Comparison of the Pathology Caused by H1N1, H5N1, and H3N2 Influenza Viruses

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Received for publication September 8, 2009; accepted September 21, 2009 (ARCMED-D-09-00424).

The spectrum of morbidity and mortality of H1N1, H5N1, and H3N2 influenza A viruses relates to the pathology they produce. In this review, we describe and compare the pathology of these viruses in human cases and animal models. The 1918 H1N1, the novel 2009 H1N1 pandemic virus, and H5N1 show inflammation, congestion, and epithelial necrosis of the larger airways (trachea, bronchi and bronchioles) with extension into the alveoli causing diffuse alveolar damage. Seasonal influenza A viruses (H3N2 and H1N1) have primarily caused inflammation, congestion and epithelial necrosis of the larger airways with lesser extension of the inflammatory process to alveoli. Localization of the inflammation and cellular damage relate to the presence of virus in different cell types. Infections with 1918 H1N1, the novel 2009 H1N1 pandemic virus, and H5N1 show virus in mucosal epithelial cells of the airways (from the nasopharynx to the bronchioles), alveolar macrophages, and pneumocytes, whereas infections with seasonal influenza viruses show viral antigens primarily in mucosal epithelial cells of the larger airways. The increased morbidity that has been encountered with the 2009 H1N1 virus is related to infection of cells in the upper and lower airways. The 2009 H1N1 virus shows similar pathology to that encountered with other highly virulent influenza A viruses such as the 1918 H1N1 and H5N1 viruses. © 2009 IMSS. Published by Elsevier Inc.

Key Words: Pathology, Influenza A virus, Novel H1N1, avian H5N1, Seasonal H3N2, Human cases, Animal models.

Introduction

Preparedness for an influenza pandemic has included surveillance for all influenza viruses but attention has been primarily focused on transmission of avian H5N1 virus to humans and further transmission of H5N1 virus among humans. Attention to the H5N1 virus started in 1997 when humans became infected during a poultry outbreak of this highly pathogenic avian influenza virus in Hong Kong (1–3). Animal studies and studies of autopsies of patients infected with the different viruses (avian H5N1, swine H1N1, and seasonal H3N2) have revealed that the cells in the respiratory tract these viruses infect are different resulting in different pathologic pictures and morbidity and mortality rates (4–11). In addition, further molecular studies of autopsy material from the 1918 pandemic lead to recognition that the virus causing disease was of swine origin (H1N1) (12,13). Reconstruction and successful animal infections with the 1918 H1N1 virus have added to the spectrum of clinicopathological outcomes that the different influenza A viruses can cause (14,17). Before the 2009–2010 season begins, a comparison of the pathology between the recent 2009 H1N1 pandemic virus (18,19) and the other influenza A viruses may help to better prepare healthcare professionals to understand the morbidity and mortality that may be encountered.

The signs and symptoms of H1N1, H5N1 and H3N2 influenza A viruses is primarily in the respiratory tract and the pathological damage encountered mirrors the clinical presentation with the most frequent pathology observed in the airways and lungs. Clinical symptoms of myocarditis and encephalopathy have been described less frequently in patients with influenza A infections, and pathology in the heart has been observed in some of these cases. It should be noted that the pathological descriptions in humans come...
primarily from autopsies; thus, our knowledge relates to the most severe cases with the poorest outcomes (20). Study of the pathology of confirmed cases is important to be able to better understand the differences in presenting signs and symptoms, duration of the disease, complications, and mortality that these viruses can produce. In addition, knowing the pathology these viruses cause will enable pathologists to perform presumptive diagnosis and seek other diagnostic techniques (PCR, viral culture) that will allow confirmation of the virus involved for specific cases.

This paper will describe and compare the pathology encountered in autopsies and animal models infected with H1N1, H5N1 and H3N2 influenza viruses.

**Pathology of H1N1**

Detailed descriptions of the pathology of cases infected with the pandemic 2009 H1N1 virus have not yet appeared in the literature. The descriptions presented here are based on the authors’ observations of four cases and a published report of one case (18). The lung pathology of deceased patients infected with the pandemic 2009 H1N1 have shown primarily diffuse alveolar damage with extensive hyaline membrane formation and intraalveolar edema (Figure 1A). The vessels in alveolar walls show thrombosis, which has lead to necrosis of the alveolar walls (Figure 1B) and varying degrees of mixed inflammatory infiltrate. Alveolar epithelial cells and pneumocytes show reactive changes, and their nuclei appear enlarged. Desquamated macrophages and pneumocytes are observed within the alveolar spaces. Prominent proliferation of fibroblasts is observed in patients with longer duration of disease. Intra-alveolar hemorrhage and erythropagocytosis have also been observed. Immunohistochemical assays have demonstrated viral antigens in pneumocytes type I and II and intraalveolar macrophages (personal communication, Dr. Sherif Zaki, Centers for Disease Control and Prevention, Atlanta, GA, 2009). Bacterial pneumonias, defined as accumulation of neutrophils in the alveoli, have been observed in 30% of cases and may be either community acquired or nosocomial (ventilator-associated) (18,19).

The trachea and major bronchi of deceased patients infected with the pandemic 2009 H1N1 have shown necrotic desquamation of the mucosa and edema and mixed inflammatory infiltrate in the submucosa (Figure 1C). The glands in these airways show loss of goblet cell and necrosis of surrounding submucosa (Figure 1D). Viral antigens have been noted in the epithelial cells and mucous glands of trachea, bronchi, and bronchioles (personal communication, Dr. Sherif Zaki, Centers for Disease Control and Prevention, Atlanta, GA, 2009). PCR demonstrating virus in tissues from the respiratory tract and lung has shown better sensitivity and specificity than immunohistochemistry. Myocardial infarction has been documented in several patients infected with the 2009 H1N1 virus (18).

The 1918 H1N1 pandemic virus is recorded in history as having caused the highest number of excess deaths (20). Multiple studies of the pathology were published at the time and newer studies have reviewed the archived pathology material to ascertain the role of viral pneumonia and bacterial superinfections (17,21–24). From the pathological standpoint, H1N1 infections during the 1918 pandemic can be divided into those patients with viral pneumonia and those with superimposed bacterial pneumonia. Those patients who died early during the infection showed viral pneumonia with focal necrosis of the alveolar walls associated with capillary thrombosis, prominent hyaline membrane formation, and intraalveolar edema. In the trachea and major bronchi, autopsies showed necrotic mucosa with edema and inflammation in the submucosa. The loss of epithelium was more pronounced in the bronchioles where the epithelial layer is thinner. In comparison, those patients with superimposed bacterial pneumonias showed abundant intraalveolar neutrophilic infiltrate associated with edema, fibrin and necrosis in a predominant lobular distribution. It is estimated that up to 90% of patients who died during the 1918 H1N1 pandemic had bacterial superinfections with *Streptococcus pneumoniae*, *S. hemolyticus*, and mixed pathogens being the most frequently isolated bacteria (17). Myocarditis was reported in patients with the 1918 H1N1 infections (22).

Several animal models have been used to study the pathology of H1N1 infections and the results vary depending on the host animal and virus used for the infection (5,8,10,14–16,25). In guinea pigs and pigs, low pathogenic seasonal H1N1 virus (A/Puerto Rico/8/34 and A/swine/Thailand/HF6/05) showed viral antigens in the mucosal epithelium associated with epithelial cell damage and could be seen from the upper airways to the bronchioles (5,10). Damage of the epithelium resulted in increased mucus production and mild inflammatory infiltrate. Inflammation surrounding bronchioles but did not spill into the lung interstitium. In ferrets, the seasonal H1N1 (A/Netherlands/26/2007) showed multifocal necrotizing rhinitis with abundant viral antigens in the nasal turbinates and focal consolidation of the lungs without evidence of viral antigens in the lower respiratory tract (25). When low pathogenic seasonal H1N1 virus (A/Puerto Rico/8/34) was used to infect mice, the lungs showed complete consolidation due to bronchitis and interstitial pneumonia, the thymus was reduced in size by 30% due to depletion of cortical lymphocytes, and the heart showed inflammatory foci (8). Thus, for the low pathogenic seasonal H1N1 viruses the extent of damage depended on the infected host.

Animal models using ferrets and pandemic 2009 H1N1 have shown more extensive consolidation of the lungs than that encountered with seasonal H1N1 virus (25). Histopathologically, ferrets show multifocal mild to moderate necrotizing rhinitis, tracheitis, bronchitis, and bronchiolitis, and viral antigens are observed from the nasal turbinates to
the bronchioles. The pandemic 2009 A (H1N1) influenza viruses replicate to higher titers in lung tissue and can be recovered from the upper and lower airways as well as the intestinal tract (26).

Infection of mice or monkeys with highly pathogenic re-constructed 1918 H1N1 viruses (1918 HA/NA:WSN or 1918 HA/NA:Tx/91) produced large areas of pneumonia that microscopically showed necrotizing bronchitis and inflammation of alveolar walls by neutrophils and macrophages (14–16). Necrotic debris was noted in the lumen of bronchioles and alveoli. Viral antigens were localized in bronchiolar epithelium and alveolar macrophages. These animals have also shown increased expression of cytokines and chemokines.

Pathology of H5N1

Even though the mortality from H5N1 infection is ~60%, there have been <15 autopsies reported in the literature (2–4,27–29). The lung pathology of deceased patients infected with the H5N1 virus has shown diffuse alveolar damage in the lungs. Depending on the duration of illness,
diffuse alveolar damage may be in the exudative early phase and show hyaline membrane formation, congestion of vasculature, and lymphocytes infiltrating the interstitium or in the later fibrous proliferative phase where fibroblast proliferation is added to the picture (Figure 2A) (3). Inflammation around bronchioles, alveolar interstitium, and in the pleura has been present. Cells involved in the pathology include presence of T-lymphocytes in the alveolar septae, macrophages in the alveolar lumen, and hyperplasia and desquamation of type II pneumocytes. Several cases have shown intraalveolar hemorrhage (Figure 2B), whereas others have shown hemophagocytosis. Hemophagocytosis has been noted not only in the lung but also in bone marrow, lymph nodes (Figure 2C), spleen, and liver. Other organs with pathology have included the spleen with depletion and apoptosis of lymphocytes, kidneys with acute tubular necrosis, and liver with necrosis, fatty change, activation of Kupffer cells, and cholestasis. Two cases have shown demyelinated areas in the brain with necrosis and reactive histiocytes. Several techniques have demonstrated

Figure 2. Histopathological characteristics of fatal cases with confirmed H5N1 virus infection showing diffuse alveolar damage in the later fibrous proliferative phase characterized by fibroblast proliferation (A) and deposition of collagen (A,B). Intraalveolar hemorrhage is seen in some cases (B) as well as hemophagocytosis (C). Hematoxylin and eosin stains; original magnifications: ×10 (A), ×5 (B), and ×63 (C). Color version of this figure available online at www.arcmedres.com.
virus that can replicate in the lung, and immunohistochemical assays have localized the viral antigens in ciliated and nonciliated epithelial cells and in type II pneumocytes. Viral sequences and antigens have been found in the brain, intestines, and placenta as well as lymphocytes and macrophages. Several animal models have been used to study the pathology of H5N1 infections and the results vary depending on the pathogenicity of the virus used for the infection (11,14,15,30). In general, those viruses that have been recovered from patients with fatal infections (i.e., A/Hong Kong/483/97) tend to produce more severe disease in the different animal models than those recovered from patients with milder disease (i.e., A/HongKong/486/97) (11,30). The animals that have been used include mice, ferrets, and primates.

Macroscopically, all animals showed various degrees of consolidation and discoloration in the lungs and hemorrhages in adipose tissue surrounding liver, intestines, kidneys, and bladder (11,30). Macroscopic changes occurred earlier in animals infected with the highly virulent viruses compared to those infected with low-virulence viruses. Microscopically, the lungs showed inflammation and edema surrounding bronchioles and spilling into the alveolar septae (11,15,30). Histopathology and flow cytometry have shown that the inflammatory infiltrate consisted of neutrophils and macrophages (14). The extent of inflammation spilling into the alveoli appeared earlier (about

Figure 3. Histopathological characteristics of fatal cases with confirmed seasonal H3N2 virus infection showing mucosal desquamation and disorganization and submucosal congestion, hemorrhage, and inflammation in the trachea (A). The lungs show intraalveolar hemorrhage and edema (B). The heart shows focal areas of mononuclear inflammatory infiltrate (C). Hematoxylin and eosin stains; original magnifications: ×10 (A), ×5 (B), and ×40 (C). Color version of this figure available online at www.arcmedres.com.
5–9 days postinfection) and was more pronounced in those animals infected with highly virulent virus compared to those infected with low-virulence virus. Similarly, the presence of epithelial necrosis has been described for those animals infected with highly virulent virus. At about 3 days postinoculation and before there are inflammatory infiltrates, viral antigens can be detected in type II pneumocytes (15). The brains of animals infected with the highly virulent viruses showed glial nodules, perivascular cuffing by lymphocytes and neutrophils, neuronophagia, and inflammation in the choroid plexus more frequently and extensively than those animals infected with low-virulence viruses (11,30). Viral antigens have been detected in neurons.

Pathology of H3N2

Publications of the pathology observed in patients infected with seasonal or interpandemic influenza have included 51 pediatric patients with fatal disease and 11 adults (six with fatal disease and five who survived the infection) (6,7,31,32). The pathological features described for H3N2 have a bias towards severe and complicated infections that have required open lung biopsies or were fatal, and an autopsy was performed to understand the cause of death. The pathological changes observed in surviving patients have ranged from acute lung injury including patchy fibrinous alveolar exudates, hyaline membrane formation, interstitial edema, and necrosis of bronchiolar mucosa to reparative changes such as proliferation of type II pneumocytes, interstitial chronic inflammatory infiltrates, and organization of airspaces and interstitium (31,32).

In autopsies of patients infected with the H3N2 virus, the most frequent and sometimes the only pathology observed has been mononuclear inflammation and congestion of the trachea, major bronchi and bronchioles commonly accompanied by necrosis of the epithelium and submucosal hemorrhage (Figure 3A) (6,7). Viral antigens may only be present in the bronchial epithelium. Changes in the lower respiratory tract include mononuclear inflammation in the alveolar septa, hyaline membrane formation and intraalveolar hemorrhage (Figure 3B). Viral antigens are rarely found in the alveoli. In 50% of the cases there were focal areas of pneumonia with Staphylococcus aureus and group A streptococci being the most frequently identified bacterial superinfections. Lymph nodes showed hemophagocytosis in half the cases. In the heart, patchy areas of mononuclear inflammation and necrosis (Figure 3C) were observed in 30% of cases.

There are several descriptions of the pathology of animals infected with the H3N2 virus that have usually been compared to animals that have been infected with seasonal H1N1 viruses. Mice, guinea pigs, pigs and ferrets have been used in these models (5,8,10,30). In all of these animal models there is desquamation and disorganization of the epithelium and inflammation of the mucosa of the airways (nasal, paranasal, sinuses, trachea, bronchus, and bronchioles) starting at 1 day postinfection. Changes in the respiratory mucosa are accompanied by the presence of viral antigens that decline sharply 3 days postinfection. Macroscopically the lungs appear consolidated, and microscopically there is neutrophilic and mononuclear inflammation in the bronchioles and alveolar intestitium. Viral antigens were detected in the epithelial cells and macrophages. Outside the lungs, viral antigens have been found in the heart and thymus. In pigs, lesions produced by seasonal H1N1 were more extensive than those produced by infections with the H3N2 virus (10).

Comparison of the Pathological Features Caused by Influenza A Viruses

The pathology caused by these viruses in humans or animal models depends on the virulence of the infecting agent and host response. All the viruses infect the respiratory epithelium from the nasal passages to bronchioles; however, high-virulence viruses (1918 H1N1, H5N1, and probably 2009 H1N1) also tend to infect pneumocytes and intraalveolar macrophages. The localization and amount of the inflammatory reaction will depend on the infected cells. Thus, low-virulence viruses (seasonal H3N2 and H1N1) cause primarily inflammation, congestion and epithelial necrosis of the larger airways (trachea, bronchi and bronchioles), whereas high-virulence viruses have equal inflammation in these localizations with additional, more extensive areas of inflammation in the alveoli. In susceptible individuals, inflammation of the alveolar walls will result in diffuse alveolar damage. It needs to be remembered that influenza A viruses do not cause inclusions or visible cytopathic effects in vivo; however, infected cells show reactive changes with enlargement of the nuclei. In addition, the pathological picture will change to intraalveolar neutrophilic infiltrate if bacterial superinfection is present. The frequency and type of bacterial superinfections that we can expect during the 2009–2010 season will be different from those encountered during the 1918 H1N1 pandemic. The use of antibiotics, supportive measures (ventilators), and vaccinations for S. pneumoniae and Haemophilus influenzae will probably reduce the frequency of these superinfections but will bring community and nosocomial bacterial infections that will have acquired antibiotic resistance.

References


