Wolters-Noordhoff Publishing; 1972. p. 287
- Palese, P.; Shaw, ML. *Orthomyxoviridae: the viruses and their replication*. In: Knipe, DM.; Howley, PM., editors. *Fields Virology*. 5th ed.. Vol. 2 2 vols. Philadelphia: Lippincott, Williams & Wilkins; 2007. p. 1647–1690.
- Pappas C, Aguilar PV, Basler CF, Solorzano A, Zeng H, Perrone LA, Palese P, Garcia-Sastre A, Katz JM, Tumpey TM. Single gene reassortants identify a critical role for PB1, HA, and NA in the high virulence of the 1918 pandemic influenza virus. *Proc Natl Acad Sci U S A*. 2008; 105(8):3064–3069. [PubMed: 18287069]
- Parrish CR, Holmes EC, Morens DM, Park EC, Burke DS, Calisher CH, Laughlin CA, Saif LJ, Daszak P. Cross-species virus transmission and the emergence of new epidemic diseases. *Microbiol Mol Biol Rev*. 2008; 72(3):457–470. [PubMed: 18772285]
- Peiris JS, de Jong MD, Guan Y. Avian influenza virus (H5N1): a threat to human health. *Clin Microbiol Rev*. 2007; 20(2):243–267. [PubMed: 17428885]
- Qi L, Kash JC, Dugan VG, Jagger BW, Lau YF, Sheng ZM, Crouch EC, Hartshorn KL, Taubenberger JK. The ability of pandemic influenza virus hemagglutinins to induce lower respiratory pathology

- is associated with decreased surfactant protein D binding. *Virology*. 2011; 412(2):426–434. [PubMed: 21334038]
- Qi L, Kash JC, Dugan VG, Wang R, Jin G, Cunningham RE, Taubenberger JK. Role of sialic acid binding specificity of the 1918 influenza virus hemagglutinin protein in virulence and pathogenesis for mice. *J Virol*. 2009; 83(8):3754–3761. [PubMed: 19211766]
- Rabadan R, Levine AJ, Robins H. Comparison of avian and human influenza A viruses reveals a mutational bias on the viral genomes. *J Virol*. 2006; 80(23):11887–11891. [PubMed: 16987977]
- Reid AH, Fanning TG, Hultin JV, Taubenberger JK. Origin and evolution of the 1918 "Spanish" influenza virus hemagglutinin gene. *Proc Natl Acad Sci U S A*. 1999; 96(4):1651–1656. [PubMed: 9990079]
- Reid AH, Fanning TG, Janczewski TA, Lourens RM, Taubenberger JK. Novel origin of the 1918 pandemic influenza virus nucleoprotein gene. *J Virol*. 2004a; 78(22):12462–12470. [PubMed: 15507633]
- Reid AH, Fanning TG, Janczewski TA, McCall S, Taubenberger JK. Characterization of the 1918 "Spanish" influenza virus matrix gene segment. *J Virol*. 2002; 76(21):10717–10723. [PubMed: 12368314]
- Reid AH, Fanning TG, Janczewski TA, Taubenberger JK. Characterization of the 1918 "Spanish" influenza virus neuraminidase gene. *Proc Natl Acad Sci U S A*. 2000; 97(12):6785–6790. [PubMed: 10823895]
- Reid AH, Janczewski TA, Lourens RM, Elliot AJ, Daniels RS, Berry CL, Oxford JS, Taubenberger JK. 1918 influenza pandemic caused by highly conserved viruses with two receptor-binding variants. *Emerg Infect Dis*. 2003; 9(10):1249–1253. [PubMed: 14609459]
- Reid AH, Taubenberger JK, Fanning TG. Evidence of an absence: the genetic origins of the 1918 pandemic influenza virus. *Nat Rev Microbiol*. 2004b; 2(11):909–914. [PubMed: 15494747]
- Schäfer W. Vergleichende sero-immunologische Untersuchungen über die Viren der Influenza und klassischen Geflügelpest [Comparative sero-immunological investigations on the viruses of influenza and classical fowl plague]. *Zeitschrift für Naturforschung*. 1955; 10b:81–91.
- Sheng ZM, Chertow DS, Ambroggio X, McCall S, Przygodzki RM, Cunningham RE, Maximova OA, Kash JC, Morens DM, Taubenberger JK. Autopsy Series of 68 Cases Dying Before and During the 1918 Influenza Pandemic Peak: Virological and Pathological Findings. *Proc Natl Acad Sci U S A*. 2011 In press.
- Shope RE. Swine influenza. Experimental transmission and pathology. *J Exp Med*. 1931; 54:349–359. [PubMed: 19869922]
- Shope RE. The Incidence of Neutralizing Antibodies for Swine Influenza Virus in the Sera of Human Beings of Different Ages. *J Exp Med*. 1936; 63(5):669–684. [PubMed: 19870496]
- Smith GJ, Bahl J, Vijaykrishna D, Zhang J, Poon LL, Chen H, Webster RG, Peiris JS, Guan Y. Dating the emergence of pandemic influenza viruses. *Proc Natl Acad Sci U S A*. 2009a; 106(28):11709–11712. [PubMed: 19597152]
- Smith GJ, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, Ma SK, Cheung CL, Raghwan J, Bhatt S, Peiris JS, Guan Y, Rambaut A. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature*. 2009b; 459(7250):1122–1125. [PubMed: 19516283]
- Smith W, Andrewes C, Laidlaw P. A virus obtained from influenza patients. *Lancet*. 1933; 2:66–68. [PubMed: 2885573]
- Subbarao EK, London W, Murphy BR. A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. *J Virol*. 1993; 67(4):1761–1764. [PubMed: 8445709]
- Tarendeau F, Boudet J, Guilligay D, Mas PJ, Bougault CM, Boulo S, Baudin F, Ruigrok RW, Daigle N, Ellenberg J, Cusack S, Simorre JP, Hart DJ. Structure and nuclear import function of the C-terminal domain of influenza virus polymerase PB2 subunit. *Nature Structural & Molecular Biology*. 2007; 14(3):229–233.
- Taubenberger JK, Hultin JV, Morens DM. Discovery and characterization of the 1918 pandemic influenza virus in historical context. *Antivir Ther*. 2007; 12(4 Pt B):581–591. [PubMed: 17944266]
- Taubenberger JK, Kash JC. Influenza virus evolution, host adaptation, and pandemic formation. *Cell Host & Microbe*. 2010; 7(6):440–451. [PubMed: 20542248]

- Taubenberger JK, Morens DM. 1918 Influenza: the mother of all pandemics. *Emerg Infect Dis.* 2006; 12(1):15–22. [PubMed: 16494711]
- Taubenberger JK, Morens DM. The pathology of influenza virus infections. *Annu Rev Pathol.* 2008; 3:499–522. [PubMed: 18039138]
- Taubenberger JK, Reid AH, Janczewski TA, Fanning TG. Integrating historical, clinical and molecular genetic data in order to explain the origin and virulence of the 1918 Spanish influenza virus. *Philos Trans R Soc Lond B Biol Sci.* 2001; 356(1416):1829–1839. [PubMed: 11779381]
- Taubenberger JK, Reid AH, Krafft AE, Bijwaard KE, Fanning TG. Initial genetic characterization of the 1918 "Spanish" influenza virus. *Science.* 1997; 275(5307):1793–1796. [PubMed: 9065404]
- Taubenberger JK, Reid AH, Lourens RM, Wang R, Jin G, Fanning TG. Characterization of the 1918 influenza virus polymerase genes. *Nature.* 2005; 437(7060):889–893. [PubMed: 16208372]
- Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, Fukuda K. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA.* 2003; 289(2):179–186. [PubMed: 12517228]
- Tumpey TM, Basler CF, Aguilar PV, Zeng H, Solorzano A, Swayne DE, Cox NJ, Katz JM, Taubenberger JK, Palese P, Garcia-Sastre A. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science.* 2005; 310(5745):77–80. [PubMed: 16210530]
- Tumpey TM, Garcia-Sastre A, Taubenberger JK, Palese P, Swayne DE, Basler CF. Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. *Proc Natl Acad Sci U S A.* 2004; 101(9):3166–3171. [PubMed: 14963236]
- Tumpey TM, Maines TR, Van Hoeven N, Glaser L, Solorzano A, Pappas C, Cox NJ, Swayne DE, Palese P, Katz JM, Garcia-Sastre A. A two-amino acid change in the hemagglutinin of the 1918 influenza virus abolishes transmission. *Science.* 2007; 315(5812):655–659. [PubMed: 17272724]
- Watanabe T, Watanabe S, Shinya K, Kim JH, Hatta M, Kawaoka Y. Viral RNA polymerase complex promotes optimal growth of 1918 virus in the lower respiratory tract of ferrets. *Proc Natl Acad Sci U S A.* 2009; 106(2):588–592. [PubMed: 19114663]
- Webby RJ, Rossow K, Erickson G, Sims Y, Webster R. Multiple lineages of antigenically and genetically diverse influenza A virus co-circulate in the United States swine population. *Virus Res.* 2004; 103(1–2):67–73. [PubMed: 15163491]
- Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. *Microbiol Rev.* 1992; 56(1):152–179. [PubMed: 1579108]
- Wright, PF.; Neumann, G.; Kawaoka, Y. Orthomyxoviruses. In: Knipe, DM.; Howley, PM., editors. *Fields Virology*. 5th ed.. Vol. Vol. 2. 2 vols. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 1691-1740.

Figure 1. Archival hematoxylin and eosin-stained tissue sections from autopsies of 1918 ‘Spanish’ influenza pandemic victims from the collection of the Armed Forces Institute of Pathology.