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Review of influenza pathogenesis and pathologic findings from experimental studies of influenza vaccination

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Influenza A virus is a major cause of year-round acute respiratory disease in swine of any age. The clinical signs of infection (a.k.a. flu) usually start in the first 24-48 hours after exposure and coincide with the nasal shedding of virus and the first microscopic lesions. The most consistent clinical sign in healthy young pigs is a fever (> 104°F or 40°C). Coughing and nasal discharge can be seen early in the disease, but can also last until 2 weeks past the initial infection, and therefore are not reliable for selecting animals for sampling. Some of the other signs that often accompany a fever, such as decreased appetite, drinking and/or activity, may indicate infection as well. At necropsy, the classic gross lesions of flu are multifocal, dark purple lobules firm to the touch in the cranio-ventral lung lobes. These areas of consolidation appear within 2-3 days of infection and directly correlate to where the most severe microscopic lesions and virus can be found.

Pathogenesis

On a cellular level, infection with flu viruses is initiated by the binding of hemagglutinin protein on the surface of the virus to sialic acid sugars on the surface of respiratory epithelial cells.³ It is generally accepted that human and swine influenzas bind to NeuAc α 2-6Gal – linked sialic acids (α 2-6) and avian influenza viruses bind to NeuAc α 2-3Gal (α 2-6) receptors.³ Once internalized, the virus replicates and many virus particles are released. The microscopic lesions indicating that this process is occurring can be seen by a pathologist within 24 to 48 hours of infection. The rate of progression and severity of the flu lesions vary among the flu viruses and can also be modified based on different host factors (e.g., level of specific immunity, age, co-infections, etc.). However, some generalizations can be made and thus the following description is a general outline of flu infection based on both personal experience with multiple viruses in an experimental setting and reports from the literature.

As shown in figure 1, a normal bronchiole (A) has a thin layer of epithelial cells (B) that have apical cilia (C) and peribronchiolar lymphoid tissue (D). The flu lesion begins primarily in the epithelial cells lining the airways 1-2 days after infection. The earliest flu lesions in figure 2 are vacuolar degeneration (E) and necrosis (F) of the

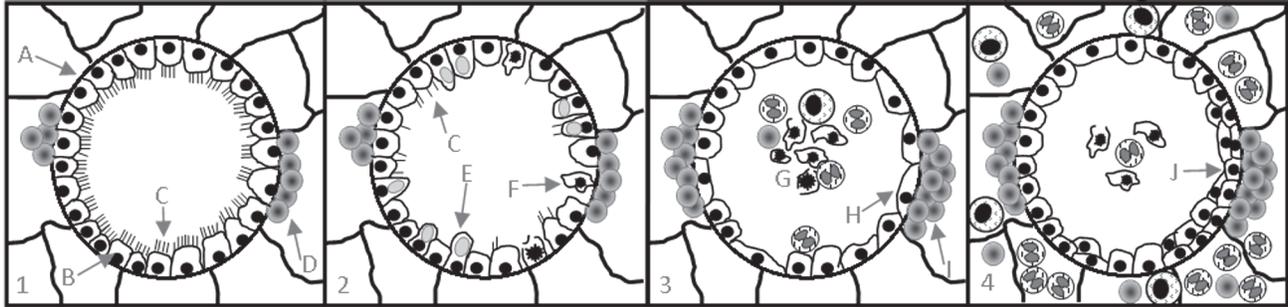
epithelial cells with loss of the apical cilia. By 3-4 days (Figure 3), the sloughing of the necrotic epithelial cells is more prominent along with a small influx of inflammatory cells (G). The remaining epithelial cells spread out to cover the basement membrane (attenuation, H) and lymphoid hyperplasia may start (I). Usually by 5-7 days after infection (Figure 4), signs of recovery in the bronchioles can be seen. These include varying degrees of epithelial hyperplasia (J) with mitotic figures in some of the epithelial cells. The inflammation in the alveolar walls and spaces can be seen as early as 2 DPI, but can take 2-5 days to consume an entire lobule or spread lobule to lobule. The amount of pure interstitial pneumonia (without the intraalveolar inflammatory component) accompanying the bronchiolar lesions is variable based on the virus, ranging from non-existent to severe, and can appear as early as 24-48 hours. If a secondary bacterial pneumonia or other complicating factor does not exist, the damaged respiratory tissues should be fully recovered on the microscopic level by 14-21 days after the initial infection.

Pathological findings in the experimental setting

The common experimental design for flu vaccine challenge studies is to give 2 vaccinations 2-3 weeks apart to healthy, recently weaned pigs and then challenge them with live virus inoculation 2-3 weeks after the second vaccination. The vaccine is usually a single inactivated virus strain with an adjuvant, but the vaccine can be commercial or autogenous, multivalent or a single virus, modified-live or killed, or DNA/recombinant subunit vaccines. The challenge virus may be homologous (same virus as vaccine) or heterologous (different virus from vaccine).

The study period is usually 5 days post infection or challenge and the data collected includes: rectal temperature, clinical signs of disease, nasal shedding (virus titers), and end-point macroscopic and microscopic pneumonia scores along with either bronchoalveolar lavage fluid (BALF) or lung tissue virus titers. Hemagglutination inhibition (HI) titers, indicating induced antibodies, are also examined pre- and post-infection. The ideal and complete protection by vaccination would result in little to no virus shedding, no microscopic or macroscopic pneumonia and no virus

Figure 1: A normal bronchiole (A) will have a thin layer of epithelial cells (B) that have apical cilia (C) and peribronchiolar lymphoid tissue (D). **Figure 2:** A bronchiole 1-2 DPI showing vacuolar degeneration (E) and necrosis (F) of the epithelial cells with loss of the apical cilia (C). **Figure 3:** A bronchiole 3-4 DPI with the necrotic epithelial cells and inflammation in the lumen (G), along with epithelial attenuation (H) and lymphoid hyperplasia (I). **Figure 4:** A bronchiole 5-7 DPI with epithelial hyperplasia (J) and inflammation in the alveolar walls and spaces.



detected in either BALF or lung tissue. Conversely, lack of protection would result in detectable virus in nasal swabs and lung tissue, and similar microscopic and macroscopic pneumonia scores between vaccinated and unvaccinated animals. A result somewhere in the middle would be suggestive of partial protection, which is the more common result.

One example of partial protection was found in an experiment conducted by the authors.¹ In this study, the efficacy of a commercial, inactivated, trivalent, swine influenza virus vaccine in pigs challenged with a contemporary alpha cluster H1N1 field isolate of North American swine-origin. The challenge virus shared < 88% HA gene similarity with the H1 vaccine viruses, thus this was a heterologous challenge. Pigs were allocated to treatment groups of vaccinated, placebo and negative control pigs and monitored for respiratory disease for 5 days post-challenge. On the challenge day and 5 days after challenge, the vaccinated pig sera had reciprocal HI titers ≥ 40 for all vaccine viruses, but ≤ 20 to the challenge virus. Gross lesions were present in the lungs of all pigs that had been inoculated with the challenge virus, but lung lesions did not differ significantly between the placebo and vaccinated pigs. However, there virus shedding was significantly reduced in nasal secretions, lungs and bronchoalveolar lavage fluid in the vaccinated pigs compared to the placebo pigs. These results indicate that the vaccinated swine were partially protected against experimental, heterologous challenge with a swine alpha cluster H1N1 virus.

In contrast to no protection or even partial protection from challenge after vaccination, there have been reports of disease enhancement after vaccination. These few instances reported in the literature of enhanced pneumonia and severe clinical signs were shown to occur in vaccinated pigs after experimental challenge. One of these cases was a DNA construct vaccine using the extracellular domain

of the matrix 2 protein (M2e) and nucleoprotein (NP). Vaccination with M2eNP and H1N1 virus challenge resulted in more severe clinical signs and death of 3/6 pigs within 48 hours of challenge.² In another study, enhanced pneumonia was found in 3/9 pigs vaccinated with a classical H1N1 killed virus vaccine and challenged with a reassortant H1N2 (γ -cluster). Interestingly, the pigs that had been previously inoculated with the identical classical H1N1 virus prior to challenge with the same H1N2 virus responded similarly to the pigs that had no vaccine before H1N2 virus challenge. This indicated that the killed virus vaccine may have induced a different response in some pigs (3/9).⁴ One other example of enhanced pneumonia was reported in 2/10 pigs that were vaccinated with a human-like reassortant H1N2 (δ 1-cluster) and challenged with pandemic H1N1 (pH1N1) virus (γ -cluster).¹ The exact mechanism of the response is unknown. In the pH1N1 challenge study, Gauger and colleagues detected HI and serum neutralizing (SN) antibodies to the priming antigen in the Vx/Ch pigs but no measurable cross-reacting HI or SN antibodies were detected to pH1N1. Thus the role of non-neutralizing antibodies may be the key to enhancing the pneumonia.

Comparative pathology to the rescue

Since influenza A virus is a shared disease of humans and animals, we can gain much knowledge for collaborative research and comparative pathology. Indeed, important insights into the mechanism of enhanced pneumonia due to influenza challenge in human patients.⁵ The results of this study suggest that in patients with severe disease, a larger proportion of serum antibodies were antibodies with no detectable neutralizing activity. The antibody avidity was also significantly higher in patients with severe disease than in those with mild disease. They conclude that higher titers of non-neutralizing antibody with higher avidity at

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the early stage of influenza virus infection may be associated with worse clinical severity and poorer outcomes.

Conclusion

While the phenomenon of enhanced pneumonia has not been a common finding, it does raise alarm to the potential hazards of using an autogenous vaccine in a herd where there is strong potential for heterologous challenge. Certainly we must learn from both the experimental setting outcomes and field observations in order to make advances in the control of influenza in swine. At the very least, we should continue to combine pathological findings with serological assays in tandem with genetic analyses of virus isolates in order to solve the continuing problems that flu presents for pigs and people.

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