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Why Revive a Deadly Flu Virus?

By Jamie Shreeve

One morning last August, Terrence Tumpey, a research scientist at the Centers for Disease Control and Prevention in Atlanta, walked into a room across a corridor from his office and took off all his clothes. He pulled on cotton scrubs and a disposable gown, two pairs of latex gloves and headgear with a clear plastic shield enclosing his face and a tube running out the back to a set of filters strapped to his waist. He walked through another door and down a hallway to a large upright freezer. Mounted beside the freezer was a retinal scanner. Tumpey, who is 6 feet tall, bent down a little to position his eyes in line with the lens. In a digital voice, the scanner asked him to step forward. Tumpey complied. "Identification confirmed," the scanner said, and a lock on the freezer clicked open.

Inside the freezer were trays and boxes containing "select agents" - highly pathogenic microbes that under the Patriot Act cannot be handled without special clearance from the Department of Justice. Tumpey wiped the frost off a box. He was the only person in the C.D.C., or anywhere else, authorized to handle this particular agent: a synthesized version of an influenza virus that, nearly a century before, killed between 20 million and 50 million people. He placed the box in a secure container, and after showering and dressing, carried the container through secure corridors to another building at the C.D.C., where he entered another suite of rooms, dressing once again according to Biosafety Level 3+ protocols, the second most stringent level of biosecurity. For the next couple of hours, he squirted the virus into the nostrils of laboratory mice. He was fairly certain they would all soon die.

Getting the flu can be a real drag. Your head pounds, your muscles ache, you lie in a bed of misery, surrounded by clammy tufts of used Kleenex you're too tired to pick up. Every year, 5 to 20 percent of the American population catches a flu virus. The elderly, very young children and people with certain health conditions are at risk for more serious complications, and annually some 36,000 of them die. Every few decades, a particularly virulent strain appears and causes a global pandemic. In the 20th century, flu pandemics occurred in 1918, 1957 and 1968. The last two killed two million and 700,000 people respectively - again, claiming most of their victims among the young, the old and the weak.

The 1918 flu virus is remarkable for two reasons. First, it caused perhaps the most lethal plague in the history of humankind. In the fall of that year it spread across the planet, perversely striking down healthy young adults. Once ensconced in their lungs, the virus triggered a havoc of inflammation, hemorrhage and cell death. Trying to draw air into such lungs was like breathing through meat. Many of the afflicted died within hours after they first began to feel a little feverish. Others succumbed more slowly to secondary bacterial infections. By the spring of the following year, the virus had disappeared as

mysteriously as it had come.

The second, and in some ways even more remarkable, thing about the 1918 flu virus is that it has literally been brought back to life. In October, a team of scientists, Tumpey among them, announced that they had recreated the extinct organism from its genetic code - essentially the scenario played out in the movie "Jurassic Park," albeit on a microbial scale. In the movie, the scientists' self-serving revivification of dinosaurs leads to mayhem and death. Tumpey and his colleagues say they hope that their resurrected microbe will help prevent a calamity, not cause one. They want to know what made the 1918 flu, which began as a virus native to wild birds, mutate into a form that could pass easily from one human to another. That question has been weighing on the minds of flu experts since 1997 - since the first fatal case in Hong Kong of the avian flu that has since killed more than 70 people in Asia. So far, all of its victims probably caught the disease from handling infected poultry and not from other people. How close is it to crossing the same lethal line that the 1918 virus did? What can be learned from the virus that caused the great pandemic that might help us avert another one?

The risks involved in trying to answer such questions are hard to calculate, because the experiment has no precedent. In essence, Tumpey and his colleagues have brought one serial killer back from the grave so that it can testify against another. How dangerous is the 1918 virus to today's population? Its genetic code is now in public databases, where other researchers can download it to conduct experiments. Scientists from the University of Wisconsin and the National Microbiology Laboratory in Canada have already collaborated to reconstruct the virus from the publicly available sequence. How easy would it be for a bioterrorist to exploit the same information for malevolent ends?

"Give me $100,000 and two months, and I can recreate it right here in my lab," says Earl Brown, a flu researcher who specializes in the evolution of virulence at the University of Ottawa. "You wouldn't be able to tell it from the real thing that was around a hundred years ago. Would it kill at the same rate as in 1918? Probably. But you really don't want to have to find that out. You don't want to give this thing a second time around."

Terrence Tumpey is not moved by such talk. Even if the virus was to get out into the population, he says he believes it would cause far less sickness than it did in 1918. And he is sure that it is not getting out, ever, at least from his lab at the C.D.C. But whatever the danger posed by the virus in his freezer, it is literal living proof that science has crossed into an uncertain new world, where the drive to know life on its most fundamental level has given birth to the means to create it.

The resurrection of the 1918 influenza virus was a team effort engaging the resources of the C.D.C. in Atlanta, an obscure military pathology lab outside Washington, D.C., an esteemed group of influenza experts at Mount Sinai School of Medicine in New York and one elderly Swede. Though the story has been told before, it is impossible not to begin with the Swede. In 1950, Johan Hultin, then a 25-year-old graduate student at the University of Iowa, was searching for a Ph.D. topic when he heard a visiting virologist

say that the only way to solve the mystery of the 1918 pandemic would be to recover the virus from a victim who had been buried in permafrost. Hultin suddenly had a topic.

After some planning, he found what seemed like an ideal site in the remote settlement of Brevig Mission on Seward Peninsula in Alaska. In a mere five days in November 1918, 72 of the 80 residents of Brevig died and were later buried in a mass grave. Hultin arrived there alone, obtained permission to dig up the grave and after two days of hacking through frozen ground came across the preserved body of a little girl in a blue dress, red ribbons in her hair. He and some colleagues eventually found four more bodies and cut out samples of their pocked and peppered lungs, keeping them frozen with dry ice exuded from fire extinguishers.

Back in Iowa, Hultin injected a solution of the lung tissue into fertilized chicken eggs - a standard method for growing flu virus - and inoculated mice, rats and finally ferrets, which have a peculiar susceptibility to human flus. Nothing worked. If the virus was there at all, it was dead. So was Hultin's Ph.D. thesis. He gave up, went to medical school and enjoyed a successful career as a pathologist in San Francisco. In his spare time he traveled all over the world, invented auto-safety equipment, restored archaeological sites, built a replica of a 14th-century Norwegian cabin in the Sierras (it took him 36 years) and did research on Mount Everest. But he never forgot about the one time in his life that he failed.

Jeffery Taubenberger, the man most responsible for resurrecting the 1918 flu virus, was looking a little sick. His face was pale and his eyes red-rimmed, and he had barely touched the pasta he ordered for lunch. He pulled out a handkerchief and sneezed hard.

"There's not a respiratory virus on earth that I don't seem to want to amplify," he told me. "If I were alive in 1918, I'd be dead."

Taubenberger is the chairman of the department of molecular pathology of the Armed Forces Institute of Pathology in Rockville, Md. His department was, in the early 90's, in the process of developing an expertise in retrieving tiny whispers of genetic code from putrefied flesh. As Gina Kolata described in her book "Flu," Taubenberger decided in 1995 to look for the 1918 virus in samples of preserved lung in the A.F.I.P.'s tissue repository, which contains about three million pathological samples dating back to the Civil War. His techniques were far more advanced than anything Hultin had at his disposal, and his goal was more modest. Taubenberger knew that flu particles are too unstable to remain intact in a frozen corpse, and he wanted only to find a remnant of the virus's genetic code, perhaps enough to reveal what made it so virulent. But for a year and a half, he, too, failed. Finally, when Taubenberger was on the verge of giving up, he recovered from a soldier's lung a tiny fragment of the killer flu's identity, like the upturned edge of a sneering mouth.

"From that moment on, I became the steward of this virus," Taubenberger said. "Whether I liked it or not, I was obligated to get the whole thing."

Taubenberger is a compact, attractive man in his mid-40's, with big, dark eyes and a quiet, precise manner of speech. He looks a bit like Frodo the hobbit in the movie version of "The Lord of the Rings," if you can imagine a middle-aged Frodo wearing a paisley tie and an oxford shirt, a cellphone strapped to his belt. Like Tolkein's hero, Taubenberger seems both obsessed with his quest and a little tired of shouldering its weight. The trace of the virus in the soldier's lung was unimaginably faint. But by using what is called the polymerase chain reaction (P.C.R.), a common method of amplifying a signal of DNA in a sample, he and his colleague Ann Reid were able to fish out a strand large enough to sequence; then they used that sequence as a hook to fish out another strand, then another, gradually overlapping pieces that matched on their ends to build increasingly longer and more coherent pieces.

"We had to tweak the P.C.R. method to its ultimate level of detection," Taubenberger said. "It wasn't simple. It was painful. Everything we did here was painful."

Almost immediately he and Reid ran into another problem: they were running out of raw material. Then, out of the blue one day in 1997, he got a letter. It was from Johan Hultin, then 72, who had read about Taubenberger's initial success in Science magazine. He told Taubenberger about his expedition to the mass grave in Brevig in 1951 and said he would be willing to go back and try to find the virus again. Hultin said he would pay for the expedition himself. If he failed, no one else need know that it had ever happened.

And that is how Johan Hultin returned to Brevig - a tall, gray-bearded figure arriving unannounced, carrying his wife's pruning shears to help him cut through bone. After again obtaining permission, he reopened the grave, and on the fourth day of digging found the body of an obese woman whose lungs were well preserved, insulated from the occasional ground thaw by her fat. He returned home with samples of her lungs and other organs and sent them to Taubenberger. The entire expedition took five days.

"Ten days later, he called me," Hultin said of the conversation with Taubenberger. "I was in my Norwegian cabin in the mountains. 'We have the virus,' he said. I'd been waiting 50 years to hear that."

A flu particle is a sphere about a millionth of an inch in diameter, containing just eight disconnected gene segments. Its surface is covered with a thicket of spikes, like a burr. The spikes are made of a protein called hemagglutinin, which sticks to receptors on the surface of cells in your respiratory tract, much as the hooked spines on a burr catch fast on fibers in your trouser leg when you're walking through high brush. In among the spikes are some other, mushroomlike protrusions of another protein, neuraminidase. These two surface proteins define the virus's identity - the face that your immune system sees and attacks. Sixteen "flavors" of hemagglutinin are known, and nine of neuraminidase. The different major families of flu are combinations of the two, hence the designation "H5N1" for the current threat. The 1918 virus was H1N1, the mother of all flus.

Flu viruses mutate very rapidly, and each season's version is a little different. But your immune system preserves a memory of its previous encounters with a flu, which are dragged up, like old photographs from the back of a closet, every time your system responds to a new flu invasion. Very rarely, a virus comes along bearing a surface protein that your immune system has never seen. Often this occurs when a single host - it could be a pig, but might also be a person - becomes infected with two strains of flu simultaneously, one from a mammalian lineage, the other from an avian one. Inside the host, the eight gene segments of the two strains are shuffled randomly into new configurations, like the symbols in the window of a slot machine. If one of these configurations happens to be both pathogenic and transmissible from human to human, jackpot: a pandemic ensues. The 1957 and 1968 pandemics both probably occurred through this kind of "reassortment." For a long time, most scientists believed the same kind of gene-shuffling triggered the far more calamitous 1918 pandemic as well.

In his hunt for the cause of the 1918 flu's virulence, Taubenberger focused first on the hemagglutinin gene. Seasonal flus are normally confined to the respiratory tract because before it can infect a cell, the hemagglutinin protein needs to be split down the middle by an enzyme found there. But some forms of avian flu - including H5N1, the one now threatening us - bear a specific mutation in their hemagglutinin gene that allows other, more ubiquitous enzymes to cleave apart the protein, freeing the virus to invade cells deeper in the lungs or even in other organs. Taubenberger looked for the same killer mutation in the 1918 virus's hemagglutinin gene, but it wasn't there. After months of more work, he and Reid decoded the gene for neuraminidase. It, too, gave no hint why this particular virus was so deadly.

Same for the next gene, and the one after that. A year went by, then another. Instead of revealing some peculiar feature that might tip off the secret of its virulence, the genetic sequence of the virus slowly emerging seemed chillingly ordinary. Among the chain of some 4,000 amino acids that made up its proteins, only 25 or 30 distinguished it from a common, nonvirulent avian flu. Rather than originating from a reassortment of genes from both an avian and mammalian source, like the viruses that caused the later pandemics, the 1918 flu most likely began as a bird-adapted strain that, with just a handful of mutations, made itself at home in human beings. To flu researchers and public-health officials, the resemblance of the 1918 sequence to those of common avian flus underscores the stark fact that there is more than one way for a virulent strain like H5N1 to make the jump and become transmissible person to person. According to Taubenberger, this suggests a new strategy for surveillance, one that would include identifying and isolating a local variant of the virus on the verge of acquiring a complete complement of the essential mutations, after which point it would become impossible to contain.

What the genetic sequence of the 1918 virus did not reveal, however, was why the virus killed so ruthlessly, or how it made that critical leap to become transmissible. For those answers, they would have to take a more drastic step. "Jeff spent 10 years of his life doing this, and it told us nothing about pathogenicity," says Robert Webster, a noted flu researcher at St. Jude Children's Research Hospital in Memphis. "That's when we realized

the sequence wasn't enough. It was necessary to put the damn thing together."

Necessary or not, the fact that it had become possible was probably enough to ensure that it would be done. In biology, the direction determined by what is possible has been downward, toward the exploration of ever more reduced levels of complexity. The progression started with the ancients, who first opened up the human body to ponder its organs and their functions. Once microscopes were developed in the 17th century, it became possible to observe the anatomy and behavior of the tissues and cells making up the organs, and with later advances, the proteins that build cells and determine their functions. In the last century we reached the level of the genes that conjure the proteins into being. Only in the last decade has automated sequencing made it possible to peer beneath genes at the individual letters of DNA constituting a complex organism's complete genome, including our own.

This is the bottom of the biological hierarchy, the fundament, where all of life rests upon the bedrock of inert information. Now that we have reached down this far, it becomes possible to use that information to do a U-turn and start back up, not just trying to understand life, but recreating and inventing it - first simple viruses, but soon bacteria and other more complex organisms. The resurrection of the 1918 flu incarnates this turning point. It is not the first virus to be reconstituted from its genetic code. But it is so far the largest, and the meanest, and the only one to be snatched back into existence from a time when we knew so much less and were so much more at its mercy.

The wonder is not that scientists could reconstitute the "damn thing" from its genetic code. The wonder, and for some the fear, is that they could do it with so little effort or expense. Biosupply companies use synthesizing machines to build tiny pieces of DNA to order, using the sequence of letters in the virus's code. When placed in solution, these chemical snippets naturally assemble into longer pieces. With the help of a copying enzyme to fill in any gaps, the DNA molecules stitch themselves together into a complete gene, which can be inserted into a stable little circle of DNA called a plasmid - packaged to go, so to speak. If you have plasmids containing all eight flu gene segments, it is a fairly simple matter to inject them along with some flu proteins into a cell and let nature take its course.

This method of building flu-virus particles from pure code is a clever application of the approach to understanding life called "reverse genetics" - that is, looking at a gene to figure out its function, rather than the other way around. But it is not one requiring some spectacular insight or technological breakthrough. The method employs fairly routine molecular biology and was developed independently by two different flu teams, one at Mount Sinai School of Medicine in New York, the other at the University of Wisconsin. Peter Palese, from the Mount Sinai team, contacted Jeffery Taubenberger and suggested that if he would supply the blueprint for the virus, Mount Sinai would function as the parts factory, putting together the genes. Another laboratory, one with the biosecurity facilities required to work with highly infectious agents, would be recruited as the final assembly plant. That role would fall to Terrence Tumpey of the C.D.C.

The team did not even have to wait for Taubenberger to finish the whole sequence of the 1918 virus to begin testing its virulence. In 2001, Adolfo Garcia-Sastre and Christopher Basler, also at Mount Sinai, reconstructed the genes for just the two critical surface proteins and sent them on to Tumpey, at that time working at the Southeast Poultry Research Laboratory in Athens, Ga. Taking advantage of influenza's innate ability to mix and match genes from two strains, he combined the two 1918 genes with others from an innocuous laboratory strain to make a complete set. Tumpey infected some lab mice, which are normally not affected much by human flus. Five days later, he came into the laboratory at around 11 at night for a quick check on their progress. All the mice were dead.

In person, Tumpey is unnervingly imperturbable; ask him what it's like handling an infectious agent that killed perhaps 50 million people, and he stares back at you and gives a little shrug. But this first demonstration of the virus's power got to him.

"I literally felt a chill go down my spine," he told me. "I knew I had this awesome virus, and I'd eventually be able to put the whole thing together."

He did not have much longer to wait. It took nearly 50 years to find a trace of the virus in preserved tissue, and nearly 10 years for Taubenberger to sequence its code, finishing the last of three genes driving the virus's replication machinery early last year. From that point, it required just a few months for the Mount Sinai group to transform the code into actual genes, and in Tumpey's lab mere days for the genes to begin assembling themselves into viable virus particles and come bursting out into the surrounding solution.

Tumpey and his colleagues were well aware that bringing such a lethal pathogen back into the world was going to cause controversy. But he was fairly certain that he had laid the groundwork to defend the decision, obtaining approvals from the highest levels at the C.D.C. and the National Institute of Allergy and Infectious Diseases, which had financed the work. He had conducted experiments showing that mice were protected from the virus by the current human flu vaccine and by Tamiflu, the antiviral drug. In any case, because a virus descended from the 1918 one has been circulating in the population since 1977, Tumpey is confident that everyone carries at least partial immunity to the 1918 virus itself.

Not everyone is as sanguine as Tumpey. "I believe that this was research that should not have been performed," says Richard Ebright, a Howard Hughes Medical Institute investigator at Rutgers University. "If this virus was to be accidentally or intentionally released, it is virtually certain that there would be greater lethality than from seasonal influenza, and quite possible that the threat of pandemic that is in the news daily would become a reality."

Neither Terrence Tumpey nor Richard Ebright really knows how vulnerable the population today would be to the resurrected virus. Nobody does. This uncertainty would

seem to limit the virus's value as a bioweapon. In theory, anyone with nefarious intent and the requisite training in molecular biology could recreate the virus from the sequence published on the Internet. But why would any sensible bioterrorist go to such lengths to create a weapon that might do no more harm than a seasonal flu bug, or, if it did prove undiminished in its virulence, would kill his own people as indiscriminately as his enemies?

Then again, common sense is not a prerequisite for membership in a terrorist organization. Accidental release of the virus cannot be ruled out, either. While few question the experience and expertise of the C.D.C. in containing dangerous microbes, other labs will be working with the virus, and there is ample precedent for accidents occurring under stringent biosecurity, including release of the SARS virus in the past few years from three separate laboratories in Asia, which led to one death. In fact, the reason those of us who were not around in 1918 still may have some immunity to that pandemic strain is that a relatively innocuous descendant H1 type was reintroduced into the environment in 1977, probably by accident in China or Russia.

Given the potential danger, Robert Webster, the esteemed flu researcher who supported the reconstruction, is among those who say it would be better to conduct future research on the 1918 virus under Biosafety Level 4 conditions - the maximum degree of security, used for working with lethal microorganisms like the Ebola virus and smallpox. But currently, only four institutions in the U.S. have functioning BSL-4 facilities, including the C.D.C. Imposing such restrictions would necessarily slow the progress of research.

This is something that Terrence Tumpey, among others, insists that we cannot afford. Earlier this month, the H5N1 virus recorded an extraordinary rash of cases, including four fatalities in Turkey, the first outside East Asia. All the victims appear to have caught the virus from eating or handling infected poultry. But most flu researchers worry that as the virus's range increases, so does the likelihood that somewhere, sometime, some random set of mutations will send it over the edge into transmissibility, unleashing a pandemic. Everyone agrees that at some point, another pandemic will come - if not from this strain, then from some other one perhaps not even yet under surveillance. The best hope of containing its impact is to understand how it works. What are its mechanisms of infection and replication? How does it foil the host's immune response and jump from a conquered host to a fresh one?

In Tumpey's view, the 1918 virus is the star witness in a murder trial, and the interrogation should proceed without unnecessary impediments. Taubenberger's sequence can help indicate what questions to ask. Experiments with individual genes can suggest some possible answers. But only the living virus can reveal the full truth. The first round of interrogation is already under way. Using reverse genetics to test the contribution of any particular gene to the virus's pathogenicity, Tumpey and his colleagues can replace any target gene in the 1918 virus with its complement from a harmless strain, then measure the effect on the virus's potency. When he replaced the 1918 hemagglutinin gene with one from a garden-variety seasonal flu, the virus replicated at less than 1100th the rate in mice; it was definitive proof of the essential role played by that gene in virulence.

Tumpey already knew that the 1918 virus did not need one of the host's own enzymes to turn traitor and cleave apart the hemagglutinin protein to help the virus infect a cell. But when he created a 1918 virus without its own neuraminidase gene, this ability was lost, revealing that the virus toted its own cleaving mechanism into the host on that gene, like a butcher who brings his own knife. Meanwhile, Peter Palese's group has shown that another gene in the 1918 virus is especially good at blunting the human immune system's initial counterattack.

"It was perfect genes, working together, that made this virus what it was," Palese said. Then he gave a little laugh. "Or what it is."

Scientists can also examine the role in virulence and transmission of particular mutations on the virus's genes. Taubenberger's sequence again offers guidance. One of the large genes driving replication, for instance, bears a single mutation that is found not only in the 1918 virus, but also in all human flus. But no bird flus have this mutation - not even H5N1. Is this mutation perhaps necessary for an avian virus to become transmissible from human to human? Combining reverse genetics with some other molecular tricks, you could insert that mutation into the gene of a nonvirulent avian flu, construct the virus and see how it behaves. The ultimate hope of such experiments is to uncover a clue to how the virus spreads or kills, and possibly a way to cripple it. Terrence Tumpey is already planning experiments with several research groups and companies that will use the 1918 virus to test possible antiviral drugs to block some universal mechanism of virulence, like the binding of hemagglutinin to the host cell. That work has added urgency, since the H5N1 flu appears to have developed resistance to one of two flu drugs currently on the market.

What may be the most informative research he intends to conduct must surely be the most dangerous as well. Tumpey's freezer contains the resurrected 1918 virus, which is lethal and highly transmissible. It also contains samples of the H5N1 virus, which is lethal but not yet transmissible. Using reverse genetics, he imagines "a great set of experiments" combining the genes of these two killers in various combinations, seeing if one might have the capacity to transmit from an infected animal model, like a ferret, to an uninfected one. This would create in the laboratory the very pandemic strain that researchers most fear may emerge at any time in nature. According to Tumpey, plans for these experiments are already "on paper." Needless to say, they will require complete approval first, and may have to be performed under Biosafety Level 4 conditions, since we would have no immunity to the recombinant organism.

For Richard Ebright, the prospect that the C.D.C. or some other lab would "jump the gun on nature" is worrisome under any circumstances. Other scientists and bioethicists are also calling for more independent, international review and control of further research on the 1918 virus and other synthetic pathogens yet to be concocted. It all comes down, of course, to whether what we can learn justifies the risk of bringing them into existence. While that debate moves forward, nature will go on conducting its own creative experiments, indifferent as always to our abilities to defend ourselves against them.

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