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What the Expert Says

Nobel Prize in physiology or medicine 2023: Development of mRNA therapeutics that paved the path of formulation of COVID-19 vaccine

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ABSTRACT

Since the concept of the vaccine was developed by Edward Jenner in 1796, vaccinology has traveled a long way with gradual improvement toward developing better methods of formulation of vaccines. In this premises in the 1990s, Karikó and Weissman joined their hands and minds aiming at understanding how different RNA species interact with the immune system. During their long journey in this direction, they performed extensive studies involving well-conceived molecular biology-based experiments that resulted in a breakthrough discovery relating to RNA therapeutics in general and messenger RNA (mRNA) therapeutics in particular. In this endeavor, they developed mRNA technology that actually paved the path leading to the development of mRNA vaccine that has many advantages. In December 2019, when the deadly virus SARS-CoV-2 emerged, which, in no time, caused a pandemic as well as an epidemic, thereby throwing a fierce challenge to the total healthcare systems worldwide, it was possible to take care of that challenge through the formulation and manufacturing on a large scale the mRNA vaccine against SARS-CoV-2 using the above technology. Thus, mRNA technology has created a strong platform that has spelled out a great promise toward controlling any pathogen infection and saving human life through the development of mRNA vaccine at a quick pace. In this short review, an attempt will be made to highlight the contributions of Karikó and Weissman and how they led the formulation of a vaccine against COVID-19, which fetched them the Nobel Prize in Medicine or Physiology, 2023.

Keywords: Molecular biology, Messenger RNA vaccines, COVID-19, Nobel prize 2023, Physiology or medicine

INTRODUCTION

The principal function of a vaccine is to train our body to develop a first line of defense, specifically called immunity, against any specific pathogen beforehand, which can prevent our body from infection by the same pathogen in the future. In a different way when our body is infected by any pathogen through natural exposure, then also our body learns to fight the same infecting pathogens in the future. The immunity thus developed in the latter case is called innate immunity, while that in the former is adaptive immunity. However, in both cases, the induction of immune response is initiated by the specific antigen component(s) that is (are) present on the surface of the pathogen in question. Since the discovery and coining of the word vaccine by Jenner in 1796,[1] the science of vaccinology has proceeded a long way with gradual improvement in relation to both formulation and application of vaccines aiming at providing the antigen to our body.[2] In the initial phases, the traditional procedure of vaccination involved the use of a pathogen in its killed or attenuated (weakened) form. When the antigenic component on the

surface of the pathogen responsible for effecting infection of the host cells through interaction with the specific receptors on the surface of the latter was identified, then it became possible to bypass the use of whole pathogenic cells and use the antigenic molecule(s) isolated from the pathogen. The progress in this direction proceeded a step further when the putative antigen-coding gene carried in DNA or RNA genome was identified, then that gene could be cloned in a suitable vector and used that to express the antigen protein in specific cells in the laboratory. When the messenger RNA (mRNA) carrying information about producing protein was discovered around 1961,[3] the attention was shifted to mRNA for its possible use as a vaccine. In this path, Karikó and Weissman started their conceptual journey about three decades ago and made various basic studies at the molecular level that paved the path leading to mRNA therapeutics in general and mRNA vaccine development in particular that actually attracted vaccinologists to plan for formulating mRNA vaccine against certain diseases with a particular interest against SARS-CoV-19 that helped control COVID-19 pandemic and epidemic in a short time. This, indeed has spelled out the promise of providing an extended healthcare benefit to humankind worldwide. In this article, an attempt will be made to highlight the contributions of Karikó and Weissman in this area, which fetched them the Nobel Prize in Medicine or Physiology in 2023.[4]

RNAs AND MRNAs

There are several types of RNA in biological materials, each having a distinct biochemical function. These RNAs include (a) mRNA, rRNAs, and tRNAs, which are involved in the processes of protein biosynthesis, and (b) self-replicating mRNA, circular mRNA, 5SRNA, siRNA, miRNA, lncRNA, antisense RNA, RNAi, etc. Each of the latter RNAs is involved in the regulation of transcription and translation in a specific manner. Having such regulatory properties, most of them have been used to develop therapeutic practices.[5,6] Excepting mRNAs that constitute about 2% of total RNAs produced by the human genome, all other RNAs are non-coding in nature. Since the discovery of mRNA,[2] molecular biologists have been studying this important biochemical tool to know how it performs the precise function of carrying information related to gene function. It has been established that the nascently transcribed eukaryotic mRNA molecules, as such are non-functional. They have to pass through certain post-transcriptional modification steps to acquire the functional form.[7] These steps are as follows: (i) splicing that removes the non-coding short intervening sequences called intron, thereby connecting two consecutive exons, (ii) addition of CAP structure at the 5' end of the stretch of untranslatable sequence (non-coding) that is followed by the open reading frame (orf), and (iii) addition of a stretch of poly A structure at the 3'-end. All these modifications of the nascent mRNAs are accomplished by specific enzymes acting through specific steps involved in all the involved processes. The mRNAs formed in the cells, in general, have relatively short half-lives that vary for different mRNAs under different conditions.[8,9] Such decay of mRNAs occurs by the action of various specific degradative enzymes present in the cytoplasm as well as in the nucleus and are highly regulated at both post-transcriptional and transcriptional levels effected through specific interactions between the cis-acting element(s) in mRNA and the proteins that bind those elements. Thus, the process of mRNA decay imposes precise control over the whole process of gene expression. In fact, the birth of RNA-therapeutics has taken place, capitalizing on those properties of RNA.

IMMUNE SYSTEM AND IMMUNITY

The primary components of the immune -system in our body are three types of specialized cells such as dendritic cells, B-cells (B-lymphocytes) and T-cells (T-lymphocytes). Of these three types of cells, the DCs capture the pathogen and pick up the antigen component and process it and then present to the B-cells which respond to produce antibodies that actually inactivate the antigen of the infecting pathogen by physical interaction thereby foiling the infection by the latter. On the other hand, the T-cells recognize specifically the cells already infected and kill them thereby preventing the infection from taking over and spreading further. During vaccinization, when an antigen enters the body, all those cells are activated to initiate their action in the above manners. Both vaccination and natural infection could also induce the production of "memory" B- and T-cells, which help our immune system prevent the illness from taking a serious form in the future.[2] As mentioned earlier, the process of vaccine formulation progressed with gradual improvement over the years and reached a time when the antigen protein(s) could be purified through various approaches and used as a vaccine.[10-12] However, the main disadvantage of those procedures was that they required large-scale cell culture and elaborate protein purification processes, which were not at all encouraging for rapid and low-cost vaccine production, especially in emergencies caused by outbreaks and pandemics. In this background, the researchers made continuous attempts to develop vaccine technologies independent of cell culture.

mRNA VACCINES: A PROMISING ALTERNATIVE

In the line of vaccine development, the use of mRNA technology soon became a matter of much interest. During the early 1990s, the Hungarian biochemist Katalin Karikó working at the University of Pennsylvania, conceived the

possible use of mRNA as a therapeutic agent. At the same time, at the same University, the immunologist Drew Weissman was interested in understanding the specific role(s) of DCs in the processes of immune surveillance and activation of vaccine-induced immune responses. Both of them started collaboration aiming at understanding how different RNA types interact with the immune system. They observed that the *in-vitro*-made mRNA could activate the DCs to release the inflammatory signaling molecules. Therapeutic processes based on mRNA might have advantages, as well as certain lacunae.[13] The advantages are as follows: (a) mRNA, as such, is unable to integrate into the host genome; hence, they are unable to generate insertional mutations. (b) mRNA could be made in quantity through *in vitro* transcription, if possible; (c) mRNA, when inside the target cell, would be able to act directly as a template for protein synthesis using host cell machinery and, thus, would provide the antigen protein with more or less continuous supply so long as it would be there thereby bypassing transcription process and saving cellular energy, and (d) mRNA is translated transiently in cells and is degraded in a relatively short time. On the other hand, the lacunae are as follows: (a) mRNAs in naked form are very sensitive to degradation by nucleases, (b) they are comparatively large and negatively charged molecules; hence, those could not be injected as such into the target cells through any of the traditional routes, (c) they fail to pass through the anionic lipid bilayer of the target cell membrane, and (d) these macromolecules possess innate immunogenicity. As the *in vitro* and *in vivo* made mRNAs induce different responses in the DCs, Karikó, and Weissman immediately conceived that this difference in the response might be originated by the presence and absence of modified bases in *in vivo* and *in vitro* transcribed mRNAs, respectively. To resolve this issue, they performed a series of experiments with *in vitro* transcribed different mRNAs, each with unique modified bases, and delivered those separately to DCs.[14] They observed that (i) the inflammatory reaction was almost abolished with the laboratory made mRNAs with incorporated modified bases, (ii) incorporation of certain bases could abolish recognition/activation specifically by some Toll-like receptors (TLRs), (iii) the base modifications enhanced RNA stability, base-pairing specificity, folding and other functional properties, and deamination of uridine in the RNA could play certain important role in DC activation, (iv) the translation efficiency as well as the stability of *in vitro* transcribed base modified mRNA could be further increased with the introduction of 5′ cap, optimized 5′- and 3′-UTRs, coding sequence and poly(A)-tail modifications into the RNA molecule,[15-18] and (v) recognition of mRNA by 2'5' oligoadenylate synthetase (OAS) and its degradation by the OAS-induced Rnase L enzyme were decreased with *in vitro* made mRNA containing modified bases[19] and that the expression of protein could be increased using purified *in vitro* transcribed mRNA.[20] Thus, Karikó and Weissman could foresee success in designing/formulating the process of therapeutic applications of mRNA bypassing the laborious process of antigen purification. Taking advantage of the above breakthrough discovery of Karikó and Weissman, Moderna developed an mRNA-based vaccine against the Middle East respiratory syndrome coronavirus using a prefusion-stabilized form of the virus spike protein made from base modified mRNA[21], and Feldman *et al*. developed the mRNA vaccines against H10N8 and H7N9 influenza viruses that could induce a good level of humoral immune responses.[22] Even then, it was felt that the success of using mRNA as a vaccine needed to develop a suitable delivery system that could push these molecules directly into the immune specific cells without causing any untoward side effects within the host.

DESIGNING AND DEVELOPING MRNA DELIVERY VEHICLES

Malone *et al*., in 1989, showed that the cationic liposome-entrapped RNA could be successfully transfected into NIH 3T3 mouse cells and that this procedure could be used to transfect RNA also into human, rat, mouse, Xenopus, and Drosophila cells.[23] Some other methods aiming at the delivery of RNA/mRNA into target cells were developed using positively charged lipids, cationic polypeptides, polymers, micelles, or dendrimers.[24,25] Based on the positive outputs from the above studies, especially those using lipid nanoparticles (LNPs)-entrapped RNA, Weissman and Karikó group formulated LNP-based vehicle and used that to successfully deliver *in vitro* made nucleoside modified mRNA to the target cells. They observed that injection of lipid nanoparticle-encapsulated mRNA complexes could increase the production of large amounts of the mRNA-coded protein in mice for varying lengths of time. This established the use of LNP tools for efficient delivery of mRNA.[26] Sometimes later, an mRNA vaccine for Zika virus was formulated.[27,28] Moderna formulated mRNA vaccine against influenza virus H10N8 and H7N9 and determined its safety and immunogenicity.[29]

mRNA DELIVERY TO DCs AND THE ROLE OF INNATE SENSING

The DCs are the first target of the immune response. Hence, the *in vitro* synthesized mRNA needed to be delivered to the DCs. In this background, Karikó and Weissman showed that the DCs given a short exposure to *in vitro* transcribed mRNA encoding the HIV-1 structural protein, Gag, could stimulate primary CD4+ and CD8+ T-cell responses[30] as well as induce DC activation and maturation.[31] DCs express both surface and endosomal TLRs, which recognize distinct molecular

structures referred to as pathogen-associated molecular patterns (PAMPs),[32] and TLR binding to PAMPs results in intracellular signaling and production of anti-viral cytokines, including type 1 interferons that detect incoming pathogens. The TLRs can distinguish different forms of nucleic acid.[33] Karikó and Weissman showed that transfection of DCs with *in vitro* transcribed mRNA stimulated a cytokine response while increasing the poly (A) length of the mRNA could reduce the production of interleukin-12 and that only polycytidylic acid could induce DC activation, leading to a better response.[34] This suggests that the nucleotide content of mRNA also determines the quality of DC activation.

mRNA VACCINES AGAINST COVID-19

Soon after the emergence of SARS-CoV-2 in China in December 2019 and spreading worldwide, creating a pandemic as well as an epidemic,[35] the development of a vaccine to control the disease better was felt very urgent. In this venture, the spike protein S on the surface of the virus particle that is involved in effecting infection of the host cell was made the target for vaccine formulation. The high-resolution structure of the spike and the protein S having 1273 amino acids was determined by Wrapp *et al*. in early 2020[36] which provided various information relating to its 3D conformation and the boundaries of various functional domains such as N-terminal domain, receptor-binding domain (RBD), transmembrane domain, and the cleavage sites for furin protease and S1/S2 junction which were capitalized for the formulation of COVID-19 vaccines as well as for the definition of neutralizing antibody epitopes and antibody escape mutations in the SARS-CoV-2 variants, especially the four variants of concern (VOC) like Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2) that appeared later.[13] In November 2021, another variant, Omicron (B.1.1.529), emerged and was listed as the fifth VOC by the World Health Organization (WHO). All these variants and the original virus SARS-CoV-2 together threw a fierce challenge to the global healthcare systems[13], requiring immediate action in the process of COVID-19 vaccine production. The S gene-specific mRNA as such or any required domain thereof could be synthesized in the laboratory using bacteriophage SP 6[37] or T7 RNA polymerase[38] and put toward the development of COVID-19 vaccine with the help of mRNA technology developed by Karikó and Weissman. In the path of vaccine design and formulation, three companies were separately founded earlier in the first decade of the 21st century and started working in collaboration with the then-academic experts with different objectives against different target diseases.[13] Those three companies were as follows: (i) CureVac (founded in 2000), which initiated work on infectious diseases and cancer; (ii) BioNTech (founded in 2008,) proceeded toward making personalized cancer vaccines; and (iii) Moderna (founded in 2010) planned to use the mRNA technology to reprogram somatic cells generating pluripotent cells and to deliver therapeutic proteins. All these three companies succeeded in developing vaccines as per their objectives. Among these three companies, CureVac and Moderna also made successful attempts to use mRNA technologies to formulate mRNA vaccines against some non-SARS-CoV-2 viruses, which have been mentioned earlier. In the COVID-19 epidemic situation, BioNTech, in collaboration with Pfizer, initiated work to develop five COVID-19 mRNA vaccines based on (i) non-modified mRNA (BNT162a1), (ii) nucleoside-modified mRNA (BNT162b2, BNT162b1, and BNT162b3), and (iii) self-amplifying mRNA (BNT162c2).[13] In their approaches, three different types of antigen were planned with nucleoside-modified S protein-coding mRNA: (i) transmembrane prefusion spike (BNT162b2), (ii) secreted spike RBD (BNT162b1), and (iii) transmembrane spike RBD (BNT162b3). Of these three, the first two were found to be very prospective; BNT162b2 encodes a full-length spike glycoprotein with two proline mutations in the S2 subunit. The WHO approved the BNT162b2 mRNA vaccine for emergency use listing[39], and the United States Food and Drug Administration officially authorized this for vaccination of individuals ranging in all age groups in October 2021.[40] However, BNT162b2 showed certain side effects like increased risk of myocarditis in some selected populations at the global level. The BNT162b2 vaccine was only 51.9% effective against the delta variant, while the same was 75.0% and 89.5% effective against beta and alpha variants, respectively.[41] The vaccine could neutralize also the Omicron variant, possibly in a dose dependent manner.[42] On the other hand, Moderna used mRNA-1273, which carries the coding information about the full-length prefusion spike protein of SARS-CoV-2 for vaccine development.[13] This vaccine has been shown to be well tolerated and safe to use in adolescents and adults with only mild adverse effects and showing an efficacy of 94.1% in the age group of 12–17 years. Another version of this vaccine, the mRNA-1273.351, was developed using the mutant spike protein of the SARS-CoV-2 beta variant, and a third formulation was designed combining both mRNA-1273.351 and mRNA-1273 that were named mRNNA-1273.211 with the prospect of using as a booster.[13] The neutralization effect of the booster against SARS-CoV-2 variants Beta, Gamma, and Delta reached a level comparable to that observed against the wild-type D614G strain. This multivalent mRNA-1273.211 vaccine appeared to be effective against the variants Beta, Gamma and Delta, while mRNA-1273 vaccine was effective against Alpha and Beta variants to the extent of 100% and 96.4%, respectively, with certain lower effectiveness against Delta (73.1%). This vaccine has been considered suitable in variant-dominated high-risk zones such as prisons or hospitals.[13] Thus, both BNT162b2

and mRNA-1273 vaccines proved to be ideal for preventing infection with Delta and other variants. Providence Therapeutics in Canada developed in 2022 a COVID-19 vaccine, PTX-COVID19-B, that is also an LNP-entrapped mRNA vaccine using the native S gene sequence from SARS-CoV-2 Wuhan-Hu-1 isolate replacing D614 with G614. It could elicit a strong immune response against SARS-CoV-2 in mice and hamsters without any adverse effects. Mouse immune sera elicited by PTX-COVID19-B were capable of neutralizing SARS-CoV-2 variants, including alpha, beta, gamma, and delta lineages.[13,43] A Chinese group developed a lipid nanoparticle (LNP)-encapsulated mRNA encoding the RBD of SARS-CoV-2 as a vaccine, which they named ARCoV. This vaccine has been shown to elicit a high level of neutralizing antibodies against SARS-CoV-2 as well as a Th1-biased cellular response in mice and non-human primates and was reported to be safe and well-tolerated. In addition, ARCoV is manufactured as a liquid formulation and can be stored at room temperature for at least one week.[13,44] From Imperial College, London, an LNP-nCoVsaRNA vaccine, COVAC 1, was formulated using a self-amplifying ribonucleic acid (saRNA of Venezuelan equine encephalitis virus) encapsulated within LNPs. In this formulation, the spike glycoprotein S of SARS-CoV-2 was stabilized in the prefusion conformation with two proline substitutions. This vaccine showed good efficacy and immunogenicity against SARS-CoV-2.[13,45] Arcturus Therapeutics based in Singapore, developed a saRNA vaccine ARCT-021, which could produce anti-spike specific antibodies.[13] This vaccine has dosage advantages compared to most approved non-replicating mRNA vaccines mentioned earlier. Furthermore, ARCT-021 could be stored in a lyophilized form that preserves its therapeutic efficacy at room temperature. Hence, ARCT-021 is advantageous over other mRNA vaccines, which are distributed as frozen liquids. Two other vaccines, one CVnCoV formulated by CureVac using COVID-19 mRNA in an LNPs-capsule that encodes full-length, pre-fusion stabilized SARS-CoV-2 spike protein, and the other MRT5500 formulated by Sanofi Pasteur in collaboration with Translate Bio using the double mutant form (2P/GSAS)of the virus S gene.[13,46] Both vaccines could induce an immune response against SARS-CoV-2 in test animals. However, the efficacy of the former was very low at 48.2%, which did not meet the required criteria laid down for successful use in vaccination, and the production volume of the latter was unable to meet the global demand at that time of emergency. Hence, both companies abandoned the vaccines formulated by them.[13]

CONCLUSION

The versatile mRNA technology developed by Karikó and Weissman has built a strong and wide platform using which any kind of pathogen infection could be successfully dealt with through the formulation of mRNA vaccine that has been exemplified by the quick control of COVID-19 by the use of mRNA vaccine formulated using the above technology. The SARS-CoV-2 virus changed its deadly character very quickly, which pushed the human population toward yet another kind of healthcare threat. The antibody generated in response to SARS-CoV-2 could also act, though in an increased booster dose-dependent manner, on most of the VOCs strains such as alpha, beta, gamma, and Delta and to some extent on omicron that emerged with time through the gain of function mutations in the various genes including the spike protein encoding S gene in the parent SARS-CoV-2 RNA genome. On the other hand, the very recently arrived strain JN 1, a sub-variant of the omicron lineage, has got one extra spike mutation L455S over those in its parent genome and has acquired significantly enhanced immune evasion capability and reduced angiotensin-converting enzyme two binding property.[47] Hence, this virus, as well as any new arrival(s), will continue to remain associated with the human population, always changing its deadly character to evade every attempt to be made to control them. In this situation, the mRNA technology has been proved to be a very convenient weapon that can be used for dissociating the above type of pathogen-human fighting association.

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