Deadly H7N9 Influenza Virus: A Pandemic in the Making or a Warning Lesson?

On March 31, 2013, the Chinese Center for Disease Control and Prevention notified the World Health Organization of the isolation of influenza A virus (H7N9 subtype) from three critically ill patients (1–3). H7N9 is normally an avian virus that is asymptomatic in avian species. However, it is a novel influenza virus in humans, and 131 humans have been infected with the virus and 32 have died as of May 8, 2013. Although the mortality rate of H7N9 infection is very high, there is as yet no indication of human-to-human transmission. This issue of the *Journal* contains two letters describing the clinical features of several patients infected with the H7N9 virus in China (4, 5).

The first cases of H7N9 were around Shanghai, but by late April 2013, other cases covered a very wide area of China. Culling of poultry and the closing of live bird markets does appear to have reduced the frequency of cases. Thus, poultry in these markets may be the main source of human infection, particularly as the vast majority of infected humans have had close associations with live birds.

The Chinese authorities deposited the sequences of the eight influenza genes, from viruses isolated from three patients, in the EpiFlu database of the Global Initiative on Sharing All Influenza Data, and an extensive analysis of the sequences commenced (1). The sequences of the viruses isolated from patients are very close to those isolated from chickens or the environment of live bird markets and indicate that H7N9 shows adaptation to mammalian hosts.

The genes for the major antigenic determinants, the spike glycoproteins hemagglutinin (HA) and neuraminidase (NA), were found to have originated from Eurasian avian influenza viruses, with the remaining six genes encoding internal proteins being closely related to avian H9N2 viruses (1).

The influenza virus genome sequence contains clues as to determinants of pathogenicity of the virus. This information in part helps to predict whether the virus will cause a disease of the severity of the 1918 Spanish influenza virus (H1N1), which is estimated to have killed 25 to 50 million people worldwide, or whether the virus will be a relatively mild infection, like the 2009pd H1N1 virus. Pathogenicity determinants derived from the genome sequences are as follows:

- HA contains two important determinants of pathogenicity. Sequence variation in HA can result in altered affinity for the different types of the cellular receptor sialic acid, preferentially binding either the mammalian or the avian variant forms. The H7N9 virus contained three mutations in the sialic acid receptor binding site that suggest specificity for binding to mammalian-like receptors (6). Additionally, HA requires cleavage by host proteases to mediate fusion. The sequence of the HA cleavage site can determine the ease with which the protein is cleaved, which in turn can influence the virulence of the strain. Highly pathogenic viruses usually contain multiple basic residues in the HA cleavage site with HA being cleaved intracellularly, whereas low-pathogenicity viruses contain HA with a single basic residue

that is cleaved extracellularly (7). H7N9 contains the low-pathogenicity HA single basic residue in the cleavage site.

- An NA stalk deletion is associated with increased virulence in mammals (8), and all H7N9 viruses encode this deletion.
- The RNA polymerase component protein PB2 amino residue 627 is a glutamic acid (E) in avian viruses and a lysine (K) in mammalian species, and PB2-627K is essential for the efficient replication of avian viruses in mammalian species (9). PB2-627K is very rare in avian H9N2 species but is found in all human viruses and is found in the H7N9 patient-isolated viruses.
- The PB1-F2 protein (encoded by the PB1 RNA segment) is associated with virulence. The human H7N9 viruses encode a full length PB1-F2 of 90 amino acids (10). The human H7N9 viruses lack the N66S mutation associated with increased pathogenicity of both the 1918 pandemic virus and the high-pathogenicity H5N1 avian viruses.
- The NS1 protein is a multifunctional protein that antagonizes the innate immune system. The H7N9 NS1 sequences lack the C-terminal PDZ domain-binding motif. This lack of a PDZ-binding motif may attenuate these H7N9 viruses in mammals (11).

Antiviral drugs are an important line of defense against the influenza virus. The human H7N9 viruses contain the mutation S31N in the ion channel protein M2, suggesting that they are resistant to the ion channel blockers amantadine and rimantidine (12, 13). The sequences of all but one of the NA proteins suggest that the NA is sensitive to oseltamivir and zanamivir. The exception is the human isolate A/Shanghai/11/2013, which contains an NA protein with an R294K mutation, a change known to confer resistance to NA inhibitors in N2 and N9 subtypes (14).

For an influenza virus to cause a pandemic, it needs to become highly transmissible from human to human, and the current evidence suggests that this is not the case. However, doubtless a stampede has begun for researchers to obtain “gain-of-function” mutations by adaptation after serial passaging the virus in ferrets. Analogous experiments with H5N1 viruses (15, 16) led to a serious analysis of the risks involved in such experimentation.

In summary, the H7N9 viruses possess several characteristic features of mammalian influenza viruses, which are likely to contribute to their ability to infect humans and raise concerns with respect to their pandemic potential. However, it cannot be predicted whether avian/human H7N9 influenza virus will remain a zoonosis in China or become a global pandemic with dire consequences.

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Additional Insights into Epithelial Secreted Phospholipase A₂ Group X in Asthma

Phospholipase A₂ (PLA₂) catalyzes the hydrolysis of the ester bond at the sn-2 position of membrane glycerophospholipids to produce lysophospholipids and free fatty acids (1). PLA₂ enzymes are implicated in diverse biological processes and exist in a variety of cytosolic and secreted forms (2). Cytosolic cPLA₂α (group IVA) may be one of the most intensively studied of the PLA₂ enzymes, as it plays a major role in releasing arachidonic acid (AA), the critical precursor of bioactive inflammatory leukotrienes and prostaglandins, from cellular membranes (3). The secreted PLA₂ (sPLA₂) family consists of at least 10 isoforms, and these enzymes act on targets accessible in the extracellular space, including cell membranes, dietary phospholipids, lung surfactant, and foreign phospholipids associated with microbial pathogens (4, 5).

Within the sPLA₂ family, the sPLA₂-X (group X) enzyme has the highest binding affinity for phosphatidyl choline and generates significant release of AA and lysophosphatidylcholine from the plasma membranes of intact cells (6). sPLA₂-X is expressed in airway epithelial cells and in inflammatory cells and is one of the major sPLA₂ isoforms detected in the airway cells of subjects with asthma (7, 8). The level of sPLA₂-X protein is low in subjects without asthma, and increased levels can be recovered from the bronchoalveolar lavage (BAL) fluid in individuals with asthma with exercise challenge (7). In the ova/ova sensitization and challenge murine asthma model, the lungs of sPLA₂-X gene-deleted PLA2G10^{-/-} mice show attenuation in several relevant airway phenotypes—lung eosinophilia, the infiltration of CD4⁺ T cells, goblet cell metaplasia, and thickening of airway smooth muscle—and in the levels of Th2 cytokines and eicosanoids (9). Interestingly, transgenic mice with overexpression of human sPLA₂-X under the control of the β-actin promoter do not have

any pulmonary defect, whereas in the PLA2G10-transgenic animals where the transgene was placed under the control of a macrophage-specific promoter, the transgenic animals died neonatally with severe lung pathology (10, 11).

In this issue of the *Journal*, Hallstrand and colleagues (pp. 42–50) studied the epithelial expression of sPLA₂-X protein recovered after induced sputum and bronchoscopic biopsy in the airways of subjects with and without asthma, with the subjects with asthma further classified into those with and without exercise-induced bronchoconstriction (EIB) (12). They found increased sPLA₂-X protein in asthmatic airways and epithelial expression of sPLA₂-X was increased particularly in individuals with asthma with EIB. When studying the primary airway epithelial cells derived from these subjects in organotypic culture *in vitro*, they found increased sPLA₂-X expression during retinoic acid-dependent epithelial differentiation and wound healing, though there were no marked differences between subjects with asthma versus control subjects. PLA2G10 gene expression was regulated by treatment with TNF, IL-13, and IL-17, and in *in vitro* studies with primary murine tracheal epithelial cells derived from PLA2G10^{-/-} gene-deleted mice, sPLA₂-X activity was found to regulate the release of AA but not prostaglandin E₂ biosynthesis.

This work is of interest for a number of reasons. Previous studies identified elevated sPLA₂-X levels in the BAL fluid of individuals with asthma, and this study localizes the increased sPLA₂-X secreted protein to the airway epithelium (7, 12). Most sPLA₂ enzymes are stored within inflammatory cells and can be released extracellularly upon cell activation, presumably accounting for a significant amount of the sPLA₂ activity detected in biological fluids during local or systemic inflammatory responses (1). This study focuses on the respiratory epithelium as a major source of airway sPLA₂-X protein in asthma,