

Mast cell lines HMC-1 and LAD2 in comparison with mature human skin mast cells – drastically reduced levels of tryptase and chymase in mast cell lines

Sven Guhl, Magda Babina, Angelos Neou, Torsten Zuberbier and Metin Artuc

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

Correspondence: Sven Guhl, Department of Dermatology and Allergy, Charité Campus Mitte, Universitätsmedizin Berlin, Charitéplatz 1, D-10117 Berlin, Germany, Tel.: 49 30 450518245, Fax: 49 30 450518900, e-mail: sven.guhl@charite.de

Abstract: To circumvent the costly isolation procedure associated with tissue mast cells (MC), two human MC lines, i.e. HMC-1 and LAD2, are frequently employed, but their relation to mature MC is unknown. Here, we quantitatively assessed their expression of MC markers in direct comparison to skin MC (sMC). sMC expressed all lineage markers at highest and HMC-1 cells at lowest levels. LAD2 cells expressed comparable high-affinity IgE receptor α (Fc ϵ RI α) and Fc ϵ RI γ but less Fc ϵ RI β than sMC and displayed slightly reduced, but robust Fc ϵ RI-mediated histamine release. Only minor differences were found for total histamine

content and c-Kit expression. Huge, and to this level unexpected, differences were found for MC tryptase and chymase, with sMC >>> LAD2 > HMC-1. Taken together, HMC-1 cells represent very immature malignantly transformed MC, whereas LAD2 cells can be considered intermediately differentiated. Because of the minute levels of MC proteases, MC lines can serve as surrogates of tissue MC to a limited degree only.

Key words: chymase – histamine – IgE receptor – mast cells – tryptase

Accepted for publication 22 March 2010. Please cite this paper as: Mast cell lines HMC-1 and LAD2 in comparison with mature human skin mast cells – drastically reduced levels of tryptase and chymase in mast cell lines. *Experimental Dermatology* 2010.

Background

Mast cells (MC) are central effector cells of allergic reactions and intimately involved in the defense against pathogens [summarized in (1–8)]. Because of the rarity of tissue MC and the costly purification procedures, human MC research mainly relies on either *in vitro*-generated MC or MC lines. Two human MC lines have been established, i.e. HMC-1 (9,10) and LAD2 (11). The cell lines have been characterized one at a time, but their direct relation to normal human tissue MC has remained unknown. Nevertheless, both cell lines are extensively used as surrogates for non-transformed primary human MC (12–24).

Questions addressed

To define the relationship of the two MC lines to their skin-derived counterparts, gene and protein expression patterns of typical lineage markers (Fc ϵ RI α , β , γ , histamine/HDC, c-Kit, tryptase, chymase) were quantified in HMC-1, LAD2 and skin MC in direct comparison. Fc ϵ RI-mediated histamine release was likewise studied.

Experimental design

MC purification from foreskin was achieved by magnetic sorting of c-Kit-positive cells, giving rise to >95% MC

purity (25). Expression and function of typical lineage markers were studied by quantitative RT-PCR, flow cytometry, histamine-analyser, protease-activity tests, ImmunoCAP™ and immunoblotting. The methods are described in detail in the supplementary online material (appendix S1).

Results

The expression of the three chains forming the Fc ϵ RI receptor complex was first investigated. The α and γ subunits were expressed at comparable levels in skin MC (sMC) and LAD2 cells, but LAD2 cells displayed diminished β chain expression (Fig. 1a). α and γ were lowest in HMC-1 cells, while β was undetectable (Fig. 1a). By flow cytometry, both LAD2 cells and sMC showed robust Fc ϵ RI α cell surface staining, whereas no expression was detected on HMC-1 cells (Fig. 1b). We also studied whether the MC subsets can be stimulated via the IgER to release histamine and whether there are differences in spontaneous histamine release. Both cell lines showed an enhanced spontaneous histamine release, when compared with sMC (Fig. 1c). On the other hand, histamine release mediated by the cross-linking of Fc ϵ RI was most pronounced in sMC followed by LAD2 cells (Fig. 1c). No stimulation above the baseline level was achieved in HMC-1, in line with the absent expression of the Fc ϵ RI complex. Substance P-mediated histamine release was, however, substantially higher in

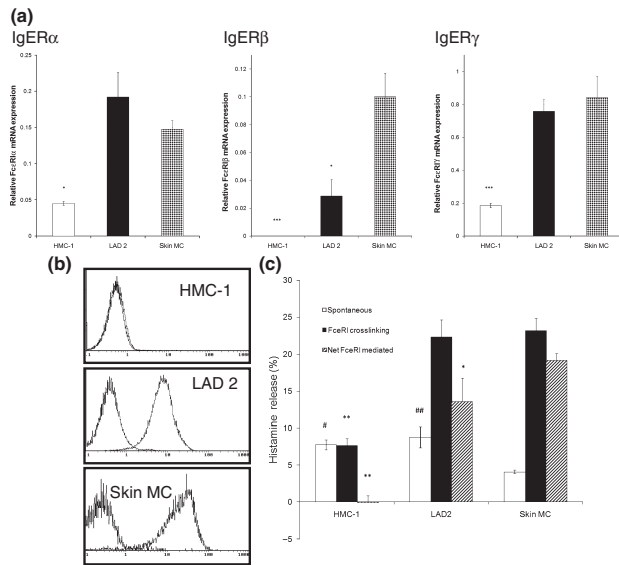


Figure 1. Expression and function of the high affinity IgE receptor FcεRI by HMC-1, LAD 2 and skin MC in direct comparison (a) Expression of the three subunits α, β and γ forming the FcεRI. Expression was determined by qRT-PCR. Relative expression normalized to the housekeeping gene β-actin is shown as the mean ± SEM of 10 (cell lines) and 16 skin MC separate cell preparations. (b) Expression of FcεRI protein at the cell surface. Staining was performed with the anti-FcεRIα antibody 29C6 (grey line) and analysed by flow cytometry, the darker line corresponds to the isotype control. Representative histogram overlays from at least 10 individual experiments are shown. (c) Spontaneous and FcεRI-mediated histamine release from HMC-1, LAD 2 and skin MC. Cell lines were sensitized with IgE overnight, stimulation was performed with anti-IgE for 30 min. Histamine release was determined as described in Methods and calculated in % of total histamine content. Spontaneous: without anti-IgE. Net FcεRI-mediated: Difference between IgE/anti-IgE and spontaneous histamine release. Data are the mean ± SEM from 8 (HMC-1), 18 (LAD 2) and 34 (skin MC) individual experiments (cell preparations). * ($P < 0.01$), ** ($P < 0.01$), *** ($P < 0.001$) significantly lower than skin MC; # ($P < 0.05$), ## ($P < 0.01$) significantly higher than sMC (Kruskal–Wallis test in a, ANOVA in c).

LAD2 than in sMC (not shown). Total histamine content was reduced in HMC-1 cells and slightly reduced in LAD2 cells compared to sMC, whereas expression of HDC (histidine decarboxylase) transcript was comparable among cells (Fig. S1). The stronger variation in histamine content versus HDC mRNA might be because of the higher spontaneous release of histamine in the cell lines. As expected, expression of c-Kit (the most important receptor tyrosine kinase of MC) was quite uniform among the cells (Fig. S2). The results for tryptase and chymase, clearly the most specific MC markers (5,26), showed a very different picture, however. qRT-PCR revealed low tryptase levels in both MC lines when compared to sMC (around 6.4% in LAD2 and 0.6% in HMC-1 cells of the amount of sMC, Fig. 2a). The level of tryptase protein, tested by two independent methods (ImmunoCAP™ and tryptase activity testing through

the cleavage of a specific substrate, respectively), was even more drastically reduced in the cell lines (reduction down to approximately 0.5–1% to the levels obtained with sMC, Fig. 2b,c). The more pronounced difference at the protein level may result, as in the case of histamine, from higher spontaneous tryptase release. In accordance with the previous data (11,27), tryptase was clearly expressed in both cell lines by flow cytometry, albeit with a lower mean fluorescence intensity than in sMC (Fig. S3a). Therefore, the cell lines express tryptase, but flow cytometry is not adequate to quantify intercellular differences; it can merely state that expression of a marker is above the level of an isotype control antibody. The drastic difference in tryptase levels among the MC subsets (as shown in Fig. 2) was further confirmed by immunoblotting (Fig. S3b). As for tryptase, chymase-specific mRNA was faintly expressed by LAD2 cells and absent from HMC-1 cells (Fig. 2d). By chymase activity testing, the expression was below the detection limit of the assay in HMC-1 and LAD2 cells (Fig. 2e). As in the case of tryptase, we could, however, detect low level chymase expression by flow cytometry in LAD2 cells (data not shown), but none by immunoblot with this exposure time (Fig. S3c). Together, tryptase and chymase are expressed at drastically reduced levels in the cell lines, and this difference can only be uncovered by methods that give truly quantitative results.

Conclusions

We report on the quantitative analysis of gene and protein expression patterns of typical lineage markers in HMC-1, LAD2 and sMC in direct comparison. As expected, HMC-1 cells express low levels of most MC markers with the exception of c-Kit and histamine. LAD2 cells express, in addition to c-Kit and histamine, high levels of functional IgER, although somewhat decreased versus skin MC. Both HMC-1 and LAD2 display, however, drastically reduced levels of tryptase and chymase compared to sMC, implying that the selective induction of MC proteases forms a late step in the differentiation process and is likely a hallmark of MC maturity. Both cell lines have been shown to express tryptase (11,27), so that the differences uncovered here could by no means be expected. Based on our data and the literature (e.g. 28), we suggest the following chronological order of MC marker appearance in maturation: c-kit–histamine/HDC–FcεRIγ–FcεRIα–FcεRIβ–tryptase–chymase (chymase only in MC_{TC} type MC, such as sMC). Together, our data show (i) that tryptase and chymase are the most reliable markers of mature (skin) MC and displayed in minute amounts by MC lines; (ii) that it is the dose rather than the presence or absence of a marker to help define the maturation stage of MC (which cannot be deduced from flow cytometric staining); (iii) that MC lines

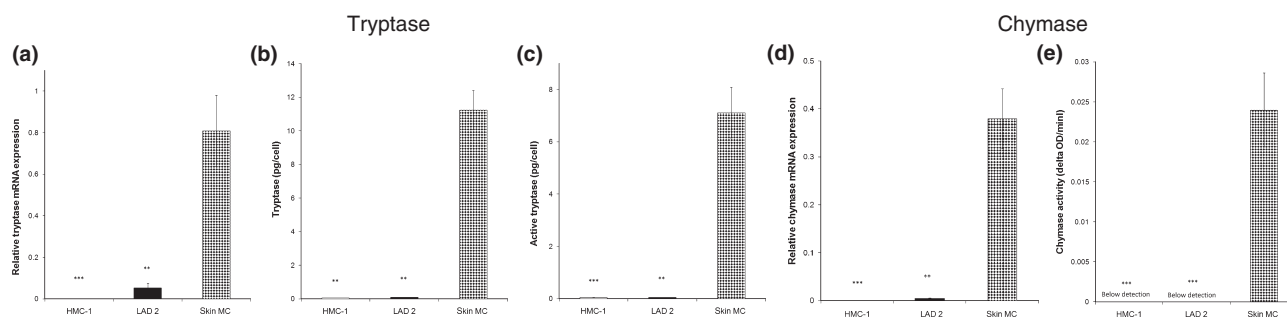


Figure 2. Drastic differences in the expression of total and active tryptase among MC subsets. (a) Expression of tryptase by quantitative RT-PCR. Normalized data are shown as the mean $\pm$ SEM of 10 (cell lines) and 16 (skin MC) separate samples. (b) Expression of tryptase protein by the ImmunoCAP technique. Results are shown as pg/cell and are the mean $\pm$ SEM of at least six cell preparations. (c) Tryptase activity in the different MC subsets. Levels of active tryptase were determined by an enzyme activity test using a specific tryptase substrate. Data are shown as pg of active tryptase per cell (determined relative to a standard curve with recombinant tryptase) and are the mean $\pm$ SEM of at least eight individual cell preparations. (d) Expression of chymase by quantitative RT-PCR. Normalized data are shown as in a. (e) Chymase activity in the different MC subsets. Levels of active chymase were determined by an enzyme activity test using a specific chymase substrate. Data are shown as deltaOD/min and are the mean $\pm$ SEM of at least eight individual cell preparations. ** ($P < 0.01$); *** ($P < 0.001$) significantly lower than skin MC (Kruskal–Wallis a, d, e, ANOVA b, c).

can serve as replacements of mature tissue MC to a limited degree only.

Acknowledgements

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. We are grateful to Drs Butterfield and Metcalfe for providing the cell lines HMC-1 and LAD2, respectively. We thank Ms Annett von Grüner, Ms Ullrike Dirla and Ms Karin Armbrust for excellent technical assistance.

References

- Kinet J P. *Annu Rev Immunol* 1999; **17**: 931–972.
- Kraft S, Rana S, Jouvin M H, Kinet J P. *Int Arch Allergy Immunol* 2004; **135**: 62–72.
- Kneilling M, Röcken M. *Exp Dermatol* 2009; **18**: 488–496.
- Heib V, Becker M, Taube C, Stassen M. *Br J Haematol* 2008; **142**: 683–694.
- McNeil H P, Adachi R, Stevens R L. *J Biol Chem* 2007; **282**: 20785–20789.
- Marshall J S, Jawdat D M. *J Allergy Clin Immunol* 2004; **114**: 21–27.
- Galli S J, Nakae S, Tsai M. *Nat Immunol* 2005; **6**: 135–142.
- Mekori Y A, Metcalfe D D. *Immunol Rev* 2000; **173**: 131–140.
- Butterfield J H, Weiler D, Dewald G, Gleich G J. *Leuk Res* 1988; **12**: 345–355.
- Nilsson G, Blom T, Kusche-Gullberg M *et al.* *Scand J Immunol* 1994; **39**: 489–498.
- Kirshenbaum A S, Akin C, Wu Y *et al.* *Leuk Res* 2003; **27**: 677–682 Erratum in: *Leuk Res* 2003; **27**: 1171.
- Shirato K, Taguchi F. *Virology* 2009; **386**: 88–93.
- Bansal G, DiVietro J A, Kuehn H S *et al.* *J Immunol* 2008; **181**: 7882–7890.
- Gilchrist M, Befus A D. *Immunology* 2008; **123**: 209–217.
- Lim S, Choi J W, Kim H S *et al.* *Life Sci* 2008; **82**: 733–740.
- Rudolph M I, Boza Y, Yefi R *et al.* *J Pharmacol Sci* 2008; **106**: 208–218.
- Meyer G K, Neetz A, Brandes G *et al.* *Infect Immun* 2007; **75**: 3868–3876.

- Moon P D, Lee B H, Jeong H J *et al.* *Eur J Pharmacol* 2007; **555**: 218–225.
- Olszewski M B, Groot A J, Dastych J, Knol E F. *J Immunol* 2007; **178**: 5701–5709.
- Castellani M L, Kempuraj D, Frydas S *et al.* *Neuroimmunomodulation* 2006; **13**: 179–186.
- Ko J, Yun C Y, Lee J S, Kim D H, Yuk J E, Kim I S. *Life Sci* 2006; **79**: 1293–1300.
- Lundeen K A, Sun B, Karlsson L, Fourie A M. *J Immunol* 2006; **177**: 3439–3447.
- Pernas-Sueiras O, Alfonso A, Vиейtes M R, Botana L M. *J Cell Biochem* 2006; **99**: 1651–1663.
- Wong C K, Tsang C M, Ip W K, Lam C W. *Allergy* 2006; **61**: 289–297.
- Babina M, Guhl S, Stärke A, Kirchhof L, Zuberbier T, Henz B M. *J Leukoc Biol* 2004; **75**: 244–252.
- Pejler G, Abrink M, Ringvall M, Wernersson S. *Adv Immunol* 2007; **95**: 167–255.
- Butterfield J H, Weiler D A, Hunt L W, Wynn S R, Roche P C. *J Leukoc Biol* 1990; **47**: 409–419.
- Shimizu Y, Suga T, Maeno T *et al.* *Clin Exp Allergy* 2004; **34**: 917–925.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Expression of histidine decarboxylase (HDC) and histamine by HMC-1, LAD 2 and skin MC.

Figure S2. Expression of the receptor tyrosine kinase c-kit by HMC-1, LAD 2 and skin MC.

Figure S3. Substantial differences in the expression of tryptase and chymase among MC subsets which can barely be uncovered by flow-cytometry.

Appendix S1. Methods is now introduced after experimental design.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Methods

Skin MC purification

For all experiments foreskin derived mast cells were used. The skin was obtained from circumcisions, with informed consent of the patients and approval of the university ethical committee. Additionally the application of human skin was conducted accordingly to the Declaration of Helsinki Principles and was approved by Institutional Review Boards of the Universitätsmedizin Charité of Berlin.

MC purification from human foreskin specimens was essentially as described (S1 /25). In brief, skin was cut into strips and treated with dispase Type I (Roche Diagnostics, Mannheim, Germany) at 0.5 mg/ml and 4°C overnight. After removal of the epidermis, the dermis was chopped in small pieces and digested with collagenase at 1.0 mg/ml (Worthington, Lakewood, NJ), for 1 h at 37 °C. Subsequently the liberated cells were separated from remaining tissue by three steps of filtration (pore sizes 250, 100, and 40 µm).

MC purification from the dispersates was achieved by positive selection using anti human c-Kit microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany), and an Auto-MACS separation device. MC purity in these preparations always exceeded 95%, as assessed by acidic toluidine-blue staining (0.1% in 0.5 N HCl).

Skin MC were kept at 5×10^5 - 1×10^6 /ml in basal Iscove's Medium supplemented with 10% FCS, 100 U/ml Penicillin, 100 µg/ml Streptomycin, 2 mM L-Glutamin, 0.002% alpha Monothioglycerol (226.4 µM) for 1-2 d or lysed immediately for downstream applications

MC lines

The leukemic MC line HMC-1 was kindly provided by Dr. J.H Butterfield (S2). Cells were cultured at 5×10^5 - 1×10^6 /ml using the same medium as for skin MC and fed three times a week.

The LAD 2 MC line, established from a patient with MC sarcoma (S3) and kindly provided by Dr. D. D. Metcalfe, was kept in Stem-Pro 34 SFM, supplemented with 100 U/ml Penicillin, 100 µg/ml Streptomycin, 2 mM L-glutamine and 100 ng/ml rhSCF (Cell Concepts/PeproTech Hamburg, Germany). Cells were cultured at 5×10^5 - 1×10^6 /ml and were semi depleted once a week.

Quantitative reverse transcription-PCR (qRT-PCR)

qRT-PCR was performed, essentially as described (S4). Briefly, total RNA was isolated using the RNeasy Total RNA kit, digested with RNase free DNase (Qiagen, Hilden, Germany), and PCR carried out with the LC Fast Start DNA Master SYBR Green kit (Roche Applied Science) using cDNA from the different MC subsets. The primer pairs were as follows.: β -actin sense 5' CTG GAA CGG TGA AGG TGA CA and antisense 5' AAG GGA CTT CCT GTA ACA ATG CA; c-kit sense 5'-CTA TGC TCT CGC ACC TTT CC and antisense 5'- CAA TGA AGT GCC CCT GAA GT ; chymase sense 5' AAG GAG AAA GCC AGC CTG ACC and antisense 5' TCC GAC CGT CCA TAG GAT ACG; HDC sense: 5' AGC TGC CTG AGA GTG CTC CTG A and antisense 5' GCA TTT TTG CCA ACC AGT CCA T; Fc ϵ RI α sense 5' CTG TTC TTC GCT CCA GAT GGC GT and antisense 5' TAC AGT AAT GTT GAG GGG CTC AG; Fc ϵ RI β sense 5' GAT CAG GAT GGT AAT TCC CGT T and antisense 5' GGA CAC AGA AAG TAA TAG GAG AG; Fc ϵ RI γ sense 5' CCA GCA GTG GTC TTG CTC TTA C and antisense 5' GCA TGC AGG CAT ATG TGA TGC C; tryptase sense 5' GGA GCT GGA GGA GCT CGT GA and antisense 5' ACC TGG GTA GGA AGC AGT GGT G. PCR was performed 40 cycles at 95° C for 20 s, 60° C (annealing temperature) for 4–5 s and 72° C for 8 s. Quantification of cDNA samples was performed automatically with reference to a standard curve. All values were normalized to the housekeeping gene β -actin. The LightCycler device Type 1.5 was used and data analysis was carried out with the LightCycler software 3.5.3 (Roche Molecular Biochemicals).

Flow-cytometry

MC were washed twice in Ca²⁺/Mg²⁺-free PBS, blocked for 15 min at 4° C with human AB-serum (Biotest, Dreieich, Germany) and thereafter incubated with either 5 μ g/ml of mouse IgG1 anti-human CD117-PE (clone A3S6E2, Miltenyi Biotech) or 2.5 μ g/ml of mouse IgG2b anti-human FC ϵ RI α -PE (clone AER-37, e-biosciences) for 30 min at 4°C. After two washes, cells were analyzed by an EPICS XL flow cytometer (Coulter Electronics, Krefeld, Germany). For negative control, cells were stained with the corresponding isotype control antibodies: 5 μ g/ml mouse IgG1-PE (clone MOPC-21, BD Pharmingen) or 2.5 μ g/ml mouse IgG2b-PE (clone eBMG2b, e-biosciences). For intracellular staining of Tryptase and Chymase cells were fixated with 4% PFA (paraformaldehyde) for 30 min at RT and permeabilised with 0,1% saponine containing PBS thereafter. After blocking with 20 % human AB serum cells were incubated with specific antibodies or isotyp controls for 30 minutes on ice The following antibodies were used : Tryptase mouse IgG1 anti human Mc-tryptase (5 μ g/ml clon AA1; #MCA-1438; Serotech), Chymase: mouse IgG1 anti human chymase (5 μ g/ml clon B7;

#MAB1254; Chemicon). After washing the binding of primary antibodies was visualised with a secondary, FITC conjugated goat (Fab) anti mouse IgG (H+L) antibody at 2µg/ml. .

Histamine content and release

Histamine release assays were performed as described elsewhere (S1, S5-6). In brief, MC lines were loaded with 1µg/ml human IgE myeloma (Calbiochem/Merck, Darmstadt, Germany) for 1h at, followed by two washes with PAG-CM in order to remove unbound IgE [PAG-CM = Piperazine-N,N-bis[2-ethanesulfonic acid]-Albumin-Glucose buffer containing 3 mM CaCl₂ and 1.5 mM MgCl₂, pH 7.4]. Cell suspensions were resuspended in PAG-CM, divided into aliquots and challenged for 30 min at 37° C with anti-IgE (1:20,000) or kept in PAG-CM for spontaneous release. Supernatants were stored at -20° C until measurement. Total histamine content was assessed upon cell lysis with 1% perchloric acid and the percentage of released histamine calculated accordingly. Histamine determination was performed by an automated fluorescence method, using an autoanalyzer (Borgwald Technik, Germany, Hamburg). All histamine determinations were performed in triplicates.

Total tryptase content by ImmunoCAP™

The determination of total tryptase content was performed by the automated ImmunoCAP™ system (Phadia GmbH, Freiburg Germany). ImmunoCAP™ is an automated ELISA system, which can be used for determination of various parameters. Such as total tryptase, total IgE or specific IgE, EC P etc. Samples are loaded to caps covered with covalently bound antibodies which detect all pro forms of α- and β-tryptase as well as mature active β-tryptase. The tryptase in the sample is bound to the immobilized detection AB's on the CAP's and after washing a β-galactosidase linked antibody against tryptase is added to form a complex. After incubation all unbound enzyme linked anti tryptase antibody is washed away and the finally bound complex is submitted to a developing agent (substrate). After stopping the reaction the fluorescence of the eluate is measured. Quantification is performed referring to a 5 point calibration standard curve ranging between 1 and 200 ng/ml. Briefly, cells were lysed in 0.02% Triton X-100 at 1.25x10⁶/ml for HMC-1 and LAD 2 or 5x10⁴/ml for skin MC. Samples were further diluted with ImmunoCAP™ dilution reagent to 5x10⁵/ml for HMC-1 and LAD 2 and 2.5x10³/ml for skin MC and subsequently analyzed by the ImmunoCAP™ system with reference to a standard curve.

Assessment of tryptase and chymase activity

For the determination of tryptase activity, cells were lysed in 0.02% Triton X-100 at 1.25x10⁶/ml for HMC-1 and LAD 2 or 5x10⁴/ml for skin MC and 50 µl samples were analyzed in triplicate, as described before (S7, S8).

In brief, enzyme activity was determined by cleavage of the peptides N-CBZ-Gly-Pro-Arg-para-nitro-anilide for tryptase or N-succinyl-Ala-Ala-Pro-Phe-para-nitro-anilide for chymase (each at 0.39 mM). To 50 µl of each sample, 150 µl of sample buffer (150 mM Tris pH 7.6 with 300 mM KCl) was added. To eliminate confounding enzyme activities, α-1 antitrypsin at a final concentration of 2 mg/ml served to suppress trypsin-like proteases in the tryptase assay, while aprotinin at 1 mg/ml was used as an inhibitor of Cathepsin G in the chymase assay. The changes in optical density per minute, caused by the cleavage of the substrates and release of para-nitro anilide, were monitored and recorded by measuring absorbance at 405 nm every 30 seconds. A standard curve of active recombinant human tryptase (Promega GmbH, Mannheim, Germany) was used to quantify the levels active tryptase in the samples.

Immunoblot

Cells were lysed at 3×10^7 /ml for primary MC and 1×10^7 /ml for HMC-1 or LAD 2 using the NE-PER nuclear and cytoplasmic extraction reagent (Pierce-Perbio, Bonn, Germany) and the protocol provided by the manufacturer. Extraction buffers contained 0.5 mM PMSF and complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany).

Protein concentrations were determined with the Coomassie plus protein determination kit (Pierce). 20 µg of cytoplasmic protein were used per lane and resolved by SDS-4-20% gradient gel PAGE. After electrophoresis, proteins were transferred to a PVDF membrane using a wet blot transfer cell and stained with Ponceau S solution to verify equal protein loadings. Membranes were blocked with 5% nonfat dried milk in PBS for 1.5 h, then probed with either 0.1 µg/ml of mouse anti-human chymase (Clone CC1, Zytomed Systems GmbH, Berlin Germany, Mob346) or 0.05 µg/ml of mouse anti-human tryptase (Clone G3, Millipore/Chemicon; Mab 1222,) over night at 4° C. After extensive washing, blots were incubated with goat-anti-mouse peroxidase conjugated secondary antibody (1:7.500, Chemicon International, Hofheim/Ts., Germany) for 90 min at room temperature, washed again, and signals detected with the ECL Plus Western Blotting detection reagents and Hyperfilm ECL (GE-Healthcare, Munich, Germany).

Statistics

The statistical significance of differences between values (LAD2 cells and HMC-1 cells versus skin MC) was assessed by one way ANOVA for unpaired samples or by Kruskal-Wallis test (when data were not normally distributed). Statistical significance was assumed at $p < 0.05$.

Supplementary References

S1 (25) Babina M, Guhl S, Stärke A, Kirchhof L, Zuberbier T, Henz B M. 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* 2004; **75**: 244-252.

S2 Butterfield J H, Weiler D, Dewald G, Gleich G J. Establishment of an immature mast cell line from a patient with mast cell leukemia. *Leuk Res* 1988; **12**: 345-355.

S3 (11) Kirshenbaum A S, Akin C, Wu Y et al. Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcepsilonRI or FcgammaRI. *Leuk Res* 2003; **27**: 677-682. Erratum in: *Leuk Res*. 2003; **27**:1171.

S4 Babina M, Schülke Y, Kirchhof L, Guhl S, Franke R, Böhm S, Zuberbier T, Henz BM, Gombart AF. The transcription factor profile of human mast cells in comparison with monocytes and granulocytes. *Cell Mol Life Sci*. 2005 Jan;62(2):214-26.

S5 Guhl S, Stefaniak R, Strathmann M et al. Bivalent effect of UV light on human skin mast cells-low-level mediator release at baseline but potent suppression upon mast cell triggering. *J Invest Dermatol* 2005; **124**: 453-456.

S6 Guhl S, Lee H H, Babina M, Henz B M, Zuberbier T. Evidence for a restricted rather than generalized stimulatory response of skin-derived human mast cells to substance P. *J Neuroimmunol* 2005; **163**: 92-101.

S7 Harvima I T, Karkola K, Harvima R J et al. Biochemical and histochemical evaluation of tryptase in various human tissues. *Arch Dermatol Res* 1989; **281**: 231-237.

S8 Kivinen P K, Kaminska R, Naukkarinen A, Harvima R J, Horsmanheimo M, Harvima I T. Release of soluble tryptase but only minor amounts of chymase activity from cutaneous mast cells. *Exp Dermatol* 2001; **10**: 246-255.

Supplementary Figure Legends

Supplementary Figure S1

Expression of histidine decarboxylase (HDC) and histamine by HMC-1, LAD 2 and skin MC

- A) Expression of HDC, as determined by qRT-PCR. Relative expression levels normalized to the housekeeping gene β -actin are shown and are the mean $\pm$ SEM of 10 (cell lines) and 16 skin MC) separate cell preparations.

- B) Histamine content in the three MC subsets, as determined by histamine analyzer. The results are shown in pg/cell and are the mean $\pm$ SEM of 20 separate cell preparations.

* ($p < 0.05$), ** ($p < 0.01$) significantly lower than in skin MC (Kruskal-Wallis test)

Supplementary Figure 2

Expression of the receptor tyrosine kinase c-kit by HMC-1, LAD 2 and skin MC

- A) Expression of c-kit transcript by quantitative RT-PCR. Relative expression normalized to the housekeeping gene β -actin is shown as the mean $\pm$ SEM of 10 (cell lines) and 16 skin MC) separate cell preparations .
- B) Expression of c-Kit protein at the cell surface. Staining was performed with the anti-c-Kit antibody A3S6E2 (gray line) and analyzed by flow-cytometry, the darker line corresponds to the isotype control. Representative histogram overlays of at least 8 individual experiments are shown. * ($p < 0.05$), significantly lower than skin MC (Kruskal-Wallis test).

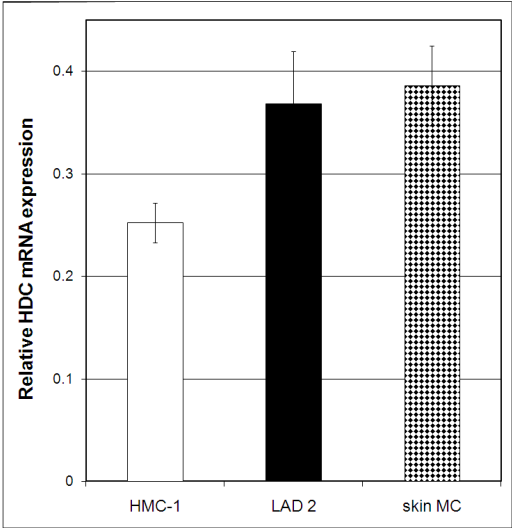
Supplementary Figure 3

Substantial differences in the expression of tryptase and chymase among MC subsets which can barely been uncovered by flow-cytometry.

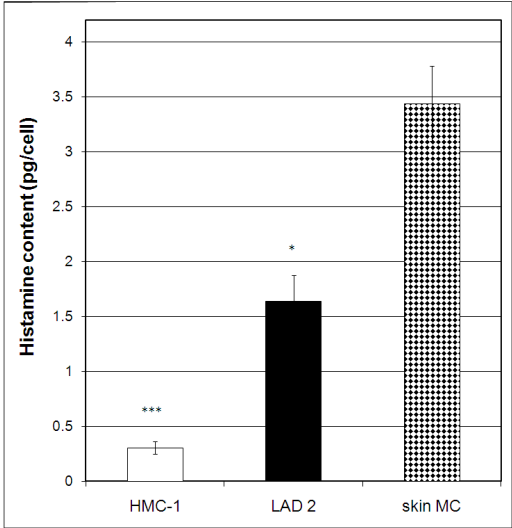
- A) Expression of tryptase by flow-cytometry. Staining was performed with the anti-Tryptase mAb AA1 (gray line) and analyzed by flow-cytometry, the darker line corresponds to the isotype control. Representative histogram overlays of at least 8 individual experiments are shown.
- B) Tryptase and C) Chymase expression by Western blot analysis. Adjacent lanes show two separate preparations of each cell subset.

Supplementary Figure 1

A

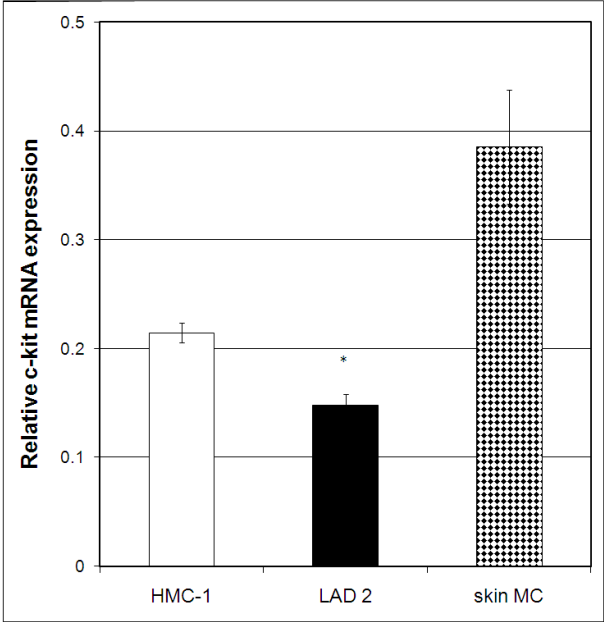


B

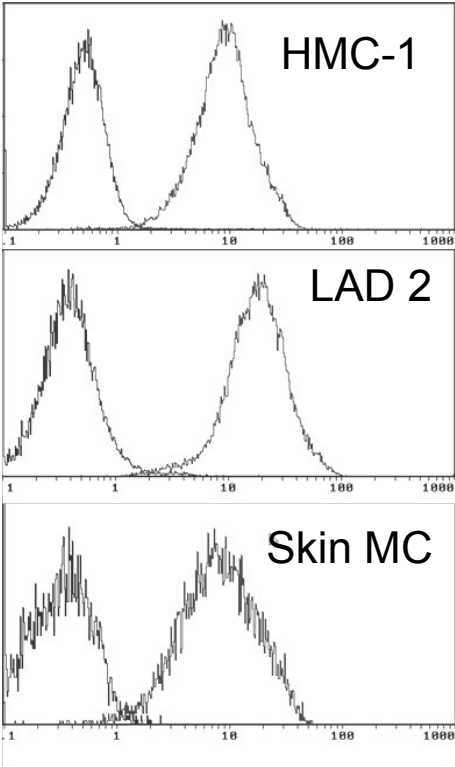


Supplementary Figure 2

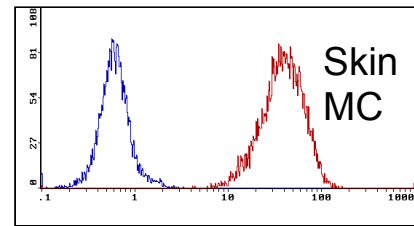
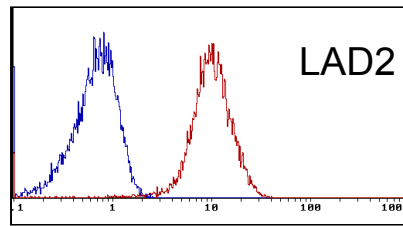
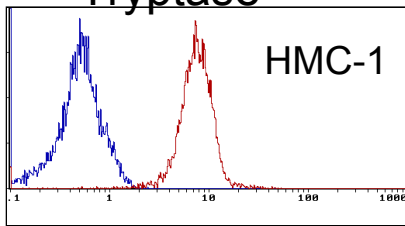
A



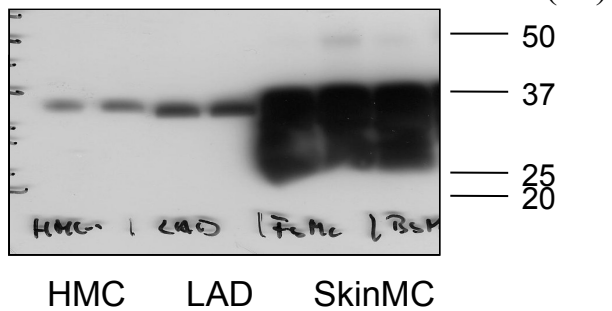
B



A Tryptase



B Tryptase



C Chymase

