An Epistemological Perspective on the Value of Gain-of-Function Experiments Involving Pathogens with Pandemic Potential

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In recent years, scientists have engaged in a vigorous debate regarding the value of so-called gain-of-function (GOF) experiments involving highly pathogenic avian influenza virus (HPAIV) and other pathogens with pandemic potential (PPP). Of particular concern have been experiments whereby something is done to PPP, such as HPAIV, and the pathogen acquires a new property, or GOF, that makes the microbe more dangerous, such as mammalian transmissibility, increased virulence, and/or the ability to defeat immunity and antimicrobial drugs. The debate has included arguments focused on biosecurity, biosafety, and ethics (1–5). Proponents of GOF experiments emphasized the utilitarian aspects of the work such as potential uses in vaccine development and strain surveillance, while opponents focused on risk (1). However, the debate has largely ignored the question of the epistemological value of such experiments, which is central to any scientific discussion of the merits of such work. Here we consider GOF experiments in the context of how information is acquired and valued in the fields of microbial pathogenesis and infectious diseases.

Epistemology is the branch of philosophy that examines the nature of knowledge, its presuppositions and foundations, and its extent and validity (6). Most scientists, and microbiologists in particular, practice their art within specialized areas that include a set of normative standards that influence the pursuit and acceptance of knowledge, and such normative standards are a major focus of epistemological research within the philosophy of science (7, 8). Normative standards in science include the methodology that is accepted for making and accepting scientific discoveries. In this regard, different fields rely on partially different methods for the questions that they pursue.

Before exploring the value of GOF experiments, we need to consider the current normative standards in the field of microbial pathogenesis that guide the approach to research. To understand the origin of such standards in the field, we must go back to the early days of the germ theory of disease. Although microbes and lack of hygiene were associated with infectious diseases by several individuals, in the mid-19th century, it was Robert Koch's demonstration that anthrax was caused by *Bacillus anthracis* that introduced the powerful concept of associating specific microbes with specific diseases. These experiments in turn established a high standard for causation, as exemplified by Koch's postulates, which themselves created a high bar for the acceptance of subsequent research (9, 10). For diseases where Koch's postulates could not be directly applied, new tools were developed such as immunological proof of causation in which immune responses in the form of serology became surrogates for making causative associations. Today new molecular tools have led to the identification of many pathogens associated with certain diseases, and the high bar established at the end of the 19th century continues to demand extraordinary rigor for association of microbes and disease (11).

The emphasis on causation led to the identification of numerous microbes as etiological agents for specific disease, and these causative associations allowed humanity to control many infectious diseases through improved sanitation, vaccination, and eventual antimicrobial drug discovery. For example, such experimental rigor led to the rapid association of HIV with AIDS within 3 years after the report of a new deadly clinical syndrome. In turn, this facilitated the development of numerous antiretroviral drugs that converted AIDS from a rapidly lethal disease to a treatable chronic condition. In fact, the germ theory of disease and the fields that originated from it, including vaccinology, are arguably the most important single scientific contribution to the increase in human life span and betterment of human health during those added years.

The standards developed for disease causation were subsequently applied to other areas of microbiological research. For example, in the late 20th century, molecular biology tools that allowed disruption of specific genes became available. In the field of microbial pathogenesis, the ability to disrupt genes led to questions of how to associate gene function with specific effects on virulence. Consequently, Stanley Falkow proposed his "molecular Koch's postulates," suggesting that associations be made only when mutating the gene in question affected virulence, and the effect could be reversed by complementation (12, 13). Such approaches in both the identification of disease causation and gene function created a set of normative standards that have dominated how investigators approach problems in the field. Consequently, research in microbial pathogenesis and infectious diseases operates with a set of normative standards that have been repeatedly tested and established to provide actionable information that can directly impact health. However, we note that microbiology is not a homogenous discipline with regard to how it pursues knowledge, and whereas microbial pathogenesis can be highly reductionist in its attempts to identify specific genes and alleles associated with virulence, environmental microbiology places high value in trying to understand how complex systems interact (14).

The types of experiments defined by Falkow, in which loss of function is used to identify determinants of virulence, are gener-

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ally not controversial because they usually result in less-virulent pathogens. GOF experiments, on the other hand, generate microbes with new functions and define new phenotypes. These new functions or phenotypes can both inform the experimenter on the possibility that such outcomes can occur and also provide insight into the mechanism as to how the new function was acquired by the microbe. We note that GOF is a neutral term with respect to the nature of the function that is acquired. For example, these types of experiments could result in useful and less dangerous new properties, such as microbial attenuation (1). Furthermore, GOF-like experiments have been extensively used in other fields such as genetics to generate variants with new phenotypes through selection for those traits, although those experiments have not been controversial because they did not involve virulent microbes. In the field of PPP research, however, GOF has come to mean the acquisition of a new property with a likelihood of making the pathogen more dangerous, such as mammalian transmissibility or virulence. In view of the current use of this terminology to mean the latter, and the high likelihood that this lexicon has already established itself, we will use it with that meaning in mind in this editorial, with the necessary cautions that we have expressed earlier (1).

When considered in the context of the normative standards of microbiology developed over the past 150 years, which place great emphasis and value on establishing causative associations, it is clear that GOF experiments are very powerful because such experiments can give direct information on cause-and-effect relationships. For example, the highly controversial experiments demonstrating that certain mutations conferred upon HPAIV H5N1 the capacity for mammalian transmissibility conclusively established that this virus had the biological capacity to generate variants that could spread from mammal to mammal. Much of the initial debate on the publication of the H5N1 papers involved the utility of public dissemination of the specific mutations associated with mammalian transmission (15). Although the publication of such data was considered worrisome by some from a biosecurity angle, the scientists involved in the work argued for their publication because the mutational data implied a mechanism for the phenomenon of mammalian transmissibility. This viewpoint emphasized the notion that the molecular changes associated with GOF directly identified the proteins that conferred this new property and that consequently, publication of the mutational data was critical for placing the observation in the context of mechanism, which is a valued position in current day science (16).

The information gained in GOF experiments involving HPAIV shed light on three layers of knowledge: (i) the new property of transmissibility, (ii) the gene(s) involved, and (iii) the specific mutations associated with the GOF (17, 18). However, the epistemological value of these layers varied. The acquisition of transmissibility was an all-or-nothing phenomenon, and the fact that it happened provided unambiguous evidence that the virus had the biological capacity to become transmissible between mammals. Short of waiting to see if such transmissible viruses appeared in nature, it is difficult to imagine another approach to address this question, which had previously been a matter of debate in the influenza research community. The identification of the genes involved provided firm information on what genes could contribute to the new property, although it remains possible that mutations in other segments of the genome might also allow for transmissibility. In contrast, the value of the specific mutational information necessary for mammalian transmission has been questioned on the basis of epistatic interactions in influenza virus genetics (2, 19, 20). In addition, questions have been raised on the usefulness of these data for surveillance purposes or prediction of transmissibility and virulence in other strains (2). However, the same questions about epistatic interactions suggest that, without a GOF type of experiment, we may not have been able to infer whether H5N1 had the capacity for mammalian transmissibility on the basis of homology comparisons with mammal-adapted strains. Hence, the debate on the value of information obtained appears to be more about the relative place in the hierarchy of the information gained rather than the GOF methodology *per se*.

The debate on the future of GOF experiments in PPP research is increasingly focusing on a risk-benefit calculation centered around biosafety concerns (1). Such a calculation could lead to outcomes that range from continuation of the work under the present biosafety framework, to a call for changes to lab design and experimental procedures, to moratoriums on what experiments could be done, to regulation of the scientific work. The risk aspect of the calculation involves the possibility and probability of laboratory accidents unleashing an outbreak and considerations on use of such agents and/or information for nefarious purposes. The broader benefit of GOF experiments has been more difficult to evaluate, given that the uses of scientific findings are often not immediately apparent.

However, a powerful argument can be made for the value of GOF experiments, because they yield information that is consistent with the normative standards of the fields of microbiology and infectious diseases, and as such, they provide information that is immediately accessible and interpretable in the context of standards in the field. When considering moratoriums or limitations on GOF experiments, it is important to take into account that there currently appear to be no alternatives to GOF experiments for seeking answers to certain biological questions. GOF experiments are human tools of inquiry that allow deep probing of biological questions and have the potential to produce information with a high probability of being true. The point is that, when one does a risk-benefit analysis of this issue, the epistemic gain from GOF experiments should be included in the bookkeeping: if one does that, the benefits of GOF experiments are potentially so great as to warrant our risking more than we otherwise might. To be sure, there are no clear, general, and universally accepted guidelines for weighing the epistemic gain against the risk of human suffering due to the pandemic potential, and the metrics that are in fact adopted will be highly context dependent.

We all think that some risks are reasonable for the gain that comes from scientific progress. Otherwise, why would scientists sometimes put themselves at risk to do a difficult or dangerous experiment or embark on a dangerous mission? For example, the individuals involved accepted the risk associated with repairing the Hubble telescope, exploring volcanoes, or doing research at the poles. Moreover, an unaware public is often put at risk, albeit minimal, as with research involving radioactive substances, where accidental release of radiation outside the laboratory is often a possibility. At another level, in clinical trials, we risk harm to the trial subjects in exchange for the knowledge that we hope to gain. But how to assess the amount of risk to others, who are not directly involved in the original activity that is justified by a given kind of possible knowledge gain, is difficult, and we proffer no general answer to that question. We merely argue that epistemic gain of

GOF experiments should be included explicitly on the benefit side of the calculation.

We therefore strongly argue against arbitrary abandonment of GOF experiments with PPP, since this would deprive these fields of a very powerful experimental tool. Instead, we suggest refocusing the discussion to articulate the type of knowledge that is needed to push these fields forward and enhancing the safety of such experiments, including the development of modified strains that would significantly lower risk. Furthermore, other innovative approaches to biosafety should be pursued. Moreover, there already exists a pharmaceutical industry that makes a different influenza vaccine every year, and it should be feasible to design vaccines against some of the experimental strains as well as invest more in the development of a pan-influenza vaccine: this would provide a ring of immunity to protect investigators and society against laboratory accidents. We urge more creativity in thought than knee-jerk responses as we deal with this crisis in science.

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