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Mast cells (MCs) are tissue-resident innate immune cells mostly present in connective and mucosal tissues, as in those constituting the skin, lung and gastrointestinal tract. MCs play key roles in host defense against pathogens and venoms but are also directly involved in the development of several pathological conditions such as allergic reactions, mastocytosis, atherosclerosis and cancer[1]. MCs express several receptors including the high affinity receptor for IgE (FcεRI), CD117/c-Kit, Mas-related G-protein coupled receptor member X2 (MRGPRX2), several Toll-like receptors (TLRs) and complement receptors, allowing them to sense and respond to several innate stimuli. According to the MC receptor involved, activation triggers the release of preformed mediators stored in granules and/or of neosynthetized mediators such as short lived prostaglandins and leukotrienes and, at a later time point, cytokines. The presence of cytoplasmic granules and the ability to degranulate within minutes allows MCs to respond faster than most immune cells upon physiological or pathological activation. MC granules contains a wide variety of molecules including bioactive amines (histamine), proteases (chymases, tryptases), anti-microbial peptides, proteoglycans and cytokines[2].

Several MC-deficient mouse models, mice with constitutive granule compound-deficiency and mice with MC-specific deficiencies for target genes have been developed to study the role of MCs *in vivo*. These tools have substantially increased our knowledge on the role of MCs in physiological and pathophysiological conditions. Moreover, experimental studies *in vitro* have provided a better understanding of the molecular mechanisms underlying MC functions such as the signaling events

involved in FcεRI-dependent degranulation. Despite the strong improvement in cell separation techniques during the past decades, purification of human tissue-resident MCs remains unpractical due to the low number of MCs in tissues and the issues related to the obtention of large biopsies. As MCs differentiate in peripheral tissues from blood progenitors arising from bone marrow, blood has been used as primary source to generate human MCs *in vitro*, giving rise to cord/peripheral blood-derived MCs (CB/PB-MCs). MC cell lines have also been generated and several human cell lines (HMC-1, LAD, LUVA, ROSA, MCPV-1) are currently available. These models display specific advantages and limits (Table 1), and no model fully recapitulating human tissue-resident MC phenotype is currently available.

In this issue of *the Journal of Allergy and Clinical Immunology*, Luo et al. described a new protocol for the generation of MCs from human induced pluripotent stem cells (iPSCs), namely iPSC-derived MCs (iPSC-MCs)[3]. Authors developed a four steps protocol where they first differentiate human iPSCs into hematopoietic stem cells (HSCs). The following steps are similar to classical protocols using HSCs from blood, in which HSCs are expanded and differentiated into MCs. Despite the additional step of iPSCs culture and its associated extra cost, the advantages of initiating the process from iPSCs are that these cells provide a self-renewing, stable and standardized material compared to blood progenitors. iPSCs are produced from somatic cells that are reprogrammed to become pluripotent and may be theoretically generated from any source, such as cells from patients harboring a specific mutation. In the past few years, iPSCs banks were generated and made accessible to the scientific community in order to share iPSC lines and improve the consistency between laboratories. Similar to MC cell lines, iPSC culture can be easily scaled up to provide a high number of MCs. iPSC-MCs may therefore be of high value for experiments requiring a large amount of cells with a high level of phenotypic homogeneity over time, such as for drug screening or mast cell activation tests (MAT). However, the greater reproducibility conferred by iPSC-MCs is achieved at the expense of genetic diversity. Primary human tissue MCs and *in vivo* experiments in animal models remain the gold standard methods to assess the biological relevance.

iPSC-MCs displayed all the characteristics of mature MCs such as the presence of abundant cytoplasmic granules containing tryptases, chymase and histamine or a high expression of the FcεRI and CD117/c-Kit. Furthermore, iPSC-MCs properly degranulated in response to IgE and substance P, although at a slightly lower level than primary skin MCs. RNA sequencing analysis of iPSC-MCs and skin MCs indicated a high similarity between these two MC models but also revealed a non-negligible divergence in the transcriptomic signature. Among the differences, Luo et al. observed a less "mature" signature in iPSC-MCs and a lower expression of FcεRI and MRGPRX2, probably underlying the reduced degranulation in response to IgE and substance P. Of note, MC cell lines and other MC models derived from progenitors, such as CB-MCs and PB-MCs, are also usually less mature than tissue MCs (Table 1), probably because the differentiation steps occurring physiologically in the tissue environment are extremely difficult to fully recapitulate *in vitro*. Moreover, MCs not only display some tissue-specificy *in vivo* but also different subpopulation coexist within the same tissue, with differences affecting granule composition[2], receptor expression[4] and response to stimuli[5] (Table 1). Various modifications in culture conditions were proposed to mimic these tissue- and subpopulation-specific phenotypes and favor the differentiation of MC with either a higher tryptase or chymase activity. Among them, in addition to stem cell factor (SCF) and interleukin (IL)-3 commonly used for MC differentiation, IL-6 or IL-4 were also employed to improve MC differentiation towards one of this phenotype. The culture conditions and the cytokines employed are critical for the

yield and maturation of MCs and probably underlie the large variability observed between groups for the same MC model. Luo et al. used SCF, IL-3, IL-6 and, in a rather uncommon way, low-density lipoproteins (LDL) as a differentiation cocktail. In fact, the same authors had previously proposed LDL as a source of lipids to support proliferation and the high requirement of phospholipids for granule formation[6]. In serum-free culture conditions, to counteract the effect of factors preventing MC proliferation and differentiation present in serum, Luo et al demonstrated that the addition of LDL strongly improved the yield of mature MC[6]. While not being totally innovative compared to published protocols using blood progenitors, Luo et al. provide a straightforward protocol using only commercial and standardized reagents to generate phenotypically homogenous MCs from IPSC lines. iPSC-MCs generated with such a protocol are mature, functional, close to tissue-resident MCs and appropriated for studying both IgE-dependent and IgE-independent activation. Whether iPSC-MCs are also suitable for studying other MC functions such as phagocytosis, IgG-dependent activation or production of extracellular traps remains to be determined.

The limited amount of mature MCs in tissues has led to the development of a large variety of *in vitro* models. MC cell lines are easy-to-grow and provide cells with an homogeneous phenotype but show several abnormalities, such as immature phenotypes and the lack of key MC characteristics[7,8] (Table 1). CB/PB-MCs are a widely used model for human studies that show a highly similar phenotype with primary MCs[3,9] (Table 1). However, CB-MCs express less FcεRI and show a reduced IgE-dependent activation than PB-MCs[10]. Unsurprisingly, iPSC-MCs, that are differentiated in a similar manner as CB/PB-MCs, also exhibit an advanced maturity and functionality close to that from tissue-resident MCs (Table 1). Both CB/PB-MCs and iPSC-MCs are interesting models that phenocopy several features of tissue-resident cells. The main limitations of CB/PB-MCs are the donor's heterogeneity and the difficulty of upscaling MCs production. Both issues can be solved by the use of IPSCs. Thus, iPSC-MCs represent an interesting alternative when a large amount of phenotypically homogenous MCs is required or for long term studies, as they provide a stable material that should help to reduce inter- and intra-laboratory variability.

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Table 1: Main characteristics of the most common *in vitro* models of MCs.

	Tissue-resident MCs	**Cell lines**	**CB/PB-MCs**	**iPSC-MCs by Luo et al.**
Availability of material	Limitation of biopsy (access/ethics, size, ...)	Easy to expand and maintain	Limitation of blood (access/ethics, volume, ...)	iPSCs easy to expand and maintain
Donor heterogeneity	Yes	No	Yes	No
Preparation time	4 to 24 hrs	Ready to use	21 to 63 days	52 days
Typical range yield	0.1 to 1 x 10^6 per g of tissue.	> 10^6 (doubling time from 48 hrs to 2 weeks	0.5 to 5 x 10^6 per 50 mL of blood	0.5 to 0.8 x 10^6 per 40 iPSC colonies
Cell surface markers				
CD117/c-Kit	++++	+++(+) (WT or mut)	++++	++++
FcεRI	++++	- (HMC-1) or ++(+)(+)	++(+)(+)	+++(+)
MRGPRX2/MrgprB2	+(+)(+)(+)	+(+)(+)	+++	+++
Granule composition				
Histamine	+++	+(+)	++++	++++
Tryptases	++++	+(+)	++++	++++
Chymases	- or ++++	- (HMC-1) or +(+)	++++	++++
Degranulation in response to				
IgE-mediated receptor cross-linking	++++	- (HMC-1) or +(+)(+)	++(+)(+)	+++
MRGPRX2/MrgprB2 receptor activation	+(+)(+)(+)	+(+)(+)	+++	+++

Note: There is currently no study comparing all these models simultaneously in the same settings. It is important to note that several protocols exist for each model giving different level of MC maturation. This table provides a summary of commonly obtained results for each model.