Effect of TRPV4 overexpression on Toll like receptor responses in murine lung epithelial E10 cells

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Background

The transient receptor potential cation channel subfamily V member 4 (TRPV4) is expressed in the lung by epithelial cells (EpC). TRPV4 is activated by heat, mechanical force, hypo-osmolarity, and arachidonic acid metabolites[2]. TRPV4 has been described as a target for treating inflammatory disease and influenza A virus (IAV) infection. There are different splice variants of TRPV4 leading to either surface or intracellular expression of TRPV4. Toll-like receptors (TLRs) are expressed on resident cells like EpCs within the lung and when activated act as a defense against pathogens. TLR3, 4 and 7 were all indicated in inflammatory responses to IAV infection[1]. Activation of TLRs can lead to increased lung pathology during IAV infection.

Aim

In this study, we aimed to investigate the inflammatory response of the murine lung EpC line E10 to TLR 3, 4, and 7 stimulation. We also investigated the effect of human TRPV4-C overexpression on the TLR 3, 4, and 7 responses.

Methods

E10 cells were grown to confluency and stable TRPV4 overexpression was achieved by infecting cells with lentiviral particles encoding for the human TRPV4-C splice variant. This variant exhibits intracellular retention. To stimulate cells, seeding media was replaced with stimulation media containing TLR3 agonist poly IC, TLR7 agonist R848, or TLR4 agonist LPS. Cells were stimulated for 2 or 24hrs for mRNA gene expression or protein expression, respectively. In selected experiments, cells were infected with IAV at 6.4 hemagglutinating unit (HAU) for 2 days. Cells were used for RNA isolation and RNA concentration was quantified by Nanodrop. The cDNA was prepared from mRNA and was subjected to qRT-PCR using primer-probe sets for mouse CXCL1 and CCL2. Mouse CXCL1 protein expression was analyzed in cell supernatant, 24 hours after stimulation by ELISA.

TRPV4 overexpression leads to increased TLR responses in murine lung EpC E10 cells.

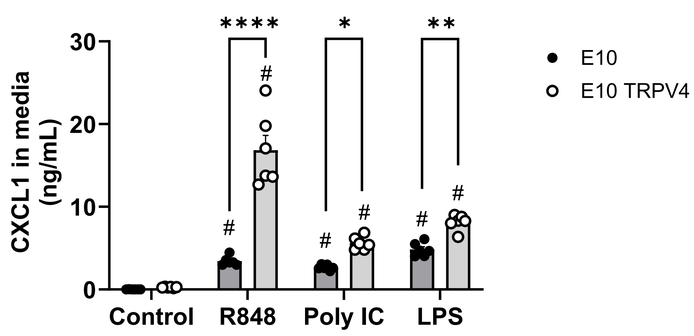

Figure 1. E10 cells (dark grey) or E10 cells overexpressing TRPV4-C (E10 TRPV4, light gray) were stimulated with the TLR7 agonist (R848, 1µg/mL), TLR3 agonist (poly IC, 10µg/mL), or TLR4 agonist (LPS, 0.1µg/mL) for 24 hours and CXCL1 protein levels analyzed by ELISA. Data (N=6) are shown as mean±SEM and analyzed by 2-way ANOVA. #P<0.05 vs control of the respective genotype. *P<0.05, **P<0.01, and ***P<0.005.

TRPV4 overexpression leads to altered CXCL1 mRNA expression in E10 cells.

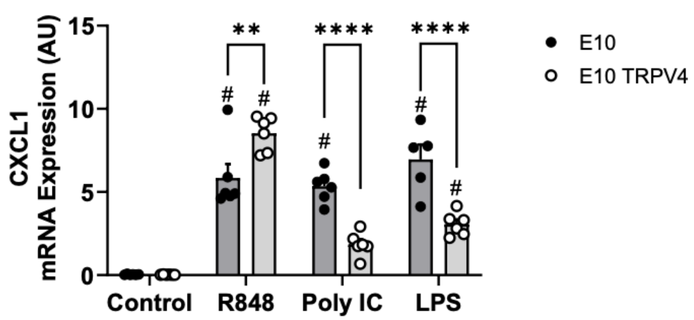

Figure 2. E10 cells (dark grey) or E10 cells overexpressing TRPV4-C (E10 TRPV4, light gray) were stimulated with the TLR7 agonist (R848, 1µg/1mL), TLR3 agonist (poly IC, 10µg/mL), or TLR4 agonist (LPS, 0.1µg/mL) for 2 hours and CXCL1 mRNA expression analyzed by qRT-PCR. Data (N=5-6) are shown as mean±SEM and analyzed by 2-way ANOVA. #P<0.05 vs control of the respective genotype. **P<0.01 and ***P<0.005.

TRPV4 overexpression leads to altered CCL2 mRNA expression in E10 cells during TLR3 and TLR4 stimulation.

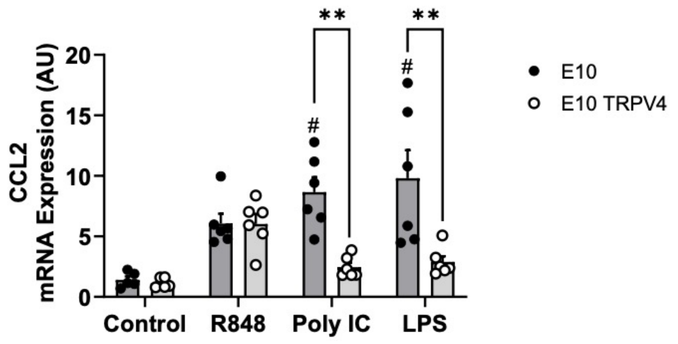

Figure 3. E10 cells (dark grey) or E10 cells overexpressing TRPV4-C (E10 TRPV4, light gray) were stimulated with the TLR7 agonist (R848, 1µg/1mL), TLR3 agonist (poly IC, 10µg/mL), or TLR4 agonist (LPS, 0.1µg/mL) for 2 hours and CCL2 mRNA expression analyzed by RT-PCR. Data (N=6) are shown as mean±SEM and analyzed by 2-way ANOVA. #P<0.05 vs control of the respective genotype and **P<0.01.

TRPV4 overexpression increased IAV infection dependent CXCL1 release.

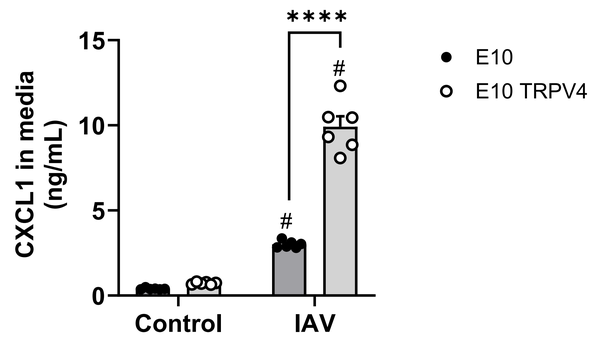

Figure 4. E10 cells (dark grey) or E10 cells overexpressing TRPV4-C (E10 TRPV4, light gray) were infected with 6.4 HAU IAV per 5 x 10^4 cells for 2 days. CXCL1 protein levels in the media were analyzed by ELISA. Data (N=6) are shown as mean±SEM and analyzed by 2-way ANOVA. #P<0.05 vs control of the respective genotype and ****P<0.001.

Results & Conclusion

Stimulation with TLR agonists poly IC, LPS, and R848 led to significantly increased CXCL1 protein levels in the media of both E10 cell lines compared to unstimulated cells. However, TRPV4 expression in E10 cells, lead to increased CXCL1 protein release compared to E10 cells. Interestingly, E10 TRPV4 cells had increased CXCL1 mRNA responses to TLR7 but reduced CXCL1 mRNA responses after TLR3 or TLR4 stimulation compared to stimulated E10 cells. With regard to CCL2 mRNA expression E10 TRPV4 cells did not show a significant increase after TLR7, TLR3 and TLR4 stimulation compared to E10 cells. TLR7 stimulation of both cell lines resulted in similar CCL2 expression. However, E10 TRPV4 cells exhibited reduced CCL2 mRNA expression after TLR3 and TLR4 stimulation. IAV challenge of E10 TRPV4 cells led to increased CXCL1 release compared to E10 cells. The data suggest that TRPV4-C expression differentially changes TLR responses with regard to protein and mRNA levels of inflammatory mediators. More studies are needed to investigate the specific mechanism behind it.

References

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