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Reconstruction of the 1918 Influenza Virus: Unexpected Rewards from the Past

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ABSTRACT The influenza pandemic of 1918–1919 killed approximately 50 million people. The unusually severe morbidity and mortality associated with the pandemic spurred physicians and scientists to isolate the etiologic agent, but the virus was not isolated in 1918. In 1996, it became possible to recover and sequence highly degraded fragments of influenza viral RNA retained in preserved tissues from several 1918 victims. These viral RNA sequences eventually permitted reconstruction of the complete 1918 virus, which has yielded, almost a century after the deaths of its victims, novel insights into influenza virus biology and pathogenesis and has provided important information about how to prevent and control future pandemics.

The “Spanish” influenza pandemic of 1918–1919 stands as the deadliest single event in recorded human history, killing approximately 50 million people worldwide (1, 2). The unprecedented burden of morbidity and mortality frustrated physicians and scientists, who were unable to identify the etiologic agent, but also spurred advances in microbiology, clinical infectious diseases, and public health practice (3). Influenza viruses were finally isolated about 15 years after the pandemic, but scientists of the early 20th century were not then capable of studying the biological basis of pandemic viral emergence, pathogenicity, or disappearance, let alone of gaining insights that might help to prevent or mitigate future pandemics. The cause of the 1918 pandemic and the determinants of its severity remained one of the most discussed medical mysteries throughout most of the 20th century.

Development of PCR technology in the 1980s, however, made it possible to recover and sequence highly degraded fragments of viral RNA retained in preserved tissues from persons who succumbed in 1918 (4–7). By use of the then-new approach of viral “reverse genetics,” these viral RNA sequences permitted eventual reconstruction of the complete 1918 virus (8). Remarkably, tiny viral RNA fragments recovered from just a few of the pandemic's many millions of victims have yielded, almost a century after their deaths, novel insights into influenza virus biology and pathogenesis and have provided important information about how to prevent and control future pandemics. This year (2012) marks the 15th anniversary of the publication of the initial 1918 influenza virus sequences (6), which led to the full genome sequence a decade later. Below, we review some of the studies performed with the 1918 virus and their implications for medical and public health practice today.

The milestone achievement of reconstructing an “extinct” pandemic virus raised a number of questions that had not been asked before. The most fundamental of these was whether it was necessary or wise to recreate by molecular means a naturally extinct virus that represented one of the deadliest infectious agents in human history. This question was answered in the affirmative when senior U.S. government scientists and officials at the De-

partment of Health and Human Services concluded that such research could play an important role in pandemic influenza preparedness (9). The U.S. government began to regulate research with the 1918 virus through the National Select Agents Registry Program at the U.S. Centers for Disease Control and Prevention (CDC). Guidelines contained in the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) manual (10) were quickly modified to support development, by institutional biosafety committees, of safe protocols for biocontainment and biosafety. Research with the 1918 virus has since gone forward at a rapid pace, leading to numerous findings, some of which are highlighted below. This important body of work exemplifies the benefits of studying pathogens that have a potential for “dual use,” provided that biosafety and biosecurity issues are adequately addressed. Dual use research is defined as work with clear benefits for society for which there also exists a theoretical potential for misuse (e.g., bioterrorism). In this commentary, we briefly review some of the important insights into influenza virus biology and public health that have already resulted from the sequencing and reconstruction of the 1918 virus. We argue that learning the most closely guarded secrets of our deadliest biological enemies is an essential means of protecting ourselves from future events of a similar nature.

PUBLIC HEALTH CONSEQUENCES OF UNDERSTANDING THE RELATIONSHIP BETWEEN THE 1918 AND 2009 PANDEMIC VIRUSES

Although the pandemic influenza viruses of 1957, 1968, and 2009 are all descended, via different pathways, from the 1918 virus, only the 2009 pandemic virus expresses an antigenically similar hemagglutinin (HA) (11). All influenza A viruses (IAV), including the

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1918 virus, possess a segmented single-stranded RNA genome and can evolve by the accumulation of selected mutations (“antigenic drift”) or through the exchange of gene segments by reassortment with other influenza viruses (“antigenic shift”). Sequencing the 1918 virus provided the basis for the subsequent understanding that the key 2009 virus HA gene, after having apparently been transmitted from humans to pigs in or about 1918, had been maintained in pigs over the past 90 years or so as a separate lineage from the 1918 human pandemic H1N1 virus (11), a lineage that has long been recognized as the “classical” swine H1N1 influenza virus. When the 2009 pandemic virus emerged in humans with a swine H1 HA gene descended from, and still closely related antigenically to, the 1918 pandemic virus, extensive cross-protection between the 2009 and 1918 pandemic viruses was demonstrated in experimental animals (12–16). Interestingly, 1918 virus-specific B cell clones could also still be recovered from very elderly survivors 90 years after their exposure to that virus but before their exposure to the 2009 pandemic virus (17). These findings provided a scientific rationale for targeting the initial 2009 H1N1 pandemic vaccine to those who needed it most, predominantly younger persons who had never been exposed to the cross-protective 1918 virus or to its early seasonally prevalent descendants. Thus, early in the 2009 pandemic, limited vaccine that might otherwise have been misdirected to the traditional risk group, the elderly (who were paradoxically at much lower risk in 2009), was instead administered to younger persons, who benefitted most.

INSIGHTS INTO NOVEL CROSS-REACTIVE INFLUENZA VACCINE APPROACHES USING THE 1918 VIRUS HEMAGGLUTININ STRUCTURE

Knowledge of the structure of the 1918 virus HA protein, as determined by crystallography, has led to a better understanding of influenza viral receptor binding and host adaptation and has also provided novel insights in an unexpected direction—structure-based vaccine design. Characterized protein motifs on the 1918 virus HA structure, conserved across many divergent HA proteins of different subtypes, are serving as a functional basis for next-generation vaccine approaches (18, 19) aimed at providing broad, cross-reactive immunity to IAV of different subtypes, including H5N1 viruses (20, 21). This knowledge has contributed to ongoing, innovative research to develop “universal” influenza vaccines that might be given infrequently yet conceivably cover all emerged and potential future pandemic viruses. It would obviously be a major advance if it became possible to circumvent the current requirement for annual vaccination that is a consequence of continual antigenic drift of viral epitopes under the pressure of immune selection.

INSIGHTS INTO INFLUENZA VIRUS EVOLUTION

The genomic sequence of the 1918 influenza virus has stimulated consideration of mechanisms of viral adaptation by which human pandemic strains emerge, including the possible role of intermediate mammalian hosts. Avian IAV have a higher guanine-cytosine (GC) content than do viral strains adapted to humans (22, 23). Gene segments from the 1918 virus have a nucleotide composition and GC content similar to those of avian IAV. Although its origin has not been fully resolved (2, 24, 25), the avian influenza virus-like genome of the 1918 pandemic virus suggests derivation from an avian virus in the decade before 1918, with or without adaptation in an intermediate host (26).

The evolution of human IAV after 1918 is better understood: all three pandemic viruses since 1918 contain gene segments derived from the 1918 “founder” virus. Consequently, the past 94 years can be considered to constitute a single “pandemic era,” with the founding 1918 pandemic virus leading to emergence of pandemic progeny viruses in 1957, 1968, and 2009 (11, 27). The realization that, since 1918, the 1918 virus has been the genetic “mother of all pandemics” has also led to reexamination of the historical record. Going back more than 500 years, evidence has suggested the existence of earlier, seemingly analogous, pandemic influenza eras (27), although for obvious reasons the genetic mechanisms remain entirely unknown. This insight has led to the hypothesis that influenza pandemic eras are initiated by extremely uncommon IAV host switch events, in which founding viruses arise *de novo* or from other yet-unknown mechanisms to cause pandemics and then, in the face of induced population immunity, prevail and seed new progeny pandemic viruses by genetic mechanisms involving mutation and reassortment. Incorporation of one or more novel gene segments into existing postpandemic viruses allows these viruses to escape population immunity while retaining those properties associated with human adaptation. This implies that viral surveillance needs to look in two directions simultaneously: for novel influenza viruses that may emerge in humans or other mammals from the avian virus gene pool and for existing human- and mammal-adapted viruses that may be involved in gene segment recycling or further adaptational evolution.

Investigation of the origins of the 1918 influenza virus with phylogenetic and bioinformatic studies has also led to comparison with many other IAV genomes from avian, mammalian, and human hosts. Thus, in certain ways, the 1918 sequencing project has served as a springboard for rapidly expanding publically available IAV genome sequences. Such sequences have already been important for understanding influenza virus evolution in humans and animals (28–31) and in responding to important unexpected events, such as the emergence of 2003 Fujian H3N2 virus (32) and the 2009 H1N1 virus pandemic (33). While practical integration of this new information into surveillance and public health preparedness is daunting, it is leading us to a greater understanding of the underlying patterns of influenza virus emergence and evolution and of the compendium of IAV strategies that result in adaptation to mammalian and human hosts. These insights in turn should lead to an enhanced ability to anticipate, detect (e.g., through targeted viral surveillance), and respond to novel influenza viral emergence in humans.

VIRULENCE, HOST ADAPTATION, AND TRANSMISSION FACTORS OF THE 1918 INFLUENZA VIRUS

The fully reconstructed 1918 pandemic virus is pathogenic—without prior adaptation—in mice (8), ferrets (34), and macaques (35) (N.B., while mice are commonly used in experimental influenza studies, disease and efficient viral replication in mice are not generally observed with unadapted human IAV strains). To combat infectious diseases, it is necessary to understand the molecular basis of each pathogen’s phenotypic properties, including infectivity, cell tropism, replication, immunogenicity, pathogenicity, and transmissibility. Numerous pathogenicity studies have been performed with the 1918 virus in animal models, including both “gain-of-function” and “loss-of-function” studies using chimeric influenza viruses carrying one or more 1918 viral genes of interest,

expressed on less pathogenic viral “reference” backgrounds. Such studies, while seeking primarily to answer questions about the 1918 pandemic, have provided insights into possible mechanisms of pathogenicity and host adaptation of future pandemic strains.

Disease severity, host adaptation, and transmission are all complex viral phenotypic properties that cannot be understood without elucidating their genetic bases, e.g., by addition of genetic information to a virus that lacks it (gain of function) or by removal of that genetic trait (loss of function). Such studies have shown convincingly that, although the innate virulence of the 1918 influenza virus is polygenic, proteins encoded by the HA and the three polymerase gene segments play key roles (8, 36–42). Despite lacking any obvious single virulence factor, influenza viruses expressing the 1918 virus HA are unquestionably pathogenic in mice and ferrets. Moreover, chimeric influenza viruses expressing each of the four known pandemic virus HA proteins (1918 H1, 1957 H2, 1968 H3, and 2009 H1 subtypes) are pathogenic in mice, based in part on their ability to replicate efficiently in lung parenchyma (41). However, the 1918 viral HA is not the only virulence factor in the 1918 virus. Several gain-of-function studies have shown that IAV expressing the genes of the 1918 viral polymerase complex are also pathogenic in mouse and ferret models (12, 36, 40, 42).

Just as evolutionary analyses have helped to clarify the relationship between the 1918 virus and its pandemic viral progeny, similar studies with the reconstructed 1918 virus have led to a fuller understanding of host adaptation. It is becoming clear that mutations associated with influenza virus host switch events may be unique and not shared with other viruses: influenza viral host adaptation appears to be a complex, context-dependent process, which likely is different for each individual virus adapting to a new host. For example, mutations associated with human adaptation in the 1918 virus have not in general been observed in different lineages of other human- or mammal-adapted influenza viruses, including the 2009 pandemic virus (37, 43–45). Therefore, in order to better predict the emergence of future pandemic viruses, structure/function correlates are critically needed to understand the biological implications of mutation patterns associated with new-host adaptation. Furthermore, understanding human influenza virus transmissibility is complicated by the lack of ideal animal models, although ferrets, guinea pigs, and mice have all been used for this purpose. Experiments with the 1918 virus have shown that changes in both the HA receptor-binding domain and the polymerase PB2 protein correlate with ferret transmissibility (46, 47). Such studies provide valuable insights into how future IAV, e.g., highly pathogenic avian influenza virus H5N1 strains, might adapt to humans and become efficiently transmissible (48, 49).

UNDERSTANDING OF INFLUENZA VIRUS RECEPTOR BINDING

Attachment of influenza viruses to target cells is mediated by HA binding to receptor glycans terminating in sialic acids that are linked in different configurations to underlying sugars. Although IAV adapted to avian hosts have specificity for α 2-3 sialic acid linkages, a few critical mutations in the HA receptor-binding domain, as seen with most human-adapted viruses, can alter specificity to cellular glycans terminating in α 2-6-linked sialic acids. Structural analyses and *in vitro* binding assays with the 1918 virus HA have confirmed that only two mutations in the receptor-binding domain determine complete alteration of viral specificity

from avian-adapted to a human-adapted configuration (50–52). Such information helps us predict what similar receptor-binding mutations might be needed for a future pandemic virus to adapt successfully to humans, thus facilitating targeted viral surveillance (53).

Recent studies using autopsy tissues of 1918 pandemic victims have led to the unexpected observation that both α 2-3 and α 2-6 receptor-binding variants of the 1918 virus cocirculated during the first year of the pandemic (54). Does this reflect early evolution from an avian- to a mammal-adapted virus? Did pandemic viral quasispecies contain both variants, and if so, was this a reflection of different cell tropisms along the human respiratory tract? Although these questions cannot yet be answered, they are stimulating further experiments to evaluate how receptor-binding variants are selected within single hosts and how these variants affect pathogenicity, host adaptation, and transmissibility.

PATHOLOGY OF INFLUENZA AND IMPORTANCE OF COINFECTIONS

The high 1918-1919 case fatality rates prompted many clinico-pathological studies in the aftermath of the pandemic. Reevaluation of 1918 autopsy tissues in the modern era (including those from which the viral RNA fragments were detected) has clarified pathological changes associated with fatal influenza pneumonias (55) and the critical role that secondary bacterial infection plays in severe disease following influenza viral infection (56). These findings have led to a burgeoning field of research in influenza virus/bacterial copathogenesis, directed at understanding the fundamental mechanisms whereby viral infections and host immune responses may potentiate secondary bacterial infections, including inhibition of respiratory cell repair (57), and how viral alterations of the immune response may affect bacterial pathogenesis (58, 59). These findings have important implications for clinical management and public health control. Awareness of the 1918 pandemic's pathologic features led physicians to anticipate and institute early antibiotic treatment of 2009 pandemic influenza virus-infected patients who might otherwise have had fatal outcomes due to secondary bacterial pneumonias (60).

THE ROLE OF HOST INFLAMMATORY RESPONSES IN DISEASE PROGRESSION

The unusual “W-shaped” age-specific 1918 death curve, with a peak of mortality in 20- to 40-year-olds, has always been one of the 1918 pandemic's most puzzling features (2). For decades, there has been speculation that a robust inflammatory response in otherwise healthy young individuals may have been a contributing factor. Interestingly, while not necessarily causally related to the unusual age-specific death curve, experimental pathogenesis studies with the 1918 virus in both mouse and nonhuman primate models support the hypothesis that aspects of the innate host response may be involved in disease progression. In mice, the 1918 virus induces a profound activation of inflammatory and cellular death receptor response mechanisms (38). Similarly, 1918 virus infection of macaques is associated with the suppression of type I interferon responses and a concomitant increase in expression of proinflammatory chemokines (35). Thus, the 1918 influenza virus is efficient at suppressing innate antiviral responses while concurrently activating potent proinflammatory responses. In recent work, the 1918 virus was used to characterize a novel open reading frame encoded by IAV. This newly identified viral protein, appar-

ently a product of all influenza A viruses, seems to modulate the host response to infection, a finding with important implications both for understanding IAV pathogenesis and in developing new ways to treat influenza viral infection (61). Together, these observations point to avenues for further research that may ultimately lead to new treatments aimed at suppressing immunopathogenic responses to IAV infection.

CONCLUSIONS

Like other fields in which studying the past helps to understand the present, virology is now finding a wealth of important information hidden in very degraded RNA viral fragments from a long-ago pandemic. The 1918 pandemic has long been a benchmark for medical historians as they seek to understand tragic events that were incomprehensible at the time. Now, virologists and allied scientists are providing the biological insights that strengthen historical understanding. But, however important understanding the past may be, it is the promise of managing the future that we insist is most compelling. Sequencing the genome of the 1918 pandemic virus has opened a once-locked door that allows us to use the past to better understand the future. The benefits of this work include not only fuller understanding of one of the deadliest of human diseases but also practical clinical and public health knowledge that can save lives and improve health today. While challenges remain, opportunities for additional discovery abound, not just in work to prevent and mitigate future pandemics but also in continuing to look backward, even before 1918, to gain insights into the larger-scale behaviors and strategies of pandemic influenza viruses as they have been played out over centuries. Although concern about dual use research was considered from the beginning of the 1918 viral sequencing project through the complete sequence and reconstruction of the pandemic virus, no adverse events have occurred. On the contrary, the benefits are obvious and manifold and have demonstrably contributed to the betterment of human health. Reconstruction of the 1918 virus has already been unexpectedly rewarding. Unquestionably, even greater rewards lie ahead.

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