NLRP3 regulates epithelial barrier integrity and protects from airway hyperresponsiveness in experimental allergic asthma

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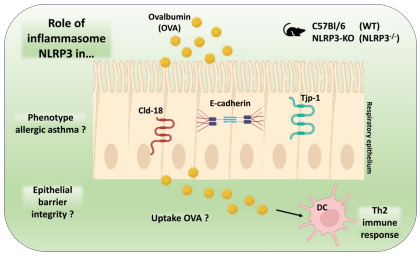
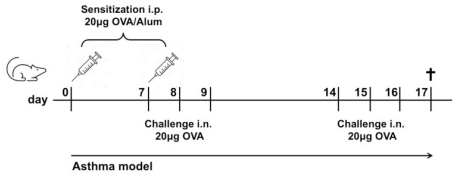
Background

The inflammasome NOD-like receptor family pyrin domain containing 3 (NLRP3) has been shown to be critical for epithelial barrier integrity. Allergic asthma diesease (AAD) is characterized by epithelial barrier dysfunction along with excessive airway inflammation, airway hyperresponsiveness (AHR), and mucus hypersecretion. Thus far, underlying mechanisms in the interaction between NLRP3 and epithelial barrier integrity in type 2-mediated allergic asthma are not well understood.



Methods

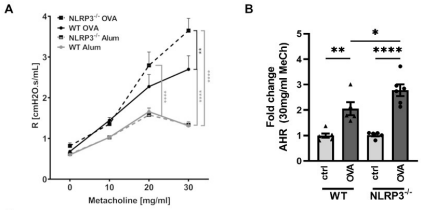
We employed an ovalbumin (OVA)-induced experimental model of AAD using wild-type (WT) and NLRP3-deficient (NLRP3$^{-/-}$) mice. At 5-6 weeks of age, mice received OVA in Alum systemically and OVA in NaCl 0.9 % intranasally (i.n.). AAD was characterized by lung function measurement (AHR) after metacholine challenge, and analysis of mucus hyperplasia with Periodic acid-Schiff (PAS) staining of lung tissue section and qPCR analysis of Muc5AC. Gene and protein expression of respiratory epithelium key players were analyzed by qPCR and flow cytometry. On a translational level, gene expression of these key players was analyzed *in vitro* in the human bronchial epithelial cell line 16HBE14o- after treatment with the NLRP3 inhibitor MCC950. *In vivo* permeability of the respiratory epithelium was functionally investigated by i.n. treatment of WT and NLRP3-KO mice with FITC-labeled OVA.



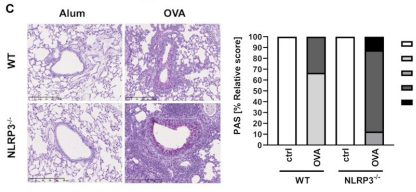
Results

NLRP3$^{-/-}$ mice exhibit an exacerbated phenotype of allergic asthma disease when compared to WT mice following OVA immunization

Airway hyperresponsiveness



Periodic acid-Schiff staining



Muc5AC

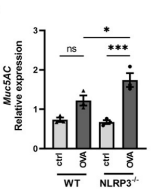
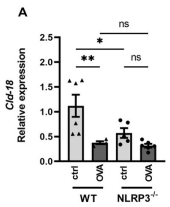


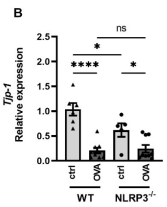
Figure 1: (A) Airway resistance (R) in OVA-immunized (OVA) and control (Alum) WT and NLRP3$^{-/-}$ mice, measured after challenge with metacholine (MeCh, n≥6/group). (B) Airway resistance values for 30 mg/ml MeCh as seen in A; Values are shown as fold change calculated relative to mean of WT control mice. (C) Levels of mucus production as determined by PAS staining of lung sections. The degree of mucus production in positive bronchi was graded as follows: 1: 5-25%; 2: 25-50%; 3: 50-75%, and 4: 75-100%. Original magnification, ×20; scale bar, 250µm; representatives of n≥8/group. (D) Gene expression of MUC5AC (n≥3/group, relative to GAPDH) in whole lung tissue, evaluated by RT-PCR. (A) Two-way ANOVA; (B&D) One-way ANOVA; mean±SEM. ns not significant, * P < 0.05, ** P < 0.01, *** P < 0.001 and **** P < 0.0001.

Expression of NLRP3 correlates with the expression of cell membrane proteins important for epithelial barrier integrity in both murine and human epithelial cells

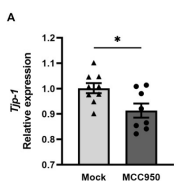
Claudin-18



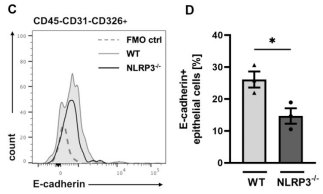
Tjp-1



Tjp-1



E-cadherin



E-cadherin

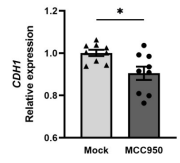


Figure 2: (A-B) Relative gene expression of Cld-18 and Tjp-1 in WT and NLRP3$^{-/-}$ mice evaluated by RT-PCR in relation to GAPDH (n≥5/group). (C) Expression of E-cadherin on CD45-CD31-CD326+ epithelial cells isolated from lungs of WT (black line) and NLRP3$^{-/-}$ mice (grey shaded line); (FMO: dotted line). One representative of n=3/group. (D) Frequency of E-cadherin+ epithelial cells in whole lung tissue of WT and NLRP3$^{-/-}$ mice (n=3/group); unpaired two-tailed Student's t-test; mean±SEM. ns not significant, * P < 0.05, ** P < 0.01, and **** P < 0.0001.

Figure 3: (A) Relative gene expression of Tjp-1 and (B) CDH1 (E-cadherin) in cell lysates of the human bronchial cell line 16HBE14o- before and 7 days after treatment with the NLRP3 inhibitor MCC950 or DMSO. Expression was evaluated by RT-PCR in relation to GAPDH (n=9/group); Mock: DMSO, NLRP3 inhibitor: MCC950; unpaired two-tailed Student's t-test; mean±SEM. * $P < 0.05$.

NLRP3 affects murine respiratory epithelial barrier permeability

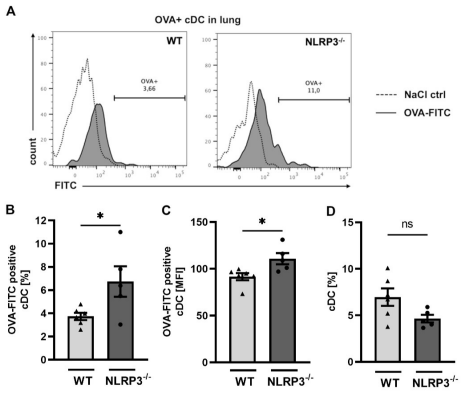


Figure 4: (A-C) OVA uptake by WT and NLRP3$^{-/-}$ lung cDCs two hours after i.n. challenge with FITC-labeled OVA respectively. NaCl 0.9% was used as control. (A) Expression of FITC-OVA+ cDCs (black tinted) or control cDCs (dashed) determined by flow cytometry. Representatives of n≥5/group; (B) Frequencies and C mean fluorescence intensity (MFI) of FITC-OVA+ WT and NLRP3$^{-/-}$ cDCs (n≥5/group); (D) Frequencies of lung cDCs in the same mice analyzed in (B) and (C); unpaired two-tailed Student's *t*-test; mean±SEM. ns not significant, * $P < 0.05$.

Summary Results

- **Absence of the inflammasome NLRP3 has a negative effect on the development of allergic airway disease in a murine experimental AAD model, as evidenced by elevated AHR and mucus production.**
- **Naïve NLRP3$^{-/-}$ mice exhibit a basal lower expression of cell membrane proteins essential for epithelial adhesion and epidermal architecture. This was validated in a translational in vitro approach utilizing a human bronchial epithelial cell line and the NLRP3 inhibitor MCC950.**
- **Functional analysis revealed that the loss of NLRP3 is accompanied by an increase in epithelial barrier permeability.**

Conclusion

The NLRP3 inflammasome is an important component in the development and maintenance of the respiratory epithelial barrier in a murine model of OVA-induced allergic asthma-like airway disease.

Outlook

Additional in-depth analysis is needed to ascertain the precise role of NLRP3 in epithelial barrier integrity. This could lead to the identification of new therapeutic approaches.

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Disclosure of Conflicts of Interest
I have no financial or non-financial conflicts of interest to declare