Molecular character of influenza A/H1N1 2009: Implications for spread and control

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Abstract The world is experiencing a pandemic of influenza that emerged in March 2009, due to a novel strain designated influenza A/H1N1 2009. This strain is closest in molecular sequence to swine influenza viruses, but differs from all previously known influenza by a minimum of 6.1%, and from prior “seasonal” H1N1 by 27.2%, giving it great potential for widespread human infection. While spread into India was delayed for two months by an aggressive interdiction program, since 1 August 2009 most cases in India have been indigenous. H1N1 2009 has differentially struck younger patients who are naïve susceptibles to its antigenic subtype, while sparing those >60 who have cross-reactive antibody from prior experience with influenza decades ago and the 1977 “swine flu” vaccine distributed in the United States. It also appears to more severely affect pregnant women. It emanated from a single source in central Mexico, but its precise geographical and circumstantial origins, from either Eurasia or the Americas, remain uncertain. While currently a mild pandemic by the standard of past pandemics, the seriousness of H1N1 2009 especially among children should not be underestimated. There is potential for the virus, which continues to adapt to humans, to change over time into a more severe etiologic agent by any of several foreseeable mutations. Mass acceptance of the novel H1N1 2009 vaccine worldwide will be essential to its control. Having spread globally in a few months, affecting millions of people, it is likely to remain circulating in the human population for a decade or more.

Introduction

The world is currently experiencing a level 6 pandemic of influenza [1]. Within the first six months of the outbreak, the World Health Organization (WHO) reported 340,000 laboratory-confirmed cases and 4100 deaths in 191 countries around the globe, due to a novel strain of influenza virus that emerged in Mexico in March 2009 [2]. These figures are gross underestimates of the number of actual cases, considered by epidemiologists to be many millions. Even in the cases of deaths, there is still underreporting, though to a lesser degree. We are just entering the first major influenza season in the winter months in the Northern Hemisphere since the virus emerged. A major increase in influenza activity is expected that will dwarf what has occurred thus far. We are in the midst of a pandemic of acute respiratory disease unlike anything seen in decades.

The etiologic agent is a new strain of influenza type A virus, A/H1N1 2009. As we previously described in early May [3], this virus is about 6% different from any known influenza virus in nature, and 27.2% different from its predecessor, the 2008 “seasonal flu” strain of H1N1. The latter difference, which has been termed a “pseudo-shift” in viral protein sequence [4], gives this influenza strain great potential for widespread human infection.

Nevertheless, by the truly gargantuan standards of past influenza pandemics, this outbreak is still relatively mild. The severity of illness, measured as morbidity and mortality, is less than in past pandemics, as is the “attack rate”, the percentage of the population affected. However, the virus has the potential to better adapt to replication in humans, and to mutate over time into a more severe pathogen.
The roots of this pandemic lie in the molecular characteristics of the genome and proteins of this novel strain of influenza, and the purpose of this article is to delineate those as they affect the prospects for continuance of the pandemic, as well as for its control.

**Defining influenza, the virus**

Influenza virus is an enveloped virus with an RNA genome, belonging to the family Orthomyxoviridae [5]. The RNA genome is segmented into 8 different RNA molecules, each with a nucleocapsid protein (NP) coat. Most segments code for only one protein of the virus, including all of those that code for the three principal structural proteins, the NP and the two surface glycoproteins. Influenza viruses are first classified into broad types by the antigenic properties of NP, into influenza A, B and C. The first of the glycoproteins, called the hemagglutinin (HA), is responsible for attachment and entry into susceptible cells, is the principal protective antigen of the virus, and, as such, is the principal target of influenza vaccines. The second glycoprotein, the neuraminidase (NA), is a mucus-digesting enzyme that releases nascent virus from the cellular surface and debris, facilitating spread of the virus through the respiratory tract. Antibody to NA also reduces the severity of influenza, and the importance of this enzyme to the virus is underscored by the fact that neuraminidase is the target of the two drugs currently licensed against influenza, Tamiflu (oseltamivir) and Relenza (zanamivir). Influenza viruses of type A are subtyped by the antigenic characteristics of the HA and NA glycoproteins. Widespread human infection has for over a century been limited to viruses with HA subtypes H1, H1 and H3, and with NA subtypes of N1 and N2 [6, 7]. Additional criteria used to identify an influenza virus include the source location, an identifying number, and the year of isolation. Thus, a compound designation is used for each isolate. For the current outbreak, the prototype isolate on 9 April 2009 is designated A/California/7/2009 (H1N1).

Three other RNA segments code for the three subunits of the influenza replicase, responsible for both replication of the genome and expression of viral messenger RNA. One of these, PB1, also codes for a second non-structural protein in reading frame 2. This protein, called PB1-F2, contains a pro-apoptotic peptide region [8–10]. Both of the two remaining genome fragments produce two proteins each, through alternate splicing. The segment 7 produces the matrix (M1) protein associated with the viral envelope. M1 and M2, present only in influenza A, a viroporin facilitating permeability of infected cells. Segment 8 produces protein NS1 that counters human interferon [11], and a nuclear export protein (NEP) that facilitates movement of influenza genomes across the nuclear membrane early in influenza replication [12]. All of the 11 viral proteins are essential in infection even in cell culture, except PB1-F2, M2 and NS1.

The impact of influenza as an infectious disease is due entirely to two major and unique molecular features of the virus. The segmented genome allows for independent reassortment of viral genes. Positive mutations in one gene are genetically segregated from deleterious mutations in another. Also, the HA and NA proteins are capable of an enormous degree of variability, up to 50%, of their protein sequence while still remaining functional [13]. This molecular plasticity, coupled with the capability for frequent genetic reassortment, can generate an enormous level of virus variation in response to its host environment, most especially the antiviral antibodies raised in humans against it.

The natural host of influenza viruses is migratory waterfowl, and avian influenza spreads separately through each of the Western and Eastern Hemispheres through natural bird migrations [5, 14]. From this avian source, pigs become infected, and this serves as an intermediate host adapting the virus to mammalian host cells, from which most human influenza arises. Swine influenza is endemic except in commercial production operations where vaccine is routinely used. The virus evolves more slowly in swine than in humans at its antigenic epitopes. Swine live a maximum of 5 years as breeding sows, and only a few months when produced for human consumption, so they have little ongoing immunologic memory. Humans, on the other hand, present a highly selective immune environment for a lifetime exceeding 70 years. Crossover of swine influenza into humans is quite rare, and almost never leads to serial infection in humans. Before 2009, the biggest exceptions to this rule were the 1918 pandemic influenza virus (which may also have had a direct avian source), and 230 cases of the 1976 swine influenza outbreak in New Jersey in the United States [15, 16]. The H1N1 2009 virus thus represents a very rare event, the crossover of swine influenza into a human with secondary spread in the worldwide population. Once this event occurred, however it occurred, swine do not contribute to the further spread of the virus in humans at all.

**Defining influenza, the illness**

The actual term used to describe the disease is “influenza-like illness” (ILI), that is, as it directly implies, a rather uncertain diagnosis. Both the WHO and its US counterpart, the Centers for Disease Control and Prevention (CDC) have launched websites to monitor the pandemic and guide public policy [see http://www.cdc.gov/h1n1flu/]. Clinical influenza