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Secretory leukocyte protease inhibitor modulates FcεRI-dependent but not Mrgprb2-dependent mastocyte function in psoriasis

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ABSTRACT

Psoriasis, which involves mast cells, is a chronic inflammatory skin disorder whose pathophysiology is still not fully understood. We investigated the role of secretory leukocyte protease inhibitor (SLPI), a potential inhibitor of mastocyte serine proteases, on mast cell-dependent processes of relevance to the skin barrier defense in psoriasis. Here, we demonstrate that the dermal mast cells of patients with psoriasis express SLPI but not those of healthy donors. Moreover, SLPI transcripts were found to be markedly upregulated in murine mast cells by mediators derived from psoriasis skin explant cultures. Using mast cells from SLPI-deficient mice and their $SLPI^{+}$ wild-type controls, we show that SLPI inhibits the activity of serine protease chymase in mastocytes. SLPI was also found to enhance the degranulation of mast cells activated via anti-IgE Abs but not Mrgprb2 ligands. Finally, we demonstrate that the expression and function of Mrgprb2 in mast cells are suppressed by a normal and, to a larger extent, psoriatic skin environment. Together, these findings reveal mechanisms underlying FcεRI– and Mrgprb2-dependent mast cell function that have not been described previously.

1. Introduction

Secretory leukocyte protease inhibitor (SLPI), a small (107-amino acid long) protein, is primarily recognized for its ability to exert anti-proteolytic effects against serine proteases [1]. In addition, SLPI has been implicated in suppressing inflammatory responses by controlling the activity of the transcription factor NFκB [2,3], restricting the deposition of neutrophil extracellular traps (NETs) to the extracellular milieu [4], and acting as an antimicrobial agent [1]. In addition to its anti-inflammatory actions, SLPI promotes regenerative tissue healing [5–7]. Thus, in restoring disrupted tissue homeostasis, its overall effects are tissue protective and beneficial to the host. However, by fostering the pro-immunogenic functions of NETs, SLPI can potentially also have the opposite effects [8].

The abundance of SLPI in mucosal secretions and its marked upregulation in chronically inflamed skin suggest an important role of this

Abbreviations: β-HEX, β-hexosaminidase; Abs, antibodies; APC, allophycocyanin; BM, bone marrow; BSA, bovine serum albumin; c-Kit, receptor tyrosine kinase; C48/80, compound 48/80; cDNA, complementary deoxyribonucleic acid; Cy7, Cyanine 7; F, female; FBS, fetal bovine serum; FcεRI, high-affinity receptor for the Fc region of immunoglobulin E; FCS, fetal calf serum; FITC, Fluorescein isothiocyanate; FMO, fluorescence minus one; HEPES, 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid; IgE, immunoglobulin E; IL-3, interleukin 3; IMQ, imiquimod; KO, knockout; M, male; Mrgprb2, Mas-related G-protein coupled receptor b2; MRGPRX2, Mas-related G Protein-coupled receptor-X2; mRNA, messenger ribonucleic acid; NETs, neutrophil extracellular traps; NFkB, nuclear factor kappa-light-chain-enhancer of activated B cells; PBS, phosphate-buffered saline; PMCs, peritoneal mast cells; PNAG, 4-Nitrophenyl N-acetyl-β-D-glucosaminide; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; RNA, ribonucleic acid; RPMI 1640, Roswell Park Memorial Institute medium 1640; SCF, stem cell factor; SLPI, secretory leukocyte protease inhibitor; SP, substance P; TLR7, Toll-like receptor 7; WT, wild type.

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protein at barrier sites such as the skin. Although SLPI levels are barely detectable in the skin at steady state, high cutaneous SLPI expression is typically observed in chronic inflammatory skin diseases, such as psoriasis and atopic dermatitis [9]. The global prevalence of psoriasis is reported to be 2% to 3% of the world's population. However, given the complexity of the disease and its various manifestations, ranging from cutaneous presentation to psoriatic arthritis, this condition may be more common [10].

The full spectrum of SLPI functions in diseased skin remains to be determined. We recently reported that SLPI knockout (KO) and wild-type (WT) mice did not differ in skin thickness and demonstrated a similar scope of neutrophil- and T-cell mediated cutaneous inflammation in an experimental model of psoriasis-like dermatitis induced by TLR7-ligand imiquimod (IMQ). However, we found that SLPI functionally intersects with the nervous system and prevents pathology-associated skin dryness in lateral skin, e.g., the cutaneous site adjacent to the skin from the IMQ-treated dorsal site [11].

Keratinocytes, the dominant cells in the outermost skin layer – the epidermis – are one of the main sources of cutaneous SLPI in psoriasis. Keratinocyte-derived protease, kallikrein peptidase 7, is inhibited by SLPI, and this interaction limits epidermal shedding [12]. SLPI is also detected in neutrophils that infiltrate psoriatic skin, where it can inhibit the proteolytic activity of neutrophil elastase and cathepsin G [4,8,13,14]. Among the potential targets of SLPI in the skin are also the serine proteases of mast cells, such as chymase [15]. However, the role of SLPI in regulating mast cell biology remains unknown.

Mast cells control many aspects of skin defense, and contribute to cutaneous inflammation [16,17]. Recently, data have emerged that suggest that mast cell maturation in the skin is maintained by microbiota via keratinocyte-derived stem cell factor (SCF). In addition, mast cell activity is suppressed by neurons via glutamate release [17,18]. The rapid responsiveness of tissue-resident mast cells to external and internal threats through degranulation, coupled with their anatomical connection to sensory afferent neurons, confers upon these cells a unique ability to initiate or modulate early inflammatory processes and strengthen skin immunological defenses under both homeostatic and skin barrier-disrupted conditions. Mast cells can be quickly activated in response to the antigen-dependent cross-linking of IgE bound to FcεRI on their surface [19]. Recent advances have demonstrated another key pathway underlying mast cell degranulation. This pathway engages different receptors on mast cells, Mrgprb2 (Mas-related G-protein coupled receptor b2, mouse ortholog of human MRGPRX2), instead of FcεRI, highlighting the heterogeneous patterns by which mast cells can be activated to mediate their functions. Activating mast cells by engaging FcεRI or Mgprb2 can result in the release of prestored, granule-loaded mediators to the extracellular milieu, including β-hexosaminidase (β-HEX) [20].

In an IMQ-induced experimental model of psoriasis-like dermatitis, mast cells have been reported to control the early onset of inflammatory skin responses [21]. Given SLPI upregulation in psoriatic skin and the potential of SLPI to inhibit mast-cell serine proteases, we hypothesize that SLPI can have an impact on cutaneous mast cells and their ability to augment skin barrier function under chronic inflammatory conditions in the skin.

Here, we demonstrate that SLPI is expressed in dermal mast cells in psoriasis patients and in the context of experimental psoriasis, where it can control chymase activity and FcεRI, but not Mrgprb2-mediated mast cell degranulation.

2. Materials and methods

2.1. Materials

All chemicals were obtained from Sigma-Aldrich unless otherwise stated. Aldara (5% imiquimod cream) and imiquimod in water (1 mg/ml) were purchased from Meda and InvivoGen, respectively. Phosphate-buffered saline (PBS) buffer was obtained from PAN Biotech. Mouse recombinant interleukin 3 (IL-3) and SCF were purchased from Pepro-Tech. Substance P (SP) was obtained from R&D Systems.

2.2. IMQ-induced psoriasis-like experimental model

All animal procedures and experiments were performed in accordance with national and European legislation after approval by the 2nd Local Institutional Animal Care and Use Committee (IACUC) in Krakow (approval # 298/2017 and 317/2018). SLPI KO and WT mice were generously donated by Dr Sharon M. Wahl [4,5]. The mice used in this study were sex- and age-matched 8- to 10-week-old littermates with a C57BL/6 background. The mice were housed under pathogen-free conditions in the animal facility at the Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University.

SLPI KO and WT mice were treated twice daily for up to 6 days with 15 mg Aldara (5% imiquimod cream) on shaved and depilated back skin, as previously described [4,22]. At the end of the experiment, the mice were euthanized with an overdose of anesthetics and perfused intracardially with ice-cold PBS. Then, the tissues were isolated and prepared for individual analyses.

2.3. Isolation and culture of peritoneal mast cells (PMCs)

Cells were washed from the peritoneal cavity using a peritoneal lavage technique with 8 ml of PBS. The peritoneal cells were centrifuged at 280 × g and resuspended in RPMI 1640 (Biowest) medium supplemented with 20% FBS (Gibco), 50 μg/ml gentamycin (Bioshop), 10 ng/ml IL-3 and 30 ng/ml SCF. The cells were further cultured in 5% CO_2 at 37 °C. On the second day of cultivation, nonadherent cells were discarded. PMCs were used for experiments between 14 and 16 days of culture. Flow cytometry analysis identified that 98% of the cells were c-Kit$^+$, and 40% of the cells were double positive for FcεRI and c-Kit and could be ranked as mast cells. The cells were then subjected to reverse transcription-quantitative polymerase chain reaction (RT-qPCR), a degranulation assay, and flow cytometry.

2.4. Stimulation of PMCs with imiquimod and normal or psoriatic mouse skin explant eluates.

Skin specimens, measuring approximately 2 cm^2, were obtained from untreated and three-day IMQ-treated C57BL/6 mice and floated dermal side down in a 6-well plate containing 2 ml of RPMI 1640 medium supplemented with 20% FCS and 50 μg/ml gentamycin and incubated at 37 °C for 24 h. The collected media were then centrifuged at 500 × g, supplemented with 10 ng/ml IL-3 and 30 ng/ml SCF, snap-frozen in liquid nitrogen, and stored at −80 °C until further use. PMCs were seeded in cell culture medium (RPMI 1640 medium supplemented with 20% FBS, 50 μg/ml gentamycin, 10 ng/ml IL-3 and 30 ng/ml SCF) or conditioned media from mouse full-thickness skin explants at a density of 2 × 10^5 cells per well in a 96-well plate with a rounded bottom. Imiquimod was added to selected wells at a concentration of 2 μg/ml. After 6 or 24 h of incubation, the plate was centrifuged at 280 × g for 5 min. The cells were then subjected to β-HEX release assay or RNA extraction followed by RT-qPCR.

2.5. RT-qPCR

Total skin and peritoneal mast cells were subjected to RNA isolation using Fenozol Plus and the Total RNA Zol-Out Kit (A&A Biotechnology). The RNA concentration and purity were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). Equal amounts of RNA were converted to cDNA (Labcycler, SensoQuest) using NxGen M−MulV reverse transcriptase (Lucigen Corporation) with random primers (Promega Corporation). RT-qPCR was performed on a CFX96 thermocycler (Bio-Rad Laboratories) using RT HS PCR Mix SYBR (A&A

Biotechnology) containing universal PCR master mix. The following primers for specific mouse gene transcripts were designed using the Primer BLAST tool (NCBI) and purchased from Genomed: cyclophilin A (housekeeper) (*Ppia*, 5′-AGCATACAGGTCCTGGCATCTTGT, 5′-CAAAGACCACATGCTTGCCATCCA), *Mrgprb2* (5′-CATTAGCATTGAGCGC TGCTT, 5′-CCTTCCAGGAGACCCAACAA), Chymase 1 (*Cma1*, 5′-CTCACTGTGCGGGAAGGTC, 5′-TTGGCTTTCTCCTTCAACTTCAG), Tryptase β2 (*Tpsb2*, 5′-TGCACCCCCACTATTACACG, 5′-GTGGGAGAGGCTCGTCATT), c-kit (*Kit*, 5′-GCCAGTGCTTCCGTGACATT, 5′-CAGGAGTGGTACACCTTTGCTC), and *Slpi* (5′-GTCCTGCGGCCTTT TACCTT, 5′-TACGGCATTGTGGCTTCTCA). Relative gene expression normalized to housekeeping gene was calculated using the $2^{-\Delta\Delta CT}$ method [23].

2.6. Immunohistochemistry and immunofluorescence

2.6.1. Hematoxylin and eosin staining

The mouse skin was fixed in PBS-buffered 4% formaldehyde (Avantor) and embedded in paraffin (Sigma-Aldrich). Ten-micron-thick skin sections were stained with hematoxylin and eosin (Thermo Scientific) using a standard protocol. Images were captured using a Nikon light microscope.

2.6.2. Toluidine blue staining

Cryo-matrix-embedded skin biopsies were sectioned into 8 μm sections, fixed in 4% formaldehyde (Avantor) and stained with 0.1% toluidine blue solution (Pol-Aura). The sections were then dehydrated in 95% and 100% ethanol (Avantor), cleared in xylene (Avantor) (2 changes) and sealed in Consul Mount Medium. Images were captured using a Nikon light microscope and analyzed by NIS-Elements software (Nikon).

2.6.3. Immunofluorescence

All human studies were performed in accordance with guidelines established by the Jagiellonian University Institutional Bioethics Committee under approved protocols (#87/B/2014; 1072.6120.30.2020) and adhered to the Declaration of Helsinki. Human samples were collected from individuals who were fully informed and had consented.

Frozen 6 μm sections were prepared from skin biopsies of psoriatic patients (age 34.5 ± 13.5; F:M 2:1; PASI 15.35 ± 3.35) and healthy donors (age 43 ± 13; F:M 2:1) and fixed in ice-cold acetone. Sections were incubated with monoclonal-biotin-mouse-anti-SLPI antibody (Abcam) or isotype control (mouse IgG1 biotin-conjugated antibody) (BD Pharmingen) followed by phycoerythrin-conjugated streptavidin (eBioscience). The mast cells were stained with FITC-conjugated avidin. The samples were counterstained with Hoechst 33,258 (Life Technologies). Images were captured using a fully motorized fluorescence microscope (Eclipse; Nikon) and analyzed using NIS-Elements software (Nikon).

2.7. Flow cytometry

Skin fragments measuring 0.5 cm^2 from both the IMQ-treated dorsal site (central skin) and the adjacent untreated site within the altered region (lateral skin) were cut into small pieces using scissors. The pieces were then incubated with a solution of 2.5 mg/ml collagenase D (Roche Diagnostics) at 37 °C while being shaken continuously for 50 min. Single-cell suspensions were obtained by mashing the tissue through 40 μm cell strainers in RPMI 1640 medium supplemented with 2% FBS. The peritoneal cells were harvested as described above. The cells were stained for viability assessment using the Zombie Aqua Fixable Viability Kit (BioLegend) and then blocked with anti-CD16/CD32 antibodies (Fc block; eBioscience) followed by staining with directly conjugated antibodies: CD45.2-APC/Cy7 (clone 104, BioLegend), CD11b-eFluor450 (clone M1/70, eBioscience), c-Kit-PE/Cy7 (clone 2B8, BioLegend), FcεRI-FITC (clone MAR-1, eBioscience), and IgE-FITC (clone RME-1, BioLegend). Singlets were selected on the basis of FCS-A vs. FCS-H. Dead cells were routinely excluded from the analysis. “Fluorescence minus one” (FMO) controls were routinely used to verify correct compensation and to set the thresholds for positive/negative events.

2.8. β-HEX release assay

The analysis of PMC degranulation was based on the levels of β-HEX activity in the supernatant. Briefly, PMCs were seeded in cell culture medium at a density of 5×10^4 cells per well in a 96-well plate with a rounded bottom. For IgE stimulation, mast cells were sensitized for 2 h at 37 °C with mouse IgE (BD Pharmingen) at a concentration of 1 μg/ml. The cells used for Mrgprb2-mediated mast cell degranulation were incubated without the presence of antibodies. After incubation, the cells were washed three times with extracellular buffer consisting of 10 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 0.4 mM Na_2HPO_4, 5.6 mM glucose, 1.8 mM $CaCl_2$, 1.3 mM $MgSO_4$, and 0.04% BSA fraction V (Gibco). Next, extracellular buffer containing either compound 48/80 (50 μg/ml; C48/80), rat anti-mouse IgE (10 μg/ml; BD Pharmingen), or SP (Roche Diagnostics) (50 μM) was added to the appropriate wells, and the cells were further incubated at 37 °C for an additional hour. The plate was then centrifuged at 280 × g for 5 min, and 50 μl of the supernatant was collected. The cells in the plate were further lysed with 0.2% Triton X-100 (Sigma-Aldrich) for 5 min, and 50 μl of the lysate was collected. Finally, 100 μl of 1 mM PNAG in citrate buffer (pH = 4.5) was added to each well containing the supernatant or cell lysate, and the 96-well plate was incubated at 37 °C for 90 min. After incubation, 50 μl of 0.4 M glycine (pH = 10.7) was added to each well to stop the reaction. The percentage of β-hexosaminidase release was assessed by measuring the absorption of the samples at 405 nm.

2.9. Preparation of mastocyte protein lysates and chymase activity quantification

Mastocyte cells (5×10^5) were pelleted and frozen in liquid nitrogen followed by further processing at 4 °C. The cells were resuspended in 150 μl of lysis buffer (0.1 M TRIS, 0.5 M NaCl, 5 mM $CaCl_2$ and 0.2% Triton X-100, pH = 8). The samples were lysed by being mechanically pipetted. After the lysis samples had been centrifuged for 10 min at 16000 × g, supernatants were collected and stored at −80 °C until use. Chymase activity was assayed in a fluorometric test using Suc-AAPF-AMC (Sigma-Aldrich) as a substrate. The assay was conducted on a 96-well black flat-bottom plate (Sarstedt). The final single reaction volume was 100 μl, while the final substrate concentration was 0.45 mM. The plate was placed in a microplate reader that had been prewarmed to 37 °C (Tecan Infinite M200). The fluorescence with an excitation wavelength of 380 nm and an emission wavelength of 460 nm was measured instantly (to measure the background level) and after 30 min of incubation.

2.10. Statistical analysis

The data were analyzed and visualized using Prism software (GraphPad Software) and are presented as either the mean ± standard deviation (SD) or the mean ± standard error of the mean (SEM). The Mann-Whitney test was used to compare two groups, while analysis of variance (ANOVA) with the Bonferroni post hoc test was used for multiple comparisons. Differences were considered statistically significant if the p value was <0.05.

3. Results

3.1. The number of mast cells did not significantly differ in psoriatic-like skin of SLPI KO and WT mice.

To determine whether SLPI plays a role in controlling the number of

mast cells and their maturation and/or function in skin inflammation associated with chronic inflammatory cutaneous pathology, we subjected SLPI KO mice and their WT littermates to IMQ treatment. In agreement with our previous report, we observed early symptoms of skin alterations such as skin thickening, redness and scaling at approx. Day 3 in both SLPI KO and WT mice [11]. These skin changes were accompanied by a robust influx of leukocytes into the IMQ-challenged skin. At Day 6 (experimental endpoint), both mouse strains developed psoriasis-like dermatitis that manifested in further progression of skin alterations, including epidermal hyperplasia (Fig. 1S).

Because mast cells are located in the skin in association with nerves and SLPI cooperates with the cutaneous nervous system to maintain skin barrier integrity [11], we first examined whether SLPI ablation affects mast cell numbers in both central (IMQ-treated) and lateral (adjacent) skin sites following IMQ challenge. As shown in Fig. 1A, skin sections from SLPI KO and WT mice revealed similar numbers of toluidine blue-stained mast cells in both the central and lateral skin of SLPI-deficient and WT mice at Days 0, 3 and 6 of IMQ treatment. A nonsignificant trend toward a lower density of mast cells was observed in the skin of both mouse types at Day 3 (Fig. 1B). These data were corroborated using flow cytometry analysis when a comparable number of c-Kit$^+$ and FcεRI$^+$ mast cells were found in the central and lateral skin samples of SLPI KO and WT mice in resting skin (Day 0), with a similar tendency toward a decreased number of mast cells, particularly in the central skin of both strains at Day 3 (Fig. 1C and Fig. 2S). In agreement with a strong infiltration of the skin by immune cells 3 days after IMQ challenge, a significant reduction in mastocytes among CD45$^+$ leukocytes was observed at this point in the central and, to a lesser extent, lateral skin, but again in a manner independent of SLPI ablation (Fig. 1C). Together, these data suggest that SLPI does not influence the quantity and percentile of cutaneous mast cells in experimental psoriasis.

3.2. *The expression levels of mast cell markers are similarly altered in experimental psoriasis in SLPI KO and WT mice.*

We next determined whether SLPI deficiency has an impact on the cutaneous levels of mast cell-associated differentiation and functional markers by RT-qPCR. These include mRNA for the genes that are primarily or exclusively expressed in the mast cells in the skin, such as the indicator of mast cell maturation *Kit* (encoding c-Kit, SCF receptor), *Tpsb2* (encoding tryptase beta 2), *Cma1* (encoding chymase), and mast cell receptor *Mrgprb2* [17,18]. As shown in Fig. 2, SLPI KO and WT mice demonstrated comparable *Kit* mRNA expression levels in the central and lateral skin over the course of IMQ treatment, with an overall tendency toward small upregulation of *Kit* at Day 6. Likewise, *Tpsb2* tended to be upregulated at Day 3 in the central skin, particularly in mice deficient in SLPI. However, in contrast to *Kit* and *Tpsb2*, the transcript levels of *Cma1* in the central and lateral skin sites were reduced at Day 3 in both SLPI-KO and WT mice, and *Mrgprb2* was markedly downregulated at Days 3 and 6 compared to baseline in both SLPI KO and WT mice. These data suggest that SLPI does not regulate mast cell differentiation, as evidenced by similar transcription levels of *Kit*, *Tpsb2*, *Cma1* and *Mrgprb2* in

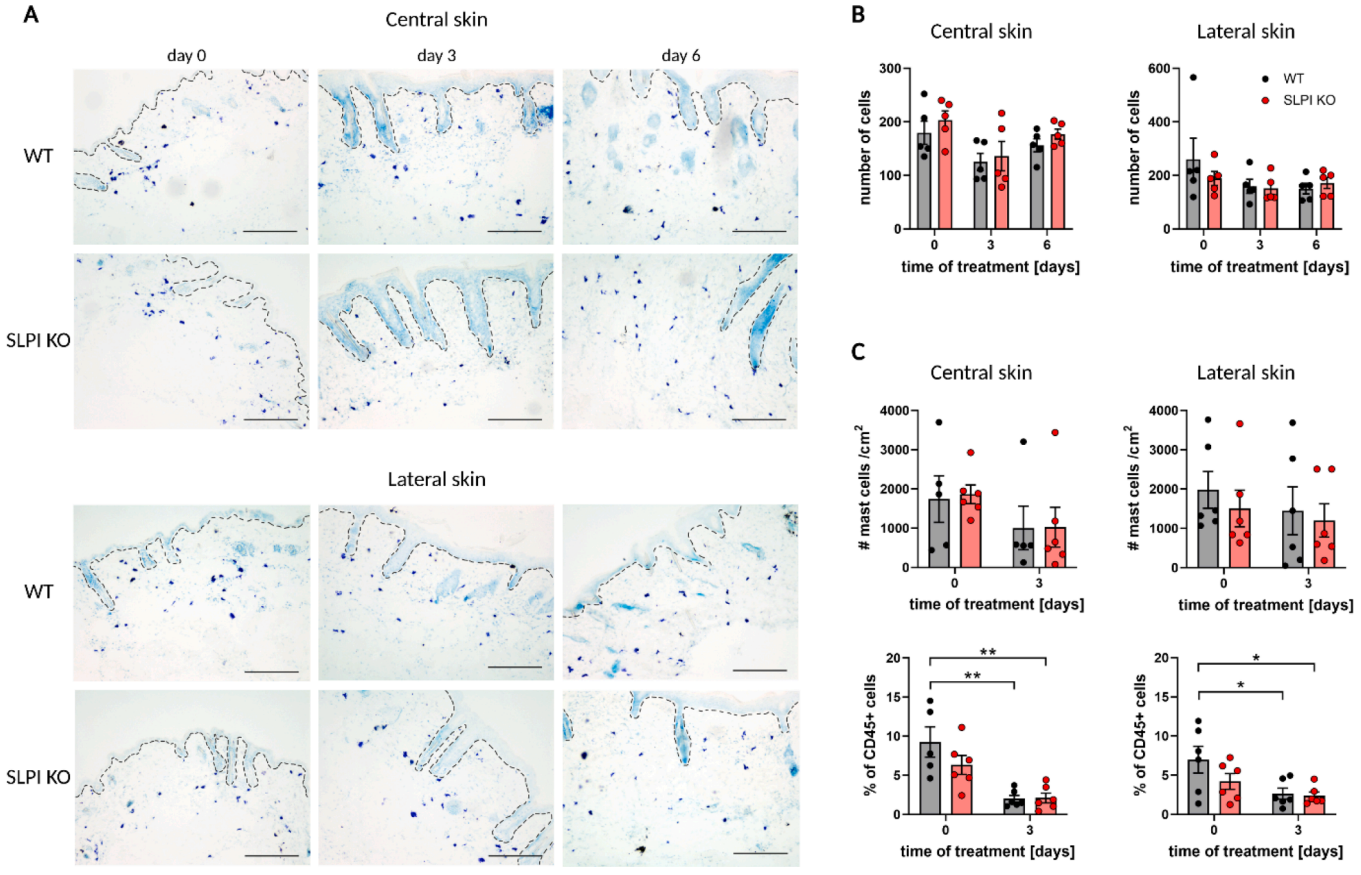

Fig. 1. In an experimental model of psoriasis, SLPI deficiency does not change the number of mast cells in the skin. WT and SLPI KO mice were subjected to IMQ treatment. **A.** Representative microscopy images of toluidine blue-stained mast cells in central and lateral skin on the indicated days. The epidermis (top) and dermis are separated by a dashed line. Scale bar = 100 μm. **B.** Quantification of mast cell numbers based on toluidine blue staining as in (A). Bars indicate the mean ± SEM, with each point representing one mouse, n = 5. For each mouse, 10 different high-power fields were analyzed. **C.** Flow cytometry analysis of mast cell counts and percentages in the central and lateral skin from WT mice (black) and SLPI KO mice (red). Data are shown as the absolute number of c-Kit$^+$ and FcεRI$^+$ mast cells in 1 cm^2 of the indicated skin regions (upper panel) and the percentage of mast cells among CD45$^+$ cells (lower panel). Each data point represents one mouse, n = 6, and bars indicate the mean value ± SEM. *, $p < 0.05$; **, $p < 0.01$ by one-way ANOVA, Bonferroni post hoc test.

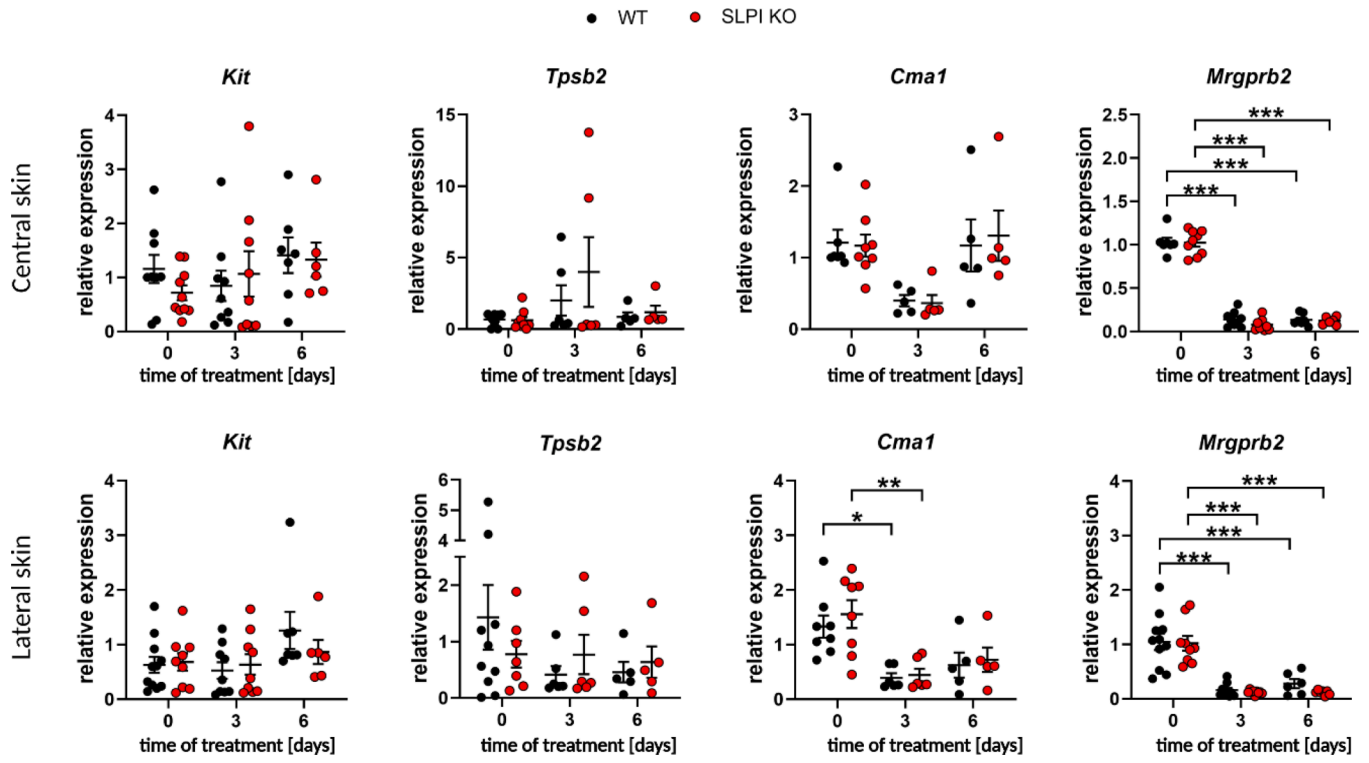

Fig. 2. SLPI does not significantly affect cutaneous mRNA expression profiles of genes characteristic of mature mast cells. WT and SLPI KO mice were subjected to IMQ treatment. The central and lateral skin were harvested on the indicated days and subjected to RT-qPCR analysis for *Kit, Tpsb2, Cma1* and *Mrgprb2* mRNA levels. Data are shown as the mean ± SEM from three independent experiments. Black dots = WT mice; red dots = SLPI KO mice; each dot indicates an individual mouse. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; by one-way ANOVA, Bonferroni post hoc test.

the skin harvested from SLPI KO and WT mice (Fig. 2). However, the expression of *Mrgprb2* and, to a lesser extent, *Cma1* changed in IMQ-challenged skin. Since the slightly reduced number of mast cells cannot solely account for such a marked downregulation of cutaneous *Mrgprb2* and *Cma1* levels, together, these data suggest that cutaneous mast cells may be functionally compromised in IMQ-induced psoriasis-like dermatitis.

3.3. *Cutaneous mast cells are a source of SLPI in experimental psoriasis.*

Despite the negligible effects of SLPI on the number of mast cells and phenotypic markers, SLPI levels greatly increased in the lesional skin of patients with psoriasis and in the experimental model of psoriasis (Fig. 3A and [4,8,11]). Therefore, this protein is strategically positioned to functionally impact mast cells in the psoriatic skin microenvironment. Although mast cells that reside in the skin might be influenced by SLPI produced by various cell types in IMQ-challenged skin, it is likewise probable that mast cells rely on their own program of SLPI expression, for example, to control intracellular proteases.

To determine whether SLPI is expressed in mastocytes in psoriatic skin, we examined human skin biopsies. As shown in Fig. 3B, avidin$^+$ mast cells in the dermis were found to costain for SLPI in psoriatic skin but not in skin from healthy donors. Overall, virtually all mast cells from healthy individuals were negative for SLPI, whereas > 93% of dermal mastocytes stained for SLPI in individuals with psoriasis (n = 3 donors in each group). Together, these data suggest that a psoriatic skin environment upregulates SLPI expression in mast cells.

In an IMQ-induced experimental model of psoriasis, IMQ and/or the secretome of IMQ-treated cells in the skin might therefore be likely candidates to affect SLPI production in dermal mastocytes. Next, we tested whether mast cells can be induced to express SLPI by these factors. This question was explored using a widely used model of murine PMCs that in many aspects resemble dermal mast cells [16]. We subjected PMCs to (i) IMQ, and/or (ii) conditioned media from normal skin explant cultures, or (iii) to the eluates of the skin *in vivo* challenged by IMQ for 3 days (referred to as the psoriasis skin explant conditioned media). Untreated PMCs were found to produce detectable *Slpi* mRNA levels by RT-qPCR (Fig. 3C). Although short-term (6 h) mast cell treatment did not change *Slpi* levels in PMCs, the transcriptional levels of this inhibitor were significantly upregulated as a result of 24 h *in vitro* mast cell stimulation with IMQ and normal and psoriatic skin-derived mediators (Fig. 3D). Moreover, IMQ and normal skin explant conditioned media appeared to exert additive effects on SLPI expression (Fig. 3D). This was also observed when PMCs were treated with psoriasis skin explant conditioned media for 24 h. These psoriasis-like skin eluates that likely contained both IMQ and psoriatic skin-derived factors were, on average, the most potent in upregulating SLPI levels in mastocytes (Fig. 3D). Together, these data suggest that IMQ and skin-derived compounds cooperate for optimal SLPI expression in mastocytes.

3.4. *SLPI controls FcεRI-dependent but not Mrgprb2-dependent mast cell degranulation.*

Thus far, our data have indicated that mast cells produce SLPI under conditions that support the development of psoriasis. To determine whether the presence of SLPI in mastocytes is functionally relevant, we first measured chymase-based proteolytic activity in SLPI-deficient and WT PMCs. As expected, SLPI KO mast cells were found to display statistically higher chymase activity than WT PMCs, suggesting that SLPI indeed inhibits chymase activity in mastocytes (Fig. 4A). Next, we compared the ability of SLPI-ablated and WT PMCs to degranulate following the activation of FcεRI or Mrgprb2. Whereas the antibody anti-IgE was used to activate IgE-bearing mast cells by cross-linking FcεRI-bound IgE, the Mrgprb2 signaling cascade was triggered by cationic C48/80. Alternatively, Mrgprb2 was activated by the natural agonist of this receptor, the SP neuropeptide. We used two ligands of

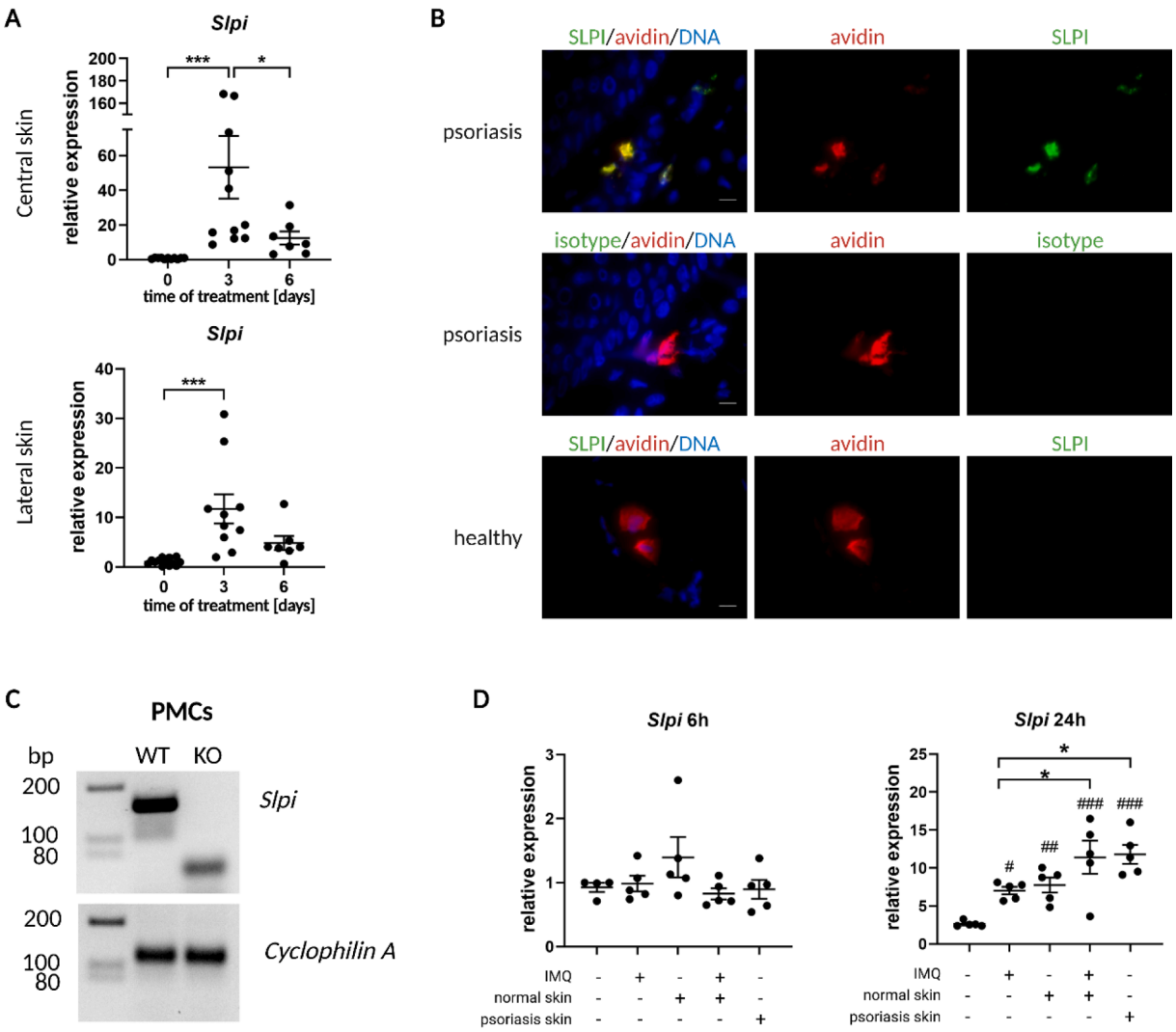

Fig. 3. SLPI is expressed in mastocytes. A. WT mice were subjected to IMQ treatment. The central and lateral skin was harvested on the indicated days, followed by RT-qPCR for *Slpi* mRNA levels. The data are shown as the mean ± SEM from three independent experiments. Each dot indicates an individual mouse. *, $p < 0.05$; ***, $p < 0.001$; by one-way ANOVA, Bonferroni post hoc test. **B.** Representative immunofluorescence microscopic image of skin samples from psoriasis and healthy donors stained for mast cells (red), SLPI (green) and DNA (blue). For each donor, 10 different high-power fields were analyzed. n = 3 donors in each group, scale = 10 μm. **C.** PMCs from WT and SLPI KO mice were subjected to RT-qPCR to determine *Slpi* mRNA levels. The resulting PCR products were then separated using gel electrophoresis. Cyclophilin A was used as a housekeeping gene. **D.** PMCs from WT mice were treated with the indicated components for 6 h or 24 h followed by RT-qPCR. Each dot indicates an individual mouse. *, #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$; by one-way ANOVA, Bonferroni post hoc test. # comparing the indicated samples with the control.

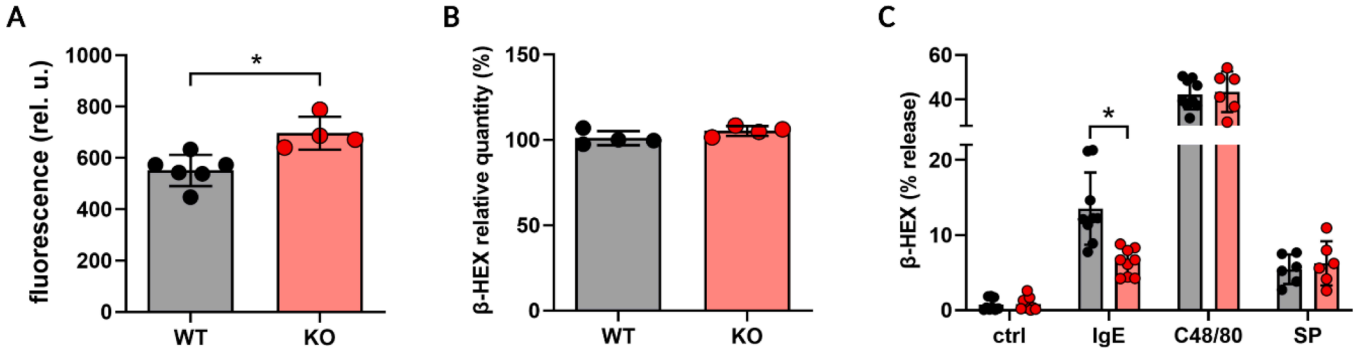

Fig. 4. SLPI affects mastocyte function. A. PMCs from WT and SLPI KO mice were analyzed for chymase activity using fluorimetry. The mean ± SD from two independent experiments is shown. *, $p < 0.05$; by Mann-Whitney test. **B.** β-HEX levels were measured in cell lysates from WT and SLPI KO PMCs, and the mean ± SD is shown, n = 4. **C.** Degranulation of PMCs measured by β-HEX release following stimulation by anti-IgE Abs (10 μg/ml), C48/80 (50 μg/ml), SP (50 μM) or with extracellular buffer alone as a control. The mean ± SD is shown, n = 6. *, $p < 0.05$; by one-way ANOVA, Bonferroni post hoc test.

Mrgprb2 since they can differ in the extent of granule release, with C48/80 being overall the stronger inducer of mast cell degranulation ([24] & Fig. 4C). We assessed the capacity of these FcεRI or Mgprb2 stimuli to mediate granule secretion from SLPI-deficient and SLPI-sufficient (WT) mastocytes by measuring the release of β-HEX, a component of mast cell granules. SLPI KO and WT mast cells did not differ in their total β-HEX content and showed an equivalent level of extracellular β-HEX following C48/80– and SP–triggered activation (Fig. 4B & C). However, FcεRI-mediated degranulation was significantly, on average, > 50% lower in SLPI KO mast cells than in WT mast cells (mean ± SD; WT: 15.3 ± 4.8% v/s KO: 6.4 ± 1.8%) (Fig. 4C). Together, these data suggest that SLPI regulates mast cell degranulation in a receptor-specific manner.

To better understand the specificity of SLPI-dependent FcεRI-mediated but not Mrgprb2-mediated mast cell degranulation, we reasoned that this might be due to the availability of the receptors for their respective agonists at the time when SLPI is highly expressed in mast cells. Indeed, RT-qPCR analysis of *Mrgprb2* and *Fcer1a* (α subunit of FcεRI) mRNA levels in mast cells subjected to IMQ and/or skin eluate treatment demonstrated that in contrast to *Fcer1a*, *Mrgprb2* showed a tendency to be or was reduced by IMQ and the skin explant cultures, respectively (Fig. 5A & B). Moreover, the combination of IMQ and skin explant cultures appeared to downregulate *Mrgprb2* expression in PMCs in an additive manner (Fig. 5A). Again, the lowest *Mrgprb2* mRNA levels were observed when PMCs were treated with psoriasis skin explant conditioned media (Fig. 5A). The diminished *Mrgprb2* transcripts in isolated mast cells subjected to IMQ and/or skin explant culture treatment are consistent with a reduction in *Mrgprb2* levels in psoriatic-like skin (Fig. 5A and Fig. 2). Dermal mast cells are the only cells known to express *Mrgprb2* in the skin [17]. Therefore, nearly abolished *Mrgprb2* levels in IMQ-challenged skin are likely due to the compromised ability of cutaneous mast cells to express *Mrgprb2* under these conditions.

Next, we examined the impact of IMQ and/or skin explant conditioned media treatment on anti-IgE Abs versus C48/80- and SP-triggered release of β-HEX from PMCs. It was likely that pretreatment of PMCs with IMQ and the skin eluate would upregulate SLPI and downregulate Mrgprb2 levels in these cells and enhance the effect of SLPI on FcεRI-mediated but not Mrgprb2-mediated degranulation. However, under these conditions, the effect of SLPI on anti-IgE-stimulated degranulation was similar to that in PMCs not treated with IMQ or not exposed to skin explant conditioned media prior to the anti-IgE challenge (Fig. 5C). In contrast, we observed a significantly reduced release of β-HEX from PMCs that had first been exposed to psoriatic skin explant conditioned media, which was likely a result of the downregulation of the surface levels of FcεRI in PMCs by psoriatic skin compounds (Fig. 5C and Fig. 3S). As expected, SLPI did not affect SP– and C48/80-dependent degranulation under any conditions (Fig. 5D & E). These data suggest that basic levels of SLPI are sufficient to promote IgE-triggered degranulation of PMCs and that upregulated SLPI expression levels do not have an immediate, measurable effect on FcεRI-dependent and Mrgprb2-mediated degranulation. However, when PMCs were treated with skin explant conditioned media prior to SP but not C48/80 stimulation, degranulation was significantly reduced in correlation with the lower levels of Mrgprb2 in these cells (Fig. 5D & E). These data suggest that a psoriatic skin environment, by downregulating Mrgprb2 levels, makes mast cells less responsive to natural cutaneous Mrgprb2 ligands, such as SP.

4. Discussion

Mast cells are implicated in a variety of pathophysiological processes in the skin, such as efficient clearance of intradermally administered *S. aureus* [16] or early onset of inflammation in chronic inflammatory skin diseases, including psoriasis-like dermatitis [21]. However, little is known about how the function of these cells is regulated in a cutaneous environment. Our data suggest that SLPI is one of the factors capable of conditioning mastocyte function. SLPI adds to recently identified regulators of cutaneous mast cells such as keratinocyte-derived SCF or neuron-derived glutamate. However, in contrast to these mediators, SLPI appears not to be involved in mast cell tissue differentiation at steady state or in maintaining skin homeostasis by inhibiting mast cell

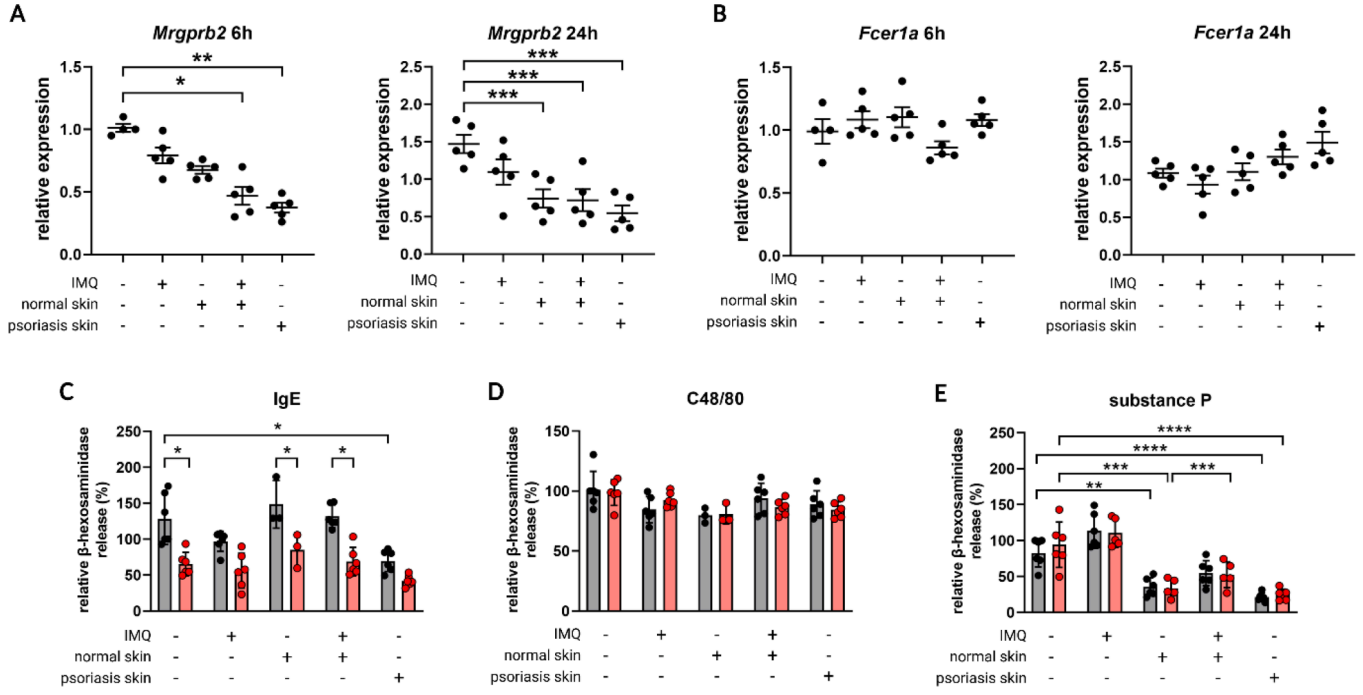

Fig. 5. Skin-derived factors do not potentiate the pro-granulation effect of SLPI in anti-IgE-treated mast cells but substantially reduce *Mrgprb2* mRNA levels and function in PMCs. WT and SLPI KO PMCs were treated with the indicated factors for 6 h and 24 h, followed by RT-qPCR for **A.** *Mrgprb2* levels and **B.** *Fcer1a* levels. Relative degranulation of PMCs measured by β-HEX release following stimulation by **C.** anti-IgE Abs (10 μg/ml), **D.** C48/80 (50 μg/ml), and **E.** SP (50 μM). The mean ± SD is shown, n = 6. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; by one-way ANOVA, Bonferroni post hoc test.

inflammatory responses [17,18]. Instead, mast cell-derived SLPI inhibits intrinsic chymase activity and facilitates mast cell degranulation in the pathway mediated by FcεRI.

There are different reports on the ability of mast cells to express SLPI [25,26]. Our studies demonstrating the expression of SLPI in human dermal mastocytes in psoriasis patients and murine PMCs are consistent with the previously reported presence of SLPI in mast cells in human tonsils and nasal mucosa detected by immunohistochemistry and in situ hybridization [26]. In contrast, murine bone marrow (BM)-derived mastocytes were previously shown not to express detectable mRNA and protein SLPI levels [25]. Distinct mast cell populations, or different functional properties of BM-derived mastocytes, such as a lower maturation status compared to PMCs and connective-tissue type mast cells, might have contributed to these different observations. By showing that the ability of mast cells to express SLPI is strongly influenced by the tissue context and greatly induced by factors produced as a result of chronic skin inflammation, our findings offer a possible explanation for these discrepancies.

Our data support the conclusion that mast cells activated by an FcεRI-dependent but not Mrgprb2-dependent mechanism rely on SLPI for their efficient degranulation. Mast cells are known to exhibit variation in the spatiotemporal features of degranulation and in the physical characteristics of the released granule structure and content, which depend on the strength and/or nature of the stimuli acting via different receptors [20,27]. Although under our experimental conditions C48/80 was the most potent stimulus of β-HEX release from mast cells, the extent of PMC degranulation was comparable for SP and anti-IgE. However, SLPI was effective as a degranulation-facilitating factor only when anti-IgE Abs were used to trigger mast cell responses. Together, these findings suggest that the effect of SLPI does not depend on the strength of the mast cell stimulation but on the nature of the degranulation strategy, mediated by the different receptors. Activation of mast cells via FcεRI or Mrgprb2 follows different dynamics and patterns of degranulation. Specifically, Mrgprb2-inducing factors are more rapid and trigger the release of smaller nonintracellularly fused secretory granules than anti-IgE Abs, which mobilize large and aggregated secretory granules [20]. These variant degranulation strategies are associated with delayed but more sustained mast cell-mediated responses *in vivo* when mastocytes are triggered by IgE antigens compared to Mrgprb2 agonists [20]. Therefore, SLPI would be expected to augment the release of the pre-stored components of mast cells in association with less-rapid but more persistent anti-IgE-triggered mast cell responses.

One possible outcome of the SLPI-dependent regulation of cutaneous mast cell degranulation could be the enhancement of skin defense that relies on IgE. At steady state, dermal mast cells carry high levels of IgE Abs on their FcεRI receptors. Moreover, topical exposure to factors that induce skin inflammation in mice, including psoriasis-like inflammation, increases IgE levels in serum and IgE-mediated surveillance, in part by binding to mast cells residing in the skin [28]. This is consistent with elevated levels of serum IgE observed in human individuals suffering from psoriasis [29] and in line with the host's need to fortify skin barrier defense that is compromised due to pathogenic skin alterations in psoriasis. Since IgE Abs that are induced by "chronic-like" experimental skin inflammation resemble in their repertoire IgE in healthy mice, these Abs might represent the elevated pool of IgE already available in the host at steady state [28]. Among the potential targets of IgE Abs are environmental antigens, including those acquired as a result of parasitic infections or skin challenge with irritants or noxious stimuli [28]. Therefore, SLPI, via its impact on mast cell degranulation, could strengthen the host's natural immune responses that are dependent on IgE. This may lead to the promotion of IgE-mediated cutaneous reactions and stronger protection against skin irritants/microbial challenge.

Our data also suggest that even basic, potentially relatively low levels of SLPI, as observed in PMCs at steady state, are sufficient to control FcεRI–mediated release of granule-loaded mediators, such as β-HEX. Upregulated SLPI expression coincided with the high presence of transcripts for FcεRI in mast cells, although the cell surface levels of the receptor were diminished as a result of PMC stimulation with psoriasis skin eluates. Nevertheless, either SLPI or FcεRI were available together in sufficiently high levels in the mast cells at steady state and in PMCs challenged with skin explant conditioned media, further suggesting a functional link between FcεRI and SLPI in mast cell responses. On the other hand, SLPI expression in mastocytes showed the opposite trend to Mrgprb2, providing a plausible explanation as to why SLPI did not influence Mrgprb2-mediated mast cell degranulation.

Our studies have primarily focused on the role of SLPI in mast cell biology. However, the marked downregulation of Mrgprb2 by skin explant conditioned media from normal and psoriasis-like skin in particular, along with the decreased response of the mast cells to the Mrgprb2 ligand SP we noticed here, suggest important implications for dermal mast cell-mediated responses. Our findings suggest that the skin environment has a strong controlling influence on mast cell Mrgprb2 function, possibly to avoid unnecessary skin responses under homeostatic conditions and to limit the effect of Mrgprb2 in psoriasis-like dermatitis. Only in the presence of a powerful Mrgprb2 activator (in our settings mimicked by high levels of C48/80) can Mrgprb2-dependent skin reactions be induced. In view of the potential of mast cell Mrgprb2 to drive early psoriatic-like skin inflammation and excite itch-sensory neurons in association with pruritic chronic inflammatory skin disorders, dysregulation of Mrgprb2 in dermal mast cells in association with psoriasis could result in less intense and more transient mast cell activation. Only the presence of the robust mast cell stimulators of Mrgprb2 could potentially overcome a strongly suppressive skin environment.

5. Conclusions

In summary, the data presented here demonstrate that SLPI can modulate IgE-dependent mast cell responses, whereas the availability of Mrgprb2 in mast cells is under the control of skin factor(s) during the steady state and to a larger extent in low-grade "psoriatic-like" skin inflammation. These findings provide previously undescribed mechanisms for the regulation of mast cell function in the skin environment. However, this study has certain limitations. First, the effect of SLPI on mast cells may not be limited solely to FcεRI but could potentially involve other receptors as well. Additionally, the functional impact of SLPI may extend beyond the regulation of mast cell degranulation. Therefore, further investigation into the regulatory activities of SLPI in human dermal mast cells represents a crucial avenue for future research.

CRediT authorship contribution statement

Patrycja Kwiecinska: Investigation, Methodology, Visualization, Writing – review & editing. **Mateusz Kwitniewski:** Investigation, Methodology, Visualization, Writing – review & editing. **Kamila Kwiecień:** Investigation, Methodology, Writing – review & editing. **Agnieszka Morytko:** Investigation, Methodology, Writing – review & editing. **Pawel Majewski:** Investigation, Methodology, Writing – review & editing. **Natalia Pocalun:** Investigation, Writing – review & editing. **Maciej Pastuszczak:** Resources, Writing – review & editing. **Marcin Migaczewski:** Resources, Writing – review & editing. **Joanna Cichy:** Conceptualization, Writing – original draft, Writing – review & editing. **Beata Grygier:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2023.110631.

References

[1] M. Majchrzak-Gorecka, P. Majewski, B. Grygier, K. Murzyn, J. Cichy, Secretory leukocyte protease inhibitor (SLPI), a multifunctional protein in the host defense response, Cytokine Growth Factor Rev 28 (2016) 79–93.
[2] C.C. Taggart, S.A. Cryan, S. Weldon, A. Gibbons, C.M. Greene, E. Kelly, T.B. Low, J. O'Neill, N.G. McElvaney, Secretory leucoprotease inhibitor binds to NF-kappaB binding sites in monocytes and inhibits p65 binding, J Exp Med 202 (12) (2005) 1659–1668.
[3] C.C. Taggart, C.M. Greene, N.G. McElvaney, S. O'Neill, Secretory leucoprotease inhibitor prevents lipopolysaccharide-induced IkappaBalpha degradation without affecting phosphorylation or ubiquitination, J Biol Chem 277 (37) (2002) 33648–33653.
[4] K. Zabieglo, P. Majewski, M. Majchrzak-Gorecka, A. Wlodarczyk, B. Grygier, A. Zegar, M. Kapinska-Mrowiecka, A. Naskalska, K. Pyrc, A. Dubin, S.M. Wahl, J. Cichy, The inhibitory effect of secretory leukocyte protease inhibitor (SLPI) on formation of neutrophil extracellular traps, J Leukoc Biol 98 (1) (2015) 99–106.
[5] G.S. Ashcroft, K. Lei, W. Jin, G. Longenecker, A.B. Kulkarni, T. Greenwell-Wild, H. Hale-Donze, G. McGrady, X.Y. Song, S.M. Wahl, Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing, Nat Med 6 (10) (2000) 1147–1153.
[6] S.S. Hannila, M.M. Siddiq, J.B. Carmel, J. Hou, N. Chaudhry, P.M. Bradley, M. Hilaire, E.L. Richman, R.P. Hart, M.T. Filbin, Secretory leukocyte protease inhibitor reverses inhibition by CNS myelin, promotes regeneration in the optic nerve, and suppresses expression of the transforming growth factor-beta signaling protein Smad2, J Neurosci 33 (12) (2013) 5138–5151.
[7] J. Zhu, C. Nathan, W. Jin, D. Sim, G.S. Ashcroft, S.M. Wahl, L. Lacomis, H. Erdjument-Bromage, P. Tempst, C.D. Wright, A. Ding, Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair, Cell 111 (6) (2002) 867–878.
[8] J. Skrzeczynska-Moncznik, A. Wlodarczyk, K. Zabieglo, M. Kapinska-Mrowiecka, E. Marewicz, A. Dubin, J. Potempa, J. Cichy, Secretory leukocyte proteinase inhibitor-competent DNA deposits are potent stimulators of plasmacytoid dendritic cells: implication for psoriasis, Journal of Immunology 189 (4) (2012) 1611–1617.
[9] G.J. de Jongh, P.L.J.M. Zeeuwen, M. Kucharekova, R. Pfundt, P.G. van der Valk, W. Blokx, A. Dogan, P.S. Hiemstra, P.C. van de Kerkhof, J. Schalkwijk, High expression levels of keratinocyte antimicrobial proteins in psoriasis compared with atopic dermatitis, Journal of Investigative Dermatology 125 (6) (2005) 1163–1173.
[10] G. Damiani, N.L. Bragazzi, C. Karimkhani Aksut, D. Wu, G. Alicandro, D. McGonagle, C. Guo, R. Dellavalle, A. Grada, P. Wong, C. La Vecchia, L.S. Tam, K. D. Cooper, M. Naghavi, The Global Regional, and National Burden of Psoriasis: Results and Insights From the Global Burden of Disease 2019, Study, Front Med (Lausanne) 8 (2021), 743180.
[11] P. Kwiecinska, B. Grygier, A. Morytko, A. Sanecka-Duin, M. Majchrzak-Gorecka, M. Kwitniewski, M. Kapinska-Mrowiecka, G. Porebski, J. Cichy, Secretory leukocyte protease inhibitor regulates nerve reflex-mediated skin barrier function in psoriasis, J Eur Acad Dermatol Venereol 36 (8) (2022) 1266–1274.
[12] C.W. Franzke, A. Baici, J. Bartels, E. Christophers, O. Wiedow, Antileukoprotease inhibits stratum corneum chymotryptic enzyme. Evidence for a regulative function in desquamation, J Biol Chem 271 (36) (1996) 21886–21890.
[13] J. Skrzeczynska-Moncznik, A. Wlodarczyk, M. Banas, M. Kwitniewski, K. Zabieglo, M. Kapinska-Mrowiecka, A. Dubin, J. Cichy, DNA structures decorated with cathepsin G/secretory leukocyte proteinase inhibitor stimulate IFNI production by plasmacytoid dendritic cells, Am J Clin Exp Immunol Cell Biol 2 (2) (2013) 186–194.
[14] J. Skrzeczynska-Moncznik, K. Zabieglo, O. Osiecka, A. Morytko, P. Brzoza, L. Drozdz, M. Kapinska-Mrowiecka, B. Korkmaz, M. Pastuszczak, J. Kosalka-Wegiel, J. Musial, J. Cichy, Differences in staining for neutrophil elastase and its controlling inhibitor SLPI reveal heterogeneity among neutrophils in psoriasis, J Invest Dermatol 140 (7) (2020) 1371–1378 e3.
[15] M. Walter, M. Plotnick, N.M. Schechter, Inhibition of human mast cell chymase by secretory leukocyte proteinase inhibitor: enhancement of the interaction by heparin, Arch Biochem Biophys 327 (1) (1996) 81–88.
[16] M. Arifuzzaman, Y.R. Mobley, H.W. Choi, P. Bist, C.A. Salinas, Z.D. Brown, S. L. Chen, H.F. Staats, S.N. Abraham, MRGPR-mediated activation of local mast cells clears cutaneous bacterial infection and protects against reinfection, Sci Adv 5 (1) (2019) eaav0216.
[17] S. Zhang, T.N. Edwards, V.K. Chaudhri, J. Wu, J.A. Cohen, T. Hirai, N. Rittenhouse, E.G. Schmitz, P.Y. Zhou, B.D. McNeil, Y. Yang, H.R. Koerber, T.L. Sumpter, A. C. Poholek, B.M. Davis, K.M. Albers, H. Singh, D.H. Kaplan, Nonpeptidergic neurons suppress mast cells via glutamate to maintain skin homeostasis, Cell 184 (8) (2021) 2151–2166 e16.
[18] Z. Wang, N. Mascarenhas, L. Eckmann, Y. Miyamoto, X. Sun, T. Kawakami, A. Di Nardo, Skin microbiome promotes mast cell maturation by triggering stem cell factor production in keratinocytes, J Allergy Clin Immunol 139 (4) (2017) 1205–1216 e6.
[19] S.J. Galli, M. Tsai, T. Marichal, E. Tchougounova, L.L. Reber, G. Pejler, Approaches for analyzing the roles of mast cells and their proteases in vivo, Adv Immunol 126 (2015) 45–127.
[20] N. Gaudenzio, R. Sibilano, T. Marichal, P. Starkl, L.L. Reber, N. Cenac, B.D. McNeil, X. Dong, J.D. Hernandez, R. Sagi-Eisenberg, I. Hammel, A. Roers, S. Valitutti, M. Tsai, E. Espinosa, S.J. Galli, Different activation signals induce distinct mast cell degranulation strategies, J Clin Invest 126 (10) (2016) 3981–3998.
[21] V. Heib, M. Becker, T. Warger, G. Rechtsteiner, C. Tertilt, M. Klein, T. Bopp, C. Taube, H. Schild, E. Schmitt, M. Stassen, Mast cells are crucial for early inflammation, migration of Langerhans cells, and CTL responses following topical application of TLR7 ligand in mice, Blood 110 (3) (2007) 946–953.
[22] L. van der Fits, S. Mourits, J.S. Voerman, M. Kant, L. Boon, J.D. Laman, F. Cornelissen, A.M. Mus, E. Florencia, E.P. Prens, E. Lubberts, Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis, J Immunol 182 (9) (2009) 5836–5845.
[23] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (4) (2001) 402–408.
[24] M. Babina, Z. Wang, S. Roy, S. Guhl, K. Franke, M. Artuc, H. Ali, T. Zuberbier, MRGPRX2 is the codeine receptor of human skin mast cells: desensitization through beta-arrestin and lack of correlation with the fcepsilonRI pathway, J Invest Dermatol 141 (5) (2021) 1286–1296 e4.
[25] S. Matsuba, T. Yabe-Wada, K. Takeda, T. Sato, M. Suyama, T. Takai, T. Kikuchi, T. Nukiwa, A. Nakamura, Identification of secretory leukoprotease inhibitor as an endogenous negative regulator in allergic effector cells, Front Immunol 8 (2017) 1538.
[26] U. Westin, A. Polling, I. Ljungkrantz, K. Ohlsson, Identification of SLPI (secretory leukocyte protease inhibitor) in human mast cells using immunohistochemistry and in situ hybridisation, Biol Chem 380 (4) (1999) 489–493.
[27] J. Meixiong, M. Anderson, N. Limjunyawong, M.F. Sabbagh, E. Hu, M.R. Mack, L. K. Oetjen, F. Wang, B.S. Kim, X. Dong, Activation of mast-cell-expressed mas-related G-protein-coupled receptors drives non-histaminergic Itch, Immunity 50 (5) (2019) 1163–1171 e5.
[28] M.D. Hayes, S. Ward, G. Crawford, R.C. Seoane, W.D. Jackson, D. Kipling, D. Voehringer, D. Dunn-Walters, J. Strid, Inflammation-induced IgE promotes epithelial hyperplasia and tumour growth, Elife 9 (2020).
[29] S.B. Paparo, M.A. Guaragna, M. Albanesi, High IgE levels in patients affected by psoriasis: review of the literature and personal observations, Clin Ter 165 (2) (2014) 91–93.