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H5N1, a wealth of knowledge to improve pandemic preparedness

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Influenza infections have been recognized in humans since ancient times, associated with the belief that it was caused by a supernatural influence (Latin *influentia*). In 1933, the causative agent of human influenza was identified and by 1955 it was realized it shared a genomic composition similar to the “fowl plague” (avian influenza) virus (Potter, 2001). Influenza viruses belong to the family *Orthomyxoridae* that include the influenza viruses type B and C, the insect viruses Togotho and Dhori, and Salmon Anemia virus. However, the most notorious members of the family are the type A influenza viruses, which are responsible for annual epidemics in humans, recurrent epidemics in swine and horses, sporadic cases in minks, seals and whales, and devastating outbreaks in poultry (Webster, 1997). The terminology “highly pathogenic avian influenza” (HPAI) was officially adopted in 1981 to designate the highly virulent forms of avian influenza. It is commonly accepted that migratory waterfowl, including ducks, sea birds, or shorebirds are the natural hosts of influenza viruses and from where they jump the species barrier (Alexander, 2000). When they do so into domestic poultry, some of these viruses can become highly pathogenic. Although HPAI viruses are primarily restricted to poultry, the world has experienced an emergence of avian influenza strains within the last 15 years that can readily jump to mammalian hosts causing disease and death. This is particularly the case of the so-called H5N1 HPAI viruses, which since 1997 have been responsible for more than 600 human infections with more than 300 deaths spanning vast geographic regions of the world, from Asia, to the Middle East, to Africa (Van Kerkhove et al., 2011). The high mortality rate of these infections, added to the evidence that many pandemic viruses emerge from the avian reservoir, make H5N1 viruses a major public health threat. Luckily, H5N1 HPAI viruses are not easily transmitted among humans or other mammals. Transmission, particularly by respiratory droplets, is considered one of the conditions for the emergence of influenza strains with pandemic potential. Another condition is to have a human population with little or no immunity against the novel strain. Expansion of these viruses into new geographical regions and wild bird hosts has been accompanied by an increase in genetic diversity through antigenic drift and reassortment, producing multiple clades. Eradication efforts have generally failed and lead to the emergence of many antiviral resistant strains. Research aimed at better understanding the ecology, evolution, pathogenesis and transmission of

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H5N1 viruses has resulted in a wealth of knowledge, including novel antivirals, alternative vaccines, and better vaccination strategies (Banner and Kelvin, 2012; Neumann et al., 2010; Sambhara and Poland, 2010). For the past 15 years, researchers have been puzzled and challenged by a virus that can poorly jump the species barrier into humans, but when it does, the outcome is uncharacteristically fatal. Early work with animal models was crucial to demonstrate that such phenomena could be reproduced in other mammalian hosts. Initial work in mice and ferrets demonstrated the high lethality of some strains of H5N1 for these animals. There has been generally good correlation between the origin of such strain, whether it was isolated from a human or a chicken, and the outcome of disease in animal models; with those isolated from the former having higher virulence than those isolated from the latter, in either mice or ferrets or both. This early work led to the realization that the polybasic cleavage site in the HA glycoprotein of H5N1 viruses plays a major role the viral pathogenesis in mammals and that key amino acid mutations in PB2 are significant contributors to virulence, although additional mutations elsewhere in the virus are important too (Hatta et al., 2001a). Early studies in ferrets also showed that, like in humans, H5N1 infections can be fatal but exhibit poor respiratory droplet transmission among cage mates (Neumann et al., 2010). These observations prompted further studies into analyzing the sialic acid receptor specificity of H5N1 viruses and better characterizations of receptor distribution in humans and many other mammalian and avian species (Kimble et al., 2010; Shinya et al., 2006; Stevens et al., 2006; Wan and Perez, 2006). Today, it is clear that it is the distribution rather than the presence or absence of receptors which determines the ability of these viruses to replicate in the upper or lower respiratory tract of a given host. It is also clear that a change in receptor specificity alone, from the typical avian-like (α 2,3-sialic acid) to the typical human-like (α 2,6-sialic acid), is not sufficient to make H5N1 strains transmissible in ferrets. Likewise, reassortment between H5N1 and human seasonal viruses does not improve transmission in ferrets (Chen et al., 2012; Jackson et al., 2009; Maines et al., 2005, 2006).

Classical gain- and loss-of-function approaches as well as systems biology approaches have revealed complex sets of viral-host interactions that modulate H5N1 disease outcome (Hatta et al., 2001b; Li et al., 2011). Exacerbated host innate immune responses, also known as cytokine storm, is triggered by infection with H5N1 and is connected to the genetic background of the host. In recent years, few genetic polymorphisms have been linked to influenza susceptibility, and it is expected that additional participating genetic elements will be revealed in the near future (Chan et al., 2005; Peiris, 2006). The contribution of host genetics is readily appreciated when comparing the susceptibility to H5N1 in *Anseriformes* versus *Galliformes*, with the former having the tendency to be more resistant to disease than the latter (Kim et al., 2009). Recent reports have shown that H5N1 viruses can “learn” to transmit by respiratory droplets in ferrets used as a mammalian model (Herfst et al., 2012; Imai et al., 2013). Most troubling is that naturally occurring viruses are just a handful of amino acid mutations away from acquiring this transmission phenotype (Russell et al., 2012).

The great progress that has been made on the basic research front with H5N1 viruses has not been accompanied by changes in agricultural practices that would have a major impact in the eradication of these and other zoonotic pathogens. Culling remains the most effective

method to eradicate highly pathogenic avian influenza viruses, but after hundred of millions of birds have been culled due to H5N1, the virus continues to circulate in vast regions of the world. Currently, H5N1 is known to circulate in Bangladesh, Cambodia, China, Egypt, Indonesia, and Viet Nam. Not surprisingly, these countries continue to report human cases with high mortality rates. Stemming from the two studies that showed respiratory droplet transmission of H5N1 and the fear that such information could be used for nefarious purposes, the “U.S. Department of Health and Human Services (HHS), a major funder of influenza research, developed a framework for guiding HHS funding decisions on individual proposals involving HPAI H5N1 research with specific attributes.” Under this framework, research proposals are reviewed for scientific merit and public health benefits prior to making a funding decision. The biosafety and biosecurity risks associated with the proposal together with risk mitigation measures that are required are all considered. It would be ideal if such initiative would translate in similar efforts to ascertain the risks associated with agricultural and animal commercialization practices. Live animal markets and animal fairs have been linked multiple times to zoonotic outbreaks. Live bird markets in Asia are undoubtedly the major contributor in the evolution of avian influenza viruses with zoonotic potential, a fact for which we seem to remain oblivious.

The accompanying review articles represent an attempt to summarize the progress made to understanding H5N1 viruses since their emergence in humans in 1997. This special issue was particularly triggered by the culmination of 15 years of work into the molecular attributes that lead to aerosol transmission of H5N1 in a mammalian model. These studies represent a major inflection point for the future of not only H5N1 research, but also in the way the research efforts should be channeled towards complete eradication of H5N1 viruses.

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References

- Alexander DJ. A review of avian influenza in different bird species. *Vet. Microbiol.* 2000; 74(1–2):3–13. [PubMed: 10799774]
- Banner D, Kelvin AA. The current state of H5N1 vaccines and the use of the ferret model for influenza therapeutic and prophylactic development. *J. Infect. Dev. Ctries.* 2012; 6(6):465–469. [PubMed: 22706187]
- Chan MC, Cheung CY, Chui WH, Tsao SW, Nicholls JM, Chan YO, Chan RW, Long HT, Poon LL, Guan Y, Peiris JS. Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. *Respir. Res.* 2005; 6:135. [PubMed: 16283933]
- Chen LM, Blixt O, Stevens J, Lipatov AS, Davis CT, Collins BE, Cox NJ, Paulson JC, Donis RO. In vitro evolution of H5N1 avian influenza virus toward human-type receptor specificity. *Virology.* 2012; 422(1):105–113. [PubMed: 22056389]
- Hatta M, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science.* 2001a; 293(5536):1840–1842. [PubMed: 11546875]
- Hatta M, Neumann G, Kawaoka Y. Reverse genetics approach towards understanding pathogenesis of H5N1 Hong Kong influenza A virus infection. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 2001b; 356(1416):1841–1843. [PubMed: 11779382]

- Herfst S, Schrauwen EJ, Linster M, Chutinimitkul S, de Wit E, Munster VJ, Sorrell EM, Bestebroer TM, Burke DF, Smith DJ, Rimmelzwaan GF, Osterhaus AD, Fouchier RA. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science*. 2012; 336(6088):1534–1541. [PubMed: 22723413]
- Imai M, Herfst S, Sorrell EM, Schrauwen EJ, Linster M, De Graaf M, Fouchier RA, Kawaoka Y. Transmission of influenza A/H5N1 viruses in mammals. *Virus Res*. 2013
- Jackson S, Van Hoeven N, Chen LM, Maines TR, Cox NJ, Katz JM, Donis RO. Reassortment between avian H5N1 and human H3N2 influenza viruses in ferrets: a public health risk assessment. *J. Virol*. 2009; 83(16):8131–8140. [PubMed: 19493997]
- Kim JK, Negovetich NJ, Forrest HL, Webster RG. Ducks: the “Trojanhorses” of H5N1 influenza. *Influenza Other Respir. Viruses*. 2009; 3(4):121–128. [PubMed: 19627369]
- Kimble B, Nieto GR, Perez DR. Characterization of influenza virus sialic acid receptors in minor poultry species. *Virol. J*. 2010; 7:365. [PubMed: 21143937]
- Li C, Bankhead A 3rd, Eisfeld AJ, Hatta Y, Jeng S, Chang JH, Aicher LD, Proll S, Ellis AL, Law GL, Waters KM, Neumann G, Katze MG, McWeeney S, Kawaoka Y. Host regulatory network response to infection with highly pathogenic H5N1 avian influenza virus. *J. Virol*. 2011; 85(21):10955–10967. [PubMed: 21865398]
- Maines TR, Chen LM, Matsuoka Y, Chen H, Rowe T, Ortin J, Falcon A, Nguyen TH, Mai le Q, Sedyaningsih ER, Harun S, Tumpey TM, Donis RO, Cox NJ, Subbarao K, Katz JM. Lack of transmission of H5N1 avian-human reassortant influenza viruses in a ferret model. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103(32):12121–12126. [PubMed: 16880383]
- Maines TR, Lu XH, Erb SM, Edwards L, Guarner J, Greer PW, Nguyen DC, Szretter KJ, Chen LM, Thawatsupha P, Chittaganpitch M, Waicharoen S, Nguyen DT, Nguyen T, Nguyen HH, Kim JH, Hoang LT, Kang C, Phuong LS, Lim W, Zaki S, Donis RO, Cox NJ, Katz JM, Tumpey TM. Avian influenza (H5N1) viruses isolated from humans in Asia in 2004 exhibit increased virulence in mammals. *J. Virol*. 2005; 79(18):11788–11800. [PubMed: 16140756]
- Neumann G, Chen H, Gao GF, Shu Y, Kawaoka Y. H5N1 influenza viruses: outbreaks and biological properties. *Cell Res*. 2010; 20(1):51–61. [PubMed: 19884910]
- Peiris M. Pathogenesis of avian flu H5N1 and SARS. *Novartis Found. Symp*. 2006; 279:56–60. Discussion 60-55, 216-219. [PubMed: 17278385]
- Potter CW. A history of influenza. *J. Appl. Microbiol*. 2001; 91(4):572–579. [PubMed: 11576290]
- Russell CA, Fonville JM, Brown AE, Burke DF, Smith DL, James SL, Herfst S, van Boheemen S, Linster M, Schrauwen EJ, Katzelnick L, Mosterin A, Kuiken T, Maher E, Neumann G, Osterhaus AD, Kawaoka Y, Fouchier RA, Smith DJ. The potential for respiratory droplet-transmissible A/H5N1 influenza virus to evolve in a mammalian host. *Science*. 2012; 336(6088):1541–1547. [PubMed: 22723414]
- Sambhara S, Poland GA. H5N1 Avian influenza: preventive and therapeutic strategies against a pandemic. *Ann. Rev. Med*. 2010; 61:187–198. [PubMed: 20059335]
- Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. Avian flu: influenza virus receptors in the human airway. *Nature*. 2006; 440(7083):435–436. [PubMed: 16554799]
- Stevens J, Blixt O, Tumpey TM, Taubenberger JK, Paulson JC, Wilson IA. Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science*. 2006; 312(5772):404–410. [PubMed: 16543414]
- Van Kerkhove MD, Mumford E, Mounts AW, Bresee J, Ly S, Bridges CB, Otte J. Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. *PLoS ONE*. 2011; 6(1):e14582. [PubMed: 21283678]
- Wan H, Perez DR. Quail carry sialic acid receptors compatible with binding of avian and human influenza viruses. *Virology*. 2006; 346(2):278–286. [PubMed: 16325879]
- Webster RG. Influenza virus: transmission between species and relevance to emergence of the next human pandemic. *Arch. Virol. Suppl*. 1997; 13:105–113. [PubMed: 9413531]