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Comparative Analysis of the Two Innovative Vaccines

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Abstract. Multiple sclerosis (MS), one of the most prevalent autoimmune diseases, poses a significant challenge for therapeutic advancements. Among these, antigen-specific therapies (ASTs) utilizing therapeutic vaccines have emerged as highly promising. Designed to induce antigen-specific tolerance without compromising the overall immune system, these vaccines offer a glimmer of hope for MS patients, despite many still being in the preclinical trial phase. This research provides an elaborate comparison of two distinct vaccine types: m1Ψ mRNA vaccines and ROS-scavenging CeNP MSN vaccines. As AST vaccines targeting MS, they exhibit notable similarities across various facets, ranging from superficial aspects such as the models employed to evaluate therapeutic effects to deeper considerations like their anti-inflammatory characteristics and key mechanistic factors. Beneath these similarities, significant differences are identified and analyzed within this article. Additionally, the advantages, disadvantages, and noteworthy features of each vaccine are thoroughly compared, accompanied by corresponding suggestions for improvement. Through this comparative analysis of the two innovative vaccines, the paper aims to enhance readers' comprehension of MS treatments. By offering humble insights, it aspires to make a positive impact on related research, ultimately contributing to the advancement of MS therapeutic approaches.

Keywords: antigen-specific therapies; multiple sclerosis; mechanism.

1. Introduction

In 1989, the Robert Malone team elaborately illustrated how they successfully transferred mRNA into tissue culture cells using a synthetic cationic lipid [1]. It is generally believed that this research marks the birth of mRNA vaccines. After that, the mRNA vaccine technology began its rapid development. Modified mRNA vaccines are one of those innumerable branches of vaccine science that is now experiencing a renaissance [2]. In these novel approaches, the nanoparticle encapsulating mRNA or the nucleosides of mRNA in vaccines are slightly modified so as to equip the vaccines with some unique features that common mRNA vaccines don't obtain, including inducing a much stronger immune reaction or, on the contrary, achieving antigen-specific tolerance [2].

Nowadays, the applications or related experiments of modified mRNA vaccines have encompassed a wide range of diseases, from influenza and genital herpes to Zika fever, multiple sclerosis, and even tumors [2]. In 2020, two synthetic RNA vaccines were approved by the FDC to fight against COVID-19, one of the most severe pandemics in decades [3]. In one of the vaccines, BNT162b2, the uridines in the mRNA molecules are replaced with N1-methylpseudouridine (m1Ψ) [3]. As mentioned above, modified mRNA vaccines also offer an opportunity for the treatment of autoimmune diseases [2]. For many years, researchers have been seeking an ideal therapy for autoimmune diseases that should be able to selectively blunt autoimmunity, or at least enable relevant repairing mechanisms to take place, without compromising normal immune function [4]. Now the researchers have realized that antigen-specific therapy (AST) might be an answer since it has the unique feature of accurately targeting only antigen-specific cells, which makes it promising to achieve the goal of suppressing only tissue-damaging autoimmune responses while simultaneously ensuring that normal immune functions are well preserved [4].

To further study autoimmune diseases and explore better treatments, many researchers focus on a neurological disease called multiple sclerosis (MS), which is usually diagnosed in young adults aging 20–40 [5]. For those who are advancing into the prime of life, being diagnosed with MS is a tragedy

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because each attack affects normal bodily functions, including those in the cognitive, emotional, motor, sensory, or visual domains [6]. Therefore, it is necessary to speed up the research on MS. In 2021, Krienke et al. Found that in EAE, with the help of their modified m1Ψ vaccine, the onset of MS can be delayed and the severity of established disease can be reduced; in the meantime, normal immune functions aren't affected, which proves that modified mRNA vaccines have the potential to treat autoimmune diseases as an AST [2]. One year later, Nguyen et al. published their research and claimed that with the help of their mesoporous silica nanoparticles (MSN) vaccine with cerium oxide nanoparticles (CeNP) can also induce antigen specific tolerization, and thus is promising in treating MS [7]. To comprehensively assess the pros and cons of mRNA vaccines, we opted to compare m1Ψ with this alternative AST that deviates from the mRNA vaccine approach, and we will place greater emphasis on m1Ψ in our analysis. In this research, these two ASTs will be compared to help the readers have a better understanding of, in the case of treating multiple sclerosis, in what ways modified mRNA outperforms MSN vaccine therapy, in what ways MSN outcompetes m1Ψ, and in what ways they have to be improved, and what potential the two vaccines possess.

2. Mechanisms

The two studies compared here exhibit several similarities. Superficially, both groups of authors chose an experimental autoimmune encephalomyelitis (EAE) to evaluate their vaccines. The EAE is a pre-clinical model that is clinically relevant for multiple sclerosis. To allow researchers to test novel therapies, the mice are injected with myelin oligodendrocyte glycoprotein (MOG) or proteolipid protein (PLP), so that in the coming days symptoms will occur. Moreover, the therapies presented in both articles take the form of therapeutic vaccines. Nguyen et al. discuss the development of an immunosuppressive vaccine for MS therapy utilizing self-antigen-loaded mesoporous nanoparticles and reactive oxygen species (ROS)-scavenging cerium oxide (CeO_2) nanomaterials [7]. In the case of Krienke et al, a vaccine with noninflammatory lipids and modified mRNA molecules, in which N1-methylpseudouridine (m1Ψ) acts as uridine, is introduced and assessed [2].

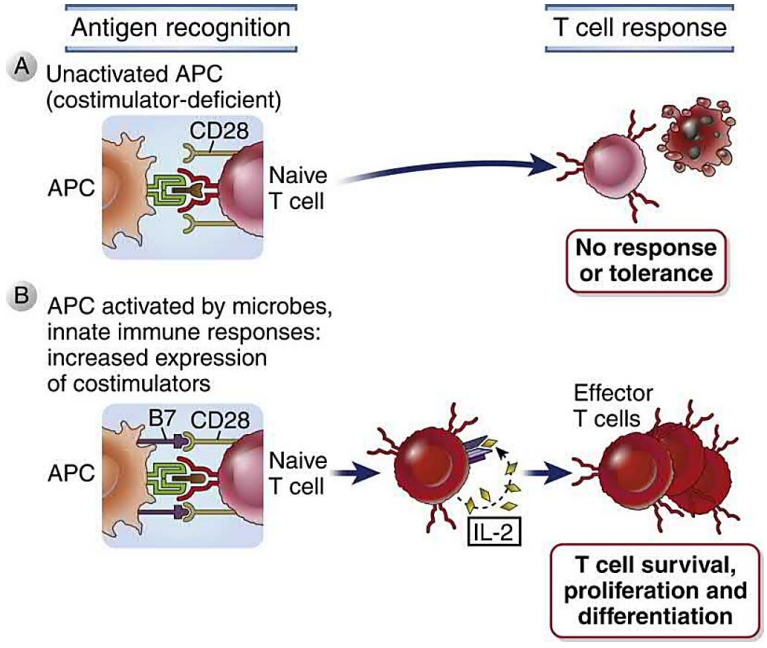

Figure 1. Functions of costimulatory in T cell activation [8].

Theoretically, the two studies also share great similarities. Their common goal is to induce antigen-specific tolerization while ensuring normal immune functions still protect the human body from other potential threats, such as pathogens or abnormal cells. In order to explain in depth how the two groups of researchers achieved this goal, the relevant mechanisms of autoimmune diseases and those of the two vaccines will be briefly summarized and analyzed here. When triggered by infections, sensitive genes may disrupt self-tolerance mechanisms, and the influx and activation of autoreactive

lymphocytes will be promoted, resulting in tissue injury [8]. To eliminate allergens, native T cells will proliferate, and then some will differentiate into effector T cells while some become memory cells, all of which happens after being activated by one antigen [8], and antigen-presenting cells (APCs) play a significant role. In addition to presenting antigen and secreting cytokines, they have another important task, namely to form a costimulation binding with the naïve T cell [8], as shown in Fig. 1.

Krienke et al. sought a breakthrough and modified the vaccine on the basis of this mechanism. As mentioned above, uridines in the mRNA molecules are replaced with m1Ψs. As a result, the modified mRNA molecules will not bind with a receptor named toll-like receptor 7 (TLR7), so that APCs cannot be activated, which will then result in failure of the process of stimulation [9]. Antigen recognition without stimulation may make antigen-specific T cells unresponsive (tolerant). In other words, if there isn't a stimulation, then autoreactive cells cannot be activated. This explains how m1Ψ mRNA vaccines function to induce T cell toleration [8].

However, if all the costimulatory are not working, it will lead to systemic immune suppression. In this case, the researchers think they have pulled off the trick, as their mRNA vaccines don't engage with TLR7, but in the meantime, they have preserved the function of inducing certain types of proteins, which are MOG35-55, the epitope of myelin oligodendrocyte glycoprotein. That means the proteins translated by this mRNA vaccine will only trigger the tolerization of certain T cells instead of affecting the whole immune system [2, 9].

For autoimmune responses, where antigen-specific autoreactive T cells are running amok, tolerogenic APCs, which are essential for inducing Treg cells (introduced below), will turn out to be of great use. To effectively induce tolerogenic APCs, Nguyen et al. came up with two powerful methods. Foremost, they take advantage of so-called MSN, which show great capacity for loading antigen, to significantly increase the number of self-antigens delivered to splenic APCs and so induce the activation of these APCs [7]. These APCs can only express a relatively low level of costimulatory molecules, which enables the related T cells to be tolerized. Besides, in order to enhance the activation of tolerogenic APCs and the following immune tolerance they induced, another efficient weapon was introduced, which has a complicated name: reactive oxygen species-scavenging cerium oxide nanoparticles, abbreviated as ROS-scavenging CeNP [9]. After adding these nanoparticles to the surface of the vaccine, the researchers found that the number of autoreactive $CD4^+$ cells was significantly decreased, which will definitely and antigen-specifically slow down the development of EAE [9].

3. Self-antigen

In both vaccines, MOG35-55 plays an important role. The Nguyen group chose to load a sufficient amount of MOG35–55 peptide into synthesized MSNs so that these self-antigens can immediately be taken up by APCs, and after that, with the enhancement provided by ROS-scavenging CeNP, trigger the following mechanisms, which, as mentioned above, include APC activation and specific T cell tolerization. In contrast, no MOG peptides can be found in the Krienke group's m1Ψ vaccine; instead, m1Ψs are responsible for the manufacture of these peptides, and the translation will begin nearly as soon as they are taken up by APCs, where peptides are produced by ribosomes and then brought to the surface of the cell by MHC molecules so as to bind with T cell receptors (TCRs) and thus activate naïve T cells. It can be briefly summarized as follows: the MOG35–55 peptides in the Nguyen group have been readily available since the vaccines were made, whereas in the Krienke group, these peptides can only be found after the vaccines are injected because they need to be translated by ribosomes using modified mRNA as a template.

4. Potential inflammatory conditions

Noninflammatory is truly a highlight that makes it unique from the massive amount of modified mRNA vaccines. This noninflammatory feature of their vaccine is mainly manifested in two different aspects. First of all, experiments have confirmed that in comparison with mRNA with uridine, m1Ψ

vaccines showcased a better quality to function while not inducing the secretions of certain inflammatory cytokines, such as IFN-α. After that comes the key factor that makes the vaccine stand out: the non-inflammatory liposomes. The delivery process shows an important impact on the therapeutic effect of the vaccine, as the inflammatory conditions could pose potential obstacles to the desirable tolerization. To overcome this difficulty, Krienke et al. designed a special liposome that is devoid of pro-inflammatory activity [9]. Unlike common liposomes which would activate APCs since their pro-inflammatory signals would be received by APCs at the injection site, this noninflammatory liposome could maximally avoid causing such substantial complications. The liposome formulation was not elaborately illustrated in their article, but it was mentioned in another research [10].

Despite not having been emphasized, the noninflammatory character can also be found in existing research. In their series of experiments, sufficient data confirmed this quality from different aspects. Microscopically, when focusing on cellular reactions, the authors pointed out that, via mechanisms including reduction of certain inflammatory cytokines, there is a potency of their MOG-MSN to induce systemic AST. Macroscopically, when focusing on the therapeutic effect, it is obvious to be observed that MSN-MOG mediated recovery from neuroinflammation, with signs including immediately and greatly hampered progression of clinical episodes, body weight recovery and regaining of mobility after paralysis [9]. Moreover, when additional doses are given, the anti-inflammatory characteristic can also be well reflected. As revealed by H&E-stained histological images of the ventral area of the white matter of the EAE mice, the number of inflammatory cells has experienced an obvious decline.

5. $Foxp3^+$ Treg

Regulatory T cells (Treg cells) are a special group of T cells that suppress the immune response in an antigen-specific way. Treg cells can be divided into two subsets more specifically, namely naturally occurring Treg cells and adaptive Treg cells. The former plays a significant role in preventing autoimmune disease, so it would be easy to comprehend that in autoimmune disease patients, these naturally occurring Treg cells are often unable to function normally. Therefore, researchers, including the Krienke group and the Nguyen group, who focus on therapies for autoimmune diseases, including MS, pin their hopes on the latter one, adaptive Treg cells. These adaptive Treg cells have the ability to secrete certain cytokines that have the function of inhibiting autoimmune reactions, i.e., by reducing the number of receptors on the involved cells surface.

$Foxp3^+$ Treg cells, which have the crucial ability to suppress $CD4^+$ T cells so as to trigger immune tolerance, play an important part in both studies, and related cytokines are also significant indicators when evaluating therapeutic effect. $Foxp3^+$ Tregs are observed to be significantly induced as a part of the cellular reactions toward the injection of MSN-MOG. Although the authors didn't elaborately illustrate the mechanism of the phenomenon, in which both numbers and frequencies of $Foxp3^+$ Tregs are increased, they provided sufficient data to prove this important fact. In one experiment after treatment with peptide-loaded MSNs, a decrease in splenic $CD4^+$ T cells and a noticeable increase in both the number and frequency of $Foxp3^+$ Tregs were observed at the same time. Nguyen et al. also pointed out that this can be seen as a desirable change to treat EAE [9].

To further prove the satisfying therapeutic efficacy attributed to Tregs, Nguyen et al. came up with a simple but convincing way. If these effects dissipate after Tregs are made unable to function normally, the conclusion can then be reasonably drawn that Treg cells play an irreplaceable part in the vaccination process. To induce a depletion of Tregs in one of the experiments, the whole vaccination was carried out under anti-Treg antibody administration. Researchers were not surprised to find out that the once-outstanding therapeutic effect had diminished. Meanwhile, the $CD4^+$ T cell infiltration resulted from the depletion of Tregs has declared the inhibited efficacy of MOG-MSN. In the research conducted by Krienke et al., $Foxp3^+$ Treg cells are also one of the crucial factors utilized to suppress $CD4^+$ T cells. Acting as a decisive indicator, its presence tells the researchers whether the results meet

their expectations. In many experiments, expansions of $Foxp3^+$ Tregs are observed, similar to the Nguyen group.

6. In What Ways Modified m1Ψ Outperforms MSN?

Most parts of the experiments presented in the two papers are conducted in EAE, but it is worth noting that the EAE model is only clinically relative, which means the therapeutic effects observed in the immunized mice don't equal the real effect on MS patients. If the EAE model is the only model utilized to illustrate the efficacy of the vaccine, then it cannot be said to be a lack of rigor in the experimental design but can be seen as a small flaw. However, the Krienke group's design was very thorough, as in addition to EAE, they also used other similar mouse models related to MS to prove the efficacy of their vaccine. Take SJL model mice, for example, where the EAE is induced by PLP139~151 epitope but not MOG35~55. Therefore, they came up with a special version of their vaccines by altering the epitope encoded by the mRNAs, i.e., replacing MOG-encoding mRNAs with PLP-encoding ones. By making this effort, they not only ensured that the successful therapeutic effect displayed in MOG-EAE mice is not a matter of luck but also indicated that the mechanism of their vaccine has a wide range of applications, allowing them to down-regulate different types of autoimmune T cells by slightly changing the modification. This feature also makes the m1Ψ vaccines even more promising when symptoms show a refractory nature in certain patients, since they may be triggered by more than one peptide. Under such circumstances, using different types of m1Ψ vaccines may achieve the goal. On the contrary, only one version of the EAE model is introduced, which is a tiny defect that doesn't affect the whole research. It would be even better if Nguyen et al. Could make use of multiple models to make their evidence even more conclusive.

As mentioned above, m1Ψ vaccines may have the potential to treat refractory MS symptoms when used in a series of mixtures of different peptide-encoding mRNAs, similar to the case of cocktail therapies. However, even more surprisingly, according to the Krienke group's research, the idea of using such cocktail therapies with m1Ψ vaccines may be practical but unnecessary. One groundbreaking phenomenon discovered in their experiments, termed the bystander effect, may be the silver bullet to optimize the therapy. As mentioned in their article, sometimes the direct target of tolerization can't be made clear, which poses an obstacle to the efficacy and specificity of the therapeutic vaccines but may be perfectly solved with the help of bystander tolerization.

Bystander tolerance is taken advantage of by the induction of Treg cells, which would suppress T cells targeting other antigens once activated. Krienke et al. conducted two experiments to elaborately illustrate the bystander effect showcased by MOG35~55 m1Ψ mRNA vaccines. In the first experiment, three groups of vaccines, namely PLP m1Ψ, MOG m1Ψ, and irrelevant m1Ψ were evaluated on mice whose EAE is induced by PLP peptide. Normally only PLP m1Ψ vaccines will function when there isn't a bystander effect, and therefore only antigen-specific tolerization is able to inhibit the disease from exacerbating. To the researcher's astonishment, the therapeutic effect of MOG m1Ψ vaccines is almost as great as the curative effect displayed after PLP m1Ψ vaccinations, indicating that bystander suppression has helped overcome the difficulty of suppressing possible non-cognate autoimmune T cells, as there is strong evidence that after both PLP and MOG M1Ψ vaccinations, antigen-specific T cells were not only notably expanded but highly activated as well. In the following experiment, a vaccine with a mixture of m1Ψ and another one encoding only MOG peptides were compared in a complicated model with EAE driven by multiple peptides, as shown in Fig. 2. Living up to the researchers' expectations, MOG m1Ψ vaccines showed a curative effect very similar to that of cocktail-encoding mixtures. By that, the results showed us a grand blueprint where a great number of subtypes of MS would be treatable with only one type of vaccine. Nevertheless, it is still too early to draw a conclusion since the research is still in its early stages, and it can't be ignored that, after all, the effect of bystander tolerization is limited. Even though the efficacy of MOG is comparable to that of cocktail therapy, it still cannot reach the level of the antigen-specific group or the cocktail group.

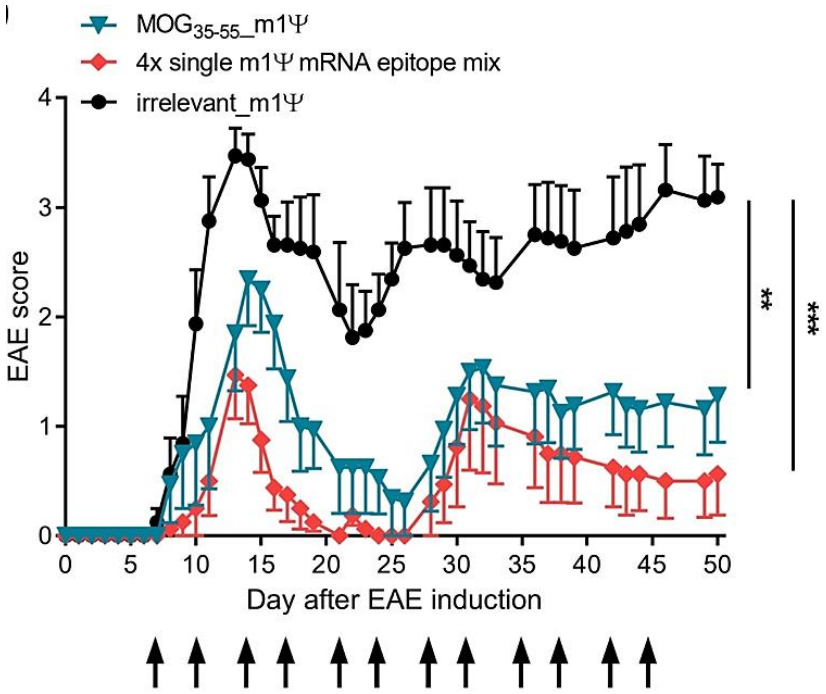

Figure 2. Induction and maintenance of antigen-encoding m1Ψ mRNA-induced tolerance [2].

It is worth noting that behind the satisfying therapeutic effect thanks to the bystander effect, a problem quietly arises. The initial goal of Krienke et al. was to induce antigen-specific tolerization without suppressing overall immune function, but bystander tolerization has broken the limits of antigen-specific in some ways. The problem is that it is still unknown if the bystander effect has the potential to pose a threat to the specificity of the target of the vaccines, as non-cognate T cells can also be affected. Though in one of the very first experiments introduced in the Krienke group's research no impairment of the capability of mounting normal immune responses was observed, it can't be proved that bystander tolerization is 100 percent harmless. Similar opinions were expressed by Onur Boyman, whose research pointed out that immune homeostasis might be affected by $CD4^+$ T-cell bystander activation [11].

7. In What Ways MSN Outcompetes m1Ψ?

On the other hand, the Nguyen group's ROS-scavenging MSN vaccine also has some special features that make it outcompete m1Ψ mRNA vaccines in some terms. As mentioned above, though not emphasized, the ROS-scavenging MSN vaccine also possesses anti-inflammatory properties. But what would happen if the vaccine itself was in an inflammatory environment after being injected? The enhancement of the therapeutic effect provided by CeNPs cannot be underestimated, since even when being interfered with by LPS, a TLR4 agonist, they were still able to inhibit the activation of APCs. When the Nguyen group's vaccine, attached to these powerful nanoparticles, was used in BMDCs cultured with LPS, the results proved that being under inflammatory conditions didn't weaken its ability to attenuate oxidative stress. On the contrary, Krienke et al. didn't conduct such experiments to find out if their vaccine could function normally in inflammatory conditions, which could be improved. Applying CeNPs to MSN vaccines was an imaginative but successful attempt. Though imaginative, it was not a matter of luck, as the idea of utilizing CeNP as an adjuvant was not random "trials and errors", but based on researchers' reasoning and hypotheses. To everyone's relief, their bold attempt brought them a surprise at last, because there are also thousands of groups of scientists whose results didn't meet their expectations. It is noteworthy that most of these adjuvants tried in numerous experiments, both the ones that succeeded and failed or had little effect, are organic compounds, especially immunosuppressive molecules [7]. However, this time they were all overcompeted by a "humble" inorganic CeNP. Just as self-commented by Nguyen et al., it was indeed "simple but effective". The promising ROS-scavenging property showcased by CeNP will definitely

broaden the horizons of scientists all around the world and draw their attention to inorganic adjuvants, in which there must be innumerable hidden potentials waiting to be dug out. Nevertheless, no such adjuvants were added to the Krienke group's modified mRNA vaccines. They would probably show even more astonishing therapeutic effects or anti-inflammatory characteristics with the help of proper adjuvants.

Furthermore, binding CeNP with MSN is also a great choice. Because of the substantial loading capacity of MSNs in comparison to alternative carriers, a minimal quantity of carriers can effectively deliver an appropriate amount of EAE-associated antigen to the APCs. The stability of the attached CeNPs was also surprising. And these nanoparticles could be maintained by the force of electrostatic interactions even after incubation in PBS for up to 3 days [7]. As sustained release was observed, we can speculate that it may have properties similar to sustained-release drugs, which may give it some unexpected attributes, such as not having too severe side effects or a longer-lasting therapeutic effect.

This article originally wanted to compare and evaluate the efficacy of the two vaccines through experimental indicators and the EAE clinical score, but later found that this comparison may not be meaningful. Although both groups of vaccines are intravenously injected and the mice used in the experiments are both C57BL/6, there are many other differences that reduce the comparability of the experiments. For example, the vaccination routines are different, which may affect the onset time and effect. Furthermore, both groups of authors designed and conducted multiple sets of experiments. In these experiments, they repeatedly changed the vaccine's ingredients, adjuvants, action environment, targeted autoantigen types, and even mouse models, striving to fully prove the practicality of vaccines or to find the most effective ingredient combination, but this also brings difficulties to systematic comparisons between different vaccines.

Overall, the vaccines produced by both groups of researchers have excellent therapeutic effects and can control the clinical score close to or even below 1 point, which can be considered extremely promising to treat or even cure multiple sclerosis.

8. Conclusion

This research compared the two ASTs, respectively MOG-m1Ψ mRNA vaccine and MOG-MSN-CeNP vaccine, providing readers with enhanced comprehension regarding the superiority of both modified mRNA over MSN vaccine therapy in treating multiple sclerosis, areas where it requires improvement, and the potential possessed by m1Ψ vaccines. For the similarities, both vaccines focus on antigen-specific tolerization, have anti-inflammatory properties, see $Foxp3^+$ Tregs as a key factor, and are evaluated in EAE during sets of experiments. While m1Ψ, as an outstanding representative of modified mRNA vaccines, shows a unique quality of bystander effect, MSN makes itself stand out with its innovative ROS-scavenging CeNPs. Despite large differences existing in both experiment design and detailed mechanism, they show equally promising therapeutic effects. And it is believed that by learning from each other, the effects of both vaccines can still be largely improved.

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