Purification and Characterization of Mast Cells from Human Saphenous Veins

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Aim

Mast cells (MC) are a heterogeneous population of cells with a unique activation profile. Often LAD2 cells or human peripheral blood derived mast cells (PBdMC) from hematopoietic stem cells are used for different studies on MC. However, because of the heterogeneous nature of MC, LAD2 and PBdMC may not always represent MC in the specific tissue of interest. MC have been detected in the vascular system but to our knowledge never been isolated and molecularly and functionally characterized in human tissue. This study aimed at developing a method for purification of MC from human saphenous veins (HSV) and further to characterize their functional and molecular profile.

Methods

HSV were obtained from surgery for varicose veins, cut thoroughly and incubated for 1 hour with 1mg/ml collagenase I and 4mg/ml collagenase II in DMEM. The tissue was further dissociated by running it through a 14G needle 25 times before filtering the suspension using a cell strainer. A cytospin of 100,000 cells was made and stained with toluidine blue to assess percentage of MC. Remaining cells were used for either total histamine, histamine release (HR) or flow cytometry. *Total histamine*: Cells were lysed by using 7%$HClO_4$. *HR:* Cells (containing 503±369MC) were stimulated for 1 hour with: 1) buffer, 2) anti-IgE (10,000ng/ml), 3) PMA/ionomycin (330/1,700ng/ml), 4) Substance P (10µM), 5) C3a (1,000ng/ml), 6) C5a (1,000ng/ml) or C48/80 (10-1,000µg/ml). Histamine was quantified fluorometrically in supernatant and lysed cells (3.5%$HClO_4$). Results are given as HR%. HR was also performed on 1 cm vein sections with the same concentrations described for suspension cells. *Flow cytometry*: Cells were tested by flow cytometry for the following markers: CD126, CD203C, TSLPR, CD117, CD123, IL-18R, ST2, CD294, CD88, FcεRIα, C3aR, CD124, CD48, CD32, CD200R, CD172a and CD300a. Isotype controls were run in parallel each time.

Results

MC were successfully isolated from HSV with a purity of 0.33±0.18% (n=21). Total histamine for HSV MC was 22.25±10.8pg/MC (n=5) which was much higher compared to LAD2 (1.2pg/MC, n=1) and PBdMC (11.1±8pg/MC, n=8). Despite the high amount of histamine/MC in the HSV MC, they only responded to PMA/ionomycin with a HR of 26.3±8% which was much lower than the response seen in LAD2 and PBdMC (72.1% (n=1) and 86.5±9.8% (n=4), respectively). HSV MC were identified on flow cytometer as CD117+FcεRI+. These were also positive for CD200R and CD203c, the latter at a higher level than LAD2 and PBdMC. Opposite to LAD2 and PBdMC no CD172a or C3aR was detected and the levels of CD32, CD300a and CD48 were lower in HSV MC. HR on 1 cm HSV sections demonstrated a heterogeneous response to anti-IgE (1 out of 3 reacted), PMA/ionomycin (2 out of 4 reacted), C3a (1 out of 4 reacted), while little or no response was observed for C5a, SP and C48/80.

Conclusion

A method for isolating mast cells from human saphenous veins was successfully established. The isolated mast cells demonstrated surprisingly high histamine content compared to both LAD2 and PBdMC. However, the process of isolation might activate the cells or reduce surface markers rendering them unresponsive to release histamine.