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Commentary

Low-dose single-shot COVID-19 mRNA vaccines lie ahead

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Without an efficient worldwide vaccination campaign, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its variants will stay endemic in many countries and continue to restrict international socio-economic activities. mRNA vaccines are an important asset in the global fight against coronavirus disease 2019 (COVID-19). The cell-free production makes the manufacturing of mRNA vaccines easier than, for example, protein- and viral vector-based vaccines. Moreover, novel antigens can be rapidly plugged-in, which makes it possible to have mRNA vaccines against new SARS-CoV-2 variants ready for production in about 3 to 4 weeks.

In this issue of *Molecular Therapy*, de Alwis et al.¹ report on a single-dose COVID-19 vaccine that is based on a self-amplifying RNA. One low dose (2 μ g) of this COVID-19 self-amplifying mRNA vaccine elicited a robust immune response and fully protected all mice against a lethal SARS-CoV-2 infection.

Moreover, in a head-to-head comparison, the COVID-19 self-amplifying RNA vaccine outperformed by far a conventional (i.e., non-amplifying) COVID-19 mRNA vaccine. In more detail, antigen-specific T cell responses and antibody titers, including virus neutralizing antibody titers, were on average 5- and 10-fold higher in mice that received the COVID-19 self-amplifying RNA. Both mRNA vaccines encoded a SARS-CoV-2 full-length spike protein and were encapsulated in the same proprietary lipid nanoparticles (LNPs). The N1-methyl-pseudouridine modification was used in their conventional COVID-19 mRNA vaccine, as it is known to increase the efficacy by tempering the type I interferon (IFN) and inflammatory responses elicited by the mRNA backbone.² The COVID-19 mRNA vaccines from

Pfizer/BioNTech and Moderna both use this modification. As in other studies, the self-amplifying RNA vaccine did not contain modified nucleosides because it is believed that such modifications may interfere with the replication.

The performance of mRNA vaccines does not only depend on the structure of the encode antigen; the sequence of the 5' and 3' UTRs, the purification procedure, the inclusion of modified nucleosides, the LNP, and the buffer are also important. Therefore, it is possible that the COVID-19 mRNA vaccines from Moderna and Pfizer/BioNTech perform better than the conventional COVID-19 mRNA vaccine used in this study, making the difference between selfamplifying and non-amplifying mRNA vaccines smaller. Nevertheless, self-amplifying RNA vaccines are just emerging and improvements, like advanced purification protocols,³ are expected to further increase their efficiency.

Similar to other reports, the superior efficacy of the self-amplifying RNA vaccine was attributed to its higher and more prolonged in vivo translation. After cytoplasmic delivery, self-amplifying RNAs instruct the cell to produce temporarily many shorter mRNA copies of the self-amplifying RNA. These short mRNA strands, which are capped and tailed, instruct the cellular ribosomes to produce the antigen, in this case the spike protein of SARS-CoV-2. Hence, self-amplifying RNA vaccines partly move the production of mRNA vaccines from the cleanroom toward the cells of the vaccinated individual. Moreover, a few copies of the self-amplifying RNA vaccine per cell are sufficient to generate efficient antigen production.

Recently other teams also reported, in mice, strong immune responses and protection

after a single low-dose of conventional COVID-19 mRNA vaccines.^{4,5} Corbett et al.,⁵ for example, found that one dose (1 μ g) of a conventional COVID-19 mRNA vaccine protected mice against viremia. However, in the study of Corbett et al.,⁵ a non-lethal challenge with a murine-adapted SARS-CoV-2 virus was used. In contrast, de Alwis et al.¹ tested their single-dose COVID-19 self-amplifying RNA vaccine in a lethal model, which involved a challenge with wild-type SARS-CoV-2 virus in hACE-2 mice.

The de Alwis et al.¹ team also investigated the contribution of T and B cells in the protection efficacy of their COVID-19 selfamplifying RNA vaccine. They did this by depleting T and B cells through intraperitoneal injection of antibodies against CD8 and CD20, respectively. However, a superficial reading of the paper can lead to the false conclusion that B cells do not contribute to the protection efficacy of this vaccine. It is important to stress that their B cell depletion efficiency was approximately 90% and not 100%. Consequently, activated spike protein-specific CD4 Thelper cells will still find B cells that can be activated and expanded. In my opinion, B cells and antibodies are important in vaccine-mediated protection against SARS-CoV-2 infection.

Single-shot mRNA vaccines that are effective at a low dose could drastically speed up the global COVID-19 vaccination campaign. Currently the COVID-19 mRNA vaccines from Pfizer/BioNtech and Moderna require two doses of 30 and 100 µg respectively. A self-amplifying RNA vaccine against COVID-19 that is effective in humans after one shot of, e.g., 10 µg would be more practical and could allow us to vaccinate at least six times faster.

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