

IL-4 and human skin mast cells revisited: reinforcement of a pro-allergic phenotype upon prolonged exposure

Magda Babina¹ · Sven Guhl¹ · Metin Artuc¹ · Torsten Zuberbier¹

Received: 29 July 2016 / Revised: 4 September 2016 / Accepted: 13 September 2016 / Published online: 20 September 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract Mast cells (MCs), unique cellular elements of the body, are commonly associated with IgE-mediated reactions and manifestations of Th2-type immunity. A key characteristic of the lineage is its heterogeneity, with subsets displaying significant variation depending on maturation stage, species, tissue, microenvironment and other. Heterogeneity also affects MC responses to extracellular cues. Indeed, IL-4, the signature cytokine of Th2-immunity, can affect MCs in opposing ways ranging from the induction of apoptosis to positive regulation of lineage characteristics. It is unknown, however, whether IL-4 alters the phenotype of terminally differentiated human cutaneous MCs. Using our well-established technique for homogeneous purification of human skin MCs, we now report that prolonged contact with IL-4 not only increases MC expansion, but also phenotypically and functionally reshapes the cells. FcεRI cell surface expression, FcεRIα-specific mRNA and FcεRI-mediated histamine release are all augmented by IL-4, while histamine release elicited by the non-immunological stimulus, substance P, remains unaffected. IL-4's potential to mold MCs is broad and similarly detectable across donors. Intriguingly, IL-4 impacts granule-associated mediators, especially histamine whose synthesis is boosted in the presence of IL-4. To our knowledge, an increase in histamine *production* by IL-4

has not been described yet for any type of MCs, but may well contribute to its pro-allergic effect given the significance of this biogenic amine to allergic symptoms. Collectively, IL-4 alters human skin MCs after long-term exposure mimicking chronic disorders by strengthening MC numbers and intensifying processes associated with allergic inflammation.

Keywords Mast cells · IL-4 · Th2 · Histamine · FcεRI · Dermis

Introduction

Mast cells are unique elements of the human body [4, 16]. In humans, these cells continue to be best understood for their role as effectors of IgE-mediated allergic reactions and the sensation of itch [4, 6, 7]. Accordingly, MCs are numerically increased and/or functionally altered in conditions like atopic dermatitis and urticaria, disorders characterized by Th2-dominance at certain stages of the respective pathology [8, 14]. Interleukin-4 (IL-4) is the signature cytokine of Th2 cells and intimately associated with protective immunity to parasitic helminths and the allergic phenotype alike [1]. IL-4 affects MCs, in which it can provoke complex and even opposing response patterns [15, 17]. The inconsistency is the result of MC heterogeneity, i.e., the variability of MC subsets across species, tissues, differentiation stages, microenvironmental conditions and others. Therefore, insights into the functional role of IL-4 on MCs in the cutaneous compartment require an experimental design, which takes into account the peculiarities of MCs in the tissue of interest, i.e., human skin.

MC purification from tissues is laborious and the starting material is not widely accessible. We have overcome this

Electronic supplementary material The online version of this article (doi:10.1007/s00403-016-1688-x) contains supplementary material, which is available to authorized users.

✉ Magda Babina
magda.babina@charite.de

¹ Department of Dermatology and Allergy, Charité Campus Mitte, Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

hurdle, and routinely purify MCs from human skin to homogeneity [3, 10, 16, 17]. Using purified skin MCs as target cells, we demonstrated a switch in their cytokine profile under the dictate of IL-4 following a 2-week-exposure [3]. Conversely, we found little impact on typical surface receptors upon shorter contact (3 days), even though IL-4 reduced lineage markers and simultaneously induced cell death in immature MC lines in the same study [17]. The lack of effect in skin-derived MCs suggested that modulations may develop slowly over time. In fact, expansion systems for MCs derived from gut and skin use a combination of SCF + IL-4 [5, 10, 12, 16]. Nevertheless, it remains currently undefined as to what extent IL-4 assists in the expansion of the cutaneous MC variety over the other supportive factors present in the culture medium, especially SCF. There is, likewise, no information on IL-4's potential influence on typical lineage characteristics like receptors, preformed mediators, and functional programs.

Our recent data from the FANTOM5 consortium highlighted a prominent expression of the IL-4 receptor gene (IL4RA) in skin MCs, combined with robust expression of crucial IL-4 network components (IL2RG, STAT6, JAK1, JAK2, JAK3) if contrasted against the highly comprehensive atlas (including ≈ 200 primary cells) [7, 16]. This rejuvenated our interest in the modulation of dermal MCs by IL-4.

We report that IL-4, indeed, makes a positive contribution to MC number and activity. Most intriguingly, IL-4 not only increases histamine *release* by Fc ϵ RI-triggering, but it also boosts cellular *production* of this biogenic amine, an effect not reported previously. Together, prolonged IL-4 skews human skin MCs towards a pro-allergic response pattern, and this is achieved through operation at several levels.

Materials and methods

Cells

MCs were isolated from human breast skin as described [3] with the modifications given in recent publications [12, 16]. The skin was obtained from cosmetic breast reduction surgeries, with informed consent of the patients and approval by the University Ethics Committee. The experiments were conducted according to the Declaration of Helsinki Principles. Skin-derived MCs were cultured for 5 weeks either in stem cell factor (SCF) alone (100 ng/ml) or in SCF plus IL-4 (20 ng/ml) [12]. Cytokines were freshly provided once a week when cultures were re-adjusted to 5×10^5 /ml. MCs were automatically enumerated by CASY-TTC (Innovatis/Casy Technology, Reutlingen, Germany) [12].

Fc ϵ RI expression

Flow cytometry was performed as described [12]. The antibody used was anti-human Fc ϵ RI α -PE at 2.5 μ g/ml (clone AER-37, eBioscience, San Diego, USA); mouse IgG2b-PE (clone eBWG2b, eBioscience) served as isotype control.

Histamine quantification

Histamine was quantified by an automated fluorescence method using an autoanalyzer (Borgwald Technik, Germany, Hamburg) exactly as described [11, 12]. All determinations were performed in triplicate, and histamine concentrations were calculated with reference to a standard curve.

Histamine release assay

MCs cultured in SCF alone versus SCF + IL-4 were tested for histamine release following Fc ϵ RI aggregation (using the anti-Fc ϵ RI α -Ab 29C6 at 0.25 μ g/ml), Substance P (at 30 μ M) or no stimulus for spontaneous release. The assays were performed exactly as described [11, 12].

MC protease activity

Detection of tryptase and chymase activity was performed as described [12, 13].

Reverse transcription-quantitative PCR (RT-qPCR)

RT-qPCR was performed using optimized conditions and primer pairs described elsewhere [12]. Values were normalized to the housekeeping gene β -actin.

Statistics

Differences between groups were assessed by paired *t* test or (when not normally distributed) Wilcoxon matched-pairs signed-rank test with the program GraphPad Prism, San Diego, CA, USA. $p < 0.05$ was considered statistically significant.

Results

IL-4 synergizes with SCF to support skin MC expansion

IL-4 is a regular addendum to an expansion system of human skin-derived MCs [10, 12, 16]. The benefit of IL-4 has not been quantified over the other components of the

expansion medium, however. Therefore, we surveyed IL-4's impact on MC expansion by employing cells derived from the skin tissue of 11 individuals. We found that SCF by itself was sufficient to maintain and expand our skin MCs, but that IL-4 heightened the cellular output in 10 of 11 samples (Suppl. Fig. S1). We conclude that IL-4 synergizes with SCF to promote the expansion of skin-derived MC.

IL-4 boosts histamine production in skin MCs

MC granules are a hallmark of the lineage, and its constituent histamine is the major target of anti-allergic drugs. Here, we surprisingly found that MCs in contact with IL-4 accumulated over 2.2-fold more histamine than their counterparts supported by SCF only (Fig. 1a). This was accompanied by a threefold increment in histidine decarboxylase (HDC) mRNA, the rate-limiting enzyme in histamine production (Fig. 1b). To our knowledge, IL-4's ability to foster the *production* of this major biogenic amine has not yet been reported.

Next, we focused on tryptase and chymase, two abundant and highly specific proteases of the skin MC granule [12, 13, 16]. While tryptase mRNA was dampened by IL-4 vis-à-vis SCF alone (to 0.71-fold), tryptase activity remained largely comparable between the groups (Fig. 2). Conversely, chymase mRNA was augmented by IL-4 (1.54-fold of expression in SCF). Chymase activity showed the same tendency, even though the increase was modest,

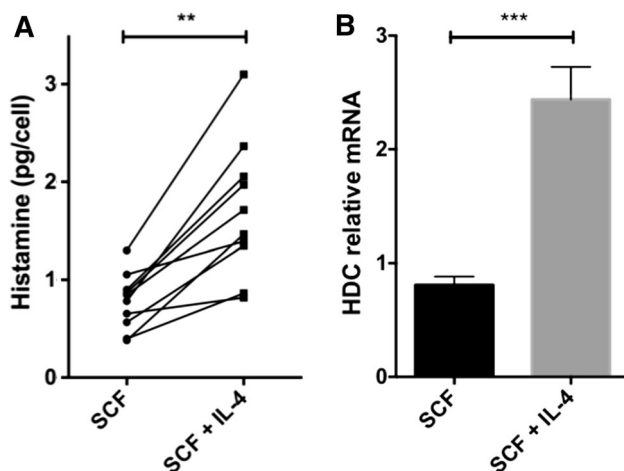


Fig. 1 MC histamine levels are elevated by IL-4. Skin-derived MCs from a number of donors were cultured in SCF alone versus SCF + IL-4. **a** Histamine was quantified by an autoanalyzer-based method and the amount per cell calculated relative to a standard curve. Data are displayed as interconnected dots, each representing an individual sample. **b** Relative mRNA expression of HDC, the histamine-producing enzyme, was quantified by RT-qPCR relative to the housekeeping gene β -actin, mean $\pm$ SEM of 11 individual preparations. ** $p < 0.01$; *** $p < 0.001$

albeit significant (mean increase to 1.18-fold of the SCF control) (Fig. 2).

Collectively, IL-4 controls the abundance of preformed mediators in MCs of human skin provenance. There is a particularly clear impact on cellular histamine contents, a finding of substantial biological relevance.

IL-4 increases Fc ϵ RI expression and supports degranulation to allergic stimulation but not to non-immunological stimuli

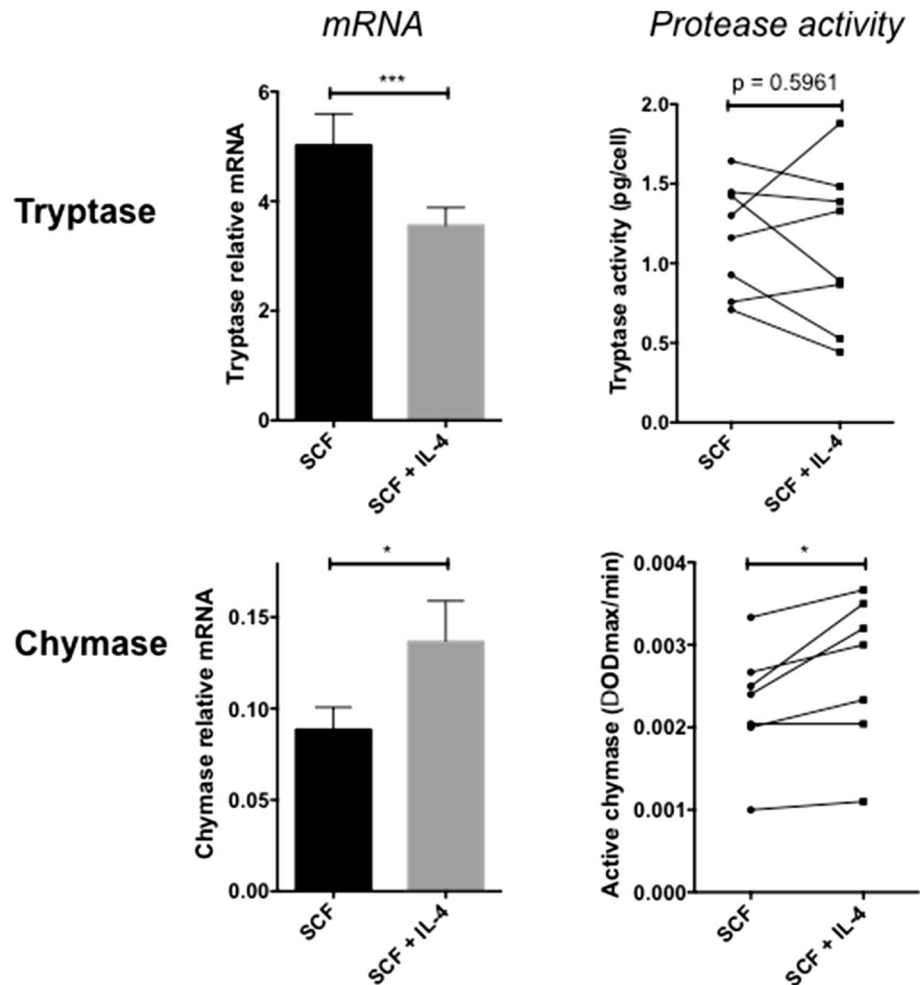
Skin MCs *ex vivo* release histamine at ≈ 20 % on average, but in a highly donor-dependent manner [2, 3, 12]. When persistently exposed to the potent cytokine “cocktail” of SCF + IL-4, MCs become high responders irrespective of this initial release [10, 12]. Here, we tested whether the boost in Fc ϵ RI-responsiveness was brought about by IL-4 or induced by SCF alone. We also addressed the question as to whether the heightened responsiveness was specific to Fc ϵ RI-triggering or duplicated by non-immunologic stimuli. As shown in Fig. 3A, MCs cultured in “SCF only” displayed profound stimulability to Fc ϵ RI-triggering (range 47.7–78.9 %). Despite this, IL-4 further enhanced this secretion in a consistent fashion (to 53.3–90.5 %) (Fig. 3a, middle). Note that the numbers given in brackets refer to the histamine *released* relative to the amount *present* in the cell prior to stimulation; so they do not result from the increased accumulation of histamine following IL-4 treatment described in the previous paragraph. The absolute *amounts* actually released are higher because they are the product of the amount per cell and the relative release, with both processes being independently augmented by IL-4.

In contrast, the low spontaneous histamine release (Fig. 3a, left) as well as substance P-mediated secretion (Fig. 3a, right) remained unaffected, suggesting that IL-4 selectively supports the IgE-dependent activation pathway. Also, IL-4 increased Fc ϵ RI cell-surface expression in the skin MC samples to 2.32-fold of SCF control (Fig. 3b), and this was accompanied by a similar increment in Fc ϵ RI α mRNA (to 1.93-fold of control expression, Fig. 3c), which reportedly correlates with the abundance of the receptor complex [2].

Discussion

This study was designed to test the hypothesis that MCs of human skin provenance are re-shaped by IL-4. Emphasis was laid on sustained exposure, because it was known from previous studies that short contact was insufficient to trigger phenotypic modulations in the cells ([17], and unpublished data). Key lineage features like preformed mediators, Fc ϵ RI, and degranulation responses to allergic

Fig. 2 IL-4 inversely regulates tryptase and chymase mRNA. *Left* Skin MCs cultured in SCF versus SCF + IL-4 were analyzed for tryptase- and chymase-specific transcripts by RT-qPCR. Data were normalized to the housekeeping gene β -actin and are given as mean $\pm$ SEM of 11 individual preparations. *Right*: Tryptase and chymase activities were measured by activity tests using substrates specific for the respective protease. * $p < 0.05$; *** $p < 0.001$



and non-immunological stimuli were surveyed. The result was that long-term exposure to IL-4 substantially strengthened skin-derived MCs numerically and skewed MC phenotype and functional competence. This suggests that in chronic scenarios of Th2-dominated skin diseases, in which IL-4 is overabundant, MCs may become functionally altered with time.

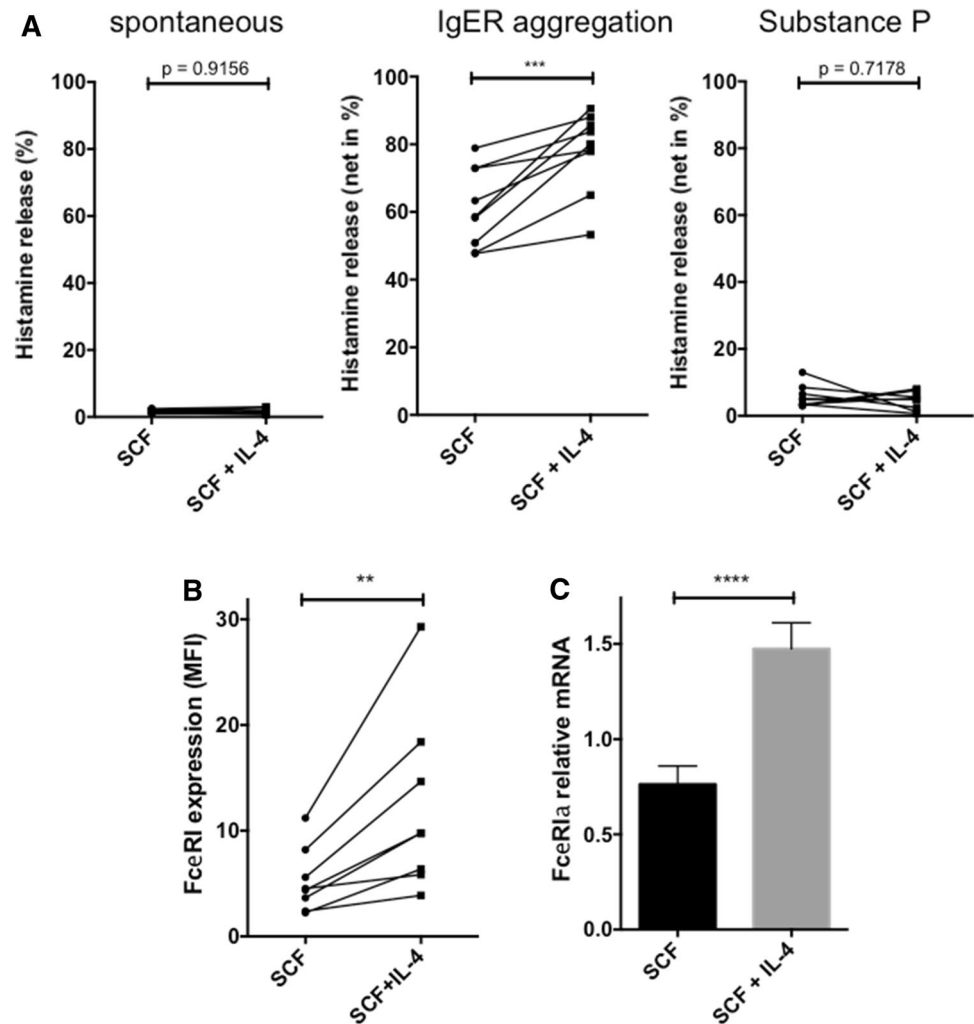
Among the molecules influenced by the Th2 cytokine, histamine is of particular interest because of its significance as mediator of acute allergic reactions, as highlighted by the wide selection and frequent use of antihistamines. The positive influence from IL-4 on histamine levels was surprising, as (to our knowledge) no such effect had been reported previously despite considerable research into IL-4's impact on the lineage. In our study, the effect was observed in MCs from all donors tested and, therefore, highly reproducible (Fig. 1). Histamine accumulation was accompanied by a similar increment in HDC expression, and a tight connection between HDC mRNA expression and cellular histamine abundance was recently reported in MCs derived from a large cohort [2]. It remains to be seen whether the histamine-promoting effect of IL-4 represents

a peculiarity of human skin MCs or is a broader phenomenon. In any case, due to the significance of histamine, our study may have uncovered an important route by which IL-4 operates in MCs to contribute to diseases of the atopic spectrum.

Also, IL-4 modulated tryptase and chymase, even if to a lesser extent and in more complex patterns. Perhaps, the most interesting finding here was the opposite regulation of the two protease genes, with tryptase mRNA being downregulated, and chymase mRNA simultaneously upregulated. The lack of agreement between tryptase mRNA and tryptase activity found herein is in accordance with our recent study [2], which conversely found a highly positive correlation between tryptase and histamine, suggesting a role for histamine in the regulation of tryptase abundance [2]. In opposition, chymase mRNA did better reflect the activity of the corresponding peptidase [2].

In this study, we also demonstrate that Fc ϵ RI-triggered degranulation reaches impressive levels in SCF alone if compared to skin MCs ex vivo ([2], Babina et al., submitted). The remarkable histamine release on persistent exposure to SCF was, however, further intensified by IL-4. This

Fig. 3 IL-4 boosts anaphylactic histamine release and FcεRI expression by skin-derived MCs, but has no impact on spontaneous or non-immunologically elicited degranulation. **a** Histamine release was assessed in paired samples of MCs cultured in SCF versus SCF + IL-4. The values given were calculated as “histamine released/total histamine of the same preparation” (in %) and are thus adjusted for the different histamine abundance in the samples prior to stimulation. **Left** spontaneous histamine release in the absence of a specific stimulus. **Middle** histamine release evoked by FcεRI aggregation. **Right** histamine release triggered by substance P. **b** FcεRI cell surface expression determined by flow cytometry. **c** FcεRIα relative mRNA, as quantified by RT-qPCR relative to the housekeeping gene β-actin, $n = 11$. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$



positive impact corresponds with other types of MCs, in which IL-4 likewise enhanced FcεRI expression and/or allergic stimulability [15, 18]. If our in vitro findings translate to the in vivo situation, allergic MC activation in an IL-4-rich environment may lead to substantially enhanced histamine levels in the adjacent tissue (and/or blood) as a result of the combined action of two distinct processes: (1) Greater *histamine accumulation* per cell, so that even with the same degree of stimulation (i.e., percent release), histamine levels in the vicinity of the degranulating MC would rise vis-à-vis IL-4-devoid conditions, (2) enhanced *responsiveness* to FcεRI aggregation, thereby increasing the portion of the total cellular histamine being released.

Contrary to the FcεRI pathway, responsiveness to substance P remained unaltered by IL-4. Substance P activates skin MCs via a neuronal, non-immunological circuit [11].

Our current findings underscore that processes tightly linked to the allergic phenotype (like FcεRI expression/functionality and histamine abundance) are boosted by IL-4. This fits the global picture of IL-4's multiple contributions to the pathogenesis of allergic diseases, where

the cytokine orchestrates Th2-type immunity by acting on several key players. For instance, IL-4 directs B cells to produce IgE, promotes the development of Th2 cells, fosters basophil functions, and induces alternatively activated macrophages at sites of allergic inflammation [7 and references therein]. The current findings are also consistent with the induction of the Th2-type cytokine IL-5 in IL-4-treated skin-derived MCs [3].

Collectively, IL-4 can shape human cutaneous MCs at a post-maturation stage, strengthening them in number and function, especially by accentuating allergy-associated processes. IL-4-mediated alterations at the level of MCs may, thus, contribute to Th2-linked pathologies in the skin including atopic dermatitis and urticaria.

Acknowledgments The authors thank Ms. Annett von Grüner for excellent technical assistance.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Funding This work was supported by the European Centre for Allergy Research Foundation (ECARF) and the Global Allergy and Asthma European Network (GA²LEN), supported by the Sixth EU Framework programme for research contract no. FOOD-CT-2004-50637.

Ethical approval This article contains studies with skin from human participants. The study was approved by the University Ethics Committee. The experiments were conducted according to the Declaration of Helsinki Principles.

Informed consent The skin, which would otherwise be disposed of, was obtained from cosmetic breast reduction surgeries, with informed consent of the patients.

References

- Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WC (2007) Protective immune mechanisms in helminth infection. *Nat Rev Immunol* 7(12):975–987
- Babina M, Guhl S, Artuc M, Trivedi NN, Zuberbier T (2016) Phenotypic variability in human skin mast cells. *Exp Dermatol* 25(6):434–439. doi:[10.1111/exd.12924](https://doi.org/10.1111/exd.12924)
- Babina M, Guhl S, Stärke A, Kirchhof L, Zuberbier T, Henz BM (2004) Comparative cytokine profile of human skin mast cells from two compartments—strong resemblance with monocytes at baseline but induction of IL-5 by IL-4 priming. *J Leukoc Biol* 75(2):244–252
- Babina M, Motakis E, Zuberbier T (2014) Mast cell transcriptome elucidation: what are the implications for allergic disease in the clinic and where do we go next? *Expert Rev Clin Immunol* 10(8):977–980. doi:[10.1586/1744666X.2014.933074](https://doi.org/10.1586/1744666X.2014.933074)
- Bischoff SC, Sellge G, Lorentz A, Sebald W, Raab R, Manns MP (1999) IL-4 enhances proliferation and mediator release in mature human mast cells. *Proc Natl Acad Sci USA* 96(14):8080–8085
- Brown JM, Wilson TM, Metcalfe DD (2008) The mast cell and allergic diseases: role in pathogenesis and implications for therapy. *Clin Exp Allergy* 38(1):4–18
- FANTOM Consortium and the RIKEN PMI and CLST (DGT), Forrest AR, Kawaji H, Rehli M, Baillie JK, de Hoon MJ, Haberle V, Lassmann T, Kulakovskiy IV, Lizio M, Itoh M et al (2014) A promoter-level mammalian expression atlas. *Nature* 507(7493):462–470. doi:[10.1038/nature13182](https://doi.org/10.1038/nature13182)
- Ferrer M, Kaplan AP (2007) Progress and challenges in the understanding of chronic urticaria. *Allergy Asthma Clin Immunol* 3(1):31–35. doi:[10.1186/1710-1492-3-1-31](https://doi.org/10.1186/1710-1492-3-1-31)
- Galli SJ, Tsai M (2012) IgE and mast cells in allergic disease. *Nat Med* 18(5):693–704. doi:[10.1038/nm.2755](https://doi.org/10.1038/nm.2755)
- Guhl S, Artuc M, Neou A, Babina M, Zuberbier T (2011) Long-term cultured human skin mast cells are suitable for pharmacological studies of anti-allergic drugs due to high responsiveness to FcεRI cross-linking. *Biosci Biotechnol Biochem* 75(2):382–384
- Guhl S, Lee HH, Babina M, Henz BM, Zuberbier T (2005) Evidence for a restricted rather than generalized stimulatory response of skin-derived human mast cells to substance P. *J Neuroimmunol* 163(1–2):92–101
- Guhl S, Neou A, Artuc M, Zuberbier T, Babina M (2014) Skin mast cells develop non-synchronized changes in typical lineage characteristics upon culture. *Exp Dermatol* 23(12):933–935. doi:[10.1111/exd.12558](https://doi.org/10.1111/exd.12558)
- Harvima IT, Karkola K, Harvima RJ, Naukkarinen A, Neittaanmäki H, Horsmanheimo M, Fräki JE (1989) Biochemical and histochemical evaluation of tryptase in various human tissues. *Arch Dermatol Res* 281(4):231–237
- Kawakami T, Ando T, Kimura M, Wilson BS, Kawakami Y (2009) Mast cells in atopic dermatitis. *Curr Opin Immunol* 21(6):666–678. doi:[10.1016/j.coi.2009.09.006](https://doi.org/10.1016/j.coi.2009.09.006)
- McLeod JJ, Baker B, Ryan JJ (2015) Mast cell production and response to IL-4 and IL-13. *Cytokine* 75(1):57–61. doi:[10.1016/j.cyt.2015.05.019](https://doi.org/10.1016/j.cyt.2015.05.019)
- Motakis E, Guhl S, Ishizu Y, Itoh M, Kawaji H, de Hoon M, Lassmann T, Carninci P, Hayashizaki Y, Zuberbier T, Forrest AR, Babina M, FANTOM consortium (2014) Redefinition of the human mast cell transcriptome by deep-CAGE sequencing. *Blood* 123(17):e58–e67. doi:[10.1182/blood-2013-02-483792](https://doi.org/10.1182/blood-2013-02-483792)
- Thienemann F, Henz BM, Babina M (2004) Regulation of mast cell characteristics by cytokines: divergent effects of interleukin-4 on immature mast cell lines versus mature human skin mast cells. *Arch Dermatol Res* 296(3):134–138
- Xia HZ, Du Z, Craig S, Klisch G, Noben-Trauth N, Kochan JP, Huff TH, Irani AM, Schwartz LB (1997) Effect of recombinant human IL-4 on tryptase, chymase, and Fc epsilon receptor type I expression in recombinant human stem cell factor-dependent fetal liver-derived human mast cells. *J Immunol* 159(6):2911–2921

Concise Communication

IL-4 and human skin mast cells revisited: reinforcement of a pro-allergic phenotype upon prolonged exposure

Running Title: IL-4 and human skin mast cells

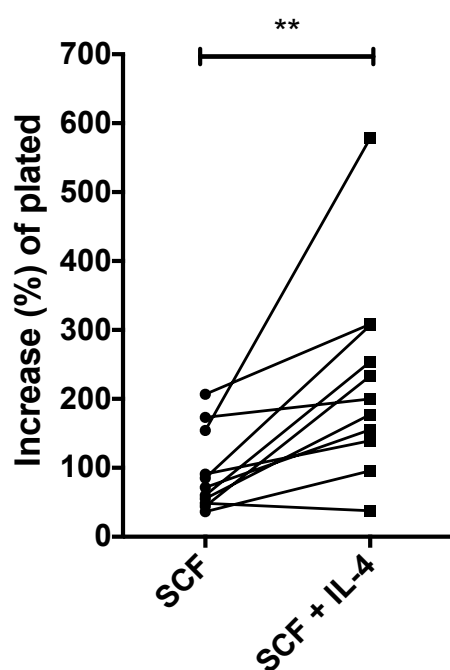
Magda Babina, Sven Guhl, Metin Artuc, Torsten Zuberbier

Department of Dermatology and Allergy, Charité Campus Mitte, Germany

Correspondence: Magda Babina, PhD, Department of Dermatology and Allergy, Charité Campus Mitte, Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

Phone No. 49-30-450518408, Fax No. 49-30-450518900, email: magda.babina@charite.de

Supplementary Figure S1



Supplementary Figure Legend S1

IL-4 makes a positive contribution to cellular output upon long-term culture

Skin MCs cultured in SCF versus SCF+IL-4 were quantitated by the automated cell counter and analyzer CASY-TTC. The data give the increase (in %) over the number originally plated (i.e. cells at the end of culture - cells at the beginning of culture/cells at the beginning of culture x 100) and are displayed as interconnected dots, each representing an individual sample. ** $p < 0.01$.