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Letter

RESEARCH LETTER

A Murine Model of mRNA COVID-19 Vaccine-Induced Myocarditis

A Shot in the Dark?

Myocarditis is a rare yet significant complication associated with messenger RNA (mRNA) COVID-19 vaccination. Despite an increasing number of clinical case reports, the underlying mechanisms of this association are still poorly understood, and experimental research in this field remains limited. To date, only 3 studies have investigated acute myocarditis following mRNA COVID-19 vaccination in murine models.[1-3] Li et al[1] reported a consistent induction (100%) of multifocal myopericarditis following high-dose intravenous administrations of the Pfizer-BioNTech mRNA vaccine (BNT162b2) in BALB/c mice. In contrast, Zirkenbach et al[2] found no adverse effects on heart inflammation in A/J and C57BL/6 mice following intramuscular BNT162b2 vaccination, even when tested in a model of experimental autoimmune myocarditis. Finally, Lee et al[3] reported increased cardiac inflammation and cytokine production after intramuscular mRNA vaccination, which was markedly augmented in a mouse model of chronic inflammation. However, their study did not demonstrate myocyte injury through histologic examination, they did not conduct immunohistochemical analysis, and sample sizes were notably small (n = 3). Given this background, we find it imperative to present our findings, which demonstrate a failure to replicate the mRNA COVID-19 vaccine-induced myocarditis mouse model reported earlier.[1]

In summary, 12-week-old male BALB/cOlaHsd mice (Inotiv) were intravenously administered either BNT162b2 (n = 27) or a saline vehicle (n = 17) using a 2-dose regimen, with a 14-day interval between doses. Each vaccine dose comprised 6 μg of mRNA diluted in saline (total volume: 60 μL). Additionally, a separate cohort of 7-week-old mice (n = 14) received only a single dose of BNT162b2. All animals were sacrificed 2 to 3 days after vaccination. All procedures adhered to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and the European Directive (2010/63/EU) on the protection of animals used for scientific purposes, with approval obtained from the University of Antwerp Ethical Committee for Animal Testing. Heart sections were stained with hematoxylin and eosin and were independently evaluated by blinded expert pathologists to assess inflammatory cell infiltration and cardiomyocyte injury.

In our study, we did not detect mRNA COVID-19 vaccine-induced myocarditis despite using an identical vaccination protocol (route, dose, and timing) and using the same substrain as previously reported.[1] Importantly, successful vaccination was confirmed by flow cytometric analysis of the spleen, unveiling a robust, spike-specific $CD8^+$ T-cell response (data not shown). A notable finding was the presence of discrete foci of cardiac fibrovascular inflammation (with limited focal cell loss) in a subset of mice, independent of vaccination status (**Figures 1A and 1B**). Intriguingly, these lesions were specifically localized in the right ventricular epicardium and predominantly consisted of M1 macrophages ($F4/80^+$ and inducible nitric oxide synthase cells; data not shown). These characteristics (eg, location and cell type) align with the histopathologic features of a late-stage manifestation of spontaneous cardiac calcinosis, a condition known to affect the BALB/c strain.[4] Although calcific deposits were not detected in our study, Li et al[1] reported the presence of pericardial calcifications exclusively in vaccinated animals. This suggests that mRNA immunization may serve as a trigger for cardiac calcinosis in susceptible mouse strains, thereby explaining the notable increases in serum troponin levels (to 1,328 ± 325 pg/mL) they observed. Conversely, our study did not reveal elevations in plasma troponin levels after vaccination (data not shown). Possible factors contributing to the divergent outcomes between both studies may include variations in the origin of the mice or differences in the sanitary status of the animal housing facilities. Interestingly, we did detect elevated cardiac mRNA levels of interferon gamma and interleukin 1β—but not tumor necrosis factor α and interleukin 6 (data not shown)—after vaccination,

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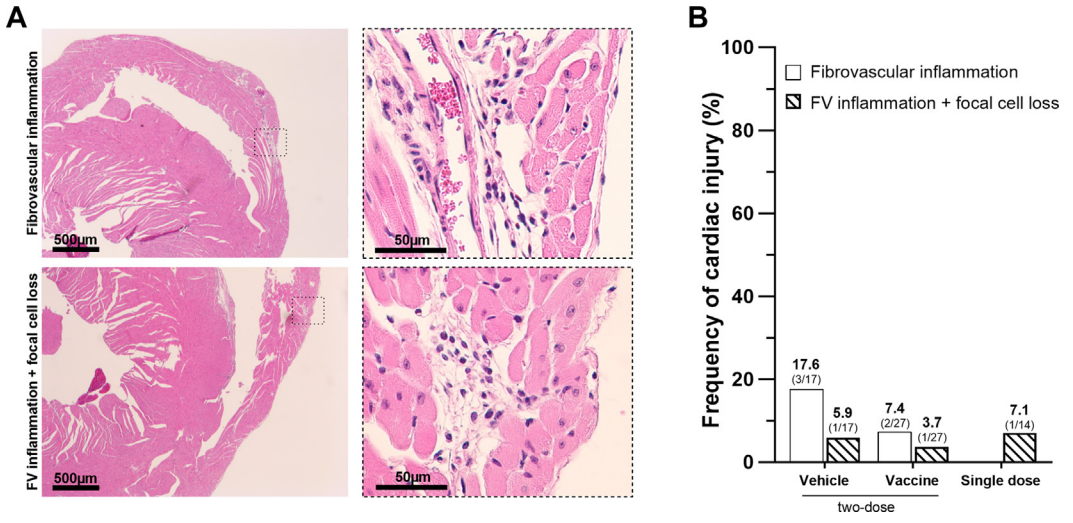
FIGURE 1 Histopathologic Evaluation of Murine Hearts Following BNT162b2 Messenger RNA Vaccination



(A) Representative image of spontaneously occurring cardiac lesions in BALB/c mice, depicting FV inflammation alone (upper) or accompanied by focal cell loss (lower). (B) Frequency of cardiac injury as quantified by histopathologic scoring of hematoxylin and eosin–stained heart sections. Group sizes: vehicle, n = 17; vaccine, n = 27; and single dose, n = 14. FV = fibrovascular.

which are indicative of mild cardiac inflammation. However, this likely reflects a systemic inflammatory response to vaccination caused by infiltrating immune cells.

Overall, our results show that mRNA COVID-19 vaccination does not elicit multifocal myocarditis in susceptible mice, even with disproportionately high doses and an intravenous administration route similar to that used by Li et al.[1] Earlier work also demonstrated no adverse effects of intramuscular mRNA vaccination, even in a sensitive model of experimental autoimmune myocarditis.[2] This raises the question of whether it is feasible to establish a reproducible experimental model of an adverse event that occurs at an exceedingly low rate in humans and likely involves a complex, multifactorial pathophysiology. Additionally, Li et al[1] reported similar rates of vaccine-induced myopericarditis in both sexes, whereas epidemiologic data indicate a clear sex bias, primarily affecting adolescent males, suggesting limited clinical translatability. Furthermore, reduced binding affinity of the SARS-CoV-2 spike protein to angiotensin-converting enzyme 2 in mice has been demonstrated, hinting that human angiotensin-converting enzyme 2-transgenic or -knock-in mice may offer a more reliable approach.[5]

In conclusion, we were unable to replicate the only mouse model of mRNA COVID-19 vaccine-induced myocarditis reported in the current literature. We hope our findings provide valuable insights to other scientists, encouraging them to explore alternative approaches, such as using different strains or species or using transgenic models. Within this context, it is paramount that negative attempts to establish a much-needed experimental model of mRNA COVID-19 vaccine-induced myocarditis are also reported.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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