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EDITORIAL

Shedding Light on Mechanisms of Myocarditis With COVID-19 mRNA Vaccines

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Myocarditis is recognized as a rare complication of coronavirus disease 2019 (COVID-19) vaccinations, especially in young adult men and adolescent boys.[1,2] Incidence is approximatel 1 to 5 cases among 100 000 in the general population, and 1 in 20 000 among those16 to 30 years of age.[2] Cases most often occur days after the second vaccine dose and are usually mild and self-limited.[2]

Article, see p 867

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines contain nucleoside-modified synthetic mRNA, encoding the viral spike glycoprotein. Current COVID-19 mRNA vaccines, BNT162b2 (Pfizer) and mRNA-1273 (Moderna), are nonreplicating mRNA vaccines that encode full-length spike proteins packaged into lipid nanoparticles, which shield from enzymatic breakdown and facilitate entry into cells, where the mRNA is translated into the spike protein, and then delivered to the cell surface, where the spike protein is expressed and processed for immune recognition.[2] mRNA vaccine injected into the deltoid muscle can result in spike protein expression in the muscle tissue, as well as in the lymphatic system and other cells after entry into the circulation.[3]

In this issue of *Circulation*, Yonker et al provide important information on persistently elevated spike protein levels in adolescents and young adults who developed myocarditis after SARS-CoV-2 mRNA vaccination.[4] Clinical presentation of myocarditis was similar to former reports[2]: the majority were men and boys with symptoms—usually chest pain—that occurred within 4 days after a second dose of the vaccination; along with elevated cardiac troponin and C-reactive protein levels.[2,4] Patients with myocarditis had persistently elevated and freely circulating full-length spike protein levels unbound by antibodies as many as 3 weeks after vaccination. Spike protein elevations were similar to those with multisystem inflammatory syndrome in children. None of the healthy controls had detectable free spike protein in their plasma at any time after vaccination. There were no significant differences in humoral or specific antibody responses (eg, anti-spike or anti–receptor-binding domain [RBD] immunoglobulin M, G, or A levels), or their neutralization capacity or ability to engage Fc receptors, self-antibodies, or other viral antibody levels in patients with myocarditis or healthy controls after vaccination. Levels did not suggest a hyperimmune or inappropriately high response, and there were only minor, nonsignificant differences in T-cell populations. There was evidence of activation of innate immunity with significant elevation of proinflammatory cytokines. Although the majority of the patients with myocarditis were men, elevated spike protein levels were present in both affected women and men. The cleaved S_1 subunit of the spike protein was not detectable in a free or antibody-bound form in adults, whereas an antibody-bound form was detectable in approximately one-third of the adolescents.[4]

These results raise the question of why circulating spike protein levels remained elevated despite adequate levels and functionality of anti-spike antibodies. Hypothetical explanations include: (1) prolonged existence of mRNA that evades destruction; (2) increased dose delivery of mRNA; and (3) the possible role of anti-idiotype

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(Ab2) antibodies resulting in the attenuation of neutralizing spike antibodies.

HYPOTHESES FOR ELEVATED SPIKE PROTEIN LEVELS

Hypothesis 1: Prolonged Existence of mRNA That Evades Destruction

Studies have estimated that vaccine mRNA transcripts would be rapidly degraded, usually within 10 to 15 minutes after injection into muscle tissue.[5] However, in a few studies, both mRNA and spike protein were detected in the plasma and lymph nodes up to several weeks after vaccination.[6,7] In these studies, free circulating vaccine mRNA was detected predominantly in the plasma, evading degradation potentially by lipid-encapsulation or nucleoside modifications.[7] If this were to be the main mechanism by which spike protein levels remain elevated, COVID-19 vaccine–related myocarditis would be seen exclusively with mRNA vaccines. Although more rare, it has also been reported with non-mRNA adenovirus-vector vaccines, which raises the possibility of other additional mechanisms.

Hypothesis 2: Increased Dose Delivery of mRNA

There has been concern about increased mRNA vaccine dose potentially playing a role in the development of myocarditis, especially in susceptible and young people. The preponderance of myocarditis cases in younger populations raises the question of dose-related toxicity.[4] Although the numbers of patients who received mRNA-1273 vaccine were too few to perform reliable comparisons with BNT162b2 in the study by Yonker et al,[4] previous studies implicated greater immune response[8] and higher rates of myocarditis with mRNA-1273 compared with BNT162b2 vaccination.[9,10] (Each dose of mRNA-1273 contains >3× the dose of mRNA of BNT162b2.) Most myocarditis cases were after the second dose,[2,4] suggesting association with re-exposure, and also raising the possibility of dose accumulation if mRNA were to persist. Additionally, although the route of vaccination is intramuscular, in a preclinical model, IV injection of a COVID-19 mRNA vaccine was associated with development of acute epimyocarditis.[11] In this study, inflammatory foci were predominantly in the right heart, suggesting a gradual bloodstream-derived exposure, which raises speculation that an inadvertently large dose of the vaccination with IV injection may contribute to development of myocarditis. Immunostaining reflected presence of SARS-CoV-2 spike–RBD protein in cardiomyocytes, infiltrating immune cells and vascular endothelial cells within the myocardium and pericardium.[11] Accumulation of unfolded spike protein was postulated to exceed the folding capacity of endoplasmic reticula, leading to stress and development of the "unfolded protein response" and triggering apoptosis in large doses.[11] Adverse effects related to a large dose may be especially important in susceptible populations (eg, young male patients and those with genetic predisposition). Head-to-head comparisons of current COVID-19 vaccines with respect to possible differences in spike protein levels, protein translation, stability, or stimulation of innate responses are not widely available.[12] While not specifically reported with COVID-19 adenovirus-vector vaccines, animal experiments have shown that adenovirus-vector DNA can remain detectable for months after inoculation in a transcriptionally active form, in contrast to rapidly degraded mRNA, and can result in persistence of antigen expression.[12] This raises the question of whether spike protein levels can also remain elevated with COVID-19 adenovirus-vector vaccines. Although the rates of vaccine-related myocarditis have been less with COVID-19 adenovirus-vector vaccines, they have been associated with increased thrombotic events.

Hypothesis 3: Role of Ab2 Antibodies Resulting in Attenuation of Neutralizing Spike Antibodies

The idiotype portions of neutralizing antibodies that bind and neutralize spike protein have distinctive sequences, which can elicit secondary antibody responses called Ab2 antibodies.[13] These can bind to the neutralizing antibody, impairing their efficacy, and result in an increase in spike protein levels. Additionally, some of the Ab2 binding regions can mirror the antigen and bind to the same target, such as the angiotensin-converting enzyme (ACE) 2 receptor, and trigger of toll-like receptors and induction of inflammatory cytokines.[13]

ROLE OF THE SPIKE PROTEIN IN THE DEVELOPMENT OF MYOCARDITIS

Central to the discussion is the potential role of the spike protein in the development of myocarditis. Spike protein has been implicated in pericyte dysfunction, endothelial cell and myocyte injury, downregulation of ACE-2 expression, unopposed ACE and angiotensin-2– mediated effects, apoptosis, and proinflammation,[3,14,15] all of which can play a role in the development of myocardial injury. Similarly, in COVID-19 infection, persistence of SARS-CoV-2 antigenemia has been implicated in the pathogenesis of multisystem inflammatory syndrome in children. Prolonged presence of spike protein can result in sustained inflammation and further injury to the endothelium and cardiac myocytes in susceptible individuals. In the study by Yonker et al, systemic levels of proinflammatory biomarkers, cytokines, and cardiac troponin were elevated. There was evidence of late gadolinium

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enhancement by MRI in myocarditis cases, but the severity of myocardial injury tissue characterization could not be ascertained because endomyocardial biopsy samples were not obtained.[4]

Molecular mimicry between the spike protein and self-antigens has also been implicated as a potential mechanism for adverse events.[16] Antibodies against SARS-CoV-2 spike proteins have been experimentally shown to cross-react with structurally similar human peptide protein sequences, including α-myosin.[16,17]

Although the reasons are not clear, similar to this study, male predominance in myocarditis cases has been described previously.[2,10] Testosterone is thought to play a role by inhibition of anti-inflammatory cells and commitment to a Th1 (T helper type 1)–type immune response.[2] Elevated spike protein levels were observed equally in female and male patients with myocarditis,[4] raising the possibility of men being more vulnerable to the potentially cardiotoxic effects of spike protein. Thus, lower vaccine dose, increased vaccine intervals, and individualized antigen-to-antibody ratios may be important to prevent high-circulating spike mRNA levels in adolescent boys or other individuals with a history of vaccine-induced myocarditis to mitigate future risk.

In the study by Yonker et al,[4] which is similar to previous reports,[2] SARS-CoV-2 spike immunoglobulin M and G neutralizing antibody levels in patients with myocarditis were not significantly different than they were in healthy vaccinated controls, presenting an argument against a hyperimmune response. Contrary to a previous case report,[2] antibodies against self-antigens were not elevated.[2] Similar to previous reports, there was no evidence of a delayed hypersensitivity reaction, such as serum sickness or eosinophilic myocarditis, antibody-dependent enhancement of immunity, thromboembolic events, disseminated intravascular coagulation, cytokine storm, hemophagocytic lymphohistiocytosis, or macrophage-activation syndrome causing myocarditis after COVID-19 mRNA vaccination.[2] There was also no evidence of acute COVID-19 or other acute viral illnesses.[2,4]

Although selected RNA molecules can be immunogenic and stimulate the innate immune system, it would likely result in destruction of the mRNA before reaching target cells, preventing spike proteins and neutralizing antibody production. Findings from Yonker et al[4] do not suggest immunogenicity of the mRNA molecule, as mRNA was not destroyed before translation into spike protein.

The study by Yonker et al[4] has several limitations. Samples were collected at different times after vaccination in myocarditis or control cases. Although the analysis for antibody responses were limited to samples collected within 11 days, such a limitation was not specified for antigen measurements. In 13 of 16 myocarditis cases, symptom onset was within 4 days of vaccination; therefore, antigen levels may have been collected during an earlier period in myocarditis cases than controls. The study sample size was small, limiting any conclusions regarding male versus female and adolescent versus adult populations, as well as feasibility of comparison between vaccine types. Myocarditis and control cohorts were not evenly balanced regarding exposure, as all adolescent controls and the majority of the myocarditis cases received the BNT162b2 vaccine. The ranges of immunoglobulin M, A, and G levels in anti-spike and anti-RBD antibodies were wider in myocarditis cases, with very low antibody levels in some patients. This raises the question of whether an antigen-to-antibody ratio could be a better variable in assessing risk at the individual level, especially in younger populations. Lack of myocardial tissue characterization and in-depth analysis of immunopathology, including T-cell subsets, nonneutralizing antibody levels were additional limitations.

Despite these rare cases of mild myocarditis, studies have demonstrated a favorable benefit–risk assessment associated with COVID-19 vaccination.[2,9,18] COVID-19 infection is associated with a markedly increased risk of myocarditis than vaccination.[18] COVID-19 infection is also associated with significant risks of myocardial infarction, arrhythmia, pulmonary embolus, deep venous thrombosis, and intracranial hemorrhage, none of which are noted in vaccination.[18] SARS-CoV-2 vaccination not only prevents COVID-19–related hospitalization and death, but complications such as myocarditis, multisystem inflammatory syndrome in children, and postacute sequelae of SARS-CoV-2 infection as well.

The study from Yonker et al sheds light on mechanisms of myocarditis with COVID-19 mRNA vaccines, and implicates elevated circulating spike protein as a potential cause or marker of myocarditis.[4] Further studies are needed to elucidate the reasons for and effects of elevated spike protein levels and whether levels can be monitored to adjust dose and frequency and mitigate individualized risk. In addition, studies are needed for the identification of risk factors (including genetic predisposition) and potential mechanisms and reasons for sex- and age-related differences, as well as the long-term impact of myocarditis after SARS-CoV-2 vaccination. Historically, rare adverse events of postvaccination myocarditis have been reported after smallpox and anthrax vaccinations, but vaccination-associated myocarditis is extremely rare with influenza, hepatitis B, or other commonly administered vaccinations.[2] Although the risk–benefit ratio favors SARS-CoV-2 vaccination for the overall population,[2] mitigating the myocarditis risk remains a high priority for research and requires further understanding of the mechanisms.

ARTICLE INFORMATION

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EDITORIAL

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