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Development of the mRNA vaccines to prevent COVID-19

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Introduction

When Pharmacokinetics and Pharmacodynamics were discussed in class recently, I was reminded of a subject that I had been curious about in the past. I used to teach at a very small school in San Juan Capistrano and I had a tradition of taking my AP Biology students on a field trip after they took their exam. This would allow them to see some real-world applications of what they had just spent a year studying. One particular year, a parent offered a tour of his company to my students and me. His company was a pharmacy, but they only sold other pharmacies, so we would be taking a tour through a manufacturing and shipping facility. I do not remember the exact year, but it was after the Patient Protection and the Affordable Care Act had been signed into law, but not yet enacted. I remember this because one of the things he discussed was the uncertainty the new law would mean to his business. He knew it would be good, but he was unsure whether or not it would be sustainable. It was on this field trip that my students saw and used a micropipette for the first time. We also toured a clean room and the warehouse, but before all that, we received presentation on the business which included a discussion of pharmacokinetics. I decided then, that pharmacokinetics would be my topic for the final project.

Pharmacokinetics and pharmacodynamics are two interrelated disciplines that together determine the effects that drugs can have on a human body.[1] Pharmacokinetics is "sometimes described as what the body does to a drug" while pharmacodynamics can be "described as what a drug does to the body".[2] In the very beginning of my research, I read about the various methods used to administer medication, pills and intravenous routes are examples. As I continued my research in an effort to narrow my topic, lipid nanoparticles came up which is a key ingredient of the subject in my article presentation, the COVID-19 vaccines. It was at this point that I decided to continue learning about the first two vaccines utilizing messenger RNA enclosed in lipid nanoparticles.

There are many reasons for the success of the COVID-19 vaccines, but I will focus on only two in this writing. The first is the use of the lipid nanoparticle. Dr. Robert Langer, a chemical engineer and professor at MIT, is credited with discovering the potential of liposomes as drug delivery systems as early as 1974 and finally published a paper on it in 1976 by Langer and Folkman. His research was focused mainly on developing methods to target and deliver drugs once inside the body.[3] He is one of the cofounders of Moderna.[4]

Around the same time Dr. Pieter Cullis, a researcher and entrepreneur at the University of British Columbia, was also focused on lipid molecules but from a different point of view. His research focused more on the individual phospholipid molecules and their chemistry.[5] He researched the various structures they would form, the conditions in which they would form and the various substances they were capable of carrying.[6] Not only did he understand their chemical behavior, but he eventually developed a method to produce vesicles of variable sizes in a matter of minutes[7] using a technology that is now referred to as microfluidic rapid mixing.[8]

A liposome is a three-dimensional structure formed by phospholipids. Very different than micelles, which are formed by a single layer of phospholipids, they were discovered when researchers learned that when the phospholipids that make them up, were placed in an aqueous environment, they would form.[9] It was later discovered that they could also be used to carry medicines by Langer. It is now known that liposomes can carry both hydrophobic and hydrophilic medications.[10] Hydrophobic medications are carried within the bilayer created by the hydrophobic tails while the hydrophilic medicines are carried in the aqueous environment created in the center when the liposome forms. Liposomes can also be made to target certain cells by adding ligands onto the phospholipids which can then dock on cells that have receptors able to recognize them. Polyethylene glycol can also be added onto the lysosomes to protect them as they enter the body.

Although both groups of researchers lead by Drs. Cullis and Langer developed a great deal of knowledge of liposomes, later known as lipid nanoparticles, and their uses as vehicles to carry medicines into the body, it was Dr. Cullis' team that improved on their performance. There was a problem with nucleic acids being able to remain or become encapsulated by the lipid nanoparticles. Dr. Cullis' developed phospholipids with a positive charge. As a result, they were attracted to the overall negative charge on a nucleic acid molecule, but the positively charged phospholipids were toxic to the body.[6] He then went on to develop ionizable phospholipids that could be made to change depending on the pH of the solution they were made in. In low pH, the phospholipids had a positive charge which allowed them to associate easily with negatively charged nucleic acids.[6] When the pH of the solution was changed to become more neutral, the phospholipids would become neutral as well by accepting protons.[6] This allowed two things. The first is a concept that is now called remote loading which involves lipid nanoparticles' acceptance of their nucleic acid cargo after their extrusion.[5] The second is that this allowed the lipid

nanoparticles to be able to travel through the blood stream where they could encounter their target cells by Leung. Once inside the cell, the pH conditions lead to deprotonation, which also leads to the escape of the nucleic acid.If mRNA, it was now accessible to ribosomes and other organelles that lead to protein production.[3] Liposomes were first discovered in the 1960's and have been researched heavily since that time. Now known as lipid nanoparticles, they have been developed into regularly used drug delivery devices.[11]

While Drs. Cullis and Langer were continuing their research, Drs. Kariko and Weissman were working on mRNA as a vaccine candidate. Dr. Katalin Kariko was born in Hungary and immigrated to the States when the Soviet Union fell apart.[12] She eventually was offered an assistant professorship at University of Pennsylvania. She had a very difficult time securing funding for her research, but she refused to give up, even accepting a demotion in order to remain at UPenn. There, she met Dr. Drew Weissman, an immunologist with prior experience working with Dr. Anthony Fauci, who was researching the development of a vaccine for HIV. Although she had experienced quite a bit of failure up until their meeting, she thought she could help in the development of a vaccine that would use mRNA.[13]

With so much research having already be completed on nucleic acids, assembling sequences of RNA or DNA was relatively simple at the time of their partnership. An expert synthesizing mRNA, she was able to create the necessary sequence which was used to make a vaccine with which they injected mice. Unfortunately, the mice developed inflammation and many of them died.[12] The problem was that mRNA created in the laboratory, known as IVT mRNA, is recognized as an antigen by mammalian cells.[14]

As is commonly known, the four nitrogenous bases that occur in RNA are adenine, guanine, cytosine and uracil. When the nitrogenous bases are attached to the sugar found in RNA, ribose, they are referred to as nucleosides[15] and are named adenosine, guanosine, cytidine and uridine.[16] It was the uridine that caused the inflammatory response in the mice.[12] In trying to determine why, they compared RNA produced in the lab to those found in mammalian cells. They found that the nucleosides in those cells had many more modifications than those of bacterial cells due to post transcriptional modifications in eukaryotic cells.[17] They found that there were no modifications in bacterial cells which helped them to understand the basis of the inflammatory response in the mice. Because bacterial RNA has no modifications, they are easily recognized by dendritic cells, which begins an inflammatory response. The change that Drs. Kariko and Weissman chose was to replace the naturally occurring uridine with pseudouridine. Not only did future attempts at a vaccine not induce an inflammatory response, but more of the protein product coded by the transcript was produced as well.

These discoveries were published back in 2005. In that publication, they discussed modifications made to the other nucleosides of mRNA and showed additional modifications that they found in uridine. Contributing to the climate were other outbreaks caused by coronaviruses. The first SARS-CoV virus caused an outbreak in 2002 that infected 8,096 people worldwide and causing the deaths of 774.[18] A second outbreak occurred in 2004 and was followed by a period of relative silence from coronaviruses. But in 2012 an outbreak of MERS-CoV caused infections in 2,266 and killed over 800 people in 27 different countries by Wang. During the ten years between the first SARS-CoV and MERS-CoV, three doctors developed expertise in understanding the spike protein on each of these viruses. They are Dr. Jason McClellan, Dr. Daniel Wrapp and Dr. Kizzmekia Corbett.

The spike protein actually consists of multiple subunits that actually can change conformation before it binds with the ACE 2 receptor.[19] This was determined using a new type of microscope called a cryoelectron microscope. The ability of the spike protein to change conformation, which the researchers called metastability, made it easier for the coronavirus to evade the immune system and bind to its receptor. It also made it difficult for the immune system to recognize the spike protein in order to make antibodies and it made it difficult to develop mRNA that could be used in a vaccine. These doctors determined that a change in the primary structure of the spike protein could lead to a rigidity in conformation which then would allow the immune system to make the antibodies that could actually work against the coronavirus.

When the pandemic caused by SARS-CoV-2 was declared and the genetic sequence of the virus made public in February 2020 (Wu, et al, 2020), this group made the same amino acid changes in the spike protein as they had in the other two. These changes were very small, substitutions of the amino acid proline in two places "in the C-terminal S2 fusion machinery" of the spike protein.[19] This resulted in the stiff conformation necessary to make the mRNA that ended up in the Moderna vaccine referred to as mRNA-1273 in two days.[20] The candidate that would eventually be used in the Pfizer-BioNTech vaccine, BNT162b1,[14] was made even faster, in a matter of hours.

Muscular vaccination causes a break in the skin which signals macrophages to the area. Waiting for those cells is the mRNA transcript enclosed in a lipid nanoparticle. The nanoparticles are taken up by the macrophages which begin making the spike protein. The spike protein is presented as an epitope to which the humoral immune responds by creating antibodies.[15] Because of research that spanned nearly fifty years, these researchers were ready with knowledge, techniques, equipment and everything else necessary to help the world get through this pandemic. Drs. Langer, Kariko and Weissman discussed the difficulties they faced as researchers trying to get funding to continue their research.[21] Dr. Langer in taking his position at MIT had to begin in an area that was outside of his specialty. Dr. Kariko discussed openly the demotion she accepted because she believed in the ability of mRNA to make a new class of vaccines. To the casual observer, me, this all seemed so new, but to this group of researchers and the myriad of people on their teams, it was an opportunity even though it was incredibly unfortunate.

There were two things that Dr. Kariko said she regretted, that the world had to suffer through a pandemic at all and that the development of these vaccines was not communicated better to the general public.[22] But, if I may, the world was different then. Maybe as a society, we could use this as a learning opportunity. Even though life is almost back to normal, maybe now the general public can be made aware of the research that allowed us to respond so quickly to this virus. I for one, will be sharing this with my students.[23–30]

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None.

Conflict of interest

The authors declares there is no conflict of interest.

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