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## Perspective

## Liposomes and mRNA: Two technologies together create a COVID-19 vaccine

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## ABSTRACT

The urgency to understand and modify immune responses has never been as great universally as during the present Coronavirus time. It has been suggested that using established techniques, a small piece of the so-called spike protein of the Coronavirus injected into humans in the form of mRNA could raise an immune response against the expressed protein, in turn killing or inactivating the invading Coronavirus. Unfortunately, however, the mRNA was found to be too vulnerable to survive in the body long enough on injection to produce the spike protein and an immune response to it. But as it happens, a solution was to hand, one waiting to be discovered.

In the mid-1960's, the biophysicist Alec Bangham and colleagues at the Babraham Institute in Cambridge observed bimolecular leaflet membrane structures formed on addition of water to dry phospholipid [1,2]. The membrane structures were initially named 'Banghasomes', then 'Phospholipid vesicles', 'Liposomes' and more recently 'Lipid nanoparticles', a name that does not seem to add significantly to its meaning as liposomes can also be of nano size. Regardless, 'Liposomes' have dominated as a name. And because of their similarity to cell membranes, liposomes were adopted as a model for the study of cell membrane biophysics.

A few years later, however, the future of liposomes was to change direction from serving as a model of cell membranes to that of a drug delivery system. The author, together with Brenda Ryman at the Royal Free Hospital School of Medicine, London, in search of a system that could deliver drugs to specific areas in the body, used liposomes to that effect. Initial work in animals confirmed liposomes as a promising drug delivery system [3–5]. It was eventually adopted by a myriad of workers, with dozens of drugs and other agents entrapped in liposomes of varying size (e.g., 20 nm to several microns in diameter), lipid composition, surface charge, ability to accommodate water soluble or lipid soluble materials and, if needed, to provide a pegylated vesicle surface, thus rendering liposomes a delivery system of multiple uses. These include the licencing of a plethora of therapeutics, for instance for cancer and antimicrobial therapy [6–9] (Fig. 1).

Soon after the liposomal drug delivery concept was established, the author, together with Anthony Allison working at the MRC's Clinical Research Centre in Harrow, London, published data from experiments in which animals were injected subcutaneously or intravenously with tetanus toxoid or with the toxoid entrapped in negatively charged liposomes

[10,11]. The antibody response to the liposomal toxoid was far greater than that of the toxoid as such, thus confirming liposomes as an immunological adjuvant for vaccines. Positively charged liposomes were superior as adjuvants to those that were negatively charged. Moreover, whereas re-injection of pre-immunised animals with tetanus toxoid (as when booster injections are required) led to serum sickness and death, animals re-injected with the liposomal toxoid remained healthy. The immunological adjuvant property of liposomes was further demonstrated using the hepatitis B surface antigen [12], and by the production of two additional liposome-based vaccines by Berna, namely Epaxal for hepatitis A, and Inflexal V for influenza, both approved for use in humans.

It was only to be expected that sooner or later, liposomes would be used in genomic vaccines as well as other liposome-based applications of nucleic acids. This was in fact predicted at the very beginning of liposome research on the drug delivery potential of liposomes [5]. Because of the vulnerability of liposomes in the circulating blood and the potential leakage of entrapped labile solutes (for example, nucleic acids), it was essential that liposomal membranes were rendered stable in the presence of blood. This was achieved by the judicious choice of liposomal lipids. We were able to show that liposomal membrane stability in blood is achieved by using a long chain phospholipid [13], for instance dipalmitoyl phosphatidyl choline, distearoyl phosphatidyl choline, or dioleoyl phosphatidyl ethanolamine, each supplemented with equimolar cholesterol [14]. Such liposomes were also supplemented with a cationic lipid, for instance 1,2-dioleoyl-3 (trimethylammonium) propane (DOTAP) which binds to the nucleic acid thus leading to high values of nucleic acid (e.g., mRNA) association with, or entrapment into, liposomes. Alternatively, one could use an equally effective ionizable

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**Fig. 1.** Alec Bangham (left) and Gregory Gregoriadis and Brenda Ryman (right) in her laboratory at the Royal Free Hospital School of Medicine, Hunter St. London, 1971.

aminolipid [15]. Our work with plasmid DNA (pRc/CMV HBS coding for the hepatitis B surface antigen, S region) entrapped in liposomes of a lipid composition identical to that just described, has shown that the injected liposomal plasmid not only expresses itself to produce the protein antigen, both humoral and cell mediated immune responses to the antigen are far greater than when the free plasmid is injected [16].

In view of the above, the following scenario for the creation of an anti-COVID-19 vaccine was made possible: mRNA coding for the protein spike of the Coronavirus, would be entrapped into liposomes that are designed to remain stable in the circulating blood [17] until they are taken up by phagocytic cells in the body by endocytosis [5]. It has been suggested that within the cytosol, there will be destabilisation of the endosomal membrane whereupon, through lateral diffusion of anionic lipids from the cytoplasm-facing endosomal monolayer, mRNA will be displaced from the complex and released into the cytosol [18]. The mRNA will then be expressed as the spike protein in turn promoting an immune response to it that will kill or inactivate the invading virus. The mechanism by which liposomes act as an immunological adjuvant to augment the immune response to the spike protein, is not clear at present.

It is a fact, however, that a liposome-based mRNA anti-COVID-19 vaccine has been created by Pfizer/BioNTech and Moderna, and already administered worldwide into millions. Both vaccines [19], developed within the last two or three years, are made of components [10,11,13,14,18] previously shown to maintain the stability of liposomes in blood and to promote immune responses. The components include distearoyl phosphatidyl choline (the lipid that provides the liposomal bilayer and hence the basis of liposomal adjuvanticity), cholesterol (which contributes to the stability of the liposomal mem-

brane in the presence of blood), and a cationic or an ionizable lipid that contributes to improving liposomal adjuvanticity [19]. The presence of the pegylated lipid in both vaccines ensures the production of liposomes free of vesicle surface to surface interactions. It follows that without the liposomal bilayers and the components within, there would not be a liposome-based anti-COVID-19 vaccine (Table 1).

Regardless of the minutiae of vesicle onomatopoeia, it is remarkable that it took fifty or so years for the two technologies, mRNA and lipid vesicles, to come together at more or less the same time of their need.

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#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Table 1**

Advantages of using liposomes to deliver mRNA into cells.

Item	Advantage
1	Liposomes are biodegradable, easy to prepare and can entrap mRNA quantitatively
2	Liposome-entrapped mRNA fully protected from nuclease attack in the blood circulation
3	Liposomal mRNA enters the cytoplasm of cells by endocytosis
4	Cationic liposomal mRNA escapes the lysosomotropic pathway to end up intact in the cytoplasm
5	Within the cytoplasm, mRNA is expressed as the spike protein whereupon, by an as yet unclear mechanism, liposomes or their remnants exert their immunological adjuvant action

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