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Two Functionally Distinct Subsets of Mast Cells Discriminated By IL-2–Independent CD25 Activities

Lorenzo Deho^{*,†}, Cristina Leoni^{*}, Tess M. Brodie^{*}, Sara Montagner^{*}, Marco De Simone[†], Sara Polletti[‡], Iros Barozzi[‡], Gioacchino Natoli[‡], and Silvia Monticelli^{*}

We identified two mast cell subsets characterized by the differential expression of surface CD25 (IL-2R α) and by different abilities to produce cytokines and to proliferate, both in vitro and in vivo. CD25 can be expressed on the surface of immune cells in the absence of the other chains of the IL-2R, which are indispensable for IL-2 signaling. We show that functional differences between the two mast cell populations were dependent on CD25 itself, which directly modulated proliferation and cytokine responses. These effects were completely independent from IL-2 or the expression of the other chains of the high-affinity IL-2R, indicating an autonomous and previously unappreciated role for CD25 in regulating cell functions. Cells genetically ablated for CD25 completely recapitulated the CD25-negative phenotype and never acquired the properties characteristic of CD25-positive mast cells. Finally, adoptive transfer experiments in the mouse demonstrated a different impact of these populations in models of anaphylaxis and contact sensitivity. Our findings indicate a general role for CD25 in contexts where IL-2 signaling is not involved, and may have important implications for all mast cell-related diseases, as well as in all cell types expressing CD25 independently of its IL-2–related functions. *The Journal of Immunology*, 2014, 193: 000–000.

Mast cells are tissue-resident cells involved in allergic responses and host defense against helminths. Their common response to stimuli consists in the release of a broad panel of both prestored and de novo synthesized inflammatory and vasoactive mediators. Despite the fact that mast cells are not considered as initiators of immune responses against pathogens, they are potent modulators of local and systemic responses, mostly because of the diversity of mediators they produce.

The current understanding of the signals required for mast cell differentiation, activation, migration, and even function is still lagging behind that of other cell types, probably because of the low number of mast cell progenitors that can be identified in vivo, and the heterogeneity of tissue factors that locally control their differentiation (1). A largely unexplored area of mast cell biology relates to diversity and plasticity; the number of stable subsets and transient functional states that might exist for this cell type is still mostly unclear. In humans, two major mast cell phenotypes have been described based on the differential expression of proteases in

the secretory granules (2): MC_T mast cells express predominantly tryptases and can be found mostly in the lung and gastrointestinal tract, whereas MC_{TC} cells express tryptases, chymase, and carboxypeptidases and can be found predominantly in the skin, peritoneum, and joints (3). However, other unique mast cell phenotypes (i.e., expressing tryptase and carboxypeptidase, but not chymase) have also been described, especially in the context of asthmatic lungs (3). In the mouse, at least two subtypes can be identified: mucosal mast cells are mainly associated with the lung and gastrointestinal tract epithelia, whereas connective mast cells are mainly found in the intestinal submucosa, peritoneum, and skin (1). Apart from their anatomic location, these two subsets are distinguished by the expression of specific proteases, with mucosal mast cells expressing primarily the chymases mMCP1 and mMCP2, and connective mast cells expressing mMCP4-to-7 and carboxypeptidase A.

Despite these attempts to classify mast cells into specific subsets, a considerable diversity and plasticity can still be observed even within the same tissue (4), and other subsets are likely to originate depending on the local microenvironmental cues, with the tissue of residence largely determining the protease profile (5). Indeed, the protease repertoire of mast cells was strongly influenced by the surrounding host tissue (6), and *Trichinella spiralis* infection led to the identification of hypogranular mast cells that could be an important source of cytokines during helminth infection (7). Potentially, other mast cell subsets with different characteristics, possibly tailored to and determined by the local environment of a given tissue or inflammatory status, await description.

In an attempt to identify molecular markers able to discriminate novel mast cell populations, we focused on CD25 (IL-2R α), because it is expressed in 93% of patients with systemic mastocytosis (8), although its role in mast cell biology remains unclear. CD25 is one of the three components of the high-affinity IL-2R, which also includes IL-2R β and the common γ -chain (γ_c) (9). Although CD25 alone is able to bind IL-2, it does so with low affinity ($K_d \approx 10$ nM) and without apparently activating intracellular signaling pathways (10). Notwithstanding its established role in IL-2 signaling, CD25

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Abbreviations used in this article: BMMC, bone marrow-derived mast cell; CD25neg, CD25-negative; CD25pos, CD25-positive; DC, dendritic cell; FPKM, fragments per kb of exon per million fragments mapped; ICC, intracellular cytokine staining; i.d., intradermally; PCA, passive cutaneous anaphylaxis; qRT-PCR, quantitative RT-PCR; WT, wild type.

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has also been shown to be often expressed in the absence of IL-2R β and to be dysregulated in certain diseases and tumors. For instance, a soluble form of CD25 is elevated in subjects with autoimmune diseases, cancer, and inflammation (11–13).

In this study, we show that CD25 identifies two different subsets of mast cells characterized both *in vitro* and *in vivo* by different transcriptional profiles, as well as by different abilities to proliferate and respond to stimuli. CD25 was able to modulate mast cell proliferation independently from the presence of IL-2 or the expression of the full IL-2R complex. Consistently, CD25KO mast cells generated quantitatively different responses compared with wild type (WT) cells in a mouse model of passive cutaneous anaphylaxis (PCA). Our data provide novel insights into the molecular network underlying mast cell biology and more generally highlight a previously unappreciated heterogeneity of mast cell populations, which might have important implications for mast cell-related diseases.

Materials and Methods

Cell cultures and surface stainings

Murine bone marrow-derived mast cells (BMMCs) were differentiated from bone marrow of C57BL/6 or CD25KO mice (14) as described (15) and cultured in the presence of IL-3 as survival/proliferation factor. Cells were fully differentiated after 3–4 wk of culture and were routinely used for experiments between weeks 4 and 9. Dendritic cells (DCs) were differentiated from bone marrow as described (16) and cultured in RPMI medium containing 10% FCS, nonessential amino acids, Na-pyruvate, antibiotics, and recombinant GM-CSF. All Abs against surface molecules (CD25, ST2) and a PE-rat-IgG1 λ isotype control to ensure specificity of the CD25 staining were purchased from BioLegend.

Cell stimulation and cytokine treatments

Unless otherwise stated, cells were stimulated with 1.1 μ g/ml IgE anti-DNP and 0.2 μ g/ml HSA-DNP (both obtained from Sigma), 80 μ g/ml LPS, or 10 ng/ml stem cell factor. Recombinant IL-2 was used at 100 U/ml, and rIL-3 was used at 10 ng/ml (PeproTech). Recombinant mouse IL-33 (BioLegend) was used at 10 ng/ml for 7 d, as described previously (17).

Intracellular staining and cytokine production

For intracellular cytokine staining (ICC) staining, cells were stimulated with IgE-Ag for 3 h or, for IL-2 detection, with PMA (20 nM) and ionomycin (2 μ M) for 5 h with addition of 10 μ g/ml brefeldin A in the last 2 h of stimulation. Cells were fixed with 4% paraformaldehyde prior to permeabilization and staining as described (18). The measurement of cytokine release in the culture supernatant was performed using the mouse Th1/Th2/Th17/Th22 13plex FlowCytomix Multiplex kit (eBioscience) according to the manufacturer's instructions. Specifically, FACS-sorted CD25-positive (CD25pos) and CD25-negative (CD25neg) BMMCs were seeded at 0.5×10^6 cells/ml in a 96-well plate. Cells were stimulated with 0.1, 0.5, or 1 μ g/ml IgE anti-DNP and 0.02, 0.1, or 0.2 μ g/ml HSA-DNP for 6 h. Alternatively, cells were stimulated with 0.1 μ g/ml IgE anti-DNP and 0.02 μ g/ml HSA-DNP for 3, 6, and 12 h. To evaluate IL-2 and IL-10 release, 2×10^6 cells/ml were stimulated with PMA (20 nM) and ionomycin (2 μ M) for 24 h. Specifically for KLF1, protein detection was performed by intracellular staining using a Foxp3/Transcription Factor Staining Buffer Set (eBioscience) according to the manufacturer's instructions. The anti-KLF1 Ab was purchased from Santa Cruz Biotechnology (F-20).

Quantitative RT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen) and was retrotranscribed using the iScript cDNA Synthesis Kit (Bio-Rad). Primer sequences for SYBR Green quantitative RT-PCR (qRT-PCR) are listed in Table II. Quantitative real-time PCR was performed with an ABI 7900HT Fast Real-Time PCR system (Applied Biosystems). TaqMan primers and probes were as follows: *Ii2R β* (Mm 00434268_m1); *Ii10* (IL 10, Mm 004391614_m1); *Klf1* (Mm 04208330_g1) and as endogenous control β 2m (Mm00437762_m1). To compare expression between different experimental conditions, the $\Delta\Delta$ CT method was used (19).

Plasmids construction and lentiviral transduction

Lentiviral vectors were generated using standard cloning techniques, and BMMCs were transduced at the second week of differentiation exactly as

described (15). Murine CD25 was cloned by PCR from T cells activated with anti-CD3 and anti-CD28 Abs. Murine KLF1 was cloned by PCR from murine lin[−]sca⁺kit⁺ hematopoietic progenitors separated from the bone marrow (20). All plasmids were verified by sequencing.

RNAi

Oligonucleotides for siRNAs against mouse CD25 (FW: 5'-CGAUUCA-CCUAACGGCUGUUU-3'; RV: 5'-ACAGCCGUUAGGUGAAUCGUU-3') were purchased from ThermoScientific. For transfection, 1×10^6 cells were transfected with 200 pmol of double-stranded oligos with the Amaxa Mouse T Cell Nucleofector kit (program X-01). Efficiency of transfection (>93%) was evaluated by transfecting cells with a fluorescent oligo (siGLO).

Cell proliferation

Cell proliferation was assessed measuring DNA replication with either ³H-thymidine or BrdU incorporation assay. To assess proliferation, IL-3 was washed out of the culture for ~16 h prior to readdition of recombinant IL-3 (10 ng/ml) with or without SCF (10 ng/ml). For thymidine incorporation assays, 5×10^5 cells/well were seeded in 96-well plates, and 1 μ Ci/ml of ³H-thymidine (GE Healthcare) was added to the culture for 7–16 h prior measurement of the DNA-incorporated radioactivity using a scintillation β -counter. BrdU incorporation assays were performed with an APC-BrdU Flow kit from BD Biosciences, and cells were allowed to incorporate BrdU for 8–12 h at 37°C.

PCA and contact sensitivity

PCA experiments and assessment of tissue-resident mast cells were performed exactly as described (18). For PCA, 1×10^6 differentiated BMMCs were injected intradermally (i.d.) in the ear pinna of mice lacking mast cells (Kit^{W-sh/W-sh}; The Jackson Laboratory) (21). After 4 wk, mast cells were sensitized with i.d. injections of IgE-anti-DNP (1.5 μ g/ml) and challenged 24 h later with i.v. injections of 250 μ g/ml DNP-HSA together with 5 mg/ml Evans blue dye to assess extravasation. The blue dye was extracted from the tissues by incubation in formamide at 63°C overnight, and its intensity (correlating with the extent of extravasation) was measured spectrophotometrically (OD₆₀₀). The presence of mast cells in the reconstituted ears was assessed by toluidine blue staining of paraffin-embedded ears. For oxazolone-induced contact sensitivity, ears of Kit^{W-sh/W-sh} mice were reconstituted as above and sensitized with 20 μ l 2% oxazolone in acetone as described (22). After 7 d, mice were challenged with 0.5% oxazolone in acetone every 2 d for six to seven challenges. Control mice were treated with acetone only. All animal studies were performed in accordance with the Swiss Federal Veterinary Office guidelines and were approved by the Dipartimento della Sanità e della Socialità, authorization numbers 03/2012 and 08/2013.

RNA-seq

mRNA-Seq library preparation from 1 μ g total RNA was performed with the TruSeq RNA Sample Prep kit (Illumina), according to the manufacturer's instructions, and sequenced on a Solexa HiSeq2000 following standard protocols. After quality filtering according to the Illumina pipeline, paired-end reads were aligned to the mm9 reference genome and to the *Mus musculus* transcriptome (using Illumina iGenome RefSeq annotation) using TopHat 1.3.1 (23). We allowed up to two mismatches and specified a mean distance between pairs (−r) of 120 bp. Transcript abundance was quantified using Cufflinks 2.0.2 (24). Differentially expressed genes (using two replicates) were identified using Cuffdiff 2.0.2 (24) using −N-u parameters. For subsequent analyses, we considered the information at the level of annotated coding genes. Those showing fragments per kb of exon per million fragments mapped (FPKM) ≥ 1 in at least one sample were retained for further analysis. Differentially expressed genes were defined as those showing at least a 1.5-fold difference and $p < 0.05$. Differentially expressed genes among CD25pos and CD25neg samples were clustered according to their FPKM values. After log2-transformation, the values for each gene were converted to z-scores. Genes were then clustered hierarchically using average linkage and 1 Pearson correlation as distance measure. Statistics for each sample are reported in Table I. Data are deposited at GEO with accession number GSE52502 (<http://www.ncbi.nlm.nih.gov/geo/>).

Statistical analysis

Statistical analysis of data was performed with Prism software (GraphPad). Data are represented as mean $\pm$ SEM, and significance was assessed by paired or unpaired Student *t* test, two-tailed, unless otherwise noted. Comparisons between groups were performed with two-way ANOVA.

Results

CD25 identifies two mast cell subsets with different phenotypes

Anti-CD25 staining of mouse mast cells, both isolated *ex vivo* (peritoneal lavage mast cells) and differentiated *in vitro* (BMMCs), showed CD25 expression in a large percentage of cells (Fig. 1A). Lack of CD25 staining on mast cells derived from *Il2Ra*^{-/-} mice (CD25KO), as well as on cells stained with an isotype-control Ab, demonstrated the specificity of the observed signal (Fig. 1A). Because CD25 is expressed by only a fraction of mast cells, we asked whether it identifies stable subsets or transient functional states with different properties. To assess systematically the ability of these cells to produce cytokines, we first sorted CD25pos and CD25neg cells and analyzed the expression of several cytokines in response to stimulation with IgE and Ag complexes. Intracellular cytokine staining for two of the most abundant mast cell cytokines, IL-6 and TNF- α , showed that the percentage of CD25neg cells expressing TNF- α and to a lesser extent IL-6 was reproducibly higher in the CD25neg fraction, although the overall magnitude of the effect was relatively small (Fig. 1B). No differences in the percentage of IL-13-producing cells could be observed by intracellular staining (Fig. 1B). Despite being small, these differences in cytokine expression were highly reproducible. Moreover, IL-6 and TNF- α were induced at a similar extent in CD25neg and CD25pos cells following LPS stimulation (Fig. 1C), suggesting that these genes are similarly competent for transcriptional activation in the two subsets.

CD25 may simply mark mast cells with slightly different activation profiles or be causally involved in the generation of mast cells with distinct properties. To discriminate between these possibilities, we analyzed CD25KO cells and compared them to CD25pos and CD25neg WT cells. Cytokine production of CD25KO cells resembled CD25neg cells, with a lower percentage of CD25pos cells expressing TNF- α (Fig. 1D). CD25pos cells also appeared to produce significantly more IL-2 compared with CD25neg cells, with CD25KO cells resembling the CD25neg (Fig. 1F). Therefore, CD25 is causally implicated in differential expression of these cytokines in distinct mast cell subpopulations.

We next assessed cytokine production in the culture supernatant at various time points after stimulation. In response to IgE and Ag stimulation, we observed higher IL-6, TNF- α and IL-13 secretion by CD25neg compared with CD25pos cells (Fig. 1E). Similarly, TNF- α production was increased in CD25neg cells compared with CD25pos also using increasing concentrations of IgE-Ag complexes (Fig. 1G). Consistent with the intracellular staining data, IL-2 was produced at higher levels in the culture supernatant from CD25pos compared with CD25neg. Vice versa, when stimulated with PMA and ionomycin, CD25neg cells appeared to express higher amounts of IL-10 compared with the CD25pos ones (Fig. 1H). Differential *Il-10* mRNA expression was also confirmed by qRT-PCR upon stimulation, with CD25KO cells resembling also in this case the CD25neg ones (Fig. 1I).

Because CD25 appeared to identify mast cells with distinct properties, we also evaluated the proliferation rate of the CD25neg and CD25pos subsets. After differentiation from bone marrow precursors, mast cells proliferate continuously for several weeks when cultured in the presence of IL-3 (25, 26). Overnight IL-3 withdrawal from mast cell cultures resulted in cell cycle exit. Readdition of IL-3 in the presence of ³H-thymidine allowed us to assess DNA replication. No ³H-thymidine incorporation was detected in the absence of IL-3 (Fig. 2A). ³H-thymidine incorporation upon IL-3 readdition was detected in all samples, but CD25pos cells proliferated significantly less than CD25neg cells did. Addition of stem cell factor, which also favors mast cell

proliferation (27), together with IL-3 did not cause major changes in the proliferation rate in both subsets (Fig. 2A). CD25KO cells resembled the CD25neg cells, in that they showed enhanced proliferation compared with the CD25pos subset (Fig. 2B). We did not observe any effect on mast cell differentiation in the absence of CD25, and CD25KO BMMCs appeared phenotypically similar to WT mast cells, as assessed both by toluidine blue staining and by the expression of the surface mast cell markers Kit and Fc ϵ RI (Supplemental Fig. 1). By week 3–4 of differentiation, all cells (WT and CD25KO) were positive by more than 90% for both Kit and Fc ϵ RI α (Supplemental Fig. 1). We also assessed IL-3-dependent mast cell proliferation at a single-cell level by BrdU-incorporation assays. Similar to the results obtained with ³H-thymidine incorporation, CD25neg cells proliferated significantly more than CD25pos cells did (Fig. 2C).

Expression of CD25 was previously described for DCs from both human and mouse, and CD25pos DCs were shown to have a distinct cytokine profile and to be more efficient in Ag presentation (28). To assess whether differences in DC proliferation correlated with CD25 expression, similar to mast cells, we differentiated DCs from CD25KO mice and WT littermates. After 1 wk of differentiation, we obtained a population of DCs that expressed CD25 on the surface (Fig. 2D, 2E). Similar to mast cells, the CD25pos DC population proliferated significantly less compared with CD25neg and CD25KO DCs (Fig. 2D–F), suggesting that expression of CD25 might generally correlate with specific proliferation abilities in different myeloid cells.

To assess the phenotypic stability of the two mast cell subsets, we sorted CD25pos and CD25neg cells to more than 92% purity, and we cultured them separately for 3 wk. Although we noticed the appearance of a small percentage of CD25pos cells in the CD25neg population (which may suggest a certain level of “conversion,” with CD25neg cells acquiring expression of CD25), these two populations remained relatively stable and did not promptly remix over this extended time period (Fig. 3A). Mast cell differentiation, proliferation, and survival can be influenced by a variety of stimuli (29), which could potentially influence CD25 expression and the ratio between the CD25pos and the CD25neg subsets. We therefore stimulated WT BMMCs with IgE-Ag complexes, LPS, and SCF, and we followed the expression of CD25 for up to 96 h. Interestingly, sustained stimulation with IgE-Ag complexes led to up to a 6-fold downregulation of CD25 expression, whereas LPS treatment led only to a transient upregulation (Fig. 3B). Importantly, treatment with IgE alone appeared to initiate a modest downregulation of CD25 expression, which was much stronger upon full stimulation with IgE and Ag (Fig. 3B, *bottom panels*). Finally, SCF treatment led to an initial modest downregulation of CD25 surface expression, which was maintained at the same level over time. IL-2 addition had no discernible effect on CD25 expression (Supplemental Fig. 2A).

IL-33 is known to promote mast cell maturation through its receptor ST2 and to induce elevated expression of mast cell proteases (17, 30–32). We therefore asked whether IL-33 treatment could influence the different phenotypes observed in the presence or absence of CD25. We first assessed surface ST2 expression on CD25pos, CD25neg, and CD25KO cells and observed similar expression on all different subsets (Fig. 3C). Consistently, IL-33 treatment led to mast cell maturation (e.g., as observed by the increased granularity, assessed by side scatter) and to a modest reduction in cell proliferation, which was similar in all subsets and not significant across multiple experiments (Fig. 3D). Our data therefore indicate that various stimuli can affect CD25 expression *in vitro*, and that inducing mast cell maturation with IL-33 does not normalize the proliferation phenotype, further pointing toward

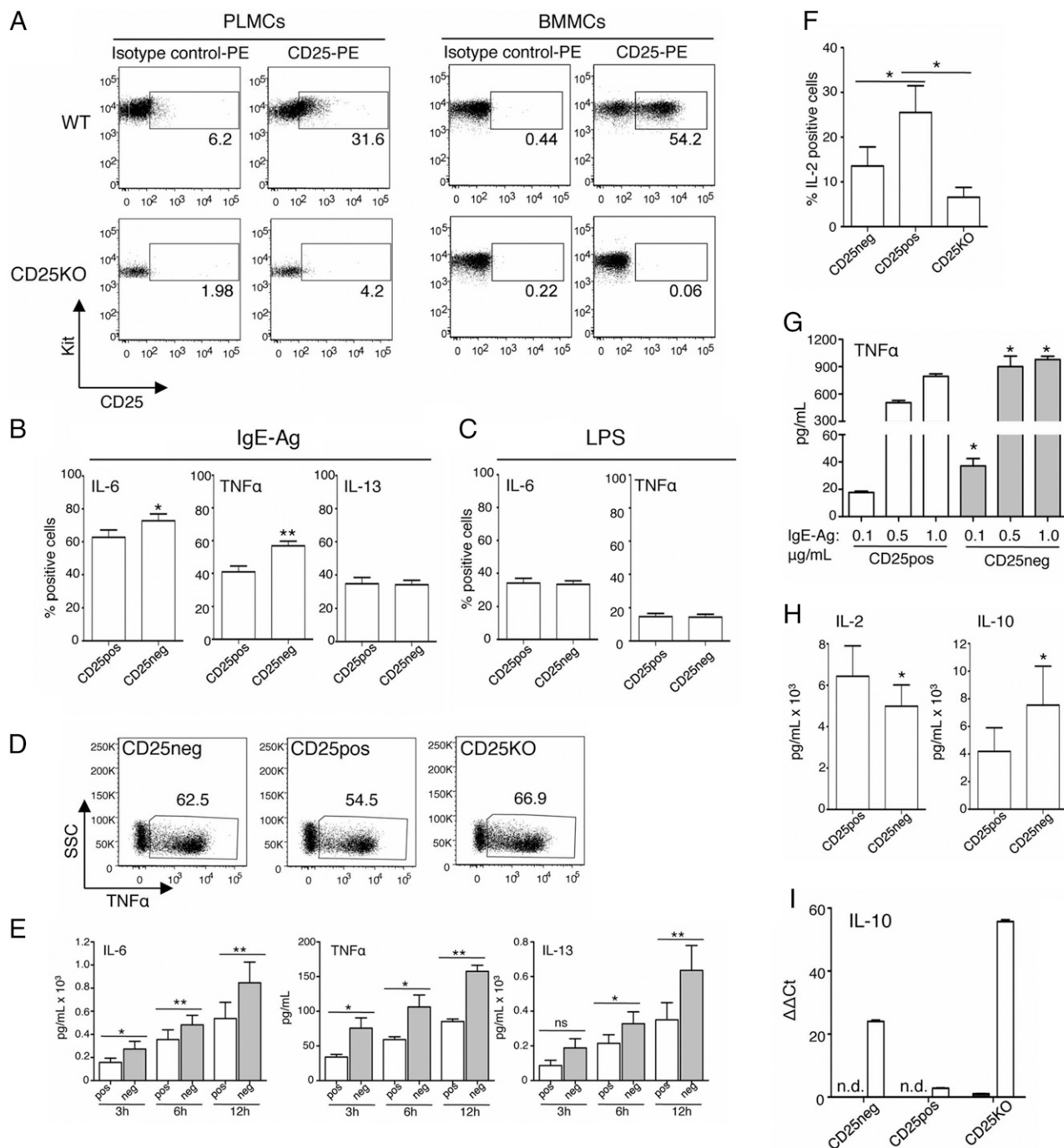


FIGURE 1. Expression of CD25 identifies two functionally distinct subsets of mast cells. **(A)** CD25 expression was assessed on mast cells from the peritoneal lavage of WT and CD25KO mice, or from in vitro-differentiated mast cells derived from the same mice. For clarity, Kit⁺ cells were first gated from the total. An Ab isotype control was used in all cases to ensure staining specificity. **(B)** Differentiated BMMCs were FACS-sorted to separate CD25neg and CD25pos cells prior to stimulation with IgE-Ag complexes and ICC staining to assess the percentage of cytokine-producing cells ($n = 5$ independent experiments for IL-6 and TNF- α and $n = 4$ for IL-13; mean $\pm$ SEM). **(C)** Cells were treated as in (B) except that they were stimulated with LPS prior ICC staining ($n = 4$ independent experiments; mean $\pm$ SEM). **(D)** BMMCs derived from CD25KO or WT littermates (the latter sorted to separate CD25neg and CD25pos cells) were stimulated with IgE-Ag complexes prior to ICC staining for TNF- α production. One representative experiment of at least three is shown. **(E)** Cells were stimulated as in (D) with IgE-Ag complexes for 3, 6, or 12 h prior supernatant collection and assessment of cytokine release using a 13-plex fluorescent bead immunoassay. One representative of three independent experiments is shown. **(F)** Cells were stimulated as in (D) with PMA and ionomycin for 5 h prior to ICC staining for IL-2 production. Shown is the compiled analysis of three independent experiments (mean $\pm$ SEM). **(G)** Same as in (E), except that cells were stimulated for 6 h with increasing concentrations of IgE and Ag (0.1, 0.5, or 1 μ g/ml IgE anti-DNP and 0.02, 0.1, or 0.2 μ g/ml HSA-DNP, respectively). **(H)** Same as in (E) except that cells were stimulated with PMA and ionomycin for 24 h prior to assessment of cytokine production. **(I)** Differentiated BMMCs derived from CD25KO or C57BL/6 mice (the latter FACS-sorted to separate CD25neg and CD25pos cells) were stimulated with IgE-Ag complexes for 8 h prior to RNA extraction and TaqMan qRT-PCR for *Il10* expression. β 2-microglobulin was used as endogenous control. * $p < 0.05$, ** $p < 0.01$. n.d., not detectable; PLMC, peritoneal lavage mast cell.

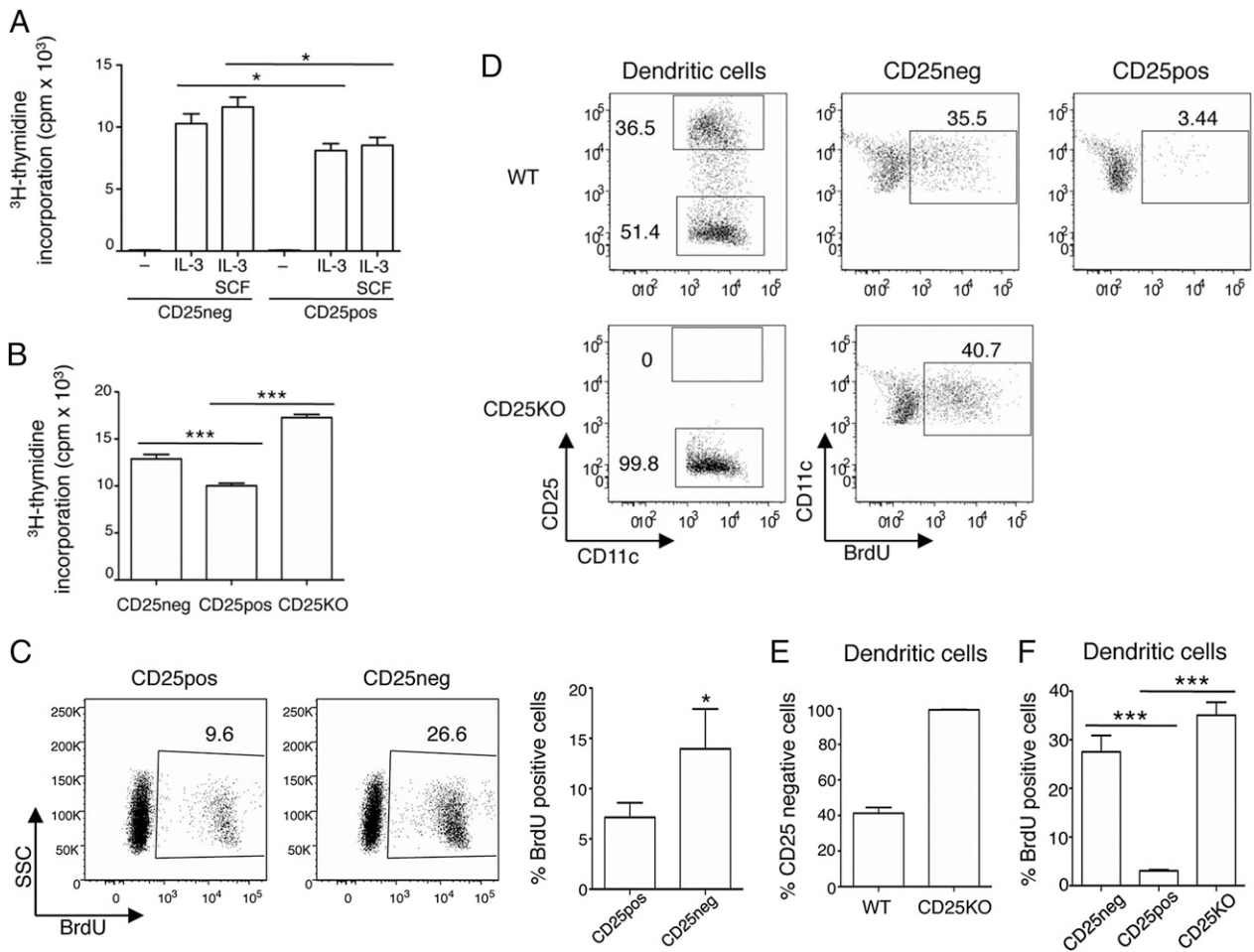


FIGURE 2. Increased proliferation in the absence of CD25. **(A)** Sorted CD25neg and CD25pos BMMCs were deprived of IL-3 overnight to arrest cell growth. IL-3 was then readded (with or without SCF) to reinduce proliferation. Proliferation was assessed by ^3H -thymidine incorporation assay. Shown is the result (mean $\pm$ SEM) of one experiment (six samples per column) representative of two independent ones. **(B)** Cells were treated as in **(A)** except that only proliferating cells (with IL-3) are shown. Shown is one experiment (eight samples per column) representative of two independent ones, mean $\pm$ SEM. **(C)** Cells were treated as in **(A)** except that DNA incorporation upon IL-3 addition was measured by BrdU incorporation. One representative experiment is shown on the left, and the compiled analysis of seven independent experiments is shown on the right (mean $\pm$ SEM). **(D)** DCs were differentiated from bone marrow of CD25KO mice or WT littermates. DCs were gated as CD11c⁺ Mac-1⁺ and then gated as CD25pos and CD25neg during the analysis. After 1 wk of differentiation, BrdU was added O/N to assess proliferation. Shown is one representative staining out of 12. **(E)** Compiled results of CD25 expression on DCs treated as in **(D)**. Shown is the percentage of CD25neg DCs obtained in our culture conditions, in both WT and CD25KO DCs. Shown are six independent surface stainings on two independent differentiations. **(F)** Compiled results of BrdU incorporation in DCs; BrdU was added overnight to DCs treated as in **(D)**. Percentage of BrdU⁺ cells in the CD25pos, CD25neg, and CD25KO populations is shown. Shown are six independent experiments on two independent differentiations (mean $\pm$ SEM). * $p < 0.05$, *** $p < 0.0001$.

a relatively stable phenotype that is not simply linked to mast cell maturation.

CD25 effects are independent of IL-2 and IL-2R signaling

To assess whether mast cells (specifically the CD25pos subset) could respond to IL-2, we first analyzed the expression of the other two components of the high-affinity receptor, IL-2R β and γ_c . Whereas γ_c showed clear surface expression, the IL-2R β subunit was undetectable (Fig. 3E), indicating that in resting conditions mast cells do not express the full high-affinity IL-2R and therefore cannot respond to IL-2. Interestingly, BMMC stimulation with IgE-Ag complexes induced a modest expression of *IL2R β* mRNA, which was not influenced by the addition of IL-2 (Fig. 3F). Although induction of *IL2R β* expression was only transient, peaking at 3 h and then disappearing (Supplemental Fig. 2B), these data suggest that at least a subset of mast cells may potentially gain the ability to respond to IL-2 during acute stimulation. To address the possibility that mast cells might respond to IL-2 upon reconsti-

tution of a functional IL-2R complex, we expressed IL-2R β using a lentiviral vector. Despite expression of surface IL-2R β (Supplemental Fig. 2C), we could not observe any response of mast cells to IL-2 (or IL-15) stimulation in any of the conditions tested, including cytokine production, proliferation, and Stat5 phosphorylation (Supplemental Fig. 2D and data not shown), likely suggesting the lack of required IL-2R signal transducers. The IL-2 endogenously produced by mast cells (Fig. 1F) did not appear to have any autocrine effect either, as addition of neutralizing anti-IL-2 Abs did not affect cell proliferation (Supplemental Fig. 2E). Therefore, although CD25 clearly identifies two different mast cell subsets, it does not appear to have a role in IL-2-dependent signaling in these cells.

To assess whether CD25 could have regulatory roles independent of IL-2, we sorted CD25pos cells and transfected them with an siRNA to knockdown CD25 expression (siCD25). Forty-eight hours after transfection, CD25 expression was strongly reduced

in siCD25-transfected mast cells as compared with cells transfected with a scrambled siRNA control (Fig. 4A). Importantly, we observed increased proliferation of siCD25-transfected cells compared with control cells, suggesting that the expression of CD25 itself might be important to directly modulate mast cell proliferation (Fig. 4B, 4C). To investigate this possibility further, we reconstituted CD25 expression in CD25KO mast cells by lentiviral transduction. As we previously reported (15, 33), efficiency of BMMC transduction was routinely 40–50%, as assessed by expression of surface CD25 (Fig. 4D). To avoid possible artifacts caused by strong overexpression, we assessed proliferation at a single-cell level by BrdU-incorporation assay specifically in cells expressing CD25 levels comparable to the endogenous ones (CD25^{low/intermediate}). Similarly to CD25^{pos} cells, CD25KO cells transduced to express CD25 showed significantly reduced proliferation compared with nontransduced cells or a control plasmid (Fig. 4E, 4F). This effect was not rescued by mutations in the cytoplasmic tail of CD25, indicating that other parts of the molecule, possibly through interaction with other proteins, are responsible for this effect (Supplemental Fig. 3). Specifically, to assess whether the cytoplasmic tail could transduce signals, we

designed mutants that were unlikely to interfere with protein synthesis and recycling from the plasma membrane, but could potentially interfere with putative phtail could transduce signals, we

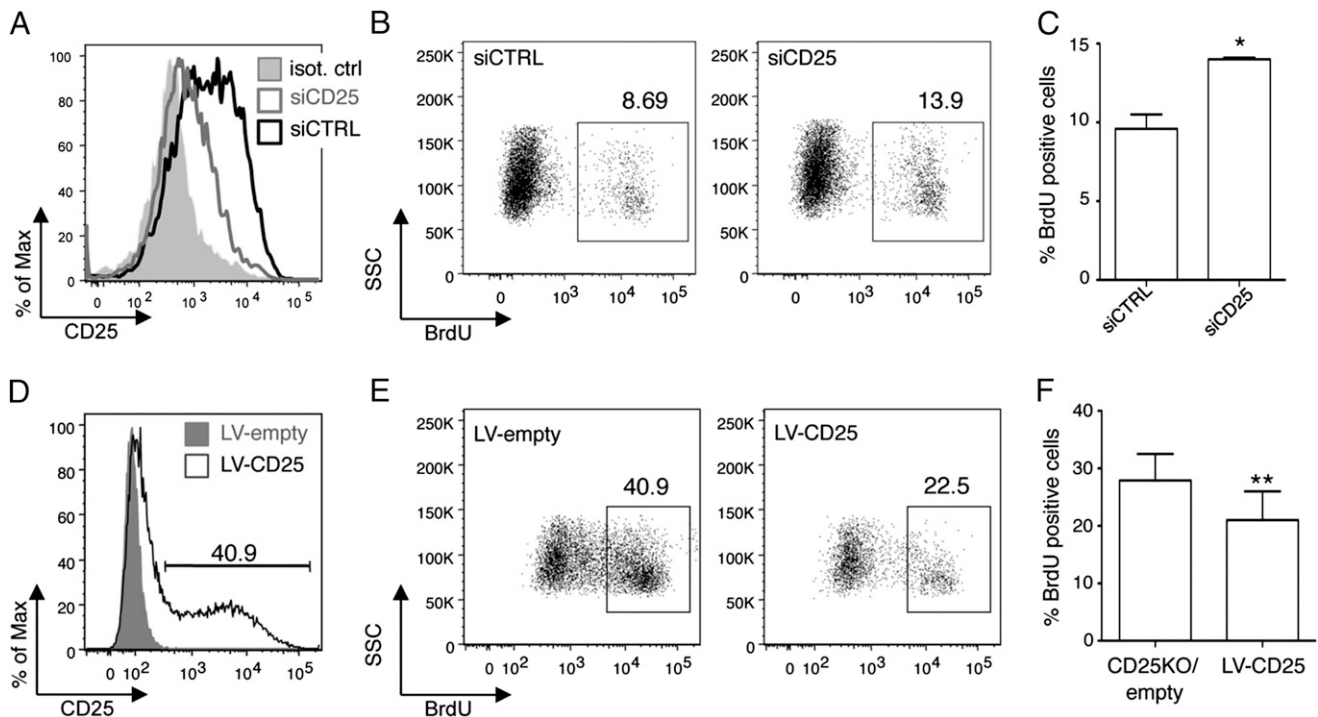


FIGURE 4. CD25 modulates mast cell proliferation. **(A)** WT CD25pos cells were sorted prior to transfection with a CD25-specific siRNA (siCD25) or a control oligonucleotide (siCTRL). Surface CD25 expression 48 h after transfection is shown (data representative of three independent experiments), in comparison with an isotype control Ab. **(B)** Same as in **(A)** except that BrdU was added 48 h after transfection for 8–12 h prior analysis. Shown is one representative result, whereas **(C)** shows the results of two independent experiments. **(D)** CD25KO cells were transduced with a lentiviral vector to reconstitute CD25 expression (LV-CD25) or with an empty control vector. Shown is CD25 expression on transduced cells. **(E)** Cells as in **(D)** were analyzed for proliferation by BrdU incorporation. Shown is one representative experiment, and **(F)** shows the compiled results of six independent experiments (mean $\pm$ SEM). * $p < 0.05$; ** $p < 0.01$.

cells (Fig. 5A, 5B, and statistics in Table I). The vast majority of expressed genes were similar in the two subsets. However, using a cut-off of 1.5-fold difference and $p < 0.05$, we identified a relatively small number of genes that were differentially expressed, including proteases (cathepsin G, mMCPI1/Prss34 trypsin, carboxypeptidase E), signaling molecules (RGS13, regulator of G protein signaling 13), chemokines and chemokine receptors (CCR3, CCL3, CCL4), as well as complement and ribosomal proteins (Supplemental Table I). Notably, most of the differentially expressed genes were expressed at higher levels in CD25pos cells, which could suggest a direct role of CD25 in promoting their expression.

Most importantly, using qRT-PCR analysis (primers listed in Table II), we found a concordant behavior of differentially expressed genes in CD25neg and CD25KO cells (Fig. 5C). The fact that cells genetically lacking CD25 appear to be phenotypically similar to CD25neg cells and to be unable to acquire properties of CD25pos cells, causally implicates CD25 as regulator of at least some of the described phenotypes. These data demonstrate that CD25 is not simply a marker of a particular physiological state that is coregulated with other genes affecting cytokine expression and proliferation. Conversely, it appears to act upstream of at least a subset of these genes and to directly control their expression.

Among the genes potentially involved in cell cycle control, the zinc finger transcription factor erythroid Krüppel-like factor (EKLF/KLF1), which is known to favor cell cycle entry in erythroid cells (34), was expressed at higher levels in CD25neg cells (Fig. 5C). To assess a potential regulatory role for this transcription factor in modulating mast cell proliferation and CD25 expression, we transduced FACS-sorted CD25pos cells with a lentiviral vector to force a higher expression of KLF1. Trans-

duced cells were selected with puromycin, and KLF1 expression was confirmed by qRT-PCR and intracellular staining (Fig. 5D, 5E). Forced expression of KLF1 reduced CD25 expression from CD25pos cells (Fig. 5E), suggesting that this factor might contribute directly to some of the phenotypes displayed by CD25neg cells. To address this point, we assessed the proliferation of CD25pos cells transduced with either KLF1 or a control vector. A BrdU-incorporation assay revealed increased proliferation of cells expressing KLF1 compared with controls (Fig. 5F), suggesting that this transcription factor is part of the regulatory mechanism that links CD25 expression to mast cell proliferation.

Differential in vivo responses of CD25pos and CD25neg mast cell subsets

We found that CD25pos/neg subsets are stable (Fig. 3A); therefore, we assessed their functions with in vivo models. We initially attempted to assess mast cell differentiation and function in vivo in *Il2ra*^{-/-} animals. However, these experiments were neither feasible nor reliable because of the severe lymphoproliferative and autoimmune disorders developed by homozygous *Il2ra*^{-/-} animals already at an early age (4–6 wk after birth) (14). We therefore used adoptive transfer models to assess mast cell functions. Specifically, we assessed mast cell responses in vivo in anaphylaxis-associated vascular hyperpermeability using a model of PCA (18). CD25KO or control WT littermate BMMCs were injected in the ear pinna of Kit^{W-sh/W-sh} mice, which normally do not have mast cells (21). After establishment of proper tissue homeostasis, IgE anti-DNP Abs were injected locally in the ear pinna prior to systemic injection of the Ag (DNP-HSA) and Evan's blue dye (Fig. 6A). As a measure of mast cell activation, we quantified the cutaneous extravasation of the dye after extraction from the tissue.

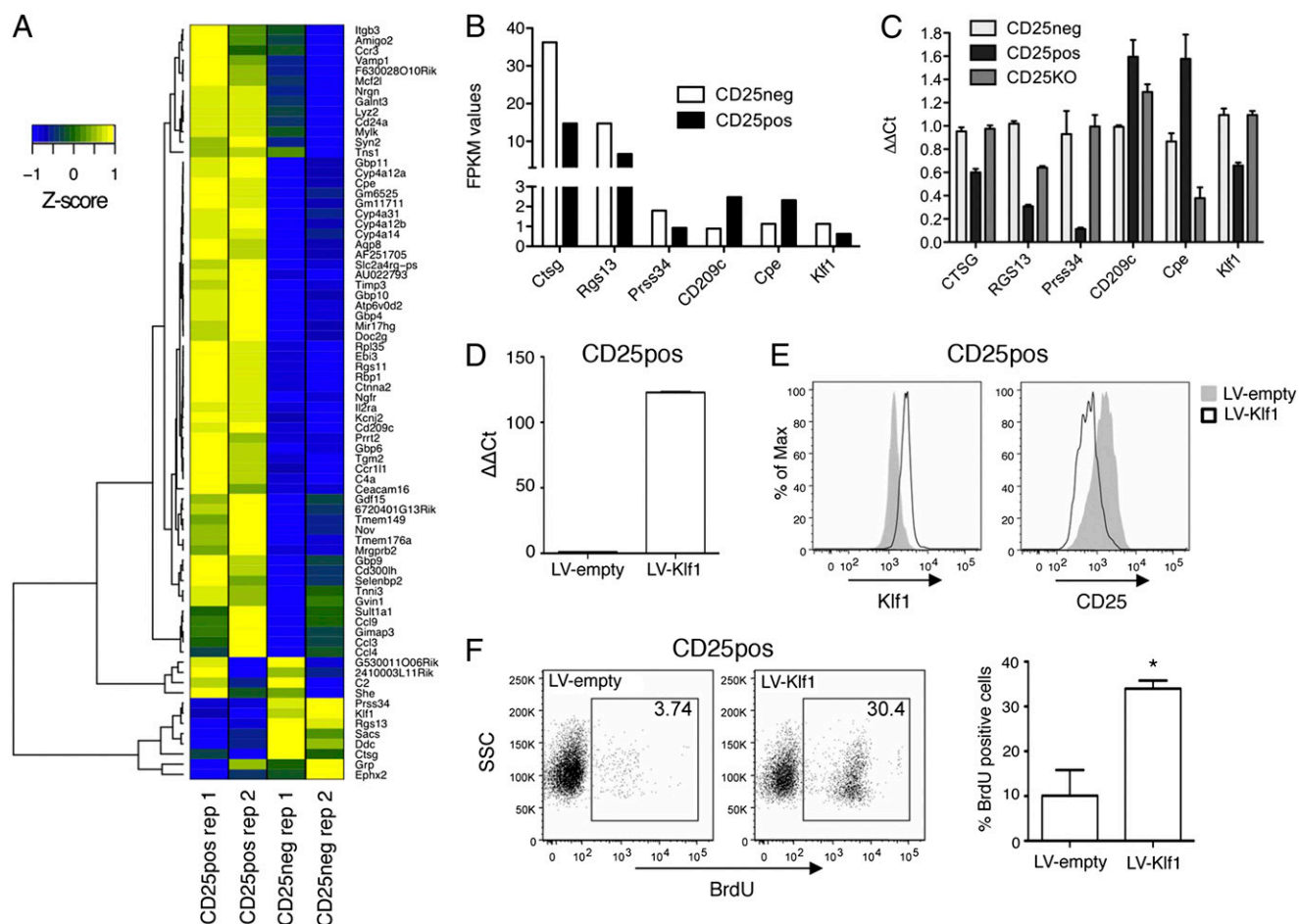


FIGURE 5. Gene expression analysis of CD25pos and CD25neg mast cell subsets. **(A)** WT BMMCs were sorted in CD25pos and CD25neg populations. After sorting, purity of the populations was >99% for CD25neg and 86–96% for CD25pos. Total RNA was extracted immediately after sorting and subjected to library preparation and RNA-seq. Two independent cultures from different mice were sequenced. Differentially expressed genes were clustered according to their FPKM values. After log₂-transformation, the values for each gene were converted to z-scores and genes were then hierarchically clustered. **(B)** FPKM values are plotted for selected genes. **(C)** The same genes as in (B) were analyzed by qRT-PCR in sorted CD25pos and CD25neg cells, as well as in CD25KO cells. **(D)** The KLF1 cDNA was cloned into a lentiviral vector used to force expression of this transcription factor in FACS-sorted, CD25pos mast cells. Upon transduction and selection of the transduced cells, KLF1 expression was verified by qRT-PCR. **(E)** Same as in (D), except that KLF1 expression was assessed by intracellular staining (left panel). CD25 expression was also evaluated on the same cells by surface staining (right panel). Representative of three independent experiments on two independent transductions. **(F)** Proliferation of cells as in (E) was assessed by BrdU-incorporation assay. Representative of three independent experiments. The compiled results of all experiments are shown in the graph on the right (mean ± SEM, unpaired *t* test). **p* < 0.05.

CD25KO cells showed a much stronger IgE response in vivo compared with WT cells (Fig. 6B). A qualitatively similar albeit quantitatively smaller difference was observed between CD25pos and CD25neg cells, which were sorted prior to tissue reconstitution, with CD25neg cells providing a stronger PCA response compared with CD25pos cells (Fig. 6C). The increased PCA response was unlikely to be determined by increased mast cell degranulation, as in vitro IgE-dependent degranulation appeared to be comparable in CD25pos and CD25neg (or CD25KO) cells (Supplemental Fig. 3D, 3E). We hypothesized that increased responses could be due to increased proliferation of the CD25neg or CD25KO subsets as observed in vitro; therefore, we assessed the number of cells reconstituting the tissues. Indeed, we observed a higher number of CD25KO cells in the ear pinna of Kit^{W-sh/W-sh} mice compared with controls (Fig. 6D). Consistent with our in vitro results, these data suggest an increased proliferation of these cells in vivo, although we cannot exclude that other mechanisms (such as increased tissue homing) might contribute to this phenotype within the complex tissue environment.

PCA experiments can measure only acute and early mast cell activation (i.e., degranulation) upon Ag recognition. Therefore, all long-term secondary effects deriving from differences in cytokine production and other de novo synthesized mediators cannot be assessed. Mast cell-derived IL-2 was previously reported to contribute to the suppression of inflammation in chronic allergic dermatitis, with a mechanism that involved the maintenance of an appropriate ratio of activated T lymphocytes to regulatory T cells at the site of inflammation (22). Because CD25pos cells produced higher levels of IL-2 than the CD25neg ones did (Fig. 1F), we set

Table I. Statistics for each RNA-seq sample

Sample	Mapped Reads	Properly Paired Reads
CD25neg_rep1	97,847,869	67,180,396
CD25neg_rep2	114,593,942	78,307,380
CD25pos_rep1	100,826,485	68,712,342
CD25pos_rep2	78,775,455	52,049,076

Table II. Sequences of primers used for SYBR Green qRT-PCR

Gene Name	Primer Sequence
<i>Ctsg</i>	FW 5'-TCTGATCCAGAGTCCAGAAGG-3' RV 5'-GAGCACTGTGATGAGTTGCTG-3'
<i>Rgs13</i>	FW 5'-GAAATATGCTTCACGAAGGG-3' RV 5'-GCATGTTTGTGAGTGGGTCACGAATG-3'
<i>Cd209c</i>	FW 5'-TGCCTACTCCACGGACAAGAAG-3' RV 5'-GGGCGGCACAGGCGTTTAT-3'
<i>Prss34</i>	FW 5'-ACGAGTGTGGCGGTTCCCTCA-3' RV 5'-GGCTCCACCTCTTTCGGCCT-3'
<i>Cpe</i>	FW 5'-CAGTGGGGCGCAGCTTCGAG-3' RV 5'-TTCACCCGGCTCATGGACCC-3'
<i>Klf-1</i>	FW 5'-CGGCGCCCCCTTCCTTCTTG-3' RV 5'-GTTCCCGGGGACAAGCCTGC-3'
<i>β-actin</i>	FW 5'-GCAGCTCCTTCGTTGCCGGT-3' RV 5'-GGCTTTGCACATGCCGAGC-3'
<i>Tbp</i>	FW 5'-CTGGAATTGTACCGCAGCTT-3' RV 5'-ATGATGACTGCAGCAATCG-3'

FW, forward; RV, reverse

out to investigate the response of these two subsets in vivo in the context of chronic oxazolone-induced contact sensitivity (35). To this purpose, we compared WT cells to CD25KO cells, as well as

sorted CD25pos and CD25neg cells. We reconstituted the ear pinna of *Kit^{W-sh/W-sh}* mice, and after 4 wk, mice underwent a first sensitization with oxazolone, followed by six to seven secondary challenges (Fig. 6E). Levels of inflammation in the ear were evaluated as an increase in ear thickness. Control mice (not shown) were treated with vehicle only and gave no response. As previously reported (22), mice lacking mast cells were much more sensitive to oxazolone-induced contact sensitivity, compared with WT mice (Fig. 6F). Dampening of the inflammatory response in this system was observed primarily upon reconstitution of the skin with WT (and specifically CD25pos) cells, whereas CD25KO and CD25neg cells had a smaller effect, which likely correlated with the reduced expression of IL-2 (Fig. 6G, 6H). These data demonstrate that CD25pos and CD25neg mast cells differentially contribute to acute IgE stimulation and to chronic inflammatory responses, likely because of their diverse abilities to proliferate and produce cytokines (Fig. 7).

Discussion

Functional diversification within specific cell lineages occurs both in lymphoid and myeloid cells, which can differentiate into phenotypically distinct but often interconvertible subpopulations (29, 36, 37). In this study we identified and characterized two novel mast

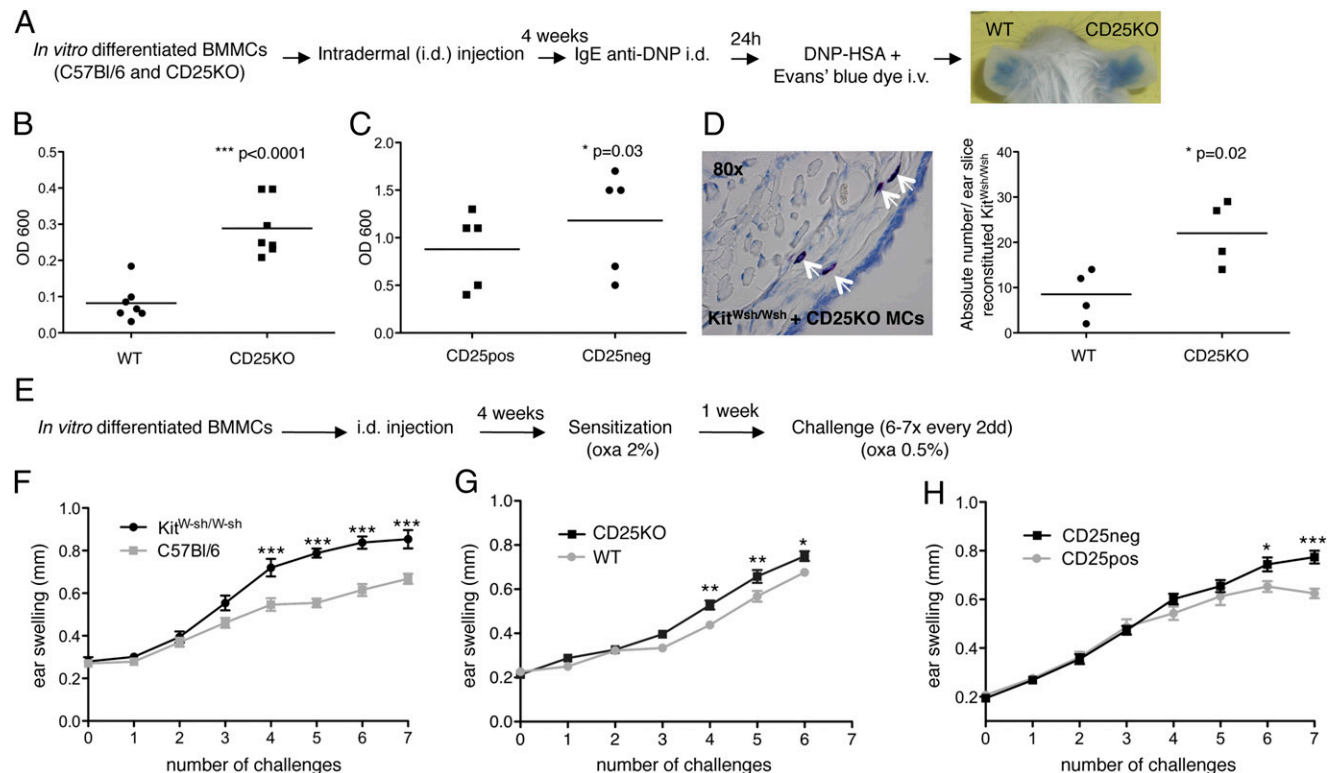


FIGURE 6. Differential in vivo responses by mast cell subsets characterized by CD25 expression. **(A)** Schematic representation of the experimental protocol for PCA. In vitro-differentiated WT and CD25KO BMMCs were i.d. injected in the left and right ear pinna respectively. Four weeks later, ears were sensitized with IgE-anti-DNP prior to challenge with HSA-DNP in the presence of Evans blue dye. One representative result is shown. **(B)** *Kit^{W-sh/W-sh}* mice were engrafted in the right ear with differentiated CD25KO BMMCs and in the left ear with BMMCs from WT littermates. The extravasated dye was extracted and measured. Each dot represents one animal ($n = 7$). **(C)** *Kit^{W-sh/W-sh}* mice were engrafted in the left ear with FACS-sorted CD25neg cells and in the right with CD25pos cells. Each dot represents one animal ($n = 5$). **(D)** To assess the presence and the number of mast cells in the engrafted tissues, ears were embedded in paraffin and stained with toluidine blue. Four ears for WT and four ears for CD25KO-engrafted mice were counted blindly (the operator counting at the microscope was not aware of the type of sample under consideration). **(E)** Schematic representation of the experimental protocol for oxazolone-induced contact sensitivity. Cells were injected i.d. in the ear pinna of *Kit^{W-sh/W-sh}* mice and allowed to reconstitute the tissue. Sensitization was performed with 20 μ l 2% oxazolone. After 7 d, mice were challenged six to seven times with 0.5% oxazolone. **(F)** *Kit^{W-sh/W-sh}* and C57BL/6 mice were used as control. Data are representative of two independent experiments and six mice (mean $\pm$ SEM). **(G)** *Kit^{W-sh/W-sh}* mice were reconstituted with WT or CD25KO cells. Challenge and sensitization with oxazolone were performed as in (E). Data include measurements from three mice (mean $\pm$ SEM). **(H)** Same as in (G) except that *Kit^{W-sh/W-sh}* mice were reconstituted with FACS-sorted WT CD25pos and CD25neg. Data include measurements of two independent experiments and seven mice (mean $\pm$ SEM). Comparisons between the groups were performed by two-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

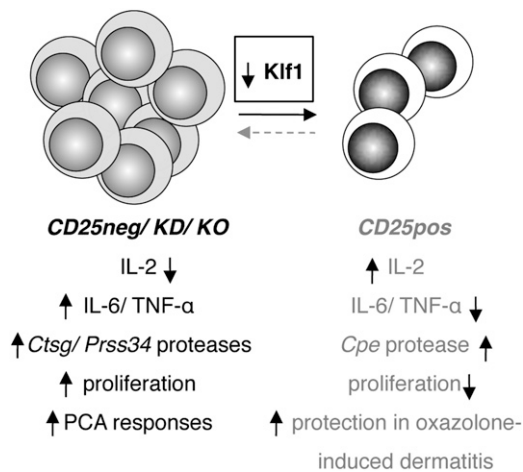


FIGURE 7. Schematic representation of the mast cell subsets and proposed role of CD25. Mast cells can be divided into two subsets characterized by differential CD25 expression, cytokine and protease profile, ability to proliferate, and importantly, showing different responses in models of disease in vivo. CD25 not only discriminates a specific mast cell subset, but also modulates cell proliferation in an IL-2-independent manner. Cells lacking CD25, either endogenously (CD25neg), genetically (CD25KO), or by RNAi (CD25KD) all show the same phenotype of increased cell proliferation compared with CD25-expressing cells. Cell expressing moderate levels of CD25, either endogenously (CD25pos) or because of reconstitution with lentiviral transduction, display reduced proliferation compared with cells lacking CD25 expression. The transcription factor KLF1 is preferentially expressed in CD25neg cells and may represent a molecular link between mast cell proliferation and CD25 expression.

cell subsets distinguished by CD25 expression and endowed with a different ability to proliferate and produce cytokines, thus leading to functional differences in their in vivo responses, which are at least in part regulated by the transcription factor KLF1 (Fig. 7). Mast cells are long-lived cells that can undergo many activation cycles with release of mediators and that can respond to many different activating signals. For these reasons, the effects of different mast cell phenotypes on responses that can be either protective or pathogenic for the host could be profound. Among the differences between CD25pos and CD25neg cells with a potentially functional effect on immune response involving mast cells, are some proteases selectively expressed by either of these subsets (CD25pos cells preferentially expressed carboxypeptidase E, whereas CD25neg cells predominantly expressed cathepsin G and mMCP11/Prss34 tryptase). Although little is known about the role of each of these specific enzymes within an immune response, they can potentially affect the type and number of inflammatory cells that are recruited at sites of exposure to pathogens (or allergens), as well as the potential extent of local tissue injury. For example, mMCP6 contributes to the recruitment of neutrophils to sites of bacterial infection (38), and it is therefore likely that other roles for the various enzymes expressed by mast cells will be uncovered in the future, including those we identified as differentially expressed between CD25pos and CD25neg cells (39).

Our data also hint at IL-2-independent roles of CD25 in regulating cellular functions. Indeed, although the phenotypes of the *Il2ra*^{-/-} and *Il2*^{-/-} animals are largely redundant, they are not completely overlapping. Consistent with our data, the phenotype exhibited by animals lacking *Il2ra* is more severe than that observed in the absence of *Il2*, suggesting that CD25 likely exerts roles that are not strictly linked to its participation in a functional IL-2R complex (14).

Because CD25 by itself cannot transduce signals, it is tempting to speculate that it may form novel transducing complexes with still

unidentified partners. In keeping with this hypothesis, an association between CD25 and ICAM-1 (40) has previously been reported. Such novel CD25-dependent complexes might be particularly important for cells like mast cells and DCs that do not express a complete and functional IL-2R and are thus IL-2 independent. Conversely, in the context of other cell types, like T cells, that respond to IL-2 and depend on IL-2 signaling for their proliferation, differentiation and function, the importance of CD25 is likely to be linked predominantly or exclusively to the IL-2R.

One possibility that remains to be thoroughly explored is whether what we provisionally identified as two different subsets of mast cells characterized by differential CD25 expression may in fact represent two different stages of mast cell maturation. In in vitro cultured BMMCs, the percentage of CD25pos and CD25neg cells remained stable over several weeks, indicating low interconversion capabilities between these two populations. However, the fact that we did not observe any decline in the CD25pos population despite their lower proliferation capabilities suggests that at least a portion of CD25neg cells could somehow acquire CD25 expression. Whether these two types of mast cells represent two stable subsets or rather plasticity between two states and how CD25 is transcriptionally regulated will be therefore the topic of future investigation.

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Disclosures

The authors have no financial conflicts of interest.

References

- Collington, S. J., T. J. Williams, and C. L. Weller. 2011. Mechanisms underlying the localisation of mast cells in tissues. *Trends Immunol.* 32: 478–485.
- Irani, A. A., N. M. Schechter, S. S. Craig, G. DeBlois, and L. B. Schwartz. 1986. Two types of human mast cells that have distinct neutral protease compositions. *Proc. Natl. Acad. Sci. USA* 83: 4464–4468.
- Dougherty, R. H., S. S. Sidhu, K. Raman, M. Solon, O. D. Solberg, G. H. Coughney, P. G. Woodruff, and J. V. Fahy. 2010. Accumulation of intra-epithelial mast cells with a unique protease phenotype in T(H)2-high asthma. *J. Allergy Clin. Immunol.* 125:1046–1053 e1048.
- Friend, D. S., N. Ghildyal, M. F. Gurish, J. Hunt, X. Hu, K. F. Austen, and R. L. Stevens. 1998. Reversible expression of tryptases and chymases in the jejunal mast cells of mice infected with *Trichinella spiralis*. *J. Immunol.* 160: 5537–5545.
- Gurish, M. F., and K. F. Austen. 2012. Developmental origin and functional specialization of mast cell subsets. *Immunity* 37: 25–33.
- Xing, W., K. F. Austen, M. F. Gurish, and T. G. Jones. 2011. Protease phenotype of constitutive connective tissue and of induced mucosal mast cells in mice is regulated by the tissue. *Proc. Natl. Acad. Sci. USA* 108: 14210–14215.
- Liu, A. Y., D. F. Dwyer, T. G. Jones, L. G. Bankova, S. Shen, H. R. Katz, K. F. Austen, and M. F. Gurish. 2013. Mast cells recruited to mesenteric lymph nodes during helminth infection remain hypogranular and produce IL-4 and IL-6. *J. Immunol.* 190: 1758–1766.
- Teodosio, C., A. C. Garcia-Montero, M. Jara-Acevedo, L. Sanchez-Munoz, I. Alvarez-Twose, R. Nunez, L. B. Schwartz, A. F. Walls, L. Escribano, and A. Orfao. 2010. Mast cells from different molecular and prognostic subtypes of systemic mastocytosis display distinct immunophenotypes. *J. Allergy Clin. Immunol.* 125:719–726, 726 e711–726 e714.
- Yamane, H., and W. E. Paul. 2012. Cytokines of the $\gamma(c)$ family control CD4+ T cell differentiation and function. *Nat. Immunol.* 13: 1037–1044.
- Wang, H. M., and K. A. Smith. 1987. The interleukin 2 receptor. Functional consequences of its bimolecular structure. *J. Exp. Med.* 166: 1055–1069.
- Akin, C., L. B. Schwartz, T. Kitoh, H. Obayashi, A. S. Worobec, L. M. Scott, and D. D. Metcalfe. 2000. Soluble stem cell factor receptor (CD117) and IL-2 receptor alpha chain (CD25) levels in the plasma of patients with mastocytosis: relationships to disease severity and bone marrow pathology. *Blood* 96: 1267–1273.
- Greenberg, S. J., L. Marcon, B. J. Hurwitz, T. A. Waldmann, and D. L. Nelson. 1988. Elevated levels of soluble interleukin-2 receptors in multiple sclerosis. *N. Engl. J. Med.* 319: 1019–1020.
- Makis, A. C., E. Galanakis, E. C. Hatzimichael, Z. L. Papadopoulou, A. Siamopoulou, and K. L. Bourantas. 2005. Serum levels of soluble interleukin-

- 2 receptor alpha (sIL-2Ralpha) as a predictor of outcome in brucellosis. *J. Infect.* 51: 206–210.
14. Willerford, D. M., J. Chen, J. A. Ferry, L. Davidson, A. Ma, and F. W. Alt. 1995. Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* 3: 521–530.
 15. Mayoral, R. J., and S. Monticelli. 2010. Stable overexpression of miRNAs in bone marrow-derived murine mast cells using lentiviral expression vectors. *Methods Mol. Biol.* 667: 205–214.
 16. Svensson, M., B. Stockinger, and M. J. Wick. 1997. Bone marrow-derived dendritic cells can process bacteria for MHC-I and MHC-II presentation to T cells. *J. Immunol.* 158: 4229–4236.
 17. Kaieda, S., K. Shin, P. A. Nigrovic, K. Seki, R. T. Lee, R. L. Stevens, and D. M. Lee. 2010. Synovial fibroblasts promote the expression and granule accumulation of tryptase via interleukin-33 and its receptor ST-2 (IL1RL1). *J. Biol. Chem.* 285: 21478–21486.
 18. Rusca, N., L. Dehò, S. Montagner, C. E. Zielinski, A. Sica, F. Sallusto, and S. Monticelli. 2012. MiR-146a and NF- κ B1 regulate mast cell survival and T lymphocyte differentiation. *Mol. Cell. Biol.* 32: 4432–4444.
 19. Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402–408.
 20. Bottardi, S., J. Ross, N. Pierre-Charles, V. Blank, and E. Milot. 2006. Lineage-specific activators affect beta-globin locus chromatin in multipotent hematopoietic progenitors. *EMBO J.* 25: 3586–3595.
 21. Grimbaldston, M. A., C. C. Chen, A. M. Piliponsky, M. Tsai, S. Y. Tam, and S. J. Galli. 2005. Mast cell-deficient W-sash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo. *Am. J. Pathol.* 167: 835–848.
 22. Hershko, A. Y., R. Suzuki, N. Charles, D. Alvarez-Errico, J. L. Sargent, A. Laurence, and J. Rivera. 2011. Mast cell interleukin-2 production contributes to suppression of chronic allergic dermatitis. *Immunity* 35: 562–571.
 23. Trapnell, C., L. Pachter, and S. L. Salzberg. 2009. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25: 1105–1111.
 24. Trapnell, C., B. A. Williams, G. Pertea, A. Mortazavi, G. Kwan, M. J. van Baren, S. L. Salzberg, B. J. Wold, and L. Pachter. 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* 28: 511–515.
 25. Nabel, G., S. J. Galli, A. M. Dvorak, H. F. Dvorak, and H. Cantor. 1981. Inducer T lymphocytes synthesize a factor that stimulates proliferation of cloned mast cells. *Nature* 291: 332–334.
 26. Kalesnikoff, J., and S. J. Galli. 2011. Antiinflammatory and immunosuppressive functions of mast cells. *Methods Mol. Biol.* 677: 207–220.
 27. Lantz, C. S., J. Boesiger, C. H. Song, N. Mach, T. Kobayashi, R. C. Mulligan, Y. Nawa, G. Dranoff, and S. J. Galli. 1998. Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature* 392: 90–93.
 28. Velten, F. W., F. Rambow, P. Metharom, and S. Goerdts. 2007. Enhanced T-cell activation and T-cell-dependent IL-2 production by CD83+, CD25high, CD43high human monocyte-derived dendritic cells. *Mol. Immunol.* 44: 1544–1550.
 29. Galli, S. J., N. Borregaard, and T. A. Wynn. 2011. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nat. Immunol.* 12: 1035–1044.
 30. Xu, D., H. R. Jiang, P. Kewin, Y. Li, R. Mu, A. R. Fraser, N. Pitman, M. Kurowska-Stolarska, A. N. McKenzie, I. B. McInnes, and F. Y. Liew. 2008. IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc. Natl. Acad. Sci. USA* 105: 10913–10918.
 31. Moritz, D. R., H. R. Rodewald, J. Gheyselinck, and R. Klemenz. 1998. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *J. Immunol.* 161: 4866–4874.
 32. Allakhverdi, Z., D. E. Smith, M. R. Comeau, and G. Delespesse. 2007. Cutting edge: The ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *J. Immunol.* 179: 2051–2054.
 33. Mayoral, R. J., L. Deho, N. Rusca, N. Bartonicek, H. K. Saini, A. J. Enright, and S. Monticelli. 2011. MiR-221 influences effector functions and actin cytoskeleton in mast cells. *PLoS ONE* 6: e26133.
 34. Tallack, M. R., J. R. Keys, P. O. Humbert, and A. C. Perkins. 2009. EKLF/KLF1 controls cell cycle entry via direct regulation of E2f2. *J. Biol. Chem.* 284: 20966–20974.
 35. Man, M. Q., Y. Hatano, S. H. Lee, M. Man, S. Chang, K. R. Feingold, D. Y. Leung, W. Holleran, Y. Uchida, and P. M. Elias. 2008. Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunologic, and biochemical changes following single versus multiple oxazolone challenges. *J. Invest. Dermatol.* 128: 79–86.
 36. Monticelli, S. 2013. MicroRNAs in T helper cell differentiation and plasticity. *Semin. Immunol.* 25: 291–298.
 37. O'Shea, J. J., and W. E. Paul. 2010. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. *Science* 327: 1098–1102.
 38. Thakurdas, S. M., E. Melicoff, L. Sansores-Garcia, D. C. Moreira, Y. Petrova, R. L. Stevens, and R. Adachi. 2007. The mast cell-restricted tryptase mMCP-6 has a critical immunoprotective role in bacterial infections. *J. Biol. Chem.* 282: 20809–20815.
 39. Stevens, R. L., and R. Adachi. 2007. Protease-proteoglycan complexes of mouse and human mast cells and importance of their beta-tryptase-heparin complexes in inflammation and innate immunity. *Immunol. Rev.* 217: 155–167.
 40. Burton, J., C. K. Goldman, P. Rao, M. Moos, and T. A. Waldmann. 1990. Association of intercellular adhesion molecule 1 with the multichain high-affinity interleukin 2 receptor. *Proc. Natl. Acad. Sci. USA* 87: 7329–7333.