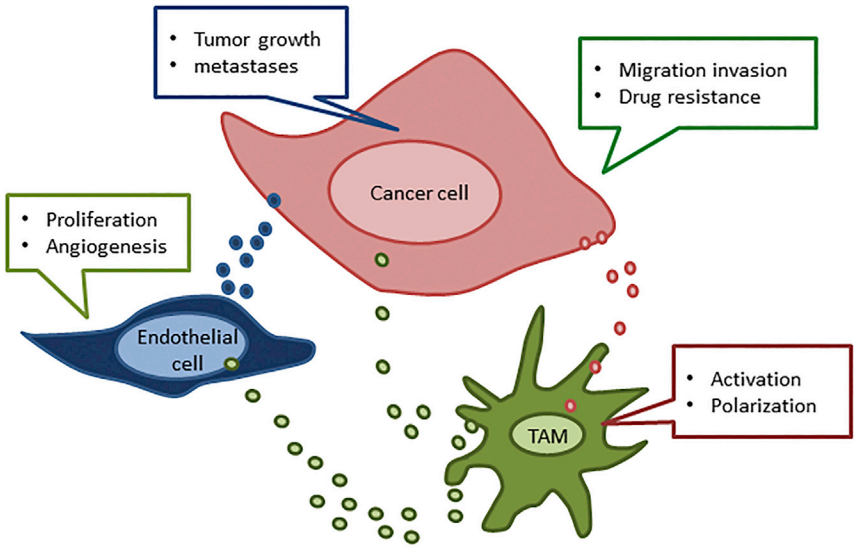

Figure 1. Tumor-Associated Macrophages (TAMs) Orchestrate Cancer-Stroma Interactions in the Tumor Microenvironment.

Exosomes secreted from cancer cells initiate M2 macrophage activation whereby, in turn, M2 macrophages secrete exosomes, which participate in the reciprocal crosstalk between TAMs to endothelial cells to cancer cells. The exosomal cargo, which includes miRNAs, affects cellular processes and thereby promotes tumor progression and angiogenesis.

plasma, and (3) targeting potential miRNAs in their cargo.

Notably, while several studies support the pro-tumorigenic role of exosomes, there are papers that suggest a role for exosomes in drug sensitization and anti-tumor immune response. Therefore, the benefits and risks of exosome depletion should be weighed for each type of cancer.

The efforts of Yang et al.[1] and other scientists to decipher the molecular mechanism involving exosome biogenesis, secretion, and crosstalk between cancer and stromal cells is beginning to bear fruit. This knowledge can be harnessed and refined, eventually allowing us to interfere with the effects of exosomes on tumor dissemination and even use them as a drug delivery platform. Initiatives should be launched to develop technologies to produce inhibitors that will interfere with exosomal biomachinery and/or to block specific molecules in their cargo.

DECLARATION OF INTEREST

The authors declare no competing interests.

REFERENCES

1. Yang, Y., Guo, Z., Chen, W., Wang, X., Cao, M., Han, X., Zhang, K., Teng, B., Cao, J., Wu, W., et al. (2021). M2 macrophage-derived exosomes promote angiogenesis and growth of pancreatic, ductal adenocarcinoma by targeting E2F2. Mol. Ther. *29*, 1226–1238.

2. Milman, N., Ginini, L., and Gil, Z. (2019). Exosomes and their role in tumorigenesis and anticancer drug resistance. Drug Resist. Updat. *45*, 1–12.

3. Binenbaum, Y., Fridman, E., Yaari, Z., Milman, N., Schroeder, A., Ben David, G., Shlomi, T., and Gil, Z. (2018). Transfer of miRNA in Macrophage-Derived Exosomes Induces Drug Resistance in Pancreatic Adenocarcinoma. Cancer Res. *78*, 5287–5299.

4. Guo, L., Akahori, H., Harari, E., Smith, S.L., Polavarapu, R., Karmali, V., Otsuka, F., Gannon, R.L., Braumann, R.E., Dickinson, M.H., et al. (2018). CD163+ macrophages promote angiogenesis and vascular permeability accompanied by inflammation in atherosclerosis. J. Clin. Invest. *128*, 1106–1124.

5. Lan, J., Sun, L., Xu, F., Liu, Lu, Hu, F., Song, D., Hou, Z., Wu, W., Luo, X., Wang, J., et al. (2019). M2 macrophage-derived exosomes promote cell migration and invasion in colon cancer. Cancer Res. *79*, 146–158.

6. Guo, W., Li, Y., Pang, W., and Shen, H. (2020). Exosomes: A Potential Therapeutic Tool Targeting Communications between Tumor Cells and Macrophages. Mol. Ther. *28*, 1953–1964.

A Tolerizing mRNA Vaccine against Autoimmunity?

Roberto Furlan[1]

https://doi.org/10.1016/j.ymthe.2021.02.003

Vaccines based on mRNA are new tools in the fight against infectious disease and have received much attention in the present pandemic. In fact, the first two approved coronavirus disease 2019 (COVID-19) vaccines in the United States are mRNA vaccines. A recent paper in *Science* now shows that this technology, which has been optimized to induce strong, protective immune responses against specific pathogens, might be adaptable to achieve just the opposite, which is to achieve antigen-specific immune tolerance to treat autoimmune diseases.[1]

As shown in the upper panel of Figure 1, the method of mRNA vaccination is typically based on injection into the muscle of a mRNA encoding a viral protein encapsulated in liposomes made of lipids with inherent pro-inflammatory activity. These nanoparticles will be taken up by antigen presenting cells (APCs) in the tissue, which will also be activated by the lipid-mediated pro-inflammatory signals and the engagement of the Toll-like receptor 7 (TLR7) by extracellular RNA that is generated in the

[1]Clinical Neuroimmunology Unit, Institute of Experimental Neurology (INSpe), San Raffaele Scientific Institute. IRCCS Ospedale San Raffaele, Milan, Italy

Correspondence: Roberto Furlan, MD, PhD, Clinical Neuroimmunology Unit, Institute of Experimental Neurology (INSpe), San Raffaele Scientific Institute. IRCCS Ospedale San Raffaele, Milan, Italy.
E-mail: furlan.roberto@hsr.it

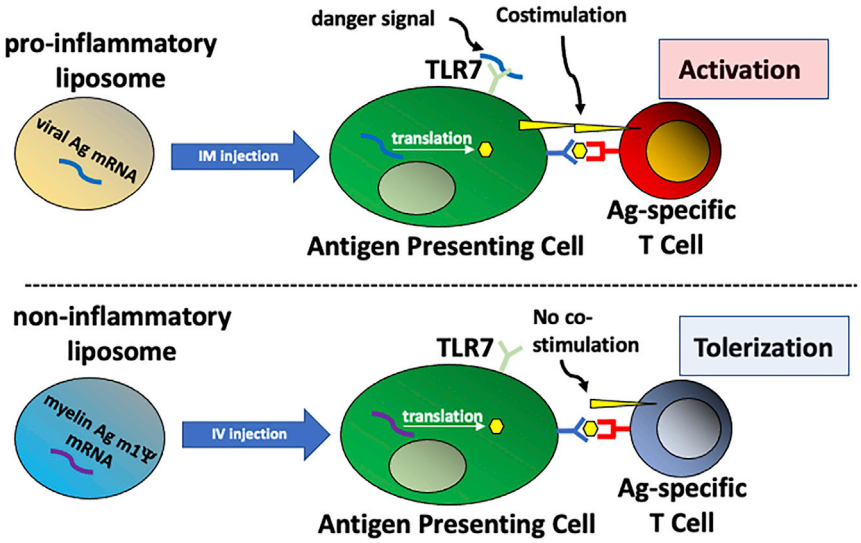

Figure 1. Principles of Activating or Tolerizing Immunization.
Liposomes constituted using pro-inflammatory lipids and carrying a viral antigen mRNA are injected in muscles to induce local antigen presenting cells (APCs) to uptake and translate the mRNA to its corresponding protein in a pro-inflammatory environment, consequently inducing downstream activation of antigen-specific T cells (upper panel). In contrast, non-inflammatory liposomes injected in the blood to deliver a mRNA that lacks danger signals results in induction of antigen-specific T cell tolerizance and inhibition of autoimmunity (lower panel).

site of injection by breakage of some liposomes. While migrating to the lymph node, APCs will subsequently translate the mRNA to its corresponding protein and load it on their major histocompatibility complex (MHC) class I or MHC class II molecules, depending on the processing and route of uptake. In the lymph node, APCs will eventually encounter a T cell specific for the viral antigen. Successful activation of the antigen-specific T cells occurs through appropriate costimulation provided by the APCs, which themselves have been activated by the pro-inflammatory signals received at the injection site. These soluble signals are most likely also drained to the lymph node along with the APCs.[2] Costimulation is crucial because, without it, antigen presentation results in antigen-specific tolerization or even clonal deletion of antigen-specific T cells.[3]

Krienke et al.[1] have exploited this latter principle to induce antigen-specific tolerization in a mouse model of the autoimmune disease multiple sclerosis. The mouse model of multiple sclerosis is called experimental autoimmune encephalomyelitis (EAE) and is based on the induction of an autoimmune reaction against brain and spinal cord oligodendrocytes by immunization with central myelin antigens. As shown in the lower panel of Figure 1, to achieve tolerization, the authors encapsulated an mRNA encoding a myelin antigen in liposomes made of lipids devoid of pro-inflammatory activity. Importantly, the authors have also synthesized the mRNA replacing the nucleotide uridine with 1-methylpseudouridine (m1Ψ), with the result that this m1Ψ mRNA can induce protein translation but cannot engage TLR7. Finally, injection of these liposomes into the blood stream has ensured the uptake by circulating or lymphoid tissue-resident APCs, but avoided the potential tissue-derived danger signals, and draining to the same lymph node as the targeted APCs. This strategy was not only highly effective in treating EAE induced by the very same antigen used to tolerize, but also inhibited EAE induced by a different antigen. It appears that cross tolerization of tissue-specific autoimmune T cells was achieved without impairing the overall ability of the immune system to mount an appropriate immune response to a new antigen. Several strategies have been explored to induce immune tolerance in autoimmune diseases, some of which have been tested also in humans with variable, sometimes paradoxical, results.[4] This novel approach has now been tested in inbred mice strains, in a very well-defined model of autoimmunity, induced by known antigens. However, it has not yet been tested in EAE induced by immunization with spinal cord homogenate, which would have led to a plethora of different autoreactivities more similar to most human autoimmune diseases. If, however, the principle of cross-tolerization holds true also in complex human autoimmunity and the tolerogenic vaccination does not result in accidental activation against the delivered antigen, then this approach would constitute a powerful and flexible new tool to treat autoimmunity. After all, there is an enormous unmet clinical need for treatment of autoimmune diseases, which are estimated to affect about 4% of the global human population.

REFERENCES

1. Krienke, C., Kolb, L., Diken, E., Streuber, M., Kirchhoff, S., Bukur, T., Akilli-Öztürk, Ö., Kranz, L.M., Berger, H., Petschenka, J., et al. (2021). A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis. Science *371*, 145–153.

2. Pardi, N., Hogan, M.J., Porter, F.W., and Weissman, D. (2018). mRNA vaccines - a new era in vaccinology. Nat. Rev. Drug Discov. *17*, 261–279.

3. Abbas, A.K., Lichtman, A., and Pillai, S. (2017). Cellular and Molecular Immunology, 10th Edition (Elsevier).

4. Steinman, L., Ho, P.P., Robinson, W.H., Utz, P.J., and Villoslada, P. (2019). Antigen-specific tolerance to self-antigens in protein replacement therapy, gene therapy and autoimmunity. Curr. Opin. Immunol. *61*, 46–53.