Supplementary File

Role of Tyk-2 in Th9 and Th17 cells in allergic asthma

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Running title: Tyk-2 regulates T helper cell number

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Results

Tyk-2 deficiency leads to a severe Th2 allergic phenotype after allergen challenge

To understand the effect of targeting Tyk-2, we analyzed Tyk-2 deficient mice in a murine model of asthma after OVA sensitization and challenge (Fig. S1a). Tyk-2 deficiency leads to severe infiltration of eosinophils into the airways of asthmatic mice, in a murine model of asthma after OVA sensitization and challenge, as shown by differential cell counts in the BALF cells from Tyk-2(-/-) mice as compared to the wild type littermates (Fig. S1 b, c). This hyper-eosinophilia was associated with elevated IL-5 production in BALF (Fig. S1d). Tyk-2 deficiency is also known to be associated with hyper IgE1. Along with hyper-IgE these mice also displayed elevated levels of IgG2A compared to wild type mice (Fig. S1 e,f). In spite of this allergic phenotype, Tyk-2(-/-) mice had the same airway hyperresponsiveness (AHR) as the wild type asthmatic mice (Fig. S1g).

When we analyzed the lung sections stained for collagen deposition (Masson-Goldner), we detected an increase in remodeling around the airways of Tyk-2(-/-) mice (Fig. S1h). This was consistent with the increased IL-13 production detected in the supernatants of lung CD4+ T cells from asthmatic Tyk-2(-/-) mice, the Th2 hallmark cytokine associated with collagen deposition (Fig. S1i).

Methods

Assessment of AHR and collection and analysis of bronchoalveolar lavage

To measure airway reactivity, we used whole-body plethysmography as previously described2,3. Data are expressed as mean values of “airway resistance [RI]/baseline RI” ± SEM. After plethysmography, bronchoalveolar lavage of the right lung was performed twice with 800 µL saline each. BALF supernatants were frozen and subsequently analyzed by ELISA. The cell pellet was resuspended in PBS and counted (CASY® TT, Roche Diagnostics, Mannheim,
Germany) and subsequently used for cytospin analysis after staining with May-Grünwald-Giemsa solution (Carl Roth, Karlsruhe, Germany).

**Histology**

Lungs were removed, fixed in 10% formalin-PBS solution, dehydrated and embedded in paraffin. 5 µm thick sections were stained with Masson-Goldner staining for quantification of collagen deposition 4.

**Isolation and analysis of lung and spleen CD4⁺ T cells and cell culture**

Lung CD4⁺ T cells were positively sorted by magnetic bead isolation (MACS, Miltenyi Biotec, Bergisch-Gladbach) according to the manufacturer’s protocol.

In some experiments lung and spleen CD4⁺ T cells were incubated with anti-CD3 (2µg/ml), anti-CD28 (2µg/ml). Antibodies were from hybridoma cell cultures. After 24 hours, or later as indicated, the supernatants were removed and analysed by ELISA and RNA extracted from the cells.

**ELISA**

Mouse IL-5 (15.6–1000 pg/ml), IgE (1.6-100 ng/ml) and IgG2a (3.1 200 ng/ml) were detected by using a specific sandwich ELISA (OptEIA; BD Pharmingen, Heidelberg, Germany). ELISA kits for mouse IL-13 (15.6–1000 pg/ml) were from DuoSet; R&D, Wiesbaden, Germany. IL-9 (30-4000 pg/ml) was determined using a Ready!Set!Go! from eBioscience (Frankfurt, Germany).
References


Supplementary Figure legends

**Figure S1. Tyk-2 deficiency induced allergic asthma in a murine model.**

- **a.** Experimental design.
- **b.** Cytopreparations from the bronchoalveolar lavage fluid of naïve (PBS, upper panels) and OVA-treated wild type (lower left panels) and Tyk-2^{+/−} (lower right) mice. BALF cells were cytospun on slides, stained with May Grünwald Giemsa and counted.
- **c.** The absolute number of eosinophils was quantified (n=4-5; p=0.01, p=0.001, p=0.005).
- **d.** BALF was retrieved and measured by ELISA for IL 5 production (n=3-4; p=0.0003).
- **e.** and **f.** Serum from these mice was tested by ELISA for concentration of total IgE (n=3-5; p=0.006; p=0.0003, p=0.002) and IgG2A (f, n=4-5; p=0.013; p=0.036; p=0.00003; p=0.029).
- **g.** Invasive plethysmography in PBS and OVA (n= 3-5).
- **h.** Lung paraffin sections were stained for Masson-Goldner and analyzed at 400x magnifications (n=3-5).
- **i.** Lung CD4⁺ T cells from naïve and OVA treated mice were isolated and cultured for 24h with α-CD3 and α–CD28 antibodies. The supernatants were analysed by ELISA for IL 13 (n=2-4; p=0.041).
Figure S2. Original Western Blot for SOCS3 and β-actin are reported in Figure 2. Upper blot SOCS3 and lower blot β-actin. From the right to the left, probes from lung proteins extracted from: 5 Tyk-2-OVA, 5 probes from wt-OVA and 4 probes from wt-PBS were loaded.