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Voices

From mRNA sensing to vaccines

The 2005 *Immunity* paper by Karikó et al. has been hailed as a cornerstone insight that directly led to the design and delivery of the mRNA vaccines against COVID-19. We asked experts in pathogen sensing, vaccine development, and public health to provide their perspective on the study and its implications.

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The cradle of basic research
The authorization and widespread distribution of two safe and highly effective mRNA-based vaccines against COVID-19 marked a critical turning point in the pandemic. Developed at unprecedented speed, these vaccines are the fruits of decades of basic research. Two pivotal researchers in this story are Katalin Karikó and Drew Weissman, who hailed from different disciplines, yet shared a determination to translate basic research into impactful biomedical interventions. A serendipitous meeting at a University of Pennsylvania photocopier led to an historic collaboration. As is often the case with basic research, their road to discovery was arduous, and their work was not widely appreciated as it was unfolding. Their hallmark 2005 *Immunity* paper dissects how RNA containing different levels of naturally occurring modified nucleosides differentially activates dendritic cells (DCs). They elegantly showed that adding certain modified nucleosides to mRNA molecules blocked DC stimulation, suggesting that modified mRNA could evade innate immune detection. This seminal finding was key to enhancing the stability of synthesized mRNAs, allowing their use as a paradigm-shifting vaccine platform. Synthesized mRNAs have potential for many biomedical applications, including mRNA vaccines for HIV, influenza, malaria, and cancer, among others. Although the success of the mRNA COVID-19 vaccines based on the meticulous work and unflinching determination of Karikó and Weissman is now internationally celebrated, we should never forget where, when, and how it all began—in the cradle of basic research.

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Unleashing DC therapeutic power
In a landmark 2005 *Immunity* paper, Karikó and colleagues described a finding that transformed the course of the deadliest viral pandemic of the 21st century. Despite several decades of effort, mRNA therapeutics were lagging behind other nucleic-acid-based therapies due to their prohibitive immunogenicity and lability. Karikó and colleagues found that synthetic mRNA activates human TLR3/7/8 and drives the release of inflammatory molecules from human DCs. Strikingly, the modification of a single nucleotide (modmRNA) was able to shut down DC activation, a result that turned out to be crucial for the clinical development of mRNA therapeutics. Subsequent studies by Karikó and others showing that modmRNA have increased translatability *in vivo* and that lipoparticle packaging enhances uptake by DCs and macrophages substantially furthered modmRNA clinical applications. The extraordinary success of lipoparticle-modmRNA-based COVID-19 vaccines is likely due to their enhanced ability to promote DC- and macrophage-mediated activation of potent viral-specific immunity, further highlighting the central role of these cells in vaccine efficacy. This technology is now being used to enhance suboptimal microbial vaccines and promote tumor-specific immunity. Even more exciting is the prospect of using lipoparticle-modmRNA to reprogram DCs and macrophages to present constitutively acquired lesional antigens into mRNA-instructed immunoregulatory or immunogenic contexts, further unleashing the therapeutic potential of DCs and macrophages across cancer and autoimmune and autoinflammatory diseases.
**Medicines for all**

Most have now heard of mRNA vaccines, but until a couple of years ago, only immunologists and scientists interested in drug and vaccine development were paying attention to the incremental advances in RNA therapeutics. Kariko and Weissman’s work in this area has been significant, and they are now getting well-deserved recognition for their contributions. The incredible speed at which mRNA vaccines were developed and deployed, their efficacy and safety, and the adaptability of this platform have made it very attractive. mRNA is now the technology of choice for vaccines against other viral infections of epidemic or pandemic potential, and it has potential for cancer treatment. There are early data on vaccines combining multiple viral antigens (e.g., influenza, SARS-CoV-2, and RSV), and development of a universal flu and universal Sarbecovirus vaccine has received an impetus. Companies are exploring tuberculosis and malaria vaccines—the challenges being identifying the immunogenic sequence(s) as well as not having good correlates of protection for these diseases. WHO has an important role to play in coordinating, facilitating, and guiding the development and deployment of these health products. A new model of R&D with public (government), private, philanthropic, and civil society participation could help accelerate the development of novel mRNA therapeutics and vaccines and also ensure fair pricing and equitable access, which will be needed to have global public health impact. Technology transfer and strengthening manufacturing capacity in underserved regions of the world can help achieve access to medicines for all populations.

**Elusive mechanisms**

How innate immune sensors distinguish non-self RNA from self RNA has been a central question in immunology over the last two decades. The findings of Kariko et al. represent a groundbreaking discovery that formed the foundation for mRNA therapeutic development and provided key clues for understanding self versus non-self discrimination by RNA-sensing Toll-like receptors (TLRs). This finding inspired numerous studies from our group and others examining the impact of RNA modifications on other RNA sensors, such as RIG-I-like receptors (RLRs), PKR, and OAS. However, in all cases, the specific mechanisms by which these receptors tolerate modified nucleotides remain unclear. Structures of many of these receptors in complex with cognate RNA suggest that nucleoside modification does not simply block RNA binding. Most of these receptors detect double-stranded RNA (dsRNA) structure by contacting the backbone with minimal interaction with bases, where RNA modification mainly occurs. Even for single-stranded RNA sensors, such as TLR7, its structure revealed that modifications that can potently suppress TLRs would not be directly sensed by TLR7. We found that many of these RNA modifications do not affect RLR’s ability to recognize dsRNA, but they suppress highly immunologic RNA byproduct formation during in vitro transcription, a common strategy to prepare RNA. Although this effect is unlikely to account for all the observed effects of RNA modification, it should be considered in future studies. Ultimately, the immune-suppressive effect of RNA modification, either direct or indirect, requires more detailed mechanistic studies.
mRNA therapeutics: COVID-19 and beyond
The path to mRNA vaccines took decades, beginning with Katalin Karikó’s seminal work in the 1990s that was aimed at the major pitfall: avoiding the massive inflammation induced by mRNAs injected into mice. Together with Drew Weissman and colleagues at UPenn, in Immunity in 2005, she determined that this inflammatory response was due to Toll-like receptors and could largely be preempted by modifying an mRNA nucleotide from uridine to pseudouridine. This finding, along with lipid nanoparticles, served as the backbone for the successful mRNA COVID-19 vaccines that achieved 95% efficacy against symptomatic infections in the pivotal Moderna and Pfizer-BioNTech large-scale trials, and over a billion doses have been administered worldwide. The FDA approved an mRNA vaccine for Ebola in 2019, and multiple other pathogens are now being targeted in clinical trials, including HIV, cytomegalovirus, rabies, RSV, influenza A, and chikungunya. In addition, an mRNA vaccine against the malaria parasite Plasmodium has been tested in non-human primates. mRNA therapeutics are expanding well beyond infectious diseases, including revving up the immune response to various types of cancer and neurodegenerative diseases. Furthermore, there is potential for downregulating the immune response to autoimmune conditions and fostering growth of new blood vessels for cardiovascular indications. What begin as a replacement for a uridine base to squash an inflammatory response in mice evolved into the basis for a broad therapeutic platform to fight both communicable and non-communicable diseases in people.

RNA sensing: More to learn
In their seminal 2005 study, Karikó, Weissman, and colleagues found that most cellular RNAs, including mRNAs, undergo a plethora of modifications that limit their recognition by RNA sensors. Building on these findings they later found that while many RNA modifications interfere with the translation of mRNA to protein, one particular modification dramatically enhances translation, generating much higher levels of protein. At the time, these findings helped explain how innate sensors distinguish foreign RNAs from self RNAs. However, what was less appreciated was the potential application of these findings to therapeutic use of synthetic RNAs. These remarkable insights paved the way for the use of informational RNA-based therapeutics, such as the Pfizer-BioNTech and Moderna mRNA vaccines now used globally to mitigate the COVID-19 pandemic. Although groundbreaking in their own right, the potential applications of these therapeutics go far beyond vaccinology to areas such as gene therapy, cancer therapeutics, and the use of RNAs to potentially limit inflammation. We still have more to learn about how RNA is sensed. Aberrant sensing of self RNA is linked to a growing number of inflammatory diseases, underscoring how modification-imposed silencing of self RNA is not always a failsafe. Self RNA instigates inflammation through TLRs and RIG-I-like receptors (RLRs) in Lupus, Aicardi-Goutières Syndrome, and additional conditions. An understanding of the mechanisms that go awry will be fertile ground for future research in immunology and RNA biology.
mRNA vaccines have proven a gamechanger against COVID-19. Key to their activity is the Goldilocks principle. Too little stimulation of the innate immune system by RNA and there is no adaptive immune response, akin to giving antigen without adjuvant. Too much and you get unacceptable reactogenicity, as well as interferon-driven shutdown of translation, whichcurtails expression of the protein encoded by the mRNA. By 2005, we had a reasonable understanding of how RNA stimulates innate immunity. Recognition of RNA by Toll-like receptors (TLRs) 3, 7, and 8 had been established and that by RIG-I-like receptors was beginning to be understood. Much effort went into understanding the features of RNA that trigger those receptors. Against this background, Kariko et al. looked for features that might reduce stimulatory activity. They found that modifications commonly present in mammalian RNA, including the presence of pseudouridine, 2-thiouridine, or methylated nucleosides, decreased the ability of RNA to stimulate TLR reporter cells and activate human myeloid cells. Although the underlying mechanism was not elucidated at the time, it suggested a practical means to bring the stimulatory capacity of mRNA into the Goldilocks range. When applied to mRNA vaccination, pseudouridine incorporation permits better expression of the target protein and, together with lipid formulation, leads to robust immune responses and acceptable adverse effects. Not too hot, not too cold mRNA is poised to revolutionize prevention of infectious disease.

Fast. Reliable. Universal
To develop a coronavirus vaccine for a potential pandemic, we needed to explore technologies that could be developed quickly, with reliable manufacturability, using an approach that was universally applicable across the coronaviridae family. Fast. Reliable. Universal. Since 2015, each time I presented our ongoing coronavirus vaccine development efforts, I placed these three tenants on an introductory slide. mRNA vaccine platforms checked all of these boxes, largely due to work by Kariko et al., which identified pivotal modifications to allow RNA to hide from host sensing. In 2017, we discovered a method to stabilize coronavirus spike proteins into the prefusion conformation, and their adjuvanted delivery elicited robust neutralizing antibody responses in pre-clinical models. The stabilizing mutations were broadly translatable to multiple coronaviruses... including SARS-CoV-2. But, with the onset of the COVID-19 pandemic, the race to a safe and effective vaccine was challenged by the speed of global viral spread. Manufacturing GMP-grade spike proteins likely would not be fast enough. So, we turned to modified RNA to deliver the SARS-CoV-2 spike antigen, turning the human body into spike protein bioreactors. mRNA-1273 entered a phase 1 clinical trial just 66 days after viral sequence release. Today, mRNA vaccines incorporating the stabilized SARS-CoV-2 spike protein are approved for use globally, making up a substantial proportion of the global vaccine portfolio, and setting the stage for vaccine development to be faster, more reliable, and even more universal in the future.
It’s about how you see self

The innate immune system senses pathogens and initiates the antigen-specific adaptive immune response. In the early 2000s, it was known that TLRs sense RNA species, but RNA is not unique to pathogens, and it was not clear how the innate immune system discriminated between foreign and self RNA. In 2005, Katalin Karikó and colleagues added a new milestone in RNA research by discovering an immunomodulatory function of RNA modifications such as pseudouridine. This and other base modifications ablated the RNA-driven activation of TLR3, TLR7, and/or TLR8. This important observation explains how the innate immune system senses foreign RNA and discriminates between prokaryotic and eukaryotic RNA. This finding fueled the investigation of RNA modifications such as 2'-O-methylated nucleotides that are now seen as an effective viral evasion strategy for immune recognition. A mechanistical understanding of how RNA modifications inhibited immune activation and to what degree single modifications differed in their immunomodulatory effect remained unclear at the time. However, despite these gaps, this finding proved fundamental for the development of pseudouridine-containing mRNA as a new class of human vaccines by rendering the RNA less immunostimulatory (with fewer side effects) and more stable (for higher protein production) at the same time. Future research should evaluate other known and synthetic RNA modifications and new delivery systems for the development of mRNA vaccines in personalized cancer treatment, as mRNAs would have to be administered multiple times and at high doses.