COMMENTARY

Impact of Gene Editing Tools, Like CRISPR/Cas9, on the Public Health Response to Disease Outbreaks

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ABSTRACT

The purpose of this communication is to explore the implications of genome editing techniques, such as CRISPR/Cas9, on public health–related responses to outbreaks of disease. The recent commercialization of genome editing techniques makes the creation and release of genetically altered pathogens a much easier task, increasing the possibility to the point of needing discussion. Three areas need to be addressed: predictions concerning potential genetic alterations, predictions and implications concerning the release of genetically altered pathogens, and the short- and long-term implications of the release of genetically altered pathogens. Full discourse on these topics among professionals in the area of public health will help to combat harm from the use of any genetically altered biologic weapons. The topics covered here include a review of the CRISPR/Cas9 gene editing technique, including a discussion of which possibilities utilize genome editing. We then address predictions about the application of gene alterations in the context of bioweapons. We discuss a few basic concepts about the evolution of an intentionally released genetically altered organism based on circumstances and patterns gleaned from observing nature in the hope that this will aid in the public health response to bioterrorism attack. (*Disaster Med Public Health Preparedness*. 2017;11:155-159)

Key Words: biodefense, biohazard release, bioterrorism, disease outbreaks, epidemics

CRISPR is a game-changing DNA editing tool that is extremely efficient, accurate, and easy to use.[1] CRISPR stands for clustered regularly interspaced short palindromic repeats. CAS stands for CRISPR-associated genes, with Cas9 being the primary nuclease (DNA cutting enzyme) in the editing process. The CRISPR/Cas9 system functions in nature as a prokaryotic immune system to edit out invading viral/plasmid/phage DNA from the bacterial genome. Its function in nature is seemingly effective at promoting bacterial survival because nearly 40% of known bacterial genomes have CRISPR sequences.[2]

In bacteria the system works by associating RNA made from the CRISPR sequence with the Cas9 nuclease. It binds to the DNA sequence on both positive and negative strands, specifically targeting the Cas9 nuclease to the CRISPR sequence where it makes a cut in the DNA. The native process serves to excise the offending viral or phage DNA from the bacterial genome. Innovative scientists recognized this mechanism as an opportunity to target cuts in DNA to specific sequences of their choice. The RNA targeting sequence associated with the Cas9 nuclease can be changed to a sequence that is complementary to the DNA where a cut is desired. Once a cut is made, a new DNA sequence of choice can be inserted (ligated) into the open space. CRISPR/Cas9 has already been used to delete genes, add genes, and regulate expression (up or down) in a variety of organisms including viruses, bacteria, fungi, insects, and mammals—even human embryos.[3-9] CRISPR/Cas9 could even function to make fusion genes, combining the DNA from 2 or more different genes to produce a novel protein previously unknown to science. Of course, the basic genetic sequences of the components would need to be known, but the combination would be unique. What CRISPR/Cas9 cannot do is create a brand new genetic sequence from scratch to produce a protein that is completely unique to science. Essentially, using CRISPR/Cas9 to alter a genome can be thought of as a genetic shift event in the evolutionary development of the organism, similar to when influenza virus acquires a novel gene from a different strain. Although all of this is academically fascinating, it has also spawned fears that this technology will be misused.[10]

Attempts are being made to regulate the use of gene editing technologies. However, the cat is out of the proverbial bag. Genome editing kits for prokaryotic systems and eukaryotic systems are currently available for purchase through a variety of scientific research supply companies. Additionally, the kits are fairly easy to use, requiring minimal scientific training. So even though the technology existed to alter genomic sequences prior to the emergence of the current editing systems, the fact that these new systems are readily available and very easy to use broadens the scope of potential users significantly. Therefore, a plan must be developed for the possible nefarious applications of this technology. Wrestling with how best to

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prevent or identify genetically altered organisms that may be released on the populations of the world and how to develop strategies to defend against such usage has become an immediate need.

PREDICTIONS CONCERNING POTENTIAL GENETIC ALTERATIONS IN PATHOGENS

One of the larger problems facing public health officials is the allocation of resources for prevention versus reacting to outbreaks. In the case of genetically altered pathogens, is it fruitful to predict what alterations might be made in what organisms and try to prevent a release? The problem of bioterrorism is too diverse to discuss in total, including humans, livestock, food crops, and other domestic animals as targets. So, for expediency, the discussion will be limited to human-targeted pathogens. Another factor limiting discussion is the very real concern of giving good ideas to bad people; thus, most examples will be limited to relatively well-known prokaryotic examples. Even limiting the discussion to bacteria that already cause disease in humans, it is still simply not prudent to attempt to identify all potential uses of gene editing technology that may create a more devastating pathogen. However, some uses are much less likely to be implemented. Thus, it is possible to narrow the scope of "potential applications of gene editing technology" to a smaller subset of "likely nefarious uses" by assuming the purpose behind making and releasing a pathogenic organism is meant to be noticed. Then the most likely use becomes the alteration of microorganisms to form a more devastating and newsworthy pathogen. Even after making such an assumption, the task is daunting. One could easily identify hundreds of bacterial pathogens that could be intensified by adding a toxin or virulence factor. Even the addition of a single gene to promote pathogenicity results in hundreds or thousands of possibilities, including known toxins, pathogenicity factors, antibiotic resistance genes, oncogenes, and prions. Add to that the task of monitoring and attempting to regulate access to materials concerning those bacteria and it becomes a huge task and cost. Quite simply, trying to predict what alterations might be made in which organisms utilizing gene editing tools is a fool's errand. However, focusing prevention efforts on obvious choices such as the simple addition of toxin-producing genes, like botulinum toxin, to common bacteria would seem prudent. After all, the use of CRISPER/Cas9 requires the use of specific RNA templates, and the amplification of the toxin gene requires DNA primers, both of which most scientists order from commercial nucleic acid sequence services. Therefore, monitoring the sequences ordered through such commercial services for a list of major toxins and pathogenicity factors may provide a thin layer of protection and would be worth the time and expense. Because trying to predict all uses of genome editing technologies to form pathogens is not an efficient use of resources or time, it appears obvious that not all releases can be prevented. Therefore, a post-release plan is also necessary.

PREDICTIONS AND IMPLICATIONS CONCERNING THE RELEASE OF GENETICALLY ALTERED PATHOGENS

Since all releases cannot be completely prevented, the development of a plan for an effective response to a release of genetically altered organisms is not only prudent but is a required aspect of preparation. To develop a response plan it is first necessary to determine what kind of release to expect and what the goal of the response should be. It is obvious that the goal of any response to an intentional release of any pathogen is to limit the scope and severity of illness. Limiting the scope and severity of illness requires quick and decisive public health measures.[11] Quick identification of intentional releases and natural outbreaks is of utmost importance for an effective response, whether involving genetically altered organisms or not. Factors important for identification of intentional release of a biological weapon are generally agreed upon and include heavy reliance on the judgment of front-line medical and laboratory personnel who make determinations about numbers of patients reporting similar symptoms as being suspicious or not.[12-14] The same initial decision-making factors and personnel can also be relied upon in the release of a genetically altered organism with the addition of a more robust pathogen identification plan. A more robust method of pathogen identification is necessary because it may not be obvious which organisms are wild-type pathogens versus genetically altered pathogens. The reason it is necessary to identify genetically altered pathogens will be discussed shortly. There are a range of outcomes for an intentional release of any pathogenic organism genetically altered or not. Placing potential outcomes for the release of genetically altered organisms into the categories described in Table 1 can facilitate the conversation on why it is necessary to quickly identify outbreaks of genetically altered organisms and think about the different possibilities as they relate to the containment status of the organism.

Even without the argument that there is a slightly stronger moral imperative to lessen harm from intentional releases, the public health response should have the same elements as any outbreak of infectious disease whether naturally occurring or intentionally released (wild-type or genetically altered). An effective response must treat the affected people and stop the spread of infection. Treating the affected people and stopping the spread require identification of the infectious organism early in the initial investigation of any outbreak. With the apparent ease of making genetic alterations it cannot be assumed that the organism is not altered. Therefore, if the infectious organism appears to be from an intentional release we need to know if the organism has been genetically modified or is a "wild-type" pathogen. It may or may not be obvious from the symptomology alone. Educated guesses could be made about the organism based on the purpose behind the release and intended target. Further identification of the perpetrator could provide clues to the level of sophistication to expect in the organism itself. However, such guesswork is not necessary in terms of

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TABLE 1

Different Outcomes for the Release of Genetically Altered Organisms

Type of Release	Description	State of Genetically Altered Organism
Prevented	Intervention by authorities prevents actual release	Organism contained
Failed	Organisms released, but no immediate harm to humans or animals is reported	Organism uncontained
Low-level	Few individuals infected with limited or no person-to-person transmission	Organism free in environment; disease contained quickly
Mid-level	Large initial exposure with limited person-to-person transmission	Organism free in environment; disease containment possible
High-level with sustained transmission	Large or small initial exposure with stable or predictable R0; person-to-person transmission	Organism uncontained

response. Regardless of the intended purpose, obvious or not, an intentional release of an organism should elicit a rapid, decisive, and robust initial response. Simply put, an assumption must be made that if an organism is released intentionally, it is not "wild-type." Therefore, we must endeavor to utilize a quick, reliable method for identification of all pathogens involved in outbreaks. Common identification methodologies, such as relying on quantitative polymerase chain reaction (qPCR) or microarray, are problematic, falling short of being able to determine whether the whole organism has been altered genetically, as they directly assess the presence of 1 or 2 genes, not the entire genome. Therefore, efforts must be prioritized to fully develop and broadly implement a methodology that identifies genetically altered pathogenic organisms with robust full genetic sequencing analysis.[15,16] Full DNA sequence identification is certainly able to fully identify organisms from raw clinical samples with no isolation or purification of the pathogenic organism. Indeed, microbiome analysis of gut bacteria has been a proof of concept, being able to identify thousands of different organisms from individual fecal samples[17,18] as well as identification of pathogens in beef.[19] Development of whole sequence identification should quickly be followed by full strategic implementation of that methodology in the Centers for Disease Control and Prevention's laboratory response network, allowing quick prioritized access for identification and analysis of suspected samples from around the globe. Speed of implementation of public health measures is of utmost importance. A full genome sequencing approach appears to be the best method to allow identification of known wild-type and unknown genetically altered pathogens with one method.[20] Quicker PCR-based identification assays should not be abandoned completely. PCR-based, pathogen-specific identification protocols can be developed on an as-needed basis once the sequence of the organism is determined to provide faster, in-field identification of the pathogen, genetically altered or not. In fact, custom PCR-based protocols are a good way of monitoring genetically altered pathogens, because such protocols can detect the presence of the alteration itself over time. Unfortunately, owing to the long-term implications of releasing genetically altered pathogens into the wild, monitoring for the alteration appears to be a necessary aspect of the public health response.

SHORT- AND LONG-TERM IMPLICATIONS CONCERNING THE RELEASE OF GENETICALLY ALTERED PATHOGENS

If the early public health response is the same, one might ask, Does it matter if the organism is altered on a genetic level? Why not respond to it like any other pathogen outbreak?

While the response to any outbreak should begin with the same elements—treating those afflicted and preventing the spread of the disease—the response should not remain identical once a genetic alteration has been identified in the pathogen. Knowing that a genetic alteration has been made to an organism allows certain assumptions to be made about the progression of the outbreak in a real-world environment. Assumption number 1: Engineered organisms are *not* wild-type organisms. They were engineered in a lab with the addition of some factor that arguably increased their virulence. This new organism has never competed for survival in the "wild." However, all genetically altered organisms will have been derived from an organism that was wild-type, that was capable of survival in the wild without the genetic alteration. Those wild-type organisms will still be able to follow the environmental path that they evolved to follow. The organism will survive and maintain itself in the environment in the context of its evolutionary history. For example, *Vibrio cholera* will endure in the environment by inhabiting the waterways, where it lives quite well with all the native genes efficiently regulated to ensure the organism will survive in a lean competitive environment. The same concept applies to normal flora, such as *Escherichia coli*, which survive in the intestines of mammals, which is where it will continue to be able to survive even once genetically altered. What effect does the proposed addition of a new gene, a genetic shift, have on this process? Evolution is a continuous process. Natural mutation rates will constantly work to make the metabolism of the organism lean or help the organism survive in some fashion. If the genetic alteration made in the organism does not significantly benefit the survival of

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the organism in a real-world setting under competitive environmental conditions, then expression of that gene becomes a metabolic burden and expression will be lost. The concept has been proven time and time again that if a gene is not currently enhancing the survival of an organism, the expression of that gene is lost in a relatively short time after exposure to the competitive natural environment, even taking into account the fact that mutation rates change as a result of a multitude of environmental conditions and other factors.[21,22] Mutation rates vary depending on a variety of factors, such as where in the genome the alteration has been made and so on.[23] When one applies this principle to an introduced genetic alteration that has limited regulatory infrastructure and that does not effectively enhance the survivability of the organism in the environment (assuming the organism survived before the alteration), a genetic mutation resulting in the loss of expression of any added gene or virulence factor will likely be an evolutionarily favored event. Thus, the new gene will be shut down to make the organism more competitive in the wild. The only variable is how long it will take for that leaner organism to outcompete the others in the race to survive. The issue remains as to what actually constitutes enhancing survivability of a human pathogen. Is simply increasing the ability to transfer to a new host—an increased R0—enough of an environmental benefit to maintain expression of an inserted gene? More studies need to be done. However, high R0 values are not the entire story in relation to fitness to survive in real-world pathogens. Influenza has an R0 of about 1 and measles has an R0 of about 15. They are essentially on opposite ends of the R0 scale; however, both are formidable pathogens with similar modes of transmission, thus making it obvious that evolution does not always result in higher R0 values. Keeping in mind the unpredictability of the impact of R0 on the survivability of an organism, it appears difficult to predict whether a genetic alteration made for nefarious purposes would benefit the survival of an organism in the wild. Even genes that directly benefit the survival of an organism, like antibiotic resistance genes, may be lost due to lean conditions. In one study, *Pseudomonas* species lost plasmid-borne tetracycline resistance in as little as 120 generations.[24] So it would appear that predicting whether the genetic alteration would benefit the organism is problematic. Interestingly, the initial purpose of the genetic alteration will likely be at odds with efficient survival of the organism. Assuming the purpose of going through the effort of genetically altering an organism and releasing it would be to affect a large-scale change of some sort, an assumption can be made that the effect of the mutation on pathogenesis would be dramatic enough to get attention. Thus, an overly dramatic pathogenesis would likely be what the releasing party is trying to achieve. It can be safe to say that an overly dramatic pathogenesis would be a metabolic drag on the organism or negatively affect the R0 by disabling or killing the host too quickly. While that concept is particularly gruesome, it also provides a window of opportunity for the public health response to catch up with the organism. In producing an organism that is not already metabolically streamlined, the makers of that organism are putting it at a competitive disadvantage in the environment and almost guaranteeing that the expression of the additional gene or genes will be lost due to natural competition in an unregulated environment. However, lost expression is not necessarily a lost gene. The gene is still in the genome of the organism and may be for a very long time. Any additional genetic material would be present whether expressed or not for "failed," "low-level," and "mid-level" releases. Expression of any additional genes will likely be turned on and off multiple times in different ways during the natural evolution of the organism. That gene will simply be another tool in the organism's toolbox waiting for the proper set of conditions and other evolutionary changes to be a permanent part of the organism's genetic regulatory scheme. If that organism comes into contact with humans during this process, a more efficient pathogen could easily emerge.

The above scenario is not a flight of fanciful assumptions. A similar pattern is being observed in natural outbreaks of multiple "novel-to-human" pathogens, some well-known and some caused by human invasion into new habitat.[25-27] The pattern usually consists of a limited exposure to the novel organism that causes a limited number of very severe illnesses before burning out. This scenario can repeat several times over many years, while the organism evolves to a version that more effectively survives in human hosts, usually losing some of its initial virulence to allow sustained transmission. The history of Ebola virus is one example of this pattern that is playing out as the world watches, with earlier outbreaks having higher mortality and less longevity in the human host.[28]

The potential to use a newly acquired gene to become a better pathogen through a longer evolutionary process is why public health response to wild-type pathogens must differ from that to genetically altered pathogens. Much effort should be made to find and destroy genetically altered organisms once they are identified with the intent to prevent the potential reemergence of the fully evolved organism at a later time. While the reemergence of the organism as a new-to-science wild-type pathogen may take some time, the potential for harm from these man-made organisms should not be underestimated. The simplest example arises from the fact that different strains of the enteric bacteria *E. coli* are the most widely used laboratory strains. If probabilities of potential outcomes are based on the easiest to get or most abundant resources, it is easy to envision a scenario involving the use of *E. coli* as the recipient of a new pathogenic gene. If that genetically altered organism is released and allowed to go through the evolutionary processes discussed, the new pathogen could become very problematic. Interestingly, the evolutionary process would occur on the newly altered *E. coli* even if the initial release failed to cause any pathology for some reason. Thus, even if considered a "failed release" from the perpetrator's perspective, because it did not cause the immediately intended outbreak of disease, the bacteria may very well be surviving and evolving in the microbiome of an infected person. From there it could at

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some future time emerge as a new and dangerous pathogen, utilizing its newly acquired pathogenicity factor gene. While there is little public health professionals can do to avert a "failed release," there are certainly some steps to take that would improve the response to outbreaks and prevent future harm at the same time.

CONCLUSION

The ready availability and ease of use of new gene editing methods open the door to a much larger potential group of people able to produce a myriad of different bioweapons. Although these organisms may only cause the intended pathology for a short time, release of these novel organisms into the population and environment is a devastating and dangerous event with consequences that may be long-term. Proactive steps should be taken to take into account these seemingly inevitable new-to-science pathogens in the public health responses to outbreaks of pathogens around the world. These steps include full genomic (and plasmid) sequencing of organisms causing outbreaks of disease, as well as plans to monitor and eradicate any genetically altered, new-to-science pathogens even if their ability to cause pathology subsides.

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