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Contemporary Feature

The Biochemistry and Cell Biology Behind Developing Vaccines Against SARS-CoV2 Virus

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The beta-Corona virus SARS-CoV-2 has caused a pandemic outbreak that has lasted for over a year and it still continues its impact in a global scale. The devastating loss of lives and livelihoods has caused socio-economic downfall across the world. The scientific community has since worked towards finding therapeutic strategies to mitigate adverse effects of the infection and vaccines to prevent the spread of the virus. The advancements and success in vaccine technologies have been tremendous and has led to the development of effective vaccine candidates under a remarkable time frame.

Pandemics and worldwide spread of viruses over the past several decades had prompted scientists to investigate more efficient alternative vaccine technologies. Beta coronaviruses have caused three outbreaks within the past 20 years: SARS-CoV (2002-2003), MERS-CoV (2012) and lastly SARS-CoV-2 (2019 till present). Since its emergence, SARS-CoV has mutated into few other strains that hold or surpass the high infection rate of the virus originated in Wuhan, China.

1. Biochemical features of SARS-CoV-2

Structurally, SARS-CoV-2 is a pleomorphic virus with a diameter between 50-200 nm. The whole viral genome is encoded in a positive-sense ssRNA, that carries a 5' cap structure and is polyadenylated at the 3' end. The polyadenylation of the genome and as well as the subgenomic mRNAs allow isolation of these molecules using oligo dTs. The whole viral genome of about 29.9 kB contains 5' and 3' untranslated regions of about 200-500 nucleotides. There are 14 open reading frames (ORFs) on the mRNA that transcribe 27 different viral proteins. Among these, non-structural proteins perform essential functions including genome unwinding, replication, capping, tailing, methylation, membrane rearrangement, etc. The structural proteins make the envelop, nucleocapsid, membrane and spike proteins. The spike protein (S) is a multifunctional trimeric transmembrane glycoprotein that plays essential roles is attachment, fusion and entry into the host cell. The S protein is where the initial mutation occurred that led to an outbreak and an eventual pandemic. The cleavage of the S protein trimer is a crucial step in the process of the viral infection. Sequence analysis of the virus had revealed insertion of 4 amino acids between S1 and S2 sub-units of the spike protein. These mutations introduce a new furin cleavage site on the S protein, that was absent in previous SARS-CoV and may have presumably led to the increased pathogenicity of the virus.

The SARS-CoV S trimer has two distinct conformations as shown by Cryo-electron micrography. The change in conformation between open and close forms, is believed to be crucial in its interaction with the human ACE2 (Angiotensin converting enzyme 2) receptors on the cell surface. The opened conformation presents the three hACE-2 recognition motifs on each of the sub-unit and renders it for interaction. ACE2 receptors are highly expressed in Type II alveolar epithelial cells, that explains the respiratory distress associated with the infection. In addition, these S proteins have an abundance of N-linked glycans that protrude out of the viral particle that play roles in protein folding, priming by host proteases and antibody recognition. Sequence analyses confirm the conservation of 20 glycosylation sequences across all SARS S proteins suggesting that

antibody presentation and interactions are perhaps comparable among the viral strains. Recent data show that S protein is more exposed to the host than the other fusion proteins on the viral surface. This suggests that the S protein may present more to the machinery of the immune system making it an ideal target for vaccines against SARS-CoV-2.

2. Milestones in developing mRNA Vaccines against SARS-CoV2

The basis of a vaccine is to mimic the entry of the virus into the host by presenting a specific immunogen to initiate a cascade of immune response driven cellular processes. There have been few immunogens that have been the focus of the vaccines. In addition, there have been several strategies that have been explored in producing a vaccine including, inactivated or live attenuated virus, virus like particles, protein or protein subunits, DNA and novel mRNA technology. Between DNA and RNA based vaccines, the latter presents an advantage by not having to cross the nuclear envelop. Among these, although novel, RNA technology is ideal if successful as it is incapable of altering the genome and its expression is maintained in a transient manner by intrinsic cellular mechanisms. In addition, synthesis of RNA molecules allows a wide array of target proteins and epitopes as immunogens in contrast to the capabilities with traditional approaches.

RNA based vaccines have made major advances over the years and have proven promising in non-human primates. RNA is able to elicit a robust innate immune response and such vaccines have been tried for HIV infections in other non-human hosts and Zika in the past. They are well tolerated in preliminary studies and in some clinical settings. One of the strengths of RNA based vaccines is that it can be produced in a large scale, in contrast to traditional approaches which makes large scale production much feasible with escalated high demand during a pandemic.

Unprotected RNA molecules are highly unstable under physiological conditions. Optimizations on the RNA molecule in prophylactic vaccines have made great advancements by introducing a capped structure to enhance translation efficiency and increase stability. Addition of 5' untranslated regions to the sequence enhanced the translation efficiency. Both the leading mRNA-based vaccines, BNT162-01 and mRNA-1273, encode the full-length spike glycoprotein where the latter with modification at two residues to prolines. The mRNAs are transcribed in vitro using a DNA template and a cap structure is introduced to increase translation capacity and stability in vivo following intra-muscular administration.

One other main challenge that was overcome was advancing cellular delivery methods to circumvent the catalytic hydrolysis of RNA by cytosolic ribonucleases. A number of strategies have been developed for RNA delivery including RNA conjugates, modified RNA, viral vectors, microparticles and nanoparticles. Among these methods, tagged or conjugated RNAs, although increasing stability, present the challenge of non-targeted binding and interactions with cellular components perhaps leading to aggregation. Despite viral vectors being a viable choice, the challenges associated with packaging the virus with the mRNA, large scale production and low immunogenicity limit its use in a prophylactic setting. Packaging genetic material into non-viral vectors with lipids, polymers, peptides and inorganic peptides have presented promising technology with an easier production pipeline. Further, the size of these particles can be altered to carry a larger load of oligonucleotides. Delivery of genetic material in lipid nanoparticles (LNP) has been through major technological advancements over the past years. LNPs may contain ionizable amino lipids, phospholipids, cholesterol and polyethylene glycol (PEG) containing lipids in their formulation. The LNP-formulated mRNA must be able to induce a marked but transient increase in pro-inflammatory cytokines at the injection site. Change in cytokines contribute to chemotaxis, recruitment and activation of specific immune cells that activate a cascade of cellular events.

The mRNA-based vaccines essentially target antigen presenting cells (APCs), which are an heterogenous group immune cells including B-cells, macrophages and dendritic cells. APCs are primarily involved in ingesting the foreign antigen and presenting the partial peptides on the cell surface along with an antigen-presenting molecule. The immunogenicity of a peptide and therefore a mRNA molecule rely on efficiency of the immunogen to act on the cell surface of the APC to generate an immune response, generating antibodies against it.

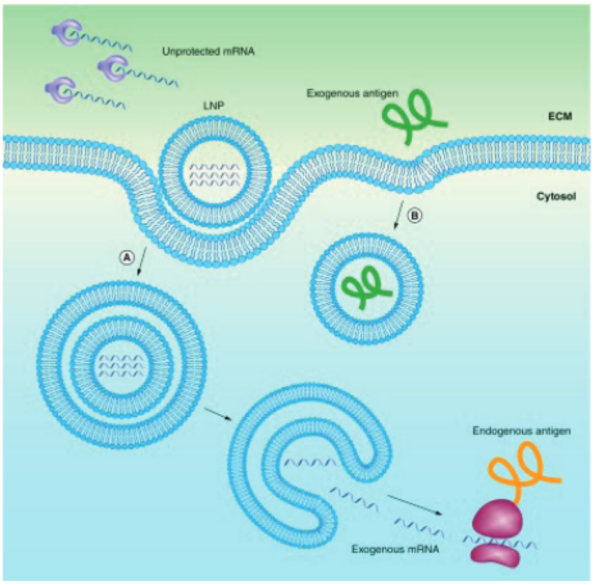

Figure 1 : mRNA molecules in lipid nanoparticles (LNP) are protected against degradation and facilitates endocytosis in to host the cell (Reichmuth et al., 2016, pp. 319–334).

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