Innate immune mechanisms of mRNA vaccines

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EMA virtual workshop on myocarditis

How do mRNA vaccines (need to) activate the innate immune system?

mRNA vaccines require no further addition of adjuvant to elicit robust immune responses

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Visiting postdoc Pieter Cullis lab, UBC
Expertise: LNPs, mRNA vaccines ,adjuvantia

Norbert Pardi

Pardi lab, UPenn
Alum of Weissman lab
Expertise: Pioneered nucleos.-modified mRNA-LNP vaccines, influenza

Michael J. Hogan

Eisenlohr's lab, CHOP
Alum of Weissman lab
Expertise: Antigen presentation, mRNA vaccines

Karin Loré

Loré lab, Karolinska Institutet
Expertise: preclinical evaluation (mRNA) vaccines in non-human primates

Immunity
CellPress
Review

Innate immune mechanisms of mRNA vaccines

Rein Verbeke,[1,2,*] Michael J. Hogan,[3] Karin Loré,[4,5] and Norbert Pardi[6,*]

Safe and effective vaccines must stimulate the innate immune system at an appropriate level such that they achieve a balance between immunogenicity and reactogenicity

Blame the Messenger

1950-60s: RNA (Viruses) – Infected cells make **"INTERFERON" (IFN)**

- IFN reaction inhibits viral replication – blocks viral RNA translation

Mechanism of Action of Interferon

I. Relationship with Viral Ribonucleic Acid

P. DE SOMER, A. PRINZIE, P. DENYS, JR., AND E. SCHONNE

Rega Institute for Medical Research, Department of Virology, University of Louvain, Louvain, Belgium

Accepted September 26, 1961

The mechanism of action of interferon has been investigated in two different cell systems, with concordant results. Interferon is able to suppress the one-cycle synthesis of poliovirus in embryonated eggs inoculated with infectious polio ribonucleic acid (RNA). This observation represents the basis of a sensitive method for quantitative bioassay of interferon activity. Interferon-treated chick embryo cells infected with Western equine encephalitis (WEE) virus do not yield detectable infectious viral RNA, as shown by extraction with cold phenol. It appears that interferon action occurs within the cell, after penetration of virus and before formation of mature virus particles. A close relationship with viral RNA metabolism is suggested.

Safe and effective vaccines must stimulate the innate immune system at an appropriate level such that they achieve a balance between immunogenicity and reactogenicity

Blame the Messenger

1950-60s: RNA (Viruses) – Infected cells make **"INTERFERON" (IFN)**

- IFN reaction inhibits viral replication – blocks viral RNA translation

Since 1990s: Discovery of **PATHOGEN RECOGNITION RECEPTORS** involved in **RNA sensing**

- **Double stranded (ds)RNA** is recognized by TLR3, RIG-I, MDA-5, PKR, OAS etc.
- **Uridine-containing RNA** is recognized by TLR7(/8 in humans)
- **Cap1 structure** is needed to avoid recognition by IFIT1 (in ssRNA) & RIG-I (in dsRNA)

PROBLEM : Anti RNA-response can jeopardize the translation, safety & efficacy of mRNA vaccines/therapeutics

Balance? The type I IFN response can **limit the antigen availability**
It can also be a potent driver of immunity - **self-adjuvant properties**

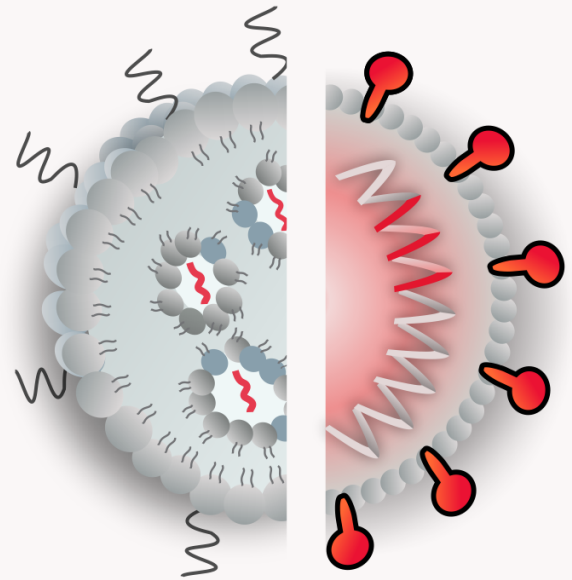
SOLUTION?

2005-… : **MODIFIED URIDINES** or uridine depletion reduce RNA sensing and improve translation

- Avoids recognition by TLR7/8, PKR, OAS… (Karikó, Weissman et al.)

CASE I: Different COVID-19 mRNA vaccine candidates

All using iLNP carriers



Uridine-modified

Moderna - Spikevax
Dose: 100 µg
Efficacy*: ~95%

Pfizer/BioNTech - Comirnaty
Dose: 30 µg
Efficacy: ~95%

Unmodified

CureVac - CVnCoV
Dose: 12 µg
Efficacy: ~47%
2nd generation

Providence - PTX-COVID19-B
Dose: 40 µg
Non-inferior to Comirnaty
in Phase 2 trial

Self-amplifying

Arcturus- ARCT-154
Dose: 5 µg
Efficacy: ~55%

Imperial - LNP-nCoVsaRNA
Dose: 0.1-10 µg
Phase 1: Low immunogenicity

*Prevention against symptomatic COVID-19 disease

Unmodified- versus Uridine-modified mRNA

Superior humoral immunity with **uridine-modified mRNA**

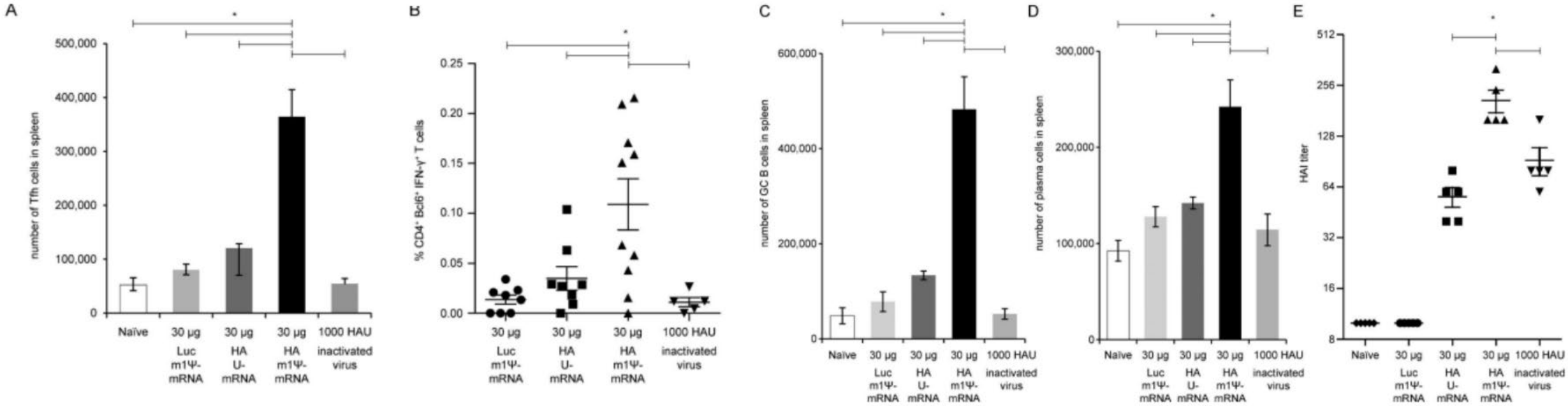


Figure 9. **A single immunization with m1Ψ HA mRNA-LNPs induces more potent Tfh cell responses, higher splenic GC B and plasma cell numbers, and higher HAI titers compared with unmodified mRNA-LNPs.** Mice were immunized with a single i.d. injection of 30 µg of Luc m1Ψ-mRNA-LNP, HA U- or m1Ψ-mRNA-LNPs, or a single i.m. immunization of 1,000 HAU of inactivated PR8 virus, and immune responses were examined 10 d later. **(A)** The total number of splenic Tfh cells was counted by staining for TCRβ$^+$CD19$^-$CD4$^+$CD62L$^-$CXCR5$^+$PD-1$^+$ T cells. **(B)** Percentage of IFN-γ producing CD4$^+$Bcl6$^+$ Tfh-like cells was measured by flow cytometry. **(C and D)** Total numbers of splenic GC B cells (C) and plasma cells (D) were determined. **(E)** HAI titers from mouse serum were determined 10 d after immunization. n = 5–10 mice, and each symbol represents values for one animal. Experiments were repeated at least two times to achieve statistical significance. Error bars are SEM. Statistical analysis: one-way ANOVA with Bonferroni correction, *, $P < 0.05$.

Pardi et al. J. Exp. Med. 2018

Unmodified- versus Uridine-modified mRNA

Superior cytotoxic T cell response with unmodified mRNA

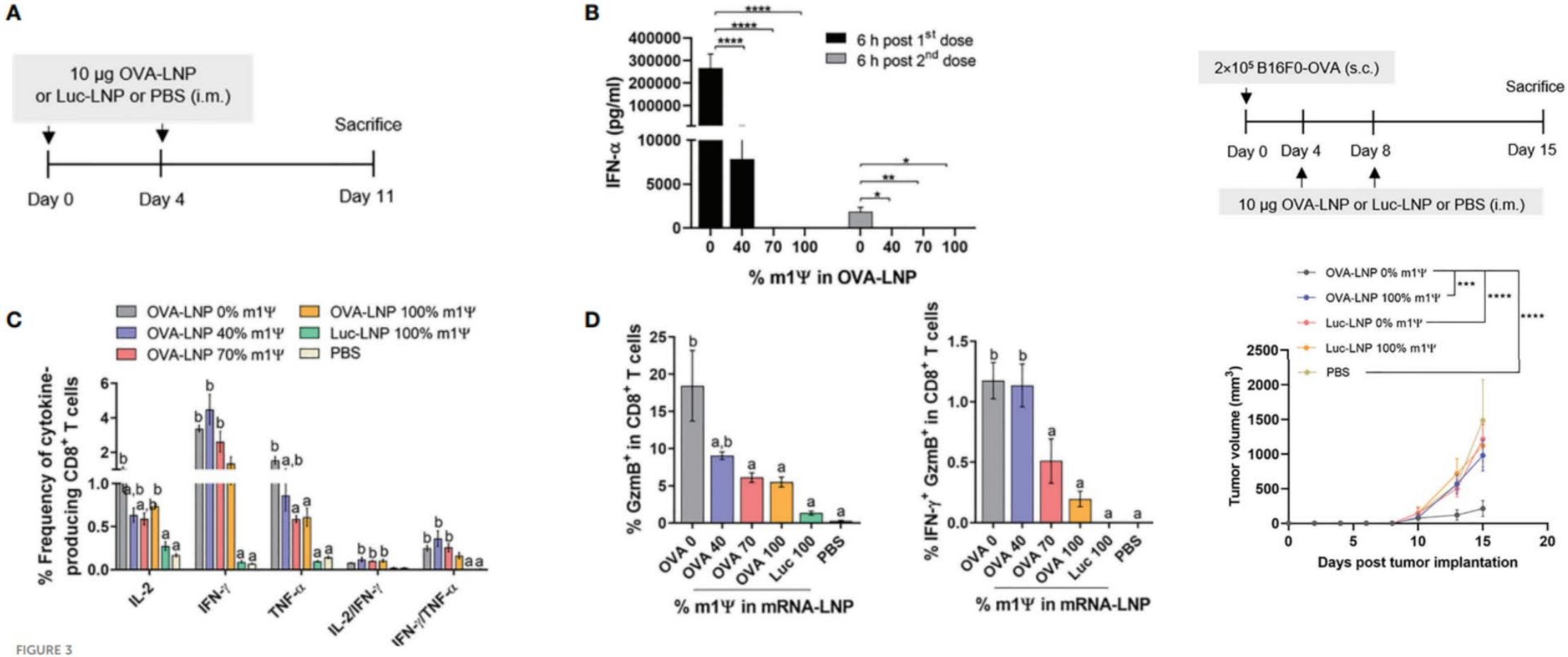


FIGURE 3

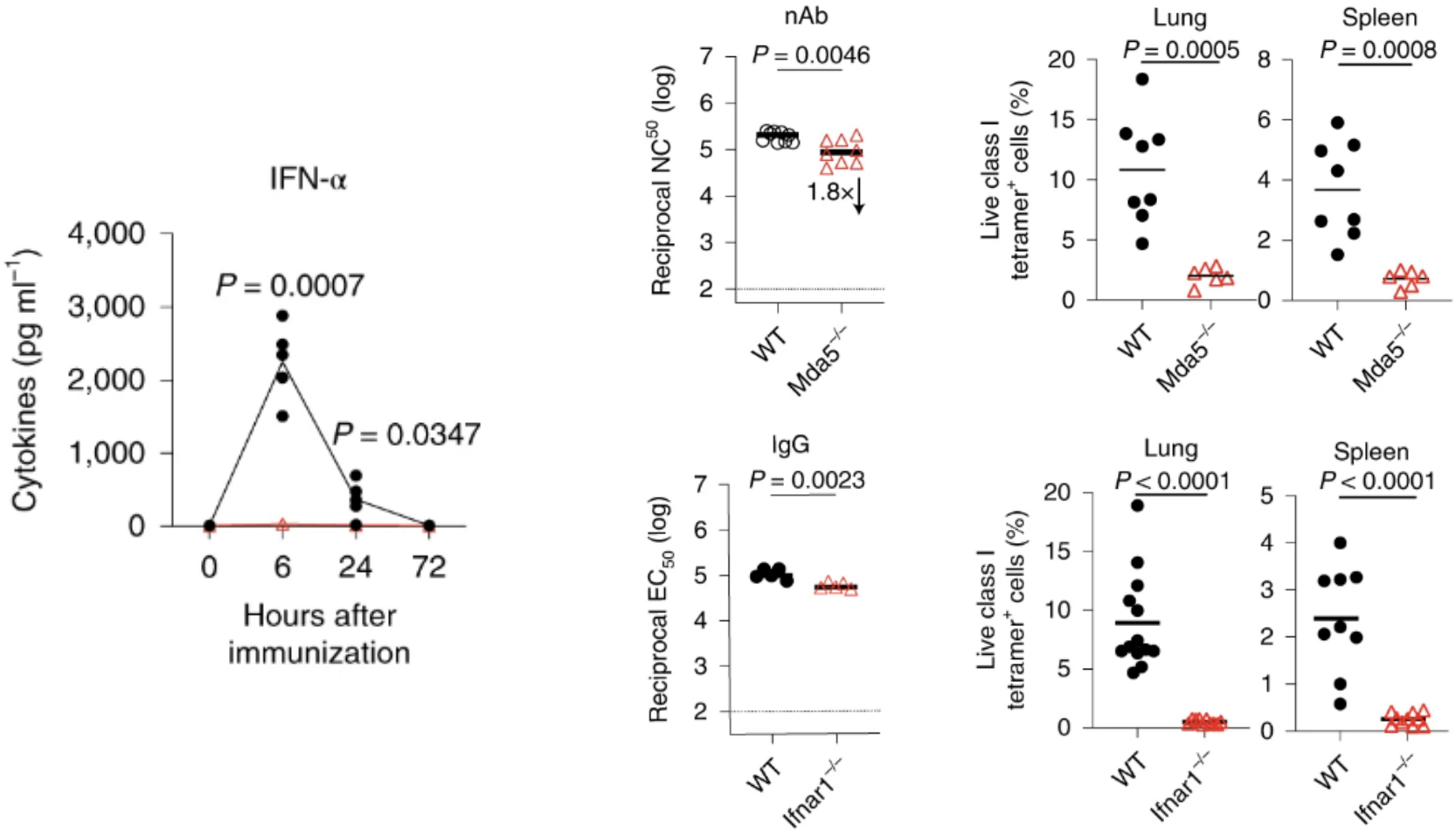
Immunization of mRNA-LNP elicits robust antigen-specific CD8+ T cell responses. **(A)** Schematic representation of the immunization regimen (see methods for details). **(B)** IFN-α concentration in the serum 6 hr after the first (day0) and the second (day4) immunizations of OVA mRNA-LNP were detected by ELISA. **(C, D)** The frequencies of IL-2, IFNγ, TNFα and Granzyme B (GzmB) and IFN-γ/GzmB-producing CD8+ T cells after 6 hr stimulation with $OVA_{257-264}$ (SIINFEKL) were measured by flow cytometry. The results are presented as the mean ± SEM, n = 4-5 biologically independent mice per group. Statistical significance: Statistical significance by one-way ANOVA with Bonferroni multiple comparisons test were indicated as (*)$p < 0.05$, (**)$p < 0.01$ and (****)$p < 0.0001$ or $p < 0.05$ compared to the unmodified target antigen: a, or control (PBS): b.

Sittplangkoon et al.
Front. Immunol. 2022

Uridine-modified mRNA-iLNP vaccines are still capable of inducing a transient type I IFN response

Fig. 7: MDA5–IFNAR1 axis is important for BNT162b2-induced CD8$^+$ T cell response.

From: Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech BNT162b2 vaccine



Li et al. Nat. Immunol.. 2022

CASE II: Seemingly similar mRNA products for different applications

moderna

Uridine-modified-LNP	Viral diseases
mRNA-1273 - Spikevax Dose: 100 µg IM Efficacy*: ~95%	**Preventation of disease/infection** • Neutralizing antibodies • T cell responses

Uridine-modified-LNP	Cancer vaccines
mRNA-4157 (Merck) Dose: 1000 µg IM Phase 2 stage	**Destroy tumors/ memory for relapse** • CD8 & CD4 T cell responses • NK cells …

Uridine-modified-LNP	Therapeutics
mRNA-3927 Dose: 600 µg/kg IV Phase 2 stage	**Protein production** • Avoid innate immunity

Keep it clean or a bit dirty

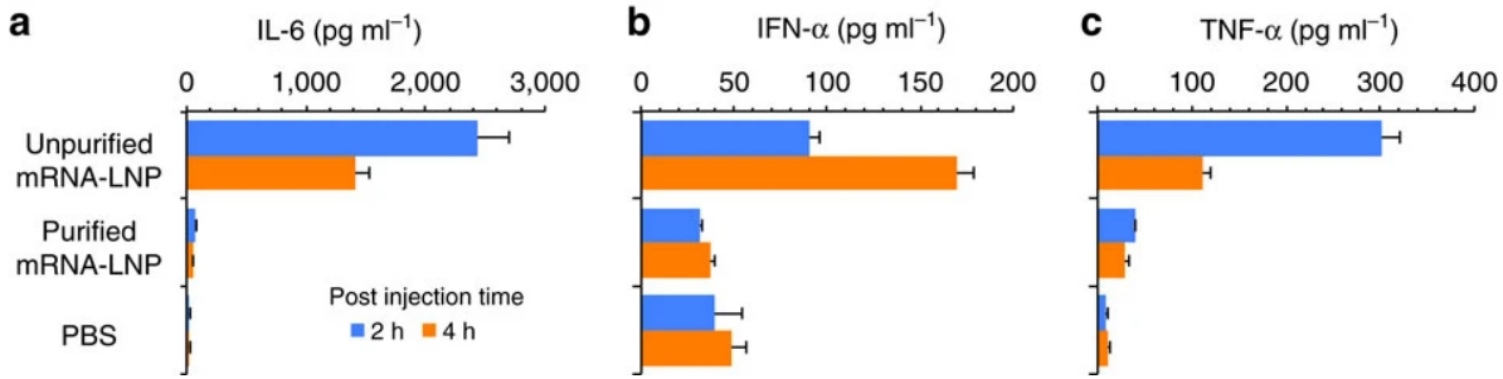
T7 polymerase gives rise to **CONTAMINANTS** in the form of short and long **double stranded RNA (dsRNA)**

- dsRNA can strongly contribute to the innate immune response
- Need for additional **purifications methods**: HPLC, Cellulose purification, RNase III
- **Alternative**: optimization of T7 IVT reaction (e.g. Moderna published on T7 mutant)

Impact of dsRNA content on mRNA-iLNP therapeutics

Figure 3: Analysis of innate immune activation by mRNA-LNPs.

From: Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge



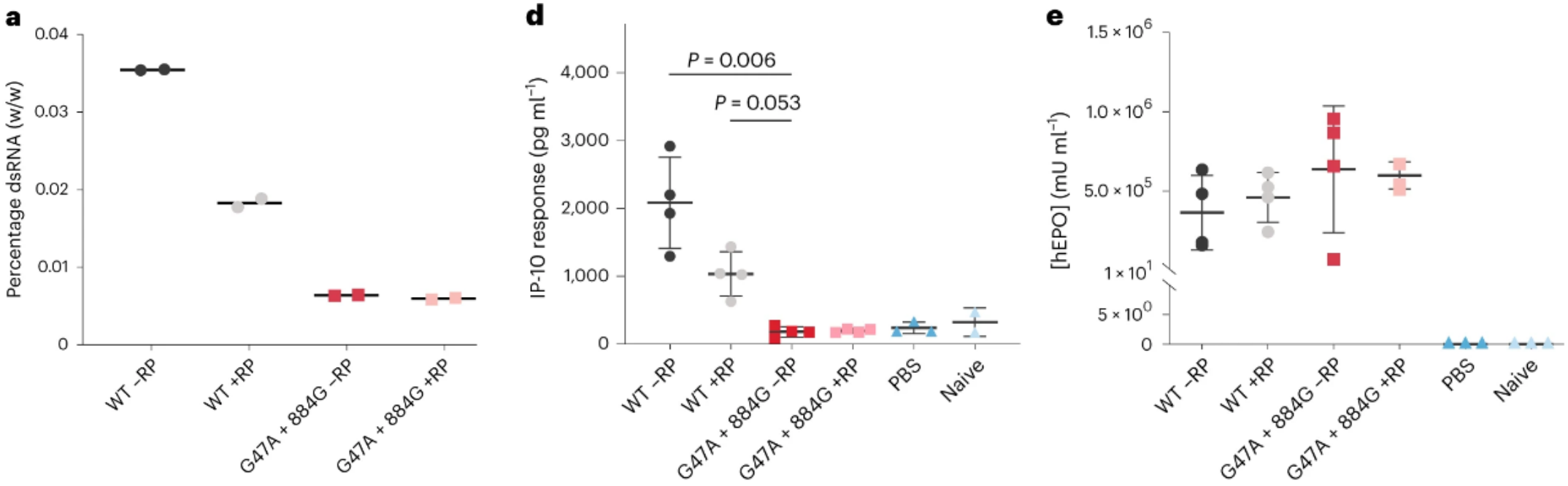
C57Bl/6 mice were i.v. injected with a 1 mg kg^{-1} dose of nucleoside-modified, FPLC-purified Luc mRNA-LNPs; unpurified, nucleoside-modified Luc mRNA-LNPs (1 mg kg^{-1}) (positive control) and phosphate buffered saline (PBS) (negative control). Animals were bled 2 and 4 h post injection and interleukin-6 (**a**), IFN-α (**b**) and tumour necrosis factor-α (**c**) levels were measured in plasma by Luminex assay. Error bars are s.e.m. Statistical analysis: one-way analysis of variance with Bonferroni correction, $P<0.01$ in comparisons of PBS to non-purified mRNA-LNPs and non-purified mRNA-LNPs to purified mRNA-LNPs. Group size is five animals.

Pardi et al. Nat. Commun. 2017

Impact of dsRNA content on mRNA-iLNP therapeutics

Fig. 5: In vitro and in vivo performance of mRNAs synthesized by WT and G47A + 884G T7 RNAPs.

From: An engineered T7 RNA polymerase that produces mRNA free of immunostimulatory byproducts



Dousis et al. Nat. Biotechnol. 2022

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Impact of dsRNA in mRNA products/vaccines

(Covid-19) Vaccines: Dirty secret ? Presence of dsRNA content? Explanation for adjuvant effects, and/or side effects?

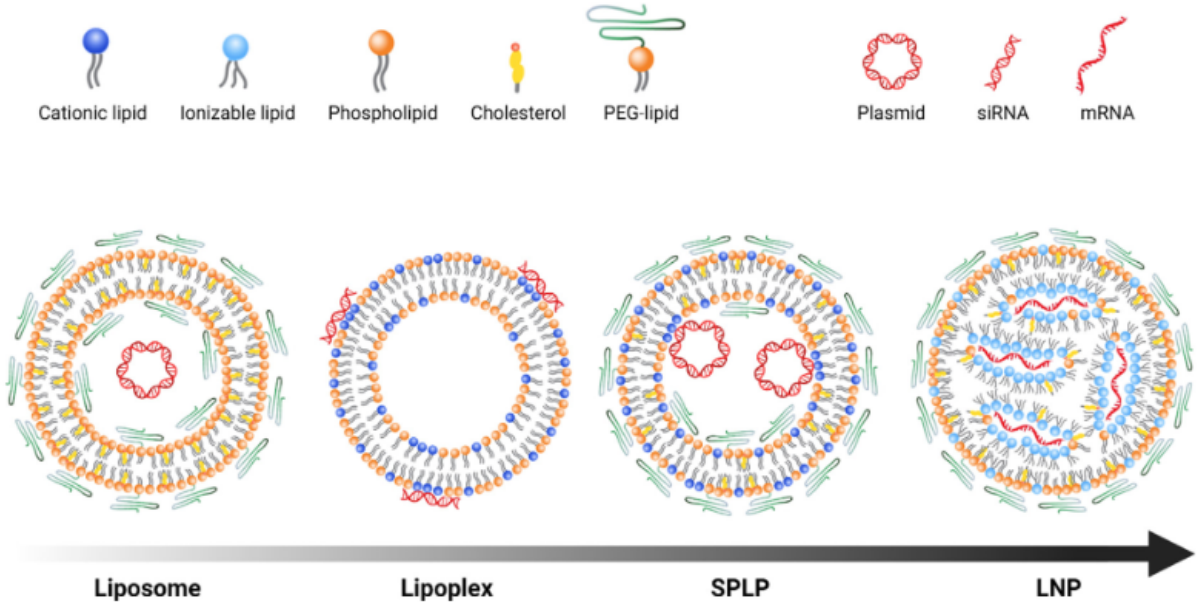
Therapeutics: **Get rid of dsRNAs !!!**

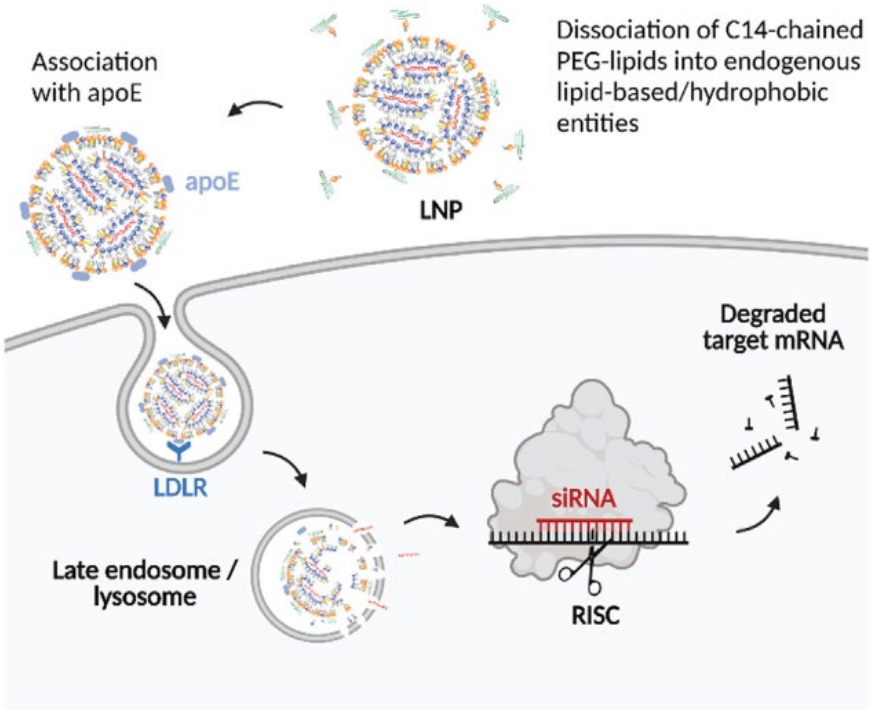
ARTICLE

Human type I IFN deficiency does not impair B cell response to SARS-CoV-2 mRNA vaccination

Aurélien Sokal[1*], Paul Bastard[2,3,4,5*], Pascal Chappert[1,6**], Giovanna Barba-Spaeth[7**], Slim Fourati[8,9], Alexis Vanderberghe[6,10], Pauline Lagouge-Roussey[6,10], Isabelle Meyts[11], Adrian Gervais[2,3], Magali Bouvier-Alias[8,9], Imane Azzaoui[6,10], Ignacio Fernández[7], Andréa de la Selle[1], Qian Zhang[2,3,5], Lucy Bizien[2,3], Isabelle Pellier[12,13], Agnès Linglart[14], Anya Rothenbuhler[14], Estelle Marcoux[15], Raphael Anxionnat[16], Nathalie Cheikh[16], Juliane Léger[17], Blanca Amador-Borrero[18], Fanny Fouyssac[19], Vanessa Menut[20], Jean-Christophe Goffard[21], Caroline Storey[22], Caroline Demily[23], Coralie Mallebranche[12,13], Jesus Troya[24], Aurora Pujol[25], Marie Zins[26], Pierre Tiberghien[27,28], Paul E. Gray[29,30,31], Peter McNaughton[31,32], Anna Sullivan[31,32], Jane Peake[31,32,33], Romain Levy[2,3,34], Laetitia Languille[10], Carlos Rodiguez-Gallego[35,36], Bertrand Boisson[2,3,5], Sébastien Gallien[37], Bénédicte Neven[34], Marc Michel[10], Bertrand Godeau[10], Laurent Abel[2,3,5], Felix A. Rey[7], Jean-Claude Weill[1], Claude-Agnès Reynaud[1], Stuart G. Tangye[31,38,39], Jean-Laurent Casanova[2,3,4,5,40], and Matthieu Mahévas[1,6,10]

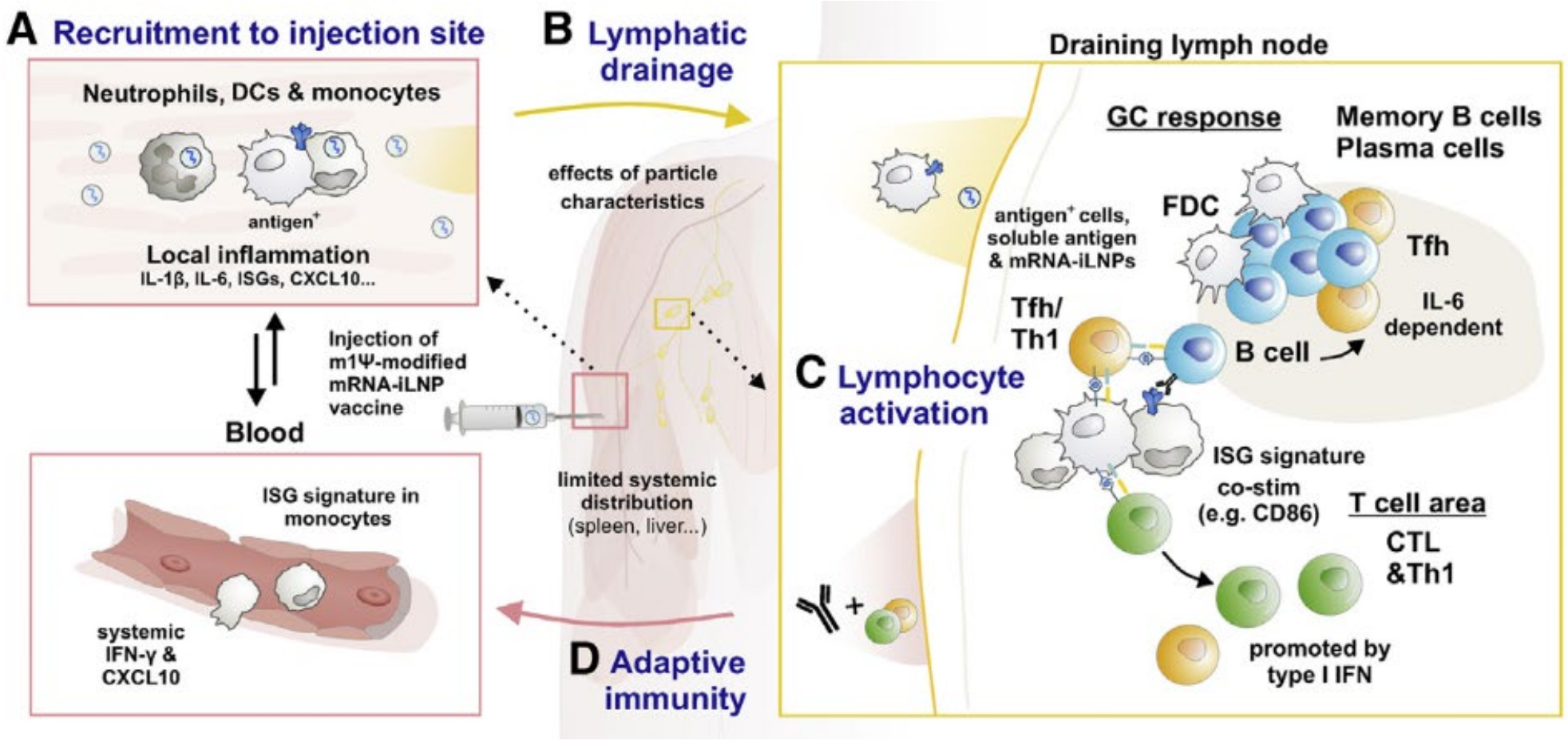
iLNPs were first optimized for i.v. delivery of siRNA to hepatocytes





Albertsen G.H. et al. Adv. Drug. Deliver.. 2022

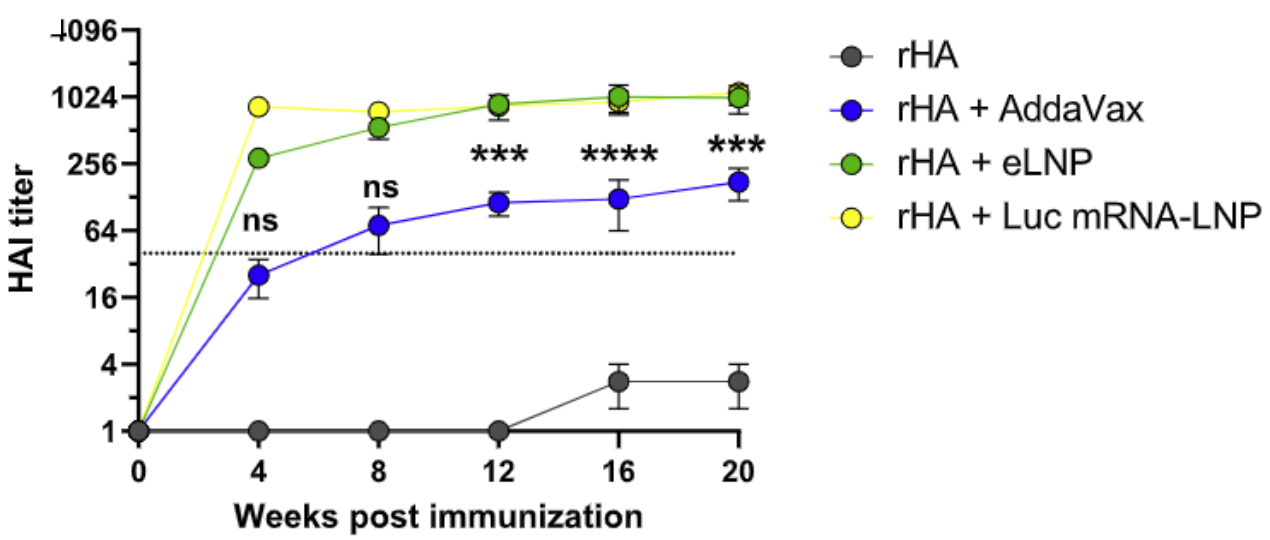
Biodistribution and innate immune cell dynamics upon administration of mRNA-iLNP vaccines

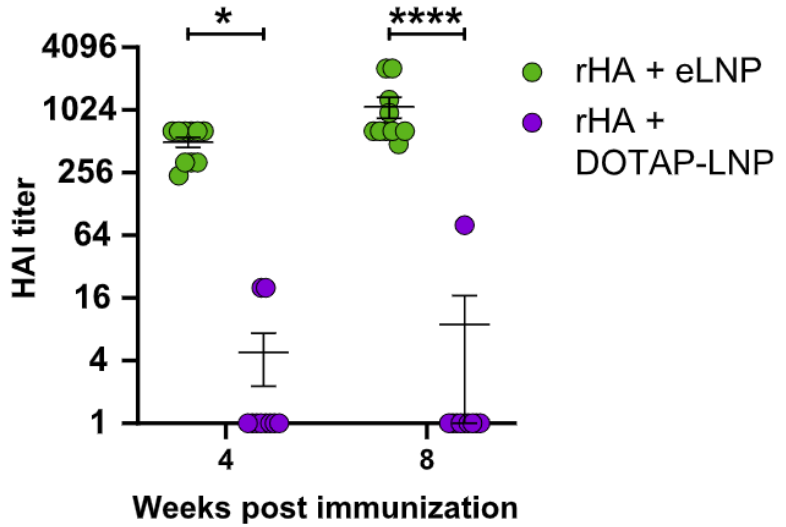


Verbeke, R., Hogan, M.J., Loré, K., Pardi, N. et al. Immunity 2022

iLNPs provide a strong adjuvant activity to (mRNA) vaccines

Combination of protein antigen with iLNPs as adjuvants



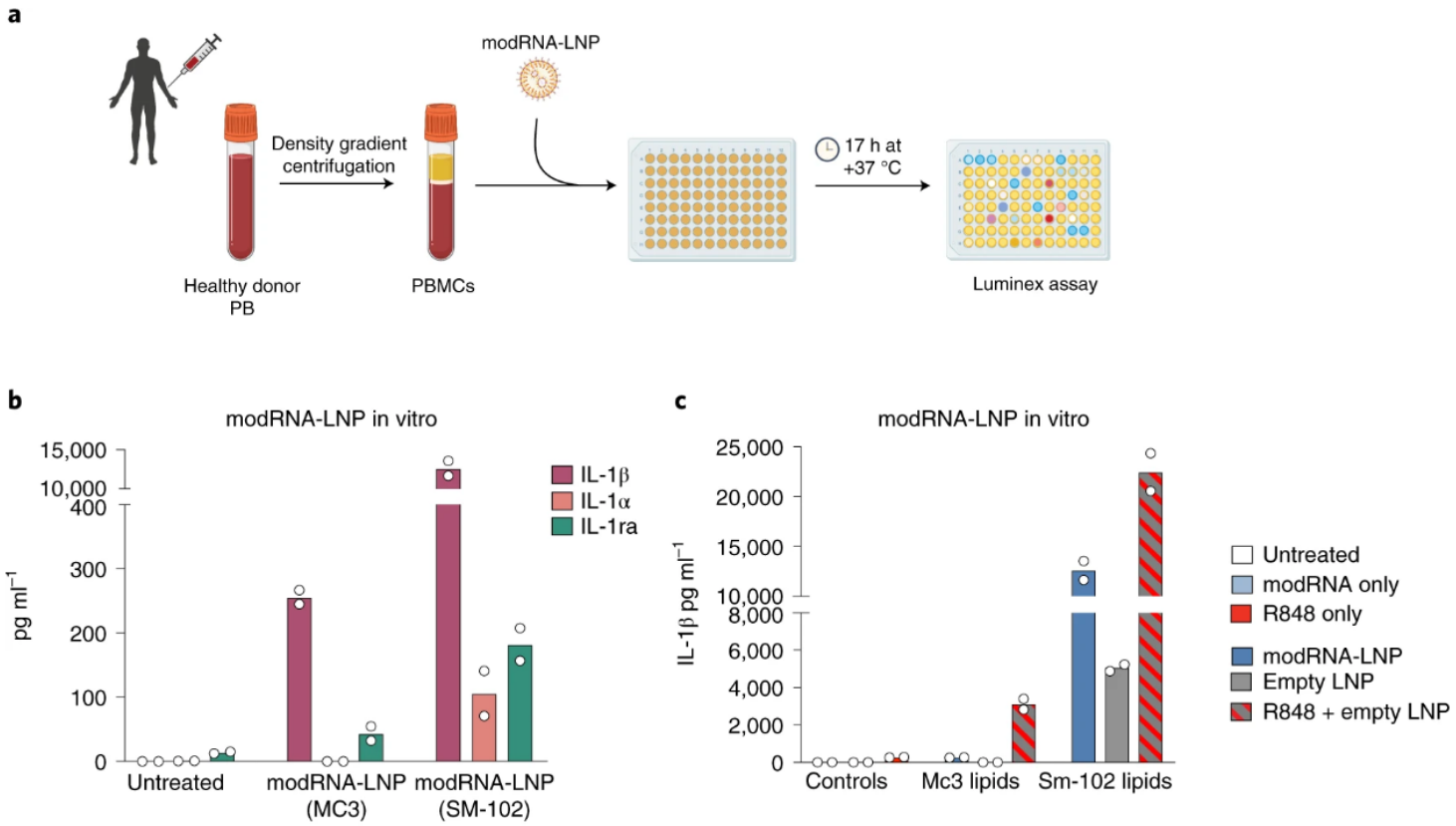


Alameh et al. Immunity 2021

Pro-inflammatory response to mRNA-iLNP vaccines and empty iLNPs

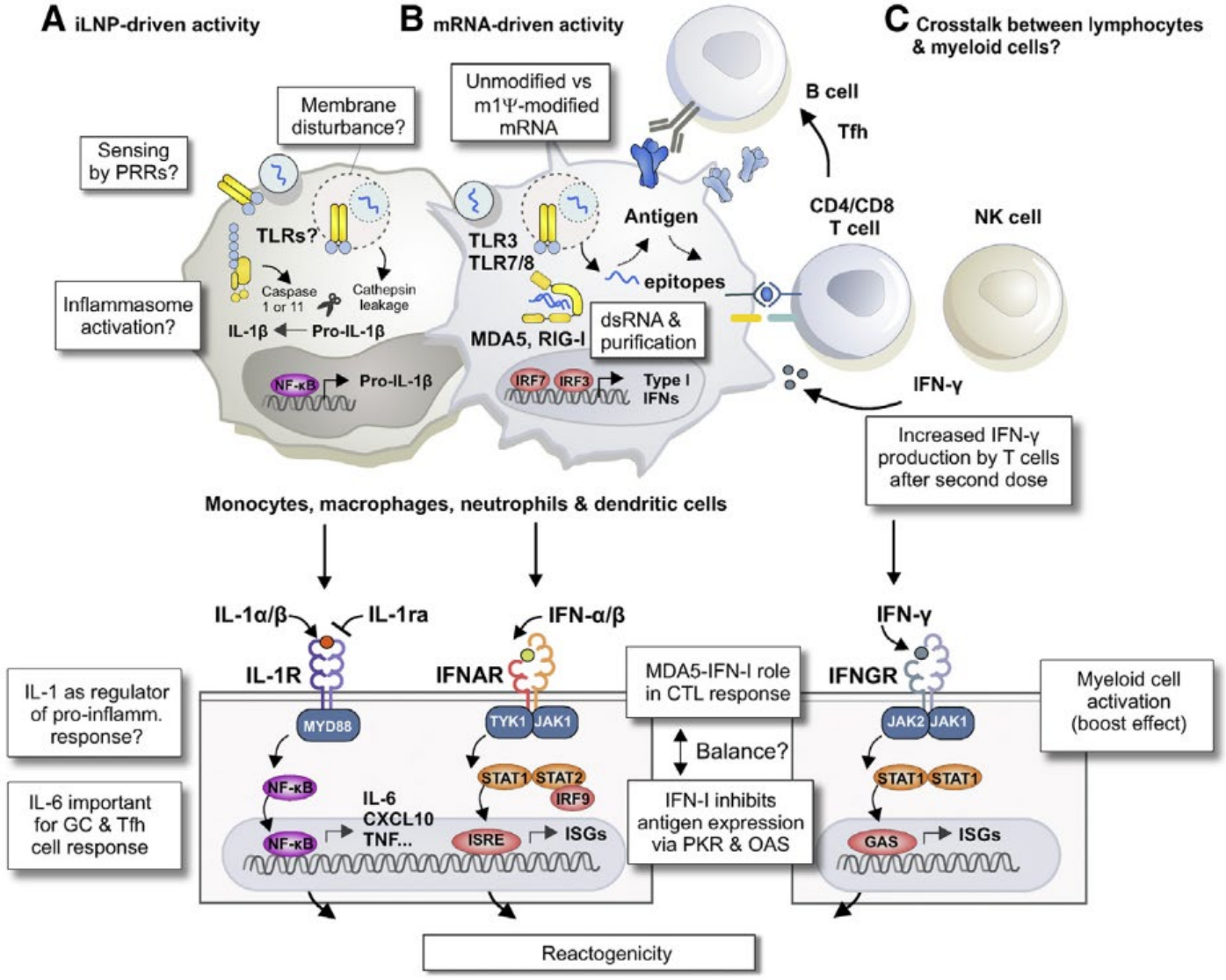
Fig. 7: modRNA-LNP induces IL-1β in human PBMCs in vitro.

From: IL-1 and IL-1ra are key regulators of the inflammatory response to RNA vaccines



Tahtinen et al. Nat. Immunol. 2022

Working model of the innate immune mechanisms of mRNA vaccines



Verbeke, R., Hogan, M.J., Loré, K., Pardi, N. et al. Immunity 2022

The path to next generation mRNA-iLNP vaccines

1) Many variables can affect the reactogenicity and immunogenicity of mRNA vaccines

Need for standardization

- **mRNA**: Modified vs unmodified, method of purification, dsRNA content, Cap structure etc.
- **Lipid carrier**: LPX ≠ LNP, LNP1, LNP2… (disclose identity of lipids, molar ratios…)

2) Which innate immune pathways contribute to protective immunity vs. which contribute to reactogenicity ?

- Are certain innate immune pathways dispensable for efficacy ?
- Are there responses that can be associated with common and rare (severe) side effects?

3) Understand the molecular mechanisms by which mRNA and iLNPs are sensed

Development of less reactogenic and more effective vaccine candidates

- Which vaccine components/features contribute to innate immune activation
  (understand combined- and potential synergistic effects of these responses)
- Multiple possible paths to develop mRNA vaccines that hit the sweet spot of immune activation
  (dependent on disease application – tailored mRNA LNP designs)

Research directions for the underlying cause of myocarditis

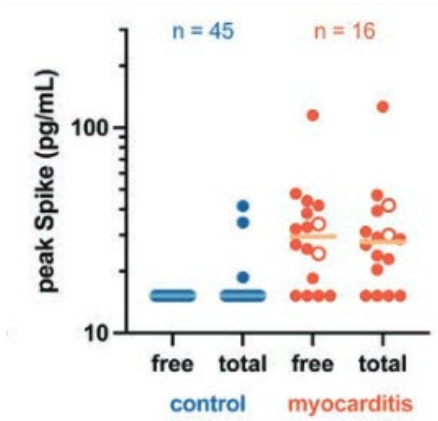
Pharmacokinetics of mRNA-LNP vaccines

Circulation

ORIGINAL RESEARCH ARTICLE

Circulating Spike Protein Detected in Post–COVID-19 mRNA Vaccine Myocarditis

Lael M. Yonker, MD*; Zoe Swank, PhD*; Yannic C. Bartsch, PhD*; Madeleine D. Burns, MS; Abigail Kane, MD; Brittany P. Boribong, PhD; Jameson P. Davis, BS; Maggie Loiselle, BS; Tanya Novak, PhD; Yasmeen Senussi, MBBS; Chi-An Cheng, PhD; Eleanor Burgess, MS; Andrea G. Edlow, MD; Janet Chou, MD; Audrey Dionne, MD; Duraisamy Balaguru, MD; Manuella Lahoud-Rahme, MD; Moshe Arditi, PhD; Boris Julg, MD, PhD; Adrienne G. Randolph, MD; Galit Alter, PhD; Alessio Fasano, MD†; David R. Walt, PhD†



mRNA modifications & dsRNA

Myocarditis and COVID-19 mRNA vaccines: a mechanistic hypothesis involving dsRNA

Gerard Milano*,[1,2], Jocelyn Gal[2,3], Anne Creisson[4] & Emmanuel Chamorey[2,3]

IL-1 responses to mRNA-LNPs

N Engl J Med. 2022 Oct 20;387(16):1524-1527. doi: 10.1056/NEJMc2205667. Epub 2022 Sep 21.

IL-1RA Antibodies in Myocarditis after SARS-CoV-2 Vaccination