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Brief Report

Low IL-13Rα1 expression on mast cells tunes them unresponsive to IL-13

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Abstract

Cytokine-mediated mast cell regulation enables precise optimization of their own proinflammatory cytokine production. During allergic inflammation, interleukin (IL)-4 regulates mast cell functions, tissue homing, and proliferation, but the direct role of closely related IL-13 for mast cell activation remains unclear. Previous work has shown that mast cells are potent IL-13 producers, but here we show that mouse mast cells do not directly respond to IL-13 by Stat6 activation, as they do not express measurable amount of IL-13 receptor α1 (IL-4Rα1) messenger RNA. Consequently, IL-4 responses are mediated via type I IL-4R (IL-4/IL4Rα/γC), and IL-4–induced Stat6 activation is abolished in γC-deficient mast cells. Type II IL-4R deficiency (IL-13Rα1 knockout) has no effect on IL-4–induced Stat6 activation. In basophils, both IL-4 and IL-13 induce Stat6 activation in wild-type and γC-deficient cells, while in type II IL-4R–deficient basophils, IL-4 signaling is impaired at low ligand concentration. Thus, mast cell and basophil sensitivity to IL-4/IL-13 is different, and in mast cells, lack of IL-13Rα1 expression likely explains their unresponsiveness to IL-13.

Keywords: allergy, basophil, cytokine, IgE, IL-13, IL-13R1, interleukin (IL)-4, mast cell, papain, Stat6 phosphorylation.

1 Introduction

Excessive mast cell activation is the root cause of many clinical manifestations observed in allergic inflammation. In antigen driven allergic inflammation, antigen-sensitization followed by re-exposure to the same antigen results in IgE-mediated mast cell activation. Pseudo-allergic reactions result in similar excessive mast cell activation, but importantly, without prior antigen sensitization. Mast cells may thus be activated by factors related to innate immune system (including TLRs [toll-like receptors] or MRGPR2 [Mas-related G protein-coupled receptor 2]) or by adaptive immune system (allergen-specific IgE crosslinking), while cytokines that regulate mast cell activation may derive from either of these immune responses.[1–4] IL-33 (e.g. in combination with Stat5 activating IL-3) can induce IL-6 and IL-13 production in murine peritoneal cavity mast cells (PMcs).[5,6] IL-13, in turn, can then either enhance allergic inflammation in tissues further,[7] or this mast cell–derived IL-13 can directly inhibit type 1 response (e.g. by decreasing IL-12 production in dendritic cells).[8]

IL-13 shares a functional receptor with IL-4 (Fig. 1A), a master regulator of CD4 T cell T helper 2 differentiation.[9] IL-4 plays a regulatory role in type 2 inflammation, while IL-13 is largely an effector cytokine, which is due to the differential expression of the receptor chains, their ligands, and differential binding energetics of IL-4 and IL-13 to their cognate receptor chains.[10,11] For mast cells, IL-4 was originally described as an important factor for proliferation and survival,[12] and IL-4 also regulates IgE expression and tissue homing.[13] While IL-4 clearly regulates mast cell biology, the role of IL-13 on mast cells is less clear.[14] However, it is of interest that in an immortalized human mast cell line (HMC-1), comparisons of IL-4 and IL-13 responsiveness have indicated that these cells do respond to IL-13.[15,16]

Here, we have studied direct response of murine mast cells and basophils to IL-4 and IL-13. We found that mouse mast cells express little or no IL-13Rα1 and thus IL-4 signaling is heavily dependent on type I IL-4R expression and mast cells respond poorly to IL-13. Basophils, on the other hand, respond to IL-13 and, quite logically, to IL-4, even in the absence of γC.

2 Results and discussion

2.1 Bone marrow–derived mast cell responses to IL-4 and IL-13

The genetic background of inbred mice may affect mast cell responses.[17] Therefore, we first analyzed Stat6 phosphorylation in bone marrow–derived mast cells (BMMCs) from B6 and BALB/c mice in response to IL-4 and IL-13.[18] Both BMMCs were sensitive to IL-4 and 0.1 ng/mL of IL-4 gave practically maximal response, but overall Stat6 phosphorylation as measured by mean fluorescence intensity was higher in B6 BMMCs (*P* values for B6 vs BALB/c at concentrations from 0.01 to 10 ng/mL were all <0.05) (*P* values provided in Supplementary Table 1). Most strikingly, 100 ng/mL of IL-13 did not induce measurable Stat6 phosphorylation in BMMCs from either background (Supplementary Fig. 1A). IL-13 preparate used in these experiments clearly induced

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pStat6 in simultaneously cultured BM-derived macrophages (Supplementary Fig. 1B), verifying the quality of IL-13 used.

Of the 2 IL-4 receptors, IL-4 signals via type I and type II IL-4 receptors (IL-4Rs), while IL-13 only signals via type II IL-4Rs (Fig. 1A). Although the functional role of IL-13Rα2 as a signal transducing receptor is still somewhat unclear, at least Stat6 is not activated by IL-13Rα2,[19] and for the remainder of this report we focus on IL-13 signaling via type II IL-4R. We first studied if IL-4 response in type I IL-4R–deficient mast cells was affected. Indeed, γC-deficient BMMCs did not show any response to IL-4, even at

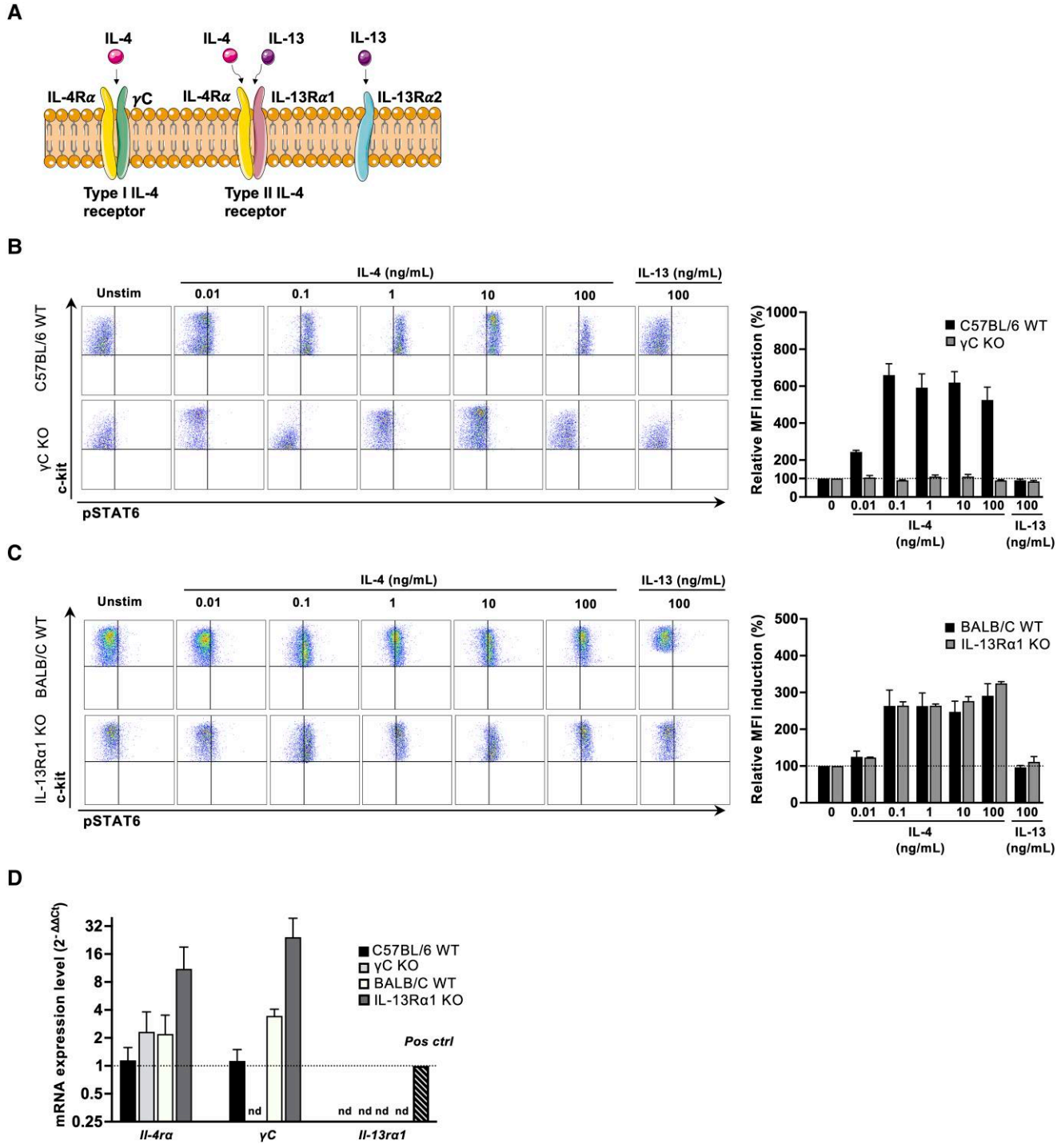

Fig. 1. Mouse BMMC signaling response to IL-4 and IL-13 and mRNA expression of IL-4 receptor components in BMMCs. (A) Organization of IL-4R and IL-13R. (B) B6 WT and γC KO or (C) BALB/c WT and IL-13Rα1 KO BMMCs were left unstimulated or stimulated with IL-4 or IL-13 as indicated for 15 min. Y641 phosphorylation of Stat6 in c-Kit/FcεRI double-positive live cells was measured by flow cytometry. Three independent experiments of BMMCs from different mice in each experiment were performed. (Right) Percent induction of pStat6 mean fluorescence intensity normalized to unstimulated sample in each experiment; mean and SEM are indicated. (D) mRNA expression of indicated IL-4R chains on day 30–60 BMMCs. Cells cultured from 3 different mice per mouse line were used; c-Kit/FcεRI positivity of cells was 95% to 99%. A positive mRNA control for IL-13Rα1 in this experiment was pooled BALB/c skin and spleen samples, treated for 2 wk with DMBA/TPA. Scale indicates *ribosomal 18S* normalized cycle number of log phase of qPCR reaction (2^−ΔΔCt method).

100 ng/mL concentration, and as in wild-type (WT) B6 BMMCs, no IL-13 response was observed (Fig. 1B), suggesting that no compensatory expression of type II IL-4R in type I IL-4R–deficient cells occurred. In contrast to γC knockout (KO) mast cells, IL-4 response in BMMCs from type II IL-4R–deficient (i.e. IL-13Rα1 KO) mice showed no defects in IL-4 signaling (Fig. 1C). All *P* values for comparing pSTAT responses are shown in Supplementary Table 1. BMMCs from γC and IL-13Rα1 KO mice developed normally and showed comparable c-Kit/FcεRI expression to WT BMMCs (Supplementary Fig. 1C). Thus, in line with type I IL-4R–dominant functional IL-4 responses in mast cells observed previously,[13] deletion of γC in BMMCs abolished IL-4 responsiveness.

One explanation for these signaling differences could be the expression of critical receptor for both IL-4 and IL-13: IL-13Rα1. For this, messenger RNA (mRNA) of *Il-4rα*, *γC*, and *Il-13rα1* in BMMCs was measured. The purity of BMMCs is shown in Supplementary Fig. 1C. The results shown in Fig. 1D indicate that *Il-13rα1* mRNA expression was not detected by quantitative polymerase chain reaction (qPCR) in any mast cell types studied, while other IL-4R receptor chains were readily expressed. A positive mRNA control for IL-13Rα1 in these experiments was pooled BALB/c skin and spleen samples, treated for 2 wk with DMBA/TPA.[20] Unfortunately, we were unable to measure mouse IL-13Rα1 protein by flow cytometry, as commercial antibodies showed insufficient specificity that was observed particularly when IL-13Rα1 KO cells were used as a negative control.

2.2 PMc responses to IL-4 and IL-13

To study mast cell IL-4/IL-13 responses in more physiological setting, we compared maximal IL-4 and IL-13 concentrations (100 ng/mL) in PMcs (PMc gating) (Supplementary Fig. 2A). In B6 and BALB/c PMcs, IL-4 resulted in clear induction of pStat6, while IL-13 induced pStat6 minimally (Fig. 2A and B). However, the *P* values for difference of IL-13 compared with unstimulated in neither B6 nor BALB/c were not significant (*P* values shown in Supplementary Table 1). In γC-deficient PMcs, both IL-4 and IL-13 (100 ng/mL each) caused slight and measurable but not statistically significant pStat6 that appeared biphasic (Fig. 2A and *P* values in Supplementary Table 1), suggesting that PMcs or a subpopulation of PMcs might have compensatory upregulation of *Il-13rα1* that may have occurred after BM development, as γC KO BMMCs did not show any response to IL-13 (Fig. 1B). It is also possible that this approximately 10% population (i.e. not present in BM-derived cultures) represents a contaminating c-Kit–positive cells (e.g. dendritic cells that become enriched in γC KO mice).[21] As said, it may also represent a currently uncharacterized subpopulation of PMcs that may be unproportionally present in γC KO mice. IL-4 responses in IL-13Rα1–deficient PMcs were slightly but not statistically significantly reduced as compared with WT control samples, while no IL-13 response was observed (Fig. 2B and *P* values in Supplementary Table 1).

2.3 Effect of mast cell activation to IL-13 responsiveness and IL-13R expression

In human lung mast cells, evidence of *Il-13rα1* mRNA and responsiveness to IL-13 was observed.[22] In fact, this IL-13 response was not evident after short-time IL-13 stimulation but appeared in a 5-day stimulation of the cells with IL-13 itself, hinting that IL-13Rα1 is not consistently expressed on cell surface, but rather

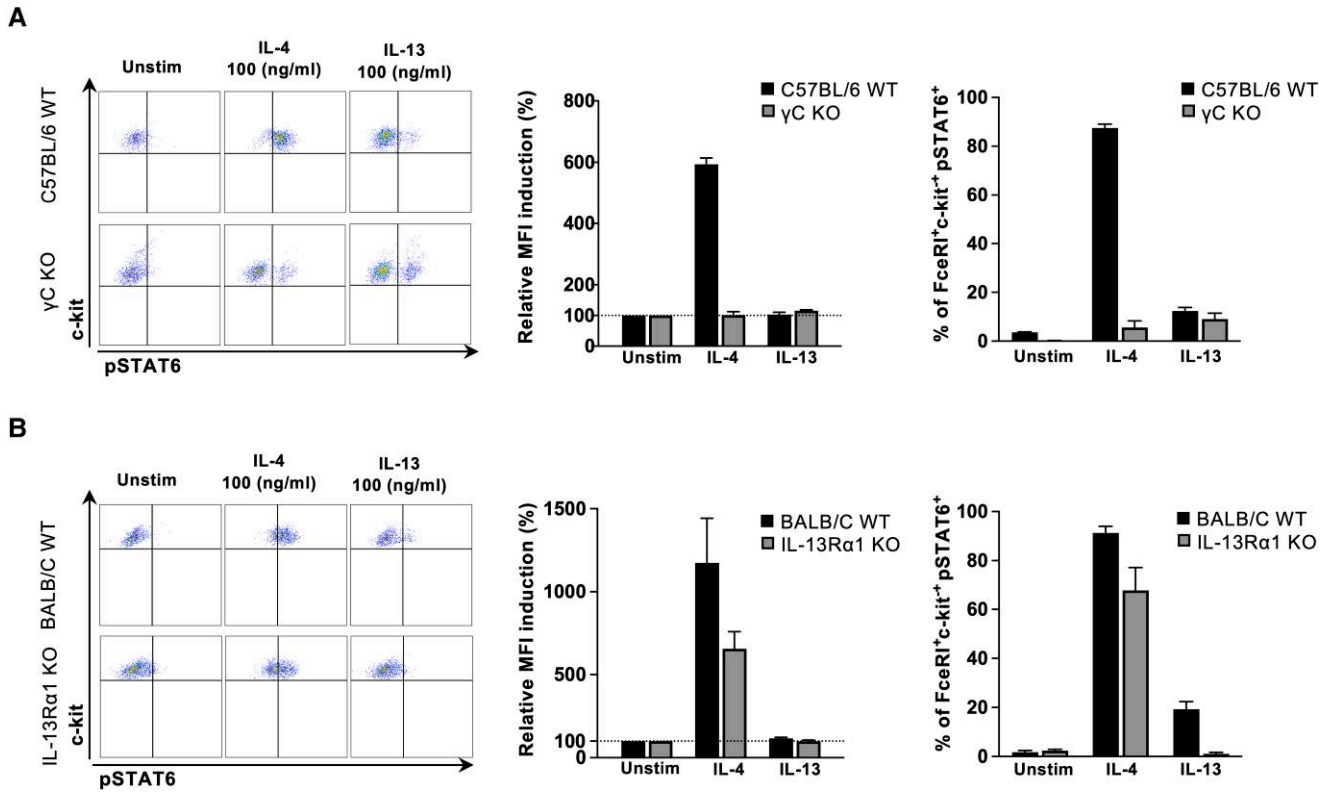

Fig. 2. Mouse PMc signaling response to IL-4 and IL-13. Peritoneal flushes from (A) B6 WT and γC KO or (B) BALB/c WT and IL-13Rα1 KO mice were left unstimulated or stimulated with 100 ng/mL of IL-4 or IL-13 for 15 min, followed by pStat6 analysis of c-Kit/FcεRI double-positive cells as in Fig. 1B. Three independent experiments were performed with pooled peritoneal flushes (n = 3 to 5 mice per experiment). (Middle) Percent induction of pStat6 mean fluorescence intensity normalized to unstimulated sample in each experiment; (right) percent pStat6-positive cells of c-Kit/FcεRI double-positive cells; mean and SEM are indicated.

is under a positive feedback loop by its ligand. In line with this, 24-h treatment of mouse BMMCs with IL-13 did not induce FcεRI expression, while IL-4 did,[13] though this could also be explained by other signaling differences such as differential IRS2 induction by IL-4 and IL-13[23] that might eventually regulate FcεRI expression differentially.

To learn if IL-13Rα1 expression might be upregulated by mast cell activation, we initially measured IL-13Rα1 in BMMCs after in vitro activation. WT and type I and type II IL-4R KO BMMCs were activated with a known IL-13 inducer in mast cells, namely IL-33. A total of 50 ng/mL of IL-33 was used to stimulate the cells for 48 h.[5,6] Because a 5-day stimulation of human mast cells with IL-13 induces IL-13 responsiveness,[22] we also stimulated murine BMMCs with IL-13 (20 ng/mL) for 5 days. We then analyzed either *Il-13rα1* mRNA or IL-4–or IL-13–induced pStat6. IL-13 or IL-33 did not induce expression of *Il-13rα1* mRNA, while the expression of *Il-4rα* and *γC* was clearly observed (Supplementary Fig. 2B). In response to IL-33, no effect on IL-13–induced pStat6 was observed (Fig. 3A and *P* values in Supplementary Table 1). For 5-day IL-13 stimulation, slight induction of basal pStat6 was observed only in B6 WT line (but the *P* value was not significant), which was somewhat confusing taking into account negative qPCR (Supplementary Fig. 2B), but when these cells were further stimulated for 15 min with IL-4, the Stat6 activation in response to IL-13 was marginal as compared with IL-4: *P* values for B6 WT, BALB/c WT, and IL-13Rα1 KO were all <0.001 (Fig. 3B and *P* values in Supplementary Table 1).

To measure in vivo *Il-13rα1* mRNA expression in PMcs, type 2 inflammation was induced by papain immunization.[24] On day 21, we harvested the peritoneal cavities and measured *Il-13rα1* mRNA in FACS-purified PMcs (purity in Supplementary Fig. 2C). Functional type 2 response was verified by IgE levels in mouse serum (Fig. 3C). While type 2 inflammation was clearly induced (the *P* value for IgE between papain or phosphate-buffered saline [PBS]–treated mice was 0.0045), *Il-13rα1* mRNA was not detected in PMcs (Fig. 3D).

One future direction for broader mechanistical studies should clarify if expression of IL-13Rα1 in various setting is a mechanism by which IL-13–mediated mast cell activation is kept in check. For example, in systemic mastocytosis, single nucleotide polymorphisms in *Il-13* cytokine promoters are associated with the disease and higher levels of serum IL-13.[25,26] It will be fascinating to learn if IL-13Rα1 expression on mast cells in systemic mastocytosis is increased. Alternatively, repopulating various mast cell–deficient mice[27] with mast cells forced to express IL-13Rα1 will be very informative.

In the context of cancer, we and others have shown that IL-13/IL-13Rα1 signaling protects mice from papilloma formation in an experimental murine model of squamous cell carcinoma,[20,28] and mast cells have been considered as one possible cellular target of IL-13 in this model. Our current results suggest mast cells are unlikely the direct target of protective IL-13 signaling in this context, albeit the *Il-13rα1* mRNA level in mast cells in this model has not, to best of our knowledge, been studied.

2.4 Basophil responses to IL-4 and IL-13; importance of type II IL-4R for basophils

Finally, IL-4 and IL-13 signaling was studied in basophils. As opposed to the BMMCs, both IL-4 and IL-13 induced clear activation of pStat6 in BM–derived basophils (BMDBs) from both B6 and γC KO mice, albeit IL-4 induced signaling was reduced (*P* values for all cytokine concentrations <0.01) (Supplementary Table 1) but not completely abolished, as in γC KO BMMCs (compare Fig. 1B and 4A). IL-4 signaling in BMDBs from type II IL-4R–deficient mice was not impaired, indicating type I IL-4R–mediated signaling was able to compensate the lack of type II IL-4R deficiency. IL-13 signaling was completely abolished as expected (Fig. 4B and *P* values in Supplementary Table 1). These data indicate that IL-4–induced signaling in BMDBs is not dependent solely on availability of either type I or type II IL-4R. Similarly, in resident tissue basophils from spleens of B6 or BALB/c mice (Gating in Supplementary Fig. 2D), both IL-4 and IL-13 did induce potently pStat6, with BALB/c basophils being far more sensitive to IL-4 than B6 counterparts in a head-to-head comparison (Fig. 4C and *P* values in Supplementary Table 1). As in BMDBs, in IL-13Rα1–deficient splenic basophils IL-4 signaling via type I IL-4R compensated the lack of type II IL-4R signaling (Fig. 4C).

In food allergy, IL-4 is a critical driver of mast cell populating the intestine, based on our results, transfer of γC-deficient mast cells in similar setting could replicate the results obtained with IL-4Rα KO.[13] Our work thus underlines the critical role of type I IL-4R for mast cell IL-4 responses and provides the molecular basis of earlier observations supporting this. In short, our results indicate that 2 developmentally and functionally related myeloid cell types that are important regulators of allergic inflammation are surprisingly different in their capability to respond to IL-13, and if availability of γC becomes limited, they are also strikingly different in their responsiveness to IL-4.

3 Methods

3.1 Mice

IL-13Rα1 KO mice on BALB/c background was provided by Regeneron Pharmaceuticals Inc, and age- and sex-matched WT BALB/c mice were used as control animals. γC KO mice (Jax line #3174) were from the Jackson Laboratory, and age- and sex-matched WT C57BL/6 (Jax line #664) mice were used as control animals. All animal maintained at Tampere University Animal Facility, following all rules and instructions posed by the facility. All experiments were performed in accordance with protocols approved by the National Animal Ethics Committee of Finland under animal permits ESAVI/23659/2018 and ESAVI/971/2021.

3.2 Cell isolation and stimulation

BM from euthanized mice was collected purified as described previously[29] and cultured in RPMI (Gibco [Thermo Fisher Scientific]) complemented with 10% fetal bovine serum (Gibco [Thermo Fisher Scientific]), 1% L-glutamine, and 1% penicillin-streptomycin and 20 ng/mL of IL-3 (PeproTech) for 10 to 12 d for basophils or 30 to 60 d for mast cells or with 20 ng/mL of macrophage colony-stimulating factor (PeproTech) for 7 d for macrophages. For peritoneal cavity cells, peritoneal cavities were flushed twice with 7 mL PBS containing 2 mM ETDA (Sigma), and the cells were then centrifuged for 5 min 350 g at 4 °C to harvest all cells. Spleens were mashed on Petri dish in PBS containing 2 mM EDTA (Sigma) and 0.1% bovine serum albumin (BSA) (Sigma), filtered through a 40-μm strainer and then centrifuged for 5 min 350 *g* at 4 °C to harvest single cells. For splenic cells and BM, red blood cell lysis was performed with ACK lysing buffer (Gibco [Thermo Fisher Scientific]). The cells were left unstimulated or stimulated with IL-33 (50 ng/mL; PeproTech) for 2 d or IL-13 for 5 d (20 ng/mL; PeproTech) and followed by the further addition of IL-4 or IL-13 (both from PeproTech) as indicated for 15 min at

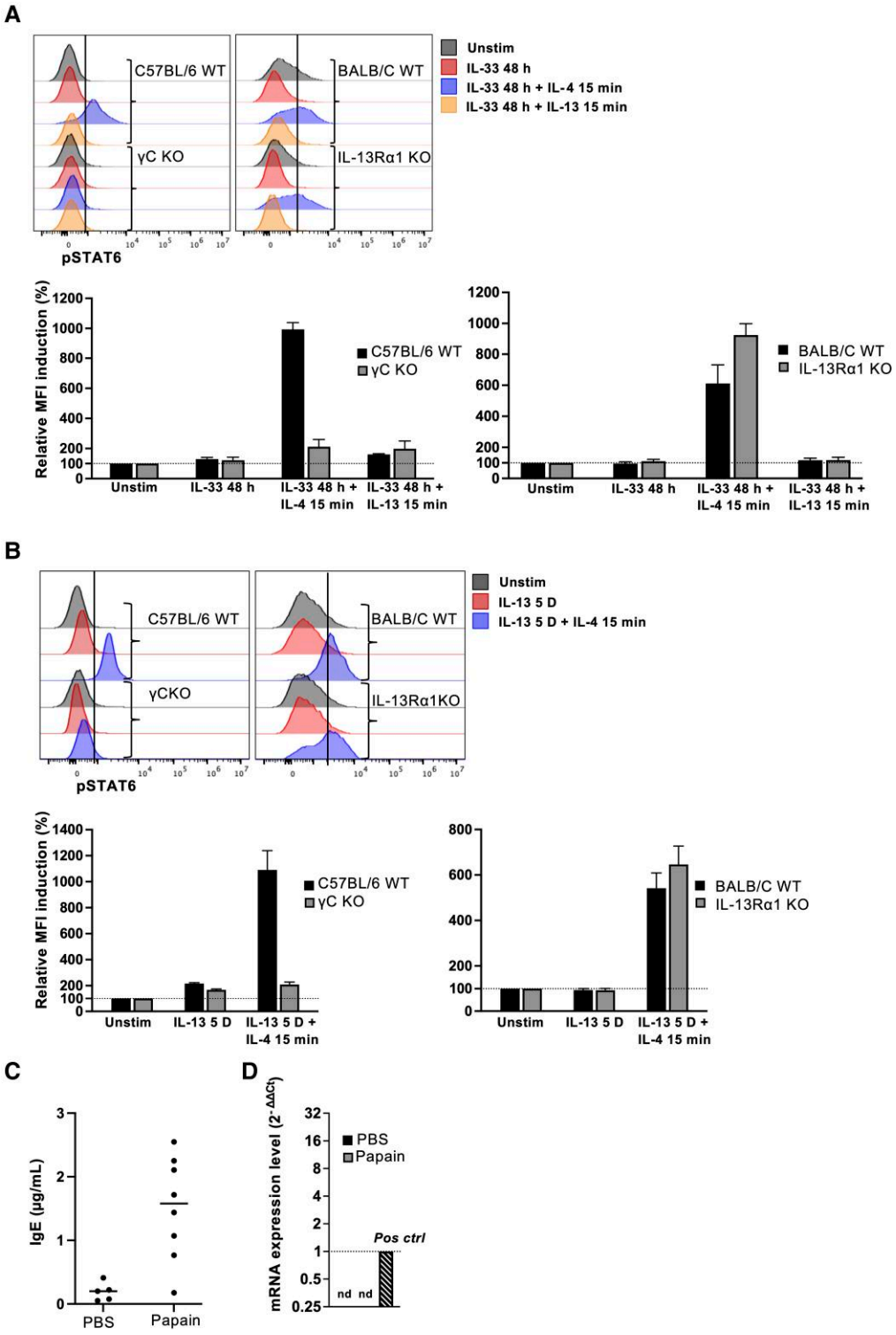

Fig. 3. Effect of in vitro activation or in vivo type 2 inflammation on IL-13Rα1 expression in mast cells. (A) BMMCs from indicated mice genotypes were either left in culture medium or stimulated with IL-33 for 2 d followed by 15 min stimulation either with IL-4 or IL-13. pStat6 was measured as in Fig. 1B. A total of 3 to 5 independent experiments with BMMCs from different individual mice in each experiment were performed. (Bottom) Percent induction of pStat6 mean fluorescence intensity normalized to the unstimulated sample in each experiment; mean and SEM from these experiments are indicated. (B) BMMCs from indicated mice were either left in culture media or stimulated for 5 d with IL-13. The cells were then either left unstimulated or stimulated with IL-4. The analysis was then performed as in Fig. 3A. (C) Papain-induced IgE levels in mouse serum. Mice were subjected to subcutaneous papain/PBS injections twice on day 0 and 14 and euthanized on day 21 (10 µg papain per mouse in 100 µL of PBS). Mice receiving 100 µL of PBS on days 0 and 14 served as control animals. After euthanasia, sera were collected and IgE levels were determined. The experiment was repeated twice using serum from PBS-treated (n = 2 to 3 per experiment) or papain-treated (n = 2 to 4 per experiment) animals. Medians are indicated by line. (D) B6 WT mice were subjected to 21-d papain immunization, and after euthanasia, the peritoneal cavity cells were collected and pooled (2 to 3 animals per pooled sample in both treatment groups). PMcs were purified by FACS, and qPCR for *Il-13rα1* was performed. A positive mRNA control for IL-13Rα1 in these experiments was pooled BALB/c skin and spleen samples, treated for 2 wk with DMBA/TPA. The experiment was repeated twice using pooled peritoneal flushes from PBS (n = 2 to 3 per experiment) or papain (n = 2 to 4 per experiment) groups.

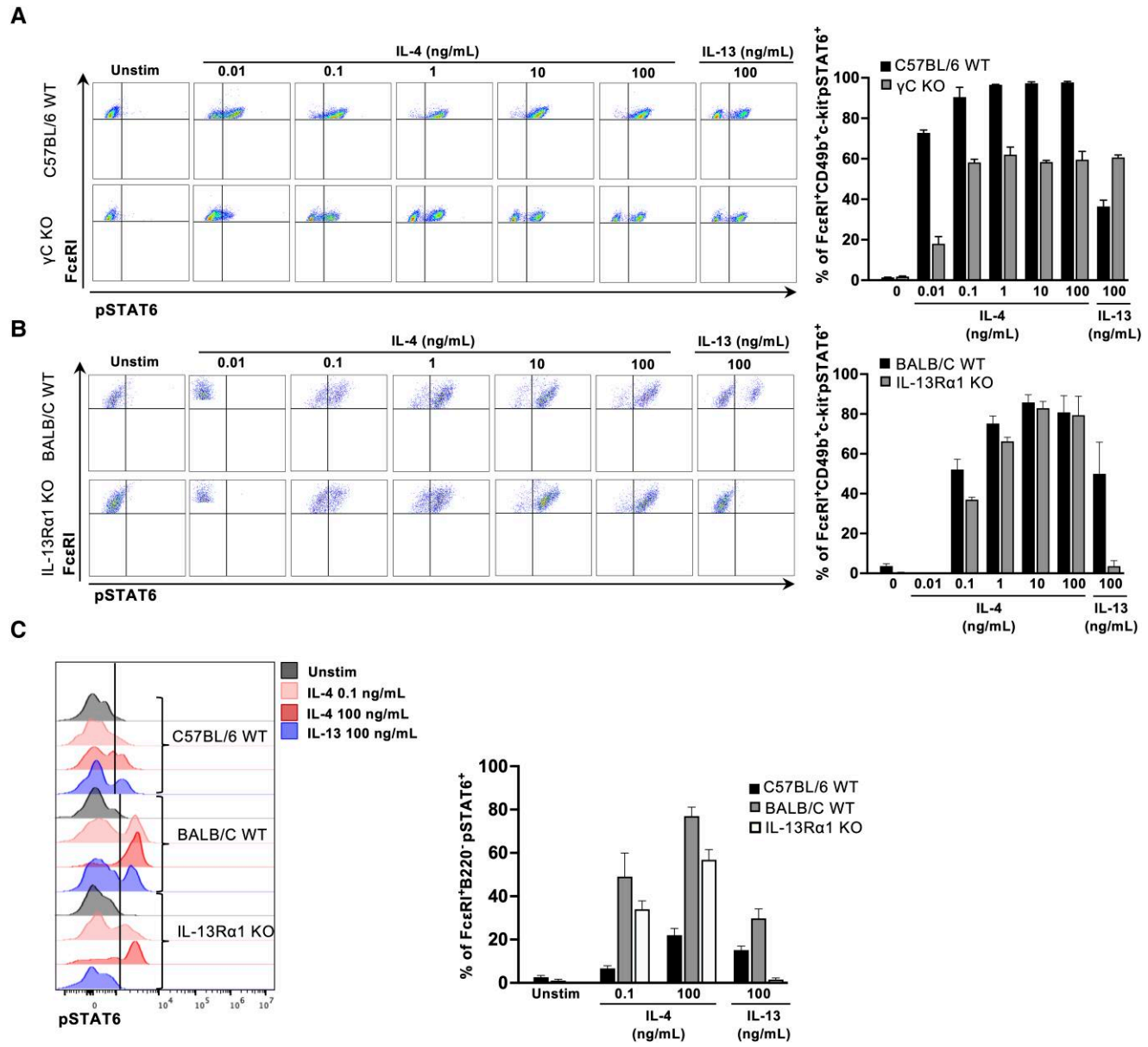

Fig. 4 . Basophil signaling response to IL-4 and IL-13. (A) B6 WT and γC KO or (B) BALB/c WT and IL-13Rα1 KO BMDBs were left unstimulated or stimulated with IL-4 or IL-13 as indicated for 15 min. Stat6 phosphorylation was measured from gated FceRI + CD49b + c-Kit- cells by flow cytometry. Three independent experiments with BMDBs from different mouse lines in each experiment were performed. (Right) Percent pStat6-positive cells of $Fc\varepsilon RI^{+}CD49b^{+}c\text{-}Kit^{-}$ cells; mean and SEM are indicated. (C) B6 WT, BALB/c WT, and IL-13Rα1 KO splenocytes were left unstimulated or stimulated with IL-4 and IL-13. Y641 phosphorylation of Stat6 in $Fc\varepsilon RI^{+}B220^{-}$ cells was measured by flow cytometry. A total of 3 to 6 independent experiments with splenocytes from different mice in each experiment were performed. (Right) pStat6-positive cells of $Fc\varepsilon RI^{+}B220^{-}$ cells; mean and SEM are indicated.

+37 °C. Samples were washed with PBS containing 2 mM EDTA and 0.1% BSA before the further analysis.

For DMBA-TPA treatment of mouse skin, the backs of mice 8 wk of age were shaved, and 48 h later, 50 μg DMBA (Sigma-Aldrich) in 200 μL acetone was applied topically on the shaved area. A week later, the back skin was treated twice a week with 5 μg TPA (Sigma-Aldrich) in 200 μL acetone. The animals were sacrificed by carbon dioxide 48 h after the final TPA application. Skin samples were harvested into RNAlater RNA Stabilization Reagent (Qiagen) and snap-frozen in liquid nitrogen. The mRNA was extracted using Invitrogen TRIzol Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. Homogenization was performed with Precellys homogenizer in CKMix tubes. The skin samples were homogenized at 6,500 rpm for 2′. The RNA extraction from the skin samples was done with E.Z.N.A. Total RNA Kit I (VWR) according to the manufacturer's instructions.

3.3 Flow cytometry

The splenic, BM-derived, and peritoneal cavity cells were incubated with mouse Fc Block (clone 2.4G2; BD Pharmingen) for 5 min at 4 °C. For fresh surface staining, the peritoneal cavity or BM-derived cells were stained against c-Kit (PE, clone 2B8 (eBioscience (Thermo Fisher Scientific)] or BV421 [BD Pharmingen]) and FcεRI (FITC, clone Mar-1; Invitrogen [Thermo Fisher Scientific]) for 20 min at 4 °C and washed twice with PBS containing 2 mM EDTA and 0.1% BSA. For sorting peritoneal cavity cells, staining against CD3 (AlexaFluor 780, clone 17A2; eBioscience [Thermo Fisher Scientific]) and B220

(AlexaFluor 780, clone RA3-6B2; BD Pharmingen) was also included. For splenic cell intracellular staining, the cells were stained against B220 (PE-Cy7, clone RA3-6B; eBioscience [Thermo Fisher Scientific]) at 4 °C and washed twice with PBS containing 2 mM EDTA and 0.1% BSA. Cells were fixed with 1% paraformaldehyde for 10 min at room temperature and permeabilized with PermBuffer IV (BD Pharmingen) according to the manufacturer's instructions. Then, cells were stained against pSTAT6 (pY641) (Alexa Fluor 647, Clone J71-773.58.11; BD Pharmingen) and FcεRI (FITC; eBioscience [Thermo Fisher Scientific]) for 20 min at room temperature. For intracellular staining of BM-derived or peritoneal cavity cells, the cells were fixed with 1% paraformaldehyde for 10 min at room temperature, washed twice with PBS containing 2 mM EDTA and 0.1% BSA, and stained against c-Kit (BV421, clone 2B8; BD Pharmingen) for 20 min at 4 °C and washed twice with PBS containing 2 mM EDTA and 0.1% BSA. Then, cells were permeabilized with PermBuffer IV (BD Pharmingen) according to manufacturer instructions and finally stained against pSTAT6 (pY641) (PE or Alexa Fluor 647, Clone J71-773.58.11; BD Pharmingen), FcεRI (FITC; eBioscience [Thermo Fisher Scientific]), f4/80 (PE, clone T45-2342; BD Pharmingen), CD11b (FITC, clone M1/70; BD Pharmingen), CD49b (PE, clone DX5; eBioscience [Thermo Fisher Scientific]), and/or c-Kit (APC or PE, clone 2B8, eBioscience [Thermo Fisher Scientific]) for 20 min at room temperature. Cells were washed twice with PBS containing 2 mM EDTA and 0.1% BSA before flow cytometric analysis.

All cells were analyzed using FACSAria Fusion (BD Pharmingen), FACS Canto (BD Pharmingen), or CytoFLEX (Becton Dickinson). Cell sorting was performed using FACSAria Fusion. Data analysis was performed with FlowJo software (version 10.8.1) (FlowJo LLC).

3.4 Statistical analysis

For statistical analysis, GraphPad Prism 9 (GraphPad Software) was used. *P* values were indicated based on the comparison setup and normality, calculated using multiple comparison analysis of variance with Dunnett's or Tukey's method, Mann Whitney *U* test, 2-tailed *t* test, or unpaired *t* test with Holm-Šídák correction. *P* values and used test methods for each comparison are shown in Supplementary Table 1.

3.5 Extraction of RNA and qPCR analysis

The mRNA was extracted using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. The amount of RNA was measured using NanoDrop One (Thermo Fisher Scientific). RNA was converted to complementary DNA (cDNA) by reverse transcription using iScript cDNA Synthesis Kit (Bio-Rad Laboratories). A total of 20 ng of cDNA was used for qPCR analysis, which was performed in MicroAmp Fast Optical 96-well reaction plate using QuantStudio 12 K Flex Real-Time PCR System (both from Applied Biosystems). The qPCR reaction was done in a reaction volume of 20 µL with Luna Universal qPCR Master Mix (New England Biolabs) according to the manufacturer's instructions with primers for *γC*, *Il-4Rα* , and *Il-13Rα1* and for the reference gene *ribosomal 18S* (all 250 nM). Used primer sequences were for *γC* forward 5′-TGTTGGTTGGAACGAATGC-3′ and reverse 5′-CCCTTTAGACACACCACTCCA-3′; for *Il-4Rα* forward 5′-GACT GGATCTGGGAGCATCA-3′ and reverse 5′-CAGTCCACAGCGCTA TCCAG-3′; for *Il-13Rα1* forward 5´-CTGAGTCAAATAGATGTT CGGGC-3′ and reverse 5′-GGAGGGGGAGTATGTGTGTTCAC-3′ and for *ribosomal 18S* forward 5′-GTGATCCCTGAGAAGTT CCAG-3′ and reverse 5′-TCGATGTCTGCTTTCCTCAAC-3′. As negative controls, no-template and no-reverse-transcriptase controls were also included. The thermal cycler profile was 1 min 95 °C, 40 × (15 s 95 °C, 30 s 60 °C + plate read). At the end of thermal program, a melting curve analysis was always performed to check unspecific PCR. The $^{\Delta\Delta}$Ct method was used to analyze the data.

3.6 Enzyme-linked immunosorbent assay

Papain from papaya latex (lyophilized powder, aseptically filled; Merck) was dissolved in PBS−/− (pH 7.2; Gibco [Thermo Fisher Scientific]). At day 0, 8-wk-old female WT C57BL/6 animals were injected subcutaneously in the scapular region of the back with 100 µL of papain at 0.1 mg/mL (10 µg per animal) or PBS−/− as control. At day 14, the same injection protocol was repeated. At day 21, animals were euthanized in a CO_2 chamber, blood samples were collected using cardiac puncture, and serums were collected using BD SST microtainer tubes (BD Pharmingen). The sera were stored in −20 °C prior the assay. IgE levels in sera were detected using IgE Mouse Uncoated ELISA Kit (Sigma) according to the manufacturer's instructions.

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Author contributions

T.S., M.I.G.-R., L.K., and L.H. performed and analyzed experiments and participated in drafting the manuscript. T.A.H.J. analyzed data and participated in drafting of the revised manuscript. I.S.J. conceived the idea, oversaw the project, analyzed results and wrote the final manuscript.

Supplementary material

Supplementary materials are available at *Journal of Leukocyte Biology* online.

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References

1. Galli SJ, Gaudenzio N, Tsai M. Mast cells in inflammation and disease: recent progress and ongoing concerns. *Annu Rev Immunol*. 2020:38(1):49–77. https://doi.org/10.1146/annurev-immunol-071719-094903
2. Theoharides TC, Tsilioni I, Ren H. Recent advances in our understanding of mast cell activation - or should it be mast cell

mediator disorders? *Expert Rev Clin Immunol*. 2019:15(6):639–656. https://doi.org/10.1080/1744666X.2019.1596800
3. Gilfillan AM, Austin SJ, Metcalfe DD. Mast cell biology: introduction and overview. *Adv Exp Med Biol*. 2011:716:2–12. https://doi.org/10.1007/978-1-4419-9533-9_1
4. Kolkhir P, Elieh-Ali-Komi D, Metz M, Siebenhaar F, Maurer M. Understanding human mast cells: lesson from therapies for allergic and non-allergic diseases. *Nat Rev Immunol*. 2021:22(5):294–308. https://doi.org/10.1038/s41577-021-00622-y
5. Junttila IS, Watson C, Kummola L, Chen X, Hu-Li J, Guo L, Yagi R, Paul WE. Efficient cytokine-induced IL-13 production by mast cells requires both IL-33 and IL-3. *J Allergy Clin Immunol*. 2013:132(3):704–712.e10. https://doi.org/10.1016/j.jaci.2013.03.033
6. Ho LH, Ohno T, Oboki K, Kajiwara N, Suto H, Iikura M, Okayama Y, Akira S, Saito H, Galli SJ, *et al*. IL-33 induces IL-13 production by mouse mast cells independently of IgE-FcepsilonRI signals. *J Leukoc Biol*. 2007:82(6):1481–1490. https://doi.org/10.1189/jlb.0407200
7. Ramalingam TR, Pescel JT, Sheikh F, Cheever AW, Mentink-Kane MM, Wilson MS, Stevens S, Valenzuela DM, Murphy AJ, Yancopoulos GD, *et al*. Unique functions of the type 11 interleukin 4 receptor identified in mice lacking the interleukin 13 receptor alpha 1 chain. *Nat Immunol*. 2008:9(1):25–33. https://doi.org/10.1038/ni1544
8. Leyva-Castillo JM, Das M, Artru E, Yoon J, Galand C, Geha RS. Mast cell-derived IL-13 downregulates IL-12 production by skin dendritic cells to inhibit the T(H)1 cell response to cutaneous antigen exposure. *J Allergy Clin Immunol*. 2021:147(6):2305–2315.e3. https://doi.org/10.1016/j.jaci.2020.11.036
9. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4+ T cell populations. *Annu Rev Immunol*. 2010:28(1):445–489. https://doi.org/10.1146/annurev-immunol-030409-101212
10. Junttila IS, Mizukami K, Dickensheets H, Meier-Schellersheim M, Yamane H, Donnelly RP, Paul WE. Tuning sensitivity to IL-4 and IL-13: differential expression of IL-4R alpha, IL-13R alpha 1, and gamma c regulates relative cytokine sensitivity. *J Exp Med*. 2008:205(11):2595–2608. https://doi.org/10.1084/jem.20080452
11. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, Keegan AD, Garcia KC. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell*. 2008:132(2):259–272. https://doi.org/10.1016/j.cell.2007.12.030
12. Brown MA, Pierce JH, Watson CJ, Falco J, Ihle JN, Paul WE. B cell stimulatory factor-1/interleukin-4 mRNA is expressed by normal and transformed mast cells. *Cell*. 1987:50(5):809–818. https://doi.org/10.1016/0092-8674(87)90339-4
13. Burton OT, Darling AR, Zhou JS, Noval-Rivas M, Jones TG, Gurish MF, Chatila TA, Oettgen HC. Direct effects of IL-4 on mast cells drive their intestinal expansion and increase susceptibility to anaphylaxis in a murine model of food allergy. *Mucosal Immunol*. 2013:6(4):740–750. https://doi.org/10.1038/mi.2012.112
14. McLeod JJA, Baker B, Ryan JJ. Mast cell production and response to IL-4 and IL-13. *Cytokine*. 2015:75(1):57–61. https://doi.org/10.1016/j.cyto.2015.05.019
15. Nilsson G, Nilsson K. Effects of interleukin (IL)-13 on immediate-early response gene expression, phenotype and differentiation of human mast cells. Comparison with IL-4. *Eur J Immunol*. 1995:25(3):870–873. https://doi.org/10.1002/eji.1830250337
16. Versluis M, Postma DS, Timens W, Hylkema MN. Effects of IL-4 and IL-13 on adenosine receptor expression and responsiveness of the human mast cell line 1. *Int Immunopharmacol*. 2008:8(6):866–873. https://doi.org/10.1016/j.intimp.2008.02.001
17. Nagashima M, Koyanagi M, Arimura Y. Comparative analysis of bone marrow-derived mast cell differentiation in C57BL/6 and BALB/c mice. *Immunol Invest*. 2019:48(3):303–320. https://doi.org/10.1080/08820139.2018.1523924
18. Goenka S, Kaplan MH. Transcriptional regulation by STAT6. *Immunol Res*. 2011:50(1):87–96. https://doi.org/10.1007/s12026-011-8205-2
19. Roy S, Liu H-Y, Jaeson MI, Deimel LP, Ranasinghe C. Unique IL-13Rα2/STAT3 mediated IL-13 regulation detected in lung conventional dendritic cells, 24 h post viral vector vaccination. *Sci Rep*. 2020:10(1):1017–1017. https://doi.org/10.1038/s41598-020-57815-z
20. Salomaa T, Pemmari T, Määttä J, Kummola L, Salonen N, González-Rodríguez M, Parviainen L, Hiihtola L, Vähätupa M, Järvinen TAH, *et al*. IL-13Rα1 Suppresses tumor progression in two-stage skin carcinogenesis model by regulating regulatory T cells. *J Invest Dermatol*. 2022;142(6):1565–1575.e17. https://doi.org/10.1016/j.jid.2021.11.013
21. Sheikh E, Tran T, Vranic S, Levy A, Bonfil RD. Role and significance of c-Kit receptor tyrosine kinase in cancer: a review. *Bosn J Basic Med Sci*. 2022:22(5):683–698. https://doi.org/10.17305/bjbms.2021.7399
22. Kaur D, Hollins F, Woodman L, Yang W, Monk P, May R, Bradding P, Brightling CE. Mast cells express IL-13R alpha 1: iL-13 promotes human lung mast cell proliferation and Fc epsilon RI expression. *Allergy*. 2006:61(9):1047–1053. https://doi.org/10.1111/j.1398-9995.2006.01139.x
23. Heller NM, Qi X, Junttila IS, Shirey KA, Vogel SN, Paul WE, Keegan AD. Type I IL-4Rs selectively activate IRS-2 to induce target gene expression in macrophages. *Sci Signal*. 2008:1(51):ra17. https://doi.org/10.1126/scisignal.1164795
24. Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat Immunol*. 2008:9(3):310–318. https://doi.org/10.1038/ni1558
25. Galatà G, García-Montero AC, Kristensen T, Dawoud AAZ, Muñoz-González JI, Meggendorfer M, Guglielmelli P, Hoade Y, Alvarez-Twose I, Gieger C, *et al*. Genome-wide association study identifies novel susceptibility loci for KIT D816V positive mastocytosis. *Am J Hum Genet*. 2021:108(2):284–294. https://doi.org/10.1016/j.ajhg.2020.12.007
26. Nedoszytko B, Niedoszytko M, Lange M, van Doormaal J, Gleń J, Zabłotna M, Renke J, Vales A, Buljubasic F, Jassem E, *et al*. Interleukin-13 promoter gene polymorphism-1112C/T is associated with the systemic form of mastocytosis. *Allergy*. 2009:64(2):287–294. https://doi.org/10.1111/j.1398-9995.2008.01827.x
27. Galli SJ, Tsai M, Marichal T, Tchougounova E, Reber LL, Pejler G. Approaches for analyzing the roles of mast cells and their proteases in vivo. *Adv Immunol*. 2015:126:45–127. https://doi.org/10.1016/bs.ai.2014.11.002
28. Rothe M, Quarcoo D, Chashchina AA, Bozrova SV, Qin Z, Nedospasov SA, Blankenstein T, Kammertoens T, Drutskaya MS. IL-13 but not IL-4 signaling via IL-4Rα protects mice from papilloma formation during DMBA/TPA two-step skin carcinogenesis. *Cancer Med*. 2013:2(6):815–825. https://doi.org/10.1002/cam4.145
29. Jensen BM, Swindle EJ, Iwaki S, Gilfillan AM. Generation, isolation, and maintenance of rodent mast cells and mast cell lines. *Curr Protoc Immunol*. 2006:3(3):23. https://doi.org/10.1002/0471142735.im0323s74