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SARS-CoV2 mRNA vaccine intravenous administration induces myocarditis in chronic inflammation

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Abstract

The current COVID-19 mRNA vaccines were developed and applied for pandemic-emergent conditions. These vaccines use a small piece of the virus's genetic material (mRNA) to stimulate an immune response against COVID-19. However, their potential effects on individuals with chronic inflammatory conditions and vaccination routes remain questionable. Therefore, we investigated the effects of mRNA vaccines in a mouse model of chronic inflammation, focusing on their cardiac toxicity and immunogenicity dependent on the injection route. mRNA vaccine intravenous administration with or without chronic inflammation exacerbated cardiac pericarditis and myocarditis; immunization induced mild inflammation and inflammatory cytokine IL-1beta and IL-6 production in the heart. Further, IV mRNA vaccination induced cardiac damage in LPS chronic inflammation, particularly serum troponin I (TnI), which dramatically increased. IV vaccine administration may induce more cardiotoxicity in chronic inflammation. These findings highlight the need for further research to understand the underlying mechanisms of mRNA vaccines with chronic inflammatory conditions dependent on injection routes.

1. Introduction

Coronavirus vaccines have been widely used as a critical measure in response to the global coronavirus infection (COVID-19) pandemic. The current COVID-19 mRNA vaccines, including Pfizer-BioNTech and Moderna, were developed and applied for pandemic emerging conditions [1, 2]. These vaccines use a small piece of the virus's genetic material (mRNA) to stimulate an immune response against COVID-19. This mRNA instructs the cells to produce a harmless piece of the virus called the spike protein. Once the spike protein is produced, the immune system recognizes it as foreign and mounts an immune response by producing

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antibodies. These antibodies can neutralize the spike protein if the person becomes infected with the virus. The mRNA vaccines effectively prevent COVID-19 infection and severe disease [3, 4]. They have undergone rigorous safety testing and have been authorized for emergency use by regulatory agencies worldwide. Although the mRNA vaccines have been generally well-tolerated, some rare side effects have been reported. However, in recent reported cases, occurrences of cardiac disorders such as myocarditis have been observed following vaccination [5–7]. These side effects have raised concerns about the safety of coronavirus vaccines and highlight the need for further research.

Myocarditis is an inflammation of the heart muscle [8–10]. According to the Centers for Disease Control and Prevention (CDC), cases of myocarditis have occurred more frequently in males under 30 years of age following the second dose of the mRNA vaccines. While the overall incidence of myocarditis following mRNA vaccination is still relatively low, it is important for healthcare providers and the public to be aware of this potential side effect [11–13]. The reason for the increased risk of myocarditis in some individuals is not yet fully understood, and further research is needed to understand this phenomenon better. The risk of myocarditis in the general population has increased after COVID-19 vaccine administration is lower than that caused by SARS-CoV-2 infection [14–16]. Thus, a more comprehensive understanding of the potential risks and underlying mechanisms is needed to provide evidence-based information for facilitating informed decision-making regarding mRNA vaccine administration to this susceptible population [17]. We discuss the possibility of myocarditis as a side effect of coronavirus vaccines. This has become an important issue due to the increasing number of reported cases of myocarditis following vaccination.

We investigate the causes and conditions under which myocarditis can occur. This includes various factors associated with the inflammation of cardiac tissues. In this study, we demonstrated the importance of research on myocarditis related to the side effects of coronavirus mRNA vaccines in the chronic inflammation model. mRNA vaccine intravenous (IV) injection develops myocarditis and pericarditis but is weak from intramuscular (IM) injection. It highlights the need for additional research and response strategies to ensure heart health and safe vaccine administration.

2. Materials and methods

2.1. Preparation of mRNA vaccine

The antigen was designed using a DNA encoding the spike protein of the SARS-CoV-2 Omicron variant. The mRNA vaccine plasmid was produced by inserting the antigen DNA into multiple cloning sites on the mRNA platform. It was produced using the EZ T7 High Yield In vitro Transcription Kit (Enzynomics, Daejeon, Korea), according to the manufacturer's protocol. Total mRNA was precipitated using lithium chloride and purified using cellulose, which was previously described [18]. mRNA was formulated using NanoAssemblr® IgniteTM (Precision Nanosystems, BC, Canada) by mixing the aqueous and organic solutions at 3:1 and a total flow rate of 10 mL/min. LNPs were formulated using the solution of LNPs [19] and were concentrated by ultrafiltration using Amicon Ultra centrifugal Filter (UFC9030, Merck Millipore, MA, USA) following the manufacturer's instructions.

2.2. Establishment of the chronic inflammatory mouse model

Male or female Balb/c and C57BL6 mice at the age of 6–8 weeks were obtained from the Dae-Han Bio link Co. (Eum-seong, Korea) and were housed in a controlled environment (inverted 12-h daylight cycle) with free access to food and water. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Samsung Biomedical

Research Institute (SBRI, #2022032201). SBRI is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) and abides by the Institute of Laboratory Animal Resources (ILAR) guide. Mice were fed a normal-fat diet (containing 5% fat) and were simultaneously treated with LPS from Escherichia (Sigma, St Louis, MO, USA). The surgical procedure for inserting the osmotic pump followed the previous report. The mice were implanted with an osmotic pump (Alzet model 1004; DURECT Corp., Cupertino, CA, USA) that was filled with either Tween-saline (0.9% NaCl and 0.1% Tween 80 in distilled water, Sigma) normal saline control or LPS diluted in Tween-saline infused at 400 μg/kg/day for 4 weeks (LPS group, n = 5) [20].

2.3. Immunization

The pump-implanted animals were randomly assigned to 6 groups for the administration of intramuscular (IM) or intravenous (IV) SARS-CoV2 mRNA vaccine or normal saline control (Groups: saline, saline pump + mRNA vaccine IM or IV, LPS pump, LPS pump + mRNA vaccine IM or IV, N = 5 each group). The mice were immunized intramuscularly or intravenously with 10 μg of Omicron Spike mRNA vaccines encoding the sequence of spike protein from the SARS-CoV-2 Omicron variant. The mRNA expression platform was previously described [18]. The immunization schedule consisted of two injections: an initial prime injection, followed by a boost injection, with a two-week interval between them. Once the immunization protocol was completed, the mice were euthanized two days post injection (dpi) after immunization and whole blood samples and tissues were collected.

2.4. Histological analysis

The heart and liver tissues of osmotic pump-implanted mice were fixed in 10% neutral formalin. After fixation, these samples were embedded in paraffin and stained with hematoxylin and eosin (H&E). We evaluated the percentage area of myocardial inflammation by computer-assisted analysis. Two different areas of each heart were quantified by ImageJ software1.45s (NIH, MA, USA) as described previously [21]. In addition, the pericarditis was evaluated by individual mouse heart surface inflammation. The procedures were carried out by investigators unaware of the sample identities.

2.5. Real-time quantitative RT-PCR (qRT-PCR)

Total RNA was extracted from lung tissue using TRIzol reagent (Invitrogen, Grand Island, NY, USA), and cDNA was synthesized from total RNA using a cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Real-time PCR was performed using the SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) to compare the mRNA levels of genes associated with inflammation, myogenesis, muscle types, and mitochondria. The primer sets used were synthesized by SFC-probe (Cheongju, Korea) for inflammation-related genes and by Bioneer (Daejeon, Korea) for the remaining genes. The primer sequences are listed in Table 1.

2.6. Enzyme-linked immunosorbent assay (ELISA)

To assess the levels of LPS, Troponin I, and LDH (Lactate dehydrogenase) in mouse serum, enzyme-linked immunosorbent assay (ELISA) was performed. In brief, the diluted mouse serum samples were then added to the wells and incubated for 2 hours at room temperature. After the incubation period, the wells were washed three times with 200 μl of PBS-T (PBS containing Tween 20). Horseradish peroxidase-conjugated anti-mouse LPS antibodies (from

Table 1. The sequences of primers used in the study.

Tissue	Gene	Forward (5′-3′)	Reverse (5′-3′)
Heart	IL-1b	TTGACGGACCCCAAAGAGTG	ACTCCTGTACTCGTGGAAGA
	IL-6	GTACTCCAGAAGACCAGAGG	TGCTGGTGACAACCACGGCC
	TNF-a	TTGACCTCAGCGCTGAGTTG	CCTGTAGCCCACGTCGTAGC
	MCP-1	ACCTGGATCGGAACCAAATG	CCTTAGGGCAGATGCAGTTTTAA
	Myh7	GCTGAAAGCAGAAAGAGATTATC	TGGAGTTCTTCTCTTCTGGAG
	Calcineurin	CGGAAACCATGAATGTAGGCACC	GAAGGCATCCATACAGGCGTCA
	GAPDH	ATCAACGACCCCTTCATTGACC	CCAGTAGACTCCACGACATACTCAGC

Abbreviations: IL-1β, Interleukin 1 beta; IL-6, Interleukin 6; TNF-a, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1; monocyte chemoattractant protein-1; GAPDH, glyceraldehyde-3-phosphoate dehydrogenase; Myh7, Myosin heavy chain 7

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Bethyl Laboratories, Montgomery, TX, USA) were added to the wells, followed by incubation at room temperature for 1 hour. The antibodies were appropriately diluted in PBS following the manufacturer's instructions. Following three washes with PBS-T, a tetramethylbenzidine (TMB) substrate was added to the wells, and the plates were incubated for 15 minutes. The reaction was then halted by adding stop solution (2 N H_2SO_4). Finally, the optical density at 450 nm was measured using a microplate reader (Molecular probe, San Diego, CA).

2.7. Statistical analysis

Statistical analysis was performed using Prism 8 software (GraphPad). The data are presented as mean ± standard deviation (SD). Significant differences between means were determined using the student's t-test, with p-values less than 0.05 considered statistically significant.

3. Results

3.1. Development of the chronic inflammation mouse model

The chronic inflammation mouse model was established by a subcutaneously embedded LPS pump, which was used to deliver a constant rate (0.11μg/h) of LPS over a period of 4 weeks. A pump infused with saline served as the control. After 28 days of pump implantation, the heart and liver were collected to assess inflammation and histological changes (Fig 1A). The LPS serum levels were significantly increased with LPS pump implantation (Fig 1B). The mRNA level of inflammatory cytokines in the heart: interleukin-1beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-a) were significantly increased in the LPS pump group (LPS, n = 5) compared to the saline control group (CON, n = 3). Additionally, monocyte chemoattractant protein-1 (MCP-1, macrophage infiltration marker) was significantly increased (Fig 1C). Furthermore, inflammatory cell infiltration was observed in H&E staining of heart cross-sections but there were no histological differences were observed in the liver (Fig 1D). A model of chronic inflammation was successfully established.

3.2. mRNA vaccine intravenous administration exacerbates cardiac inflammation

The impact of the mRNA vaccine administration routes was observed in the developed chronic inflammation model. The mRNA vaccine was administrated intramuscularly (IM) or intravenously (IV) on days 14 and 28 after LPS pump implantation. Then, the mouse was sacrificed 2 dpi second vaccine administration. The serum LPS level was detected by an ELISA

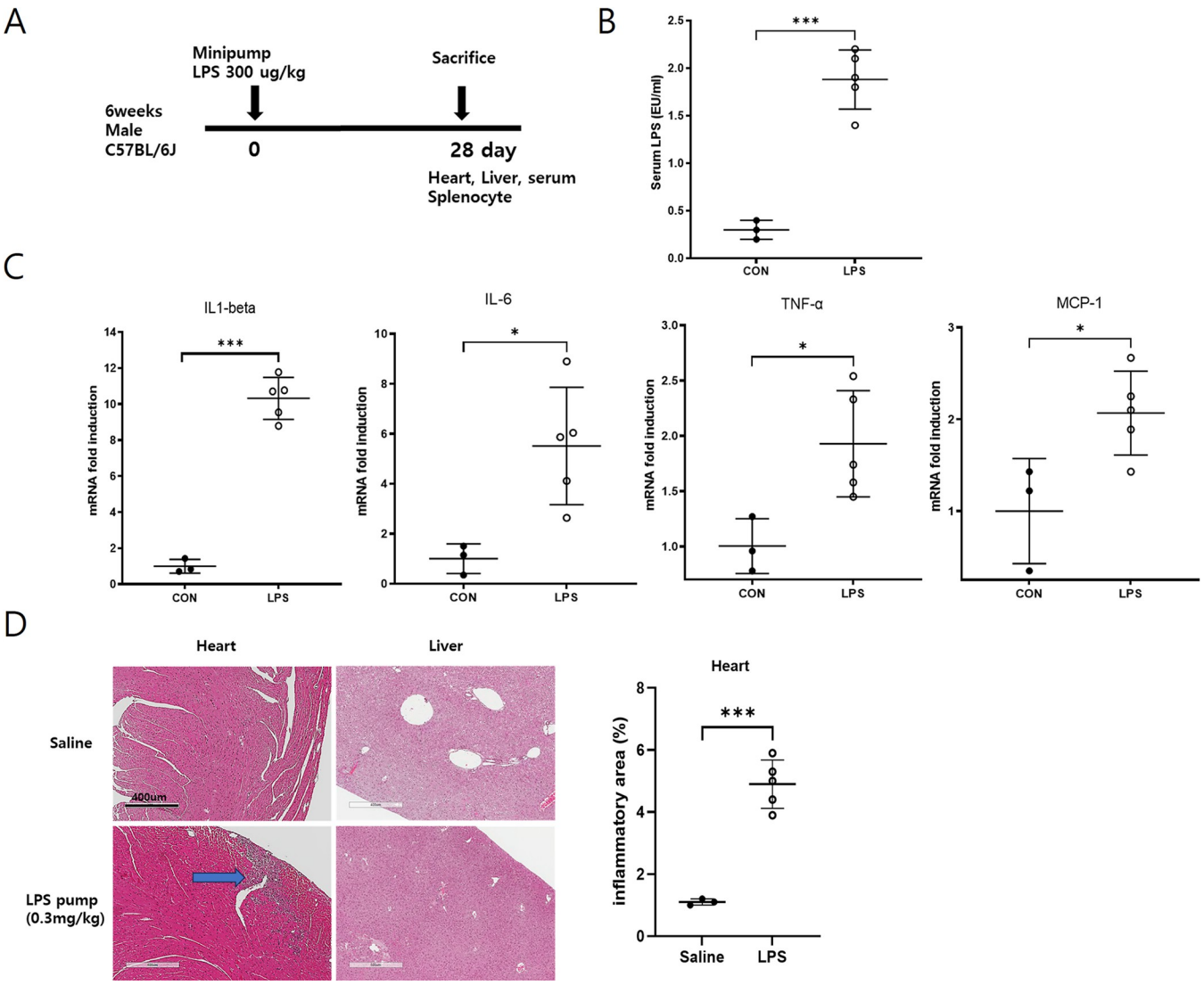

Fig 1. Generation of chronic inflammation mice model. (A) Schematic diagram of animal experiment. The LPS pump was implanted for 30 days and then sacrificed to collect tissue samples for analysis. (B) The LPS concentration in the serum was measured with an ELISA kit. (C) RNA was extracted from the mice hearts of CON (n = 3) and LPS (n = 5) groups, and then RNA was applied for quantitative RT-PCR probed by IL-1beta, IL-6, TNF-alpha, and MCP-1. (D) Histologic findings showed focal inflammatory cell infiltration in LSP pump-implanted mice hearts (blue arrow). The area of inflammation was quantified from each mouse heart. All data are the mean ± standard deviation (SD) from independent experiments. *P < 0.05, **P < 0.01, and ***P < 0.001 by two-tailed Student's t-test.

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(Fig 2A and 2B). The pericarditis was observed from each group's heart. mRNA vaccine IV administration induced pericarditis with (3/5) or without LPS pump (3/5). LPS pump-induced chronic inflammation is not different from the development of pericarditis (Fig 2C). The blood lymphocyte levels were slightly increased by mRNA vaccine administration. Even more, it increased in chronic inflammation mice compared to the saline group but not different in monocytes (Fig 2D). The serum inflammatory cytokine IL-6 was maximized at 8hr then decreased until 24hr after IM or ID (intradermal, n = 5 for each group) mRNA vaccine administration (S1 Fig).

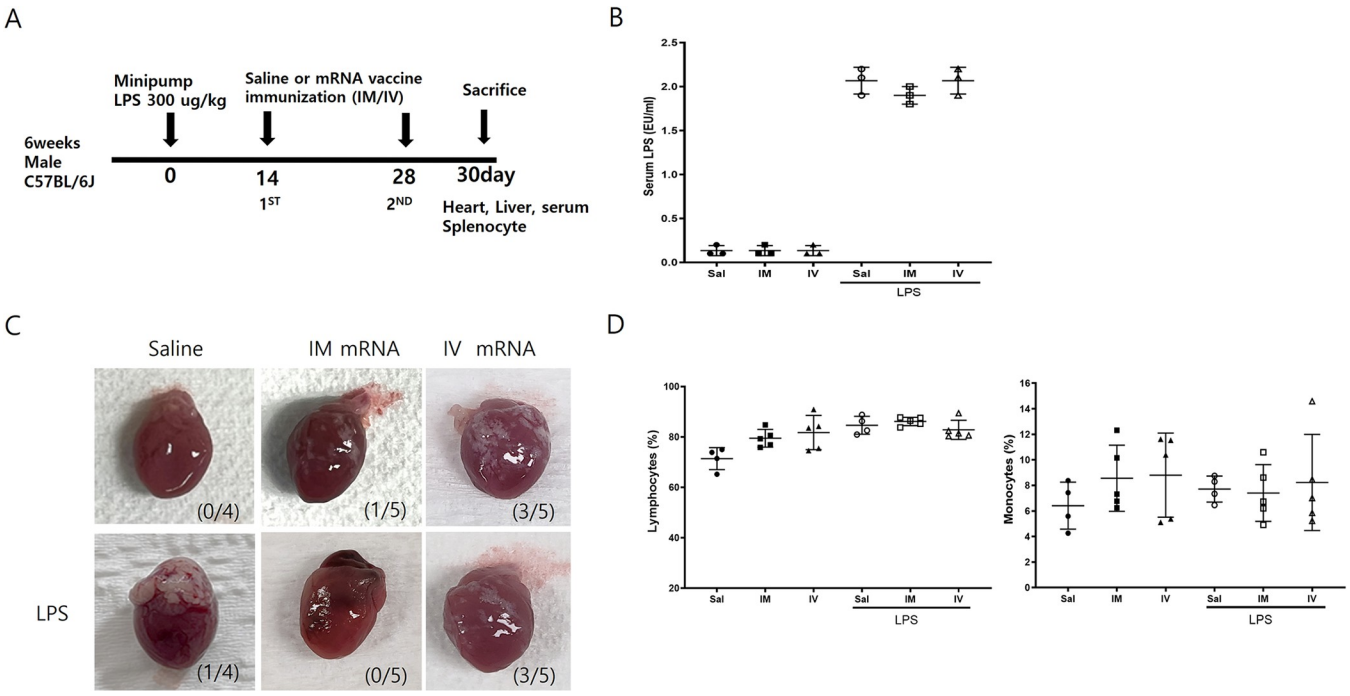

Fig 2. mRNA vaccine administration in chronic inflammation mice model. (A) Schematic diagram of animal experiment. (B) LPS level was measured in the serum two days after boosting immunization. (C) The pericarditis was observed by the surface calcium accumulation of individual mice hearts. Intravenous mRNA vaccine administration induced three mice out of five with or without LPS chronic inflammation. (D) Peripheral blood was subjected to hematological analysis. Lymphocyte and monocyte numbers were counted and presented as blood percentages.

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3.3. mRNA vaccine intravenous (IV) administration induces more myocarditis

Histopathological changes in the heart at 2 dpi after the second dose of vaccine immunization. mRNA vaccine IM and IV immunization only induced myocarditis compared to the saline group (Fig 3A–3C). The percentage of inflammatory cell area was significantly increased in mRNA vaccine immunization with the LPS pump implantation (Fig 3D–3F). Interestingly, vaccine IV administration significantly increased the immune cell infiltration in the heart with or without LPS pump implantation (Fig 3G). These results demonstrated that mRNA vaccine immunization induced cardiac damage and myocarditis in chronic inflammatory conditions. Moreover, IV vaccine injection dramatically increased the inflammatory cell infiltration in the heart.

3.4. mRNA vaccine intravenous (IV) administration induces cardiac Inflammatory cytokine transcription

Quantitative RT-PCR (qPCR) analysis showed mRNA expression levels of inflammatory cytokine IL-1β, IL-6 and macrophagic chemokine MCP-1. Gene transcription was enormously increased in the LPS pump with the IV mRNA vaccine group compared to those without the LPS pump IV mRNA vaccine group (Fig 4A). Myosin light chain7 (Myh7) and calcineurin, which is one of the cardiac hypertrophy and damage markers, were significantly increased in the heart tissue of mRNA-administered groups with or without LPS pump implantation. Moreover, IV vaccine administration dramatically increased these gene expressions (Fig 4B). In addition, myocardium damage was confirmed by serum levels of troponin I and lactate

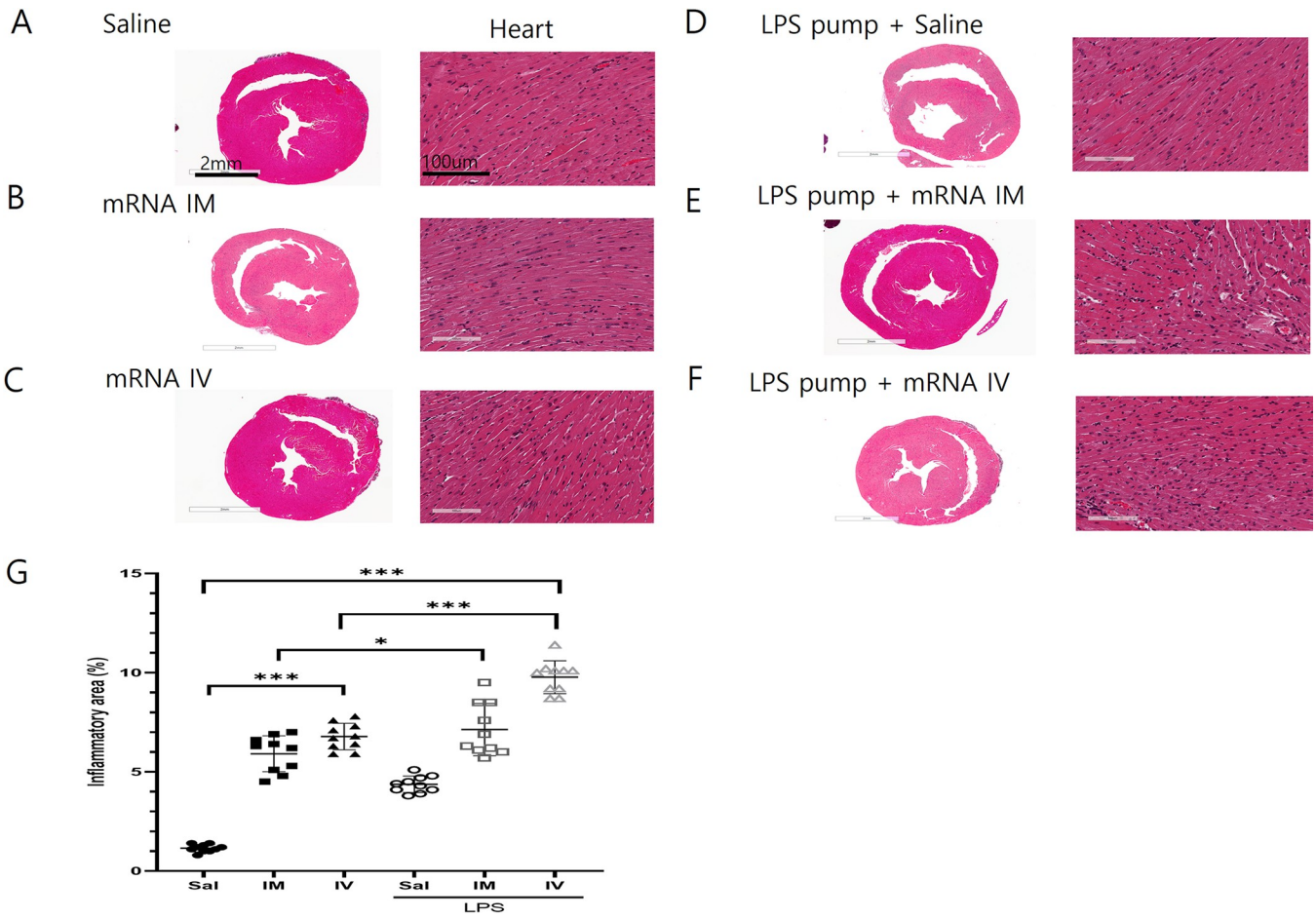

Fig 3. mRNA vaccine IV administration induces significant impacts on heart tissue, the presence of chronic inflammation. (A-F) Heart tissue was collected two days after boosting immunization and subjected to the histological finding. The heart inflammation level was observed by hematoxylin and eosin stains. (G) The inflammatory percent area was quantified from three or four fields of each heart using NIH-ImageJ software (n = 5 in each group). All data are the mean ± SD from independent experiments. *P < 0.05, **P < 0.01, and ***P < 0.001 by two-tailed Student's t-test.

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dehydrogenase (LDH). The IM and IV mRNA vaccine injection exhibited enhanced serum levels of troponin I and LDH. The troponin I and LDH were significantly increased by the LPS pump implantation group compared to the saline pump group (Fig 4C). These results indicated that IV mRNA vaccine administration induced inflammation in the LPS pump chronic inflammation model. Moreover, mRNA vaccine IV injection induced myocardium damage with or without an LPS pump (Fig 5).

4. Discussion

We investigate the impact of mRNA vaccines on low and sustained levels of inflammation, such as atherosclerosis. We established an animal model that induced chronic inflammation by using an LPS mini-pump system for the constitutive release of LPS for 4weeks. We successfully established a chronic inflammation model (Fig 1) and mRNA vaccine administrated through IV or IM injection. In a Balb/c mouse with male mice, IV but not IM administration of COVID-19 mRNA vaccine in chronic inflammatory condition induced acute myocarditis and/or pericarditis with increased inflammatory cytokine transcription, cardiomyocyte damage and inflammatory cell infiltration in the heart within 2-day post injection (dpi) of the

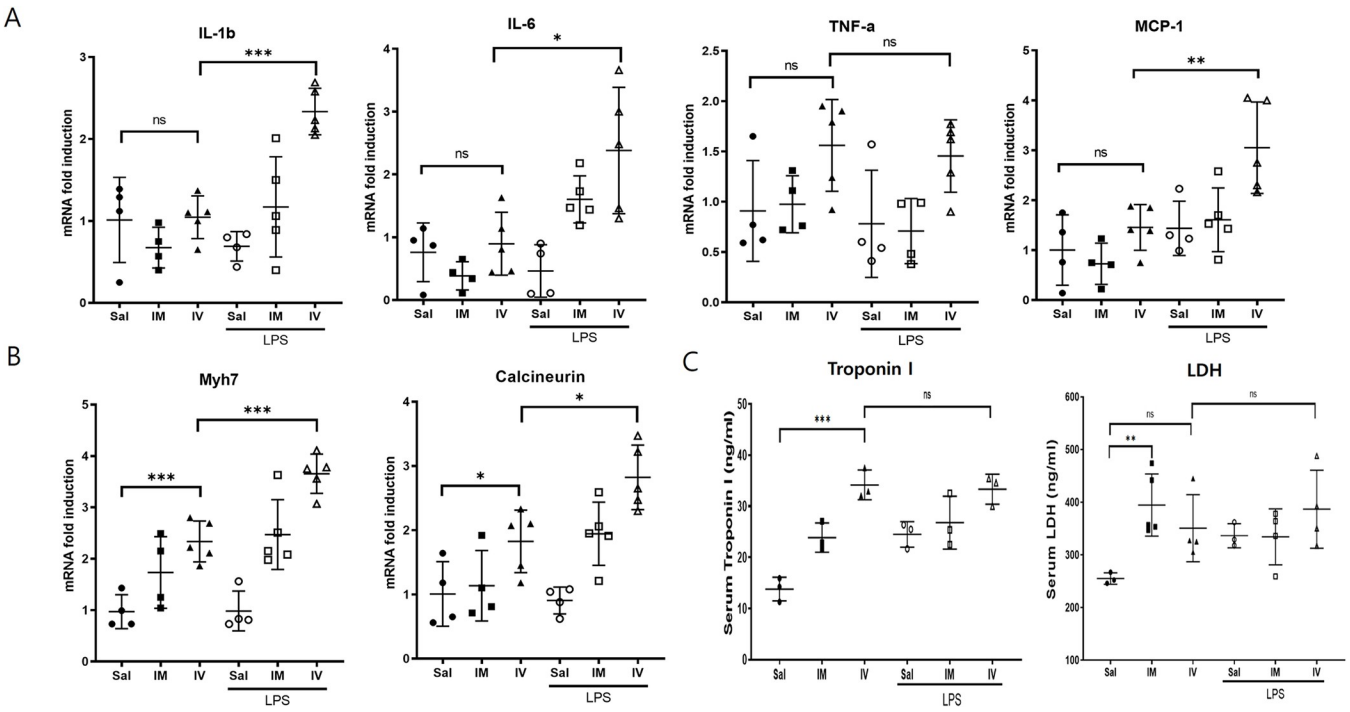

Fig 4. Effects of chronic inflammation on the different mRNA vaccines injection route. (A-B) RNA was extracted from the mice heart of each group (n = 4 each group). Inflammation marker (IL-1beta, IL-6, TNF-alpha, and MCP-1), and heart damage marker (Myh7 and calcineurin) mRNA levels were confirmed by quantitative RT-PCR. (C) The cardiac muscle damage marker Troponin I and LDH serum levels were measured by ELISA kit following the manufacturer's protocol. *P < 0.05 **P < 0.01 vs. saline group. All data presented are mean ± SD.

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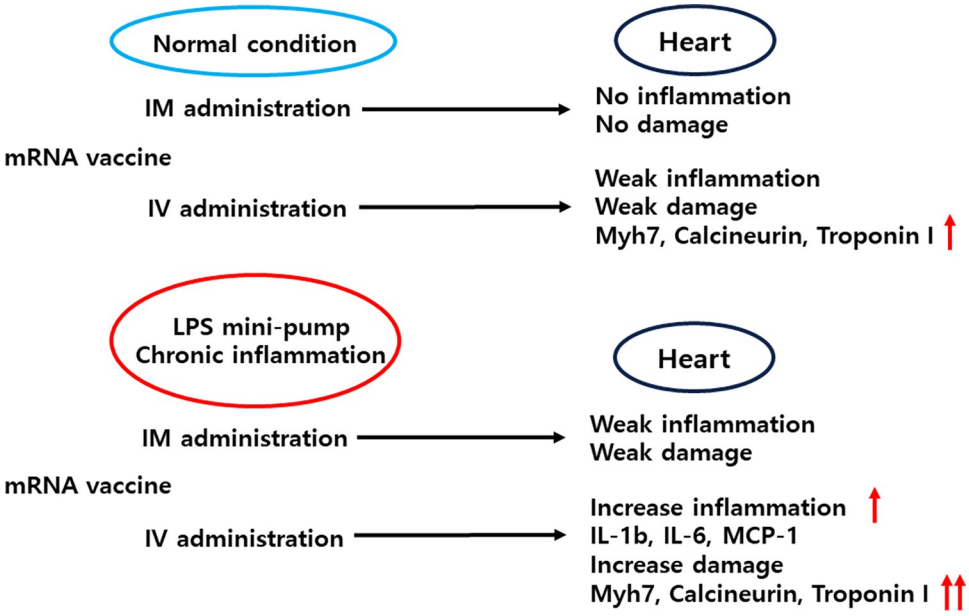

Fig 5. Key finding illustration. IV mRNA vaccine administration induced inflammation in the LPS pump chronic inflammation model. The heart inflammatory cytokine (IL-1beta, IL-6, and MCP-1) level was increased with LPS pump. Moreover, mRNA vaccine IV injection induced myocardium damage (Myh7, Calcineurin, and Troponin I) with or without an LPS pump.

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second vaccination. IL-1beta, IL-6, and MCP-1 expression levels generally increased significantly from 2 dpi in the IV group compared to the IM group in the presence of chronic inflammation. Can Li, previously reported that mRNA vaccine IV injection only induced acute heart pericarditis and inflammatory cell infiltration [16]. However, our data showed that chronic inflammatory conditions increased inflammation and myocardium damage in both IV and IM injections of the mRNA vaccine.

Our results showed that mRNA vaccine IV immunization induces inflammation and inflammatory cytokine production in the heart more than IM immunization. Moreover, the pericarditis and myocardium damage dramatically increased in the mRNA vaccine IV injection with the chronic inflammation condition. However, there are no different levels of lymphocyte and monocyte in IV and IM mRNA vaccine injection. Previous reports have shown that elderly patients with chronic diseases like diabetes Mellitus, hypertension, and hypercholesterolemia have a higher risk of COVID-19 infection [22], but there is no clear evidence of mRNA vaccine immunization-induced myocarditis in elderly patients with chronic diseases. However, myocarditis has been reported significantly more frequently after the second vaccine dose in males and people younger than 30 years of age [23, 24].

COVID-19 mRNA vaccines were associated with pericarditis at a rate of 12.6–24 cases per million following a second dose [11]. The enhanced cardiac damage and myocarditis following mRNA vaccine administration in our chronic inflammatory mouse model raises important questions regarding the potential risks of mRNA vaccines in individuals with preexisting inflammatory conditions [25]. Thus, the inflammatory milieu created by chronic inflammation may contribute to cardiac damage exacerbation following mRNA vaccine immunization [26, 27]. Here, we found that chronic inflammation increases the risk of adverse events induced by mRNA vaccines, particularly in the heart. Interestingly, the IV mRNA vaccine administration affects heart pericarditis and inflammation more. However, the mice experiment has a lot of limitations to show the relevance in the human model because nobody is getting IV administration for the mRNA vaccine. Dr. Knowlton commented that vaccines uptake into the bloodstream accidentally, which may cause systemic acute inflammation and myocarditis [28]. Myocarditis is a rare serious event in humans associated with COVID-19 mRNA vaccines, but the mortality among myocarditis patients was low (2 cases per 10,000,000 vaccination). We are successfully able to manage COVID-19 vaccine-induced myocarditis with early detection and treatment [29–31].

In conclusion, our findings suggest that mRNA vaccine IV injection dramatically induces cardiotoxicity and inflammation in the mouse heart, with chronic inflammation that may occur during the immunogenic process of the mRNA vaccine. It is important to note that future applications of the murine model system will likely require further investigation into how well this model represents the rare complication of myopericarditis following COVID-19 mRNA vaccination in humans. For example, it is often challenging to correlate the dose of a drug when adjusted to body weight between a small mouse and a much larger human. In this case, the mouse received 0.25 μg per gram weight. The Pfizer mRNA vaccine dose in humans is 0.4×10^{-3} μg per gram for a 70-kg person [28]. These findings give us a very important message that the limitation of mice model experiment. However, still, the chronic inflammatory conditions must be considered as risk factors in younger age male persons.

Additional research is required to define the cardiac toxicity of mRNA vaccines in populations with chronic inflammatory diseases. It is essential to define post-vaccination myocarditis structurally and functionally, using molecular techniques such as immunochemistry, EKG, biomarkers, and imaging. In addition, the patients presenting with prolonged symptoms must be followed up to identify the effects of myocardial inflammation. It will give an important answer to preparing the next mRNA vaccine for future pandemics.

Supporting information

S1 Fig. Serum IL-6 expression in Covid-19 mRNA vaccine administration. Serum was collected from each time point (1, 4, 8, 24hr) after COVID-19 mRNA vaccine IM or ID administration (n = 5, each time point) and then applied for ELISA to measure IL-6 protein concentration.
(DOCX)

S1 Data.
(XLSX)

Author Contributions

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Writing – review & editing: Ha-Eun Jeon, Seonghyun Lee, Jisun Lee, Jae-Hwan Nam, Byung-Kwan Lim.

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