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Current state of knowledge on the excretion of mRNA and spike produced by anti-COVID-19 mRNA vaccines; possibility of contamination of the entourage of those vaccinated by these products

Helene Banoun[1*]

[1]Pharmacist biologist, Former Inserm researcher, Member of the Independent Scientific Council, Marseille 13000, France.

[*]**Corresponding to:** Helene Banoun, Pharmacist biologist, Former Inserm researcher, Member of the Independent Scientific Council, Marseille 13000, France.
E-mail: helene.banoun@laposte.fr.

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Abbreviations
LNPs, lipid nanoparticles; MMR, measles/mumps/rubella; EVs, extracellular vesicles; VEGF, vascular endothelial growth factor; pDNA, plasmid DNA; IM, intramuscular; VLP, virus like particles; RBD, receptor binding domain (spike).
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Abstract
The massive COVID-19 vaccination campaign is the first time that mRNA vaccines have been used on a global scale. The mRNA vaccines correspond exactly to the definition of gene therapy of the American and European regulatory agencies. The regulations require excretion studies of these drugs and their products (the translated proteins). These studies have not been done for mRNA vaccines (nor for adenovirus vaccines). There are numerous reports of symptoms and pathologies identical to the adverse effects of mRNA vaccines in unvaccinated persons in contact with freshly vaccinated persons. It is therefore important to review the state of knowledge on the possible excretion of vaccine nanoparticles as well as mRNA and its product, the spike protein.

Vaccine mRNA-carrying lipid nanoparticles spread after injection throughout the body according to available animal studies and vaccine mRNA (naked or in nanoparticles or in natural exosomes) is found in the bloodstream as well as vaccine spike in free form or encapsulated in exosomes (shown in human studies). Lipid nanoparticles (or their natural equivalent, exosomes or extracellular vesicles (EVs)) have been shown to be able to be excreted through body fluids (sweat, sputum, breast milk) and to pass the transplacental barrier. These EVs are also able to penetrate by inhalation and through the skin (healthy or injured) as well as orally through breast milk (and why not during sexual intercourse through semen, as this has not been studied). It is urgent to enforce the legislation on gene therapy that applies to mRNA vaccines and to carry out studies on this subject while the generalization of mRNA vaccines is being considered.

Keywords: COVID-19 vaccine; vaccine shedding; COVID vaccine adverse effects; Lipid nanoparticles; LNPs; mRNA vaccine; exosome; exosome excretion route; gene therapy; spike protein; LNPs excretion routes; exosomes penetration

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Introduction

Why are we interested in this hypothesis, which may seem conspiracist?

The expression "vaccine shedding" classically refers to the possible excretion of a virus by a person who has been freshly vaccinated against that virus; this is valid only for live attenuated virus vaccines (measles/mumps/rubella (MMR), chickenpox, rotavirus, nasal spray influenza).

No COVID-19 vaccine uses this formula. Therefore, there is no risk that a vaccine recipient will transmit a vaccine virus. However, mRNA-based COVID-19 vaccines are the first to be used commercially in humans on a global scale and no studies have been conducted regarding the possible excretion of the vaccine itself (lipid nanoparticles containing mRNA) of the vaccine mRNA or of the vaccine product, the spike protein translated by the cells of the vaccinee.

The COVID vaccination started in December 2020. The first published testimony of vaccine shedding that I saw dates from December 2021 and is that of Dr Ray Sahelian [1]: he reported cases of medical or scientific colleagues who had observed symptoms close to those of the adverse effects of the vaccine after having been in contact with freshly vaccinated persons; he proposed an excretion of the products of the vaccine by the skin and the respiratory tract and asked for complementary studies.

At the beginning, this type of testimony did not seem very credible to me, but they accumulated and in October 2021, I received a testimony from a group of French caregivers: they observed a stroke in a 7-year-old child with no risk factors and whose parents had been freshly vaccinated. There are Telegram groups listing testimonies from patients and doctors. All of these testimonials report symptoms or conditions reported in the COVD-19 vaccine adverse event databases : the adverse effects of mRNA vaccines against COVID-19 are now recognized by regulatory agencies (see VAERS and Eudravigilance databases, as well as the ANSM, France).

The vaccines are all based on the spike protein, which has since been recognized as the main responsible for the pathogenicity of SARS-CoV-2 [2-6]. Therefore, in the event that the vaccine or its product (the spike) passes from vaccinated to unvaccinated, the adverse effects of the vaccine should be found in some unvaccinated people in contact with vaccinated people. The exploration of vaccine-related pathologies in non-vaccinated age groups in contact with vaccinated people could give indications in the sense of vaccine shedding, but it does not give significant results (unpublished). As there are more than 400 pathologies related to adverse vaccine reactions in the pharmacovigilance reporting databases (see for example, the UK data, spontaneous notification data for Pfizer vaccine in May 2021 [7]), this large number dilutes the signals that could appear in non-vaccinated age groups.

On the other hand, an analysis of European, Israeli and US data shows that for the non-vaccinated 0-14 age group, most of the associations between mortality and vaccination in adults are positive: the excess mortality in non-vaccinated age groups when vaccination campaigns begin could be explained by a transmission phenomenon of the vaccine or its products. This pattern of positive correlations increases from the week of vaccination to week 18 after vaccination and then disappears. It indicates indirect negative effects of adult vaccination on mortality in children aged 0-14 years during the first 18 weeks after vaccination [8].

What is the biological plausibility of transmission of the vaccine or its products from vaccinated to unvaccinated?

To answer this question, we need to explore the possibility and routes of excretion of the vaccine or its products and the routes of their possible penetration.

Concerning the vaccine and its products, it may be the transmission of the circulating spike in the vaccinated (in free form or included in exosomes or EVs), the transmission of circulating naked mRNA or complete lipid nanoparticles (LNPs).

Therefore, the ability of LNPs, mRNA and vaccine spike to be excreted by different possible routes and then the ability of the same products to enter by different routes into the body of unvaccinated in close contact with vaccinated should be explored.

The excretion of mRNA-containing LNPs, the excretion of modified spike-encoding mRNA, and the excretion of spike produced by vaccinees have not been studied in the trial phase of the vaccines, contrary to the recommendations of the regulators concerning gene therapies. Pharmacokinetic studies of nanoparticles in general have not explored the excretion of the transporters or the transported molecules. This area should be explored.

Pfizer documents obtained by FOIA [9] show that only the excretion of some components of the LNPs (ALC-0315 and ALC-0159) was studied in the urine and feces of IM injected rats.

Regulations regarding the excretion of gene therapies by regulatory agencies

There was no regulation of mRNA clinical trials prior to RNA vaccines, yet there is strict regulation of gene therapy products. It is difficult to justify that mRNA vaccines are not considered in the same way as gene therapies regarding this regulation; indeed the only difference is that they are supposed to protect against a disease and not cure it. Gene therapies are intended for a small number of people in poor health, whereas vaccines are used on a large scale on healthy people: it would therefore be wise to apply stricter rules to them. However, the description of gene therapy products provided by the regulatory agencies does include mRNA and adenovirus vaccines.

The 2015 FDA document on Gene Product Shedding Studies [10] concerns gene therapies, which are defined as "all products that exert their effects by transcription and/or translation of transferred genetic material and/or by integration into the host genome and that are administered in the form of nucleic acids, viruses or genetically modified microorganisms". In this sense mRNA vaccines are indeed gene therapy products and should have been submitted to these excretion studies.

Excretion studies must be conducted for each VBGT (virus or bacteria-based gene therapy products), first in animals but also in humans, especially when there is a risk of transmission to untreated individuals. According to this document, clinical excretion studies are not stand-alone studies but are integrated into the design of a safety or efficacy trial. The term "shedding" refers to the release of VBGT products from the patient by any or all of the following routes: feces (feces); secretions (urine, saliva, nasopharyngeal fluids, etc.); or through the skin (pustules, lesions, sores).

The NIH guidelines [11] provide biosafety principles specifically for "synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can pair with naturally occurring nucleic acid molecules"; these are molecules of more than 100 nucleotides with the potential to be transcribed or translated. This April 2019 document is about modified and unmodified synthetic nucleic acids. Any experiment involving the deliberate transfer of a nucleic acid to a human must be preceded by Institutional Biosafety Committee approval (which is confirmed here [12]), but approval was not given because of the emergency clearance given to mRNA vaccines.

Based on an EMA document on excretion of gene products [13], mRNA vaccines meet the definition of GMTPs (gene therapy medicinal products), however their designation as a "vaccine" has allowed them to escape the clinical trial requirements for gene products that relate in particular to excretion potential, biodistribution, pharmacodynamics, genotoxicity, insertional mutagenesis (page 36 : Pharmacokinetic studies should be performed when a protein is excreted into the bloodstream). The expression of the nucleic acid sequence (its translation into protein) should also be studied (page 37). Excretion is defined as the dissemination of the vector through secretions and/or feces and should be addressed in animal models (page 30).

Therefore, according to the regulations of the American and European agencies, mRNA vaccines correspond to the definition of

gene therapy products and should have been subjected to excretion studies by all secreted fluids (urine, saliva, sputum, nasopharyngeal fluids, semen, breast milk), feces and skin (healthy or injured). These studies should have concerned the nanoparticles containing the mRNA, the naked mRNA and the product of the vaccine after translation (the spike protein).

An example of an excretion study corresponding to this regulation of gene products can be found in a report submitted to the EMA to authorize a drug intended to treat an orphan disease; it is a product based on LNPs with a composition close to that of mRNA vaccines. Here the LNPs contain siRNA. The regulations require extensive studies for this gene therapy, unlike those for mRNA vaccines, which are similar. However, studies on the excretion of these LNPs give little information. In animals, the radioactivity of LNPs is found in the urine (50%) and in the feces (between 10% and 24%). In humans, no study with radioactive LNPs has been performed, but the components of LNPs are found in the urine for less than 1% of the plasma concentrations. It is assumed that elimination is via the feces but this has not been proven. There have been no studies on excretion in milk or other body fluids [14].

Reference to possible vaccine shedding in Pfizer documents

The protocol for the Pfizer Phase I/II/III trial of COVID-19 mRNA vaccines (which began in May 2020) mentions the possibility of passage of the study product through inhalation or skin contact and passage through semen from a man exposed through inhalation or skin contact and passage through breast milk; the possibility of an adverse vaccine reaction from these exposures is also mentioned [15]. Pfizer's data clearly indicate that a pregnant woman may be exposed to “the intervention studied due to environmental exposure.” Environmental exposure can occur through “inhalation or skin contact.” Examples of environmental exposure during pregnancy include: -A female family member or health care provider reports that she is pregnant after being exposed to the study intervention through inhalation or skin contact. -A male family member or health care provider who was exposed to the study intervention by inhalation or skin contact subsequently exposes his female partner before or around the time of conception. This clearly means that any contact, including sexual contact with someone who has received the vaccines, exposes those who have not received the vaccines to the “intervention”, i.e. mRNA. Exposure during breastfeeding had also to be immediately notified during the trial: it is assumed that the investigator is concerned that a breastfeeding mother could transmit the experimental mRNA to her baby if she received the vaccines directly or if she is “exposed to the study intervention by inhalation or skin contact.”

Structure and function of extracellular vesicles (EVs) or exosomes and lipid nanoparticles (LNPs)

Natural extracellular vesicles (EVs or exosomes) are generated by most living cells, they are spherical bilayer proteolipids ranging in size from 20 to 4,000 nm and they can contain various molecules (lipids, proteins and nucleic acids, like signaling RNAs). EVs are natural carriers in the human body and are involved in intercellular communications, they can serve as transporters for different molecules that can thus pass from cell to cell, resulting in a marked response from the target cell [16]. Synthetic mRNA vaccine LNPs have the same structure as the natural exosomes they seek to mimic [17, 18]. Naturally produced exosomes can carry spike or vaccine mRNA as discussed below. LNPs have the ability (like natural exosomes) to fuse with cell membranes and release their cargo into the cytosol.

LNPs used for mRNA vaccines are nanosized (less than 1 micrometer) lipid systems made of 2 or more (usually 4) lipids at varying ratios. The most typical lipid composition used for mRNA-LNP systems consists of a cationic/ionizable lipid, a phospholipid “helper lipid”, cholesterol and/or a poly(ethylene glycol) (PEG) associated lipid. LNPs can be administered IM, subcutaneously, intradermally, intratracheally, orally, ophthalmically and even topically. LNP injected by all of these routes is capable of driving translation of mRNA to protein for several days [19]. The size of LNPs in COVID-19 mRNA vaccines is reported to be between 60 and 100 nm [20].

This trafficking of EVs is bidirectional during pregnancy (EVs cross the fetomaternal barrier and uterine cells constantly secrete exosomes) and EVs can be used to deliver drugs to the fetus during pregnancy [21].

EVs have a potential advantage for use in vaccine therapies because they are the body's natural antigen carriers and can circulate in body fluids to distribute antigens even to distal organs [16].

Little is known about the pharmacokinetics of mRNA vaccines

Nanoparticles in animals

According to a study by researchers independent of mRNA vaccine manufacturers, in mice, mRNA-carrying LNPs injected IM pass from the injection site into the lymph nodes and then into the systemic circulation, accumulating primarily in the liver and spleen. LNPs pass first into the lymphatic circulation and then into the bloodstream (LNPs smaller than 200 nm pass directly into the lymph, while those between 200 and 500 nm are transported into the lymph by dendritic cells). Unintentional direct injection into a blood vessel may also occur during intramuscular (IM) injection [22].

Nanoparticles in humans

Exposure of the human body to nanoparticles can occur accidentally through inhalation, skin contact, or ingestion. In the case of inhalation, the possible routes of transfer of nanoparticles are the bloodstream (systemic), the lymphatic vessels, the gastrointestinal tract and the central and/or peripheral nervous system [23].

Excretion of PEG-coated LNPs is primarily through feces and urine and primarily through feces when they are $>$ 80 nm in diameter. LNPs can be excreted through saliva, sweat, and breast milk [24].

LNPs of size $<$ 5 nm are rapidly excreted by the kidney. Nanoparticles that are between 5 and 200 nm tend to have extensive blood circulation. Larger LNPs have prolonged blood circulation and little renal excretion. Because of the size of LNPs, inhalation is the most direct route of entry into the pulmonary system. Exposure can be intentional, as in the case of targeting or therapeutic nanoparticles, or unintentional, through inhalation or dermal exposure, due to the increasing number of industrial applications of nanoparticles [25].

The mRNA

Persistence of viral mRNA after viral infections. The viral RNA of some viruses persists for a long time in the brain, the eyes, the testicles: this has ben showed for the measles virus, the Ebola virus, Zika and Marburg. SARS-CoV-2 persists in the respiratory tract and intestine. Viral RNAs are also detected in secretions, blood, or tissue. Prolonged shedding of these RNAs in the respiratory tract, feces, sweat, conjunctival fluid, and urine is common. Studies have shown that full-length viral RNA can persist over the long term. This persistent RNA can be translated into protein even if no viable virus can be assembled.

In patients who later develop long COVID, viral RNA is found in the blood in the acute phase of the disease [26].

Fate of vaccine mRNA. Huge amounts of mRNA are injected compared to the circulation of a virus during a natural infection: up to 10 to 7 times more, according to Professor Jean-Michel Claverie [27].

Vaccine mRNA is present from day one and persists in the bloodstream for at least 2 weeks after injection; its concentration starts to decrease after 4 days. This lifetime is much longer than was claimed by the manufacturers on the basis of brief studies in rats. The transported mRNA is encapsulated in LNPs but is found in plasma (i.e. not associated with white blood cells). This mRNA is capable of being translated into spike protein in susceptible cells and tissues [28].

mRNA packaged in LNPs is able to escape from LNPs and form extracellular vesicles that transport it to other cells: these vesicles are secreted after endocytosis of mRNA-loaded LNPs. These EVs protect

the mRNA during transport and distribute it intact to the recipient cells, the mRNA is functional and can then be translated into the protein of interest. The inflammatory response is lower after transfection with EVs than with LNPs. The uptake pathways of EVs differ from those of LNPs and are not likely to trigger the autophagic-lysosomal pathway, as they release their contents into the cytoplasm presumably without undergoing lysosomal trapping. Moreover, because of their small size, EVs can escape rapid phagocytosis and routinely transport and deliver RNA into the circulation, crossing the vascular endothelium to target cells [29].

The presence of extracellular vesicles in all biofluids is attested. They can contain nucleic acids. In sweat, we find EVs containing nucleic acids from bacteria, viruses, skin fungi but also from human cells. These EVs can also contain viruses (hepatitis C, for example). Small mRNAs (20 to 200 bp) are found in these sweat EVs; they are functional (can be translated), RNAs are protected from skin nucleases in the EVs [30].

Note that the vaccine RNA comprises 4,284 nucleotides (Pfizer) [31]. Thus, the possibility of RNAs of this size being excreted through sweat should be explored.

EVs may contain “signal” molecules such as miRNAs. It is possible that EVs contain full-length mRNAs, which are key mediators of intracellular communication. Blood and sweat RNA analyses are correlated: the EVs found in sweat reflect the circulation of EVs in plasma. Bare RNAs are also found in sweat (not encapsulated in EVs). MiRNAs are selectively selected and enriched in sweat EVs from blood and do not passively circulate in any blood or sweat fractions [32].

An increase in sweating after the COVID vaccine has been noted [33] and people who have received the vaccine have complained of increased sweating, particularly at night [34].

The possibility of exudation of extracellular vesicles from the skin has been shown: keratinocytes are able to exude extracellular vesicles capable of carrying miRNAs. In psoriasis, EVs excreted by keratinocytes pass from cell to cell: from keratinocyte to neighboring keratinocyte. In patients with lichen planus (inflammatory rash) extracellular vesicles carrying miRNAs are excreted in saliva [35].

Nanoparticles are naturally present in sputum [36]: RNA-containing exosomes were isolated from sputum of mild asthmatic patients [37].

Passage of vaccine mRNA into milk. Vaccine mRNA was found in the milk of 1/10 women studied (4/40) in the first week after vaccination with mRNA vaccine (either after dose 1 or dose 2). Amounts can reach 2 ng/mL of milk [38]. This amount may seem small compared to the 30 micrograms of mRNA injected with the vaccine, but it can be enough to produce a significant amount of spike. Indeed, an infant makes several feedings per day, for approximately 240 to 360 mL per day and a total over a week of 1680 to 2,520 mL in the first week. The newborn, weighing between 2 and 5 kg, could therefore be exposed to a dose of 5 µg of mRNA in its first week. This seems disproportionate compared to the 10 µg injected into children aged 5 to 11 years who weigh approximately 18 to 35 kg respectively [39]. The method used in the latter study is more sensitive than that of Golan et al. who did not find mRNA in milk [40]. This same team had also explored the passage of vaccine mRNA into milk by indirectly searching for PEG contained in LNPs. PEG was searched for in the milk of 13 women at varying times after vaccination: Figure 1 of the article shows the detection of vaccine PEG in milk between 24 hours and one week after injection. However, the authors concluded without specifying that these quantities were not significant [41].

Another study investigated whether COVID-19 vaccine mRNA can be detected in the expressed breast milk of breastfeeding individuals who received vaccination within 6 months of delivery. The presence of mRNA was investigated in free form and encapsulated in extracellular vesicles. EVs were isolated by centrifugation of milk.

Vaccine RNA was found within 48 h of vaccination and in higher concentrations in EVs than in whole milk. The highest concentration found was 17 pg/mL in EVs and the lowest was 1.3 pg/mL in whole milk. The priority presence of mRNA in EVs and not in whole milk may explain why Golan et al. did not find it [42].

It has been known for some years that mRNA encapsulated in extracellular vesicles is protected from gastric juices and can transfect intestinal cells [43, 44]. A recent review by Melnik and Schmitz confirms that milk EVs survive the extreme conditions of the gastrointestinal tract, are internalized by endocytosis, are bioavailable, reach the bloodstream, and penetrate peripheral tissue cells [45].

Transplacental passage of nanoparticles? In mice, LNPs of the same type as those used in COVID-19 mRNA vaccines have shown their ability to transfect mRNA after injection into a fetal vein or in utero [46].

In an attempt to immunize fetuses against neonatal herpes in pregnant mice by injection of mRNA-loaded LNPs into the mother, the possibility that transplacental passage of LNPs would explain both fetal immunization and maternal passage of induced Ig is not discussed [47].

Studies have shown that it is very possible that nanoparticles of comparable size to those used for mRNA vaccines are capable of transplacental passage in humans [48, 49].

The delivery of LNP-based therapies during pregnancy poses risks that should be investigated. Detection of transplacental passage depends on the sensitivity of the detection methods: for some types of nanoparticles embryotoxicity has been observed while no absorption by the fetus was observed; this absorption does not seem to correlate with the type, size or surface electric charge of the nanoparticles. Translocation of nanoparticles is likely to depend on the different stages of pregnancy. During the first trimester, the placental barrier is very thick to protect the developing embryo and becomes thin at term when large amounts of nutrients are needed to support fetal growth. However, in animals, placental transfer appears to be higher in early pregnancy. There is a need to develop human models for NP transfer studies in early pregnancy. Comparison with animal studies is essential, as the placenta is the most species-specific organ [50, 51]. 240 nm nanoparticles are able to cross the human placental barrier [52].

All these publications underline the difficulty of extrapolating animal studies to humans concerning the transplacental passage of nanoparticles. From a 2022 review [53], nanoparticles can transit through ordinary placental transcellular transport mechanisms such as pinocytosis, active transport, facilitated diffusion and passive diffusion. RNA cargo exosomes are also able to cross the human placental barrier. PEG-coated LNPs are reported to have less diffusion across the placental barrier than liposome-based formulations, but are able to deliver some of their cargo to the fetus [54].

All of these data cannot rule out that LNPs from mRNA vaccines are capable of reaching the fetus of a vaccinated mother during pregnancy.

Excretion of LNPs into the sperm? I have not found any studies regarding the possibility of LNPs passing into the sperm; however, the effect of nanoparticles on fertility and sperm quality has been widely studied in animals [55]. The toxicity of nanoparticles on male reproductive function is well established, gold nanoparticles have been shown to act only by interacting with the surface of sperm cells but not penetrating them. No data is available on the possible penetration of LNPs into the sperm.

According to a confidential Pfizer document obtained through the FOIA [56] concerning pharmacokinetic studies in rats, LNPs concentrate in the ovaries and to a lesser extent in the testes.

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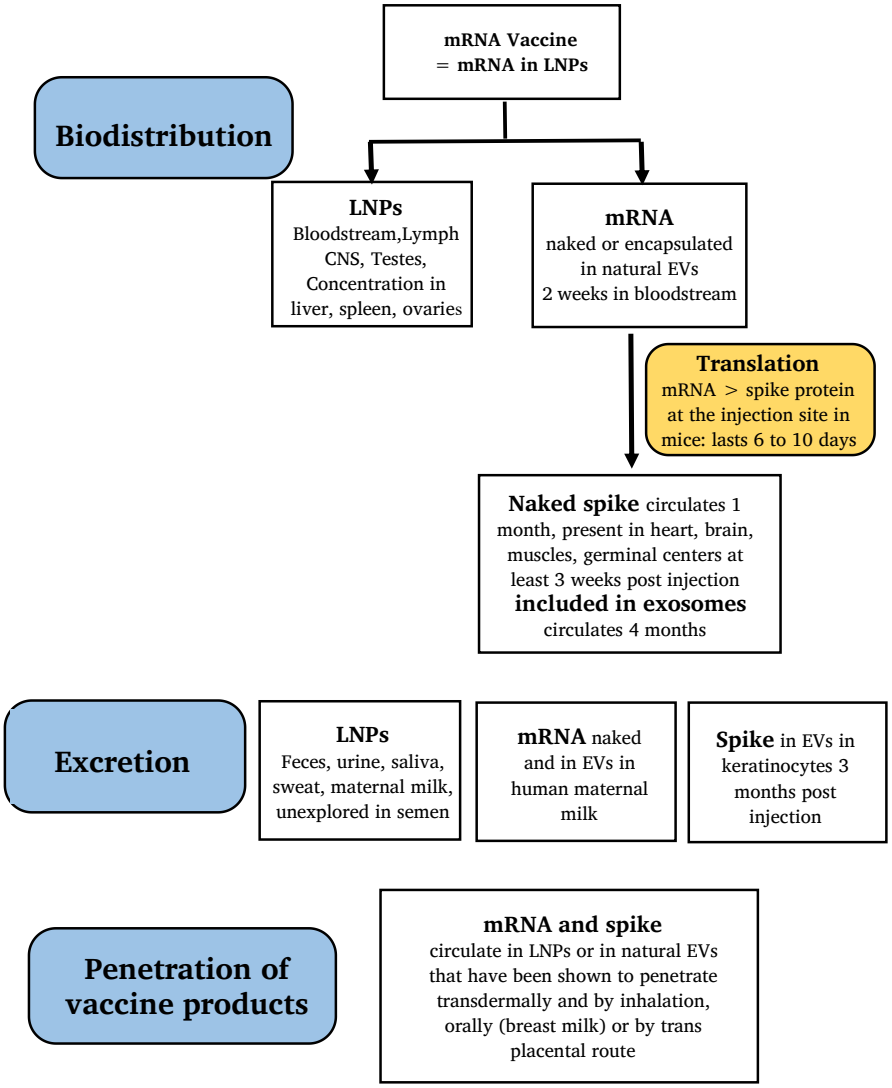

Figure 1 State of knowledge on excretion of mRNA vaccines

Fate of spike protein after mRNA translation

A CDC-sponsored news site accessed on July 21, 2021 notes that the lifespan of spike in the bloodstream is "unknown and may be a few weeks." [57]. Injection of LNPs containing pseudouridine-modified mRNA by IM, subcutaneously and intradermally results in protein production at the site of injection, the duration of active translation is 6 to 10 days in mice. Intradermal injection produces a lower initial amount of protein but over a longer period of time than the IM route. By the intradermal route, the half-life of protein production is the longest compared with other injection routes (IM, subcutaneous, IV, Iperitoneal, intra-tracheal). By IM delivery, the majority of translation ceased in the liver at day 2 post-injection but lasted for up to 8 days in muscles [58].

In humans, the spike protein could persist for a long time in vaccinees, monitoring of vaccine adverse effects should therefore be extended [59]. Comparison of spike concentrations achieved during disease and after vaccination shows that during severe COVID-19 the median concentration observed is 50 pg/mL with maximums at 1 ng/mL. During severe Covid infection, levels of up to 135 pg/mL of S1 spike can be detected, most commonly between 6 and 50 pg/mL. After vaccination with mRNA vaccine concentrations up to 150 pg/mL are commonly observed but may reach 10 ng/mL in individuals with vaccine-induced thrombocytopenia [60].

The same team [61] also shows that spike protein persists for a long time in free form: vaccine-induced spike mRNA circulates in plasma as early as D1 after vaccination and up to 14 days, with the peak occurring at D5 with 68 pg/mL of S1 sub-unity detected; full-length spike is detected up to D15, with a peak at 62 pg/mL. After the 2nd dose, free spike is no longer detected as it would be bound to antibodies; the study does not detect antibody-spike immune complexes.

Another team also showed that, after vaccination with mRNA, spike protein enters the bloodstream, persists for more than a week and is completely eliminated within 1 month. The increase in blood spike concentration after vaccination is rapid (1 to 3 days) [62].

According to an autopsy, vaccine spike is found up to three weeks after injection in different organs (heart, brain, muscles, germinal centers, etc.) and particularly in the endothelium of capillaries [63].

Circulating spike-containing exosomes

After COVID-19 infection, spike circulates as exosomes. Exosomes are released from cells into the extracellular environment under normal and pathological conditions. Exosomes are an important tool for intercellular communication, as they serve as shuttles for the transfer of biologically active proteins, lipids, and RNA. EVs can incorporate pathogenic proteins and/or viral RNA fragments from infected cells to

transport material to target cells, an event that plays an important role in responses to viral infections. SARS-CoV-2-S protein or derived fragments were clearly present in the exosomes of COVID-19 patients. SARS-CoV-2-S-derived fragments are present in exosomes from all COVID-19 patients [64].

Spike also circulates in exosomes after mRNA vaccination in humans. Authors have proposed that after LNP internalization and mRNA release, antigen sorting and trafficking can induce the release of S protein-containing exosomes. The events presented would occur in the apical and/or basolateral surfaces of polarized (e.g. epithelial) cells [65]. Indeed, the vaccine spike is spontaneously enveloped in exosomes or extra-cellular vesicles: Vaccination with mRNA and translation of the mRNA induces the production of exosomes carrying the spike protein and circulating in the blood 14 days after injection and up to 4 months after vaccination. Injection of these exosmoes into mice induces the synthesis of anti-spike antibodies [66].

Vaccine spike was found in keratinocyte vesicles of dermal endothelial cells from a patient with skin lesions 3 months after vaccination with Pfizer-BioNTech vaccine. This patient had varicella-zoster virus infection. A plausible hypothesis was that RNA stabilization by methyl-pseudouridine substitution at all uridine nucleotides for BNT162b2 could result in long-term production of the encoded spike from any cell, persistently affecting the microenvironment of the protective immune system, including the skin [67].

All these data indicate that vaccine LNPs or exosomes naturally formed after vaccination could contain mRNA or spike and could be present in body fluids. Are these nanoparticles capable of entering from these fluids into the bodies of unvaccinated individuals in contact with freshly vaccinated individuals?

Ability of LNPs or natural extracellular vesicles (EVs or exosomes) and mRNA to enter through different pathways

Use of nanoparticles for therapeutic purposes by inhalation, transdermal, in utero, conjunctival route

In a review dedicated to the safety of nanoparticles in biomedical applications, we learned that exposure to LNPs can occur through ingestion, injection, inhalation and skin contact. Some exposures are unintentional, such as pulmonary inhalation of NPs in the environment or at manufacturing sites [68].

Nanosystems are increasingly being exploited for topical and dermal delivery, including therapeutic peptides, proteins, vaccines, gene fragments, or drug carrier particles [69]. Intradermal administration of mRNA encoding vascular endothelial growth factor (VEGF) has been shown to result in functional protein expression in the skin even in the absence of lipid nanoparticles [70]. According to Palmer et al [71], in a lipid nanoparticle formulation, liposomes increase the transdermal passage of molecules used to treat skin diseases. Skin penetration of siRNAs has been demonstrated in the form of nano-carriers, these siRANs transfect cells and express the targeted gene of interest. Nanocarriers have been tested for use in transdermal vaccination [72].

Extracellular vesicles are used to deliver therapies other than vaccines: clinical studies are underway by local route (periondontitis, ulcers, epidermolysis bullosa) and by inhalation (ongoing trial against Alzheimer's disease) [73]. Lipid nanoparticles with a lipid bilayer are able to pass the skin barrier and carry genetic material. These particles can penetrate the skin through hair follicles or directly into keratinocytes due to their similarity to cell membranes [74].

Intranasal, oral, and intraocular and subconjunctival administration of extracellular vesicles capable of carrying drugs has been successfully tested.

Intranasal administration represents the second most frequently reported route. It is effective in transporting drugs into the CNS, into the lungs. Most of the protective effects were obtained in a similar way for intravenous and intranasal administration. Oral administration has been described for EVs from bovine milk in a mouse model. Six hours after administration, vesicles were localized in the liver, heart, spleen, lungs, and kidneys. Intraocular and subconjunctival injection of MSC-derived EVs (stem cells) delivered vesicles to the retina in a rabbit model of diabetes-induced retinopathy [75].

Nanovesicles naturally produced by plants are morphologically and functionally identical to their mammalian analogues. A review on plant nanovesicles brings together knowledge on the transdermal, transmembrane, and targeting mechanisms of these vesicles. Experiments on mice have shown that it is possible to deliver RNA into a brain tumor via these intranasally introduced nanovesicles. These nanovesicles would also be able to efficiently transport their cargo through the skin and into the skin cells [76].

Lipid nanoparticles are a potential carrier for delivering molecules to the posterior chamber of the eye: they have demonstrated excellent ocular permeation characteristics and penetration-enhancing capabilities, while exhibiting high drug loading and entrapment efficiencies [77].

Nanoparticles in vaccination and gene therapy trials (LNPs containing nucleic acids) via the respiratory route

Nucleic acid cargo nanoparticles are capable of transfecting airway cells in animals and humans by local administration (instillation or nebulization). The DEFUSE project [78], submitted by Eco Health Alliance in response to a DARPA call for proposals, deals with the transcutaneous administration of vaccines in animals using nanoparticles. For therapeutic purposes, the LNPs formulation was optimized for lung penetration by inhalation and it was verified that mRNA is efficiently translated in the lung after nebulization (mouse assay) [79].

The intranasal route has also been studied for vaccination with mRNA cargo LNPs as well as for gene therapy in cystosis fibrosis with mRNAs encapsulated in LNPs by the intranasal route by instillation in the nostrils of mice: the mRNA transfects the nasal cells and expresses the protein of interest in cells that did not express it because of a genetic defect [80].

In humans, liposomal DNA-containing nanoparticles administered locally by nebulization have transfected airway cells. A recent phase 2b trial of cystic fibrosis transmembrane conductance regulator (CFTR) DNA delivery using a liposomal delivery system showed that after repeated monthly nebulizations for one year, the cystic fibrosis patient groups experienced a stabilization of lung function, while the placebo group experienced a decline [81].

Clinical trials for influenza prevention have shown the efficacy and safety of inhaled mRNA vaccines: Bare mRNA or mRNA enveloped in lipid particles (especially PEG-based as in the anti-COVID mRNA vaccines), is able to be inhaled in an aerosol and transfect lung epithelial cells [82]. In utero administration of lipid nanoparticle formulations containing mRNA can be applied to deliver mRNA to mouse fetuses, resulting in protein expression in the fetal liver, lungs, and intestines [70].

Testing LNPs for transcutaneous vaccination

In a review [72] on the possibility of transcutaneous vaccination with LNPs, we learn that undamaged human skin is impermeable to micro- and nanoparticles but there is evidence of some dermal penetration into viable tissues (mainly in the stratum spinosum of the epidermal layer, but also possibly in the dermis) for very small particles (less than 10 nm). When using intact skin penetration protocols, there is no conclusive evidence of skin penetration into viable tissues for particles with a primary size of about 20 nm and larger. But there is no information appropriate for skin with impaired barrier function, e.g., atopic skin or sunburned skin. Some data are available on psoriatic skin. There is evidence that some mechanical effects (e.g., bending) on the skin may affect nanoparticle penetration. But it has been shown that nanoparticles accumulate in follicular openings, sebaceous glands or skin folds. An aqueous suspension of nanoparticles as well as a hydrogel formulation of these particles, applied to pig ear skin in vitro, penetrated deep into the hair follicles. These particles can release various encapsulated compounds that then penetrate the skin.
There is evidence in the literature that the trans-follicular route can be

used: topical application of naked plasmid expression vectors to intact mouse skin induced antigen-specific immune responses. HBsAg-specific cellular and antibody responses were induced in the same order of magnitude as those produced by intramuscular (IM) injection of recombinant HBsAg polypeptide vaccine. In contrast, no immune response could be induced in nude mice: the presence of normal hair follicles was a prerequisite for the induction of a response. The particles were approximately 150 nm in size. LNPs in mRNA vaccines are between 100 and 400 nm [22].

One system that has been clinically tested transdermally is the DermaVir HIV-1/AIDS patch. It contains a plasmid DNA (pDNA) vaccine encoding all major HIV-1 antigens and viral-like particle formation.) The pDNA is formulated as mannosylated polyethyleneimine nanoparticles (80-400 nm) similar to those of pathogens. This study involved 12 people immunized with the vaccine: they developed higher and broader levels of $CD8^+$ T cells compared to placebo, although it had no effect on $CD4^+$ T cell numbers [72].

Naked RNA could also be used via skin passage and inhalation

RNA oligonucleotides can penetrate intact skin and retain their biological activity, penetration through the skin does not depend on the size of the molecule under study (12.5 to 29.3 kDA) [83].

The feasibility of inhaled RNA for passive transfection has also been demonstrated in a number of studies. Mechanistically, inhaled RNA can lead to passive synthesis of non-infectious spike proteins using cell transfection machines, thus leading to immunization of the individual [84].

Therapeutic and vaccine LNPs in COVID-19

Given that vaccine LNPs are synthetic exosomes it is not surprising that COVID therapeutics and vaccines with natural exosomes as vectors are being tested. Nebulization of exosomes for inhalation therapy has been tested against COVID-19. Clinical trials are underway to deliver aerosolized anti-viral therapies in EVs in COVID-19. Currently, over sixty clinical trials are underway to study the effects of MSCs (mesenchymal stem cells) and EVs (containing these MSCs) in COVID-19 patients. A phase 1 clinical trial to evaluate the safety and efficacy of inhaled exosomes derived from allogeneic adipose MSCs for the treatment of COVID-19 pneumonia has been completed. 3 clinical trials used aerosol as the route of administration [16]. In 2022, MSCs exosomes showed efficacy for nebulization therapy in COVID-19 patients [85].

Natural exosome vaccines against SARS-CoV-2: plantar or inhalation route

Exosome vaccines carrying mRNA have been considered against SARS-CoV-2 [86]. Vaccine trials injected as exosomes into the footpad of mice showed induction of antibodies against spike [87]. Spike RBD exosomes (nanoparticles) are capable of nebulizing and inhaling antigen into mouse lung cells and inducing an immune response. They are virus-like particles (virus like particles (VLP)) naturally obtained from lung cells and carry RNA from their parent cell as well as various proteins expressed on their surface [88]. Also by inhalation, exosomes containing mRNA or spike protein are able to immunize mice or non-human primates against SARS-CoV-2 and natural EVs are more effective than synthetic EVs [89].

The possible reinterpretation of a study may support vaccine shedding

Scientists compared unvaccinated children living with unvaccinated parents with children who were also unvaccinated but living with vaccinated parents [90]. The children of vaccinated parents have anti-COVID IgG in their nose and the difference with the children of unvaccinated parents is significant. The authors think that this is due to antibody shedding by droplets: what is transferred would be the IgG antibody itself in the saliva droplets. But it is possible that children develop intranasal IgG because other vaccine byproducts or exosomes are excreted by their vaccinated parents. This could be due to lipid nanoparticles of mRNA that could be excreted and transferred through saliva, sputum or skin. Children would develop an immune response to the nanoparticles (or vaccine by-products) instead of IgG being transferred directly from parents to children. The antibodies sought are IgG and IgA against the RBD of the spike and not against the nucleocapsid of the virus, which is a pity because the authors have developed both types of tests [91]: this does not allow to distinguish children who would have been naturally infected by the virus (and would have anti-RBD and anti-N antibodies) from children who would have developed antibodies following their parents' vaccination (and would only have anti-RBD and no anti-N because not induced by the vaccine).

Conclusion

There are many testimonies of non-vaccinated persons who experienced symptoms identical to the adverse effects of the vaccine after having been in contact with freshly vaccinated persons. A study shows an excess of mortality in the non-vaccinated age groups when vaccination campaigns begin, which could be explained by a phenomenon of transmission of the vaccine or its products.

It is important not to neglect these testimonies because the required studies of pharmacokinetics and in particular of excretion of the vaccine and its products have not been carried out in spite of the regulations in force for gene therapies, which include mRNA vaccines according to the definition of these gene products. Moreover, the doubt about the possible transmission of the vaccine creates an unhealthy climate of suspicion of the non-vaccinated towards the vaccinated: a clarification would therefore be welcome.

The vaccines are all based on the spike protein, which has since been recognized as the main responsible for the pathogenicity of the SARS-CoV-2 virus: if transmission of the vaccine or of the spike is possible, it is logical to find the adverse effects of the vaccine in non-vaccinated people in contact with vaccinated people.

Little is known about the pharmacokinetics of the vaccine. Vaccine LNPs are very similar to natural EVs or exosomes, whose structure and function scientists have tried to mimic as closely as possible. According to the few studies conducted by manufacturers and independent researchers, mRNA vaccine LNPs circulate in the blood and accumulate in the spleen and liver of mice (and to a lesser extent in many organs including ovaries and testes, bone marrow,..). Translation into spike protein persists 6 to 10 days in mice at the injection site and 8 days in the muscles.

The route of excretion of LNPs varies according to their size, in the case of LNPs of mRNA vaccines excretion should be mainly by the feces but also by the urine. The quantitative results of these studies suggest that other routes of excretion than feces and urine should be explored. Studies prior to mRNA vaccines suggest that EV excretion is possible through saliva, sweat, and breast milk.

Studies have shown that it is very possible that nanoparticles of comparable size to those used for mRNA vaccines are capable of transplacental passage in humans. Natural nanoparticles (EVs) are naturally present in all body fluids (including sputum, saliva, and sweat) and in keratinocytes and can carry nucleic acids that are thus protected from nucleases. Certain types of RNA (miRNAs) are selectively selected and enriched in sweat EVs from blood. No studies have been found regarding the possibility of passage of LNPs into semen; given the biodistribution in all organs and fluids, such passage is a priori possible and should be explored.

Viral RNA of many viruses is found in blood, secretions and tissues. Vaccine mRNA is injected in quantities orders of magnitude greater than the viral RNA produced during natural infection. This mRNA is found in the blood as early as the first day after injection and persists for up to 15 days. It is able to escape from LNPs and to be encapsulated in EVs, it is functional and can be translated into protein. Vaccine mRNA naked or encapsulated in EVs is found in breast milk within the first week after injection; it is protected from gastric juices and can transfect neonatal cells.

RNA embedded in EVs or even naked is capable of transfecting cells by inhalation or transdermal passage. Intranasal, oral, transdermal intraocular and subconjunctival administration of extracellular drug-carrying vesicles has been tested: LNPs can be administered through the skin, intranasally, intraconjunctivally and by inhalation; experiments have shown that mRNA included in these LNPs is capable of transfecting cells Vaccination trials against COVID by inhalation of EVs containing mRNA or spike protein have shown positive results in mice and nonhuman primates. Natural EVs are more effective than synthetic EVs.

Spike protein translated from vaccine mRNA persists for months in large quantities in vaccinees; it is found in free form in plasma and encapsulated in EVs that form spontaneously from the cells where spike was produced. These EVs can deliver their cargo to different cell types, in particular to fetal cells of vaccinated mothers. Spike can be found in keratinocytes of the skin.

Specifically against coronaviruses, gene therapy and vaccination trials (especially with mRNA) have shown the possibility of transfecting cells transcutaneously, nasally and by nebulization from LNPs and even from naked mRNA. Spike or mRNA RBD vector exosomes have been tested by inhalation in animals for anti-COVID-19 immunization.

All these studies show that EVs carrying mRNA and spike could therefore be excreted by different body fluids and could enter by transcutaneous or inhalation route in unvaccinated individuals (as well as by breast milk in infants and by transplacental passage in fetuses and why not by semen). Naked mRNA could also be excreted and entered.

The mRNA (and adenovirus) vaccines correspond exactly to the definition of gene therapy given by the health agencies (FDA, NIH and EMA). According to the regulations of these agencies, these products should be subject to additional pharmacokinetic studies (in particular excretion studies) as a matter of urgency as the widespread use of mRNA technology becomes apparent. Indeed, Sanofi launched clinical trial of the first mRNA-based seasonal flu vaccine candidate [92], Moderna launched phase 3 trial of mRNA influenza vaccine [93]. For these flu vaccines, emergency approval should not be applied and the requirement for these additional studies should not be exceeded.

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