Establishment and application of human Precision-cut lung slices for analysing the immune response to adjuvants

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1. Abstract

Respiratory tract infections remain a major global health burden. Therefore, the development of new vaccines is an ongoing area of research, in particular intranasal vaccines have gained increasing attention. To improve vaccine efficacy, adjuvants can be used. However, only limited numbers of adjuvants are currently licensed for use. Therefore, the aim of this study was to establish a human *in-vitro* model using precision-cut lung slices (PCLS) to investigate the immune response to adjuvants in the lower respiratory tract. During the experiments, three adjuvants, GLA-SE, 3M052-LS, and GLA-3M052-LS, were tested. The results showed increased cytotoxicity in PCLS treated with GLA-SE. Furthermore, cytokine measurements indicated that immune cells remained present within the PCLS.

2. Introduction

Lower respiratory tract infections are the fifth leading cause of death worldwide and resulted in 1’458 cases of infection in Switzerland in 2023 [1]. Vaccination remains one of the most effective measures to prevent severe respiratory infections [2] and consist of biological material designed to induce an immune response against a specific pathogen [3]. Intranasal vaccination in particular is non-invasive and painless, as it does not require the use of needles [4].

Adjuvants are substances that are added to vaccines to enhance the immune response. Numerous studies have shown that adjuvants can strengthen, improve, and prolong immune responses. They exert their effects primarily by activating toll-like receptors (TRL) on innate immune cells, thereby promoting the development of a specific adaptive immune response. [5]. In this study were the following adjuvants tested: GLA-SE is composed of Glucopyranosyl lipid A (GLA), a synthetic TLR4, formulated in a stable oil-in-water emulsion (SE) [6]. 3M052 is a synthetic TLR7/8 agonist belonging to the imidazoquinoline class of compounds, formulated as a liposomal adjuvant (3M052-LS) [7]. GLA-3M052-LS is a combined adjuvant consisting of GLA and 3M052, enabling the simultaneous activation of TLR4 and TLR7/8 and formulation as a liposomal adjuvant [8].

Precision-cut lung slices (PCLS) are small, uniformly cut sections of lung tissue obtained from either animal or human sources. They enable the investigation of the LRT, as they preserve the complex 3D architecture of the lung, including all relevant cell types as well as resident immune cells. Diseased tissue can be used, for example, to study conditions such as chronic obstructive pulmonary disease (COPD) or asthma. In contrast, healthy tissue is particularly suitable for investigating lung biology, as well as for drug testing and the development of new vaccines [9].

3. Aim and leading Question

Aim 1: Establishment of a human *in-vitro* model using precision-cut lungs slices (PCLS) to investigate the immune response to adjuvants.
Question: Can a reproducible and viable human PCLS model of the lower respiratory tract be established in our lab?

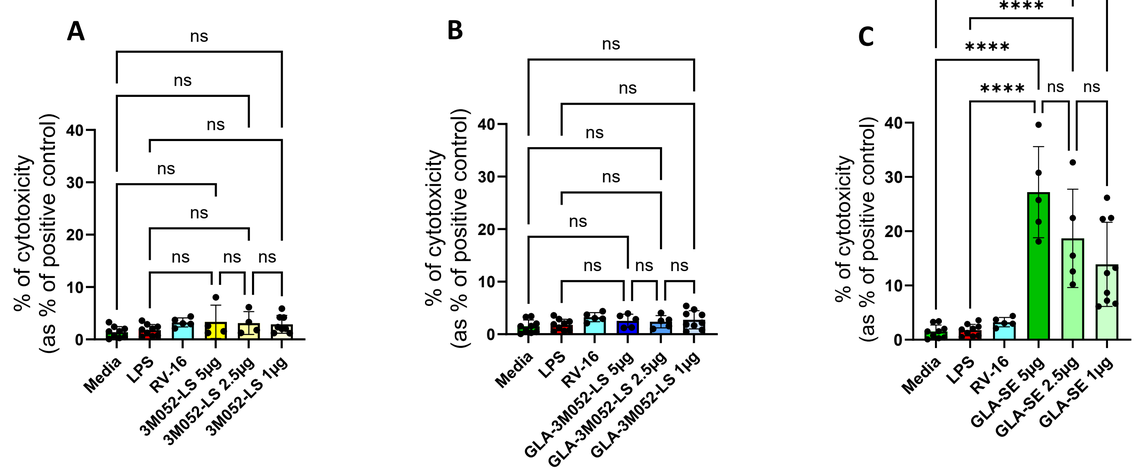
Aim 2: Investigation of the innate immune responses in PCLS after treatment with GLA-SE, GLA-3M052-LS and 3M052-LS.
Question: “Does treatment of PCLS with these adjuvants lead to increased LDH release in the supernatant which correspond in cytotoxicity?” and “Can a difference be detected when the cytokines IL-6, CCL2, CCL5, TNF-α and type 1 IFN-β are measured in the supernatant in the adjuvants used?”

4. Method and Material

To generate PCLS, donated lung tissue got infiltrated with agarose and cut with the Compressome® VF-310-0Z. The PCLS had a diameter of 4 mm and thickness of 400 µm and where treaded with GLA-SE, 3M052-LS and GLA-3M052-LS at dose of 5 µg, 2.5 µg and 1 µg and incubated for 24h. After incubation supernatant were collected for Cytotoxicity and Cytokine measurement and PCLS were fixed with Paraformaldehyde for later Immunofluorescence staining.

5. Results

Cytotoxicity:

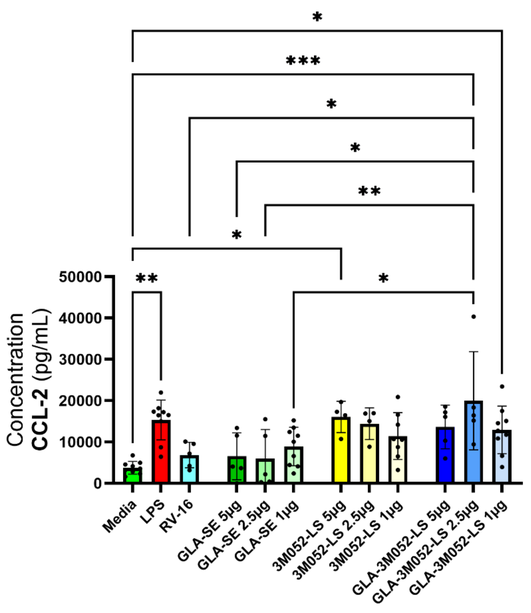


Figur 1: Adjuvants compared to negative & positive control and with each dose. **A:**.3M052-LS in dose of 5 µg, 2.5 µg and 1 µg compared to Media, LPS and each other. **B:** GLA-3M052-LS in dose of 5 µg, 2.5 µg and 1 µg compared to Media, LPS and each other, **C:** GLA-SE in dose of 5 µg, 2.5 µg and 1 µg compared to Media, LPS and each other. Statistical significance was evaluated with Ordinary on-way ANOVA test. ns: not significant, *p-value <0.05, **p-value <0.01, ***p-value <0.005, ****p-value <0.0001 (Holzer, 2026)

GLA-SE showed a significant difference compared to the controls but not between the three tested dose. However, 3M052-LS and GLA-3M052-LS did not exhibit any significant differences compared to the controls or between the tested doses.

Cytokine:

CCL-2 levels were measured in all treatment groups. LPS showed a significant difference only compared to the media control. Treatment with 3M052-LS at a dose of 5 µg and GLA-3M052-LS at a dose of 1 µg resulted in significant differences compared to the media control. Furthermore, GLA-3M052-LS at a dose of 2.5 µg showed significant differences compared to GLA-SE at doses of 5 µg, 2.5 µg, and 1 µg, as well as compared to RV-16 and the media control.



Figur 2: CCL-2 production: All treatments compared to one other. Statistical significance was evaluated with Ordinary on-way ANOVA test. ns: not significant, *p-value <0.05, **p-value <0.01, ***p-value <0.005, ****p-value <0.0001 (Holzer, 2026)

6. Discussion

- In-vitro model works but needs further adaption and a lot of practice
- Dose for GLA-SE need to be adapted, as cytotoxicity is still too high at 1 µg
- Epithelial cells and immune cells were still present within PCLS and capable of producing cytokines in response to adjuvant stimulation

List of references

[1] WHO, 2024, in chapter “Leading causes of death globally” and “Respiratory infections”
[2] Mettelman et al., 2022, p. 759
[3] Pollard & Bijker, 2021, p. 83–85
[4] Kehagia et al., 2023, p. 3590-3599
[5] Zhao et al., 2023, p. 1–3
[6] Seydoux et al., 2018, p. 98-99
[7] Yang et al., 2022, p. 16
[8] Naz et al., 2025, p. 1
[9] Viana et al., 2022, p. 1–7

Figure: