VACCINE FORMULATIONS FOR PROTECTION AGAINST COVID-19 INFECTION

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Before 2003 only twelve animal or human coronaviruses were identified

2003
Severe acute respiratory syndrome coronavirus (SARS-CoV)
8096 cases and 774 deaths

2012
Middle East respiratory syndrome coronavirus (MERS-CoV)
2,442 cases and 842 deaths

2019
Wuhan, China, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged
>78M cases and >1.7M deaths

SARS-CoV-2 likely crossed over from bats at a wet market in Wuhan, although Pangolin’s are also likely a host.
SARS-CoV-2 BINDS THROUGH SPIKE PROTEIN TO ACE2

SARS-CoV-2

Spike protein

Angiotensin-converting enzyme (ACE) 2 receptors

Host cell

ACE2 Host Receptor

S1-NTD

S1-CTD/RBD

S2

RBD: Receptor Binding domain

NTD: N-terminal domain

CTD: C-terminal domain

S protein: Spike protein

ACE2: Angiotensin-converting enzyme 2 - Host receptor where spike protein binds
**COVID-19 IMMUNOPATHOLOGY**

Can include hyper-immune responses

- A cytokine storm that leads to immune cell infiltration of the lungs
- Alveolar damage can lead to pulmonary failure
- Can result in acute respiratory distress syndrome (ARDS)

SARS-CoV-2 infection is thought to act in part through antibody-dependent enhancement (ADE).

Non-neutralizing antibodies formed after infection or vaccination could lead to enhanced virus uptake in cells.

ADE with SARS-CoV-2 infection has not been characterized in humans.

**Th1 AND Th2 RESPONSES**

Th = T helper cells (CD4+)

Generally “Pro-inflammatory”-type response
- **Th1**, Th17

Generally anti-parasite response
- **Th2**

A Th1 response, over a Th2 response
- Indicates a strong cellular (T cell) AND humoral (e.g. antibody) response
- Thought to help overcome ADE and adverse immunopathology

Image: https://immunobites.com/2020/04/13/the-many-flavors-of-t-cells/
**CLINICAL TRIALS**

**Preclinical**
- Results are those collected from animal models

**Phase 1 Trials**
- Relatively small trials with ~20-100 healthy patients
- Primary objective is simply to establish safety
- A range of doses to the patients
- May collect correlates of protection (e.g. neutralizing antibodies and T cell responses)

**Phase 2 Trials**
- To establish a dosage that is most likely to achieve the desired endpoint of the vaccine (e.g. protective efficacy).
- Examines the effect of the dose on different biomarker correlates of protection (e.g. neutralizing antibody, T cell responses)

**Phase 3 Trials**
- Evaluation of the vaccine to achieve a certain clinical endpoint.
- For COVID-19, this endpoint is protection against COVID-19 from SARS-CoV-2 exposure.

In the UK, some companies such as hvivo are conducting human challenges with SARS-CoV-2 infection.

TYPES OF VACCINES

- Safety concerns because vaccine components could become virulent.
- Reduced efficacy because the immune system needs a ‘danger’ signal to activate it, which something like a protein does not normally have.

Although this lists viruses, it could also be bacteria or other pathogens.
THE VERY FIRST VACCINE WAS SIMILAR ENOUGH TO SMALLPOX TO BE PROTECTIVE

Edward Jenner noticed that milk maids did not have smallpox scars and thought they were exposed to a pathogen that protected them.

Vaccination with cowpox provides protection against both smallpox and cowpox.
ATTENUATED PATHOGEN

Genetically altered original pathogen to be non-infective

Will not cause significant infection.  Will cause significant infection.

Vaccination with attenuated pathogen provides protection against pathogenic pathogen.

Cannot be taken by patients with a suppressed immune system (e.g., HIV+, TB+, organ transplant, cancer patients).

Protective immunity through attenuated organism with elements ‘knocked out’ that result in reduced pathogenicity. (e.g. the dark green spikes allow for infection and absence of them prevents infection)

Examples:
- Measles
- Polio (Sabin)
- Rotavirus
- Varicella zoster (Chicken pox)
- Yellow Fever
- Influenza (nasal)
**COVID-19 LIVE ATTENUATED VIRAL VACCINE**

Mutations in the 2'O methyltransferase non-structural protein 16 (NSP16) as well as the exonuclease NSP14 of SARS-CoV-2

2'O methyltransferase NSP16 and exonuclease NSP14 is involved in the RNA cap creation

A single mutation could still alter to infect aged populations, the second mutation was required for safety

In hamsters, it prevented infection in the lungs, but not nose. Decreased immunopathology compared to infection.
TYPES OF VACCINES

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REPLICATING AND NON-REPLICATING VIRAL VECTORS

- Uses virus packaging to deliver RNA or DNA
- Viral vector could replicate for short period of time, an explicit number of replications, or not at all
- Can elicit potent immune responses

Adenovirus, Rabies, Measles, Vesicular stomatitis virus (VSV), and HIV based vaccines have all advanced to clinics wherein spike protein is expressed.

Obvious safety concerns with immunocompromised individuals.
ASTRAZENECA AND OXFORD’S ADENOVIRUS FORMULATION

Vector derived from a chimpanzee adenovirus and encoding the S protein (ChAdOx1 nCoV-19)

Schedule: Day 0 (prime) and 28 (boost)
Notes: Co-administered with acetaminophen (Tylenol) to limit local and systemic reaction to the vaccine

With prime:
91% of patients (32/35) had neutralizing titers

With boost:
100% had neutralizing responses
T cell responses to the spike protein peaked at day 14

Average Vaccine Efficacy: 70%
MIX-UP DURING TRIAL

Interestingly, one trial site gave a half-dose prime followed by full-dose boost and noted 90% efficacy. Another site gave two full doses and noted 62% efficacy.

Results of this dosing change indicates an issue with viral vectors, that a neutralizing response can be generated against the vector, limiting its repeated use.

Also, an issue with the clinical trial design was that the more efficacious group did not include patients >55 years old.

The Italian manufacture of the vaccine had used PCR to verify viral titer.

Oxford used a significantly less exact spectroscopic based method to confirm titer (e.g. nanodrop), which was confounded by polysorbate 80 (Tween).
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INACTIVATED OR KILLED VIRUS

Viruses are killed and can not replicate, but capsid and other proteins are enough to generate a protective immune response

Killed through heat or chemical means (e.g. formaldehyde is used to kill influenza vaccines in chicken eggs).

Examples:
- Hepatitis A
- Influenza
- Polio (Salk)
- Rabies

Protective immunity from the killed organism with immunogenicity as effective as live organism
INACTIVATED VERSION OF SARS-CoV-2

Beijing Institute of Biological Products Company and Sinovac

BIBPC: Isolated patient strains

- Grew in vero cells for best yield
- After 10 passages in cells, S-protein showed good homology to patient samples
- Inactivated with the addition of β-propionolactone

Sincovac formulation
Schedule: Day 0 (prime) and 14 (boost)

With prime: 97.4% of patients had seroconversion (produced antibodies against the virus)

Interestingly, the day 28 boost had better seroconversion, but the day 14 schedule is progressing to phase 3 clinical trials.
## ADJUVANTS LIKE ALUM ARE USED TO BOOST VACCINE RESPONSES

<table>
<thead>
<tr>
<th>Component</th>
<th>Clinical Vaccine Candidates Containing Adjuvant (Antigen Type)</th>
<th>Description</th>
<th>Skew</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advax-SM</td>
<td>Vaxine Pty/Medytox (Recombinant Protein)</td>
<td>Delta-inulin (water-insoluble polysaccharide) microparticles mixed with CpG 1018</td>
<td>Th1 skew (No skew without CpG)</td>
<td>Unknown, antigen-presenting cell-dependent</td>
</tr>
<tr>
<td>Alum</td>
<td>Sinovac (Inactivated Virus), Sinopharm (Inactivated Virus), Bharat Biotech (Inactivated Virus), Clover (With CpG 1018, Recombinant Protein), FBRI SRC VB VECTOR (Peptide Subunit), West China Hospital/Sichuan University (Recombinant Protein)</td>
<td>Aluminum salts (aluminum hydroxide or aluminum phosphate)</td>
<td>Th2 Skew</td>
<td>Multifaceted</td>
</tr>
<tr>
<td>AS03</td>
<td>Clover (Recombinant Protein), Medicago (VLP), Sanofi/GSK (Recombinant Protein)</td>
<td>Squalene and DL-α-tocopherol oil-in-water emulsion stabilized by polysorbate 80</td>
<td>Th2 skew</td>
<td>Unknown, potentially innate immune recruitment and activation</td>
</tr>
<tr>
<td>CpG 1018</td>
<td>Vaxine Pty/Medytox (Included in Advax-SM, Recombinant Protein), Medicago (VLP), Clover (With Alum, Recombinant Protein), Medigen/NIAID/Dynavax (Recombinant Protein)</td>
<td>Unmethylated oligodeoxynucleotide (ODN)</td>
<td>Th1 skew</td>
<td>Toll-like receptor 9 (TLR9) stimulation</td>
</tr>
<tr>
<td>Ionizable Lipid (various proprietary versions)</td>
<td>Moderna/NIAID (mRNA), Pfizer (mRNA and replicon RNA), Arcturus (replicon RNA), PLA Academy of Military Sciences/Walvax Biotech</td>
<td>Lipid molecules containing amino groups which become cationic at acidic pH.</td>
<td>Th2 skew in absence of other adjuvants</td>
<td>Unknown, potentially TLR2/TLR4 stimulation</td>
</tr>
<tr>
<td>Matrix M</td>
<td>Novavax (Recombinant Protein)</td>
<td>Lipid nanoparticles containing cholesterol and immunostimulatory Quillaja triterpenoid saponins Matrix-A and Matrix-C in an 85:15 ratio</td>
<td>Balanced Th1/Th2 skew</td>
<td>Unknown, potentially innate immune recruitment and activation</td>
</tr>
<tr>
<td>MF59</td>
<td>Anhui Longcom (Recombinant Protein), Queensland/Seqirus/CSL (Recombinant Protein)</td>
<td>Squalene oil-in-water emulsion stabilized by polysorbate 80 and sorbitan trioleate</td>
<td>Th2 skew</td>
<td>Unknown, potentially innate immune recruitment and activation</td>
</tr>
</tbody>
</table>

Alum, CpG and MF59 are FDA approved in other vaccine formulations. AS03 is approved for an influenza pandemic vaccine.
TYPES OF VACCINES

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VIRUS-LIKE PARTICLES (VLPs)

Protein elements from viruses self-assemble into a particle. FDA approved formulations include:

Hepatitis B
- GlaxoSmithKline's Engerix
- Merck and Co., Inc.'s Recombivax HB

Human papillomavirus
- Gardasil
  - Adjuvanted with Alum
- Cervarix
  - Adjuvanted with Alum and monophosphoryl lipid A (MPL; a lipopolysaccharide from Salmonella enterica Serovar Minnesota)
  - Pulled from US markets

Image: Mohsen et al. Immunol Rev. 2020
MEDICAGO’S PLANT BASED VLP

Medicago uses plants as a source to produce recombinant proteins that self-assemble into VLPs

- *Nicotiana benthamiana*, a close relative of the tobacco plant indigenous to Australia

Entering Phase I trials (NCT04450004) with COVID-19 vaccine

Phase II trial with quadrivalent Influenza vaccine

- Increased pain at injection site compared to placebo, that resolved in 1 day
- The addition of alum did not increase antibody response
- Illustrated cross-reactive antibody titers and cellular response

![Medicago's Plant-Derived Virus-Like Particle (VLP) of SARS-CoV-2](https://www.hospimedica.com/)
TYPES OF VACCINES

Reduced efficacy because the immune system needs a ‘danger’ signal to activate it, which something like a protein does not normally have.

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Subunit vaccines use parts of the pathogen and an adjuvant.

Subunit elements alone are not typically immunostimulatory enough and an adjuvant is often required.

An adjuvant promotes a ‘danger’ signal, and the antigen creates a target for that signal

With live-attenuated, inactivated and viral vectors the ‘danger’ signals are still present in the formulation as is the antigen. These separate parts are added together for a subunit vaccine.

**Examples:**
- Hepatitis B
- Influenza
- *Haemophilus influenza* type b (Hib)
- Pertussis
- Pneumococcal
- Meningococcal
- Human papillomavirus
SUBUNIT COVID-19 VACCINES

Clover Biopharmaceuticals
- Tetrameric spike protein nanoparticle adjuvanted with TLR9 agonist CpG and alum
- Entered Phase 1 trials in Australia with a stabilized spike protein trimer (NCT04405908)
- Partnership with GSK

University of Queensland in partnership with CSL and Seqirus
- Spike protein stabilized with HIV-1 GP160 protein and adjuvanted with MF59
- Phase 1 has stopped in Australia (ACTRN12620000674932) since patients registered false-positives for HIV because of the used of GP160
TYPES OF VACCINES

Reduced efficacy because the immune system needs a ‘danger’ signal to activate it, which something like a protein does not normally have.

Although this lists viruses, it could also be bacteria or other pathogens.

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BACKGROUND OF DNA VACCINES

In early 1990’s it was observed that plasmid injected in skin or muscle induced a humoral response

- Simple approach to a vaccine since plasmid is easier to manufacture than protein or other vaccine approaches

In late 1990’s clinical trials began

- HIV-1, cancer, influenza, human papillomavirus (HPV), hepatitis, and malaria.
- The DNA vaccines were safe and well tolerated, but they proved to be poorly immunogenic
  - Antibody titers were very low or nonexistent
  - CD8⁺ T-cell responses were sporadic
  - CD4⁺ T-cell responses were of low frequency
- Typically, plasmid was injected or via skin electroporation

Second generation DNA vaccines

- Better delivery of plasmids
- Better identification of antigen
- Inclusion of adjuvants
DNA BASED COVID-19 VACCINES

Inovio Pharmaceuticals
- Electroporation (Cellectra-5P Adaptive Constant Current Electroporation Device) to deliver DNA
- Phase I against MERS
  - 50% had detectable neutralizing antibodies at one or more timepoints
  - 88% of the patients had T cells that produced IFN-γ in the presence of the S-protein
- Started Phase 1 for COVID in April 2020

Genexine
- Commonly deliver DNA for antigen and for Fms-like tyrosine kinase-3 ligand (FLT3L) an immune cell growth factor
- In mice, S protein without the S2 portion (pGX27-SΔTM) had better neutralization than the full-length S protein (pGX27-S)
- In rhesus macaques, a prime-boost-boost illustrated strong humoral and cellular response with reduced viral load and immunopathology

Osaka University, AnGes, and Takara Bio
- Use a pyro-drive jet injector, which detonates a small amounts of explosive powder to propel plasmids into the skin at variable, controllable depths

Image: Miyazaki et al. Pharmaceutics, Drug Delivery And Pharmaceutical Technology. 2019
Image: http://www.bioelectrochemical-soc.org/
HISTORY OF RNA VACCINES

In 1990 mRNA was injected IM and a local production of protein was observed as well as induction of immune response

On a molar basis mRNA has comparable response to DNA

Illustrated good pre-clinical activity against both infectious diseases and cancer

- Given IM, ID, SubQ, IV, intraspenic, intranodial, and transdermal via gene gun
- Also, ex vivo pulsing of dendritic cells and re-introduction

Advantages of RNA vaccines over DNA

- DNA could potentially integrate into the host genome, although not yet seen clinically
- DNA must go into the nucleus opposed to mRNA which can have influence in the cytosol
- +/- RNA antigen expression can be shorter time frame than DNA expression

Ulmer et al. Vaccine 30 (2012) p. 4414
**TYPE OF RNAs THAT CAN BE USED IN VACCINES**

<table>
<thead>
<tr>
<th>Type</th>
<th>Abbreviation</th>
<th>Function</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Messenger RNA</td>
<td>mRNA</td>
<td>Codes for proteins</td>
<td>All organisms</td>
</tr>
<tr>
<td>Ribosomal RNA</td>
<td>rRNA</td>
<td>Translation</td>
<td>All organisms</td>
</tr>
<tr>
<td>Signal recognition particle RNA</td>
<td>7SL RNA or SRP RNA</td>
<td>Membrane Integration</td>
<td>All organisms</td>
</tr>
<tr>
<td>Transfer RNA</td>
<td>tRNA</td>
<td>Translation</td>
<td>All organisms</td>
</tr>
<tr>
<td>Transfer-messenger RNA</td>
<td>tmRNA</td>
<td>Rescuing stalled ribosomes</td>
<td>Bacteria</td>
</tr>
</tbody>
</table>

mRNA is threatened by rapid degradation by ubiquitous extracellular ribonucleases before being taken up by cells.

CureVac was the first mRNA nanoparticle vaccine to get to clinical trials for Rabies. It uses mRNA complexed to protamine. (NCT02241135 2014-18)

However, their COVID-19 formulation is lipid based.
POLYPLEX FORMULATION

Polyplexes use cationic polymers to bind to anionic nucleic acids

- **Advantages**: Neutralization of charge as well as particle size can facilitate uptake by APCs. Nucleotides maybe partially or fully protected by polymer.
- **Challenges**: Cationic polymers can adduct host DNA and can have toxicity concerns.
- **FDA Approval**: Polyplexes are approved for topical application as wound dressing.
- **Technology**: Branched Poly(β-amino ester) (PBAE) to deliver mRNA encoding spike protein for pulmonary delivery (Translate Bio).

![Diagram of PBAE Polymer, mRNA, and Polypex](Image: Zhao et al. JCR 2016)
LIPID NANOPARTICLES (LNPs)

Cationic and neutral lipids can be used to complex anionic DNA. These are not necessarily liposomes, they are lipid complexes.

- **Advantages**: Charge neutralization and size can facilitate APC uptake.
- **Challenges**:  
  - Nucleotides potentially available for degradation.  
  - Pre-existing or induced immunity against PEG and phosphorylcholine.  
  - Cationic lipids can adduct host DNA and have toxicity concerns.  
  - Often requires **very low storage temperatures**.
- **FDA Approval**:  
  - Liposomes and lipid complexes have been approved for decades, and internationally inactivated virus in lipids has been approved as a vaccine.  
  - siRNA based lipid carriers are FDA approved.
PFIZER AND BIONTECH LNP s

0.43 mg ALC-0315  
Cationic lipid

0.05 mg ALC-0159  
PEGylated lipid

0.09 mg Distearoylphosphatidylcholine (DSPC)  
Cationic lipid

This is added with 0.2 mg cholesterol and 30 µg nucleoside-modified messenger RNA (modRNA) encoding the full-length viral spike (S) glycoprotein of SARS-CoV-2.

95% at preventing symptomatic COVID infection in patients who were part of the clinical trial

FDA approved December 11, 2020 for emergency use

Schedule of day 0 prime and boost (day 21)
SM-102 is a version of sphingomyelin, with the general structure given here. Moderna’s version is termed an **ionizable lipid**.

Distearoylphosphatidylcholine (DSPC)

Cationic lipid

DMG-PEG 2000

1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000

**MODERNA LNPs**

**Ionizable lipids**
- Contain an amine group, a linker group, and a lipid tail
- Their amine group allows for a positive charge to complex with nucleic acids

**Modernar vaccine was 94.1% effective in preventing symptomatic COVID-19 infection in patients in the clinical trial.**

December 16, 2020

Moderna’s formulation was FDA approved for emergency use
MANUFACTURING OF LIPID NANOPARTICLES

Pfizer’s and Moderna’s fabrication methods are proprietary. This is an example of how these particles can be made in a scalable fashion where each mixer scales linearly.

siRNA is pictured here, but these formulations would use mRNA.

Microfluidics T-mixer or similar can be used to form the particles.
The importance of storage conditions

The cold chain means that there is a temperature-controlled environment from vaccine processing to use
- Manufacturer
- Transportation to distributor
- Delivery to and storage at provider
- Administration to patient

Cold chain storage is a significant limitation for application in resource limited settings

Pfizer’s requires storage at -70 °C
- This would require a -80 °C freezer that would primarily be available only at most hospitals

Moderna’s at -20 °C
- This would be available at hospitals, pharmacies, and most doctor’s offices

CureVac reports an LNP which has three-month thermostability at 5 °C
- This would be available at hospitals, pharmacies, and most doctor’s offices
POLYETHYLENE GLYCOL (PEG) BACKGROUND

Also known as: poly(ethylene oxide) (PEO), polyoxyethylene (POE) and Carbowax®

It is a polyether

MW commercially available from 300 to 10,000,000 g/mol
• Viscosity increases with chain length

Low toxicity

Highly miscible in most solvents and water (not common for polymers)

Very hydrophilic
• It is highly thermodynamically favorable for water molecules to surround PEG in a liquid environment
  • Resist protein absorption
  • Resists cell adhesion

Not biodegradable
PEG HYPERSENSITIVITY

PEG is ubiquitous in food, cosmetics and medicine.

- Used as surfactants, emulsifiers, cleansing agents, humectants, and skin conditioners.

A Case Report of Angioedema and Anaphylactic Shock Induced by Ingestion of Polyethylene Glycol

Amy Rossi, MD  
Lesley Osborn, MD

University of Texas at Houston, McGovern Medical School, Department of Emergency Medicine, Houston, Texas

CDC confirms 6 cases of severe allergic reaction to Pfizer’s COVID vaccine — out of 272,000 shots given so far

By NELSON OLIVEIRA  
NEW YORK DAILY NEWS | DEC 20, 2020 AT 1:09 PM

CASE REPORT | ENDOSCOPY

Polyethylene Glycol (PEG)-Induced Anaphylactic Reaction During Bowel Preparation

David Gachoka, MD

Department of Internal Medicine, University of Toledo, Toledo, OH

Image: https://www.amazon.com
ANAPHYLAXIS IS A TYPE OF HYPERSENSITIVITY REACTION

On Sunday 1/17/21, California saw a spike in apparent allergic reactions, with 10 in a 24-hour period.

Rate of anaphylaxis is expected to be about 1:100,000

Anaphylaxis is Type I hypersensitivity response. Other hypersensitivity responses have been reported.

https://www.foxnews.com/health/california-official-calls-for-pause-on-moderna-vaccine-lot-after-possible-allergic-reaction
Image: https://www.downstate.edu/Karp/intoallerg1633.html (Janeway originally)

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**WHICH TYPE OF PEG IS IMMUNOGENIC?**

Most patients have low levels of anti-PEG antibodies, mostly IgM.

Formation of neutralizing IgG typically occurs when PEG is bound to a tetrameric protein (e.g. Oncaspar, PEG-uricase). The protein serves as a *hapten*.

Also, PEG on a nanoparticle also seems to invoke a stronger antibody response than unbound PEG.

**PEGylated nanoparticle (e.g. Doxil)**

**Unbound PEG**

Grenier et al. Journal of Controlled Release 287. 2018 p. 121
**PEG AND REACTIONS CAN START WITH IGM ANTIBODIES**

**IgM**
- Largest antibody
- Produced after 1st exposure to antigen
- Weak binding strength and with low specificity
- Functions to cross-link B-cell receptors to create plasma cells (antibody releasing B cells)

**IgG**
- 75% of serum Immunoglobins (Igs)
- Several different isotypes (e.g. IgG1, IgG2a)
- With repeated exposure to antigens and T cell help, B cells generate IgG that bind more tightly and with increase specificity

This is illustrative of a typical IgM to IgG response using COVID-19 infection as a model. This is not available for PEG, but the polymer may have a similar response.


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ANAPHYLAXIS IS A SEVERE HYPERSENSITIVITY RESPONSE & PEG HAS A CONTINUUM OF RESPONSES

***There is no conclusive study that indicates 100% this is what happens in patients***

Repeated B cell activation can lead to IgE production.

Kozma et al. ACS Nano 2019, 13, 8, 9315–9324
Zhou et al. The Journal of Allergy and Clinical Immunology: Nov 2020,
Kristy Ainslie, PhD – UNC Eshelman School of Pharmacy – Jan 18, 2021
LNPS CAN INDUCE ANTI-LIPID AS WELL AS ANTI-PEG ANTIBODIES

There is also evidence of antiphosphorylcholine (PC) IgM

Besin et al. Immunohorizons 2019 (Moderna)
Image: Guevara et al. Front Chem 2020

sIgM-/- are IgM KO mice
WT are C57BL/6J mice
COMBINED ANTI-PEG AND ANTI-PS EFFECT WITH LNPS

Note protein expression

Besin et al. Immunohorizons 2019 (Moderna)

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B1 = in response to Lipid (PS)
B2 = in response to PEG
Artificial Antigen Presenting Cells (APCs)
Shenzhen Geno-Immune Medical Institute
K562 cells are modified with lentivirus to express SARS-CoV-2

Bifidobacterium longum is used as a commensal bacteria to deliver plasmid encoding spike protein (Symvivo).

Delivery of adenovirus encoding spike protein via oral tablet (VaxArt).
WHERE DID THIS INFORMATION COME FROM?

Considerations for size, surface charge, polymer degradation, co-delivery, and manufacturability in the development of polymeric particle vaccines for infectious diseases

Christopher J. Genito, Cole J. Batty, Eric M. Bachelder, and Kristy M. Ainslie*

C. J. Genito, C. J. Batty, Prof. E. M. Bachelder, Prof. K. M. Ainslie
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Other sources are cited as well as image sources. Some images were from Wikipedia or generated via BioRender or Clipart.
PLACES TO FIND MORE INFORMATION ON THE IMMUNE SYSTEM

- *How the Immune System Works* by Lauren Sompayrac
  - 152 pages
  - Used ~$15

- *Immuno Biology* by Kenneth Murphy
  - 924 pages
  - Used ~$125

- *Fundamental Immunology* by William E. Paul
  - 1312 pages
  - Used ~$125

Increasing cost, time, and level of intensity