Mast cells derived from systemic mastocytosis exhibit an increased responsiveness to hyperosmolarity

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Abstract

This is a "Letter to the Editor" with no abstract.

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To the Editor,

Systemic mastocytosis (SM) is a disease characterized by increased number of aberrant mast cells in one or several organs and increased systemic levels of mast cell mediators [1]. Indolent SM (ISM) is the most common form of SM constituting approximately 80% of the patients diagnosed with SM. Individuals with ISM often have mediator mediated symptoms, most commonly from the skin, the gastrointestinal tract, cardiovascular and respiratory system, but also in the form of anaphylaxis [2]. Although basal mediator levels, including serum tryptase and metabolites of histamine and prostaglandin D_2 in the urine, are increased at steady state[1,3,4], the symptoms often come as spells without any obvious trigger suggesting an intrinsic defect causing a hyper-reactive state of the mast cells, or an endogenous trigger.

We have previously addressed the hypothesis of a hyper-reactive mast cell phenotype in ISM by *in vivo* provocation of mast cells in the skin and respiratory tract by skin prick application of morphine and inhalation of mannitol, respectively [3]. None of these triggers mounted a response that was different between ISM patients and healthy volunteers (HV). To further investigate the hypothesis of a hyper-reactive mast cell phenotype we also developed mast cells *in vitro* from 14 ISM patients and 13 HV (same subjects as included in[3]). Peripheral blood was obtained, and a CD34-selection was performed. The CD34-positive progenitor cells were cultured using a protocol published by Lappalainen et al.[5] (for details see supplement). When the cells were mature, they were plated and exposed to IgE-receptor activation, morphine or mannitol-induced hyperosmolarity, representing three distinct activation pathways (see supplement). As a read out for mast cell activation we measured the release of histamine (as a measurement of degranulation) and PGD_2 (newly synthesized lipid mediator); i.e., two prominent mast cell mediators, released through two different routes, that are increased in ISM.

The growth and development of mast cells *in vitro* from CD34-selected progenitor cells was followed over a 6-week period. At no point did we observe any difference between cells from ISM patients or HV (Figure 1). This result stands in contrast to a study where a significant increase in mast cell growth from CD34-selected progenitor cells from ISM patients was described [6]. An explanation could be the different culture protocols used in the two studies but could also be a result of the low numbers of ISM patients included by Carter

et al. (n=4), and the big variation in growth[6]. The *in vitro* developed mast cells were plated and exposed to different mast cells secretagogues: the calcium ionophore A23187 (1μM), morphine (1 and 10 μg/ml), anti-IgE (0.02-20 ng/ml) and mannitol (0.3 and 0.7 M). The release of histamine was comparable between mast cells derived from ISM and HV in response to all tested secretagogues (Fig 2A). In contrast, mast cells derived from ISM showed a significantly increased release of PGD_2 in response to mannitol, but not to the other tested triggers (Fig. 2B). It has been reported previously that the release of β-hexosaminidase (released through degranulation) after IgE-receptor activation is the same from mast cells derived from ISM as from HV [6]. However, in that study they neither investigated the secretion of PGD_2, nor other type of secretagogues.

Our study provides the first evidence that mast cells derived from ISM exhibit an aberrant response profile to mannitol-induced hyperosmolarity, with no change in degranulation but an increased synthesis and secretion of PGD_2, the main eicosanoid produced by mast cells. A hyper-reactive mast cell phenotype in ISM is still elusive, but our data indicate that an intrinsic defect in these cells could affect other signaling pathways than the commonly studied downstream of the IgE-receptor, and that other mediator releasing systems than degranulation, i.e., newly synthesized mediators, should be studied. Finally, the increased levels of PGD_2 in ISM support the presence of an increased reactivity of their mast cells to osmolarity changes.

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Conflict of interest

The authors report no conflict of interest

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Figure legends

Figure 1. *In vitro* growth and maturation of mast cells over a 70 days period. CD34-positive peripheral blood cells from healthy volunteers (n= 13) or individuals with indolent systemic mastocytosis (n=14) were cultured under conditions that promote mast cell development. Mean ± SEM.

Figure 2. Release of histamine and PGD_2 from activated*in vitro* developed mast cells. Mast cells were treated for 30 minutes with calcium ionophore A23187, morphine, anti-IgE or mannitol and the release of histamine (A) and PGD_2 (B) was measured in the cell free supernatant. Healthy volunteers (open boxes) (n=6-10) and systemic mastocytosis (filled boxes) (n=7-13). Results are shown as box and whiskers; the box extends from the 25th to 75th percentiles and the whiskers min to max. * $p<0.05$

Figure 1.

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Figure 2.

A

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B

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