Human Whole Blood Assay Investigations of Lipid Nanoparticle mRNA Immune Stimulation and Mitigation

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Abstract

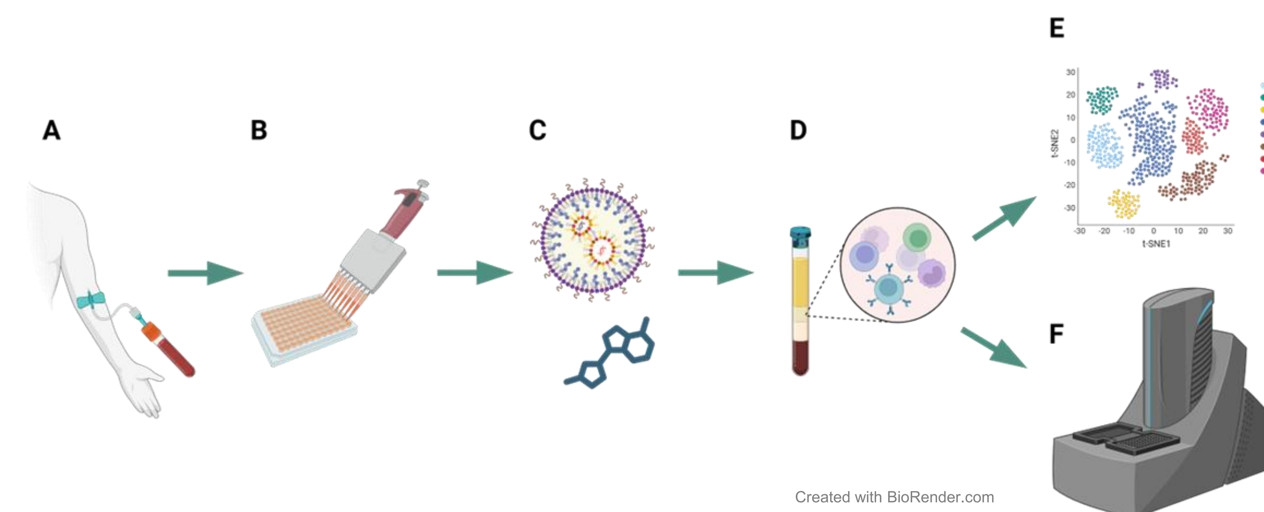
Infusion-related reactions (IRRs) are a common clinical finding associated with the intravenous administration of various products, including therapeutic antibodies, parenteral nutrient lipid emulsions, and lipid nanoparticle (LNP)-based nucleic acid products. The use of LNP technology has increased substantially in recent years with the advancement of gene and epigenetic editing technologies, and early clinical data indicates IRRs remain a clinical consideration. Human whole blood assays have been used to evaluate the safety of therapeutic antibodies and recently, LNP. We optimized a human whole blood assay to assess the contributions of LNP components, such as mRNA payload (e.g., quality, modified vs. unmodified) and lipid, to immune stimulation. Additionally, this assay was used to screen clinically relevant mitigating agents that could dampen IRRs, be more specific than steroids, and be more amenable to repeat dose regimens of LNP.

Commercially available or fresh whole blood from healthy donors was collected in blood tubes containing either heparin, citrate or EDTA as an anticoagulant. Whole blood was diluted 1:1 with normal saline and aliquoted into culture treated 96-well plates. LNP, empty LNP, vehicle control, and PBS were added to the diluted blood and incubated under culture conditions for 24 hours. Plasma was isolated, and inflammatory biomarkers were quantified using Meso Scale Discovery (Meso Scale Diagnostics, LLC, U-Plex, Rockville MD, USA). When screening mitigating agents, the agents were added to the peripheral blood cells an hour before treatment with LNP. Peripheral blood cells were treated with LNP containing a lipophilic dye and carrying GFP mRNA payload. The cells were then analyzed by flow cytometry for uptake (DiI+) and expression (GFP+) of mRNA-LNP.

To determine the optimal conditions to assess the immune stimulatory effect of LNP, we tested a variety of conditions. The secretion of a panel of 10 inflammatory biomarkers in response to treatment was quantified and compared to saline control. Using LPS as a benchmark inflammatory molecule, we found that heparin was the most suitable anti-coagulant as it does not interfere with divalent cations required for immune cell signaling, subsequent inflammatory biomarker secretion, and presumably complement cascade activity at the concentrations of heparin used. While commercial human whole blood is widely available, we found that the immune response was largely muted compared to that of fresh whole blood. Under these conditions, we determined that unlike our benchmark inflammatory molecule, LPS, stimulation of whole blood cells with LNP required a longer 24-hour co-incubation to achieve peak response. Screening dozens of donors from a pool of healthy adults revealed that responses to LNP varied. Donors were classified based on the total inflammatory biomarker output in response to mRNA-LNP, empty LNP, and saline control, which offered the least amount of data manipulation and maintained the uniqueness of the donor responses. We screened mitigating agents including those that are the current standard of care for LNP infusion using dexamethasone as the benchmark. Peripheral blood cells were also assessed for uptake of LNP and expression of a GFP mRNA payload by flow cytometry to help understand cytokine responses and which cells may be responsible.

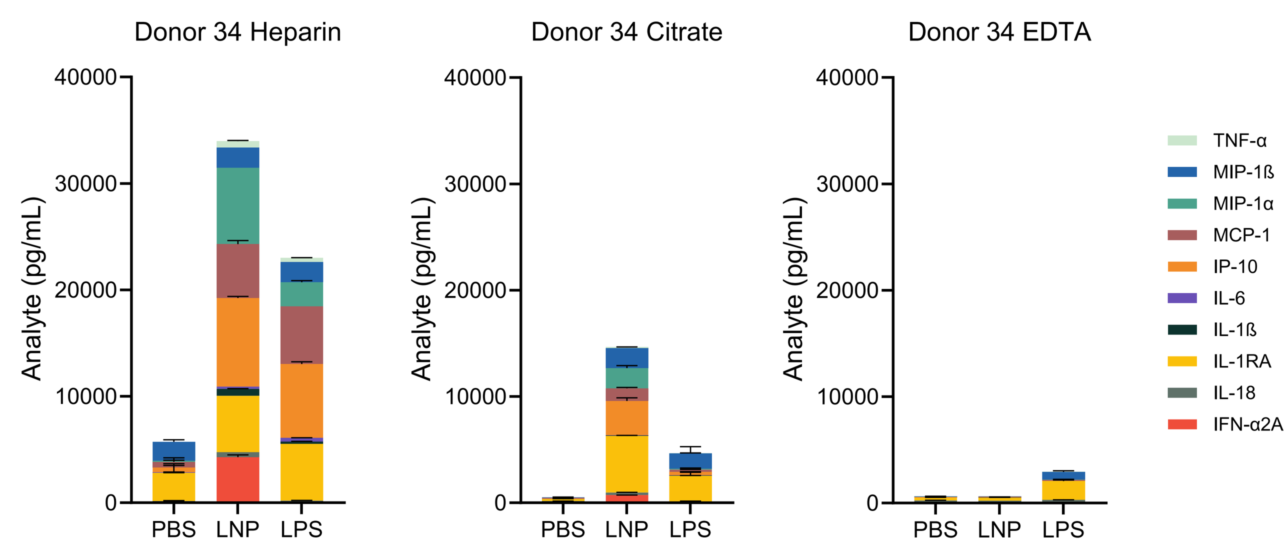
The current standard of care for the prevention of IRRs relies on the use of broad-spectrum immunosuppressors such as dexamethasone. Use of more targeted and specific pre-medications offers a more effective means of mitigating adverse immune stimulation to LNP infusion and enables repeated dosing without the long-term effects of steroid use. As new therapeutics advance and evolve, so should the co-medications and treatments used to mitigate adverse reactions such as IRRs.

1 Whole blood assay workflow



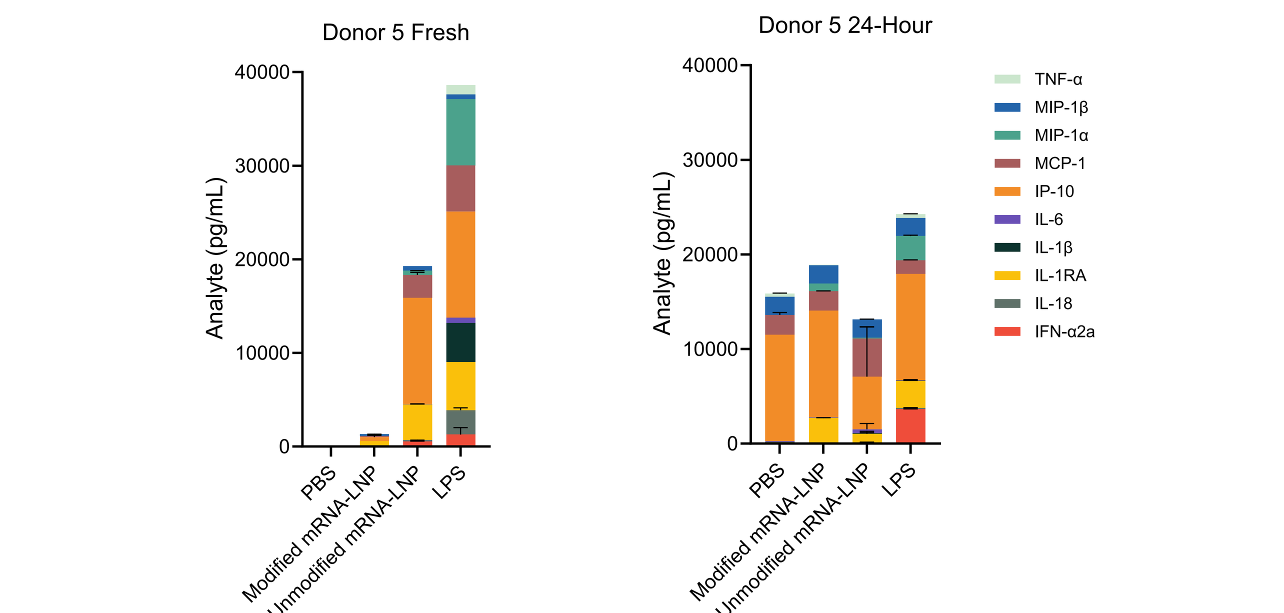
Fresh human whole blood is drawn into sodium-heparin blood tubes (A). Whole blood is diluted 1:1 with normal saline and plated into a deep-well 96-well plate (B). Diluted blood is treated with 5-10 µg mRNA-LNP based on mRNA content and then incubated under cell culture conditions for 24 hours *(Optional: diluted whole blood is pre-treated with mitigating agents, inhibitors or agonists for 1 hour under cell culture conditions)* (C). Plasma is isolated from blood cells (D) *(Optional: blood cells are processed and single cell RNA sequencing or flow cytometry is performed (E))*. Immune stimulation and efficacy of mitigating agents is assessed via Meso Scale Diagnostics (F).

2 Heparin is an optimal anti-coagulant



Fresh human whole blood was drawn into sodium-heparin, sodium citrate, or K_2EDTA blood tubes. Diluted whole blood was treated with PBS, 10 µg/mL mRNA-LNP (unmodified/unpurified payload), or 0.1 ng/mL LPS and the cumulative immune biomarkers were measured after 24 hours. Heparin enabled robust cytokine and chemokine production. EDTA chelates divalent cations required for immune cell signaling, cellular processes and complement activation resulting in muted immune biomarker production. Citrate also chelates but the interaction with divalent cations is weaker and reversible.

3 Fresh whole blood is required



Whole blood from one donor was drawn into sodium-heparin blood tubes and either used immediately in a whole blood assay or stored at 4°C for 24 hours prior to use. Immune biomarkers produced by fresh blood cells in response to different treatments is very clear; LPS and unmodified/unpurified mRNA-LNP induce a robust immune response while those produced in response to PBS and modified mRNA-LNP were minimal in comparison. In contrast, blood stored for 24 hours shows little difference between treatments, suggestive of non-specific immune responses.

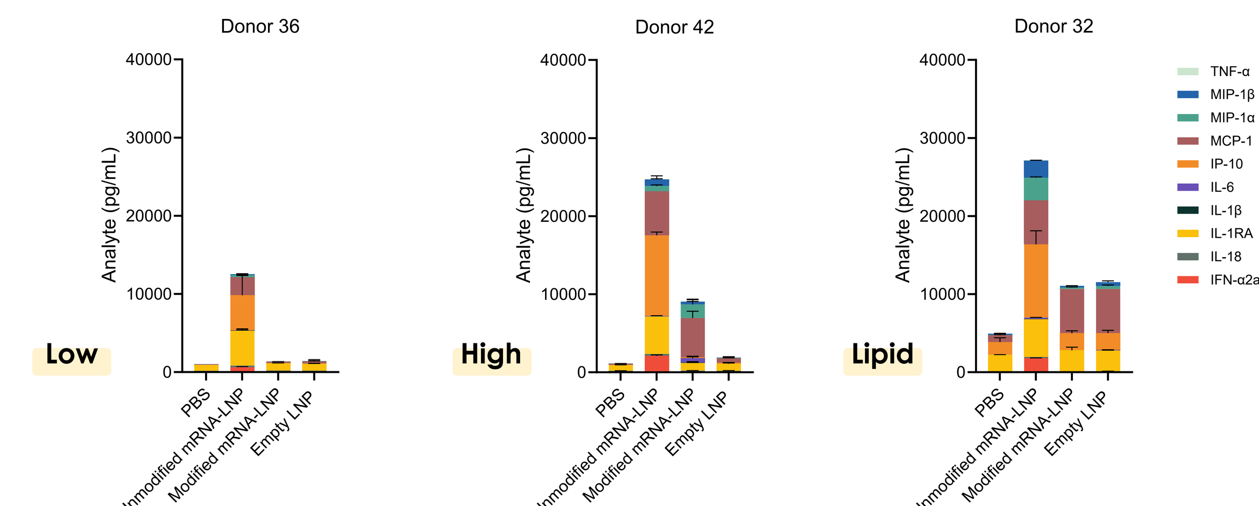
4 A 24-hour incubation is required

Whole blood was treated with 5 µg/mL mRNA-LNP (unmodified/unpurified payload) and incubated under cell culture conditions for the durations indicated.

At 4 hours post-treatment MIP-1a, MIP-1β, IP-10 and IL-1RA were detectable, however, it took over 6 hours until IFN-α2A, IL-6, MCP-1 and TNFa become detectable.

Under these conditions a robust interferon inflammatory response is not detected until 24 hours post-treatment.

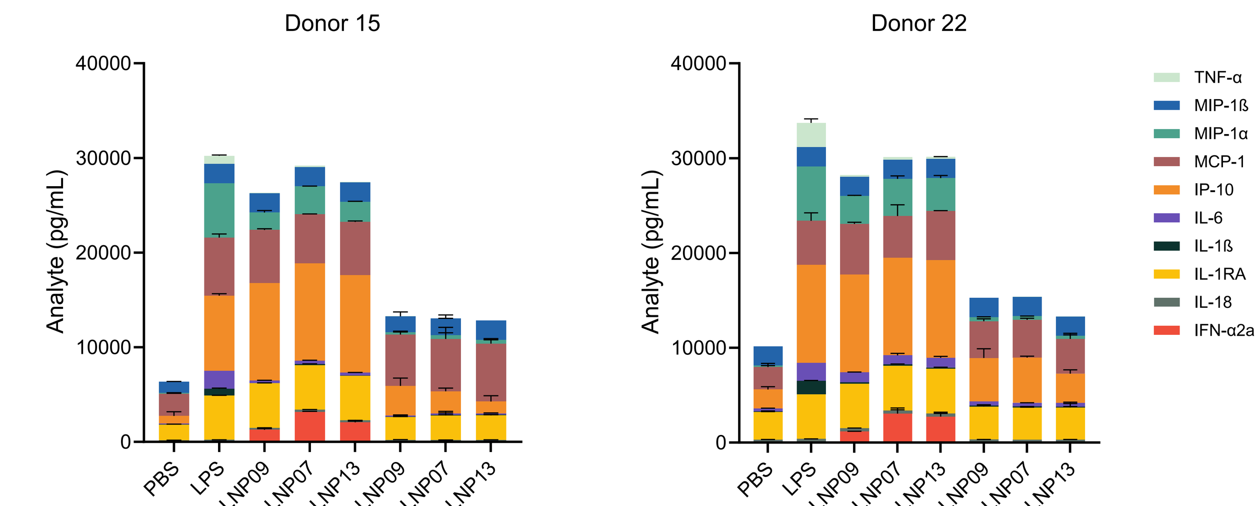
5 Large variation in individual responses to mRNA-LNP & LNP



Whole blood donors were classified based on the cumulative total immune biomarkers detected upon treatment with unmodified/unpurified mRNA-LNP and empty LNP. Lipid responders demonstrated equivalent immune stimulation when treated with empty LNP compared to mRNA-LNP.

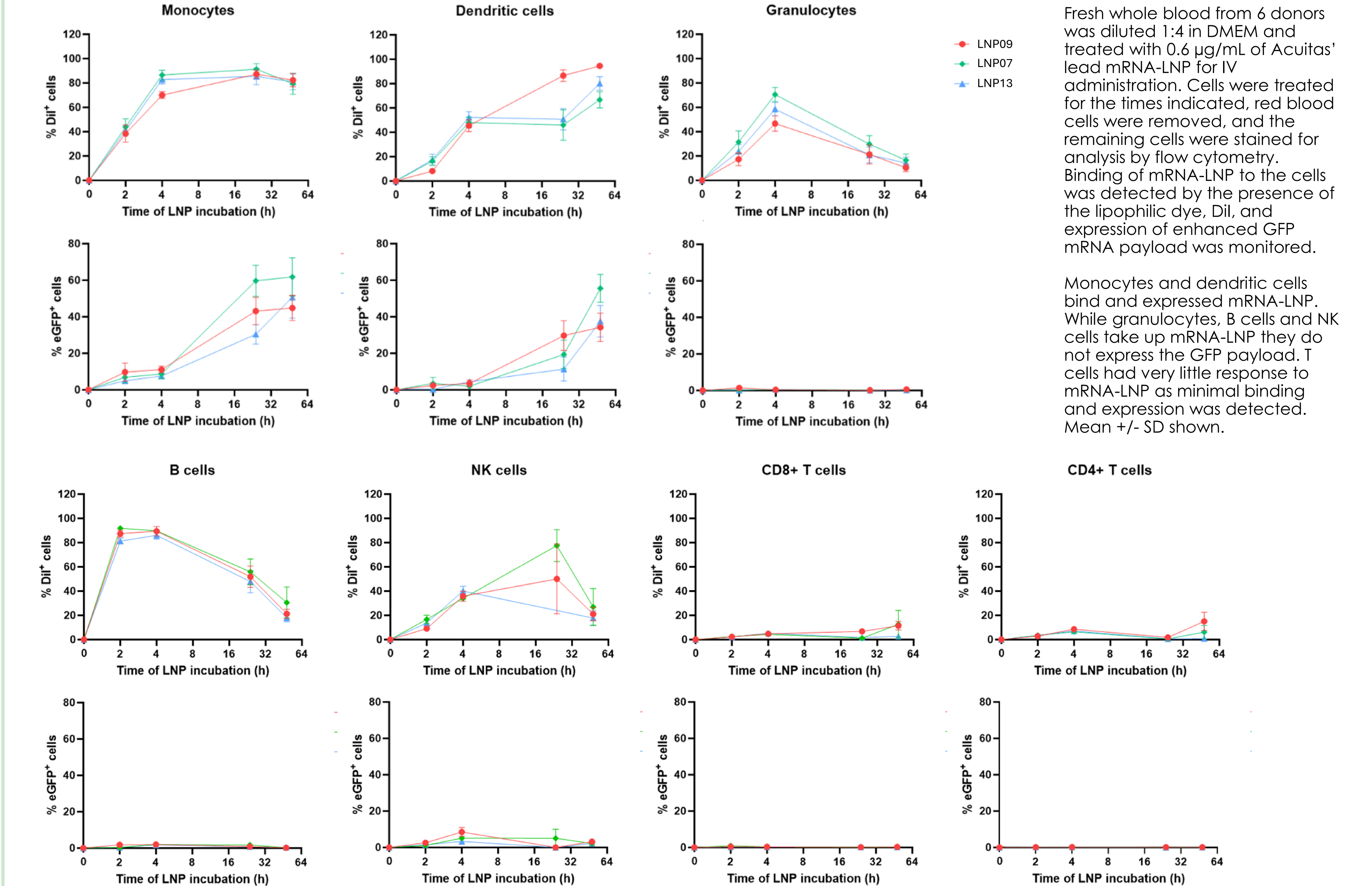
<10,000 = Low   >25,000 = High   Equal response to LNP +/- modified mRNA = Lipid

6 Acuitas' lead ionizable lipids for IV administration have similar immune profiles



Whole blood was treated with 5 µg/mL mRNA-LNP (unmodified/unpurified payload) or the lipid equivalent empty LNP. LNP07, 09 and 13 induce similar levels of cumulative immune biomarkers. LNP09 induced slightly less IFNa2A but interferon-associated IL-1RA, IP-10 and IL-6 are similar. The immune response to empty LNP was much lower than mRNA-LNP and all empty LNP had a similar immune biomarker profile.

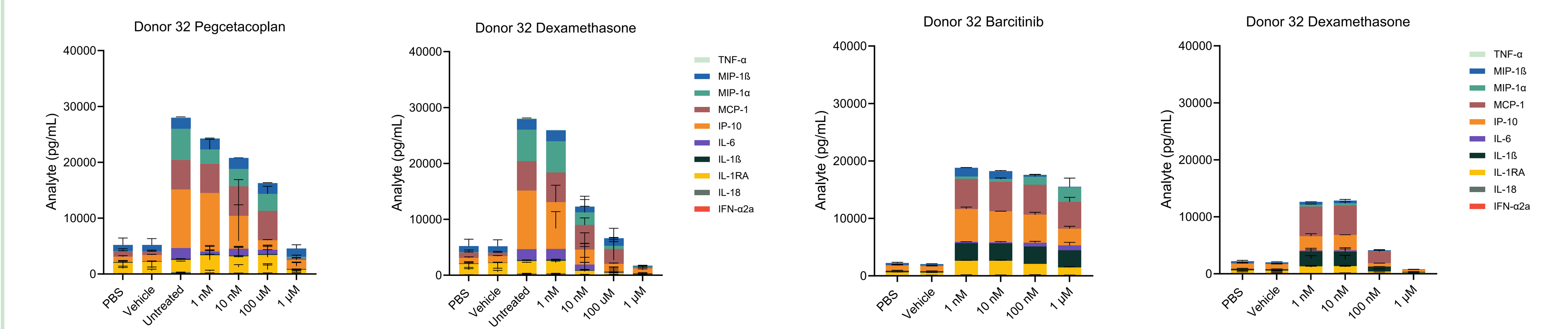
7 Monocytes and dendritic cells bind, take up and express mRNA-LNP



Fresh whole blood from 6 donors was diluted 1:4 in DMEM and treated with 0.6 µg/mL of Acuitas' lead mRNA-LNP for IV administration. Cells were treated for the times indicated, red blood cells were removed, and the remaining cells were stained for analysis by flow cytometry. Binding of mRNA-LNP to the cells was detected by the presence of the lipophilic dye, DiI, and expression of enhanced GFP mRNA payload was monitored.

Monocytes and dendritic cells bind and expressed mRNA-LNP. While granulocytes, B cells and NK cells take up mRNA-LNP they do not express the GFP payload. T cells had very little response to mRNA-LNP as minimal binding and expression was detected. Mean +/- SD shown.

8 Clinically-relevant compounds mitigate mRNA-LNP immune stimulation almost as well as steroid



Blood was pre-treated for 1 hour with agents at the concentrations indicated. mRNA-LNP were added at a concentration of 5 µg/mL. Pegcetacoplan is an approved complement inhibitor that blocks C3/C3b function (IC50 in vitro 15.6/21.3 nM) . It is used to treat paroxysmal nocturnal hemoglobinuria thrombocytopenia in clinical settings. Barcitinib is an approved dual JAK1/2 inhibitor (IC50 in vitro 5.9/5.7 nM) and has been used to treat inflammation caused by rheumatoid arthritis, Covid-19 and severe dermatitis.

Both mitigating agents were effective inhibiting immune stimulation of blood cells from a **high/lipid responder** when treated with mRNA-LNP (modified payload). Mitigating agents were dissolved in DMSO (vehicle).

Summary

1. There is a broad range of immune responses to mRNA-LNP in the population
2. The optimized whole blood assay can be used as a tool to interrogate the immune response of patients
3. Acuitas' lead and clinically relevant LNPs for IV administration elicit similar immune responses
4. Modification and purity of mRNA payload has a large impact on immune stimulation
5. Monocytes and dendritic cells take up and express mRNA-LNP
6. Clinically relevant complement and JAK1/2 inhibitors effectively mitigate this immune response

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