Culture of human mast cells – the influence of progenitor source and culture conditions

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ABSTRACT

Mast cells (MC) are widely distributed throughout the body and have been showed to participate in various cellular mechanisms. Allergic reactions are the most examined, but recent investigations have focussed on the role of MC interactions in diseases such as mastocytosis, atherosclerotic plaque, cardiomegali, SIDS, hypertension and renal diseases. The knowledge of these interactions and understanding of MC differentiation and function has increased considerably in the past centuries through studies on rodent mast cells or human mature mast cells obtained from lung-, skin- or intestinal biopsies as well as human MC lines. However, cell yields and MC phenotype vary to a great extent between these model systems, which emphasises the need for in vitro generation of mature human MC comparable to the MC residing in tissues in vivo.

Initially in this PhD project, experiments were conducted with cord blood derived CD133+ progenitors cultured for 12 weeks. The MC generated were metachromatic stained with Alcian Blue, contained histamine, and were tryptase and chymase positive. Flowcytometry analysis showed low FcεRI expression, and only modest histamine release upon anti-IgE stimulation of the IgE-sensitized MC was observed. Thus, further optimization was required to investigate receptor mediated activation in detail. Thus, we developed a 7-week protocol for generating functional human mast cells from CD133+ progenitors isolated from peripheral blood and cultured under the influence of stem cell factor (SCF), IL-6 and FCS. MC contained tryptase and expressed functional levels of FcεRI. Anti-IgE stimulation induced significant release of histamine and PGD2.

The differences in MC phenotype and functionality observed during the development of the protocol above encouraged us to investigate the effect of various culture conditions used for generation of mature MC. Next, the effect of culture time, cell density, media renewal, and addition of IgE for MC maturation was investigated. Most parameters investigated were demonstrated to influence MC phenotype or function or both and this led to the comparison of MC established from peripheral or cord blood progenitors cultured in parallel.

During the last two decades different scientific groups have investigated the phenotype and function of in vitro generated human MC. The cells have been shown to display variable surface markers and functional characteristics. To investigate the significance of different progenitors, we compared MC generated from CD133+ progenitor cells from cord blood (CBMC) or peripheral blood (PBMC). MC were tryptase+, regardless of the source of progenitor cell. The density of ckit/CD117 receptors on CBMC was significant higher than that of PBMC. The density of CD203c and FcεRI was significantly higher on PBMC. PBMC contained and released significant more histamine and PGD2 upon ligation of FcεRI. Culture with IL-4 increased expression of tryptase, FcεRI, CD117 and CD203c, secretion of histamine and PGD2 of PBMC, while only histamine secretion was increased for CBMC. Thus, cord and peripheral blood may give rise to different types of MC.

The question addressed in the individual investigation should determine the choice of progenitor cell and protocol.