mRNA Vaccine: Determinants of Clinical Efficacy, and Optimization of Pharmacological Effects

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ABSTRACT

The messenger ribonucleic acid (mRNA) vaccine has proven to be beneficial in containing the severe acute respiratory syndrome coronavirus. However, some school thought that it is not as effective as proposed, even though many others attest to its efficacy. The study employed scoping review on online journal and book publications to reveal the pharmacokinetics and pharmacodynamics of the mRNA vaccine. In this work, we have discussed the mechanism of action of the self-amplified messenger ribonucleic acid vaccines, the determinants of the clinical efficacy, and the possible reasons behind the varying efficacies that different populations may experience. We provided possible ways to tackle this challenge. In providing these solutions, we discussed pharmacogenetics viz-a-viz epigenetics. The study also used figures to elaborate on the factors that determine the clinical efficacy of mRNA vaccines. We are confident that caregivers and public health officials in the tropics would find the information interesting and invaluable.

Key Words: *mRNA vaccine, Codon optimization, Polyadenylation, Nucleosides, Epigenetics, Pharmacogenomics*

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INTRODUCTION

The pharmacokinetics of the messenger ribonucleic acid (mRNA) vaccines are influenced by the route of administration, delivery vehicle, and adjuvants [1]. Unlike some conventional vaccines that use live-attenuated viruses that revert to their pathogenic form, mRNA vaccines do not revert to any pathogenic form. This is because they are in the synthetic form, which cannot replicate inside the body. The mRNA vaccines also undergo a metabolic decay a few days after the inoculation of these vaccines [2]. The factors critical to obtaining enhanced clinical efficacy include the 5' cap structure, which is essential for the efficient translation of proteins [3, 4]. The open reading frame (ORF) enhances this translation [4]. The poly adenine (A) tail, also called the poly(A) tail, helps in the stability of the mRNA [4, 5]. The untranslated regions (UTRs) help in the regulation of the translation of mRNA [3, 4]. The modified nucleosides, a synthetic form of nucleotide, aids in preventing the triggering of deleterious immune reactions [4, 6]. An impure protein elicits an immune reaction, hence the need for high purity of the mRNA to avert this reaction.

In this review, we analyzed the pharmacokinetics and pharmacodynamics of the mRNA vaccine by elaborating on factors that determine its clinical efficacy. We also discussed the effects of pharmacogenetics and epigenetics. After which, an expose was done on the factors that may limit the pharmacologic efficacy of the vaccine and possible ways to overcome this challenge.

MATERIALS AND METHODS

There was a review after an online search for journal and book publications, to reveal the pharmacokinetics and pharmacodynamics of the mRNA vaccine. The study also used figures to elaborate on the factors that determine the clinical efficacy of mRNA vaccines.

RESULTS AND DISCUSSION

The literature review revealed much useful information about the mRNA vaccine with regards to the

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pharmacological effects and optimization of the clinical efficacy of vaccines. To present clearly and concisely, the study used illustrations to communicate the ideas. **Figure 1** is an illustration of the way the self-amplified mRNA (SAM) vaccine works. **Figure 2** is a summary of the critical factors that determine the clinical efficacy of the vaccine at the molecular level. **Figure 3** is the summary of the epigenetic factors and the way our immune system is affected.

Pharmacokinetics of mRNA vaccines

Pharmacokinetics can be described as the movement of pharmacological agents through the body. It is how the body processes the pharmacological agent. The pharmacokinetics of vaccines are significantly impacted by their route of administration, delivery vehicle, and to a lesser extent, adjuvants. These three factors also profoundly affect the strength of immune response generated by mRNA vaccine as these factors are the spatiotemporal determinants of the efficacy of vaccines during preclinical studies [1]. The SAM vaccines rarely need adjuvants due to their replicase machinery; their efficiency in terms of pharmacokinetics is enhanced when they are directly injected into the antigen-presenting cells.

The route of administration and delivery vehicle

The administration of vaccines using routes such as the intramuscular (IM), intradermal (ID), or subcutaneous (SC) injection, benefit so much from the adept nature of antigen-presenting cells (APC) such as the dendritic cells. These APCs are densely packed in the skin tissue [7] and skeletal muscle [8]. Hence, these regions are mostly the sites of choice when administering vaccines. For antigen-specific immunity to occur, there is a need for mRNA to be inoculated into the antigen-presenting cell (APC), such as the dendritic cell. These dendritic cells help to present the vaccines to the immune system.

The mRNA vaccine needs the delivery vehicle to overcome some challenges associated with degradation by endogenous RNases and the difficulty of the mRNA vaccine passing through the cell membrane due to its large and polyanionic molecules. The delivery vehicle is vital to circumvent the entrapment and degradation of mRNA in the endo-lysosomal compartments. It also precludes the activation of anti-viral host defense mechanisms, obviating the recognition and enzymatic degradation of mRNA, while it optimizes intracellular translation of the mRNA and the expression of associated proteins [9, 10]. The delivery vehicles also allow for better biodistribution, cellular targeting, and cellular uptake mechanism, and subsequently, an improved outcome of the vaccines [11].

The aphorism that like charges repel, while opposite charges attract which guides electrostatic interactions partly contributes to the performance of many vaccines at the molecular level. An example is the delivery vehicles such as the polyplexes, which help in transporting nucleic acids. While many delivery vehicles are polycationic at physiological pH, the nucleic acids (RNA or DNA) are polyanionic [12]. The efficiency of polyplexes depends on the molecular weight, surface charge, and hydrophilicity of the used Polyethyleneimine (PEI) or its derivatives concerning the ratio of the polymers in the complex responsible for the size, surface charge, and hydrophilicity of the resulting nanoparticles. The efficiency of transfection rises as the molecular weight increases, despite the significant limitation associated with its cytotoxicity [11, 12].

The cationic lipids, polymers, and dendrimers have proven themselves a versatile means of transporting exogenous mRNA. These lipid-based nanoparticles mostly have four functional components: an ionizable cationic lipid, used for self-assembly into virus-sized (~100 nm) particles and for permeation of encapsulated mRNA into the cytoplasm; lipid-linked polyethylene glycol (PEG), is to enhance the half-life of formulations; cholesterol is to serve as a stabilizing agent, while naturally occurring phospholipids is for biocompatibility with the lipid bilayer of mammalian cells and biodegradability; all of them function in unison with the mRNA to look like the lipid bilayer of a cell membrane [13, 14]. The benefits of this type of delivery vehicle are high temporal and thermal stability, high loading capacity, low production costs, ease of preparation, large-scale production, reduction in therapeutic doses, diminished toxicity, and drug resistance, with enhanced targeted specificity and bioavailability of drugs to the tissues. Classic examples are Nanostructured lipid carriers (NLC), solid lipid nanoparticles (SLN), liposomes, and the cationic peptide protamine [11, 13].

Studies have shown that systemically administered mRNA–LNP complexes have the liver as its primary target due to the binding of apolipoprotein E and subsequent receptor-mediated uptake by hepatocytes. Similarly, intradermal, intramuscular, and subcutaneous administration has demonstrated a localized prolonged protein expression [13].

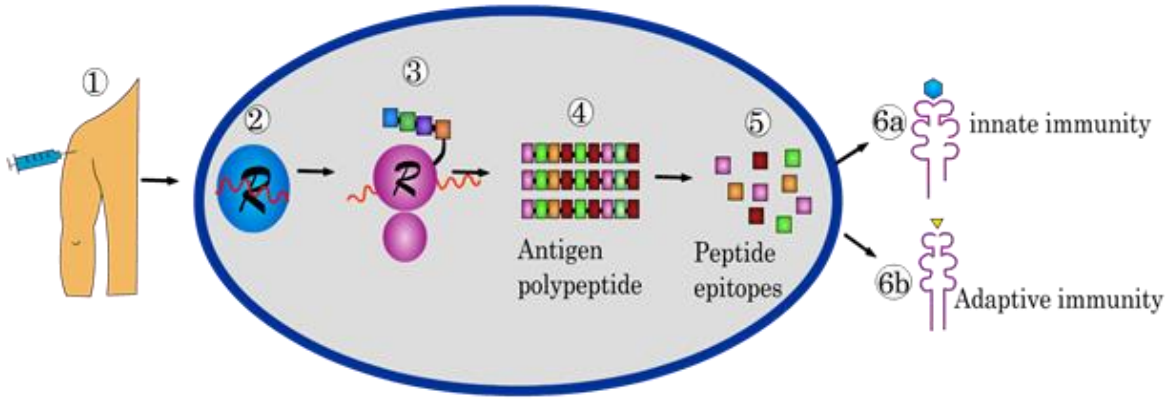

Figure 1. An illustration of the way the SAM vaccine works.

The SAM vaccine is injected into the deltoid muscle due to the abundance of APCs (step 1). This SAM vaccine which contains the replicase (R) and the mRNA (red spiral), gets into the cytosol via endosomal release (step 2). The Ribosomes modify the vaccine via post-translational modification (step 3). This modification induces the replicase machinery to produce a copious amount of the desired protein called antigen polypeptide (step 4). The antigen polypeptide is broken down into peptide epitopes (step 5). The dendritic cell processes and presents the peptide epitope to the innate immunity on first exposure and the adaptive immunity on subsequent exposures (step 6). This mechanism applies to most SAM vaccines, including those designed against COVID-19.

Critical factors that determine the clinical efficacy of mRNA vaccines

The hallmark of every mRNA vaccine is its ability to execute its intended purpose, which is seen in its efficacy and potency. In this section, the factors that are crucial to achieving this clinical efficacy and potency are discussed. These factors include the 5' cap structure, the ORF; the poly adenine (A) tail, also called the poly(A) tail; the untranslated regions (UTRs), the modified nucleosides, and the purity of the mRNA. **Figure 2** gives a summary of these factors.

The 5' cap structure

The natural form of the 5' cap structure in Eukaryotic mRNA is known as cap0. The conjugation of the inverted 7-methyl guanosine (m7G) to the first nucleotide of the mRNA by a 5' to 5' triphosphate bridge during the process of transcription causes it's formation. The 5′ cap is needed for efficient translation and blocks 5′–3′ exonuclease-mediated degradation (3, 4). The cap0 interacts with cap-binding proteins (CBPs), which is essential for the mRNA nuclear export, and also interacts with the initiation factor, 4E (EIF4E), of translation in the cytoplasm, which is crucial for the translation initiation.

Cap0 could also serve as an immune marker that distinguishes between cellular RNA and viral RNA, enhancing the sensing of viral RNA by the immune system, a scenario that requires the modification of these native caps (4). This difference in the cap structures of the virus and the cellular RNA partly contributes to their specificity, thus making specificity critical in protein production and immunogenicity. An example can be seen when an incompletely capped 5′ triphosphate and Cap0 structures stimulate retinoic acid-inducible gene I (*RIG-I*). Melanoma differentiation-associated protein 5 (MDA-5) also detects the absence of 2′O-methylation on RNA and stimulates an immune response. Hence, to prevent the immune response stimulation by RNA sensors such as MDA-5 or RIG-1, IFIT-1 or 2′O methyltransferase (2′O MTase) is used for methylation [3].

The two types of synthetic 5'cap increasingly used in molecular biology are the cap1 and cap2. The human enzyme that can aid in generating these caps is specific to the methyl-7-Guanine (m7G), also called the methylguanine-methyltransferase. The m7G-specific 2′O MTase methylates the second or third ribonucleotide at the 2′O or 3′O position of the ribose generating cap1 and cap2 structures, respectively. Cap2 and Cap1 are less reactogenic in humans than cap0, and they maximize the efficiency of translation. Thus, introducing cap2 or cap1 in the design of IVT mRNA is crucial in obviating immunogenicity, but the cap0 incorporation could be more useful in therapies in which there may be the need for potent immune responses [4].

The open reading frame (ORF)

The ORF is the codon composition of the region that codes for the protein sequence [4]. The ORF benefits *in vitro* via codon optimization, which follows the age-long law or theory of use and disuse seen in evolutionary biology. According to Gómez-Aguado *et al*., Codon optimization is a well-studied process employed to enhance the efficiency of *in vitro* translation and synthesis of proteins among various organisms aided by the functionality of transfer

RNAs (tRNAs). The tRNAs are naturally conferred with two significant properties: firstly, it represents the single amino acid to which a part of it is covalently linked; secondly, the tRNA bears the anticodon, a trinucleotide sequence that recognizes and binds the codons on the mRNA via complementary base pairing [15]. This recognition enables the transfer of amino acids to the part of the ribosome, where they are utilized to elongate the polypeptide chain during the translation procedure. The disparity with which the tRNA aids in this elongation led to a phenomenon called codon bias usage, which favors codon optimization [16]. This natural evolution is due to the degenerate nature of these codons, which are the 61 different codons and a set of 3 stop codons in coding for only 20 recognized amino acids, making some of them be preferentially used by many organisms, while some are rarely or never used [16]. These similar codons are called synonymous codons because they code for the same amino acids at different frequencies. Among these synonymous codons, the codons rarely used are termed rare codons [17]. Synonymous codons occur at varying frequencies in different organisms, and their substitution potentiates protein expression [5].

The elimination or depletion of the rare codons such as UA and UU dinucleotides in the ORF of mammalian cells has proven to protect the IVT mRNA. This is because endoribonucleases such as RNase L preferentially cleaves single-stranded mRNA-coding regions inside UA and UG. This occurs in several mRNAs during acute viral infections; thus, the need to reduce or eliminate these dinucleotides to circumvent the endoribonuclease-mediated cleavages of these IVT mRNA in mammalian cells [18]. Optimizing a codon is mainly based on substituting multiple rare codons with other more frequent codons which encode the same amino acid. This helps to improve the rate and translation efficiency of the recombinant protein produced, making it cost-effective [4, 5]. An example is an optimization of guanine and cytosine (GC) content which significantly enhanced the quality of some DNA vaccines [3]. However, mutations that modify the coding sequence of the RNA or DNA without affecting the amino acid sequence of the protein produced, called synonymous mutations, may alter the quantity of the proteins produced via changes in translation efficiency, making the clinical application of this strategy in humans a daunting task [4, 19].

The poly adenine (A) tail

The inclusion of a poly adenine (A) tail known as poly(A) tail to the 3′ end of mRNA is called polyadenylation. This process is catalyzed by poly(A) polymerase, and it is an integral part of the processes leading to the production of mature mRNA [20]. The poly(A) tail serves as the docking site for poly(A)- binding protein (PABP), which is exported alongside the mRNA from nucleus to cytoplasm, where this complex binds to and recruits proteins that translate, such as translation initiation factor 4F (EIF4F). The normal range of the length of the poly(A) tail in a natural form of eukaryotic mRNA comprises 100 to 250 residues of adenosine. This length determines the stability of the mRNA molecule to a large extent, as its depletion or removal hastens the destruction of mRNA via enhanced exonucleotide digestion [20]. By interacting with poly(A)-binding protein, the poly(A) tail provides stability for mRNA and abrogates the degradative effects of nucleases [4]. The poly(A) tail can be added to the 3′ terminal of IVT mRNA post-transcriptionally using recombinant *E. coli* poly(A) polymerase (E-PAP) I, or it could already be encoded in the DNA template vector of the mRNA [20, 21].

The length of the poly(A) tail enhances the translation efficiency and stability; however, this depends on the target cell. In the *HeLa* human epithelial cells, the adenosine residues extension in the poly(A) tail from 14 to 98 boosted the protein expression, while the dendritic cells (DCs) require 120 adenosine residues to enhance the efficiency of translation and protect and stabilize the IVT mRNA [3, 4].

The untranslated regions (UTRs)

The UTRs flank the ORF at both ends of 5′ and 3′. The UTRs are non-coding regions that do not play a direct role in the codification of proteins, but their length, sequences, and secondary structures are critical for regulating the mRNA translation and the subsequent protein expression. While 5′ UTR participates in the translation initiation, a step considered the most intricate step among the whole process, 3′ UTR influences the stability of mRNA and the degree of protein expression [3, 4].

The internal ribosomal entry sites (IRES) in the 5’ UTR recruits the ribosome and initiate a cap-independent translation. Thus, optimizing the translation efficiency of IVT mRNA requires that 5′ UTRs containing IRES from viral origin be incorporated. In events of this nature, translation is not dependent on EIF4E, as it is seen in the case of microbes depending on cap0 (4). Also, the expression of IVT mRNA is extended to cells where levels of EIF4E are low. Nevertheless, the 5′ cap is still needed to protect the mRNA against nucleases; thus, most IVT mRNAs contain both IRES and 5′ cap in their structure [4]. Also, the Kozak consensus sequence found in the 5′ UTR contributes to the translation process initiation. The Kozak sequence presented as RCCAUGG, where *R* is a purine (A or G), was taken as the preferred sequence for the start of a translation in eukaryotes. The importance of the nucleotides on this sequence varies as the −3 and the +4 positions are more critical than the adenine of the starting codon AUG. The G nucleotide should be in the position

+4, and A/G nucleotides should be at −3 to efficiently enhance the start codon (AUG) recognition [4].

The presence of specific sequences of β-globin and α-globin mRNAs in the region of 3′ UTR enhances the duration of protein expression and the stability of IVT mRNA, respectively. Additionally, regulating the localization of proteins is influenced by modifying the length of the 3′ UTR sequence, such as is seen in the case of CD47 membrane protein, where long 3′ UTR initiates the protein expression on the cell surface; while the short 3′ UTR leads to the localization of the protein in the endoplasmic reticulum [4].

The modified nucleosides

Modified nucleosides which are synthetic nucleotides, strategically aim at preventing immunogenicity. Modified nucleosides such as pseudouridine or N-1-methylpseudouridine have been employed to abrogate intracellular signaling and trigger protein kinase R (PKR), leading to enhanced antigen expression and adaptive immune responses [3, 22]. Exogenous mRNA stimulates innate immune responses by interacting with pattern recognition receptors (PRRs), which include Toll-like receptors (TLRs) and cytoplasmic RNA sensors, such as retinoic acid-inducible protein I (RIG-I). The uridine residues activate TLR7, while GU- and AU-rich RNA strands activate TLR7 and TLR8. However, the incorporation of modified nucleosides into the transcript, (i.e., pseudouridine (Ψ), N1-methylpseudouridine (N1mΨ), 5-methylcytidine (m5C), 5-methyluridine (m5U), N6-methyladenosine (m6A), or 2-thiouridine(s2U)), circumvents the activation of TLRs. The Ψ and s2U, also obviate *RIG-I* activation. The addition of m6A in the 5' UTR has also been proposed as an alternative to IRES to favor cap-independent translation [4, 6]. This m6A can trigger cap-independent translation by binding to the eukaryotic initiation factor 3 without EIF4E activity [6].

Purity

The *in vitro* generation of an mRNA involves generating the DNA, after which the mRNA is generated. To obtain pure DNA, the starting material that yielded the DNA needs to be purified. Equally, the process leading to mRNA generation must involve a purification process to obtain a pure mRNA. These purification processes help clear the impurities and other unwanted substances during the production of the mRNA vaccine. The primary aim of this purification process is to enhance protein expression and reduce the immunogenicity induced by exogenous materials (33).

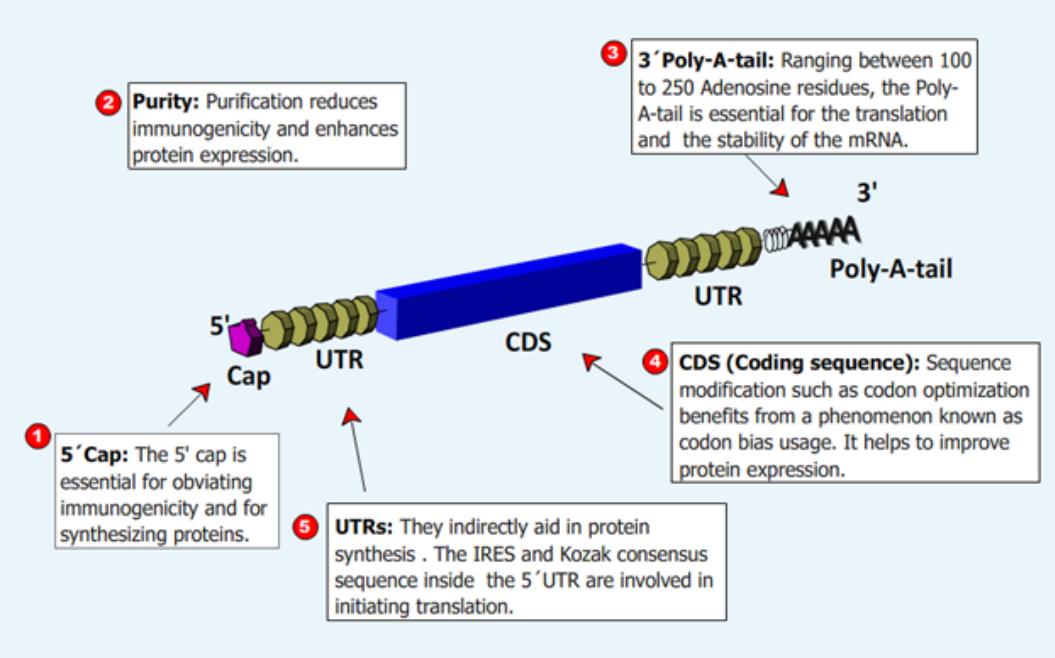

Figure 2. A summary of the critical factors that determine the efficient expression of the gene of interest by the mRNA construct.

Five elements influencing the efficacy of the pharmacokinetics of mRNA are 5′ capping efficiency and structure; UTR length, structure, and regulatory elements; coding sequence modification; poly-A-tail properties; and mRNA purity.

Pharmacodynamics of mRNA vaccine

Pharmacodynamics can be described as the effects of pharmacological agents on the body. It is what the drug does to the body. The interplay of pharmacodynamics and pharmacokinetics often culminate in both the intended

effects (pharmacologic effects) and unintended effects (side effects).

The mRNA vaccine administered for the prevention of COVID-19 has shown promising results. While the FDA and the manufacturers of these vaccines posit that it is over 90% efficient, some schools of thought opine that it is not as effective as the FDA proposes. Because the production of the mRNA vaccines shares a similar process with other mRNA therapeutics, it would be beneficial to perceive these vaccines as pharmacological agents and critically analyze some factors that must have led to the variations in their efficacy. While doing this, some of the components of the mRNA vaccine are considered in line with a few of the effects known to be indirectly or directly associated with these vaccines. Some factors which may be contributing to these effects are also reviewed.

Reports have it that a two-dose regimen of the mRNA vaccines resulted in 94-95 % protection against COVID-19 in people ages 16 and older. Common mild localized side effects include heat, pain, and redness. Rare systemic side effects are fatigue, myalgias, fever, headache, arthralgias, and hypersensitivity reaction. Systemic side effects were more prominent in vaccine recipients younger than 55 years, with an increased side effect of headache, chills, fatigue, and fever within two days of the second dose. Rare adverse events are vaccine administration-related shoulder injury, right axillary lymphadenopathy, paroxysmal ventricular arrhythmia, right leg paraesthesia, arteriosclerosis, cardiac arrest, hemorrhagic stroke, and myocardial infarction. Anaphylatoxic reactions seen here are facial, labial, and glossal edema coupled with Bell's palsy [23].

The variation of the efficacy of the mRNA vaccine could be hypothesized based on the role of the varieties of genes among different populations and the interplay of epigenetic factors.

Pharmacogenetics and pharmacogenomics

Humans respond to pharmacological agents, whether for curative or prophylaxis, differently. A heterogeneous response that may be due to variations in genes is seen in pharmacogenetics. The gene has been estimated to contribute to 20 to 95 percent of the disparity in the effects and toxicities of drugs. This, resulting from sequence variants, could be linked to drug targets, metabolizing enzymes, receptors, or delivery vehicles [24]. Pharmacogenetics and pharmacogenomics deal with the genetic basis in favor of this variety of responses to pharmacological agents in individual patients. The conventional approach of pharmacogenetics is based on studying sequence variations in candidate genes which may be implicated in the disparity of responses [25].

Pharmacogenomics on the other hand looks at the total effects of all the genes implicated in this response. These studies are done to optimize the efficacy of pharmacological agents based on the genetic composition of patients as an individual or as a group [25]. Pharmacogenomics focuses on the alterations in the pharmacokinetics of drugs such as absorption, distribution, metabolism, and elimination. It also considers the modulation of drugs via pharmacodynamics, including altering a drug's target or its metabolic pathways [26-28]. Drugs whose effects are mediated via the ACE receptors sometimes display disparity in response, and the vaccines may not be an exception [26]. It is essential to note that most vaccines used during this COVID-19 pandemic elicit their action through the ACE 2 receptors.

The heterogeneous responses of different populations to the mRNA vaccines could be partly attributed to variation among humans. This variation could be due to the disparity in how these enzymes metabolize this vaccine across the different populations. The enzymes which may be implicated in this case include nitric oxide synthase 2, Adenosine Monophosphate (AMP)-activated protein kinase, arginase 1, indoleamine 2,3-dioxygenase 1, hexokinase, and phosphofructokinase [29-31]. The inability of the dendritic cells to optimally process the antigen peptides may affect the efficiency with which these antigen peptides are presented to the immune system.

The human body can be conductive due to the presence of a large number of electrolytes (charged ions) dissolved in an aqueous solution, such as blood [32, 33]. The large size of vaccines, whether DNA or RNA, makes it difficult to permeate the cell membrane and reach the cytosol, where it undergoes post-translational modification. Delivery vehicles, also called drug transporters, are used to facilitate this permeation [14]. When the polycationic delivery vehicles complex with the polyanionic nucleic acids, net charges which are mostly electrons are produced. This net charge conjugates with the ion-filled body fluids to produce static electricity via the nerves. These charges may increase the chances of some types of bulbs being lighted, even though the specific cause of the lighting may be unconnected to these charges. This lighting intensity partly due to genetic disposition may be absent or present in various degrees among vaccine recipients due to the molar weight, polarity, and hydrophilicity of these delivery vehicles [34]. However, this calls for further research.

Epigenetic and epigenomics

Considering the peculiar nature of vaccines, there are two main ways in which their pharmacological effects could be optimized. It is either done by increasing the dose of the inoculum or via the manipulation of epigenetic factors. The former may benefit those with an intact, healthy body system, while the latter may favor those whose system has been compromised by age, disease state, and lifestyle. Epigenetic factors may be used in conjunction with

pharmacogenetics to determine the type of drug and the required dose needed to attain an optimized pharmacological effect [35].

In 1942, the British embryologist, Conrad Waddington, coined the term epigenetics; this is the study of mitotically or meiotically heritable phenotypic changes in chromosomal or gene function; without a change in gene sequence (code) of a DNA. In humans, it is mediated mainly via DNA methylation and histone modification. These two mechanisms act like switches on top of the DNA to turn it "off" or "on" in adaptation to certain external stimuli [36-39].

Factors that mainly utilizes these two mechanisms to influence epigenetic phenomenon in humans are called epigenetic factors. These epigenetic factors are environment, lifestyle, age, disease state, cross-immunity, Food, and drugs [38, 40, 41]. These epigenetic factors contribute to a great extent to the variation in pharmacological effects of most drugs which by extension includes the vaccines, hence determining their optimal efficacy [26].

The environment of humans influences their ability to resist infections via the immune system. Human epigenetic patterns can be adversely impacted by exposure to organic pollutants, air pollution, metals, benzene, and electromagnetic radiation. Chemical compounds and xenobiotics in water or the atmosphere are other potential elements posing a threat to humanity by altering the epigenetic mark [42].

Lifestyle factors that might negatively modify epigenetic patterns include sedentation, diet, obesity, alcohol consumption, tobacco smoking, psychological stress, and job-related stress such as night duties have a way to dampen our immunity against infections [43]. Although, physical training may be of numerous benefits to the immune system.

Cross-immunity has a way to offer some protection against infections and prevent debilitating conditions. People living in Nigeria do not take yearly vaccination against the seasonal flu because it is not available in the national program for immunization. In this case, cross-immunity seems to make their immune system adept at handling most types of flu. However, aging has some adverse effects on the immune system; conspicuous among them is a decline in the synthesis of B-cells and T-cells. It could also negatively influence cross-immunity.

Widely described examples abound in the use of diets or supplements in inducing epigenetics. The study of the intake of supplements containing folate and other methyl donors during antenatal care accents to this. Due to the loss in the capacity of *de novo* synthesis of Vitamin B9, or folic acid, utilized in the biosynthesis of tetrahydrofolate by the human body, it is necessary to obtain it via diets. This applies to vitamin B6 and B12, which are both co-factors. These B vitamins and folate also influence the epigenome to prevent cancer in animal models for diseases [44]. A similar scenario is also seen with vitamin C [45-48].

The use of pharmacological agents in treatments can also induce epigenetic changes. Because epigenetic changes help determine whether genes are turned on or off, they modulate the production of proteins in cells. This modulation contributes to ensuring that only the proteins needed for a particular function are produced. Such is the fate of the antigen protein, which is processed by the dendritic cells. Patterns of epigenetic modification vary among cells within a tissue and also among different tissues within an individual. Epigenetic regulation of genes responsible for drug-metabolizing enzymes has remained an important concept not only for drugs but inadvertently for vaccines [42, 44].

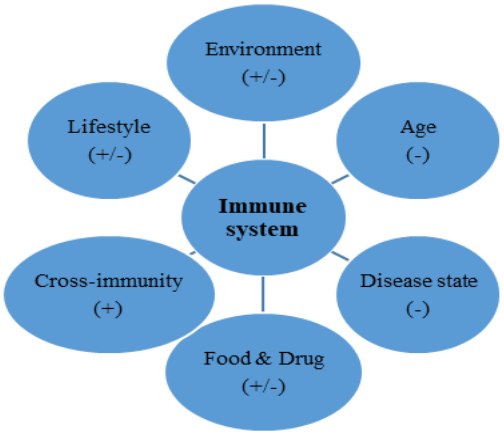

Figure 3. A summary of epigenetic factors and their effects on our immune system.

The positive sign indicates positive impact, while the negative sign indicates negative impact.

Optimization of pharmacologic effect of vaccines: a future with epigenetics

Based on observational studies in clinical immunology, one with experience would deduce how pharmacological agents, especially the mRNA vaccines, could be optimized using epigenetics. The following paragraphs will elucidate more on a few of the ways to optimize these vaccines.

The function of ascorbic acid (vitamin C) in biosynthetic reactions can never be overemphasized. Vitamin C has also been identified as a co-factor for newly characterized hydroxylases that regulate the transcription of genes and signaling pathways. These hydroxylases are classified under the ubiquitous family of Fe-containing 2-oxoglutarate-dependent dioxygenases, a group of enzymes involved in biosynthesis, post-translational modification of proteins, and oxidative demethylation of methylated histone residues and methylcytosine. These hydroxylase enzymes include γ-butyrobetaine dioxygenase, *N*-

trimethyl lysine hydroxylase involved in carnitine synthesis, and the prolyl-, lysyl- and arginine hydroxylases that are involved in modifying the collagen and the alpha regulatory subunit of the hypoxia-inducible factors associated with the immune system [49].

Vitamin C is known to modulate T cell maturation and dendritic cell-mediated T cell polarization. Vitamin C as an epigenetic modulator has been shown to stabilize and improve the expression of the Treg master regulator gene Foxp3 conferring positive effects on the generation of induced Tregs (iTregs). Studies also show that Vitamin C can trigger the expression of Foxp3 on CD4+ Foxp3− T cells in a TGF-β-dependent fashion. As an optimizer of the expression of Foxp3 on Treg, vitamin C is inductively able to optimize the actions of T-cells and dendritic cells [50]. Studies have shown that vitamin C, which modulates the epigenome, could prevent and manage COVID-19 as prophylaxis, adjunctive therapy, or home remedy [45-48].

CONCLUSION

While pharmacogenomics and pharmacogenetics generally focus on the disparities in efficacy, epigenetics concentrates on adaptation and survival, by enhancing these efficacies in various individuals. This work hypothesizes the need to galvanize the benefits of epigenetics in optimizing the pharmacological effects of the vaccines. Performing the experiments based on this hypothesis requires a step-wise approach using both the mammalian cell culture and animal models for *in vitro* and *in vivo* studies, respectively. The various governments and their respective public health institutions could take a cue from this hypothesis to review how they administer these vaccines to their citizenry, especially those with compromised immunity.

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