Ferrets and the Challenges of H5N1 Vaccine Formulation

Alan W. Hampson
Australian Influenza Specialist Group, Richmond, and School of Applied Sciences and Engineering, Monash University, Clayton, Victoria, Australia

(See the article by Govorkova et al., on pages 159–67.)

It has been more than 6 decades since the discovery that influenza viruses can infect ferrets and that they can be readily cultivated in the allantois of embryonated chicken eggs. Remarkably, since that time, no better animal model of influenza infection in humans has been found, and the embryonated chicken egg has yet to be successfully replaced as a medium for cultivating vaccine virus. As demonstrated by Govorkova et al. in this issue of the Journal of Infectious Diseases [1], ferrets and embryonated eggs may still provide the basis for developing influenza vaccines that will be effective in the face of a contemporary influenza pandemic threat.

It is widely accepted that a future influenza pandemic is inevitable. The ongoing spread of H5N1 in the world’s domestic poultry and associated sporadic transmission to humans is thought by many to be a prelude to a pandemic, in that it provides repeated opportunities for the virus to gain the capacity for human-to-human transmission, through either mutation or genetic reassortment. This has been the main incentive for accelerating national and international pandemic preparedness planning.

Vaccines are, without doubt, the most effective means of reducing the excess morbidity and mortality associated with both interpandemic and pandemic influenza. Despite extensive research and development on new vaccines and alternative production processes and the potential promise that they may hold [2], the overwhelming bulk of today’s influenza vaccines remain inactivated viral vaccines produced from virus grown in embryonated eggs. However, there have been subtle changes in the nature of these vaccines since the evolution of the last pandemic virus in 1968. Influenza A vaccine strains are now rapidly adapted to the required uniform morphology and increased growth potential by genetic reassortment with a laboratory-adapted virus, vaccines are formulated to a standard antigen content on the basis of an immunological assay, and vaccine virus is prepared in a much purer form by use of methods such as zonal ultracentrifugation.

More importantly, though, there has been a progressive replacement of whole-virus vaccines with split-product or purified-subunit vaccines, to further reduce the low level of residual systemic reactogenicity associated with purified vaccines. It is now generally considered that whole, split, and subunit vaccines have comparable efficacy but differ in terms of reactogenicity, particularly in young children. However, data to support efficacy have, for the most part, been generated in immunologically primed adults during an interpandemic period. Experience gained during trials of vaccines containing the A/New Jersey/76 (H1N1) virus, thought by US authorities to constitute a pandemic threat [3], and the reemergent A(H1N1) subtype A/USSR/77 virus clearly demonstrates the superior immunogenicity of whole-virus vaccines in immunologically naive individuals [4, 5]—a fact that, as noted by Hilleman [6], has gone largely unheeded.

The current global capacity to produce egg-based influenza vaccines is limited; it is estimated to be in the vicinity of 300 million doses of trivalent inactivated virus vaccine annually for normal seasonal use [7], and production schedules have a protracted time frame. Production of the more recently introduced living attenuated (cold-adapted) vaccine currently contributes little to the overall supply. Hence, in the event of a pandemic, if we assume the use of a monovalent vaccine, a requirement for 2 doses in a naive population [8], and the usual 15-μg hemagglutinin (HA) dose, there may be in the vicinity of 450 million doses available to fully immunize 225 million people in the first year. Consequently, there is growing interest in increasing the potential vaccine supply and the speed with which this could be made available, including precautionary stockpiling of vaccine prepared from an H5N1 strain.

However, vaccination against the H5N1 viruses appears to be problematic. From the time of the first observed human cases...
in Hong Kong in 1997, it was found that the hemagglutination inhibition (HI) test was a serological test for immunity against influenza that was insensitive for detecting serological evidence of infection with H5N1. Instead, a more sensitive microneutralization test was required. Shortly after this, a small human trial was conducted with an H5N3 vaccine in which the H5 HA was antigenically closely related to that of the H5N1 Hong Kong viruses. Of the vaccinees receiving 2 doses of conventional surface antigen vaccine, even at a 30-μg HA antigen dose, only a few seroconverted, as assessed by the HI test or the more sensitive microneutralization and single radial hemolysis (SRH) tests. Vaccine formulated with a proprietary oil adjuvant, MF-59, induced putative protective levels of antibody as determined by the SRH test but not by the HI test [8]. Subsequently, it has been demonstrated that an HI test in which avian erythrocytes (chicken or turkey) are replaced by horse erythrocytes has increased sensitivity for detection of H5 antibody in humans [9]. Although the sensitivity of antibody detection tests presents an issue for regulators, particularly where there is no accepted correlate of protection against influenza A viruses for other serological tests. Although the ferret challenge model has been little used to assess vaccine effectiveness recently, there is substantial literature from the University of Sheffield Medical School in the 1970s regarding the ferret as a model of human influenza, including studies assessing new split and subunit vaccines [12, 13].

Protection of ferrets against intentional challenge, therefore, has the potential to provide useful additional data for assessment of vaccine formulations where human serological data alone are inadequate. An important finding in early ferret studies was the need for either prior priming of the animals by infection with an unrelated influenza subtype or, in the absence of this, use of adjuvants if a measurable antibody response was to be achieved with inactivated virus preparations. The priming appears to have been fortuitously achieved in the Goverkova et al. study by the use of animals that predominantly demonstrated serological evidence of prior A(H3N2) infection. It is unknown whether similar priming may occur to any extent in humans.

Somewhat surprisingly, in the Goverkova et al. study, ferrets achieved significant and measurable homologous HI antibody titers, measured using chicken erythrocytes, after vaccination with inactivated H5N1 vaccine, even with a single dose of 7 μg of HA without adjuvant. There are insufficient published data on H5N1 immunization in ferrets to know whether this is a consequence of using whole-virus vaccine or of some inherent difference between the responses of ferrets and humans to H5N1. Certainly, the improved response to whole virus seen in human H1N1 trials some years ago [4, 5] suggests that this could be a significant factor, as may more-recent human clinical trials of H2 and H9 vaccines [14].

Possibly the greatest significance of Goverkova et al.’s study is the demonstration of a significant cross-strain protective effect even in the presence of minimal antibody levels. This, together with human serological data generated with an H5N3 vaccine [15], strengthens the argument for stockpiling vaccines prepared from currently available H5N1 vaccine strains. It would now be valuable to evaluate vaccines prepared under commercial-production conditions in the ferret model, particularly those that have been evaluated in human clinical trials, and to compare the potency of the whole-virus and split or subunit preparations. This would not only allow comparison between the human serological response and the ferret protective effect but also ensure that the results obtained in the ferret are not influenced by differences between small-scale laboratory preparations and vaccines manufactured under full good manufacturing practice conditions. Regrettably, because of the requirement for high-level biological containment, there are few facilities where such challenge experiments are possible, particularly those that have the capacity and expertise to utilize ferrets.

References


