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Perspective

Before Virus, After Virus: A Reckoning

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SUMMARY

The 2020 Lasker Awards, a celebration of one of the most prestigious international prizes given to individuals for extraordinary contributions to Basic and Clinical Medical Research, Public Health, and Special Achievement, was cancelled because of the COVID-19 pandemic. Typically, essays on the awardees and their scientific and medical contributions are solicited and published in *Cell* in collaboration with the Lasker Committee. This year, the Lasker Committee commissioned an essay to reflect on the historic contributions that scientists and physicians have made to our understanding of immunology and virology, and future directions in medical and basic research that have been highlighted by COVID-19 pandemic.

"If you think research is expensive, try disease."
—Mary Lasker

THE STRUGGLE

In the summer of 1882, a Russian professor of zoology, Elie Metchnikoff (also called Ilya Mechnikov) quarreled with his colleagues at the University of Odessa. He was a temperamental man with a depressive streak, with scientific interests that ranged from the embryology of cuttlefish to the digestive system of flatworms. But he was often in conflict with his colleagues, and in '82, he moved to Sicily, where he set up a private laboratory (Gordon, 2008). In Messina, where the warm, shallow, windy beaches yielded a constant wealth of marine animals, Metchnikoff began to experiment with starfish. Alone one evening—his wife and children had gone to watch the local circus—Metchnikoff devised an experiment that would change our understanding of immunity. The starfish larvae were semi-transparent; he had been watching cells move about in the bodies. He was particularly interested in the movement of the cells after injury. What if he stuck a thorn in one of the starfish's feet?

He spent a sleepless night and returned to the experiment the next morning. A group of motile cells—a "thick cushion layer"—had accumulated busily around the thorn. He had, in essence, observed the first steps in inflammation and immune response: the recruitment of immune cells to the site of injury. The immune cells moved toward the site of inflammation actively—i.e., on their own. "[T]he accumulation of mobile cells round the foreign body is done without any help from the blood vessels or the nervous system," he wrote, "for the simple reason that these animals do not have either the one or the other. It is thus thanks to a sort of spontaneous action that the cells group round the splinter" (Mechnikov, 1967).

By the mid-1880s, the splinter of the idea—immune cells being recruited actively to inflammatory sites to launch a response—led to a series of monumental experiments. The immune cells, he found, tried to ingest—*eat*—the infectious agent or irritant that had accumulated at the site. The phenomenon was called "phagocytosis"—or eating (of an infectious agent) by an immune cell (Metchnikoff, 1884). In an extraordinary series of papers published in the mid-1880s—a body of work that would eventually win him the Nobel Prize (Table 1)—Metchnikoff described the relationship between an organism and its invaders as "Kampf"—a "drama unfolding within organisms" that was like a perpetual struggle. He wrote, "A battle takes place between the two elements [i.e., the microbe and the phagocytic cells]. Sometimes the spores succeed in breeding. Microbes are generated that secrete a substance capable of dissolving the mobile cells. Such cases are rare on the whole. Far more often it happens that the mobile cells kill and digest the infectious spores and thus ensure immunity for the organism" (Mechnikov, 1967).

As I write this, we are in mid-struggle against a miniscule, deadly pathogen that has swerved the course of human history. What words does one use—what phrases—to adequately capture the difference in living in the BV versus the AV—Before Virus and After Virus? To witness the sights and sounds of this struggle is to realize that life has been pushed off its known orbit forever: the constant beeping of alarms in the wards that eventually merged together into a mind-numbing wall of sound; the terror and confusion written across the brow of a (masked) cancer patient who was told that he had the virus; and, above all, the hideous damnation of dying alone, with a handheld camera as the only fragile connection with your family—"dying on iPhone," as one doctor friend described it.

This is not a moment to celebrate, but to reflect and recalibrate; it is a moment of introspection, perhaps even of revision. We need to look back to move forward. And so this essay looks back at history—of virology, vaccinations, and immunology—and asks: what have we learned, and what must be revisited?

Table 1. Immunology and Virology Researchers Recognized through Lasker and Nobel Awards

Name	Award	Year	Field
Ilya Metchnikoff	Nobel	1908	Physiology or Medicine
Paul Ehrlich	Nobel	1908	Physiology or Medicine
Emil Von Behring	Nobel	1901	Physiology or Medicine
Max Cooper	Lasker	2019	Basic Medical Research
Linus Pauling	Nobel	1954	Chemistry
Frank McFarlane Burnet	Lasker	1952	Basic Medical Research
	Nobel	1960	Physiology or Medicine
Niels Jerne	Nobel	1984	Physiology or Medicine
Joshua Lederberg	Nobel	1958	Physiology or Medicine
Gerald Edelman	Nobel	1972	Physiology or Medicine
Rodney Porter	Nobel	1972	Physiology or Medicine
Susumu Tonegawa	Nobel	1987	Physiology or Medicine
	Lasker	1987	Basic Medical Research
Leroy Hood	Lasker	1987	Basic Medical Research
Phil Leder	Lasker	1987	Basic Medical Research
Jacques Miller	Lasker	2019	Basic Medical Research
Rolf Zinkernagel	Lasker	1995	Basic Medical Research
	Nobel	1996	Physiology or Medicine
Peter Doherty	Lasker	1995	Basic Medical Research
	Nobel	1996	Physiology or Medicine
Emil Unanue	Lasker	1995	Basic Medical Research
Ralph Steinman	Lasker	2007	Basic Medical Research
	Nobel	2011	Physiology or Medicine
Bruce Beutler	Nobel	2011	Physiology or Medicine
Jules Hoffman	Nobel	2011	Physiology or Medicine

Only researchers who have been discussed in this article have been included in the table. They are presented in the order in which they are discussed in the text.

IMMUNITY BEFORE IMMUNOLOGY

We knew about immunity long before we knew about the immune system. As early as 1500, medical healers in China had realized that those who survived smallpox did not catch the illness again (survivors of the disease were enlisted to take care of new victims) and inferred that the exposure of the body to an illness must protect it from future instances of that illness. Chinese doctors ground smallpox scabs into a powder and insufflated it into a child's nose with a long pipe (Jannetta, 2007). Vaccination with live virus was a tightrope walk: if the viral inoculum in the powder was too large, the child, instead of acquiring immunity, would acquire a full-fledged version of the disease—a devastation that occurred about one in a hundred times. If all went well, the child would have a mild, local experience of the disease, and be immunized for life.

In the seventeen-sixties, traditional healers in Sudan practiced *Tishteree el Jidderee* ("buying the pox"); a healer, typically a woman, haggled with a mother over the price of her sick child's ripest pustules (Bayoumi, 1976). It was an exquisitely measured art: the most astute among the healers recognized the lesions that were likely to yield just enough viral material, but not too much. The differing sizes and shapes of the pustules led to the European name for the disease: *variola*, from variation. The process of immunizing against the pox was called "variolation."

In May 1796, a young physician named Edward Jenner proposed a safer approach to smallpox vaccination. He used material from pustules of *cow*pox—a disease caused by a virus related to smallpox—harvested from a young dairymaid, Sarah Nelmes, and inoculated the son of his gardener, an 8-year-old boy named James Phipps, with it. In July that year, he inoculated the boy again, but this time with material from a smallpox lesion. Although Jenner had breached virtually every boundary of ethical human experimentation (there is, for instance, no record of informed consent, and the subsequent "challenge" with live virus might well have been lethal to the child), it apparently worked: Phipps did not develop smallpox. After facing initial resistance from the medical community, Jenner increased his vaccination efforts and became broadly celebrated as the father of vaccination (even the word "vaccine" carries the memory of Jenner's experiment; it is derived from "vacca," Latin for cow) (Riedel, 2005).

Yet even this story, retold and recycled in textbooks, is riddled with misattributions (history, too, has its revisions). The virus carried in Sarah Nelmes' pox lesions may have been horsepox, not cowpox (even Jenner acknowledged the fact: "the Disease makes its progress from the Horse [as I conceive] to the nipple of the Cow, and from the Cow to the Human Subject," he wrote). Nor, perhaps, was Jenner the first vaccinator: in 1774, Benjamin Jesty, a prosperous farmer from Yetminster village in Dorset, convinced by the stories of dairymaids who frequently got cowpox and seemed immune to smallpox, supposedly harvested lesions from the udder of an infected cow, and inoculated his wife and two sons. Jesty became an object of ridicule among physicians and scientists—but his wife and children survived the smallpox epidemic without catching the disease (Hammarsten et al., 1979).

But how did inoculation generate immunity, particularly long-term immunity? Some factor produced in the body must be able to counter the infection and also retain a "memory" of the infection over multiple years. In 1888, the biochemist Paul Ehrlich (Ehrlich, 1891) was traveling to Egypt when he heard an extraordinary (and possibly apocryphal) story of a snake-charmer who, having been repeatedly bitten by a cobra during his childhood, had become resistant to subsequent attacks by cobra venom. Ehrlich believed that an "antivenin" substance must have been generated in the snake-charmer's body. In 1890, in Berlin, Emil Von Behring and Kitasato Shibasaburo launched a series of experiments to understand how immunity to toxins and venoms might arise. Among the most dramatic of these experiments was the demonstration that the serum of an animal exposed to tetanus, or to diphtheria toxin, could be transferred to another animal and confer immunity to tetanus or diphtheria (Behring and Kitasato, 1890). In a rather desultory footnote to the diphtheria paper, Von Behring first used the word "antitoxisch"—or anti-toxin—to describe the activity of the serum (Lindenmann, 1984). In 1891, in a wide-ranging, speculative paper entitled "*Experimental Studies on Immunity*", Ehrlich pushed scientists to imagine the material nature of this "activity." He boldly coined

the word "Anti-Korper"—anti-body. The word "korper"—from corpus, or body—signaled his growing conviction that an "antibody" was an actual chemical substance—a "body" generated to defend the body.

Where did these antibodies come from? In the 1940s, the Danish physiologists Mogens Bjørneboe and Harald Gormsen and their Swedish colleague, Astrid Fagraeus (Fagraeus, 1947), showed that the serial inoculation of rabbits with vaccines or toxins caused a particular cell type, called plasma cells, to expand and secrete antibodies. The origin of these plasma cells was traced back to a particular class of white blood cell called a B cell (Bjørneboe and Gormsen, 1942).

Drawing on this early work, Max Cooper, a young biologist working with Robert Good in Minnesota, followed the trail of a report first published in a poultry journal and demonstrated that in chickens, B cells were generated in an organ called the Bursa of Fabricius, found near their cloaca (the organ had been described by the medieval anatomist Hieronymus Fabricius). When Cooper removed the bursa in irradiated hatchlings, there were no B cells, and no antibodies. In humans, though, there was no bursa (Cooper et al., 1965). Instead, B cells were eventually found to originate in white-blood progenitors, typically found in the bone marrow.

But the puzzle of how a plasma cell might learn to produce a specific antibody to bind an antigen—a biological molecule that was a yang to an antigen's yin—remained unsolved until the late 1950s. In the 1800s, Ehrlich had proposed a magnificent theory. Every cell in the body, he argued, displayed an immense set of unique proteins—"side chains," as he called them—attached to its surface. The side chains were shaped in the form of cognate opposites, or inverted shapes, to the toxin or antigen—like a lock to a key, or a mold to a statue. When a toxin or pathogenic substance bound to one such side chain in a cell, the cell increased the production of that side chain. With repeated exposures to the antigen, Ehrlich speculated, the side chain was ultimately released into the blood, thereby producing an antibody. But the theory required every immune cell to come pre-loaded with side chains carrying an inverted universe of all molecules—a mind-boggling cosmos of antibodies that had to be present in every immune cell. Decades later, the chemist Linus Pauling proposed an even more rococo theory: the specificity of an antibody for its cognate antigen was created by an antibody folding around an antigen and acquiring the inverted shape of the antigen. The antigen, in short, was like a mold that "instructed" an antibody how to form around it.

But the "instruction" and the "infinite side chain" theory were both conceptually implausible: proteins couldn't be made to fold around antigens, like medieval drapery, nor could a cell display an infinite variety of side chains, awaiting release. The most plausible solution to the conundrum of how antibodies were generated, and how they became antigen specific, was eventually proposed in an obscure paper published in 1957 in the *Australian Journal of Science* by a Melbourne scientist, Frank MacFarlane Burnet (Burnet, 1976), who drew on earlier work by Niels Jerne and David Talmage. What if, Burnet reasoned, every B cell expressed only *one* antibody? In short, a massive "repertoire" of antibodies was already present in the immune cells of the body, and it was the antibody-expressing *cell*—not the antibody itself—that was selected, and grew, when it bound the antigen. "[I]t is tempting to consider that one of the multiplying units in the antibody response is the cell itself," Talmage had written. "[But] *only those cells are selected for multiplication whose synthesized product has affinity for the antigen injected*." (The italics are mine.)

Burnet, following this line of thought, reasoned that it was this *clonal proliferation* of an immune cell—a cell stimulated by the binding of an antigen—that enabled the antibody response. At Oxford, James Gowans discovered that the "Burnetian repertoire" (as it came to be called) was carried by circulating small lymphocytes that divided rapidly in response to antigens. When he transferred these active lymphocytes—later found to be B cells—from an antigen-exposed animal to a naive animal (an ingeniously simple experiment), Gowans found that he could transfer antibody-mediated immunity as well. As the geneticist Joshua Lederberg wrote with remarkable prescience (yet without experimental evidence), "Do antigens bear instructions for antibody specificity [as Pauling had argued] or do they select cell lines [that are specific for the antigen—i.e. by clonal selection]"? Lederberg clearly favored the second theory (Lederberg, 1959).

The molecular "shape" of an antibody was also soon solved: between 1959 and 1962, Gerald Edelman (Edelman and Poulik, 1961) and Rodney Porter (Porter, 1959), working at the Rockefeller University in New York and Oxford University (REFS), respectively, discovered that most antibodies are Y-shaped molecules (some subclasses of antibodies have modifications to this shape). The two outer tines of the Y bind to the antigen, each acting like a prong. The shaft, or the stem, of the Y, serves many functions. Macrophages use shaft to capture antibody-bound microbes, viruses, and peptide fragments and swallow them, much like the shaft of a fork is used to pull food into the mouth. This, indeed, is one mechanism of "phagocytosis"—cells eating microbes—the phenomenon that Metchnikoff had observed. The shaft or stem of the Y has yet other purposes: it also attracts a cascade of toxic immune factors to attack microbial cells.

The genetics of how immune cells make such a diverse repertoire of antibodies—a unique antibody type per cell—was worked out, piece by piece, by Susumu Tonegawa, Leroy Hood, and Phil Leder. It involved the regulated shuffling of DNA within the B cell—the recombination of genetic modules, followed by more mutations to create a "mature" antibody—a strategy that Lederberg had loosely, and presciently, proposed years earlier.

IMMUNOLOGY ENCOUNTERS CELLULAR IMMUNITY

In 1961, a thirty-year-old PhD student in London, Jacques Miller, discovered the function of a human organ that most scientists had long forgotten. The thymus—named because it vaguely resembles the lobe-shaped leaves of the thyme plant—was, as Galen described it, "a bulky and soft gland" that sat above the heart. Even Galen noted that it slowly involuted as humans grew older. And when the organ was removed from adult animals, nothing significant happened. A dwindling, dispensable, involuting organ; how could it possibly be essential for human

lives? Scientists began to think of the thymus as a vestigial detritus left behind by evolution—an appendix or a tailbone hanging, incidentally, above the heart.

But might it have a function during fetal development? Using minute forceps and the thinnest silk sutures, Miller removed the thymus from neonatal mice about sixteen h after birth. The effect was unexpected and dramatic. The lymphocytes in the blood—the white cells in the blood that were not macrophages or monocytes—dropped dramatically, and the animals became increasingly susceptible to common infections. B cells dropped in number, but some other white cell—some previously unknown type—was even more dramatically diminished. Many of the mice died of the mouse hepatitis virus; many had bacterial pathogens colonize their spleens. By the mid-1960s, Miller had realized that the thymus was the site of maturation for a different kind of immune cell—not a B cell, but a T cell, from the word "T-hymus" (Max Cooper, working independently, had also established that two kinds of lymphocytes existed, and that the thymus was the maturation site for T cells). But if B cells generate antibodies to kill microbes, what do T cells do (Miller, 2020)?

In the 1970s, Rolf Zinkernagel and Peter Doherty, immunologists working in Australia, provided the first clue. They began with so-called killer T cells: these T cells would recognize the virus-infected cells, perforate their cell membranes, and douse them with toxins, forcing the infected cells to shrivel and die, thereby purging the virus within the cell as a result. These T cells would be eventually known as cytotoxic (i.e., "cell killing") T cells, and they carried a marker on their surface: CD8 (Zinkernagel and Doherty, 1974).

But the peculiar thing about these CD8-positive T cells, Zinkernagel and Doherty discovered, was that they had a capacity to recognize viral infections only in the context of the "self"—i.e., *only if the T cell and the infected cells came from the same strain of mouse*. It was as if the T cell was capable of computing a kind of dual logic. First: does the cell that I am surveying belong to my body? And second: is it infected with a virus or a bacterium?

Using genetic techniques, Zinkernagel and Doherty tracked the detection of the "self" to a molecule called major histocompatibility complex (MHC) Class I—a protein that comes in thousands of variants. Each of us carries a unique combination of MHC Class I genes. It is this "self" MHC that the T cell first detects. It is as if the MHC protein is a frame. Without the right frame, or context, the T cell cannot even see the picture.

The Zinkernagel-Doherty experiments had solved one half of the logic problem. But how does a CD8 cell find a self-cell with a virus embedded within it? My doctoral mentor, Alain Townsend, first at Mill Hill in London, and then at Oxford, took up this question in the 1990s. Townsend began his experiments with CD8 killer T cells and influenza virus. Some of these killer T cells elicited by flu infection, researchers had found, were detecting the presence of the influenza protein, called NP, inside a flu-infected cell (Townsend et al., 1986).

But that's where the mystery began. "That protein, NP, never makes it to the cell surface intact," Townsend told me recently. We were sitting in a London taxi cab, returning from a lecture. It was London dusk, with its mix of smog and rain and sudden shards of oblique English light, and the streets, as we sped through them—Old Bond, Bury Street—were full of houses with partially lit windows and closed doors. How could you detect a resident inside one of these houses, unless the resident happened to poke his head outside?

"NP is always inside the cell," Alain continued. He performed the most sensitive tests—assay upon assay, week upon week—to find the NP protein on the flu-infected cell's surface, where a T cell might detect it. But it wasn't there. "As far as cell surface proteins are concerned, there is nothing for a NP-detecting T cell to see. It's invisible on the cell surface—*it isn't even there*—and yet it's perfectly visible to the T cell" (A. Townsend, personal communication).

How, then, was the T cell detecting NP? The crucial discoveries came in late 1980s. The CD 8 killer T cells, Alain found, was not recognizing intact NP, poking its face outside the cell. Rather, the cells were detecting viral *peptides*—small pieces, or fragments, of the viral protein, NP. And crucially, these peptides had to be "presented" to the T cells in the right "frame"—in this case, carried, or loaded, by the Class I MHC protein—the very protein that Zinkernagel and Doherty had implicated in the killer T cell response. The Class I protein was actually a carrier, a peptide-bearer—and thus the "frame" required for the recognition by a CD8 T cell.

In the 1990s, working in parallel, Emil Unanue began to explore the immune detection of microbes that are internalized by cells—*a la* Metchnikoff. Once phagocytosed, the microbes and their debris are targeted to compartments, such as the lysosome, chock-full of degrading enzymes, that can chop the proteins into peptides. And analogous to what Townsend had found, these peptide fragments from the microbes are bound by a related class of protein carriers—called Class II MHCs—that present the peptides, as if on a special molecular platter, to the T cell (Harding and Unanue, 1990).

But it's here that the immune response diversifies and forks; it assumes a second wing of attack. A second subclass of T cells, called CD4 positive cells, senses these MHC-II carrier-mounted peptide fragments. Instead of killing the infected cell, the CD4 T cell incites B cells to start synthesizing antibodies. It secretes chemical substances, including cytokines, that amplify the macrophage's capacity to become mobile and phagocytose; it causes an upsurge of local blood flow and summons yet other immune cells to challenge the infection. In the absence of the CD4 cell, the transition between the detection of a pathogen and antibody production by B cells falls apart. For all these properties—and especially for supporting the B cell antibody response—this type of cell is called the "helper" T cell.

There's a final type of immune cell that deserves mention. In 1973, Ralph Steinman, working at the Rockefeller University in New York, looked down a microscope and found cells in lymph nodes that "assume a variety of branching forms, and constantly extend and retract many fine cell processes"—like a mobile, many-branched tree. "Dendritic cells," as Steinman named them (after the Greek work for "tree") are professionally designed to present antigens to T cells and jumpstart an immune response (Steinman and Cohn, 1973).

In a sense, the discovery of dendritic cells brings us back, full circle, to the Kampf between pathogens and the immune system and to the origins of immunology. The history of immunology forms a strange circle: it returns to rediscover its origins. The

century that followed Metchnikoff's discovery of macrophages—from the 1880s to 1980s—was dominated by antibodies, B cells, and T cells. These responders to infection are "adaptive", i.e., they arise, on command, to attack specific pathogens. But in evolutionary terms, this adaptive immunity is a relative newcomer. Amidst the buzz and excitement of B and T cells, a more ancient wing of the immune system—the so-called "innate" system—was largely forgotten and ignored. Dendritic cells and macrophages, among several other cell-types, are part of this innate immune system.

These cells possess receptors, including a family called toll-like-receptors, or TLRs, that do not recognize specific pathogens but molecular "patterns" common to pathogens in general. These patterns are chemicals carried or released by viruses and bacteria when they enter the body or infect a cell, including components of the bacterial cell wall or forms of viral RNA (these pathogen-induced, pattern-recognition receptors and the signals activated by them were described and discovered by many scientists. Among them, Bruce Beutler, Jules Hoffman, Charles Janeway, and Ruslan Medzhitov deserve special mention). Prompted by signals from these pattern recognition receptors, the cells of innate immune system release specific signals and chemicals—interferons, among them—to stir up an anti-viral and inflammatory response. They are the first responders to infections—and yet, ironically, among the last to be fully acknowledged, or understood, as essential parts of the organismal physiology of the immune response.

VIRAL IMMUNOLOGY IN MID-STRUGGLE

I am an immunologist-turned-virologist-turned-internist-turned-oncologist-turned-writer-turned-historian (which is to say: I have mastered the science of lack of expertise). But I am also a New York doctor who experienced the devastating brunt of the SARS-CoV-2 epidemic through the stories of my patients, nurses, and colleagues. I present this history—cursory, abbreviated, and familiar, perhaps, to many readers—with due humility to capture two contrasting points. First: to illuminate how richly the past century of immunological research has contributed to our understanding of the typical response to viruses and some pathogens. But second, and conversely: to highlight how poorly we understand the physiological consequences of the immune response to SARS-CoV-2. The power of science lies in its ability to dissect physiological phenomena into their component pieces. But the SARS-CoV-2 pandemic has illustrated that *reassembling* those pieces to understand immune physiology at an organismal level remains elusive, particularly for this virus.

Take, for instance, just three of the many mysteries of SARS-CoV-2 infection that we are still trying to solve.

First: what determines the strength and durability of an immune response to the virus? It's a question of seminal importance to vaccine developers, and yet, definitive answers are missing. In a paper published in *Nature*, Michel Nussenzweig and his colleagues dissected the immune response to SARS-CoV-2 infection (Robbiani et al., 2020). Nearly one-third of infected patients, they found, produced very low amounts (or "titers") of neutralizing antibodies to the virus. I asked Nussenzweig, one of the most knowledgeable immunologists in the field, about the relevance of these sluggish antibody responses. Do individuals with low-titer antibodies have fewer memory B cells to combat a future infection? Can they be re-infected—and if so, would they suffer milder disease? And could such re-infected individuals carry enough virus to infect the immunologically naive population?

Or take an even more basic question that has enormous epidemiological and public policy significance: is there a level, or threshold, of viral load that a patient must carry in order to infect others? In other words, is there a difference between the *infected* and the *infectious* (if so, more stringent isolation protocols might be deployed on those that are infectious until they clear the virus)? Nussenzweig doesn't know—and nor, of course, does the whole field.

And what about T cells? Some of the vaccines currently in late-phase trials elicit T cell responses, while the nature and strength of the T cell response for some vaccine candidates remains unknown. Does it matter? Does it influence the efficacy or durability of the vaccine? We don't know. And there's an odd finding that keeps cropping up: some people—up to forty percent in some studies—possess T cells that "cross-recognize" SARS-CoV-2-infected cells because these people have been previously infected by other, related common-cold coronaviruses that share genetic similarities. Could these people be partially protected? We don't know. More generally, why do infections by some viruses, or inoculation with some vaccines, precipitate durable, long-term responses, while the immunity to others wanes over time, causing re-infections, and requiring "boosters" for continuous immunity? We don't know. Despite decades of research on the immune response to viruses, fundamental questions about vaccine development, immune durability, and the physiology of the anti-viral response in the human organism, remain unsolved.

Second: why do some people recover from infection, while others progress to a fulminant, deadly disease? Are there host factors that predict severe disease? An intriguing Dutch study implicated one gene: TLR7. This X-linked gene was mutated in two pairs of brothers who suffered an atypically severe form of COVID-19 for their age (one pair was found to have a deletion of the gene, while the other pair had a single amino acid change) (van der Made et al., 2020).

TLR7 is one of the receptors involved in the innate immune response to viruses. When cells from the peripheral blood of these brothers were challenged with chemical signals that activate TLR7, the production of interferons (particularly a subtype termed type I), and interferon-related genes, was blunted, especially in the pair of brothers with the deletion in TLR7.

A separate study from a team in Paris converged on similar results (Hadjadj et al., 2020). The team profiled fifty virus-infected patients and eighteen controls. And again, in patients with most severe forms of the disease, the expression of type I interferon was blunted, while the blood levels of other inflammatory cytokines, such as interleukin 6 and tumor necrosis factor α, were increased. Akiko Iwasaki's group at Yale also profiled a large cohort of patients with moderate or severe infection and compared them to healthy controls (Lucas et al., 2020). The sustained activation of certain patterns of chemokines and cytokines was correlated with severe illness—a phenomenon that Iwasaki has termed "immunological misfiring." A more recent

paper, published in *Cell*, also implicated dysfunctions in innate immune cells, particularly myeloid cells such as neutrophils and monocytes, in patients with severe COVID infection (Schulte-Schrepping et al., 2020).

To read these papers is to glimpse a code, or a pattern, behind them—but to be unable to find the code-breaking algorithm. The Rosetta stone is missing. One possibility is that type 1 interferons produced by lung cells (possibly by lung-resident immune cells, including dendritic cells) are necessary for initial resistance. A blunted response fails to control the virus and predicts worse disease. Once the infection progresses, though, innate cells such as monocytes produce the dysfunctional cytokine storm—the immunological misfiring that Iwasaki describes. I asked Iwasaki and Medzhitov to reconcile these various studies. "There appears to be a fork in the road to immunity to COVID-19 that determines disease outcome," Iwasaki told me. "If you mount a robust innate immune response during the early phase of infection, you control the virus and have a mild disease. If you don't, you have uncontrolled virus replication in the lung that result[s] in misfiring of the immune response that fuels the fire of inflammation leading to severe disease" (A. Iwasaki, personal communication). But overall, the data suggest that innate cells, interferons, and a dysregulation of the intricate networks of signals that connect immune cells are somehow involved. Again, though, these studies illustrate the fact that our understanding of the organismal physiology of this viral infection lacks the detail and resolution that are required to understand SARS-CoV-2 infection at a granular, mechanistic level.

Finally: what about the diffuse, systemic manifestations of SARS-CoV-2 infection? There are systemic physiological effects of CoV-2 infection that remain mysterious. Some infected children experience an autoimmune illness similar to Kawasaki's disease (Jones et al., 2020). Why? We don't know. Microstructural changes have been found in the brains of some affected patients (Filatov et al., 2020); there are cardiac, vascular, and autoimmune sequelae of the infection that we don't understand. Many infected adults have blood clotting disorders that require the use of anti-clotting medicines (Al-Samkari et al., 2020).

The pandemic has energized us, yes, but it has also provided a necessary dose of humility. It has also been a call to action. It is time, as Mary Lasker would have it, to return to research, to reflection, to revision ("[i]f you think research is expensive, try disease"). We have learned so much. We have so much left to learn.

DECLARATION OF INTERESTS

This article was commissioned and paid for by the Lasker Committee. The paragraph on the practice of the Chinese and Sudanese practice of variolation is adapted from the *New Yorker* (Mukherjee, 2020) and will appear in a forthcoming book by S.M. S.M. is a co-founder of Vor, Myeloid, Immuneel, Faeth, and Cura Therapeutics and serves on the Boards of Frequency, Trialspark, Equillium, Cellenkos, and Puretech.

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