An Avian H7N1 Gain-of-Function Experiment of Great Concern

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ABSTRACT Inappropriately named gain-of-function influenza research seeks to confer airborne transmission on avian influenza A viruses that otherwise cause only dead-end infections in humans. A recent study has succeeded in doing this with a highly pathogenic ostrich H7N1 virus in a ferret model without loss of virulence. If transposable to humans, this would constitute a novel virus with a case fatality rate ~30 greater than that of Spanish flu. A commentary from three distinguished virologists considered the benefits of this work to outweigh potential risks. I beg to disagree with conclusions in both papers, for the underlying science is not as strong as it appears.

An avian influenza A virus "gain-of-function" (GOF) experiment has been performed whereby a highly virulent ostrich H7N1 strain has been selected for efficient airborne transmission between ferrets. The recently published paper in the *Journal of Virology* (1) represents a quantum jump between danger levels, for the acquisition of airborne transmission was achieved without loss of virulence—3/5 ferrets infected by the airborne route died, just as with the parental strain. The paper was accompanied by two commentaries (2, 3) from editors of the *Journal of Virology* and ASM top brass, publishers of the journal. There are many aspects of the experiment that need commenting on, while one of the commentaries gives cause for concern (2).

First, the science. There has not been a single lab-confirmed human case caused by this avian combination of an H7 hemagglutinin and N1 neuraminidase. In contrast, human infections with very little onward transmission to humans have been described for avian H7N2 (6 human cases), H7N3 (58 cases), and H7N7 (90 cases; see reference 4) viruses and of course for the ongoing H7N9 outbreak (450 cases; http://www.who.int/influenza/human_animal_interface/influenza_h7n9/Risk_Assessment/en/). In short, H7N1 is not a threat to humans, as H5N1 and H7N9 have proven themselves to be. It could be argued that it is a model for H7N2, H7N3, H7N7, or H7N9, but then it would have been more apposite to have used one of these avian strains. Of course, avian H7N1 viruses could one day become a threat to humans, but then that could be postulated for all avian influenza viruses and indeed numerous nonhuman viruses and is in the realm of speculation. If such a postulate is approved, it opens up the field to selection of transmissible strains of the remaining 120-plus avian influenza virus H,N combinations, not to mention other animal viruses. The only influenza viruses that have successfully crossed over to humans in the last 100 years are H1N1, H2N2, and H3N2.

Second, adaptation took place without any mutations in the part of the hemagglutinin that binds to the human α2-6 sialylated glycans that are receptors for the virus in the upper respiratory tract. Many virologists in the field would have predicted mutations in the H7 protein's receptor binding pocket along the lines of the H5N1 GOF studies or as a reading of a recent paper on the avian H10 hemagglutinin posits (5). A structure-guided study of the H7 protein from a human H7N9 strain showed that the single G228S substitution conferred better binding to the human upper tracheal tissue sections (6). As this was performed on an isolated H7 protein and not in an infectious virus and GOF format, we do not know whether this "anticipated" finding is a feature of the experimental system or whether it reflects the genetic background of the H7, as has been observed for H5 mutations, a phenomenon known as epistasis (7).

Third, flu viruses are constantly mutating due to lack of replication and proofreading mechanisms. Lineages come and go, as can be seen, for example, for H5N1 (8). Fine. Then why use an H7N1 strain isolated in 2000? Why not use a circulating H7 virus? H7 hemagglutinin sequences from viruses isolated in 2010 to 2014 vary by 3 to 18% at the protein level compared to the 2000 H7 ostrich sequence. While H7 proteins show remarkably conserved antigenic variation (9), this tells nothing about how individual H7N1 strains with similar or different internal genes might adapt. Accordingly, the pertinence of this paper to interpreting the adaptive potential of circulating avian H7N1 strains is lacking.

Last, the majority of ferrets infected through the airborne route died from symptoms paralleling those of the parent virus when it is mechanically inoculated into ferrets—3/5 animals died after having been infected by the airborne route. In other words, there was no trade-off between virulence and transmission, as was heard following publication of the H5N1 GOF papers (10, 11). This is why the present H7N1 paper is stunning.

Such a trade-off is a highly fallacious argument in this precise context. To date, avian H5N1 viruses have caused 650 symptomatic human infections, with 386 deaths (case fatality rate [CFR], ~60%; http://www.who.int/influenza/human_animal_interface/EN_GIP_20140124CumulativeNumberH5N1cases.pdf?ua=1). Let's do a thought experiment and imagine an H5N1 virus engineered to become transmissible by the airborne route between humans, yet with a human CFR of ~0.6%, reduced by a factor of 100 from that of the parental avian virus. Even though the virus is transmissible between humans, we limit the total number of infections worldwide to a million people. Despite the much-reduced CFR, the number of dead would be ~6,000 which is vastly greater than that seen for natural H5N1 infections over the last 17 years. Hence, a trade-off is by no means good news.

The number of animals used in GOF influenza experiments, generally 3 or 4 animals per point, sometimes 5, has rightly been

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criticized as too small to yield statistically robust results (12). There is no way that such experiments have the power to quantitate the reduction of virulence. To properly identify a drop in the case fatality rate in ferrets of an engineered avian flu virus from ~60% to, say, 2%, which characterized H1N1 Spanish flu, huge numbers of ferrets would be necessary. As such numbers of animals will not be forthcoming, it makes no sense to posit a trade-off that is effectively an unfalsifiable postulate.

We are left with a highly pathogenic H7N1 virus that is transmissible via the airborne route. As a general rule of thumb, a pandemic influenza A virus that is transmissible between humans is generally transmissible between ferrets. Hence, it is easily possible that this lab-engineered H7N1 strain would constitute a novel danger for humans if ever it escaped. In an accompanying article, Dermody et al. note that "Highly pathogenic avian influenza viruses have been sporadically introduced into humans . . . these strains are more likely more virulent in humans than the 1918 influenza virus A H1N1 pandemic strain . . . the virulence of this strain in birds suggests that it could be similarly virulent in humans" (2). The transmissible H7N1 ostrich virus is arguably covered by this remark, which comes from three very well known U.S. virologists, including the editor in chief of the *Journal of Virology* and the chair of the ASM Journals Board.

These distinguished authors go on to explain the internal review process. We learn that prior to submission to the *Journal of Virology*, the University of Maryland Biosafety Committee (UMBC) (representing the host institution where the work was performed) and the NIAID (the funder) studied the issue of whether the work constituted dual-use research of concern, or DURC. On two counts there was agreement, but they differed on a third count—"does the knowledge, information, products, technologies meet the definition of DURC." Basically, they disagreed on what constitutes DURC, while "neither group recommended against publication." When the work was submitted to ASM editors, they "did not reach a consensus about whether the work represents DURC" (2).

This 11th-hour disagreement between interested parties and then among ASM editors is staggering. If the work represents DURC, and I think that it does, then I would have expected the parties to have sorted out definitions before embarking on the work, especially given the precedent provided by the H5N1 debate that started in the fall of 2011. This alone represents a colossal lack of oversight. As the study was published despite this disagreement, it means that there was, and is, no mechanism in place that could claw back any controversial or particularly dangerous finding that could have emerged unexpectedly. This is akin to no oversight at all.

Despite the publication of a novel human pathogen that, in the words of three seasoned virologists, "could be similarly virulent in humans" (2)—hardly a minor affair—the evaluations that presumably UMBC and the NIAID made during institutional review go unreferenced. ASM editors "concluded that the benefits of publishing the paper outweighed any potential risks, and ASM decided to move forward with publication" (2). Again, no sources are cited; where is the risk-benefit analysis that such a statement implies? This would constitute a first; people like myself would be eager to study the arguments that led them to such a conclusion. As scientists we are data driven; we need the facts to see if there were any flaws in the arguments of one side or the other or even both. Publication constitutes the most grueling peer review.

A recent paper lays out the arguments from both sides in the GOF influenza debate for the first time (13). What we learn is that risk-benefit analyses are very difficult. In terms of logic, if virologists are split as to the benefits of GOF influenza research, and they are, then it is not possible to generate a consensus. The corollary is that risk-benefit analyses are not presently possible. Then how is it that ASM representatives concluded that the benefits of the H7N1 GOF research outweighed the risks (2)? They have simply given their opinion, but once again, where are the data?

The ASM has not organized a wide-ranging conference on this topic, nor one including the societal and legal ramifications, before coming to a conclusion, nor, apparently, consulted its membership. Very recently, the ASM issued a statement on dual-use research of concern and biosafety, oriented toward the GOF controversy (https://www.asm.org/index.php/publicpolicy-2/statements-testimony/137-policy/documents/statements-and-testimony/93024-durc-7-31-14). Toward the close, we read, "The ASM recommends that the National Academy of Sciences should be asked to consider whether the current scope of GOF research offers the benefits that make taking the risks inherent in performing that research. . . ." Referring the risk-benefit issue up and away implies that they have no in-house analysis.

Statements of authority don't wash in science, which is data driven. Time and time again, the flu community has touted the benefits of GOF research, but not once has anyone done any hypothesis breaking. It has fallen to nonflu scientists to do the breaking, and the arguments are out there (14–18). Incidentally, Dermody et al. reiterate claims that GOF transmission work on these avian viruses may "guide preparation of influenza vaccines, and lead to identification of new antiviral drugs." While they used the conditional tense, these claims have been shown to be wanting (14–18). It cannot be said that there is a consensus as to the benefits of this work.

Only one risk analysis has been published (19). No wide-ranging and independent risk or liability analysis has been published, while few learned societies have even discussed the matter followed by a position paper—which is surprising given the resulting hoo-ha since this hot topic emerged back in the fall of 2011. Like it or not, virology is at the Asilomar moment foreseen as far back as 1975.

As it happens, there are some pretty clear guidelines out there. The InterAcademy Panel (IAP) on international issues represents 76 national science academies, including the U.S. National Academy of Sciences and European equivalents. The first article of the 2007 IAP statement on biosecurity reads, "scientists have an obligation to do no harm." It goes on to say that scientists should "recognize that individual good conscience does not justify ignoring the possible misuse of their scientific endeavor," which is rather clear (http://www.interacademies.net/File.aspx?id=5401). Most virologists I have spoken to see their responsibility ending with publication, which shows how out of touch they are with their peers.

In the section on oversight, we read, "*Scientists with responsibility for oversight of research or for evaluation of projects or publications* should promote adherence to these principles by those under their control, supervision or evaluation and *act as role models* in this regard" (my italics). This clearly identifies funders, their reviewers, and journal editors. Given the lack of up-front oversight from the funders, journal editors are dealing with GOF manuscripts as best they can and by their own admission on an *ad hoc* basis (3). Yet the IAP statement, published in 2007, before influenza GOF research was in motion, recommends that they should take a long, measured view and then get the word out, rather than have journal editors handle DURC manuscripts—nor even decide whether the work represents DURC or not—at the 11th hour, on a case-by-case basis, which is the current

situation. Despite this advice from the world's major academies, I have found only a handful of scientists who know about this statement. Those that did were invariably academy members. In short, although the IAP statement is a good document, no effort was made by the academies to get their advice known to principal investigators and postdocs at the bench.

To many scientists, blocking a publication at the last moment appears cruel and unfair, even though the security of society may be at stake. As a bench scientist, I commiserate. Nonetheless, this very issue has been addressed. In 2010, the Max Planck Society issued a code of rules that gives the president of each Max Planck Institute the power to stop publication of a manuscript if the findings are deemed to hurt the Society at large or the institute where the work was performed (http://www.mpg.de/232129/researchFreedomRisks.pdf). The document is full of common sense and stresses the importance of the researcher, who by definition is closer to the issues than most.

Fortunately things are now moving. The Dutch Academy of Arts and Sciences published a report in late 2013 that recommended setting up a standing committee to evaluate DURC (http://www.bureaubiosecurity.nl/dsresource?type=pdf&disposition=inline&objectid=rivmp:246499&versionid=&subobjectname). Equally, in early 2014, the German Ethics Council, at the request of the German Parliament, came out with a very complete report with an executive summary in English (http://www.ethikrat.org/files/opinion-biosecurity-summary-and-recommendations.pdf). They too recommended setting up a DURC committee. This theme is picked up in the second paper by Casadevall et al., accompanying the H7N1 GOF paper (3). In short, the three independent documents all push in the same direction. These are courageous calls that I strongly subscribe to.

The Dutch Academy of Arts and Sciences has held a debate in June 2014, and a 1-day conference is slated for November 2014. A half-day conference on legal aspects of the work was held at the University of Freiburg in July 2014. The U.S. National Academy of Science will be holding a 1-day symposium on the issue in November, while the Volkswagen Foundation and the Max Planck Society are organizing a 2-day conference in December 2014 (http://www.volkswagenstiftung.de/dualuseresearch). On a topic of such importance to society and given the oft-used, almost hackneyed phrase "that microbes do not respect borders," it is not for one group to decide for the rest of the world's infectious disease community, let alone the public.

The ostrich H7N1 GOF paper is far from the last. Some insiders have spoken of a tidal wave of viral GOF studies working their way toward publication. Some researchers are even engineering human influenza viruses such that they can escape extant vaccine coverage. I urge funders, universities, and learned societies to foster discussion on this topic from a diverse spectrum of thinkers. And fast, for we are already up against an airborne route-transmissible H7N1 virus that is "more virulent in humans than the 1918 influenza virus A H1N1 pandemic strain" (2). As there appear to be no checks in place, is it wise to continue such work until such a discussion has taken place? I think not.

In the meantime, we can all do something simple and take a mental Hippocratic oath.

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