The ferret as a model organism to study influenza A virus infection

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Influenza is a human pathogen that continues to pose a public health threat. The use of small mammalian models has become indispensable for understanding the virulence of influenza viruses. Among numerous species used in the laboratory setting, only the ferret model is equally well suited for studying both the pathogenicity and transmissibility of human and avian influenza viruses. Here, we compare the advantages and limitations of the mouse, ferret and guinea pig models for research with influenza A viruses, emphasizing the multifarious uses of the ferret in the assessment of influenza viruses with pandemic potential. Research performed in the ferret model has provided information, support and guidance for the public health response to influenza viruses in humans. We highlight the recent and emerging uses of this species in influenza virus research that are advancing our understanding of virus-host interactions.

Introduction

Influenza viruses are responsible for seasonal epidemics and sporadic pandemics of varying severity in humans. In a typical year, seasonal influenza viruses cause 250,000 to 500,000 deaths globally (approximately 36,000 in the United States), with >90% of these deaths occurring in the elderly (Thompson et al., 2004). Rates of infection and mortality can increase greatly during a pandemic. The influenza pandemic of 1918-1919 was the worst pandemic of modern times, which eventually infected about one-third of the world’s population, killing 20-50 million people (Johnson and Mueller, 2002). Although the recent 2009 H1N1 pandemic virus did not achieve similar levels of mortality (probably owing to both immunological reasons and advances in modern medicine over the past century), both pandemic viruses targeted young, healthy adults – a demographic not usually associated with seasonal influenza-related mortality (Dawood et al., 2009). Highly pathogenic avian influenza (HPAI) H5N1 viruses have caused over 560 human infections, with a 60% mortality rate; among many of these fatalities were young, previously healthy, adults (WHO, 2011). The heterogeneity of influenza viruses in their natural reservoir of wild waterfowl, and the growing diversity of influenza virus lineages affecting mammalian species (including swine, equine, canine and others), suggests that future human infection with viruses of pandemic potential will continue to occur. For these reasons, the availability of small mammalian models to study the virulence of emerging influenza viruses, in addition to understanding the molecular determinants that confer heightened pathogenicity and transmissibility among selected strains, is essential.

Ferret models (Mustela putorius furo) have been established for numerous viruses that cause respiratory infections, including human and avian influenza viruses, coronavirus, nipah virus, morbillivirus and others (Bossart et al., 2009; Martina et al., 2003; Smith and Sweet, 1988; Svitek and von Messling, 2007; Zitzow et al., 2002). Ferrets are an attractive mammalian model for these studies owing to their relatively small size and the fact that they emulate numerous clinical features associated with human disease; this is especially the case with regard to influenza. Ferrets and humans share similar lung physiology, and human and avian influenza viruses exhibit similar patterns of binding to sialic acids (the receptor for influenza viruses), which are distributed throughout the respiratory tract in both species (Maher and DeStefano, 2004; van Riel et al., 2007). The high susceptibility of ferrets to influenza virus, and the ability of sick ferrets to transmit virus to healthy ferrets, was first demonstrated in 1933 (Smith et al., 1933). Since this time, the use of the ferret model has been indispensable in experimental studies to elucidate virus-host interactions following influenza virus infection (Belser et al., 2009; Smith and Sweet, 1988). In this Primer article, we discuss the advantages of using the ferret for research with influenza A viruses, emphasizing the effectiveness of working with a model that can present both the pathogenic and transmissible features of influenza virus infection. We further highlight recent studies that are expanding the utility of the ferret to address novel research questions in this model.

Advantages of the ferret as a small animal model for influenza virus pathogenesis and transmission

Several mammalian models are available for influenza virus research, with their use depending on the specific research questions being addressed. Mice have traditionally been used as a model for influenza virus pathogenesis and offer many advantages over other laboratory species, including low cost, broad availability of reagents and the availability of transgenic mice with targeted gene disruptions (Belser et al., 2009). However, most human influenza strains do not replicate efficiently in mice without prior virus adaptation, limiting the utility of mice as models in which to study this group of

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Recent advances and future uses of the ferret model in influenza research

A greater understanding of influenza viruses and ferret biology as well as the continued development of sophisticated laboratory equipment has led to the ability of scientists to examine numerous properties not previously studied in the ferret model. Below we describe selected areas of investigation that are likely to expand our understanding of influenza virus infection in the coming years.

Aerosol delivery systems

Intranasal administration of virus in a liquid suspension has long been the traditional method for influenza virus inoculation in the ferret model. However, this practice is not truly reflective of ‘natural’ virus infection in humans, which commonly occurs following direct and indirect contact with contaminated surfaces. Exposure of naïve ferrets to aerosolized influenza virus has been performed using a nose-only inhalation exposure system (Lednicky et al., 2010; Tuttle et al., 2010). However, only recently has a rigorous method of ensuring standard doses of virus inoculum during aerosol delivery been reported, and a comprehensive comparison of intranasal and aerosol methods of inoculation has been conducted (Gustin et al., 2011). The development of a method to measure the number of viable influenza viruses in droplets and droplet nuclei exhaled from infected animals has furthered the utility of this model (Gustin et al., 2011). The development of influenza virus aerosol delivery systems that rigorously control for virus challenge inoculums, as well as the development of analytical systems for the ferret model, allow a more accurate recreation of natural influenza infection in the laboratory to study influenza virus transmission and perform risk assessments of emerging influenza viruses that pose a threat to public health.

Induction of immune responses

Reports of hypercytokinemia following H5N1 virus infection in humans, resulting in a deleterious ‘cytokine storm,’ have underscored the need to study the induction of innate immune responses in severely infected hosts (de Jong et al., 2006).
Moreover, knowledge about specific immune responses to vaccines, which correlate with protection against infection, will help to improve the design of novel vaccine candidates. In vivo assessments of immune function (i.e. innate and adaptive responses) following influenza virus infection have been primarily limited to the mouse model owing to a lack of ferret-specific reagents and incomplete genome sequencing of this species. However, the regulation of ferret proinflammatory mediators has been measured by real-time PCR and microarray analysis (Cameron et al., 2008; Svitak et al., 2008). Canine reagents have also been used successfully to assess ferret host responses, owing to the high levels of homology between canine and ferret nucleotide sequences, and numerous mouse monoclonal antibodies have demonstrated cross-reactivity with proteins expressed by ferret lymphocytes (Fang et al., 2010; Rutigliano et al., 2008). Continued immunology-based research in the ferret will result in a valuable outbred mammalian model for the study of innate and adaptive immune responses, leading to an improved understanding of human infection.

Laboratory abnormalities
Severe human infection with influenza viruses can result in substantial abnormalities of the lymphohematopoietic system, including lymphopenia, leukopenia, anemia and quantitative alterations of numerous serum analytes. Although lymphopenia has been previously demonstrated in ferrets, comprehensive analysis of white and red blood cell populations in whole blood from severely infected ferrets has only recently been performed (Belser et al., 2011; Zitzow et al., 2002). Furthermore, comprehensive analyses of serum chemistry profiles have allowed for detailed measurement of numerous parameters in the ferret that are frequently observed in severe human influenza virus infection (Belser et al., 2011). The ability to measure these parameters throughout the duration of an experiment by collecting samples during scheduled anesthesia of ferrets makes this an important contribution towards the standard pathotyping of viruses in this model.

Opportunistic infections
The 1918 H1N1 virus was extraordinarily virulent, but much of the mortality associated

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Table 1. Clinical, laboratory and virological features shared by humans and small mammalian models following influenza virus infection

<table>
<thead>
<tr>
<th>Human clinical feature</th>
<th>Ferrets</th>
<th>Mice</th>
<th>Guinea pigs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Present following infection with human and avian influenza viruses</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Sweet et al., 1979; Zitzow et al., 2002</td>
</tr>
<tr>
<td>Nasal secretions</td>
<td>Present following infection with human and avian influenza viruses</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Van Hoeven et al., 2009a; Zitzow et al., 2002</td>
</tr>
<tr>
<td>Coughing</td>
<td>Sneezing detected more frequently among highly transmissible viruses</td>
<td>Not observed</td>
<td>Generally not observed</td>
<td>Maines et al., 2006; Sun et al., 2010</td>
</tr>
<tr>
<td>Gastrointestinal complications (rare)</td>
<td>Present during infection with the 2009 H1N1 virus strain</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Belser et al., 2011; Maines et al., 2009; Sun et al., 2010</td>
</tr>
<tr>
<td>Serum abnormalities</td>
<td>Present; more pronounced following infection with virulent viruses</td>
<td>Not examined</td>
<td>Not examined</td>
<td>Belser et al., 2011</td>
</tr>
<tr>
<td>Neurological complications (rare)</td>
<td>Present following infection with highly pathogenic avian influenza viruses</td>
<td>Similar to ferret model</td>
<td>Not detected</td>
<td>Lu et al., 1999; Maines et al., 2005</td>
</tr>
<tr>
<td>Weight loss and/or anorexia</td>
<td>Present; more pronounced (&gt;10%) following infection with virulent viruses</td>
<td>Similar to ferret model</td>
<td>Minimal (&lt;10%) even following infection with virulent viruses</td>
<td>Lu et al., 1999; Van Hoeven et al., 2009a</td>
</tr>
<tr>
<td>Lethargy</td>
<td>Present; more pronounced following infection with virulent viruses</td>
<td>Similar to ferret model</td>
<td>Not detected</td>
<td>Maines et al., 2005; Van Hoeven et al., 2009a</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>Present; more pronounced following infection with virulent viruses</td>
<td>Similar to ferret model</td>
<td>Not examined</td>
<td>Tumpey et al., 2000; Zitzow et al., 2002</td>
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<tr>
<td>Hypercytokinemia</td>
<td>Divergent innate immune responses detected following infection with virulent viruses</td>
<td>Present following infection with virulent viruses</td>
<td>Not examined</td>
<td>Svitak et al., 2008; Tumpey et al., 2000</td>
</tr>
<tr>
<td>Transmission to susceptible contacts</td>
<td>Present following infection with most human, but not most avian, influenza viruses</td>
<td>Not reliably detected</td>
<td>Present, generally similar to ferret model</td>
<td>Herlocher et al., 2001; Lowen et al., 2006; Maines et al., 2006; Schulman and Kilbourne, 1963</td>
</tr>
<tr>
<td>Distribution of sialic acid (SA) receptors in respiratory tract</td>
<td>Predominance of α2-6 SA in ferret upper respiratory tract</td>
<td>Predominance of α2-3 SA differs from human respiratory tissue</td>
<td>High presence of α2-6 SA in upper respiratory tract</td>
<td>Ibricevic et al., 2006; Leigh et al., 1995; Sun et al., 2010</td>
</tr>
</tbody>
</table>

Shaded regions represent advantageous characteristics of a model.
with this pandemic was due to bacterial pneumonia that developed post-infection (Morens et al., 2010). Studies in mouse and ferret models corroborate the finding that previous infection with influenza virus can render a host more susceptible to secondary bacterial infection (Peltola et al., 2006). Although research in the ferret model has been limited to date, recent work has further demonstrated that prior infection with influenza virus can influence the severity and transmissibility of Streptococcus pneumoniae in ferrets, a finding that corroborates historical data in humans documenting that the incidence of pneumonia increases during pandemic influenza (McCullers, 2006; McCullers et al., 2010). Although histopathology provides evidence of bacterial involvement following wild-type virus infection, further work is needed to more precisely measure the onset of naturally occurring opportunistic infections following influenza virus infection in this model.

### Immunocompromised hosts
Humans with immunocompromised immune systems represent an especially susceptible population to influenza virus infection, and pose numerous challenges with respect to effectively preventing and treating infection. Although ferrets have not been used extensively to research influenza virus infection in the context of immunosuppression, this remains an understudied area that could benefit from such a model. Immunocompromised ferrets (achieved either by treatment with chemotherapy drugs or by depleting CD8+ and/or CD4+ T cells) were found to safely tolerate live, attenuated influenza vaccines (Huber and McCullers, 2006; Min et al., 2010). Although the research in this model is largely limited to drug- or antibody-induced immunosuppression, additional future studies evaluating influenza virus pathogenicity and transmissibility in the context of immunosuppression should allow for a greater understanding of influenza virulence in immunodeficient populations.

### Conclusion
Influenza viruses continue to emerge and cause human infection, so there remains a need to characterize the pathogenicity and transmissibility of these viruses in animal models. This information serves a vital role in public health with respect to evaluating the efficacy of novel vaccines and therapeutics against currently circulating strains. As described in this Primer article, the ferret is an excellent small mammalian model that can recapitulate many of the hallmarks of human influenza virus infection. Given the wealth of information that can be generated while studying respiratory virus infection in this model, ferrets offer numerous advantages over other small mammalian models and are an ideal laboratory species to measure the most crucial aspects of influenza virus infection in the context of improving public health.

### References
Influenza A virus infection in ferrets


