What Can We Learn from Reconstructing the Extinct 1918 Pandemic Influenza Virus?

Commentary

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Introduction

The influenza pandemic of 1918/1919 was a unique event in recorded history, costing on the order of 50 million lives in less than a year (Johnson and Mueller, 2002). Even though this number of deaths was horrendous, the overall mortality rate for those infected in the U.S. and in Europe was only about 2%. Thus, a very large percentage of the two billion world population became infected and suffered some degree of illness.

Indirect evidence obtained several decades later suggested that the pathogen responsible for this illness was an influenza virus belonging to the H1 hemagglutinin (HA) subtype and that it (or its descendants) continued to circulate in the human population as well as in pigs. The first human influenza virus isolates date from 1933, and these H1 viruses circulated until 1957, only to reappear in 1977 (Nakajima et al., 1978). Except for this short interruption of 20 years, H1 viruses have been circulating in the human population since 1918 up until the present and have undergone continuous antigenic change (or "antigenic drift"). From 1957 to 1968, influenza viruses of the H2 subtype were prevalent, and in 1968, another subtype change occurred when viruses with an H3 HA appeared (Figure 1).

It is thought that human influenza viruses undergo a major subtype change (or antigenic shift) by acquiring novel HA genes from animal (avian) influenza viruses by reassortment. Because the population has little or no herd immunity to such a new virus, pandemics or global epidemics can result. This genetic exchange leading to a new reassortant most likely occurs when the same cell is simultaneously infected by a human and an animal influenza virus and a reshuffling of the segmented RNA genome of these viruses takes place. Compared to the 1918 pandemic, the pandemics caused by the 1957 (H2N2) and 1968 (H3N2) viruses were relatively mild, with estimates of one million and half a million deaths worldwide, respectively, which corresponds to an overall mortality rate of less than 0.02% and 0.01%.

Reconstruction of the 1918 Influenza Virus

The advent of reverse genetics techniques for negative-strand RNA viruses makes it possible to introduce DNA-derived sequences/genes into influenza viruses (Luytjes et al., 1989) and to reconstruct infectious influenza viruses entirely from commercially available oligonucleo-

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tides (Fodor et al., 1999; Neumann et al., 1999). This is done by transfecting into cells (1) expression plasmids that transcribe the eight RNA segments of the influenza virus and (2) helper plasmids that express the RNA-dependent RNA polymerase of the virus. Based on the nucleotide sequence that was obtained from RNA fragments present in lung samples of victims of the 1918 influenza virus (Taubenberger et al., 2005), we succeeded in reconstructing the extinct pandemic virus (Tumpey et al., 2005). This virus turns out to be highly virulent in the mouse model, more so than any other human (i.e., nonmouse-adapted) influenza virus strain tested. It is also highly pathogenic for chicken embryos, capable of killing these embryos at very low doses (less than 100 tissue culture infectious particles). Finally, the virus is also able to grow in human tissue culture cells to high titers (almost 10^9 plaque-forming units/ml, which is a ten times higher titer than that observed for other human influenza viruses), and it can replicate in cells in the absence of trypsin, which may also indicate high virulence (Tumpey et al., 2005).

Questions for the Immunologist

What Is the Immunological Basis for the W Shaped Curve of Case Fatality Rates in the 1918 Influenza Pandemic? One of the benefits of having the 1918 influenza virus "in hand" is that we can begin to study in the laboratory the mechanisms that may have contributed to the high morbidity and mortality associated with the 1918 pandemic. Based on the experiments performed so far, the 1918 virus is indeed unusually virulent and certainly seems to be a real "winner" as far as replication is concerned. In 1918, two age groups had an unusually high case fatality rate as a result of influenza: the very young (less than 1 year) and healthy young adults (ages 25–35). Interestingly, the mortality rate dropped beyond about age 35, only to again rise in people older than 65 years (Figure 2). This W shaped curve is unusual and is not seen in pre-1918 years. For example, in 1915, a U shaped curve for mortality is observed (with few fatalities in the age group 5–50). Such U shaped curves are typical for previously exposed populations, and indeed, most interpandemic years follow a U shaped distribution. The high mortality W shape for the 1918 virus may result from the combined effects of (1) the intrinsically greater virulence of the virus (as compared to other pandemic influenza viruses) and (2) the fact that it struck a population that was mostly immunologically naïve (Zamarin and Palese, 2003; Palese, 2004). This hypothesis further suggests that the virus might have been even more devastating (i.e., to the 35–65 age group) had there not been a similar H1-like virus in circulation before 1889. This may have provided partial crossprotection in the older age group born before 1889. Without this prior exposure of the older population, the case fatality rate for the 1918 pandemic would have followed a V shaped curve, with many more adults dying or becoming seriously ill.

What Are the Factors Determining a V Shaped Curve for Fatality Rates? Most lytic virus infections in immunologically naive humans have such a V shaped curve of
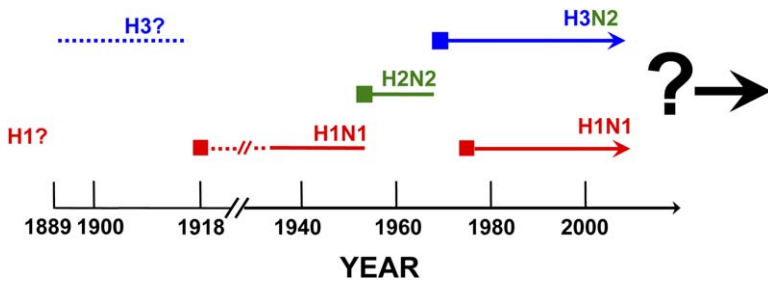
Figure 1. Influenza A Virus Subtypes Circulating in the Human Population

Currently H1N1 (hemagglutinin, subtype 1; neuraminidase, subtype 1) and H3N2 subtypes are prevalent. From 1957 to 1968, H2N2 viruses circulated, and before that, starting in 1918, H1N1 strains were observed. The broken line indicates that no isolates are available. H1 subtype strains are postulated to have circulated until 1889, and indirect evidence (Dowdle, 1999) suggests that H3 strains were subsequently introduced.

case fatality rates (and/or serious complications) when plotted against age groups. For example, the case fatality rate of measles on the Faroe islands in 1846 (when measles virus was not endemic) follows a V like shape with a trough in the 10–19 year group. Infants under 1 year of age and adults between 40 and 49 years had a more than 100-fold and 15-fold higher mortality rate, respectively, due to measles than the group 10–19 years (http://www.deltaomega.org/PanumFaroeIslands.pdf). Another example concerns infections by smallpox virus. Again, in immunologically naive populations, the mortality rates follow a V like shape, as was discovered when the indigenous populations of the Americas first came into contact with viral agents brought to the Western hemisphere by the Europeans. A recent manifestation of the V shaped curve of viral infection in an immunologically naive host is the smallpox vaccination program. This biodefense-driven program had to be discontinued
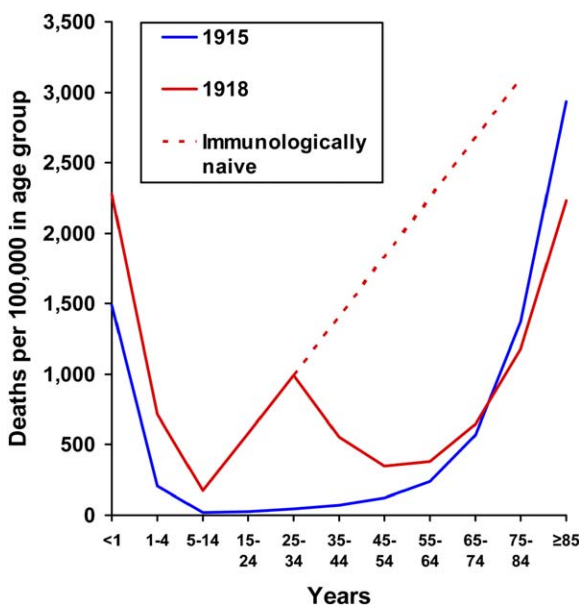

Figure 2. Specific Death Rates Caused by Influenza/Pneumonia for 1915 and 1918 in the United States

U and W shaped curves for influenza/pneumonia deaths for the years 1915 and 1918, respectively. Specific death rate is for 100,000 persons in the age group indicated (Linder and Grove, 1947). It is hypothesized that a V shaped curve would have been observed had the entire population in 1918 been immunologically naïve (dotted line).

because of the number of serious complications occurring in immunologically naive age groups older than children (trough of the curve).

Does the group of 5–14 year olds possess a better innate immune response against influenza viral infections than younger or older individuals? Are there age-dependent mechanisms involving clonal exhaustion of T cell responses during viral infections (Welsh and McNally, 1999)? These are general questions that should be asked (and hopefully answered) for influenza and for many other viral diseases, including measles, mumps, poliomyelitis, and smallpox.

Was There Immune Memory in the Population of 1918?
Was there indeed a partial immune protection against influenza H1 subtype viruses in the population born before 1889? Two pieces of indirect evidence support such a hypothesis. First, when in 1977 an H1N1 virus reappeared that was genetically very similar to strains circulating in 1950 (Nakajima et al., 1978), people younger than 25 years of age were most affected, suggesting that there may be long-lasting immunity in people who were infected by an earlier virus. Second, mice challenged with viruses carrying the 1918 HA and neuraminidase (NA) were shown to be largely protected from death by prior immunization with a vaccine containing the current H1N1 component (Tumpey et al., 2004). This experiment suggests that in mice partial immune protection can be achieved by immunizing with a virus belonging to the same subtype. Thus, we should ask whether current preexisting immunity in humans may preclude the outbreak of a highly virulent 1918-like H1 virus (the same question could be asked about H3N2 viruses, to which we also have antibodies). Can we develop new quantitative measurements (immune surrogate markers) that go beyond the classical hemagglutination inhibition tests in identifying the immune status of the individual?

Does Recycling of HA Subtypes Occur? If indeed H1-like viruses were prevalent before 1889 (see above) and H3-like viruses before 1918 (Dowdle, 1999), we need to try to understand how influenza viruses (or influenza virus genes) recycle in such a way that viruses with similar antigenic makeup occasionally reappear. It is possible that human influenza viruses recur once the herd immunity in humans wanes and that these human viruses are circulating in animals in the meantime. This hypothesis might be explored by more intense surveillance of influenza viruses, involving not only avian species but also other species as well. This should also include a better

understanding of the immunological response pattern of different avian species to (influenza) virus infections. In addition, samples from human cadavers and animal species should be further examined for the presence of influenza virus RNAs, and their sequences should be obtained and compared with sequences of other influenza viruses, including that of the 1918 strain. In this context, it should be noted that more than 28 million nucleotides were recently sequenced from material obtained from a Siberian woolly mammoth that died more than 25,000 years ago (Poinar et al., 2005). This was achieved by a novel emulsion polymerase chain reaction and pyrosequencing technique. Such sequencing techniques may help in identifying influenza viruses from different species and from earlier time periods. Based on this information, the question of recycling of HA subtypes may be more fully answered in the future.

Does the Influenza Viral Antagonist of the Innate Immune System Affect Species Specificity and Tissue Tropism? Experiments with influenza viruses lacking the NS1 gene have shown that the NS1 protein has interferon antagonist activity (Garcia-Sastre et al., 1998). Analysis of the NS1 protein of the 1918 virus suggests that the 1918 virus-derived NS1 protein shows optimal activity in human cells (Basler et al., 2001; Tumpey et al., 2004). Is it possible that the NS1 proteins of different strains determine differential virulence in one species versus another? Another question concerns the restriction of influenza virus infections to the respiratory tract. Might this be because respiratory cells are less likely to react to viral infections by a vigorous antiviral (interferon) response, so as not to induce a highly inflammatory and thus detrimental response in the respiratory tract? Nonrespiratory tissue would induce a more vigorous cytokine response (innate immune response) and thus, in most instances, prevent a transfer of influenza viruses to other tissues/organs.

Which Factors Influence the Pathophysiology of Influenza Virus Infections? Which factors in a virulent virus such as the 1918 virus determine whether the host is able to mount a beneficial immune response or whether its response leads to a detrimental cytokine storm (Kobasa et al., 2004)? What determines the well-known clinical symptoms of myalgia at sites where no virus replication can be detected? Very little is understood about the mechanism leading to this manifestation. Even the phenomenon of "antigenic sin" (i.e., the specific immune recall response to earlier influenza virus strains after infection or vaccination with later variants) is not well understood (Gulati et al., 2005). The generation and maintenance of memory, both for humoral immunity as well as for memory T cells (Masopust et al., 2004), remains an intensely studied topic in immunology. Studying the mechanisms of memory in humans will be important for understanding the disease outcome of influenza and for improving vaccine designs against the ever changing virus.

Last, but not least, major efforts will have to be made to better understand the parameters determining transmission. The 1918 virus had an unusual ability to transmit from human to human. At the other end of the spectrum are the H5N1 viruses, which cause devastation in poultry and can also be transmitted to humans, with more than 70 human deaths having been reported (http://www. who.int/csr/disease/avian_influenza/country/en/). However, these H5N1 strains are not effectively transmitted from person to person. Thus, it will be important to define the immunological and pathophysiological characteristics of a strain (gene) that determine transmission in humans.

Universal Influenza Virus Vaccines? It has been shown that mice can be protected against influenza viruses containing the 1918 HA and NA by using conventional vaccine methodologies (Tumpey et al., 2004). It has also been shown that the 1918 virus possesses an NA and an M2 protein that make it sensitive to the FDA-approved NA inhibitors and amantadines, respectively (Tumpey et al., 2002). Thus, there are tools available to attenuate influenza virus infections. However, because of logistical and financial constraints associated with the use of antivirals and because of the emergence of resistance (de Jong et al., 2005; Le et al., 2005), the only realistic public health measure against both epidemic and pandemic influenza is vaccination. The currently available technology would allow the development of effective vaccines, if industry could be given sufficient incentives and the regulatory agencies would be willing to embrace newer technologies, including the use of tissue culture, adjuvants, and reverse genetics (Fauci, 2006).

The challenge in the short run will be to facilitate rapid public health responses by developing sufficient industrial capacity and providing the right regulatory and commercial climate to allow unencumbered use of the vaccine. In the long run, we will have to address even more complex challenges. One direction to explore is the development of crossreactive vaccines that would protect against strains resulting from antigenic drift. Could this be achieved by selecting monoclonal antibodies that are highly crossreactive and then identifying the epitopes responsible for such reactivities? We might be able to then base future crossreactive vaccines on these epitopes. Could vaccines based on cellular immune responses that are made longer lasting be a solution for developing crossreactive (universal) vaccines? Or will completely new approaches, which do not build on any present-day techniques (or adjuvants), be necessary? Another approach may be directed at developing vaccines that could be effective against viruses of all known 16 HA subtypes (Fouchier et al., 2005). Are there structural elements shared by all HAs (NAs) that could serve as the basis for a universal vaccine? Would (local) interferon inducers (immune enhancers) be a solution rather than specific vaccine preparations? Can we hope for the introduction of new and safe adjuvants that go beyond the effectiveness of those currently being used (and which may allow for the immune induction of universal epitopes)? The synergistic knowledge of virologists and immunologists as well as geneticists should bring us novel medical interventions (Nabel, 2004) and advances in the field of vaccines.

Conclusion
The influenza pandemic of 1918/1919 was a unique event, and reconstructing the virus has helped us to begin understanding why the virus was extraordinarily virulent and what contributed to the unusual mortality pattern in the population. Questions still need to be answered as to how the 1918 virus and other influenza viruses interact with the innate immune system, and

new technologies will have to be developed for quantitatively measuring immune parameters after infections and leading to protection. These include both cellular and humoral immune responses. The availability of the 1918 virus will help us to understand the mechanisms by which pandemic influenza viruses are transmitted from human to human and from one species to another. Efforts underway in many laboratories will effectively expand our knowledge of the biological and molecular properties of pandemic influenza viruses, and this research will provide us with better preventions and treatment strategies for the future.

Acknowledgments

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