PERSPECTIVE

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Engineering H5N1 avian influenza viruses to study human adaptation

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Two studies of H5N1 avian influenza viruses that had been genetically engineered to render them transmissible between ferrets have proved highly controversial. Divergent opinions exist about the importance of these studies of influenza transmission and about potential 'dual use' research implications. No consensus has developed yet about how to balance these concerns. After not recommending immediate full publication of earlier, less complete versions of the studies, the United States National Science Advisory Board for Biosecurity subsequently recommended full publication of more complete manuscripts; however, controversy about this and similar research remains.

Knowledgeable observers operating within a legitimate framework for the public good have expressed divergent opinions about the importance and public safety implications of two papers, one recently published[1] and one soon-to-be published[2], describing the production of ferret-transmissible H5N1 influenza viruses, and about related influenza transmission and pathogenesis research[3–7]. Some have emphasized that understanding the underlying principles of influenza virus host adaptation and transmission can lead to better prevention and control of viruses that arise naturally, whereas others have drawn attention to 'dual use' implications—that is, bioterrorism—or to accidental release of potentially deadly viruses. The most commonly mentioned public safety concerns relate to three assumptions: (1) H5N1 viruses are currently highly lethal to humans but are poorly transmissible; (2) genetic manipulation of H5N1 viruses to increase transmissibility in mammals such as ferrets will create variant viruses that remain highly pathogenic and that become transmissible in humans; and (3) if accidentally or intentionally released, such a virus could precipitate a historically severe influenza pandemic. How do these assumptions hold up against scientific data? In this perspective, we address research evidence related to the epidemic/pandemic potential of genetically engineered H5N1 viruses, and discuss limitations in understanding how influenza viruses become pathogenic, transmissible and potentially pandemic in humans.

Background

Influenza is among the leading global infectious causes of death, periodically causing pandemics that can kill millions of people. Countless influenza A viruses circulate globally in a reservoir that consists of hundreds of avian species. Rarely, one of these viruses undergoes changes that enable it to switch hosts to infect mammals, including humans, although it is not clear whether human transmission can result directly from adaptation of an avian influenza virus (this has not been documented to occur), or only indirectly via further adaptation of pre-existing human or mammalian-adapted viruses, the mechanism that has been associated with all known pandemic and seasonal viruses after 1918. The factors underlying all such emergences are poorly understood[8]. In the past 80 years of influenza virology, three pandemics have resulted from reassortments of pre-existing human-adapted or mammalian-adapted viruses with one or more avian-influenza-derived genes, but no purely avian influenza virus has emerged to cause a pandemic or human outbreak, or has even become stably adapted to humans. However, because avian influenza viruses have adapted to other mammals, it is considered plausible that such an emergence could occur in humans.

Among many other important research areas related to influenza, it is therefore critical to study the mechanisms by which influenza viruses emerge from birds to become adapted to mammals and ultimately humans, and to learn how the phenotypic properties of such evolving viruses may be associated with human transmission and disease. Among the many subtypes and strains of avian influenza A viruses that exist in nature, those that have at least occasionally infected mammals (for example, H5N1, H7N7 and H9N2) are of interest because they might theoretically be more likely than other influenza A viruses to adapt directly or indirectly to humans. Highly pathogenic avian influenza (HPAI) H5N1 viruses have been of particular interest with respect to theoretical pandemic potential because they have been unusually pathogenic in domestic poultry and have infected and killed several hundred people over a 15-year period.

In seeking to understand such influenza viruses, a research approach used widely in virology is to engineer specific genetic mutations into naturally occurring viruses, and then study the resulting viral phenotypic properties in animals, including infectivity, cell tropism, viral replication, pathogenicity and transmissibility. These types of experiments can potentially provide clues about whether and how a virus might adapt to humans, and what prevention and control options might be useful if that virus did emerge. Much H5N1 research of this type has already been published, including viral genetic engineering to evaluate properties such as pathogenicity and transmissibility in ferrets and other animal models. In the context of this published research literature, we comment on questions relevant to the two papers under discussion[1,2].

H5N1 infectivity for humans

The ongoing HPAI H5N1 enzootic continues to cause 'spill-over' human infections. World Health Organization (WHO) data indicate that since 2003, HPAI H5N1 viruses have infected 603 people and killed 356 (ref. 9). Technically, the term 'highly pathogenic' refers only to the effects of certain H5 and H7 influenza viruses in poultry, not in humans or other mammals; most such viruses either cannot infect, or are relatively harmless in, humans. HPAI H5 and H7 phenotypes are both associated with mutations in the haemagglutinin (HA) gene that usually result from insertion of a sequence of codons encoding multiple basic amino acids at the location where the two linked protein domains comprising the

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mature HA are cleaved during infection[10]. This cleavage site change leads to disseminated viral replication in multiple organs of avian species, resulting in high mortality. However, in humans, who cannot be easily infected with most low pathogenicity or HPAI viruses, if they can be infected at all, efficient replication outside the respiratory tract generally does not occur. Therefore, despite the current unusual situation with H5N1 viruses in humans since 2003 (see below), neither H5N1 nor other HPAI viruses would necessarily replicate systemically or cause extreme pathogenicity should human adaptation occur. Although the basis of HPAI H5N1 viral pathogenicity in severe and fatal human cases remains unknown, there is no evidence suggesting that it results from changes known to be associated with viral adaptation to gallinaceous poultry; in fact, no human-adapted or pandemic influenza virus contains genetic changes indicative of prior poultry adaptation.

Solely on the basis of publicly available information about pathogenicity of intranasally inoculated H5N1 virus (the model for natural human and animal infection), the laboratory-derived H5N1 viruses produced in the two papers under discussion[1,11–15] do not have enhanced pathogenicity in ferrets compared to the 2009 pandemic H1N1 virus, which is considered to be mildly pathogenic for humans[14,15]. An apparent misconception has nevertheless arisen in recent public discussions of these studies, namely that the engineered, ferret-transmissible H5N1 viruses were extremely pathogenic in ferrets after intranasal inoculation or aerosol transmission. This notion seems to have resulted in part from misunderstandings about a technique—intratracheal inoculation—used in a separate sub-study reported in the manuscript by the Fouchier group[14,15], a method that is not directly relevant to viral transmissibility or natural pathogenesis. As documented since the 1940s[16], intratracheal inoculation of influenza viruses is not a 'model' for natural viral pathogenicity; influenza viruses that are otherwise considered to be of low pathogenicity often induce severe and even fatal disease in animals when administered by this route, including the 2009 pandemic H1N1 virus[15]. The presentation of transmissibility studies alongside high-dose intratracheal inoculation pathogenesis studies in the Fouchier manuscript seems to have suggested (incorrectly) to some that the engineered transmissible H5N1 virus is deadly after intranasal inoculation or aerosol transmission between ferrets and, by extension, might be both transmissible and deadly for humans, that is, a virus of deadly pandemic potential. No evidence has yet been presented to support this, although the possibility that additional unspecified genetic changes might do so cannot be excluded.

Potential for human adaptation of H5N1 viruses

It is questionable to what extent HPAI H5N1 is adapted to, or capable of adapting to, humans. It is not clear why one evolving lineage of avian HPAI H5N1 viruses, out of a large and genetically divergent pool of H5 and other avian influenza viruses that rarely infect humans (much less cause severe human disease), has recently infected hundreds or perhaps thousands of people. It may be that the human cases are a result of unusual high-dose exposures or rare individual genetic susceptibilities. Alternatively, H5N1 viruses may be beginning to do something no other HPAI virus has ever been documented to do—adapt directly to humans. And if H5N1 did adapt, could it cause a pandemic?

No HPAI virus in the historic record has ever been efficiently transmitted between humans, let alone caused a pandemic. Even when avian influenza virus genes have been imported by reassortment into existing human influenza viruses, as happened for example in 1957 with H2N2 influenza and in 1968 with H3N2 influenza, the sources seem to have been circulating low-pathogenicity avian viruses, not poultry-adapted viruses such as HPAI viruses[10]. Conceivably, the considerable host-switching mutations associated with adaptation of wild bird viruses to gallinaceous poultry, or at least of wild viruses to HPAI poultry viruses, represent an evolutionary pathway divergent from those pathways associated with mammalian adaptation, seemingly presenting an additional challenge for poultry-adapted influenza viruses to achieve efficient mammalian adaptation[17]. After 15 years of high-density enzootic circulation in domestic poultry around the world, no human-adapted H5N1 virus has emerged from a natural reservoir, suggesting the existence of unknown biological barriers.

Despite circulation of influenza A viruses of 16 HA subtypes in billions of birds over a very long time span, the four pandemics in the last century have been restricted to influenza viruses bearing HA subtypes H1, H2 or H3. Decades ago, many experts predicted that influenza pandemics could be explained by 'recycling' of a small number of HAs in new human generations; more recently, this belief has been expanded to posit that the other HA subtypes (including H5) are fundamentally incapable of adapting to humans, being selected against by biological constraints or unappreciated selection pressures[18–20]. Despite widespread influenza virus circulation and dynamic evolution at the human–animal interface, with many billions of quasispecies, mutations and gene constellations circulating, only four influenza pandemics have occurred in the last century, and in the three of those with a known viral origin the viruses resulted from reassortment of pre-existing human or swine viruses[21], not by mutation or adaptation of existing avian viruses.

This suggests that *de novo* emergence of a human pandemic influenza virus is an extremely rare event that is not easily achieved in nature[10], and presumably would not be easily achieved by engineering a small number of laboratory mutations. As some of the key engineered H5N1 mutations in the two studies occur spontaneously during normal laboratory passage[22], or have been found singly or in combination in natural H5N1 and in other influenza viruses[23–26], including strains from wild birds, it remains unclear whether or how the engineered viruses in question create or increase the risk of a pandemic.

Engineering H5N1 phenotypic changes

Serial passage of a virus in intact animals or in tissues derived from a particular species often results in enhanced species-specific virulence, which can be applied to establish an animal model with measurable morbidity and/or mortality outcomes useful for evaluating antiviral therapeutics, passive immunization and vaccines. Influenza viruses, SARS coronavirus and Ebola virus have all been passaged in mice to enhance virulence; the resulting host-adapted viruses have been studied biologically and used to evaluate strategies for control and prevention. However, adaptational mutations resulting from serial passage tend to be host-specific and may not produce the same outcomes in other species. For example, the classical swine influenza virus, A/swine/Iowa/1930 (H1N1), is very pathogenic in ferrets and mice but poses no threat to humans[27]. Another example is mouse-adapted Ebola virus, which is lethal for mice and guinea pigs but attenuated for nonhuman primates[28,29]. Ferrets are susceptible to a wide range of viruses including influenza viruses, SARS coronavirus, canine distemper virus and some parvoviruses, many of which do not infect humans or other mammals. A number of influenza viruses that replicate efficiently in ferrets[30–33] seem poorly able, or unable, to infect humans, even after experimental challenge[34]. Thus, pathogenicity and transmissibility of any influenza virus in ferrets cannot be used directly to predict what type and severity of disease the same virus might produce in humans and human populations.

Predicting human transmissibility

It is unclear whether genetic manipulation of an H5N1 virus to achieve transmissibility in a particular mammal such as a ferret can predict human transmissibility. Because natural history and viral challenge studies cannot always be performed in humans, they have been conducted in experimental animals including mice, guinea pigs, ferrets, non-human primates and various other mammals. Unfortunately, there is no perfect animal model capable of reproducing all of the important variables involved in human influenza infection, although each animal model may be useful in understanding some aspect of influenza biology. Unlike most other mammals, ferrets generally can be infected with many or most avian, mammalian and human influenza viruses without prior viral adaptation, and often transmit efficiently between them[35], providing useful general information about the viral genetic basis of phenotypic properties such as infectivity, pathogenicity, transmissibility and

immune responses[35], even though the findings cannot necessarily be directly applied to human infections[36–38]. Furthermore, in decades of research, using a large number of different avian and mammalian influenza viruses, severe or fatal disease has not often been observed in ferrets following intranasal inoculation or aerosol exposure.

These useful traits of easy infectability and mildly symptomatic infection have rendered the ferret a 'permissive' influenza model. Specifically, many naturally occurring influenza viruses that infect, and often transmit between, ferrets are not known to infect people or cause human disease[27,30–33,39,40]. Ferrets are thus an imperfect model for predicting human infectivity or transmissibility, let alone the high level of transmissibility characteristic of pandemic spread. On the basis of public presentations by the senior authors of the two studies in question, neither of the engineered H5N1 viruses was as efficiently transmissible in ferrets as the human-adapted 2009 pandemic H1N1 virus[14,15]. Phenotypic properties such as replication, pathogenicity and transmissibility are likely to be polygenic traits driven by mutations that are independent and possibly competing[10,41]. Transmissibility is a complex phenotype that probably requires cooperative changes in more than one gene segment, and these may differ greatly between different viruses that become transmissible. Mutations that confer transmissibility in a ferret may be species-specific and irrelevant to other hosts[42]. There are probably multiple unique virus-specific pathways to transmissibility for particular viruses infecting particular hosts[43]. For example, transmissibility of the 1918 pandemic H1N1 virus has been linked to changes in the genes encoding HA and PB2 proteins[36,37], whereas transmissibility of the 2009 pandemic H1N1 virus, which has a closely related HA, has been linked to gene segments encoding the neuraminidase and matrix proteins[44].

Moreover, because determinants of viral pathogenicity may be lost upon adaptation to a new host, H5N1 viruses, whether or not transmissible, do not always cause severe disease in ferrets or non-human primates[45–47]. For these reasons viral phenotypes observed in animal models like the ferret may not predict what would happen in humans. Indeed, given that many influenza viruses that are non-pathogenic for humans easily transmit and may cause illness in ferrets, the 'ferret model' does not reliably predict human transmissibility or pathogenicity, although the model remains valuable for understanding the principles and dynamics of infection.

In addition to data from experiments in mammals, it is noteworthy that of the many mammalian-adapted influenza viruses that infect and transmit explosively among pigs, among horses and among dogs, few infect humans and none are transmitted between them[48]. Although swine influenza viruses caused sporadic human infections before 2009[49,50], and caused the 2009 H1N1 pandemic[21], outbreaks associated with human influenza viruses are rare in pigs. It is even conceivable that H5N1 viruses have already become adapted to mammals without causing severe disease or onward transmission to humans. Evidence from China's Qinghai Lake, for example, shows 13.4% H5N1 seropositivity and 3.4% active infection in apparently healthy, live-caught rodents called pikas[51]. Clearly, adaptation of an influenza virus to a specific mammalian host does not necessarily predict its infectiousness, pathogenicity or transmissibility in other mammals, even though valuable insights into mechanisms of disease, host responses, and prevention and treatment may be obtained by studying these particular animals. Such insights can provide valuable clues in designing countermeasures against deadly epidemics and pandemics.

H5N1 case–fatality rate

Belief that an H5N1 virus could produce a 59% pandemic case-fatality rate is the most frightening aspect of the current controversy surrounding aerosol transmission of H5N1 virus in ferrets. In 500 years of observation, no influenza pandemic is believed to have caused a case-fatality rate over about 2% (ref. 52); pandemic and seasonal circulation of H1, H2 and H3 influenza viruses over the past century have all produced lower overall mortality rates[53]. The widely cited 59% figure is not a mortality rate but a case-fatality rate computed from cumulative cases reported to WHO since 2003[9]. (Case fatality refers to fatal cases divided by all fatal plus non-fatal cases combined.) By general consensus, the WHO figure probably overestimates actual mortality. Among other concerns common to epidemiological data, the WHO case definition[54] features diagnostic severity criteria (evidence of an acute pneumonia on chest radiograph plus evidence of respiratory failure) that constitute a self-fulfilling prophecy for fatality; as with many illnesses studied epidemiologically, severe diseases are more likely to receive optimal diagnostic work-up ('detection bias'); and most H5N1 cases have been reported from countries with limited resources for identifying milder cases, if they occurred. These factors together could combine to erroneously inflate case-fatality calculations by over-counting severe cases and under-counting mild cases[55].

However, potentially more important clues to actual H5N1 pathogenicity and human case-fatality rates come from epidemiological studies, which taken together suggest to us that H5N1 may not be highly lethal except in people with rare susceptibilities. Forty-six published H5N1 seroprevalence studies of various exposure categories (household contacts, healthcare workers, poultry workers, and so on) show generally low H5N1 seroprevalence (mean, 1.7% of 21,435 persons examined in all 46 studies combined (a bibliography of these studies is available from the authors on request)). Given intense poultry and other exposures in many study areas, these low rates at first seem perplexing, especially when compared to the much higher seroprevalence rates in humans for other avian influenza viruses such as H9N2 (ref. 56). When such information is considered in light of statistically significant clustering of non-human-transmitted (that is, presumably avian-acquired) household cases in genetically related versus unrelated persons[57,58], a reasonable explanation seems to us to be that H5N1 is so poorly adapted to humans that exposure does not normally lead to infection or even the development of a detectable immune response[57,59], except in persons with specific but undefined genetic susceptibilities, many of whom become cases[60–62]. There are few data on what the basis of such genetic susceptibilities may be, although recent evidence has linked severe human influenza to a minor *IFITM3* allele[63], supporting the suspicion that genetic determinants of influenza infection and replication in humans do exist.

A published meta-analysis of selected seroprevalence studies implies that the actual H5N1 case-fatality rate may be far below 1% (ref. 56), and thus probably far below the case-fatality rates for seasonal influenza. This has been disputed because it has been difficult to find mild cases, and because of the possibility that some low-level antibody titres ($<$1:80) might be false positives[5]. On the other hand, rapid disappearance of human H5N1 vaccine-induced antibody[64] suggests that the opposite problem of false negatives could be occurring and, if so, might be especially problematic in cross-sectional studies in which the time since infection is not known, and which could in some cases be long enough for antibody titres to wane to sub-threshold or undetectable levels.

Given such confusing information, there has been little agreement so far on the important question of asymptomatic and undetected H5N1 infections. But whatever the case, unless healthy seropositive people detected in seroprevalence studies temporally and geographically associated with H5N1 cases are all falsely seropositive, their addition to exposure denominators greatly decreases case-fatality determinations. For example, the 1997 Hong Kong H5N1 outbreak case-fatality rate of 33.3% (ref. 65) drops to around 3% with the addition of exposed seropositive persons detected in the related seroprevalence studies. Similar recalculations of other data would yield far lower rates, and wider seroprevalence studies would undoubtedly lower case-fatality rates even further.

Thus, an explanation for the apparent case-fatality rate/seroprevalence paradox may not be purely one of missing cases. Like other poorly adapted viruses that rarely infect humans[34], the H5N1 virus may simply be productively infecting too few of the people exposed to it, even in situations of widespread human contact, leaving minimal immunological evidence of exposure at the population level, while at the same time 'finding' and

infecting those occasional persons with unusual susceptibilities to it; that is, cases[59]. Even so, it should be remembered that limited spread of a deadly H5N1 virus, or pandemic H5N1 spread associated with a far lower case-fatality rate, would still be of public health concern.

The dangers of information release

Owing to global concern over a possible H5N1 influenza pandemic, the pathogenicity, immunogenicity and transmissibility of naturally occurring and laboratory-derived H5N1 viruses have been examined extensively and safely using high-containment facilities and appropriate regulatory and safety oversight (see later). The two H5N1 studies under discussion[1,2] build upon and are the logical extensions of dozens of similar published studies performed in the wake of the 1997 Hong Kong H5N1 outbreak. This research includes another published study in which genetic engineering of the H5N1 virus was able to newly create transmissibility in ferrets[66], a similar study in which increased ferret transmissibility was not documented[67], and a study in which transmissibility was restored and arguably increased in guinea pigs[68]. None of these publications, including the prior publication of engineered H5N1 transmissibility in ferrets, led to concern among scientists, federal agencies or the public.

Such studies feature numerous pathogenicity-associated, and sometimes transmissibility-associated, mutations affecting the HA-receptor-binding site, including changes that enhance receptor affinity for α2-6-linked sialic acid receptors, thought to be important for human adaptation[25,69–71]. Other studies have examined mutations associated with changing antigenicity[72,73], changes associated with fusion[25,74], changes associated with the polybasic HA cleavage site[75–77], and virulence factors in the polymerase proteins, crucial for viral replication[24,68], and in the non-structural protein (NS1), involved in antagonizing host type I interferon responses[78–80]. This widely available body of published research complicates determination of what to do with these two and with similar research manuscripts that seem likely to continue to appear. Withholding or redacting them does not prevent anyone from piecing together the basic information that they contain. Most of this information is generally known and relatively obvious, has already been published, and is now being widely publicized and discussed as a result of increased attention[11,13,14,81,82].

Some would argue that even this background research should not have been done, or should henceforth be classified and made available only to 'approved' scientists who would be vetted by yet-to-be-determined mechanisms[83,84]. But had these former studies not been made available in the open literature, the field of influenza research would have been considerably impeded and our current state of knowledge and readiness for responding to future outbreaks and/or pandemics would be lessened. Some proposed that 'censoring' this information actually increases the risk of bioterrorism[85]. The two studies under discussion[1,2] can help augment surveillance to detect naturally emerging viruses with pandemic potential and expand our knowledge of the principles underlying host adaptation. Although the dangers of 'information release' in the case of these two studies is probably small or nil—because all or most of the critical information is widely available anyway—it nevertheless remains important to rethink larger questions about balancing safety (accidental or deliberate release of an influenza virus or any dangerous pathogen) with the need to study such viruses to learn enough of their biology to prevent and control them. These are important issues that should be discussed broadly among scientists, policy makers and the public.

Biosafety and biosecurity concerns

As novel pathogens emerge, scientists must be able to continue to work with them safely and appropriately in teams using the talents of many highly trained researchers. Numerous layers of robust biosafety and biosecurity protection and oversight are in place to safeguard the scientists and the public alike, including rigorous safety training, biocontainment practices, regulations and oversight, select agent rules, background investigations and biosurety oversight[86]. The H5N1 studies under discussion[1,2] were both performed in high containment laboratories with rigorous and appropriate oversight and biosecurity measures[15], as is the case for all such research in the US.

Few disagree that it is crucial to continue research with H5N1 and other emerging infections, including investigation of how emerging pathogens adapt to new hosts and cause disease. However, it is important to ask whether some types of infectious diseases research should not be done, or not published openly. If so, we need criteria to identify such research in advance, and processes to balance the importance of the research knowledge with the importance of preventing adverse consequences of the research[87]. Even with the eventual publication of the two H5N1 studies, questions about how such research should be approved, evaluated and made public remain unanswered[83,84]. The biomedical field is built on more than a century of openness and full publication/broad discussion of all findings; it is unclear how redacted publications of future scientific data can be accomplished, and what effect such a system would have on science and scientific progress.

These complex questions have been asked and answered in the past[87], and are being asked again in the context of these two papers. Continued discussion and decisions about how to deal with this research will be of importance to scientific progress and public health. We believe that it is important to consider the broader context of research aimed at understanding how influenza viruses adapt to humans. H5N1 is only one of many avian influenza viruses. If, as we believe existing data suggest, pathways to human adaptation are many, virus-specific, and with few common denominators, it will be important to study not just H5N1 but a wide range of avian and mammalian- and human-adapted viruses, including studies that feature backward genetic engineering to remove phenotypic determinants of adaptation, studies in nonhuman primates and, when safe and appropriate to do so, in human challenge studies[88].

Future directions

The H5N1 controversy underscores how little is known about determinants of human influenza pathogenicity and transmissibility, which are among the most fundamentally important questions in infectious disease research because of the huge burden of influenza.

In the past two decades the question of pursuing and publishing potential 'dual use' infectious disease research has always been decided in favour of conducting and publishing the research; for example, delineating the genomes of smallpox[89] and SARS viruses[90], defining the pathogenicity of neuraminidase-inhibitor-resistant influenza viruses[91,92], genetically altering and making ferret transmissible both a more pathogenic pandemic H2N2 influenza virus[93] and an H9N2 avian influenza virus[40], and resurrecting from RNA fragments, recreating and studying *in vivo* the 1918 pandemic influenza virus[94–96]. In the latter case, important findings already have markedly enhanced our understanding of the emergence, transmissibility and pathogenicity of that important virus, helping us to prepare for and respond to the emergence of other influenza viruses. Examples include using the 1918 HA crystal structure in vaccine design[97], investigating the role of the host immune response in disease[98,99], identification of mutations associated with pathogenicity and host adaptation[36,100,101], understanding influenza evolution[21], and helping guide and target the response to the 2009 H1N1 pandemic[53,102]. All of this work has been done safely with appropriate oversight, and without negative consequences.

In considering the threat of bioterrorism or accidental release of genetically engineered viruses, it is worth remembering that nature is the ultimate bioterrorist. Indeed, H5N1 mutations, including some of those made in the two studies under discussion[1,2], occur spontaneously in nature, probably at a high rate, although they have not yet led to a pandemic. Given the relative rarity of pandemics caused by newly emerging influenza viruses, their explosive transmissibility may result from unique and virus-specific mutational changes that arise at very low frequency. For past pandemics, we have had limited ability to detect such changes by surveillance or by animal model experimentation. Thus, our best hope in

preventing and controlling the microbial agents that continually challenge us is to increase fundamental knowledge about the mechanisms by which they emerge, spread and cause disease, so that we can develop countermeasures such as enhanced surveillance, better diagnostics, vaccines and drug therapies. In moving forward we need to be safety conscious and to have consensus safety measures and policies in place, while at the same time using all available tools to seek broad understanding about the complex relationships between viruses and hosts. It is only this knowledge that stands between us and the devastation of future influenza pandemics. In reconsidering the proper balance between progress and safety, the critical importance of advancing scientific knowledge needs to be kept front and centre.

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