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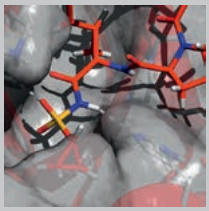

Medicinal Chemistry and Chemical Biology Highlights

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Vaccines against Covid-19, Different Strategies towards the Same Goal

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Since the end of the 18th century, and the work of Edward Jenner, several techniques have been developed to elicit protective priming/training of the immune system. Jenner discovered that the inoculation of vaccine, a non-pathogenic-in-human bovine virus, that shares enough similarity with smallpox, was able to confer protection against smallpox infection in 'vaccinated' people. Later, Louis Pasteur improved the technique by using 'attenuated' viruses to initiate a protective immune reaction. By exposing some virus-contaminated biological material to air, he could attenuate *Rabies lyssavirus* particles to the point where they could not replicate and therefore not generate a disease, but would still confer resistance to further infections from non-attenuated viruses.

In principle, the vaccination is using the capacity of the immune system to learn and adapt to the encountered pathogens. While the natural immune system is able to recognize classes of molecules like double-strand RNA (dsRNA), beta-glucoses or lipopolysaccharides, through toll-like receptors (TLR), the adaptive immunity can recognize small peptide sequences or antigens. This requires the selection and amplification of competent clones able to recognize these specific sequences. The time required to recruit and clonally expand these competent immune cells vary depending on the individual, and of course on the starting amount of these competent cells. It is then a race between the immune system and the virus. On one hand, the immune system is scanning its portfolio of lymphocytes looking for the most competent one, and on the other hand, the virus is hijacking host cells for its replication at the expense of the host's health. In this race the competitive advantage of pre-existing 'knowledge' of the virus by the immune system is crucial.

The original principle of vaccination as it was practiced for decades is the inoculation of inactivated viral particles. These viral particles are unable to infect host cells and replicate, so they allow the immune system to pre-amplify competent clones without the risk of getting sick. The challenge is inoculating sufficient amount of particle to generate an immune response to obtain a strong clonal expansion that will efficiently protect the host. One alternative to the injection of large number of particles, is the use of adjuvants. These molecules that are efficiently recognized by the immune system draw attention towards their accompanying virus particles and elicit a more efficient detection. Such use of adjuvants is widely discussed and the cause of many anti-vaccine campaigns (*e.g.* aluminum salts). While being beyond the scope of this short communication, it is interesting to note that dsRNA and ssRNA can actually be used as adjuvant themselves, due to their high immunogenicity.[1]

The sudden outbreak of the SARS-CoV-2 in 2020 put a lot of pressure on the scientific community to rapidly come up with vaccine solutions to the pandemic. A traditional approach was used with inactivated viral particles. The challenge, here, lies in virus culture *in vitro* for expansion to obtain viral particles while maintaining an unaltered strain that will resemble the strains found in the real-world (avoiding genetic drift). Several techniques are available for the inactivation: boiling, which induces denaturation and release of viral bricks in the medium (Fig. 1, panel 1) or air/chemical exposure, that can alter permanently the surface and reduce infection ability (Fig. 1, panel 2). Finally, it is still possible to inactivate the viral particles by chemical alteration of the nucleic acids contained in the virus using beta-propiolactone. Inactivated viral particles has been used mainly in China to rapidly produce vaccinal solutions (Sinovac, Sinopharm). After injection of the vaccine, the inactivated viral particles are then recognized and internalized by the antigen presenting cells (APC) of the immune system (*e.g.* macrophages). After digestion, antigens are processed into small peptides (12 to 15 amino acids) that are later exposed associated with MHC-II complexes to recruit the competent lymphocytes responsible for the adaptive immune response, ultimately resulting in antibodies production.

Another approach is the use of purified proteins that will elicit a similar immunological reaction. This strategy is widely used in laboratories to produce antibodies for research purposes. In this case, to produce a vaccine, the gene responsible for the coding of the spike protein (surface protein of the SARS-CoV-2 virus) is used *in vitro* to prepare a concentrated solution of the protein injection in the patients. There, the challenge is to produce high titer of protein in a native configuration. Since the spike protein is a membrane protein, it is usually more stable when associated with a plasmatic membrane. Another technical challenge is then the agglomeration of the protein. Detergent- or heat-denatured proteins could be used, but these treatments result in reduced immunogenicity. The vaccine Novavax was developed using purified proteins. The injected protein is taken up by immune cells, digested and processed by APC to recruit immune cells. With this vaccine format, the immune system will only produce antibodies against this protein and not another viral molecule that could be found inside the particle (nucleocapsid or viral replicases).

With the recent progresses in genetic engineering, another strategy has been used to produce vaccines: viral vector, or genetically modified virus. Firstly, a virus (vector) is genetically modified to render it innocuous by removing one essential protein required for its amplification, generally the enzyme responsible for the viral genome replication or replicase. Secondly, the vector is modified by inserting the gene coding for the targeted protein from the targeted virus. The genome size of a virus being

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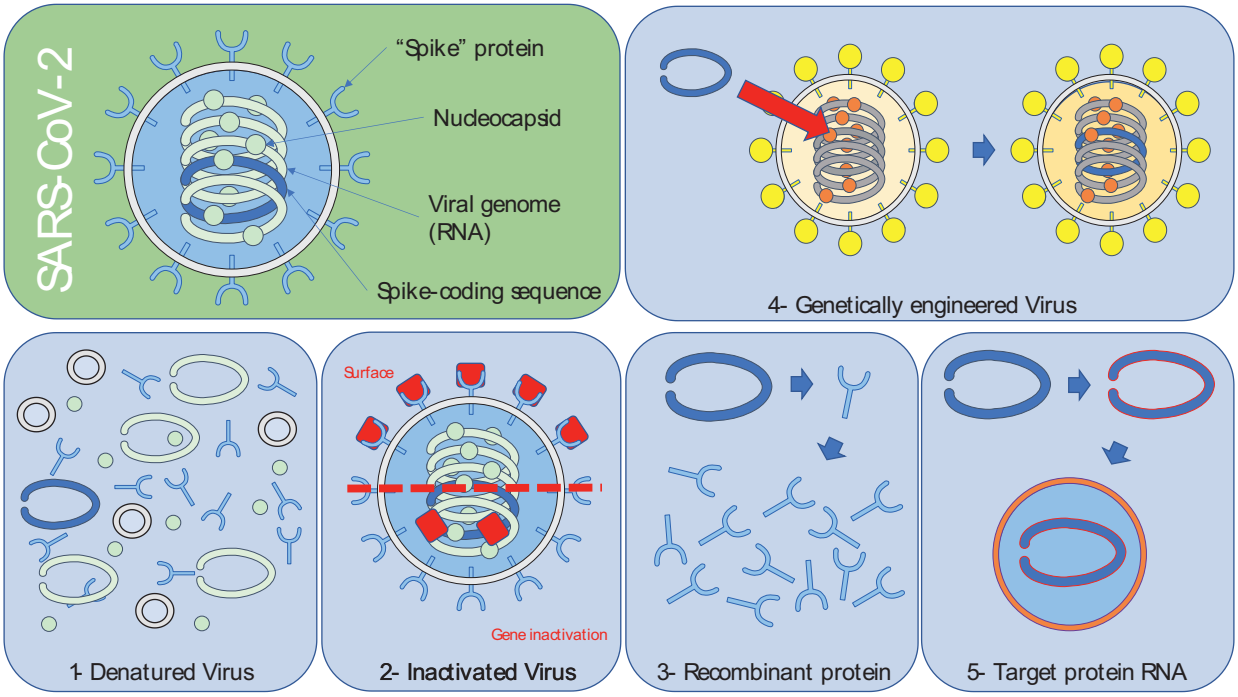

Fig. 1. Schematic representation of vaccine options for SARS-CoV-2.

limited to only small essential genes, the two modifications must be done concomitantly by exchanging the replicase gene by the target gene (spike gene). In the case of SARS-CoV-2, several vectors (strains) were used, almost all derived from heterologous recombinant adenovirus (rAd). For example, Astra-Zeneca (Chimpanzee; ChAdY25), Janssen (Human, Ad26), Sputnik V (combination of Human Ad26 and Ad5). These adenoviruses have been selected because they trigger a strong immune response. The challenge with such approach is that such incompetent viruses require co-culture with a helping virus that will provide the missing enzymes or genetically modified cell lines modified to express the missing enzymes. They are on the other hand fairly inexpensive to produce and stable at room temperature.

The most recent advances in RNA technologies have led to a new generation of products, that revolutionized the vaccine market: the mRNA vaccines. Interestingly, many advances made with RNA vaccination were previously aimed at targeting cancer cells. In principle, any mRNA, even exogenously introduced ones, could result in production of a protein.

Recent research has shown that exogenous circulating RNA transcripts can be processed by the dendritic cells (DC). DCs are part of the immune system and play an important role in the response against viruses.[2] They act as APC in collecting exogenous material and presenting it to the cells of the adaptive immunity system by combining them to a MHC-II complex. This allows the recruitment of competent lymphocytes. DCs are expressing several TLRs. Among them, TLR3 and TLR7 / 8 which recognize dsRNA and ssRNA respectively, can subsequently activate various transcription factors that, in turn, cause the release of multiple proinflammatory cytokines to attract other immune cells and induce maturation of the DCs.[3] These matured DC will then elevate their membranal expression of MHC-II and other coactivators required for the activation of the lymphocytes. The exact mechanism by which RNA-derived peptides are presented is not completely understood. It seems that unlike macrophages completely digesting their prey, DCs can recover part of the pathogens for immunity training.[4] One could think that upon entry of this exogenous RNA, part of it will trigger DC maturation, and another part will interact with the cellular machinery to translate the coding sequence into the target protein. In the case of the spike protein, it will be expressed on the plasmatic membrane. Upon endocytosis, these spike proteins will then be processed, cleaved in smaller peptides and re-expressed at the membrane, associated with MHC-II complexes for B-cell selection.

Given the short half-life of RNA in blood, the two most successful RNA-based vaccines (Moderna, Pfizer/BioNTech) used PEGylated liposomes[5] to efficiently deliver RNAs to the DCs. In addition, to improve translation, RNA sequences were flanked by a 5' CAP and a 3' poly-A tail, both modifications having also a protective function by preserving the ends from exonucleases. Finally, one of the most critical modifications made to enhance RNA stability was the replacement of all uridines in the sequence by N1-methyl-pseudouridine (N1mψ). Interestingly, this modification increases the translation of the sequence[6] and reduces immunogenicity.[7] This obtained balance between immunogenicity and protein expression seems to be extremely successful in terms of vaccination potential.

While being a world-wide challenge, the pandemic has been a strong booster for innovation in vaccine development and exposed RNA technology advances and nanomedicine under the spotlight opening the way to new innovations in pharmaceutical science.

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