The purinergic P_2Y_2 receptor is crucially involved in innate immune activation in an in vitro model of acute respiratory distress syndrome

Michael Fauler, Eva Wirsching and Manfred Frick
Institute of General Physiology, Ulm University, Germany

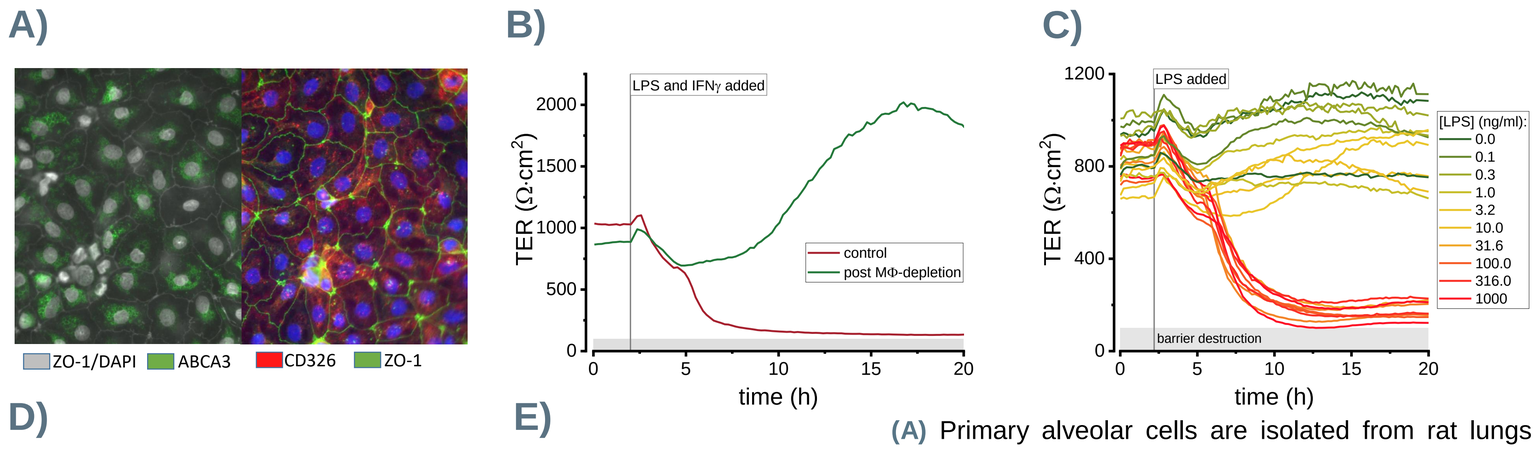
Introduction

ALI/ARDS (Acute Lung Injury/Acute Respiratory Distress Syndrome) is a frequent cause of respiratory failure in critically ill patients suffering from various illnesses, including Covid-19, or following direct or indirect lung trauma. ALI/ARDS aggravates the disease course in the need for intensive care. Despite intense basic and clinical research, mortality remains high. ARDS is caused by an exuberant activation of the innate immune response. This leads to the breakdown of the alveolar air-blood-barrier and formation of an intra-alveolar exudative oedema.

We have developed an *in vitro* model of the alveolar barrier, composed of rat primary alveolar epithelial cells and alveolar macrophages. Upon stimulation with *Lipopolysaccharide* (LPS) the model displays a threshold-like activation of macrophages. In a pharmacological screening approach we tested for factors potentially involved in this activation process.

Within the initial panel, *suramin*, a non-specific inhibitor in the purinergic system, was able to prevent the LPS-induced barrier-breakdown. Subsequent experiments identified the P_2Y_2-receptor as being critically involved.

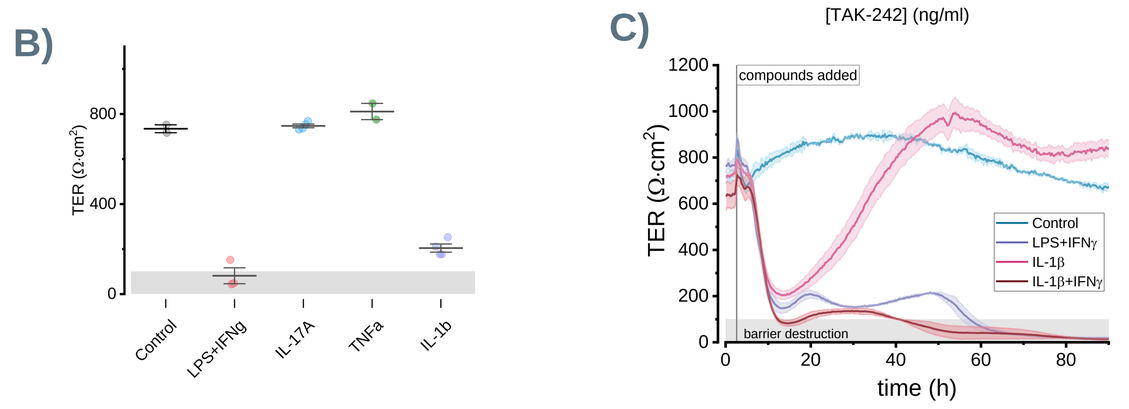
The *in-vitro* cell model



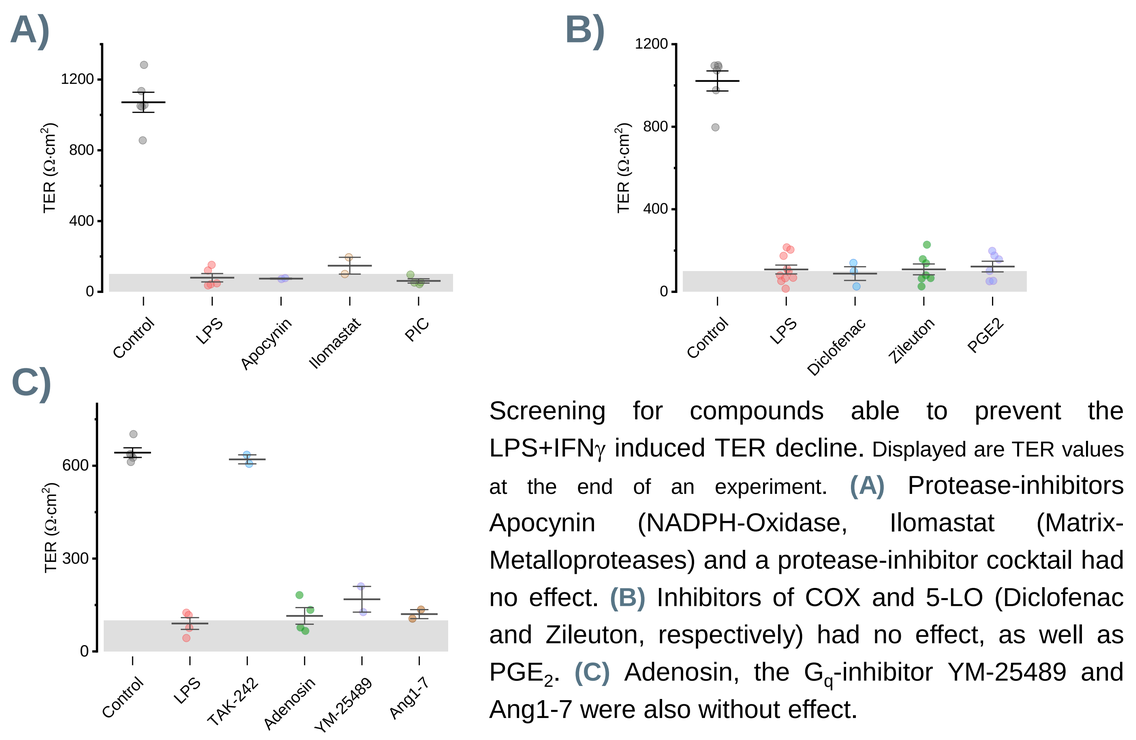
(A) Primary alveolar cells are isolated from rat lungs and cultured for 3 days on cell culture inserts until they establish a tight epithelial cell layer. **(B)** They are treated with Clodronate-Liposomes to remove remaining macrophages. **(D)** Bronchoalveolar lavage cells are seeded on top of the epithelium. **(E)** These cells are >99% $CD68^+$ macrophages. Purple: Isotype control, red: CD68. **(C)** Upon stimulation with LPS and IFNγ cell layers display a threshold-like activation leading to a strong decline of the transepithelial electrical resistance.

LPS and Cytokines

(A) Resatorvid (*TAK-242*), a specific inhibitor of *Toll-like Receptor 4* (TLR-4) signalling, prevents the LPS-induced TER-decline in a concentration-dependent manner. Lowest TER values at the end of an experiment are shown. **(B)** Within the cytokine panel only IL-1β elicited a response. **(C)** The IL-1β effect was transient. Similar to LPS the addition of IFNγ was necessary to evoke a sustained and profound TER reduction.

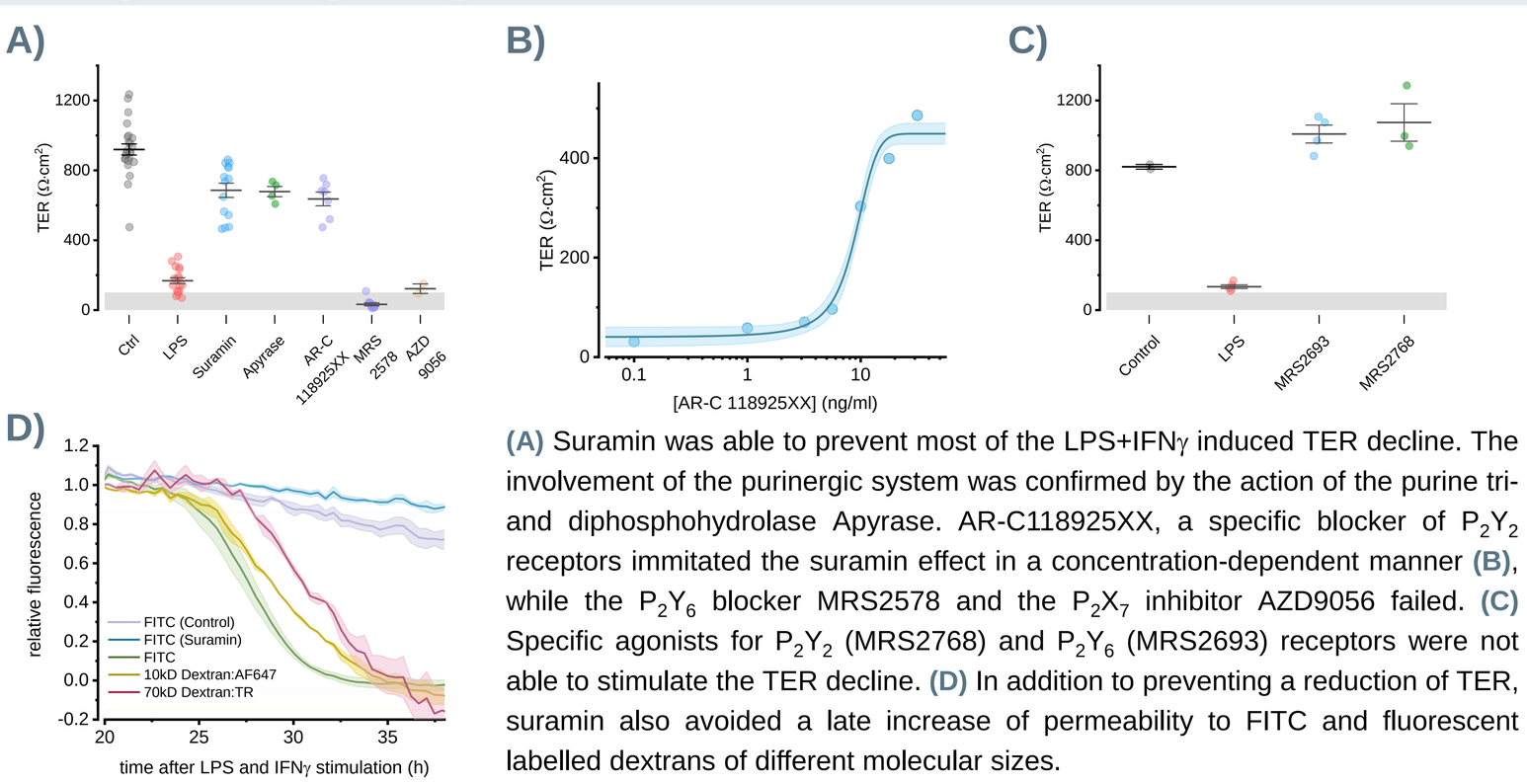


Inhibitor screening panel



Screening for compounds able to prevent the LPS+IFNγ induced TER decline. Displayed are TER values at the end of an experiment. **(A)** Protease-inhibitors Apocynin (NADPH-Oxidase, Ilomastat (Matrix-Metalloproteases) and a protease-inhibitor cocktail had no effect. **(B)** Inhibitors of COX and 5-LO (Diclofenac and Zileuton, respectively) had no effect, as well as PGE_2. **(C)** Adenosin, the G_q-inhibitor YM-25489 and Ang1-7 were also without effect.

The *purinergic* system



(A) Suramin was able to prevent most of the LPS+IFNγ induced TER decline. The involvement of the purinergic system was confirmed by the action of the purine tri- and diphosphohydrolase Apyrase. AR-C118925XX, a specific blocker of P_2Y_2 receptors immitated the suramin effect in a concentration-dependent manner **(B)**, while the P_2Y_6 blocker MRS2578 and the P_2X_7 inhibitor AZD9056 failed. **(C)** Specific agonists for P_2Y_2 (MRS2768) and P_2Y_6 (MRS2693) receptors were not able to stimulate the TER decline. **(D)** In addition to preventing a reduction of TER, suramin also avoided a late increase of permeability to FITC and fluorescent labelled dextrans of different molecular sizes.

Summary

- Activation of BAL-derived macrophages seeded on top of a primary rat alveolar epithelial cell culture displays a threshold phenomenon with respect to response elicitation measured as barrier integrity breakdown (reduction of TER).
- The response depends on the presence of macrophages.
- Specific blocking of the purinergic P_2Y_2 receptor prevents the barrier breakdown in a concentration dependent manner.
- Stimulation of P2-purinergic receptors alone is insufficient to elicit the response.

Therefore, we conclude that the LPS-induced epithelial barrier damage depends on the presence of macrophages, and on P2Y2-signalling, which is necessary but not sufficient.