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Skin mast cell phenotypes between two highly divergent cohorts – more pronounced variability within than between groups

Running Title: Mast cell diversity between skin compartments

Magda Babina, Sven Guhl, Metin Artuc, Torsten Zuberbier

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

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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

Abstract

Mast cells (MCs) mature in organs like the skin. Here, we studied whether MCs from foreskin (fs) and breast skin (bs) tissue displayed general differences in lineage traits. In-group variation for FcεRI expression and function was pronounced independently of the skin

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subset. Granule mediators showed less variability within groups, and some difference between groups, with bsMCs containing less histamine than fsMCs. Some inter-group difference was found for tryptase and chymase. In conclusion, while the skin compartment can shape its MCs in a donor-independent way, the impact of individualities seems more decisive than sex, age and common attributes of the location.

BACKGROUND

Mast cells (MCs) are hematopoietic cells that complete their differentiation exclusively in tissues (1,2). These unique elements of the human body are the main effector cells of IgE-mediated allergic disorders, but may additionally contribute to various other processes (1-4). Our current knowledge of human MCs is limited, partially as a result of the heterogeneous nature of the cells with diversity across types, tissues, subcompartments and differentiation stages [(5) and references therein]. We recently reported that foreskin MCs display inter-individual variability but with clear-cut graduation among traits (5). The skin itself is not a homogeneous organ, but displays diverse features in distinct locations. Thus, the specific requirements of each skin compartment are potential variables to shape its resident MCs.

QUESTIONS ADDRESSED

We investigated whether MCs from two highly divergent skin subsets (female adult breast skin versus male juvenile foreskin) would display similar degrees of variability within each cohort. We also asked if the skin subset influenced its MC population, i.e. to what extent MC features were tissue-imprinted (including specific location, age and sex) and to what degree they resulted from individualities beyond these parameters.

EXPERIMENTAL DESIGN

MCs were isolated from either foreskin (fs) or breast skin (bs) tissue, yielding >98% pure MC suspensions (5-7). Cells were analyzed for FcεRI expression (flow-cytometry), tryptase and chymase activity, tryptase abundance (ImmunoCAP™), histamine content, and histamine

release by FcεRI-crosslinking. All experimental procedures are detailed in the online supplement.

RESULTS

Morphology and MC size

MCs from the two groups were morphologically indistinguishable. However, fsMCs had a slightly, yet significantly greater average diameter than those from breast skin tissue (median values of 10.82 versus 10.61 μm, respectively; Fig. S1A-B). Both skin MC subsets stained bright purple with acidic toluidine blue (Fig. S1C).

FcεRI expression and function display inter-individual variability

As shown in Fig. 1A, FcεRI expression varied strongly among individuals and this applied to fsMCs and bsMCs alike (CV 105.2% and 117.6%, respectively). Note that the CV is defined as SD/mean, whereas the median is given in the figures because the data were typically not normally distributed. FcεRI-triggered histamine release likewise differed substantially and both cohorts displayed a similar level of variance (Fig. 1B, CV 61.9% for fsMCs, 63.0 for bsMCs). Collectively, bsMCs (like fsMCs) from certain subjects are highly susceptible to FcεRI-elicited responses, while those from other individuals are fairly resistant to this classic type of MC stimulation, as recently reported for fsMCs (5).

Variability in either FcεRI expression or in stimulated histamine release was not accompanied by general differences between the compartments, substantiating that individual manifestation of these traits may be driven by factors not primarily afforded by the skin site itself (including different sex or age).

Granule-associated mediators

MC granules are the most salient feature of the lineage, and the mediators they harbor are relevant to many MC functions and clinical settings (2,8).

Focusing on the cellular amount of histamine, the primary target of anti-allergic drugs, we found relatively little variation within each cohort (CV bsMCs: 34.7% vs. fsMCs: 21.7%), but a difference between fsMCs and bsMCs. In fact, bsMCs contained $\approx 70\%$ or $\approx 63\%$ of the fsMC amount by mean or median, respectively (fsMCs vs. bsMCs: mean 2.94 vs. 2.05 pg/cell; median 2.94 vs. 1.87 pg/cell). The MC specific proteases, which are barely expressed by non-MCs (3,8), displayed intermediate variability with CVs of 34.3, 39.2, 32.7% (fsMCs) and 55.9, 57.6, 35.4% (bsMCs) for chymase, tryptase activity and tryptase protein, respectively.

Both proteases showed statistically tangible differences between the cohorts, yet in opposite ways. Chymase activity was lower in bsMCs vs. fsMCs (84% by mean, 68% by median), while tryptase protein appeared higher in bsMCs (196% by mean, 212% by median), although the number of samples was fairly low. Tryptase activity tended to be higher in bsMCs, even if less pronounced (150% by mean, 118% by median). Together, granule mediators seem to be influenced by the skin compartment, and this primarily applies to histamine.

CONCLUSIONS

We show that similar levels of MC variability exist in different skin locations. For all features investigated, the variance was equal or (slightly) higher for bsMCs, but this partially reflects the lower number of bsMC samples included in most analyses. Importantly, the variability in IgER expression and function, as observed recently (5), is not restricted to a particular skin location, age range or sex, but seems a general phenomenon brought about by individual factors. Consistently, no general differences in skin MC number or distribution due to sex or age could be detected in an earlier study (9). Because there is diversity *within* each group, while no difference is found *between* the groups, general traits of a particular skin

compartment do not seem dominant contributors to IgER-triggered releasability. Since MCs from all donors can become high responders in optimal environments (7), this likewise precludes genetic differences of the MCs themselves as the sole regulators of FcεRI responsiveness. Regardless of the molecular background, it seems plausible that variability in the FcεRI pathway potentially contributes to the divergent proneness of sensitized individuals to manifest allergic disorders.

The micromilieu provided by a particular skin compartment may nevertheless also shape its MCs in a general, donor-independent way, as exemplified by the granule constituents, especially histamine, which showed significant differences between groups. Interestingly, chymase and histamine were both higher in fsMCs, whereas the opposite was true for tryptase. Because of substantial overlap, the differences for tryptase and chymase seem rather subtle, however, and were revealed thanks to the large amount of samples available for the study.

Collectively, inter-individual MC variability can be found in different skin compartments. It is particularly pronounced for the IgER pathway. MCs are likely molded by multiple factors both emanating from the immediate vicinity within the skin and provided systemically. The future challenge will be to pinpoint the major contributors, which create the inter-individual differences.

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Figure legends

Figure 1

Expression and function of FcεRI display tremendous variation among individuals in both skin compartments

Left: Quantification of FcεRI protein expression at the cell surface by flow-cytometry in multiple MC preparations from the two skin subsets. MFI = Mean fluorescence intensity. Note that a logarithmic scale is used to account for the full range of variability. Right: Net histamine release mediated by FcεRI aggregation in fsMCs and bsMCs. Each dot depicts MCs from one donor, the bar gives the median.

Figure 2

Granule constituents are differentially expressed by bsMCs versus fsMCs in a mediator-dependent manner

Histamine was quantified by an auto-analyzer based method, while chymase and tryptase activities were measured by cleavage of specific substrates, and total tryptase was quantified by the Immuno-CAP system, as described in suppl. Methods. Each dot corresponds to one MC preparation (donor); the bar shows the median. ** p < 0.01, **** p < 0.0001.

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Figure 1

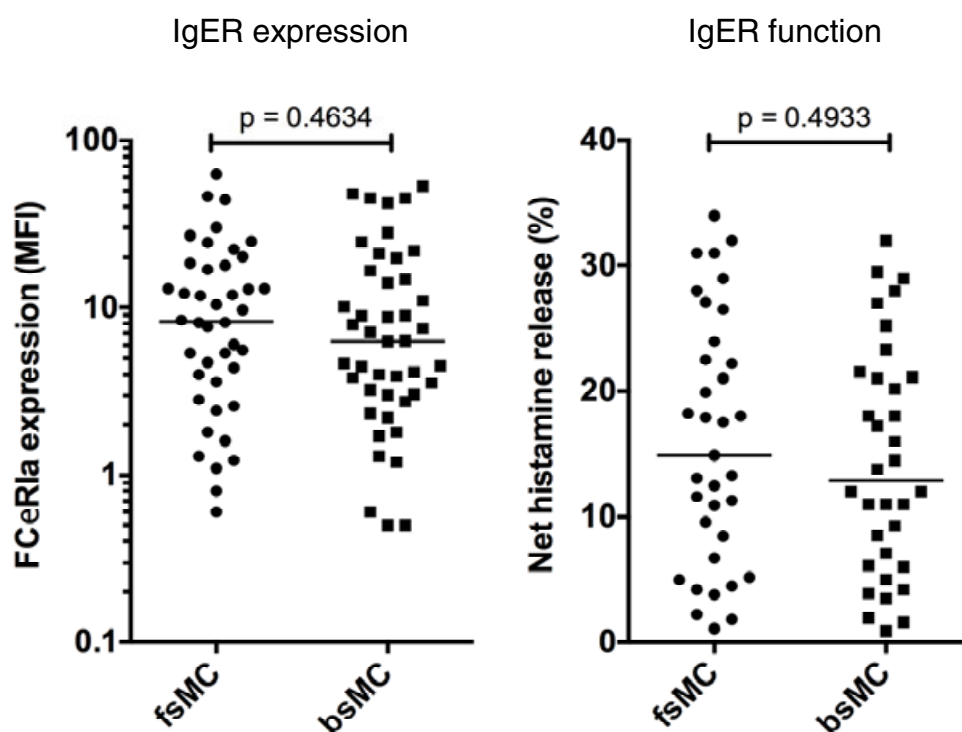


Figure 2

