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mRNA Vaccine Applications in Global Health: Promise and Challenges

The global COVID-19 pandemic has focused intense attention on the potential benefits and continuing challenges of vaccine development. In the twentieth century, a large number of important vaccines were developed empirically, without clear understanding of molecular immunology. Mass vaccination with these products successfully eliminated the scourge of smallpox, and greatly reduced and contained the global burden of other diseases such as measles, pertussis, chicken pox, and polio. Nevertheless, many important global challenges remain unresolved, and there is a tremendous unmet need for safe and effective vaccines against diseases such as AIDS (Acquired Immune Deficiency Syndrome), parasitic infections, and tuberculosis (TB).

2020 was the year when many people first became aware of mRNA vaccine technology and its potential benefits to public health. The promise of this technology is clear. Importantly, the success of those COVID-19 vaccines was only possible because of decades of foundational work by industry and academic scientists. Some of that work was specifically envisioned as the basis for a response to future pandemics. Other foundational work comes from longstanding efforts in AIDS vaccine development.

Notably there are important similarities and differences in the nature of the public health need represented by acute COVID-19 infection compared to that pertaining to AIDS and TB, for example. SARS-CoV-2 infection causes an acute symptomatic infection typically followed by natural clearance of the SARS-CoV-2 virus from an infected person. In contrast, the causative agents of AIDS and TB produce long, slow latent infections. For diseases in this category, effective vaccines will probably need to provide stronger, broader, and more durable responses. In addition, the complex lifecycles and immune evasive properties of these pathogens make successful vaccine design significantly more difficult. Ongoing work is investigating the promise of mRNA in vaccine development for HIV and other infections of global importance. New RNA technologies under development, in combination with complementary vaccine approaches, will also add to the toolbox of approaches to meet the challenges of these global infectious diseases.

In infectious disease medicine, a prophylactic vaccine is a medicinal product intended to prime immunological memory and protect against infectious disease caused by specific viruses, bacteria, or other pathogens. An antigen is the molecular target of an immune response – for example a viral protein. A vaccine always includes one or more antigens or genetic material encoding such antigens. Each vaccine also needs a vehicle or vector to make sure the antigens are delivered to the correct cellular and anatomical locations. Vaccines also often include a drug substance intended to activate the immune response to the antigen, called an adjuvant.

Historically, many vaccines were developed using preparations of the target infectious agent that had been killed by heat or chemical exposure. Other vaccines incorporated live pathogen strains that were attenuated (unable to cause disease in healthy people). The Salk- and Sabin-type polio vaccines respectively are examples of killed and live-attenuated type vaccines, both still in clinical use. More recently, vaccines have been developed that incorporate artificial recombinant protein antigens plus chemically designed adjuvant compounds; examples of recombinant protein vaccines in clinical use include hepatitis B and herpes zoster or shingles vaccines.

Within the past few years, we have seen FDA approval of vaccines incorporating modern synthetic biology and gene transfer technology. This includes vaccines based on viral vectors – artificially engineered viruses that deliver and express a target antigen in a manner designed to provoke a protective immune response. This category also includes vaccines using messenger RNA (mRNA). In normal cell biology, mRNA plays the critical role of encoding genetic information transcribed from chromosomal DNA, and carrying that encoded "message" to ribosomes, which are the protein factories of the cell. Thus any properly encoded mRNA sequence can be expressed as a protein. Using synthetic biology, artificial RNA messages can be manufactured to encode any protein sequence. This allows rapid production of an mRNA product encoding any desired viral antigen sequence.

Both of the RNA vaccines with current FDA approval are comprised of mRNA molecules encoding a viral antigen, with the mRNA enclosed in a protective particle of synthetic lipid – aka a lipid nanoparticle or LNP. When a person receives one of these vaccines, the LNPs deliver the mRNA cargo into cells near the injection site, which immediately begin to synthesize the mRNA-encoded antigen. In turn, the expressed antigen is taken up by specialised immune cells to prime the antiviral immune response.

Vaccine-induced immune memory has two functional components: humoral immunity and cellular immunity. Humoral immunity is mediated by antibodies, which are complex soluble proteins secreted by B cells. Antibodies can exert a variety of antiviral effects. Antibodies that can directly block viral entry into a target cell are called neutralising antibodies. Cellular immunity is mediated by T cells, which can promote a variety of antiviral effects when they come into contact with infected cells or cells displaying fragments of viral proteins. Initiation and control of humoral and cellular immune responses depend on complex interactions among B cells, T cells, and other categories of white blood cells.

COVID-19 is caused by the SARS-CoV-2 coronavirus.[1] In most cases, a person becomes infected with SARS-CoV-2 through inhalation of airborne aerosols or droplets containing viral particles. Each viral particle is decorated with external viral spike proteins that can grab onto and bind a protein called ACE2 on the surface of human

airway cells. After binding to the ACE2 receptor, the virus can enter a human cell and hijack the host cell biology, turning the cell into a factory to make more SARS-CoV-2 particles. Upon release, these particles spread to neighbouring cells in a self-amplifying process of replication. In the upper airways, this viral replication can lead to mild-to-life-threatening flu-like symptoms. If the virus spreads to lower airways it can cause pneumonia. The extent and significance of SARS-CoV-2 spread to other organ systems is the subject of ongoing investigation. Over a period of days to weeks, an infected person's immune system learns to fight back against the virus, typically leading to clearance of the virus from the body and resolution of the acute disease. Before immune clearance, while the virus is spreading in the airways, it also becomes airborne in exhalant air, resulting in transmission to new human hosts. The degree to which SARS-CoV-2 may persist in some infected persons after acute disease is also a matter of ongoing investigation.

The primary challenge for COVID-19 vaccine designers in 2020 was to come up with a vaccine that could induce immune responses capable of slowing the viral replication process in an exposed person, to prevent the occurrence of severe acute disease. Secondary goals included: i) inhibition of SARS-CoV-2 transmission to another person; and ii) total blockade of SARS-CoV-2 infection in the vaccinated person, such that no viral replication could occur. Achievement of the primary goal was highly significant and a primary rationale for FDA approval; benefits of vaccination with respect to the secondary goals were also observed.[2]

Due to heroic efforts of vaccine developers, the medical community, government agencies, and volunteer participants in clinical trials, the primary goal of 2020 COVID-19 vaccine programs was achieved in an extraordinarily short time frame, leading to FDA approvals of two mRNA vaccines and to mass vaccination of global populations.

This historic success was made possible by a broad range of medical and social factors. On the level of immunology and virology, vaccine developers had several factors working in their favour:

1. An essential viral component to be targeted by the immune system was readily apparent: the viral spike protein. Based on prior studies of other coronaviruses, the likely role and vulnerability of the viral spike protein to immune attack was understood from the beginning.
2. Vaccine developers already had spent many years studying how mRNA could be delivered in test animals and in human research participants to generate robust antibody responses.
3. SARS-CoV-2 typically has a naturally limited, acute-clearing infectious cycle in each infected person.
4. Large numbers of participants volunteered to participate in global COVID-19 vaccine clinical trials and as the SARS-CoV-2 viral "attack rate" was high, vaccine efficacy could be measured rapidly.

The success of COVID-19 mRNA vaccine development has raised interest in ongoing efforts to apply mRNA vaccine technology to other global infectious disease challenges. There is no doubt that mRNA can play a very important role in the future of global health. However, many of the factors that allowed COVID-19 vaccines to be rapidly developed and commercialised are not equally applicable in the case of other specific global health concerns, where additional challenges are present.

AIDS is a disease caused by one of two closely related viruses: Human Immunodeficiency Virus-1 or Human Immunodeficiency Virus-2 (collectively "HIV"). HIV is transmitted by exposure to body fluids from an infected individual, mostly at mucous membranes through sexual activity, by contaminated blood products, or via shared needles during intravenous drug use. Analogous to the spike protein expressed on SARS-CoV-2, HIV particles carry envelope proteins that bind to receptors on human target cells and mediate infection. HIV specifically infects a subset of white blood cells – "CD_4+ T cells" – vital for maintenance of the human immune system. After initial acute HIV infection, an infected person's CD_4+ T cells are gradually eliminated over a period of years, resulting in eventual loss of immune function and symptomatic AIDS. Without antiviral drugs to suppress HIV replication, persons with AIDS eventually die of secondary infections due to immune failure.

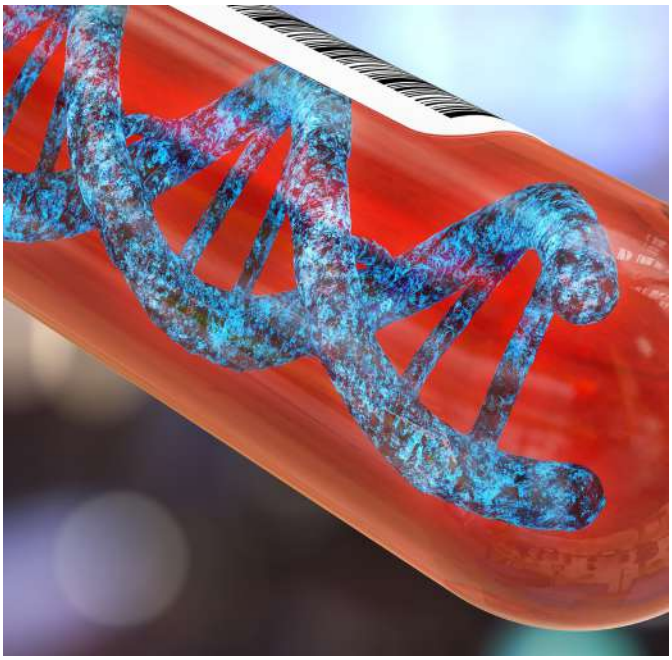
Similar to the SARS-CoV-2 spike protein binding to the ACE2 receptor on a target cell, a component of the HIV envelope protein, GP120, binds to the CD_4 protein on a target CD_4+ T cell. GP120 binding to CD_4 is followed by a complex series of molecular interactions resulting in HIV entry and productive infection of the target cell. These broad similarities in the viral life cycle, depending on specific interaction of a viral surface protein with a cellular receptor, raise the question of whether the impressively rapid success of mRNA vaccine development for COVID-19 can be replicated with AIDS. Decades of effort spent on AIDS vaccines have yielded incremental but significant advances toward a globally effective HIV vaccine. While extensive research has shown that the relatively simple type of antibody responses associated with COVID-19 vaccine efficacy are inadequate to protect against HIV infection at the population level, new findings point the way to more effective anti-HIV responses.

Some factors that make the HIV envelope protein a difficult target are as follows:

1. Globally, HIV-1 exists in genetically distinct "clades" with different susceptibility to various kinds of antibodies. In addition, replication of the HIV genetic code is intrinsically error-prone, resulting in a much greater degree of genetic diversity within replicating HIV viral populations compared to SARS-CoV-2. As a result, HIV infection takes the form of a "viral swarm" containing many genetic variants, some of which will be capable of evading any typical vaccine-induced immune response.
2. Like SARS-CoV-2, HIV incorporates a kind of sugar molecule into the envelope proteins expressed on the viral surface. These glycan sugar moieties serve as a screen or camouflage to keep host antibodies away from essential targets on the viral particle.
3. Whereas the spike proteins on the surface of SARS-CoV-2 are present in a target-rich array, the envelope proteins on HIV are sparse and spread out on the viral surface. This sparsity interferes with certain close molecular interactions of attacking antibodies that contribute to antiviral efficacy.

New approaches to mRNA vaccine design have the potential to overcome these challenges and promote production of "broadly-neutralising" antibodies against HIV.

Aside from these differences in viral particle structure, there are other important differences in viral biology that make HIV vaccine development a more difficult challenge. Importantly HIV is a retrovirus, which means that after infecting a target cell, HIV goes through a stage where the HIV genetic code is integrated into the host chromosomal DNA. This retroviral DNA functionally becomes part of the host cell genome. The latency time from integration to reactivation and production of new viral particles may be as short as a few hours or as long as many years. During these extended latency periods, the retrovirus exists essentially as a short sequence of DNA



code in the chromosome of the infected cell, functionally invisible to the immune system. Upon reactivation, even a small number of these latently infected cells is sufficient to seed active systemic infection and eventually lethal AIDS in the infected person.

This key life cycle factor makes the "victory conditions" of HIV vaccine development much more stringent than those applied to the COVID-19 pandemic. Whereas a COVID-19 vaccine can be deemed a success if it significantly inhibits viral replication during the acute disease phase and prevents severe disease, the primary goal of HIV vaccine studies generally includes prevention of latent infection that may reactivate weeks or months after initial exposure. A COVID-19 vaccine can provide significant public health benefits simply by keeping infected people out of the hospital during the acute phase of infection. To be really useful, an HIV vaccine must induce a much more suppressive antiviral immune response and prevent viral replication occurring.

As mentioned above, adaptive immunity involves more than just humoral antibody responses and the B cells that produce them. T cells also play critical roles in protection from infectious disease. Broadly speaking CD4+ "helper" T cells direct and modulate immune responses, whereas CD8+ "killer" T cells directly eliminate virally infected cells. Many lines of evidence from *in vitro* studies, animal models, and human clinical research demonstrate potentially critical roles for T cells in antiviral immune responses induced by viral infection.

The first mRNA vaccines to be commercialised are relatively simple and consist of an LNP encasing an mRNA strand encoding one viral protein. In some cases products of this type may not meet the challenge of protection against diseases that cause latent and chronic longterm infections, where viral replication continues to occur. Importantly, however, the potential contribution of RNA products to effective vaccines extends far beyond the current first-generation modalities. Examples of emerging concepts in development for RNA vaccines include the following:

- Utilising protein modelling and molecular engineering to create more stabilised and immunogenic synthetic antigens.
- Heterologous prime-boost vaccination plans including mRNA vaccine plus "booster" doses from other categories of recombinant or live-attenuated vaccine products. Many years of preclinical research indicate that a rationally designed mix-and-match approach with multiple types of vaccines for the same infectious agent can provide synergistic benefits in terms of maximising immune protection and minimising unwanted effects such as "immunodominant" off-target responses.
- RNA vaccines encoding multiple synthetic antigens, each optimised to promote a different branch of immune memory (antibodies, helper T cells, killer T cells).
- Whereas first-generation mRNA products are linear strands that persist for only a short time after vaccination, newer classes of mRNA products are under development, some of which have circular structures and others are capable of self-replication, or have other features designed to promote persistent antigen expression after vaccination. These approaches are intended to lead to stronger, broader, and more durable immune responses.
- LNPs currently in use do not provide targeted delivery of mRNA cargo to particular anatomical or immune compartments in the body. Rapid advances in the field of nanomaterials provide the potential for more complex, engineered nanoparticles that can deliver RNA products to specific tissues or cell types with a regulatable timing to enhance vaccine responses.
- For infections that originate from exposure at a mucosal surface, there is growing evidence that the most protective immune responses may engage special mucosal immunity functions. Vaccines designed to stimulate immune responses at a mucosal surface (such as the nasal mucosa) may promote mucosal immune responses with enhanced protective capabilities specifically for mucosal infections. Any or all of the above technologies can potentially be engaged to enhance design of mucosal vaccines.

In conclusion, the utility of RNA vaccines to provide significant public health benefit in the context of the COVID-19 pandemic health emergency has now been clearly demonstrated. The molecular design and formulation of these first-generation products produce limitations in the protective efficacy of resulting immune responses. Newer technologies and combinations of multiple vaccine approaches are likely to extend the efficacy and range of applications for RNA vaccines.

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