CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Amplifying RNA Vaccine Development

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In the early 1990s, direct injection of nucleic acids (RNA or DNA) into the muscles of mice led to in vivo expression of proteins encoded by the injected nucleic acid.[1] This finding, together with studies showing the elicitation of immune responses and protection against infection by means of the delivery of DNA that encodes pathogen proteins into the skin or muscle of mice, seeded the field of vaccinology such that only the coding sequence of a gene encoding a protein of a pathogen is necessary to create a vaccine. Early studies showed that both DNA and RNA vaccines induced immune responses. Delivery by plasmid (a small, circular extrachromosomal DNA molecule) initially emerged as the dominant strategy, and although the first clinical studies involving humans were mostly disappointing, advances in delivery and in the incorporation of immunostimulatory sequences (genetic adjuvants) have spurred new clinical trials and have informed strategies to develop vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (Covid-19).

Recent interest in messenger RNA (mRNA) vaccines has been fueled by methods that increase mRNA stability and protein production and improve delivery. These methods include the use of modified nucleosides as well as the development of nanoparticle-delivery technologies that stabilize mRNA, enhance cellular uptake, and improve the bioavailability of the mRNA once it is inside the cell. Avoidance of the risk of integration into the host genome is considered a comparative advantage of mRNA (with respect to DNA vaccines), although extensive studies have eased this concern about DNA vaccines. A clear advantage of mRNA vaccines is that, unlike DNA vaccines, they do not need to enter the nucleus to express the antigen. Instead, once inside the nucleus, a DNA vaccine will produce many copies of mRNA molecules, resulting in the production of more antigen per transfected cell. Of interest, then, are self-amplifying RNA vaccines, such as those involved in the strategy described by Beissert et al.[2] to increase the yield of antigen expressed by mRNA vaccines.

Self-amplifying RNA vaccines are derived from the genome backbone of an alphavirus in which the genes encoding the viral RNA replication machinery are intact but those encoding viral structural proteins are replaced with a transgene encoding the vaccine antigen.[3] A self-amplifying RNA vaccine can be delivered in the form of plasmid DNA, viruslike RNA particles, and in vitro transcribed RNA (Fig. 1) and can elicit substantially stronger immune responses than mRNA.[4] This immunogenicity, coupled with the ability to deliver self-amplifying RNA with the use of synthetic formulations in a cell-free and potentially highly scalable manner, makes the approach particularly attractive. DNA plasmid–based self-amplifying RNA vaccines combine the advantages of a more stable DNA nucleic acid product with greater levels of antigen expression of self-amplifying RNA vaccines to elicit stronger immune responses in preclinical models than conventional DNA vaccines.[5]

Beissert et al. describe a strategy that is based on two RNA vectors — one retaining the replicase-encoding gene and the other encoding the antigen. The replicase machinery is therefore provided "in trans" (i.e., two genes acting together but on different RNAs) by a self-amplifying RNA or a nonreplicating mRNA and mediates replication of the antigen-encoding RNA. The authors found induction of robust and protective neutralizing antibody responses in mice after immunizing them with antigen-encoding RNA expressing the influenza protein hemagglutinin at nanogram doses, although comparatively high numbers of replicase-encoding RNAs

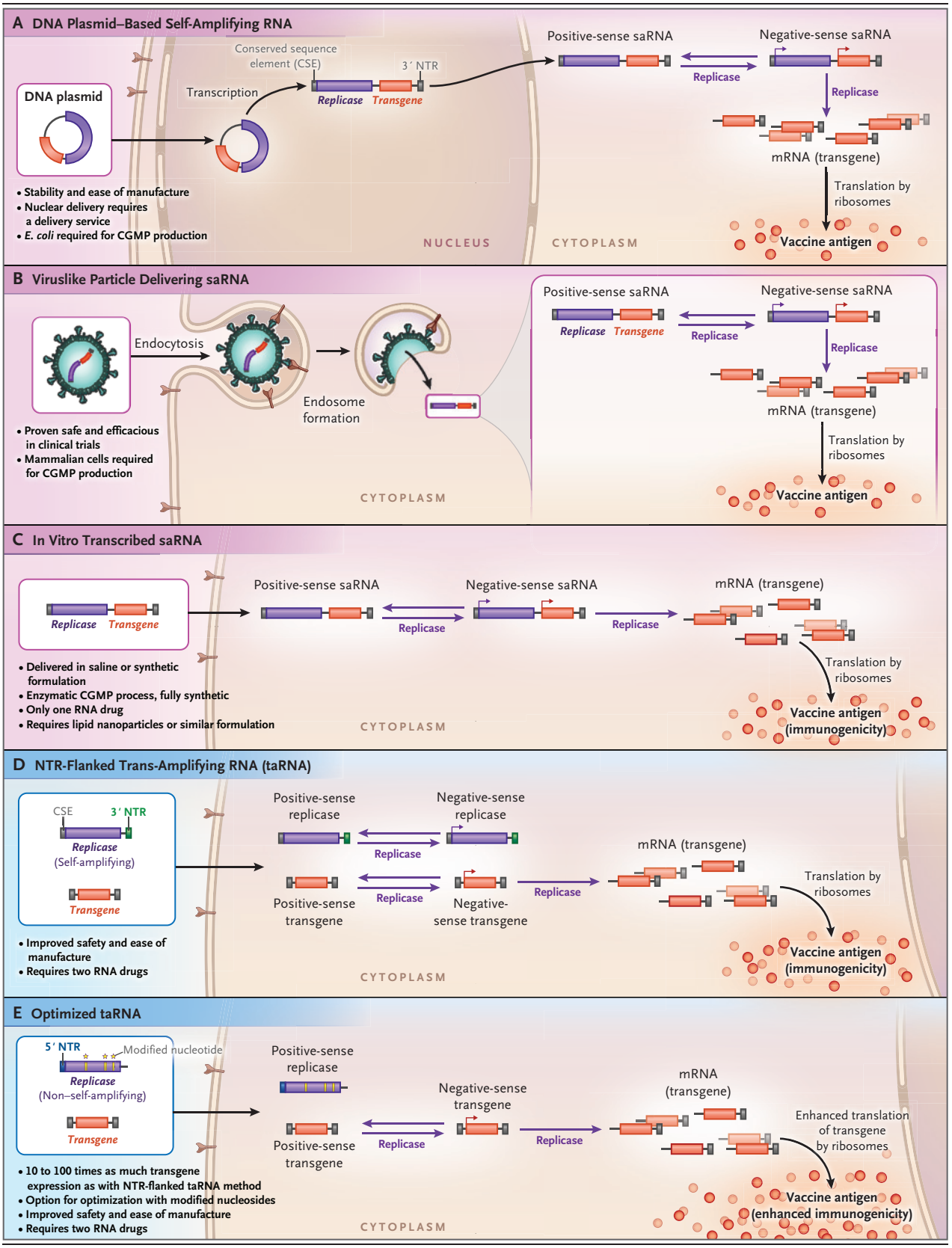

Figure 1 (facing page). Obtaining Antigen Expression by Alphaviral Replicon RNA.

Plasmid DNA carries replicase genes (encoding proteins that replicate RNA) and the transgene (which encodes the vaccine antigen) into the nucleus, where it is transcribed, generating replicon RNA (the part that encodes replicase proteins). Replicon RNA is then transported to the cytoplasm, which is then followed by RNA self-replication (also called self-amplification), messenger RNA (mRNA) production, and translation of vaccine antigen (red) (Panel A). Viruslike RNA particles that are produced in a separate packaging step (not shown) deliver replicon RNA to the cytoplasm by means of receptor-mediated endocytosis (Panel B). In vitro transcribed replicon RNA is delivered to cells either in saline or in synthetic formulations (Panel C). Common to each approach, the replicase protein complex is translated from the upstream two thirds of the replicon RNA genome (purple). The replicase initiates RNA-dependent RNA polymerase–mediated transcription of a negative strand (–RNA) using the 3′ nontranslated (NTR) region (green) and, using the –RNA as a template, also transcribes a positive strand (+RNA) from the 5′ NTR region (green), as well as a subgenomic promoter (arrow) to initiate transcription into mRNA. Many antigen proteins (Ag) are translated directly from the mRNA by cytoplasmic ribosomes. A dual strategy was described recently by Beissert and colleagues[2] in which a replicon RNA encodes the replicase machinery "in trans" to the co-delivered antigen-encoding RNA (Panel D). The authors found immunogenicity when the replicase genes were flanked by NTR regions to facilitate intracellular replication (Panel E). They observed enhanced immunogenicity when the replicase genes were optimized for translational efficiency (and lacked flanking regions). CGMP denotes Current Good Manufacturing Processes, and *E. coli Escherichia coli*.

were required. This approach offers key advantages, as compared with conventional self-amplifying RNA vaccines, in its potential for increased safety, manufacturability, and ease of optimization. The safety benefit stems from the fact that the use of two separate RNAs avoids the risk incurred with self-amplifying RNAs that are engineered to express budding-competent viral glycoproteins that could, in theory, find their way into extracellular vesicles and transfer to new host cells. With regard to manufacturability, scaled-up production can be a challenge for very long RNA transcripts, such as self-amplifying RNAs, whereas the trans-amplifying approach permits shorter lengths of RNA, albeit with two potential drawbacks: the requirement to manufacture two RNA drugs and the added complexity that is due to a need for efficient in vivo delivery of both into the same cell. Finally, as Beissert et al. point out, this approach can be further improved by implementing new strategies in mRNA technology such as nucleoside modifications, stabilizing sequences, and codon optimization of the entire replicon gene — strategies that are not yet possible for conventional self-amplifying RNA.

With the emergence of the Covid-19 pandemic, an mRNA vaccine was the first to enter clinical trials, with the first volunteers receiving the vaccine within 10 weeks after the genetic sequence of SARS-CoV-2 was released (www.modernatx.com/modernas-work-potential-vaccine-against-covid-19). Nucleic acid vaccines are now a major hope for solving this pandemic crisis. This comes as no surprise. From their earliest conception, nucleic acid vaccines were recognized as a possible solution for a rapid pandemic response. The need for only the sequence of a pathogen in order to generate the vaccine and its simplicity in manufacture have long been recognized as superpowers in nucleic acid vaccines with regard to the delivery of a rapid response to an emerging epidemic. The ability of self-amplifying RNA vaccines, and now trans-amplifying RNA vaccines, to provide amplified and durable production of antigen in vivo, coupled with potent inherent innate immune-stimulating properties, adds to these powers and may provide the dose-sparing (i.e., getting the same immune responses with smaller doses of vaccine) that will probably be needed to meet global demands. We can only hope that their deployment will render the Covid-19 pandemic crisis into a more manageable challenge, saving lives and decreasing morbidity.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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