Interleukin-2 – induced vascular leak syndrome: clinically relevant *in vitro* recapitulation with a patient-derived lung-on-chip

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AlveoliX
In-vitro models inspired by nature

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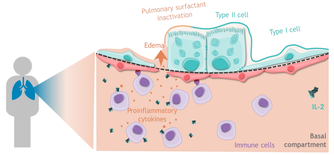
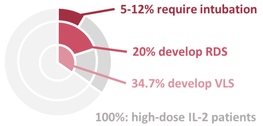
Introduction

Systemic administration of **Interleukin-2 (IL-2)** at high doses in cancer immunotherapy has been associated with **vascular leak syndrome (VLS)** and pulmonary edema. The complex mechanism of action underlying this adverse effect is not yet well understood, however it has been proposed to result from different events involving induction of cytokine release to the bloodstream, generation of complement-activation products, activation of neutrophil and endothelial-cell adhesion molecules. The vascular leakage across the **alveolar-capillary barrier** leads to leukocyte infiltration and cell death, resulting in alveolar epithelial **barrier disruption**. This is associated to an abnormal accumulation of intravascular fluid in the alveolar air space, and creation of a highly **inflammatory environment**, typical of pulmonary edema.

However, the majority of patients present mild pulmonary adverse effects, while one third (34.7%) of the patients undergoing high-dose IL-2 immunotherapy develops VLS [1]. More specifically, approximately 20% develop respiratory distress syndrome (RDS) and 5-12% require intubation and mechanical ventilation [2,3].

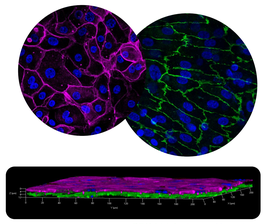




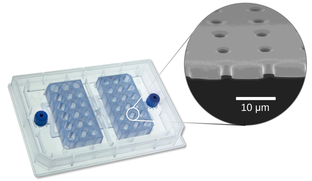
It is therefore crucial to consider this variability and be able to recreate both the **overall-population response** as well as the specific **donor-to-donor variation**.
Within this purpose we propose a personalized-medicine approach, where we aim at recapitulating the within-donors variation in response to IL-2 treatment, as well as a second model more **robust and reproducible** for overall response assessment.

Methods

To generate the patient-derived alveolar barrier model, human primary alveolar epithelial cells (hAEpC) freshly isolated from donors undergoing lung resection are seeded on the apical side of the porous membrane of the AlveoliX Lung-On-Chip [4,5], and endothelial cells (HUVEC) are seeded at the basal side. The cells are then cocultured until barrier formation (TEER > 800 Ω·cm2), when they are treated basolaterally with coculture medium containing immune cells (PBMC) and/or IL-2 at 1 µg/ml. Treatment is maintained during 72h and read-outs are subsequently performed to assess IL-2 induced effects.



PECAM-1 Actin Hoechst



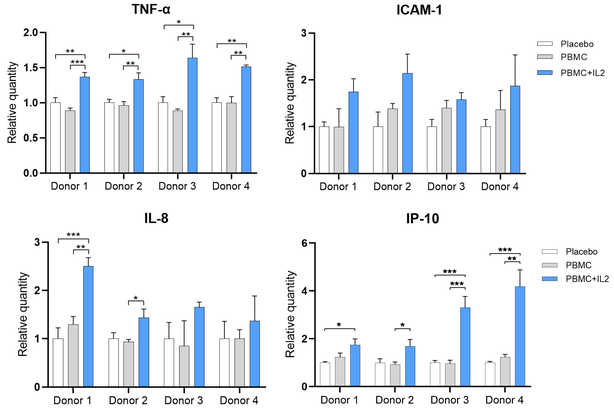
A more robust model suitable for pharmacological safety testing was further developed. Immortalized human alveolar epithelial cells are cocultured with human primary lung microvascular cells (hLMVEC), following the same culture procedures. The direct contact is done by flipping the chips upside-down for 24h.
A Toxic T-Cell Bispecific (TCB) at 1 µg/ml was used as positive control for barrier disruption and cytotoxicity.

Results

Personalized approach: donor-derived model reproduces the clinical variability among patients

Inflammatory response: IL-2 induces cytokines release in all donors

In presence of PBMC, cytokines such as TNF-α, ICAM-1, IL-8 and IP-10 are found in higher amount in the culture supernatants, witnessing an inflammatory response triggered in all the donors. Of particular interest, IP-10 was clearly increased upon IL-2 treatment in all donors, matching the clinical data reporting increased blood levels of IP-10 associated with IL-2 induced VLS [6].

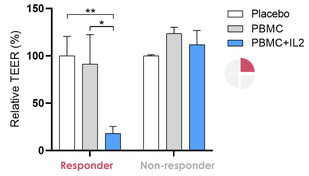


n=4, mean + SEM, One-way ANOVA for each donor, α=0.05

Alveolar leak and toxicity

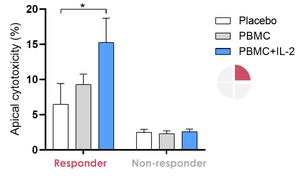
Despite the inflammatory environment depicted by the cytokines panel, alveolar leak and toxicity are found only in one donor out of four. This is in accordance with the clinical data, where only a minority of IL-2 treated patients is reported to develop severe lung conditions.
The *Responder* donor (Donor 3) and a *Non-responder* are shown as representative data. In the *Responder* donor barrier function is impaired upon IL-2 treatment in presence of PBMC, showed by the drastic drop in TEER.
Interestingly, apical cytotoxicity is observed in the *Responder* donor, suggesting a specific toxicity to the alveolar epithelial cells.

Barrier tightness drops



n=4, mean + SEM. Two-way ANOVA, α=0.05

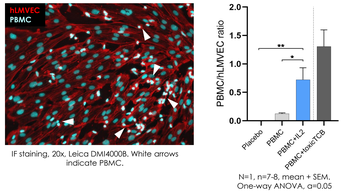
Epithelial cytotoxicity increases



n=4, mean + SEM. Two-way ANOVA, α=0.05

Towards a robust model for reproducible pharmacological safety testing

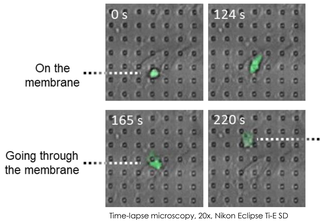
Direct contact between immune cells and endothelium



IF staining, 20x, Leica DMI4000B. White arrows indicate PBMC.

N=1, n=7-8, mean + SEM, One-way ANOVA, α=0.05

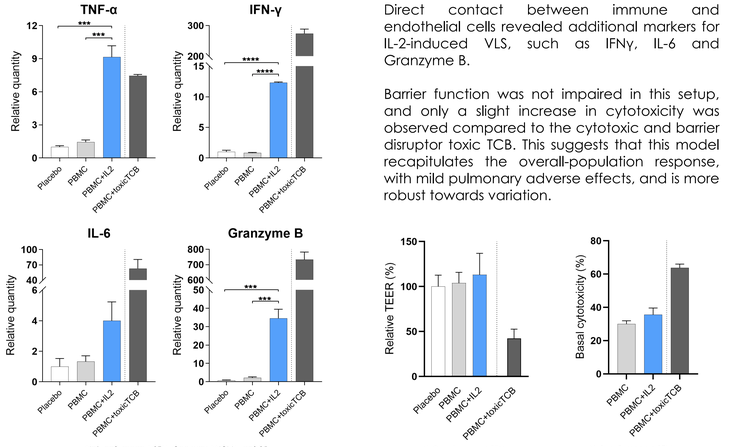
When allowing direct contact, presence of IL-2 increases the number of PBMC attaching to the activated endothelial cells.



Time-lapse microscopy, 20x, Nikon Eclipse Ti-E SD

PBMC can roll on the endothelial cells lying on the membrane and eventually cross towards the apical side through the pores.

Cytokine response and barrier functionality



N=1, n=3, mean + SEM, One-way ANOVA, α=0.05

Direct contact between immune and endothelial cells revealed additional markers for IL-2-induced VLS, such as IFNγ, IL-6 and Granzyme B.

Barrier function was not impaired in this setup, and only a slight increase in cytotoxicity was observed compared to the cytotoxic and barrier disruptor toxic TCB. This suggests that this model recapitulates the overall-population response, with mild pulmonary adverse effects, and is more robust towards variation.

N=2, n=6, mean + SEM

N=1, n=3, mean + SEM

Conclusions

- Our models allow **direct contact of immune cells with endothelium** and crossing towards the apical side of the barrier, for more accurate physiologically-relevant studies.
- The **donor-derived model** reproduces the individual specific response reflecting the **donor-to-donor variability**: IL-2 triggers a proinflammatory environment but leads to barrier disfunction and cell death only for a minority of donors.
- We have developed a second model that recapitulates the **overall-population response**, with mild pulmonary adverse effects, and is more **robust** towards variation and therefore reproducible and suitable for standard **pharmacological safety testing**.

References

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