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REVIEW ARTICLE

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Autoimmune inflammatory reactions triggered by the COVID-19 genetic vaccines in terminally differentiated tissues

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ABSTRACT

As a result of the spread of SARS-CoV-2, a global pandemic was declared. Indiscriminate COVID-19 vaccination has been extended to include age groups and naturally immune people with minimal danger of suffering serious complications due to COVID-19. Solid immuno-histopathological evidence demonstrates that the COVID-19 genetic vaccines can display a wide distribution within the body, affecting tissues that are terminally differentiated and far away from the injection site. These include the heart and brain, which may incur *in situ* production of spike protein eliciting a strong autoimmunological inflammatory response. Due to the fact that every human cell which synthesises non-self antigens, inevitably becomes the target of the immune system, and since the human body is not a strictly compartmentalised system, accurate pharmacokinetic and pharmacodynamic studies are needed in order to determine precisely which tissues can be harmed. Therefore, our article aims to draw the attention of the scientific and regulatory communities to the critical need for biodistribution studies for the genetic vaccines against COVID-19, as well as for rational harm-benefit assessments by age group.

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COVID-19 genetic vaccines; spike protein; antigen presentation; autoimmunity; histopathology; immunohistochemistry

1. Introduction

As a result of the spread of SARS-CoV-2, a global pandemic was declared by the World Health Organisation (WHO). The head of the WHO declared an end to COVID-19 as a public health emergency on May 5th, 2023; however, he stressed that it does not mean the disease is no longer a global threat [1]. The worldwide response to the outbreak focused on mass and indiscriminate vaccination using novel genetic platforms. Invoking emergency regulatory pathways to expedite market introduction, and the inherent public trust in traditional vaccines (based on inactivated or attenuated viruses), facilitated the use of lowered regulatory standards of safety and efficacy and circumvention of critical pharmacodynamic, pharmacokinetic and genotoxicity tests typical for drugs and gene therapies. Thus, billions of people were vaccinated despite a paucity of data regarding biodistribution or bio-persistence in humans, which only emerged from independent research or Freedom of Information disclosures after the administration of billions of doses. The speed at which the genetic vaccines were developed, manufactured and released was presented to the public as an achievement made possible by the scientific prowess of the pharmaceutical industry working in partnership with global governments for the greater good. However, in the words of the recently retired head of vaccine R&D at Pfizer, Dr. Kathrin Jansen: "*We flew the aeroplane while we were still building it*" [2]. This "achievement" involved scientific imprudence that must be subject to increased scrutiny as evidence of safety signals, negative vaccine efficacy and immune escape continues to accumulate.

The rationale behind this review article is to address the critical issue of the off-target distribution exhibited by the genetic vaccines against COVID-19, with a particular focus on the immunohistochemistry findings from histopathological studies. In fact, recent and conclusive sources of histopathological evidence demonstrate that the genetic vaccines against SARS-CoV-2 exhibit a distribution beyond the injection site that may involve terminally differentiated tissues subject to severe symptomatic injury. On the basis of a rational and unbiased assessment of the scientific evidence,

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and declaring that we have no conflicts of interest, we raise serious concerns regarding the safety of vaccination, especially for younger age groups and naturally immune who have a negligible theoretical benefit from vaccination. In fact, the former have a very low infection fatality rate [3], and the latter have a much higher protection against re-infection and severe COVID-19, conferred by natural immunity [4]. Therefore, the purpose of this article is to call the attention of the scientific and regulatory communities to the absolute necessity for pharmacokinetic and pharmacodynamic studies, as well as for rational harm-benefit assessments by age group.

2. The immunisation mechanism of the genetic vaccines

Many medical doctors and scientists currently recommending the COVID-19 genetic vaccines, might overlook key immunological mechanisms and underestimate the potential autoimmune consequences. Although this fact cannot be implicitly associated with hazard, even Pfizer does not fully understand how their vaccine works as Senior Vice President for Vaccine Clinical R&D Dr. William Gruber stated at FDA's VRBPAC meeting of June 15th, 2022: "*we don't have a complete understanding of the nature of the way that the vaccine works in terms of producing immune response*" [5]. The genetic vaccines against COVID-19 that were authorised for emergency use in the USA and in the European Union are the mRNA (nucleoside-modified) vaccines (produced by Pfizer/BioNTech and Moderna) and the adenoviral vector vaccines (produced by AstraZeneca and J&J/Janssen) [6,7]. These vaccines contain genetic information that hijacks human host cell machinery to synthesise the spike protein of SARS-CoV-2 and present it on the surface of cells as the immunogen [8–10]. Most likely, once translated by the ribosomes, the spike protein gets processed by the Golgi apparatus, and presented to the immune system in two ways: i) as the entire protein displayed on the cellular membrane, which can be recognised by B cells and T-helper cells; and/or ii) as protein fragments loaded on the major histocompatibility complex I (MHC I) [8,9,11].

All nucleated cells display the MHC I on their membranes, which present endogenous antigens, derived from the proteasomal degradation of intracellular proteins, to $CD8^+$ T lymphocytes [12–14]. This mechanism enables the immune system to monitor the proteosynthetic activity of all nucleated cells, in order to identify whether a cell is producing mutant, viral and/or non-self proteins, in general. The MHC II displays fragments of exogenous antigens that have been phagocytised throughout the body to $CD4^+$ T lymphocytes, and it is found on the membranes of professional antigen-presenting cells (APCs) [12,13]. When the immune system recognises a viral antigen as foreign, it triggers an inflammatory reaction which leads to the death of the antigen-presenting cell [12,13]. Consequently, the genetic vaccines, by inducing human cells to synthesise a viral protein, intrinsically rely on an autoimmune reaction mediated by T-cells to elicit an immune response.

3. Biodistribution beyond the injection site

Considering that every cell that synthesises viral proteins is perceived as a threat by the immune system and killed [11], it becomes crucial to determine the exact biodistribution of the genetic vaccines within the organism. Some authors pointed out the need for accurate pharmacokinetic and pharmacodynamic assessments [11,15–18]. However, despite the fact that pharmacokinetic studies are a fundamental part of drug safety assessment, according to European Medicines Agency (EMA) policy, they are generally not required for vaccines [16]. Thus, classifying these platforms as "traditional vaccines" allowed for such evaluations to be skipped [16,19]. It is well known that even "traditional vaccines" can cause the immune system to target its own cells throughout the immunisation process. However, there are some major differences between the genetic vaccines and the "traditional vaccines" for which the biodistribution evaluation is not "generally required". As was relayed in the letter by Polykretis and McCullough, the vaccines based on inactivated or killed viruses mainly involve presentation to APCs that phagocytose the virus particles and present the viral antigens to the immune system [20]. Such cells, which undergo a continuous turnover, perform this specific function within the organism, making them somewhat expendable. Regarding the vaccines based on attenuated viruses, they have a weakened virulence, resulting in the infection of a minor number of human cells in order to trigger an immune response.

A biodistribution study performed by Pfizer on rats and submitted to drug regulatory agencies, first released by Japan's Pharmaceuticals and Medical Devices Agency (PMDA), showed that the lipid nanoparticles (LNPs) containing the mRNA accumulate beyond the injection site, mainly in the liver, the adrenal glands, the spleen, the ovaries and other tissues [21]. Based on the findings of the aforementioned study (study No. 185350), regarding the biodistribution examined in rats injected with radiolabeled LNPs and luciferase modRNA, the Comirnaty (Pfizer/BioNTech) assessment report of the EMA, dated February 19th, 2021, on page 47, states: "*The radiolabeling data, measuring distribution to blood, plasma and selected tissues, of IM injection of a single dose of 50 µg mRNA over a 48-hour period is considered more sensitive than the bioluminescence method and indicate a broader biodistribution pattern than was observed with bioluminescence. Over 48 h, distribution from the injection site to most tissues occurred, with the majority of tissues exhibiting low levels of radioactivity*" [10]. Therefore, the EMA was aware that in rats a biodistribution beyond the injection site was occurring and that it was involving "*most* (selected) *tissues*". There is additional evidence that genetic vaccines can persist in the blood; Fertig et al. discovered that vaccine-associated synthetic mRNA stays in the bloodstream for at least two weeks following injection [22]. Notably, blood samples from children and young adults who developed post-mRNA vaccination myocarditis revealed the presence of circulating free spike protein [23]. Exosomes with spike protein have been detected in blood on day 14 after vaccination and increased after the

booster dose, lasting until four months [24]. Due to the principles of chemical kinetics and passive diffusion, the prolonged persistence of the LNPs containing the genetic material encoding the spike protein in the systemic circulation could enable it to reach even distant tissues. In support of this, the vaccine mRNA was detected even in secretions, such as breast milk [25]. Furthermore, it is noteworthy that the vaccine mRNA can persist in the lymph nodes up to 8 weeks [26], instead of "*few days*" as stated initially by the CDC [27].

4. A Role for exosomes

One mechanism by which the mRNA and the spike protein could be distributed throughout the body is *via* extracellular vesicles, particularly exosomes. A study preceding the release of the mRNA vaccines found that human cells exposed to mRNA nanoparticles were able to release fully intact mRNA molecules into exosomes, and that these exosomes could be taken up by recipient cells that then synthesised fully functional protein from the mRNA code [28]. Furthermore, an *in vitro* study demonstrated that human cells transfected with the mRNA nanoparticles coding for spike protein released the spike protein into exosomes that could then be taken up by microglia in the brain, triggering an inflammatory response [29]. In studies on biodistribution, very high concentrations among organs are found in the spleen. Immune cells in germinal centres in the spleen release exosomes as an essential step in antibody production [30]. Exosomes protect their mRNA cargo from degradation, and, furthermore, they not only travel freely *via* the vasculature and the lymphatic system, but they also easily navigate nerve fibres. *Via* travel from the spleen along the splanchnic nerve and the vagus nerve, they could reach major organs such as the heart, the liver and the brain [31].

Exosomal transport of genetic material also plays an important role in reproductive tissues such as the testes, where it has been demonstrated that a phenomenon known as Sperm-Mediated Gene Transfer (SMGT) occurs. This is the process by which genetic material from somatic cells in males can be passed on to progeny in an inheritable mosaic fashion, at low copy number, without needing to be stably integrated into the genome [32–35]. Recently, this phenomenon has also been shown to occur with gene therapies injected directly into mouse brain, where about a third of embryos inherited the transgene from the male being injected prior to mating [36]. The liposomes that traffic the genetic vaccines into the cells of the host also act as exosomes, delivering the genetic code for the spike protein into the cells in the testes and ovaries where the spike proteins could be synthesised in cells important for reproduction. An autoimmune inflammatory reaction against the cells synthesising the spike protein in either of these tissues, could result in sterility or decreased fertility due to the death of the germ cells. Furthermore, since there are *in vitro* data that suggest that the vaccine-derived genetic material can be reverse transcribed to DNA in a human liver cell line [37], we must not only be concerned about possible reactions against host tissues, including reproductive tissues, but we should also be concerned that these sequences may be passed on to the progeny, and we should thoroughly investigate such possibility.

5. Histopathological data

Strong histological evidence from biopsies and autopsies have demonstrated that the vaccine-derived spike protein was synthesised in terminally differentiated tissues [38–42]. Baumeier et al. detected the vaccine-derived spike protein on the cardiomyocytes of 9 out of 15 patients with clinical suspicion of myocarditis (which were negatively tested for SARS-CoV-2), proving that the viral protein has been synthesised in the heart tissue and suggesting an autoimmunological response due to the vaccination [38]. Schwab et al. describe the histopathological findings from standardised autopsies performed on 25 people who had passed away unexpectedly and within 20 days from vaccination (all nasopharyngeal swabs were negative, and none of the deceased persons had a recognised or symptomatic SARS-CoV-2 infection prior to vaccination) [39]. Both the aforementioned studies support the idea that vaccine-induced myocardial inflammation was a consequence of excessive T-lymphocytic infiltration, predominantly $CD4^+$ T-cells, which are the main drivers of autoimmunological myocardial injury. Mörz described the expression of the vaccine-derived spike protein in the brain and the heart of a patient who developed multifocal necrotising encephalitis upon vaccination with the Pfizer/BioNTech vaccine [40]. A 14-year-old Japanese girl died two days after receiving the third dose of the Pfizer/BioNTech vaccine and since there was no preceding infection, allergy, or drug toxicity exposure, the patient was diagnosed with post-vaccination multi-organ inflammation [41]. The histopathological findings clearly showed T-lymphocytic and macrophage infiltration in the lungs, pericardium, myocardium, liver, kidneys, stomach, duodenum, bladder, and diaphragm. It has to be specified that in this study no specific anti-spike immunostaining has been used; however, the T-cell infiltration displays a similar pattern with that observed in the abovementioned studies, and in the histopathological findings presented by Prof. Arne Burkhardt on September 18th, 2022, during the 2nd Medical Symposium, "Current Findings on Vaccination Adverse Reactions" [42]. Moreover, immunohistochemistry also revealed the expression of the vaccine-encoded spike protein in the vesicular keratinocytes and the endothelial cells in the dermis [43].

6. Additional causes of inflammation

A series of neurological disorders, including chronic inflammatory demyelinating polyneuropathy (CIDP) and multiple sclerosis (MS), have been firmly diagnosed and attributed directly to mRNA based COVID-19 vaccination [44–47]. Although routine clinical diagnostic measures cannot confirm the presence of SARS-CoV-2 spike protein, generated

by the vaccinal mRNA in these cases, they can be explained *via* Long Interspersed Nuclear Element-1 (LINE-1) and Human Endogenous Retroviral (HERV) mediated insertion mechanisms to support spike protein translation within the affected neural tissues [48]. Additionally, the mechanisms of p53 overexpression due to the spike protein toxicity in neurons has been recently revealed [49]. Dysregulated levels of p53 are strongly associated with the emergence of a dysregulated inflammatory response and development of autoimmunity [50]. Additionally, it has been demonstrated that mRNA vaccines induce the production of autoantibodies in extent that appears to be directly correlated with the number of vaccine exposures, supporting the notion that immune system hyperstimulation may result in autoinflammation [51].

The presence of free spike protein in the blood [52–54] constitutes an additional source of hazard since it may dysregulate the renin-angiotensin system *via* ACE2 binding [55–57], and could cause endothelia-platelet interactions [58], harming the cardiovascular system.

7. Conclusions

Numerous studies report the onset of autoimmune reactions following COVID-19 vaccination [47,59–76]. The histopathological data provide indisputable evidence that demonstrates that the genetic vaccines exhibit an off-target distribution, causing the synthesis of the spike protein and thus triggering autoimmune inflammatory reactions, even in tissues which are terminally differentiated and subject to symptomatic damage [38–40,42]. Despite the fact that the mechanisms of the antigen processing and presentation and the consequences for cells synthesising viral proteins are largely known and have been characterised for decades [13], the genetic vaccines were rolled out in the absence of accurate biodistribution and bio-persistence evaluations in humans, and the vast majority of the scientific community accepted that without raising concerns. Indeed, page 20 of Pfizer's non-clinical overview submitted to FDA in 2021 stated: "*No RNA or protein metabolism or excretion studies will be conducted*" [77]. Further, the question posed by VRBPAC member Dr. Jay Portnoy on June 15th, 2022 regarding the number of cells producing spike protein, and the amount and persistence of spike protein production after mRNA dosing, was dismissed as "academic" by Pfizer representative Dr. William Gruber [5]. A similar question asked by ACIP's Dr. Pablo Sanchez on June 23rd, 2022 was answered by the Moderna representative: "*The spike protein availability, I believe, is on the order of days, but like less than a week. But I will confirm that with our tox folks as well*" [78]. To our knowledge, this has not been made available.

Moreover, the guidance against performing autopsies, ostensibly to limit viral transmission, implemented by many countries worldwide during the pandemic, severely limited the ability to gather more clinical information regarding direct evidence of injuries in tissues which may have led to vaccine-related deaths [79]. The association of COVID-19 vaccination with the development of serious cardiovascular complications, especially amongst the younger and healthier age groups, has been widely recognised [23,80–83]. In a growing number of studies, it has been determined upon autopsy that vaccine-induced conditions were the cause of death [39,41,84,85]. In general, the potential risks of a genetic vaccine that induces human cells to become targets for autoimmune attack cannot be fully assessed, without knowing the exact distribution and kinetics of LNPs and mRNA, as well as the production of spike protein. Since the human body is not a strictly compartmentalised system, this is a matter of serious concern for every genetic vaccine (current or to be developed in the future) which induces human cells to synthesise non-self antigens. In fact, for some tissues, such as those terminally differentiated, the loss of cells results in irreversible damage with a potentially fatal prognosis. In conclusion, in light of the undeniable evidence of off-target distribution, the administration of genetic vaccines against COVID-19 should be halted until accurate pharmacokinetic, pharmacodynamic and genotoxicity studies are performed, or they should only be delivered in circumstances when the benefits greatly outweigh the risks.

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Author contributions

Conceptualisation, P.P. and P.Am; writing-original draft preparation, P.P.; writing-review and editing, P.P., A.D., J.C.L, D.W., A.K., M.M., P.B., M.F., S.S., P.Am; supervision, P.P. and P.Am All authors have read and agreed to the published version of the manuscript.

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