Gain-of-Function Research and the Relevance to Clinical Practice

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(See the editorial commentary by Frank et al on pages 1359–61.)

The ongoing moratorium on gain-of-function (GOF) research with highly pathogenic avian influenza virus, severe acute respiratory syndrome coronavirus, and Middle East respiratory syndrome coronavirus has drawn attention to the current debate on these research practices and the potential benefits and risks they present. While much of the discussion has been steered by members of the microbiology and policy communities, additional input from medical practitioners will be highly valuable toward developing a broadly inclusive policy that considers the relative value and harm of GOF research. This review attempts to serve as a primer on the topic for the clinical community by providing a historical context for GOF research, summarizing concerns about its risks, and surveying the medical products that it has yielded.

Keywords. gain of function; potential pandemic pathogens; coronavirus; influenza; science policy; health policy.

Gain-of-function (GOF) research typically involves mutations that confer altered functionality of a protein or other molecule. These types of mutations have been used as powerful tools to understand basic bacterial and viral biology and pathogen-host interactions. Despite the recency of a public debate, GOF research has constituted a common, long-standing practice in the discipline of microbiology. In recent years, a public discussion has surfaced, centering on the application of GOF research to highly pathogenic and potentially lethal viruses [1]. Despite the emergence of this public dialogue, much of it has been steered by members of the microbiology and policy communities. There remains room for additional input from clinical and public health practitioners, who are often the end users of the products GOF research yields. As the results from GOF research are salient to both the improved understanding of disease pathogenesis and the development of medical countermeasures to infectious diseases, the debate over its safety and value is of direct relevance to medical and public health practitioners. This review article will provide a historical context for the current debate, describe the potential risks and benefits of this type of experimental study, and present some examples of how GOF research translates into tangible products of use to practicing clinicians.

GOF: AN HISTORICAL PERSPECTIVE

Genetic mutations can be classified in many ways, one of which is by their impact on protein function. In the simplest terms, mutations can result in a protein’s loss of function or GOF. The distinction between the 2 phenotypes is not always clear. GOF research, in this context, usually results in the introduction of changes to biological agents that might increase their ability to infect a host and cause disease by enhancing their transmissibility or pathogenicity [2]. In recent years, this class of research has provoked controversy, particularly in the setting of dual use research of concern (DURC). DURC is a subset of microbiological research that, as defined by the US government, “can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security” [3, p. 1]. Some of the potential consequences of DURC that have been cited include the manipulation of pathogens for use as biological weapons and the development of mechanisms by which pathogens can evade countermeasures. DURC currently pertains to the select agents and toxins defined by the US Centers for Disease Prevention and Control and the US Department of Agriculture [4]. Among these pathogens, highly pathogenic avian influenza virus (HPAI) is of high concern to both public health and agriculture authorities.

Public discourse on the controversies of influenza virus research is about a decade old, beginning in 2005 with the reconstitution of the 1918 influenza A(H1N1) [5–7]. The more recent debates over the safety and merits of GOF research first surfaced in 2010, in the context of studies on the transmission dynamics...
of HPAI A(H5N1) (Figure 1). Laboratories at the University of Wisconsin (Madison) and Erasmus University Medical Center (EMC; Rotterdam, the Netherlands) performed a series of experiments [8, 9] that involved the mutation of 2 influenza A(H5N1) strains through multiple passaging. The two laboratories identified specific amino acid changes that enhanced airborne transmissibility of the virus between ferrets—a standard animal influenza model that exhibits a natural history and pathology similar to what is observed in humans. The potential translation from ferrets to humans raised concerns among funders (ie, the National Institutes of Health [NIH]) and the broader biosecurity policy community that the research could be used for intentionally harmful purposes or result in an accidental release of pathogens from the laboratory into the general population.

In 2011, the Department of Health and Human Services (DHHS) convened the National Science Advisory Board for Biosecurity (NSABB)—an independent federal advisory committee chartered to provide advice on the biosecurity oversight of dual use research. The NSABB was asked to weigh in on whether the GOF studies should be published in the public domain. After initial review of 2 manuscripts, one submitted to Science (by investigators at EMC) and the other to Nature (by investigators at the University of Wisconsin), the NSABB requested that study authors and the journals withhold from publication the details about the study methods [10]. Consequently, the influenza research community voluntarily implemented a year-long moratorium on GOF research. In March 2012, the NSABB recommended publication of both studies, with some minor changes to the EMC manuscript [11]. These deliberations led to the creation of a US framework for DURC studies [3, 12] and further stimulated a debate on GOF research within the scientific community [13].

Recently, influenza virus researchers laid out a rationale for GOF experiments in the context of influenza A(H7N9) [14, 15]. These arguments were met with some criticism [16–18], especially with respect to the risks of accidental or intentional release of this HPAI. Given the growing concern over this and other HPAI subtypes, the White House Office of Science and Technology Policy and the DHHS announced a moratorium, on 17 October 2014, on all new funding for GOF research on all influenza viruses, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) [2]. Additionally, the US government called for a voluntary moratorium on all such research, irrespective of funding source, while the risks and benefits of such experiments could be assessed. On 15 and 16 December 2014, the National Academy of Sciences, National Research Council, and Institute of Medicine convened experts from the disciplines of infectious diseases, research ethics, and science policy to discuss the potential risks and benefits of GOF research in a public forum to help inform the federal government on how best to proceed in regulating GOF research on potentially dangerous biological agents [19]. Shortly after the meeting, the NIH notified a subset of researchers affected by the research pause that their work could resume [20]. Specifically, 5 research projects on MERS-CoV animal model development and 2 on HPAI were cleared to continue.

The discussion on the merits and risks of GOF research has not been limited to the United States, as the Dutch Court of Appeals recently handed down a verdict concerning EMC’s objection to export license regulations regarding the publication of HPAI GOF research [21]. Export licenses in the European Union are in place to prevent the proliferation of weapons of mass destruction and, thus, apply to specific biological agents, chemical agents, and technologies. In 2012, the Dutch government ruled that EMC had to apply for an export license to publish their GOF work, which they did to expedite publication. However, EMC later filed an objection, maintaining that GOF research in this context was for “basic scientific research.” The Dutch Court of Appeals ruled that EMC had no legal standing to contest the export license regulations but did not address the legality of the export license itself, leaving the issue open for continued debate. Currently, all GOF research within the European Union requires export licenses for publication.

A deliberative review process, headed by the NSABB, is currently underway [22] to evaluate the potential impacts of GOF research and to set criteria for what types of research can be conducted and made available in the public domain. A large part of the risk analysis will likely involve the potential for these pathogens to be misused either intentionally or accidentally. Attempts have been made to anticipate the likelihood of the latter scenario, resulting in wide-ranging estimates [1, 19, 23]. The recent safety lapses at the Centers for Disease Control and Prevention and the NIH that could have resulted in exposure to anthrax and smallpox, respectively, have diminished public confidence in the ability of even high-containment laboratories to mitigate the risk of accidental release of pathogens of potential harm. Though the actual risk of accidental release of highly pathogenic viruses may be low, public tolerance of that risk may be the ultimate determinant of what types of research are allowed to proceed.

Increasing attention has been brought to the use of alternative methods of investigation in areas that have historically been studied through GOF research. Some of the alternatives that have been proposed rely heavily on in silico technologies, such as computational modeling and disease forecasting [24–26]. The relevance of these other methods is an important consideration for the scientific community, medical practitioners, and the general public, as the risks and benefits of each approach and the tangible outcomes they yield will vary according to the interests and needs of each sector. All of these factors are being considered by the NSABB, which will decide how to proceed with the current moratorium and the future of GOF.
Figure 1. Historical perspective on recent debates associated with gain-of-function (GOF) research. Abbreviations: DHHS, Department of Health and Human Services; EMC, Erasmus University Medical Center; HPAI, highly pathogenic avian influenza virus; MERS-CoV, Middle East respiratory syndrome coronavirus; NIH, National Institutes of Health; NSABB, National Science Advisory Board for Biosecurity; SARS-CoV, severe acute respiratory syndrome coronavirus; USG, US government.
research. As the GOF debate has transpired to date, the ramifications of this research for the practicing clinician have not been made clear.

**CLINICAL APPLICATIONS OF GOF RESEARCH**

**Animal Models**
The development of novel prophylactic and therapeutic interventions invariably requires evaluation in animal models that, at least partially, recapitulate the disease in infected humans. Many emerging and reemerging zoonotic diseases lack relevant animal models that closely recapitulate human disease [27]. In these instances, GOF experiments are often needed to adapt virus isolates from humans to different, sometimes unnatural, mammalian hosts. Adaptation to a new host inherently involves the alteration of pathogens through mutation. As the development of appropriate animal models can be a rate-limiting step in the evaluation of prophylactic and therapeutic interventions, GOF modifications to viral strains can be an important tool toward accelerating the product development pipeline.

Coronaviruses such as SARS-CoV and MERS-CoV require meaningful small-animal models that elucidate viral pathogenesis and immunity. The human isolates are manipulated either through natural evolution, targeted mutation, or repeated exposure to human factors in nonhuman hosts. One of the more reliable SARS-CoV murine models was developed by modifying a human isolate through 15 serial passages, after which it was lethal to young mice [28]. This mouse-adapted virus strain contained 6 coding mutations that conferred increased virulence, approximating many features of SARS-CoV disease in humans and thus providing a robust and reproducible challenge model for testing vaccines, antivirals, and other interventions [29]. The development of an appropriate animal model for MERS-CoV, on the other hand, provides unique challenges because the viral receptor used for cell entry is radically different in mice. Models thus far have included transient transfection [30] and transgenic mice [31], although it is still unclear whether these models accurately recapitulate human infection. Approximating human disease in these small-animal models might require further passaging in the presence of a humanized receptor, thus creating a potential for the development of GOF phenotypes.

**Vaccines**
Many live-attenuated vaccines, including some of the most successful vaccines ever developed, have been generated through GOF research. From polio to smallpox to influenza, live-attenuated vaccines elicit immunity against authentic epitopes on whole pathogens without causing disease. The live-attenuated measles vaccine was created by passaging the virus until mutations arose that altered virus tropism—a technique that could be considered, by current definitions, GOF research [32]. New research on highly pathogenic viruses has emphasized the different ways GOF mutations can generate even-more-effective live-attenuated vaccines. Mutations within RNA virus polymerases, for example, modify replication fidelity to generate higher or lower mutation rates during viral replication. These fidelity mutants could potentially alter viral tropism, modify key antigens, and increase resistance against novel therapeutic interventions or antibody responses, but they could also lead to a virus that is less fit [33, 34]. These particular types of experiments have been carried out on a range of viruses, including alphaviruses [35, 36] and picornaviruses [37]. The introduction of GOF mutations not only attenuates the virus but also provides improved understanding of the mechanics of viral replication, thus potentially uncovering new strategies in the development of vaccines against emerging pathogens.

**Therapeutic Interventions**
The generation of escape mutants in the presence of an investigational agent is common practice for the evaluation of antibiotics, antivirals, and other monoclonal antibodies. GOF experiments with HPAIs and highly pathogenic human influenza viruses, for example, have identified specific mutations that can confer multidrug resistance [38, 39]. GOF experiments are necessary in this context because naturally occurring resistant strains may not yet exist or the complex background of naturally occurring mutations may preclude identification of the amino acid residues that are critical to resistance [40]. These GOF studies are equally important in research on antivirals and antibiotics and can help inform the development of combination therapies. Passive immunotherapy, which often includes a combination of products, is particularly dependent on GOF experiments for evaluating efficacy [41–43], as seen in the current Ebola outbreak that has prompted a robust program to evaluate combination monoclonal antibody therapies [44, 45].

**Disease Surveillance**
In the past half-century, GOF research has contributed to an improved understanding of the epidemiology of emerging pathogens and has informed efforts to conduct surveillance for future outbreaks. In the context of influenza, data, derived from GOF research, on the relative transmissibility of hemagglutinin mutations has aided in the interpretation of molecular surveillance data [46]. Specifically, the initial influenza A(H5N1) [8, 9] and later influenza A(H7N9) experiments identified amino acid changes in influenza virus hemagglutinin or RNA polymerase through viral passaging or site-directed mutagenesis. This research elucidated mechanisms by which naturally occurring influenza virus strains might evolve to replicate more efficiently and transmit more easily within mammalian hosts [47, 48]. The results of these experiments can be used to cross-reference traits found among circulating strains and help predict transmission patterns and pathogenicity [49]. As the field of disease surveillance evolves to accommodate a growing repository of viral sequences, GOF research will also play an important role in assessing the public health significance of genotypic variation.
Though current understanding of the relationship between genotypic data and phenotypic expression is suboptimal, the increasing reliance by the clinical community on molecular diagnostic tools may help to reduce that uncertainty. As costs of whole-genome sequencing continue to decrease, data from these techniques are likely to become more central to disease surveillance programs. The results of GOF experimentation can also help inform decisions about countermeasure selection and stockpiling, particularly in the context of influenza surveillance programs [50]. The improved understanding of how HPAs evolve to transmit more efficiently has also factored into decisions about the creation of pandemic vaccine stockpiles.

**THE ROLE OF CLINICIANS IN THE GOF RESEARCH DEBATE**

The world has been witness to a number of emerging infectious disease pandemics over the past several decades. Each time, clinical and public health practitioners were on the front lines, providing care and treatment and finding ways to interrupt transmission, and were ultimately responsible for containing the outbreak. Healthcare providers require effective medical countermeasures and epidemiologic information to assess risk and support decisions about treatment and prevention. Recent outbreaks of infection due to Ebola virus, MERS-CoV, and pandemic influenza virus, however, continue to demonstrate that medical and public health readiness for emerging infections is not always optimal and could benefit from more research and development. As outlined above, GOF research plays a significant role in ensuring that clinicians have the tools they need to respond to infectious disease outbreaks. Therefore, the clinical community is directly affected by policy decisions on what types of research are and are not allowed to continue. There are also risks associated with GOF research, of which the clinical community will have to be acutely aware. As recent lapses at high-profile laboratories have illustrated, there remains the potential that bacterial and viral strains can escape even the most secure environments. Should a pathogen escape, whether it is naturally occurring or the product of GOF research, the clinical community will have an important role in detecting and responding to such incidents. Because of their unique role as both beneficiaries of the products of GOF research and mitigators of its risks, clinicians have a vital stake in the public debate on how GOF research should proceed.

**Notes**

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