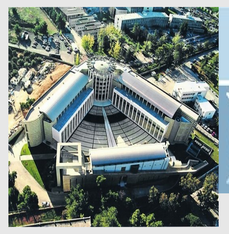
Delineation of the molecular mechanisms underlying TSLP-driven non-type 2 inflammatory responses in severe asthma



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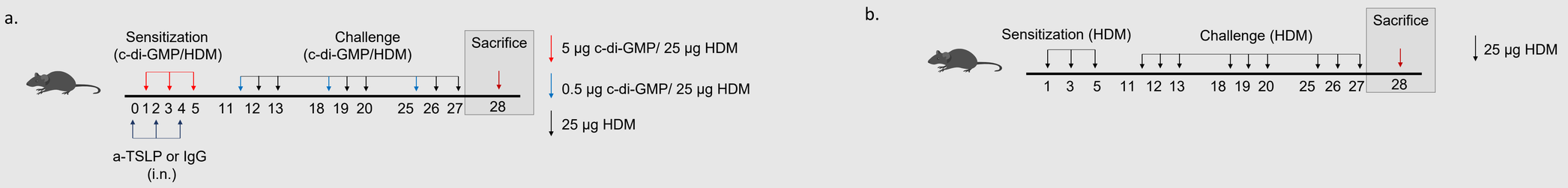
Abstract

Patients with severe asthma (SA) represent a group of asthmatics that is poorly responsive to standard of care treatment thus leading in some cases, in life-threatening disease exacerbations. Currently-available biologic therapies display superior efficacy mainly in patients with mild-to-moderate allergic or eosinophilic asthma. Hence, targeting factors that hold broader effects on airway inflammation than existing biologics could constitute an attractive therapeutic approach for SA patients. Thymic stromal lymphopoietin (TSLP) is an upstream initiator of the inflammatory cascade thus representing one such appealing therapeutic target. Still, the precise role of TSLP in SA pathogenesis remains elusive. Our aim was to investigate whether inhibition of TSLP in vivo can restrain excessive non-type inflammatory responses that prevail in SA and ameliorate disease phenotype. 8-12 week-old female C57BL/6 were sensitized with HDM and c-di-GMP intranasally (i.n.) on days 1, 3, and 5. Mice were then rested for 5 days and subjected to 3 challenge sets involving 3 consecutive challenges with HDM and c-di-GMP with a rest of 4 days between challenge sets. c-di-GMP was administered i.n. along with HDM, on the first day of each challenge set, followed by HDM administration in the next 2 challenges. For the preventive protocol, anti-TSLP or the respective isotype control were given i.n. for three consecutive days prior to allergen sensitization. Mice were sacrificed 24 hours after the last challenge. BALF, lungs and serum were isolated from all experimental groups. BAL inflammatory cell counts, peribronchial and perivascular inflammation, mucus production and cytokine release were measured in serum, BALF and lung homogenates by ELISA. Mice with SA were also compared with mice with mild-to-moderate asthma that received HDM i.n. on days 1, 3, and 5, then rested for 5 days and subjected to 3 challenge sets involving 3 consecutive challenges with HDM. We observed significantly increased TSLP levels in the BALF, lung homogenates and serum of mice with SA compared to mice with MMA and to a greater extent to control mice (naïve). In vivo blockade of TSLP before allergen sensitization significantly decreased the levels of peribronchial and perivascular inflammation, mucus production by goblet cells as well as total numbers of BAL infiltrating inflammatory cells and especially neutrophils. We also detected decreased levels of IL-17, IFN-γ and IL-13 in the serum, BALF and lung homogenates of SA mice that received anti-TSLP compared to SA and to a greater extend MMA mice. Finally, in vivo ablation of TSLP also diminished the expression levels of type1, 2 and 17 cytokines in mediastinal lymph node cell culture supernatants upon ex vivo allergen stimulation. Our data reveal that inhibition of TSLP before allergen sensitization in a well-established murine model of SA restrains pulmonary inflammation and attenuates key asthma features. Our studies may pave the way for delineating the molecular mechanisms through which TSPL orchestrates non type inflammatory responses that prevail in SA.

Methods



a) Experimental design of the protocol utilized. Mice were sensitized and challenged with c-di-GMP/HDM and 24 hours after the final allergen challenge, mice were euthanized and analyzed for different end points. Some groups of mice were administered a-TSLP or IgG i.n. for three consecutive days prior to allergen sensitization. b) Experimental design for MMA protocol. Mice were sensitized and challenged with HDM and 24 hours after the final allergen challenge, mice were euthanized and analyzed for different end points.

Results

Figure 1. TSLP expression levels increase during SA progression.

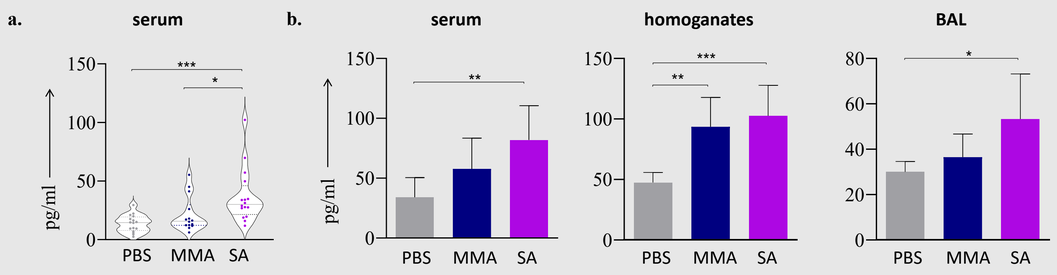


Figure 1. **a.** TSLP expression levels in serum of SA, MMA and HC (n=14-16). Statistical significance was obtained by the Mann–Whitney U test. **b**. TSLP expression levels in serum, lung homogenates and BAL fluid of control (PBS), MMA and SA mice (n=8-10). Statistical significance was obtained by unpaired Student's t-test; *p <0 .05, **p < 0.01 and ***p < 0.001.

Figure 2. *In vivo* blockade of TSLP before allergen sensitization in severe asthma mouse model ameliorate lung inflammation and reduce the total numbers of BAL infiltrating inflammatory cells.

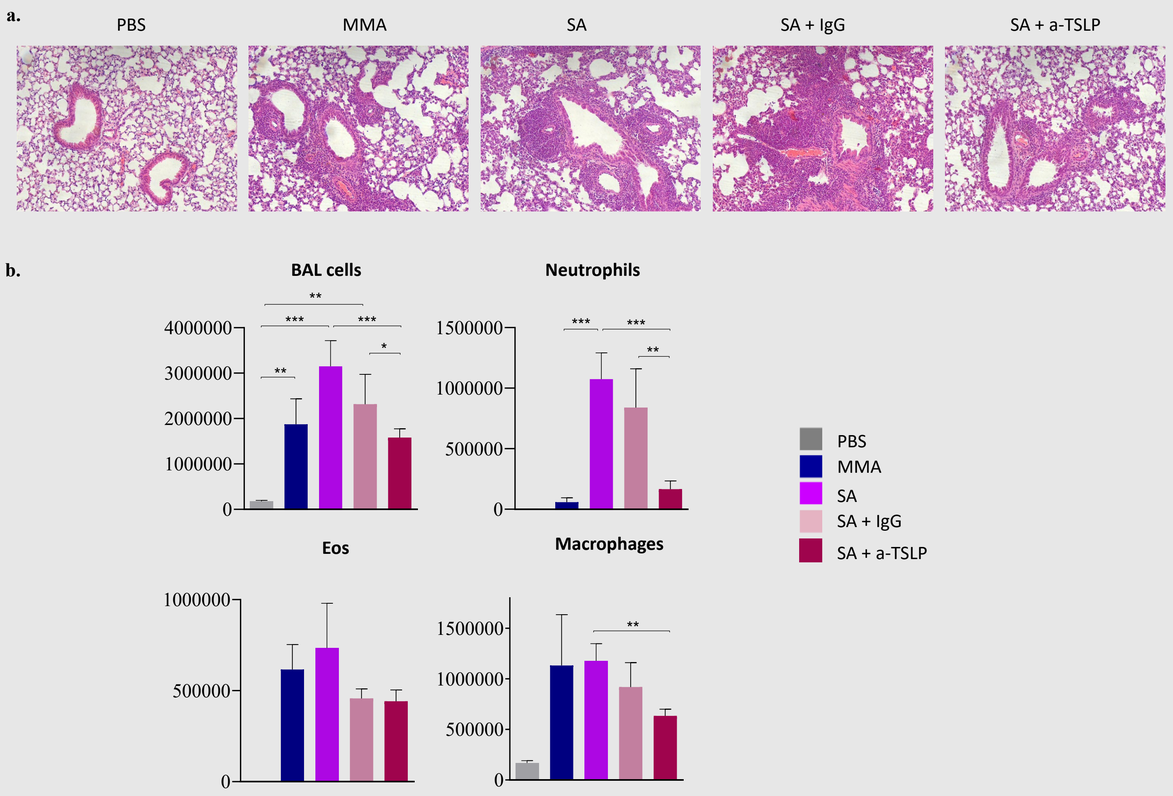


Figure 2. **a.** Representative photomicrographs of H&E-stained sections of PBS, MMA, SA, SA+IgG and SA+a-TSLP mice. **b.** BAL differentials from PBS, MMA, SA, SA+IgG and SA+a-TSLP mice (n=7-9). Statistical significance was obtained by unpaired Student's t-test; *p < 0.05, **p <0 .01 and ***p < 0.001.

Figure 3. *In vivo* blockade of TSLP before allergen sensitization in severe asthma mouse model decrease inflammatory cytokine release in lung homogenates and in BAL.

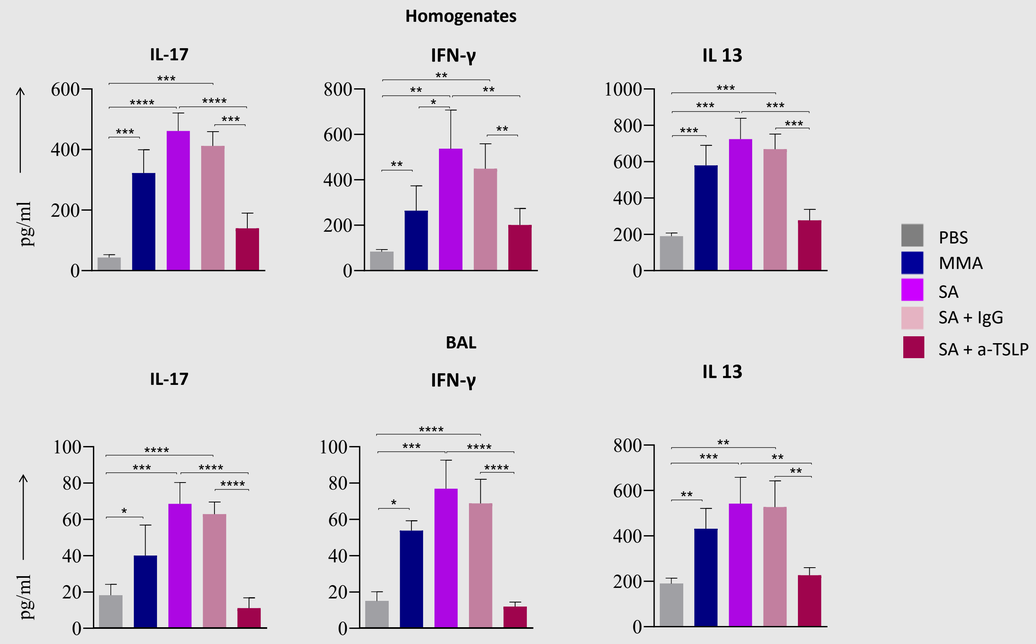


Figure 3. IL-17, IFN-γ and IL-13 expression levels in homogenates (upper panel) and BAL fluid (lower panel) of PBS, MMA, SA, SA+IgG and SA+a-TSLP mice (n=7-9). Statistical significance was obtained by unpaired Student's t-test; *p < 0.05, **p <0 .01 ***p < 0.001 and ****p < 0.0001.

Conclusions

(a) TSLP expression levels are higher in the serum of severe asthmatic patients compared to mild moderate asthmatic and healthy controls.

(b) Administration of **anti-TSLP *in vivo*** before allergen sensitization significantly decreased the levels of peribronchial and perivascular inflammation, mucus production by goblet cells as well as total numbers of BAL infiltrating inflammatory cells and especially neutrophils.

(c) Also we observed decreased levels of IL-17, IFN-γ and IL-13 in the serum, BALF and lung homogenates of SA mice that received anti-TSLP compared to SA and to a greater extend MMA mice.

Our data reveal that inhibition of TSLP before allergen sensitization in a well-established murine model of SA restrains pulmonary inflammation and attenuates key asthma features. Our studies may pave the way for delineating the molecular mechanisms through which TSPL orchestrates non type inflammatory responses that prevail in SA.