Letter 46: Here We Go Again

October 2, 2023

Dear Daughters:

BOOSTER TIME AGAIN

Today your mother and I got yet another COVID-19 booster shot, and I urge all three of you to do the same as soon as you can. This is a song I have sung before, but please don't let my repeated refrain stop you from protecting yourselves with the best technology available. It seems most Americans have grown fatigued with repeated injections. Only 17% got last fall's updated shot, according to the CDC, compared with nearly 70% who got the primary series.

Why Do We Need Another Booster?

We need another booster shot because the COVID-19 virus keeps evolving new forms that are better able to infect us. The initial very effective vaccines were directed at the original Wuhan strain of the virus. As the vaccine's effectiveness faded after about six months, a "booster" (another shot of the same stuff) was recommended to maintain protection. When the Omicron variant appeared in December 2021, the initial vaccine offered far less protection against it, so a second formulation of the vaccine was developed. This one was a "two-fer" bivalent vaccine targeting both the original Wuhan stain and the Omicron subvariant called BA.4 which was the most dominant variant last winter. This is the updated booster we all got last spring.

Playing COVID-19 Whack-A-Mole

But the virus keeps evolving new more-infective forms (can you believe most Americans still don't believe in evolution?). Maintaining protection from COVID-19 is like playing a game of Whack-A-Mole! The most common version of the virus circulating last spring, called XBB.1.5, was selected by the CDC in June for a new booster, the one your mother and I got today.

Of course, the virus will keep evolving. Today XBB.1.5 has already been replaced as the most common variant in the United States by another quite similar but even more infective variant

called EG.5, with another quite different variant called BA.2.86 (it has more than 30 changes!) rapidly overtaking EG.5 in frequency. Both Pfizer and Moderna say their testing of their new vaccine found that the shots worked as well against EG.5 as XBB.1.5. Preliminary data indicate that it also protects against BA.2.86, but the evidence I can find is still slim.

You don't have to be a rocket scientist to see where this is going -- annual reformulations of our COVID-19 vaccines. Like we do with the flu shots we get every fall, we are going to need to adjust our protection to match the current form of the virus.

Stated simply, COVID-19 is not going to go away. Instead, a problem that was once pandemic is now chronic.

INVENTING mRNA VACCINES WON THE NOBEL PRIZE TODAY

I have always been fascinated by the Nobel Prize, which was announced today for the year 2024. Reading news accounts, it is easy to misunderstand the nature of the prize. Only 1-3 individuals can win a Nobel Prize, but major technological advances are often achieved by teams of scientists collaborating, rather than by single individuals. The human genome was sequenced by literally hundreds of scientists sharing their data and ideas. Pointing out this seeming discrepancy – hundreds of scientists but only one to three winners -- an opinion piece published in the New York Times this week states that the Nobel Prize was an outdated concept, and should be redesigned to award large research collaborations. But collective scientific advances are not what the Nobel Prize is about. It awards original insight, the discovering of a new way of thinking.

This year's Nobel Prize in Physiology or Medicine is a case in point, a beautiful example of how a key insight by two scientists has led to major advances that impact all of science. It was awarded to Hungarian-American Katalin Kariko, now a professor at Szeged University in Hungary (the first woman to win a Nobel Prize since 2015), and to her collaborator, American Drew Weissman, a professor at the University of Pennsylvania.

Dr. Kariko's Story

This year's prize is also a fantastic story about a woman's career in science, one I am going to tell you in some detail because it is also a revealing account of one woman's struggle and eventual triumph in the male-dominated world of biomedical research.

Kariko, the daughter of a butcher, had come to the United States in 1985 when her research program in Hungary ran out of money. She was studying mRNA, the molecule copied from DNA which is used by cells to direct how their proteins are made. A dark corner of molecular biology at the time, mRNA was not being studies in very many labs in the 1980s, and few research grants were being funded for mRNA research. Almost all research into genes was being directed at DNA, which because of its double-helix structure was quite stable and easy to work with. mRNA, on the other hand, was single-stranded and quite unstable, making it very difficult to use in laboratory experiments.

Arriving in Pennsylvania in 1985 (that's her in the photo with her husband and daughter shortly after arriving) she found a post-doctoral position at Temple University that would let her continue her research on mRNA. These were early days, and little was known of mRNA. Even the most basic laboratory tasks had not yet been sorted out: How do you make RNA molecules in the lab? How do you get mRNA into the cells of a living body?

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In 1989, after four years as a post-doc investigating the basics of mRNA at Temple, Kariko landed a job with a cardiologist at the University of Pennsylvania. She was a research assistant professor, a low-level faculty position never intended to lead to a tenured permanent position. She was supposed to be supported by grant money, but none ever came in. But the work was exciting. She set out to insert mRNA into cells, seeing if this would induce them to make new proteins. This had never been done before. She set out to insert mRNA containing the gene

sequence of a protein called the urokinase receptor into mouse cells growing in tissue culture. These cells did not have that gene and so should never be able to make that protein . After mRNA insertion, the cells made the urokinase receptor! This suggested to her that mRNA could be used to direct any cell to make any protein she chose. *"I felt like a god,"* Kariko recalls.

But the cardiologist soon left the university to join a biotech firm, leaving Kariko without a lab or financial support. "*They expected I would quit,"* she says. But one of the junior faculty remembered her from when he was a resident at Temple University, and urged the head of the neurosurgery department to give Kariko' research a chance. Now she again had a place to work. For a few years she looked for ways to use mRNA to get blood vessel cells to make nitric oxide (a dilator). No success. Then her friend and the department chairman both left the university. Kariko was AGAIN without a lab and without funds for research. A scientist without a lab and without research funds is like a baseball player without a field to play in or bat with which to hit the ball.

But her luck was about to change. At a photocopy machine she encountered Dr. Drew Weissman, a new faculty member who had for several years been frantically and unsuccessfully trying to make a vaccine against H.I.V. AIDS was at that time a major killer, and Weissman, a physician and virologist, was one of many researchers seeking an effective vaccine. Kariko said simply "*I can do it*." So Weissman offered her space in his lab to prove it.

A New Approach to Making Vaccines

Like all other researchers at the time, Weissman was attempting to construct a vaccine that worked by introducing into a patient bits of the disease-causing virus. Making antibodies to these bits, the patient would become immune to any future attack by the virus (You may recall we discussed these approaches in Pandemic Letter #8, May 16, 2020). Kariko suggested that she and Weissman take a completely different approach: insert mRNA from the AIDS virus into the patient's cells, instead of inserting viral proteins into the patient's bloodstream. The patient's cells would then manufacture the AIDS proteins themselves, releasing these newly-made proteins into the body where they could induce protective antibody production. *This is much more like what happens in a real infection*, she argued – *and I am sure we can do it!*

It didn't work, though. Kariko had no trouble getting mouse cells growing on a petri dish to take up mRNA, but the cells of a living mouse would not. Any mRNA introduced into living animals was instantly destroyed. Kariko and Weissman wrote research proposals describing the potential of the approach, but grant reviewers were not impressed. Weissman's lab relied instead on seed money that the university gives new faculty members to get started. Countless experiments failed, every promising approach a dead end. This went on for years. Whatever Kariko and Weissman tried, animal immune systems interpreted the mRNA they were trying to add as an invading pathogen and attacked it. Until they could find a way to make introduced mRNA acceptable to living animal cells, there was never going to be any vaccine.

But they kept at it, month after month. Kariko's husband, manager of an apartment complex, calculated that her endless workdays meant she was earning about a dollar an hour.

Breakthrough

You can imagine their frustration. Every living cell in an animal's body makes mRNA, and the immune system doesn't destroy it. Why is the mRNA Kariko was making different? The answer came from that invaluable tool of the serious researcher – a control experiment. While their introduced mRNA molecules produced an immediate immune system attack, the control molecules (another form of RNA called transfer RNA or tRNA) did not.

That difference was the key that unlocked the puzzle. How is tRNA different from mRNA? Uridine, one of the four kinds of chemical nucleotides in tRNA, is slightly modified to form pseudouridine. A quick experiment revealed that naturally occurring mRNA had pseudouridine too. Bingo – pseudouridine is what protects natural mRNA from destruction!

So in order to protect introduced mRNA molecules from immune attack, what the researchers needed to do was chemically treat their mRNA to convert some of its unidine to pseudouridine. When Kariko and Weissman then introduced this chemically-tweeked mRNA into mice, the mice manufactured the protein encoded in the mRNA in prodigious amounts!

Success at last. Now Kariko and Weissman were able to introduce any mRNA they wanted into any cell. This was the basic scientific discovery for which Kariko and Weissman got the Nobel Prize today.

The Long Wait

Fame and fortune now, right? Nope. The grants they wrote to explore this exciting result were not funded. Reviewers said mRNA would not be a good therapeutic agent because it was so unstable, and thus was not worth investigating further. The leading journals *SCIENCE* and *NATURE* rejected their work. They finally succeeded in getting the niche journal *IMMUNITY* to publish their findings in 2005, but the paper got little attention.

As you might expect, this didn't slow Kariko and Weissman much. They continued to put in long hours despite little encouragement. They understood that a mouse is a far cry from a human, and that many things that work in mice do not in humans. so they set out to check their pseudouridine-tweaking method of mRNA cell entry with an animal more like us. They introduced the mRNA for the protein erythropoietin (a protein that stimulates the body to make red blood cells) into a monkey. The monkey's red blood cell soared. Now a monkey is not a human – but it is close. If they could get mRNA into a living monkey's cells, this was a strong indication they would be able to introduce mRNA into the cells of human patients just as easily

Now the path was open to attempt their novel mRNA vaccine. They wished to take a very direct approach: inject a patient with mRNA made in the lab that had the nucleotide sequence of a virus surface protein, tweaked to contain pseudouridine. With luck, they felt, the patient's cells would manufacture the virus protein, and the body's immune system would produce antibodies to these proteins. Immune protection.

But how to get the considerable money required for clinical trials of a vaccine? "*We talked to pharmaceutical companies and venture capitalists. No one cared*," Weissman said. "*We were screaming a lot, but no one would listen."*

Off To The Races

Finally Kariko and Weissman succeeded in attracting the interest of two biotech companies: Moderna in the United States and BioNTech in Germany which partnered with Pfizer for clinical trials. These companies set out to make a mRNA flu vaccine, with clinical trials to be underway soon (Dr. Kariko eventually became a senior vice president of BioNTech). Things were progressing slowly, with clinical trials being planned by BioNTech for several viruses.

Then came the pandemic. Worldwide, biomedical researchers rushed to study the coronavirus causing the rapidly-spreading COVID-19 disease. Researchers already knew that the spike proteins of coronaviruses protrude out from the virus surface. When the gene sequence of the Wuhan strain of COVID-19 was posted by Chines researchers in January 2020, it immediately became possible to manufacture in the laboratory a mRNA with the gene sequence of the COVID-19 spike protein. This virus was a perfect candidate for a mRNA vaccine! Within days BioNTech and Moderna had designed mRNA vaccines, both using the pseudouridine modification discovered by Kariko and Weissman.

It took almost a year to complete clinical trials. On November 8, 2020, the first results of the Pfizer-BioNTech clinical trials came in. The mRNA offered powerful immunity to the COVID-19 virus! "*Oh, it works,"* Kariko said to her husband. "*I thought it would*." She and Dr. Weissman were vaccinated on December 18, 2020. Almost exactly three years later, on December 10, 2023, they would receive the Nobel Prize.

Was it worth the long years of largely unsuccessful experiments, clinging to the fringes of academia with little respect or reward? To me Kariko and Weissman are heroes. About 400 million doses of the Pfizer-BioNTech vaccine and 250 million doses of the Moderna vaccine have been administered in the United States, and many more millions globally, saving countless lives. Every one of these saved individuals lives in debt to these two quiet dogged researchers.

Sorry for the long story, but I want my daughters to know this hard-fought journey.

Stay safe. Get boosted. Vote.