

Mast cell regulation of epithelial TSLP expression plays an important role in the development of allergic rhinitis

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Epithelial cell-derived thymic stromal lymphopoietin (TSLP) is a master switch for asthma or atopic dermatitis by inducing a dendritic cell-mediated Th2-type allergic inflammation. Allergic rhinitis is also pathologically characterized by Th2-type allergic inflammation. This study demonstrates that mast cells regulate the epithelial TSLP expression in allergic rhinitis. TSLP expression was found to be up-regulated predominantly in the nasal epithelium in the ovalbumin (OVA)-sensitized and -nasally challenged mouse model of allergic rhinitis, which was abolished in mast cell-deficient WBB6F1-W/W^v in comparison with control WBB6F1-+/+ mice. Similarly, the epithelial TSLP expression was reduced in Fc receptor γ chain (Fc γ R)-deficient mice, where the high-affinity IgE receptor (Fc ϵ RI) is not expressed on mast cells, in comparison with control C57BL/6 mice. Furthermore, the administration of neutralizing TSLP antibody during the challenge phase of OVA inhibited the development of allergic rhinitis. These results suggest that the direct stimulation of epithelial cells by antigens alone may not be sufficient to induce TSLP expression in the nasal epithelium, and that mast cell regulation of epithelial TSLP expression, possibly via Fc ϵ RI, plays an important role in the development of allergic rhinitis.

Key words: Allergic rhinitis · Epithelial cells · Mast cells · TSLP

Introduction

Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine, which binds to a TSLP receptor (TSLPR) consisting of the IL-7 receptor α -chain (IL-7R α) and a common γ receptor-like chain (TSLPR- γ) [1, 2]. TSLP was originally identified as a factor derived from a thymic stromal cell line that could support the growth of a mouse B cell line [3]. However, recently, TSLP, derived from epithelial cells, has been shown to be capable of activating CD11c⁺ myeloid DC to up-regulate costimulatory molecules, leading to the differentiation of CD4⁺ T cells into Th2 cells. Therefore, it plays a

key role in the development of allergic diseases such as asthma or atopic dermatitis [1, 2, 4]. For instance, transgenic mice expressing TSLP in keratinocytes or in lung epithelial cells have been shown to develop atopic dermatitis- or asthma-like inflammation in the skin or the lung, respectively, while TSLPR null mice failed to develop an inflammatory lung response to inhaled antigen [5–7]. In humans, TSLP has been shown to be expressed by keratinocytes in atopic dermatitis and by bronchial epithelial cells in asthmatic airways [8, 9].

In contrast to the pro-allergic action of TSLP, regulation of TSLP expression in epithelial cells has not been fully elucidated. Previous studies suggest that TSLP expression in mouse keratinocytes is regulated by vitamin D and retinoic acid signaling [10]. Most recently, TNF- α , IL-1 β , and ligands for Toll-like receptors

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such as LPS were shown to up-regulate TSLP expression via NF- κ B pathway in cultured human bronchial epithelial cells and keratinocytes [11–13].

Mast cells play a critical role in allergic inflammation including asthma and allergic rhinitis via multiple mechanisms [14–16]. Mast cells in patients with allergic rhinitis and asthma produce Th2-type cytokines, induce IgE synthesis in B cells, and can autoactivate themselves via the mast cell-IgE-high affinity receptor (Fc ϵ RI) cascade [17]. In addition, mast cells up-regulate the production of a variety of cytokines/chemokines in epithelial cells and fibroblasts, and induce the recruitment of basophils, T cells, and eosinophils into sites of allergic inflammation as well as their own intraepithelial accumulation. However, it remains unclear whether mast cells contribute to the epithelial TSLP expression at the site of allergic inflammation.

In this study, a direct assessment of the roles of mast cells on epithelial TSLP expression was conducted using a mouse model of allergic rhinitis and two types of genetically mast cell-deficient mice (WBB6F1-*Kit*^{W/W^v mice and C57BL/6-*Kit*^{W-sh/W-sh} mice).}

Results and discussion

TSLP expression in nasal epithelium requires mast cells and Fc γ R in a mouse model of allergic rhinitis

WBB6F1-^{+/+} mice with a mutation of c-kit are deficient for mast cells [18] and Fc γ R-deficient mice showed no expression of FcR (Fc γ RI, Fc γ RIII, and Fc ϵ RI) [19] because the expression of FcR requires a homodimer of the subunit Fc γ R for surface expression and signal transduction in the mouse [20]. C57BL/6 and WBB6F1-^{+/+} mice challenged with OVA showed an infiltration of leukocytes in the nasal submucosa, whereas WBB6F1-*W/W^v* mice and Fc γ R-deficient mice challenged with OVA did not show any infiltration of leukocytes into the nasal submucosa, as previously described [21, 22] (data not shown). Immunohistochemical staining with anti-TSLP antibody revealed little positive immunoreactivity of TSLP in the nasal mucosa obtained from the PBS-challenged C57BL/6 mice, but the positive immunoreactivity of TSLP increased predominantly in the nasal epithelium after the induction of allergic rhinitis in C57BL/6 and WBB6F1-^{+/+} mice (Fig. 1 and 2). In contrast, the increase of TSLP-positive cells in the nasal epithelium after the induction of allergic rhinitis was absent in WBB6F1-*W/W^v* mice and Fc γ R-deficient mice (Fig. 2). Immunohistochemical staining with control IgG antibody showed negative staining in the nasal mucosa after the induction of allergic rhinitis in C57BL/6 mice (Fig. 1). Therefore, TSLP expression is induced in the nasal epithelium in a mouse model of allergic rhinitis and the presence of mast cells and Fc γ R is necessary for the allergen-induced epithelial TSLP expression.

To further clarify the roles of mast cells and Fc γ R in mast cells in epithelial TSLP expression in a mouse model of allergic rhinitis, mast cell-deficient C57BL/6-*Kit*^{W-sh/W-sh} mice [23, 24] were reconstituted with BM-derived mast cells (BMMC) derived from wild-type C57BL/6 mice or Fc γ R-deficient mice with C57BL/6

genetic background. As shown in Fig. 2, an increase in the number of TSLP-positive cells in the nasal epithelium after the induction of allergic rhinitis was observed in the C57BL/6-*Kit*^{W-sh/W-sh} mice reconstituted with wild-type BMMC, but not with Fc γ R-deficient BMMC. Therefore, mast cells and Fc γ R in mast cells plays a critical role in the epithelial TSLP expression in a mouse model of allergic rhinitis.

TSLP is critical for the development of allergic rhinitis

To determine whether TSLP, expressed in the nasal epithelium, plays a critical role for the development of the mouse model of allergic rhinitis, the effects of TSLP neutralizing antibody on the development of allergic rhinitis were clinically and pathologically evaluated by periodic acid-Schiff (PAS) staining to demonstrate goblet cells.

C57BL/6 mice treated with control IgG antibody during the challenge phase with OVA showed a significant increase in the nasal rub counts (Fig. 3). The mice also showed an infiltration of leukocytes in the nasal submucosa, and the number of PAS-positive cells in the nasal epithelium (goblet cells) and submucosa (gland cells) also increased in these mice (Fig. 3 and data not shown). In contrast, the mice treated with anti-TSLP neutralizing antibody showed a significant reduction in the frequency of nasal rubs with little infiltration of leukocytes in the nasal submucosa, and a decreased number of PAS-positive cells in the nasal epithelium and submucosa (Fig. 3 and data not shown). Interestingly, immunohistochemical staining revealed the TSLP levels in the nasal epithelium to be relatively up-regulated after treatment with anti-TSLP antibody (Fig. 3). To exclude the possibility that a trivial deposition of anti-TSLP antibody on the tissue may affect the TSLP staining, the nasal mucosa specimens obtained from C57BL/6 mice treated with anti-TSLP antibody during PBS challenge were stained with anti-TSLP antibody and only slight immunoreactivity for TSLP was confirmed in these tissue specimens (data not shown). These results indicated that the blockade of TSLP activity inhibited the development of the mouse model of allergic rhinitis.

The current results suggest that the direct stimulation of epithelial cells by antigens alone may not be sufficient to induce TSLP expression in the nasal epithelium because the deficiency of mast cells and Fc γ R in mast cells abolished epithelial TSLP expression regardless of the stimulation with the antigen (OVA) on the nose. This is consistent with recent *in vitro* studies, which suggest that TSLP expression in epithelial cells requires proinflammatory cytokines such as TNF- α [11–13]. Therefore, it is possible that mast cell-derived inflammatory cytokines including TNF- α generated at the site of allergic rhinitis may play an important role for the induction of TSLP expression in the nasal epithelium. Because the mast cell release of TNF- α or other proinflammatory cytokines can occur immediately following the antigen stimulation in the nose of patients with allergic rhinitis [25] and Fc γ R was also shown to be critical for the epithelial TSLP expression (Fig. 2), we therefore speculate that mast cells (possibly

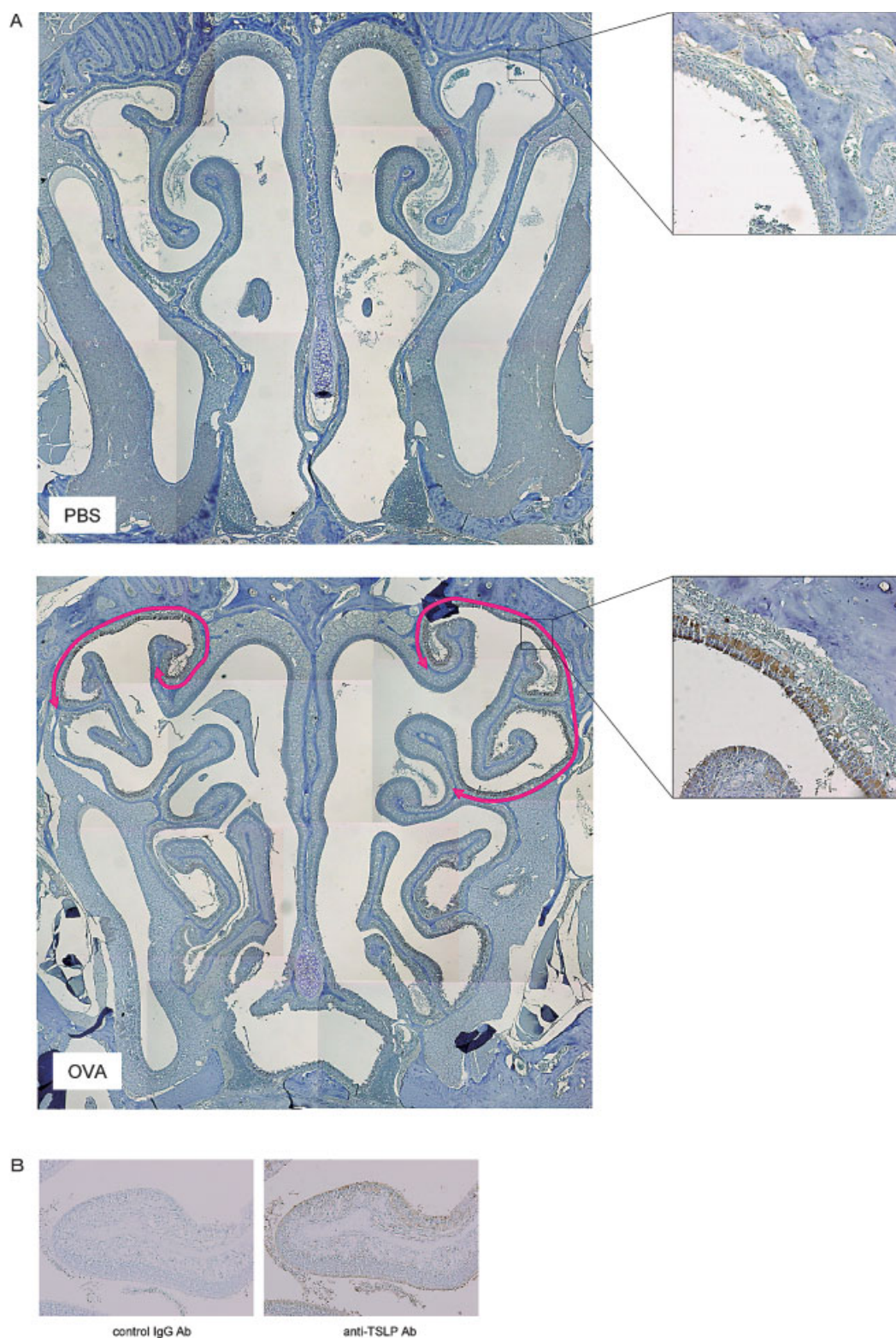


Figure 1. Repeated nasal exposure to OVA in OVA-sensitized C57BL/6 mice induces TSLP expression in the nasal epithelium. (A) Representative pictures of TSLP expression in the nasal mucosa of OVA-sensitized C57BL/6 mice repeatedly challenged with OVA (OVA) or control PBS (PBS) are shown. The curved lines indicate positive staining area. (B) Representative pictures of the nasal mucosa specimens stained with control IgG antibody (control Ab) or anti-TSLP antibody (anti-TSLP Ab) after the induction of allergic rhinitis.

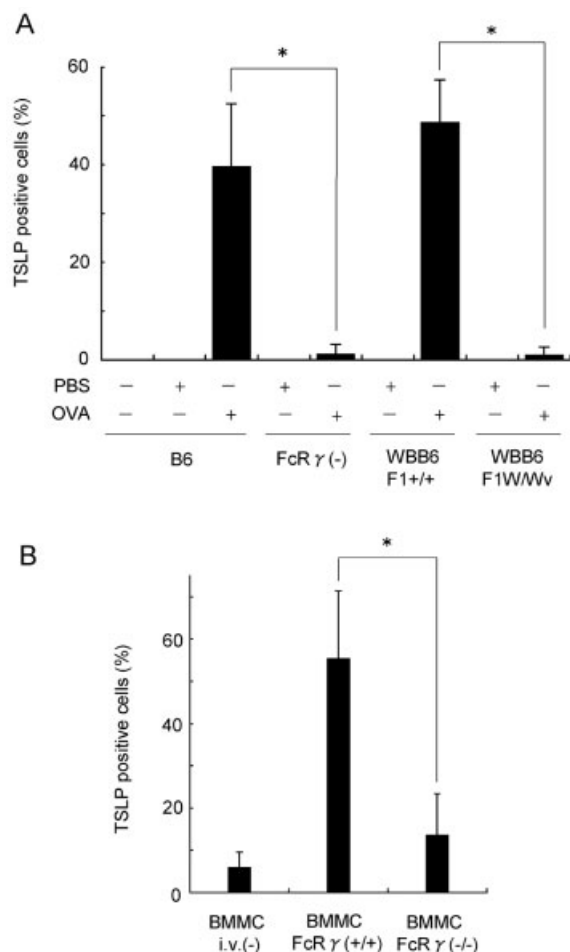


Figure 2. Deficiency of mast cells and Fc γ R abolishes TSLP expression in the nasal epithelium during allergic rhinitis. (A) A quantitative analysis of TSLP expression in the nasal epithelium during allergic rhinitis using C57BL/6, Fc γ R-deficient, WBB6F1-+/+, WBB6F1-W/W^v mice as described in the text. (B) A quantitative analysis of TSLP expression in the nasal epithelium during allergic rhinitis using C57BL/6-KitW-sh/W-sh mice reconstituted with wild-type BMMC (BMMC Fc γ R(+/+)) or with Fc γ R-deficient BMMC (BMMC Fc γ R(-/-)), as described in the text. BMMC i.v.(-): C57BL/6-KitW-sh/W-sh mice without the transfer of BMMC. Values represent the mean + SD of eight mice in each group. **p* < 0.05.

via Fc ϵ RI-mediated release of TNF- α) might play a critical role in the initial phase of allergic rhinitis through the induction of epithelial TSLP expression.

Conversely, TSLP has been shown to stimulate the production of high levels of Th2 cytokines by human mast cells synergistically with IL-1 and TNF- α [12]. Therefore, it is possible that there is an important interaction between epithelial cells and mast cells via TSLP and proinflammatory cytokines for the development of allergic rhinitis. Mast cells may induce TSLP expression in the nasal epithelium following the allergen challenge, and then epithelial-derived TSLP could stimulate mast cells to produce Th2 cytokines to further augment allergic inflammation.

Treatment of the mice with anti-TSLP neutralizing antibody inhibited the development of allergic rhinitis (Fig. 3). These results

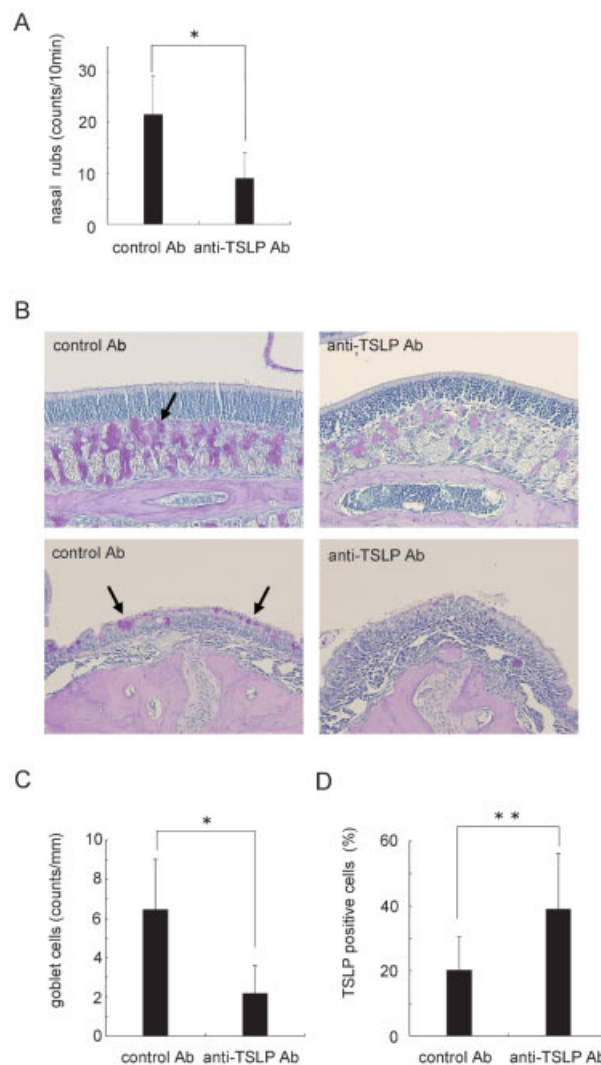


Figure 3. Anti-TSLP neutralizing antibody inhibits the development of allergic rhinitis. The OVA-sensitized mice were intranasally challenged with 100 μ g OVA in 10 μ L PBS twice per day for 1 week (total 14 times/week). The mice were challenged intranasally with PBS in a similar manner for the negative control. During the OVA challenge, rat anti-mouse TSLP neutralizing antibody or the isotype control rat IgG2a (15 mg/kg per mouse) was administered intraperitoneally every other day. (A) Appearance of nasal symptom (nasal rubs). The frequency of nasal rubs was counted for 10 min after the last intranasal challenge with OVA. Control Ab: mice treated with control IgG antibody, anti-TSLP Ab: mice treated with anti-TSLP antibody. (B) Representative PAS staining of the nasal mucosa obtained from C57BL/6 mice treated with anti-TSLP antibody (anti-TSLP Ab) or control IgG (control Ab). Upper panels: submucosa; lower panels: epithelial area. Note the decreased number of PAS-positive cells in the nasal submucosa and epithelium. The arrows indicate the PAS-positive cells. (C) A quantitative analysis of the epithelial PAS-positive cells. (D) A quantitative analysis of the epithelial TSLP-positive cells. Values represent the mean + SD of eight mice in each group. Both * and ** represent *p* < 0.05.

are consistent with the previous *in vivo* studies using mouse models of asthma and atopic dermatitis [5–7]. Thus, the current results also support the importance of TSLP in allergic inflammation. In addition, the findings that treatment with anti-TSLP neutralizing antibody up-regulated TSLP expression in the nasal epithelium (Fig. 3) may suggest the presence of negative feedback regulation in the epithelial TSLP expression.

Concluding remarks

In summary, this study provides significant evidence that mast cells play a critical role in TSLP expression in the nasal epithelium in allergic rhinitis and subsequent development of the disease. FcεRI, expressed on mast cells, is likely to be involved in the epithelial TSLP expression. To our knowledge, this is the first study suggesting TSLP to play a potential role in allergic rhinitis.

Materials and methods

Mice

Female 4–6 wk C57BL/6 mice, WBB6F1-+/+, and WBB6F1-W/W^v mice [18] were purchased from Japan SLC (Tokyo, Japan), FcγR-chain deficient mice (C57BL/6 background) and mast cell-deficient KitW-sh/W-sh mice on the C57BL/6 background were previously described [19, 23, 24], and were bred under specific pathogen-free conditions.

Allergic rhinitis model

An allergic rhinitis model was established as previously described with some modifications [26]. Briefly, the mice were actively immunized i.p. with 10 μg OVA (Sigma Aldrich, St. Louis, MO) in 4 mg aluminum hydroxide on days 1 and 7. Starting on day 14, they were challenged intranasally with 100 μg OVA in 10 μL PBS twice per day for 1 week (total 14 times/week). The mice were challenged intranasally with PBS in a similar manner for the negative control. For some experiments, rat anti-mouse TSLP neutralizing antibody or control rat IgG2a (15 mg/kg per mouse, R&D Inc., Minneapolis, MN) were administered intraperitoneally every other day, starting on day 14 until sacrifice. The dosage of the antibody (15 mg/kg) was based on previous experiments [27]. The animal experiments were approved by the Institutional Review Board of the University of Yamanashi.

Histological examination

At 12 h after the final nasal challenge, mice were killed with carbon dioxide. The heads were removed, fixed, and decalcified. Coronal nasal sections were visualized by staining with hema-

toxylin and eosin (H&E) or PAS/hematoxylin (to demonstrate goblet cells). For immunohistochemistry, the coronal nasal sections were deparaffinized and stained with anti-TSLP antibody or control IgG antibody (Santa Cruz Biotechnology Inc.) through the use of peroxidase-based Vectastain ABC kits with DAB substrate (Vector Laboratories, Burlingame, CA).

Nasal symptom

Nasal rubs were observed for 10 min after the last intranasal challenge using a video recorder and the frequency of the nasal rubs were counted by investigators who were blind to the treatment protocol.

Assessment and quantification of histological examination

The length of the positively TSLP-stained epithelial layer in the total nasal epithelial lining of the coronal sections was measured and expressed as percentage of the total length of the total nasal epithelial lining of the coronal sections (% of the total length). The number of PAS-positive cells in the total nasal epithelial lining of the coronal sections was counted microscopically in a blinded manner and it was expressed as the number per the total length of the total nasal epithelial lining of the coronal sections (number/mm). Two or four specimens of the TSLP- or PAS-stained coronal sections from one mouse were selected. The mean score was counted, and then the final mean scores were calculated from eight animals.

Reconstitution of KitW-sh/W-sh mice with BMMC

BMMC were generated from the femoral BM cells of mice and maintained in the presence of 10% pokeweed mitogen-stimulated spleen-conditioned medium as a source of mast cell growth factors as previously described [28]. Mast cell deficiency of KitW-sh/W-sh was reconstituted by the intravenous injection, 4 weeks after starting the culture, of 2×10^6 BMMC derived from C57BL/6 or FcγR-chain deficient mice, as previously described [23, 24]. The mice were used for experiments 5 wk after the injection of BMMC. The reconstitution of the mast cells was confirmed by toluidine blue staining of the nasal mucosa.

Data analysis

Data are represented as the mean + SD. The statistical analysis was performed by unpaired Student's *t*-test. A value of $p < 0.05$ was considered to be significant.

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Conflict of interest: The authors declare no financial or commercial conflict of interest.

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Abbreviations: BMMC: bone marrow-derived mast cells · Fc ϵ R: Fc receptor ϵ chain · Fc γ R: Fc receptor γ chain · PAS: periodic acid-Schiff · TSLP: thymic stromal lymphopoietin

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